THE INFLUENCE OF OXYGEN ON THE ISOLATED HUMAN UMBILICAL ARTERY

A Thesis presented for the degree of Doctor of Philosophy

By

Stephen John MacLennan, BSc.

Institute of Physiology,
University of Glasgow.

December, 1986.

Table of Contents

List of tables

In the following list "low Po_2" (low oxygen tension) and high Po_2 refer to Po_2's of ~15mmHg and ~120mmHg respectively. Precise values are given in the text.

List of figures

In the following list, "low Po_2" (low oxygen tension) and high Po_2 refer to Po_2's of ~15mmHg and ~120mmHg respectively. Precise values are given in the figure or in the figure legend.

Abreviations

Term	Abreviation
Human umbilical artery	HUA
Concentration-response curve	CRC
Oxygen	O_2
Oxygen tension	Po_2
5-hydroxytryptamine	5-HT
5-carboxamidotryptamine	5-CT
Molar	M
Cyclo-oxygenase inhibitor	COI
Confidence limits	C.L.'s
Prostacyclin	PGI_2
Calcium	Ca^{++}
Extracellular calcium concentration	$[Ca^{++}]_e$
Antagonist-resistant	AR
Antagonist-sensitive	AS
Intra-uterine growth retardation	IUGR
Patent ductus arteriosus	PDA

Acknowledgements

My principal thanks are extended to my supervisor Dr. J.C. McGrath for giving me the chance to be able to work on this project and for his enthusiastic help and interest in the subsequent three years. Thanks also to Dr Martin Whittle of the Queen Mothers' Hospital, Glasgow, for his suggestions during the project and for his help in co-ordinating the collection of tissue with the staff of the labour suite, the Queen Mothers' Hospital, to whom I am also indebted.

I must also thank the others, past and present, of THE AUTONOMIC PHYSIOLOGY UNIT, who, under Ian McGrath, have provided a stimulating research environment. They are: Alison, Andy, Christine, Craig, Eddie, Funmi, Ian, Jacky, Janette, Tom and Vince.

Lastly, and certainly not least, I have to thank my fiance, Alison, for her constant support throughout the last three years.

Declaration

The experimental work and other research which is contained within this thesis was undertaken wholly by myself. None of the material has previously been presented for any other degree. Some of the results have been published during the period of this study, details of which are given below.

Publications:

McGrath, J.C., MacLennan, S.J., Mann, A.C. & Stuart-Smith, K.(1984). Analysis of the contractile responses of human umbilical blood vessels to 5-HT and oxygen. Br. J. Pharmac., **82**, 290P.

McGrath,J.C., MacLennan, S.J. and Stuart-Smith, K. (1985). Characterization of the receptor mediating contraction of the human umbilical artery by 5-Hydroxytryptamine. Br. J. Pharmac., **84**, 199–202.

McGrath, J.C., MacLennan, S.J., Mann, A.C., Stuart-Smith, K. & Whittle, M.J. (1986). Contraction of human umbilical artery, but not vein, by oxygen. J. Physiol., **380**, 513–519.

McGrath, J.C. and MacLennan, S.J. (1986). Oxygen modifies the potency of 5-HT, and of 5-HT antagonists, in human umbilical artery. Br. J. Pharmac., **88** 320P.

MacLennan, S.J. and McGrath, J.C. (1986). Evidence for "5-HT_1-like" receptors in human umbilical artery. Br. J. Pharmac., **89**, 587P.

Summary

This study is an investigation of two aspects of the effect of oxygen on the isolated human umbilical artery. The physiological environment of the artery, with respect to the blood, in utero, is both acidic and de-oxygenated, i.e. pH ~7.28, Po_2 ~15mmHg. The major part of this work uses longitudinal strips of the artery which were initially set-up under these physiological conditions.

The experimental work is divided into three chapters. Chapter 1 is an assessment of the 3 main techniques used to measure smooth muscle contraction of isolated preparations, i.e. isometric and isotonic contraction, and constant flow perfusion pressure. It was found that the potency of 5-hydroxytryptamine (5-HT), bradykinin and adrenaline to cause contraction was similar using any of the techniques. At low Po_2 (the physiological level) 5-HT was more potent than bradykinin while adrenaline weakly contracted only the perfused artery. The order of potency was 5-HT>bradykinin>>adrenaline. At high Po_2 (~120mmHg) adrenaline produced constriction of the artery when assessed by any of the techniques. The order of potency was 5-HT>bradykinin>>adrenaline.

The vasoconstrictor effect of raising the oxygen tension above the physiological level was similarly assessed and it was found that the sensitivity of the smooth muscle was similar using isometric or isotonic contraction, or constant flow perfusion pressure. However, when using the latter technique the artery required to be under longitudinal tension for oxygen to cause contraction. From this it was concluded that oxygen may act on the longitudinal muscle alone. Since the potency of 5-HT, bradykinin and adrenaline, and of raising the oxygen tension, was similar in the three techniques there seemed to be no advantage in using any one technique for assessing contraction of the longitudinal muscle, in preference to the other two. Isometric

contraction was used in all subsequent experiments. This comparative study of the techniques used to assess contraction of the human umbilical artery failed to resolve differences between the potency of various agonists, as reported in the existing literature.

In chapter 2 a further investigation is made of the vasoconstrictor effect of oxygen. Raising the oxygen tension above 15mmHg evoked concentration-related contractions. In the initial experiments a threshold for contraction was predicted: at pH 7.28 this was 36mmHg and the maximum response occurred at 297mmHg. However, in further experiments raising the oxygen tension from 0mmHg to 15mmHg caused a contraction. This argued against a threshold for contraction. This effect of oxygen was mediated by prostaglandins: the cyclo-oxygenase inhibitor, indomethacin, inhibited oxygen-induced contractions at nanomolar concentrations. Flurbiprofen was approximately equipotent to indomethacin, while aspirin was more than 10,000 fold less potent.

From a study of the influence of gestational age on the effect of oxygen it could be concluded only that the smooth muscle of the umbilical arteries, from infants at least of 27 weeks gestation, could synthesise constrictor prostaglandins since this was the earliest gestational age at which oxygen caused contraction. Only one other preparation of a lesser gestational age (26 weeks) and which was not contracted by oxygen, was examined. No significant correlation was found between gestational age and sensitivity to oxygen, or the size of the maximal oxygen-induced contraction. Umbilical arteries were similarly sensitive to 5-HT at all gestational ages which were tested, from 26 weeks to 41 weeks.

In chapter 3 the receptors for 5-HT and adrenaline are characterized. At the physiological Po_2, methysergide and ketanserin were potent competitive antagonists of the contractile response to 5-HT

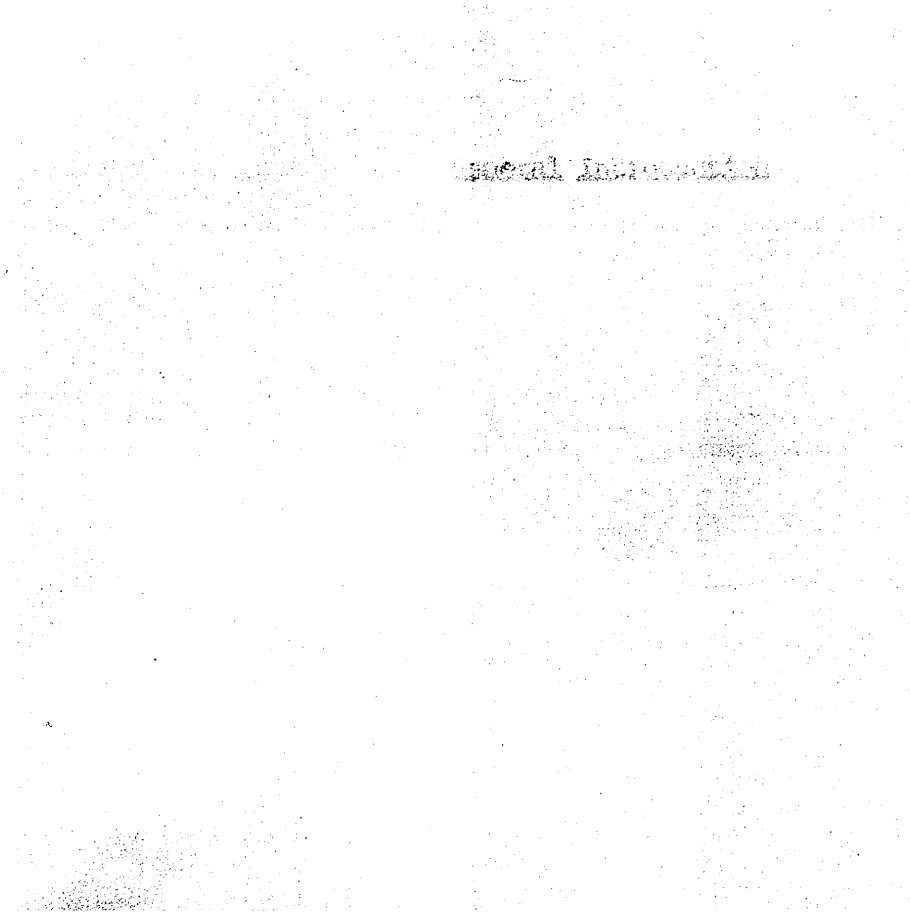
whose affinities for the vascular receptor and at the 5-HT_2 ligand binding site in brain tissue were similar. It is this evidence which primarily allows the receptor in the human umbilical artery, at the physiological oxygen tension, to be called 5-HT_2. Adrenaline did not contract the artery at physiological oxygen tensions.

At high oxygen tensions (~120mmHg) concentration-response curves to 5-HT in the presence of ketanserin were biphasic: low concentrations of 5-HT were resistant to blockade by ketanserin but were antagonised by cyclo-oxygenase inhibitors. The response to low concentrations of 5-HT was mimicked by an analogue, 5-carboxamidotryptamine (5-CT) with a greater potency than 5-HT. These two observations suggest that at high Po_2 5-HT acts at a receptor which is not 5-HT_2, but which can be called 5-HT_1-like. The functional expression of the 5-HT_1-like receptor requires prostaglandins. The response to higher concentrations of 5-HT was sensitive to blockade by ketanserin, but not by cyclo-oxygenase inhibitors. This component of the response to 5-HT was mediated by the 5-HT_2 receptor.

It was found that both 5-HT_2 and 5-HT_1-like receptor mediated responses to 5-HT were antagonised by phentolamine, a non-selective alpha-adrenoceptor antagonist, and by Wyeth 26703, a selective $alpha_2$-adrenoceptor antagonist (with regard to adrenoceptor sub-types).

At high Po_2 there is a small functional population of $alpha_1$ adrenoceptors: The response to adrenaline was competitively antagonised by the $alpha_1$ antagonist, prazosin, while the selective $alpha_2$ antagonist, Wyeth 26703, did not cause blockade of the response to adrenaline. This conclusion was reinforced by the evidence of selective agonists: phenylephrine ($alpha_1$) was ~30 fold less potent than adrenaline while xylazine ($alpha_2$) and UK 14304 ($alpha_2$) did not cause contraction.

In the general discussion a detailed scheme is put forward, by which closure of the umbilical artery may occur at birth. Central to this proposal is the increase of the oxygen tension of umbilical arterial blood at birth. It is suggested that the increase in oxygen tension ultimately leads to constriction of the umbilical arterial smooth muscle via prostaglandins, thromboxanes and 5-HT.



General Introduction

Interest in the modulatory role of oxygen (O_2) on smooth muscle activity, both direct and indirect, can be traced back to at least the 1920's. As Garry (1928) observed "it (was) often tacitly supposed that an inadequate supply of O_2 causes a fall of tone and a cessation of movement in surviving smooth muscle. Yet, in the physiological literature...there is no unequivocal evidence in support". His paper represented the first quantitative assessment of the effect of O_2 lack on smooth muscle. Garry replaced the O_2 aerating the saline solution with nitrogen, and demonstrated that spontaneous contractions of smooth muscle preparations were abolished and that the general tone declined. This was found almost without exception in uterine and intestinal preparations from several species. He also demonstrated that in the cat uterus adrenaline was without effect in nitrogen and in O_2 caused a marked contraction.

These two effects of O_2 on smooth muscle, an effect on the tone and by modifying the action of agonists, shall be discussed.

Direct effect of O_2 on smooth muscle tone

In general the effects of O_2 on smooth muscle have been studied by reducing the Po_2 of the bathing or perfusate saline from a value greater than or equal to the physiological level, to a value less than the physiological normal, i.e. hypoxia-induced responses. The common finding has been that vascular strips (with inherent tone or where tone is induced pharmacologically or by depolarisation) respond to graded reductions in bathing solution Po_2 with graded relaxation. Evidence for this apparent direct effect of O_2 on vascular smooth muscle has come from numerous studies on strips isolated from large vessels. These studies have proposed several mechanisms for this behaviour. This has been reviewed by Pittman (1981).

It has been suggested by many investigations (Garry, 1928; Smith

and Vane, 1966; Detar and Bohr, 1968) that hypoxic vasodilation is due to a limitation of energy. However this view has not gained any general acceptance apart from the case where substrate availability in the bath limits ATP production (Paul, 1980). Garry (1928) found that the effects of reducing the Po_2, and of cyanide, were similar in causing relaxation and hence suggested that the effects were on oxidative processes, as had earlier been shown in striated muscle (Hill, 1928a). Coburn et al (1979) have shown that strips of rabbit aorta retain some sensitivity to O_2 after tissue respiration is abolished by cyanide. These findings show that mechanisms other than a limitation of ATP to the contractile apparatus contribute to the decline in tension observed in hypoxia. As discussed by Paul (1980), vascular smooth muscle exibits a considerable capacity for aerobic glycolysis. Under hypoxic conditions, glycolysis is strongly stimulated in some muscles and appears to be capable of satisfying the energy needs of the muscle at the level required before hypoxic relaxation.

An alternative proposal put forward by Coburn et al (1979) is that oxygen might influence vascular contraction by acting on some specialised "O_2 receptor" on the vascular smooth muscle cell and that the surface membrane may be involved in the transduction process. Their conclusion is based upon a comparison of the relation between isometric force and O_2 consumption when the latter is lowered by graded decreases in Po_2 or graded increases in cyanide concentration. The qualitative relationships between force and O_2 uptake were quite different in the two situations, but, as pointed out by Pittman (1981), an unambiguous interpretation of the experiments rests on the determination of the specificity of cyanide for cytochrome oxidase.

Detar (1980) has proposed that the sensing of Po_2 by vascular smooth muscle cells involves the sarcolemmal Na^+-K^+ active transport mechanism. This hypothesis was based on indirect evidence from

experiments on isolated rabbit skeletal and cardiac muscle arteries: ouabain or Li substitution and low temperature (27°C), conditions known to inhibit Na^+-K^+ATPase activity, were found to reverse both hypoxia- and K^+-induced depression of noradrenaline contraction, and this depression occurred only when the external K^+ concentration was in the range of 3–4mM. Detar suggested two schemes to account for hypoxic reversal of contraction, each of which is yet to be tested. The first involves an electrogenic mechanism whereby hypoxia causes an increase in membrane permeability to Na^+. The resulting increase in intracellular Na^+ concentration stimulates the Na^+-K^+ pump leading to an increasing hyperpolarisation and depression of contraction. In the second scheme hypoxia causes an increase in Na^+-K^+ATPase activity and/or a decrease in Na^+ permeability and thus there is a decrease in intracellular Na^+ concentration. This leads to an increase in Na^+-Ca^+ exchange and subsequently to depression of contraction. An electrogenic mechanism may operate in this, as in the first scheme: a decrease in intracellular Na^+ concentration would cause there to be a transient hyperpolarisation of the membrane until Na^+-Ca^+ exchange began.

Paul et al (1979) have presented evidence however, which suggests that Na^+-K^+ transport in vascular smooth muscle is specifically coupled to glycolysis, while oxidative metabolism is coupled to the energetics of the contractile process. These observations taken together imply that if oxygen sensing is accomplished through Na^+-K^+ transport processes, the link is likely to be an indirect one.

The other main theory is that hypoxia changes the production rate of some vasoactive substance. Among those substances which have been suggested are those implicated as mediators of local blood flow, e.g. adenosine and prostaglandins, (see Sparks and Belloni, 1978). Van Harn et al (1977) showed that the rate of formation of adenine nucleotide

derivatives (adenosine, inosine and hypoxanthine) in pig carotid artery strips was increased in hypoxic media relative to the formation rate under well-oxygenated conditions. Pittman and Quinn (1979) subsequently tested the hypothesis that increased endogenous adenosine formation in anoxia was responsible for the depression in active isometric force relative to that for well-oxygenated strips of pig carotid artery. Hypoxia-induced relaxation could be fully reversed by adding adenosine deaminase to the bathing medium. This enzyme catalyses the conversion of adenosine to inosine and ammonia, and no evidence was found that either of these products, in the expected concentration range, could reverse the anoxic relaxation, and they postulated that adenosine deaminase removed the smooth muscle cells from the relaxant influence of adenosine.

Kalsner (1976) has reported that hypoxic vasodilation of the isolated bovine coronary artery is mediated by an increased production of a vasodilatory prostaglandin. Reducing the bathing solution Po_2 from 580mmHg to 47mmHg resulted in an eightfold increase in prostaglandin production which was coincident with a fall in tone. Aspirin was added at Po_2=47mmHg and the hypoxic vasodilation was completely reversed. It was suggested that the vasodilatory prostaglandin was PGE_1. It is probably more likely that the prostaglandin was PGI_2 however, since (1) the most abundant precursor for prostaglandin biosynthesis is arachadonic acid which gives rise to prostanoids of the "2 series", e.g. PGE_2, PGI_2. (2) PGI_2 is the major prostanoid metabolite of vascular smooth muscle and which, in the coronary artery (and in most other vascular tissue), is a vasodilator (Moncada and Vane, 1980).

Evidence from other studies suggests that prostaglandins are mediators of both the intrinsic tone of smooth muscle preparations, and of their sensitivity to O_2. Smith and Vane (1966) found that the tone of isolated strips from various intestinal and vascular smooth muscles,

including the rat stomach, varied directly with the Po_2 of arterial blood from donor animals, or of a Krebs solution, with which they were superfused. Eckenfels and Vane (1972) showed that the intrinsic tone of the rat stomach strip was reduced by indomethacin and that the normal contraction induced by raising the Po_2 was abolished by indomethacin. Thus, endogenous prostaglandins were implicated as mediators of O_2-induced smooth muscle tone.

Farmer et al (1974) have similarly shown that the intrinsic tone of the guinea-pig trachea, present at a Po_2 of ~150mmHg (Tyrode solution was aerated with room air) was mediated via prostaglandins, since either indomethacin or the prostaglandin antagonist, SC19220, reduced the tone of the preparations in a concentration-dependent manner.

Smooth muscle responsiveness and Po_2

The limited amount of evidence from earlier studies, after Garry (1928) showed that hypoxia drastically reduced the contractile responsiveness of smooth muscle, can perhaps be most profitably considered by an examination of the evidence presented by Chang and Detar (1980). Their results not only embody the findings of these earlier studies but which also present some novel and important observations.

In their study, isolated rabbit arteries of varying diameter were contracted by adrenaline ($1X10^{-7}M$) at different Po_2's and the contraction (contractile responsiveness) noted. They found that the contractile responsiveness was depressed in the largest arteries (greatest wall thickness) when the bath Po_2 (Pbo_2) fell below ~130mmHg, and in the smallest arteries when the Pbo_2 fell below ~60mmHg. However, from measurements of the luminal surface Po_2 (Pso_2), and knowing such parameters as O_2 consumption, diffusion constants, they were able to

calculate that the critical Po_2 in the vessel wall (Pwo_2), below which contractile responsiveness was depressed, was for either small or large arteries ~50mmHg. Three important conclusions could be drawn from these findings. (1) The sensitivity to hypoxia of vascular smooth muscle cells is similar for large conducting arteries and small parenchymal arteries. (2) The sensitivity to hypoxia falls well into the range of O_2 pressures that is physiologic in situ. (3) The depression of contractile responsiveness of small artery samples observed when Pbo_2 falls below 60mmHg, and that of large artery samples observed when Pbo_2 falls below 130mmHg cannot be explained by an anoxic core hypothesis as previously suggested (Pittman abd Duling, 1973).

Two theories were discussed to try to account for the hypoxic depression of contraction. (1) A limitation of energy supply. However, they had found that the hypoxic depression was greatest at lower, rather than higher, levels of stimulation (stimulation being the concentration of agonist), i.e. the hypoxic depression was greatest when demands on energy production were lowest. This argued against a limitation of energy supply as the basis for the hypoxic depression. Other reasons (as discussed earlier) such as the ability of vascular smooth muscle to sustain hypoxic glycolysis also argue against this theory. (2) The second hypothesis they considered was simply that when Pwo_2 falls below ~50mmHg, some aspect of the contractile apparatus is affected. It is therefore apparent that the manner in which hypoxia depresses contractile responsiveness is still unclear.

Although hypoxia depresses responses in smooth muscle, why are pharmacological experiments routinely carried out in hyperoxia, i.e. when 95% O_2 or 100% O_2 is employed to aerate the saline? Presumably this is (1) to avoid an anoxic core and because (2) it is tacitly assumed that a high Po_2 will do no harm. However these presumptions may

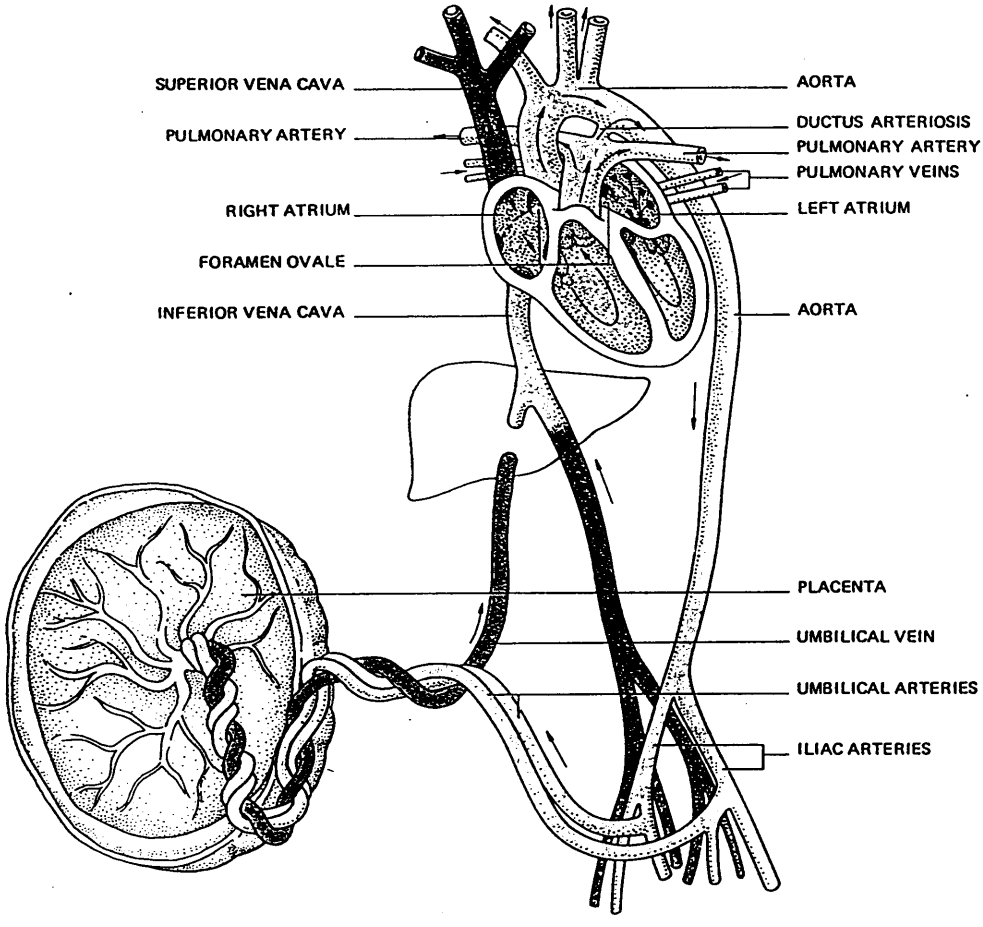


Figure 1 A diagram showing the fetal circulation. The arrows represent the direction of blood flow. Oxygenated blood from the placenta flows in the umbilical vein. This largely bypasses the liver via the ductus venosus (not shown) and then mixes with de-oxygenated blood from the lower extremities in the inferior vena cava. This mixed blood bypasses the right side of the heart via the foramen ovale. De-oxygenated blood from the upper extremities in the superior vena cava enters the right side of the heart. This is then pumped from the right ventricle and joins the output from the left ventricle in the aorta, having largely bypassed the lungs via the ductus arteriosus. The blood flowing in the aorta, and hence umbilical arteries, is therefore de-oxygenated and acidic due to the mixing of umbilical venous blood and de-oxygenated blood from the extremities.

not be valid. (1) If the presumption, that a contracting muscle will have an anoxic core unless there is a high partial pressure of O_2 in the bath, stems from Hill's (1928b) classic considerations on the diffusion of O_2 into muscle, then this presumption is invalid since (a) Hill's calculations were based on skeletal muscle which has a much higher metabolic rate than vascular smooth muscle and (b) Chang and Detar (1980) have shown that even the thickest of vascular smooth muscles (200uM) will have an anoxic core (Po_2=0mmHg) only when the Pbo_2 approaches ~80mmHg. (2) Fasehun et al (1985) have shown in various smooth muscles from the rat (anococcygeus, portal vein and tail artery) that although agonist-antagonist interactions at $alpha_1$ receptors did not vary between normoxia (16% O_2) and hyperoxia (95% O_2), the nature of the agonist response changed. In normoxia the response was biphasic while in hyperoxia the response became monophasic.

In this study I have investigated two aspects of the effects of O_2 in the isolated human umbilical artery. (1) Does O_2 modify the tone of the vessel, and if so by what mechanism (chapter 2) and (2) Does O_2 modify the response to agonists, and the interaction between agonists and antagonists, with special reference to the characterization of the receptors for 5-hydroxytryptamine (chapter 3). Chapter 1 comprises a study of the various techniques used to assess smooth muscle contraction.

The fetal circulation is shown in figure 1. The greatest part of the work which has established our knowledge of the fetal blood-gas status can be attributed to Wulf (1964) and Pearson (1976). The pH, Pco_2 and Po_2 of the umbilical venous and arterial blood are respectively: 7.34, 40mmHg and 30mmHg; and 7.26, 50mmHg and 15mmHg (Wulf, 1964; G. Dobbie, personnal communication).

Methods and Materials

All of the experiments outlined in this study were carried out on isolated preparations of the human umbilical artery.

1 Collection and storage of umbilical cords

Lengths of umbilical cord (5-30cm) were cut from the placental portion as soon as practically possible after delivery but normally within 45 minutes. From this point until setting up the tissue in organ baths all possible steps were made to exclude excess oxygen from the preparations. The cords were placed in de-oxygenated Krebs-bicarbonate saline in specimen jars and the temperature maintained at 4°C by standing the specimen jars in cooled water from a thermocirculator (Conair Churchill, model 05/CTCV). The cords were stored under these conditions for up to 48 hours, before transferring the cords to the laboratory in de-oxygenated, ice-cold (4°C) saline. The saline was de-oxygenated by pre-gassing with 8% CO_2 in Nitrogen.

Arteries were dissected free of the surrounding Whartons jelly in de-oxygenated Krebs solution, to reduce the oxygen tension as low as possible. Care is required in the dissection as it is known that prostaglandins are released from damaged tissue (Piper and Vane, 1971) which can cause constraction of the isolated human umbilical artery (Hillier and Karim, 1968).

2 Techniques for measuring smooth muscle contraction

In a preliminary set of experiments three different techniques were employed to assess smooth muscle contraction: (i) isometric contraction; (ii) isotonic contraction; (iii) constant flow perfusion pressure.

(i) Isometric recording. Longitudinal strips of artery of length 1-1.5cm were suspended within 40ml organ baths containing Krebs at 37°C under a tension of 1g. The tension was monitored using Grass FT03c transducers and a Grass model 7 polygraph.

(ii) Isotonic recording. For "isotonic" recording the Grass isometric transducers were modified by using springs of force constant 1g/cm: the maximum tension change with this method was 0.4g at the baseline of 1g (as compared with 1.5–2g maximum under isometric contraction). This method is therefore, strictly speaking, auxotonic.

(iii) Perfused preparations. One end of a 2-3 cm length of artery was cannulated with a polythene cannula (outside diameter 1.34mm) and then placed within a 40 ml organ bath containing Krebs at 37°C. In those preparations held under tension the vessel was double cannulated. To the distal portion of the cannula a thread was attached. This thread was passed around a pulley and tied to a 2g weight hence allowing a tension of 2g to be put on the vessel. Thus, any longitudinal shortening of the vessel would be due to an isotonic contraction.

Krebs at 37°C was perfused through the artery by a peristaltic pump (Watson-Marlow flow-inducer, model 502S/R) from a heated reservoir. Inflow pressure was monitored via a side-arm using a pressure transducer (Elcomatic, model EM 751). The volume of the heated perfusate reservoir was maintained at approximately 100ml by means of a "drip-feed" system from a second reservoir. By keeping the volume of the perfusate reservoir low, and gassing with 6-8%CO_2, balance N_2, the oxygen tension was 20mmHg despite the natural tendency for equilibration of such an open system with atmospheric gas tensions.

When starting the perfusion, the resistance to flow was very great: to avoid excessive pressure the flow rate was initially set at 5ml/min. Over the next 2-2.5 hours the tissue relaxed and the flow rate was gradually increased to 25ml/min. This flow was maintained for the course of the experiment and produced a baseline pressure of ~30mmHg.

The gas mixtures used to aerate the organ baths were also used to

aerate the perfusate reservoir so allowing the simultaneous change of gas mixtures to the organ baths and perfusate.

Solutions of 5-HT, bradykinin and adrenaline were made up in Krebs immediately prior to starting the perfusion of each agonist. These were heated to 37°C and aerated until the desired gas tensions were reached before commencing the perfusion. Each concentration of agonist was perfused for six minutes before increasing the perfusate agonist concentration in a cumulative manner. The agonist was not added as a bolus to the bath. After six minutes of perfusion, given the volume of the bath and the perfusion flow rate, the concentration of drug in the bath was equal to that in the perfusate. The agonist concentration was sequentially increased. Peak inflow pressure at each concentration was noted.

In all experiments the tissues were equilibrated for 2-2.5 hours before any experimentation, gassed with 2.5% O_2, 8% CO_2, balance N_2, to mimic the gas tensions and pH of umbilical arterial blood, *in utero*, i.e. Po_2, 15mmHg; PCO_2 50 mmHg ; pH 7.28. (Wulf, 1964; Pearson, 1976). Po_2, PCO_2 and pH of the organ baths were monitored by withdrawing samples and analysing them on an IL 1302 blood-gas analyser.

In all experiments the order of addition of agonists was randomised, as was the order in which the two levels of oxygen tension were investigated.

A modification of technique (i) - isometric contraction - was used in all the experiments after this preliminary investigation. Longitudinal strips of artery (1-1.5cm) were suspended under 1g tension. Isometric tension was recorded using transducers (Grass FT03c) and a recorder (Grass model 7 polygraph or Linseis TYP 2065). Organ baths (40ml) were designed to incorporate an oxygen electrode (Instrumentation Laboratories, model 1302) , (figure 1). This

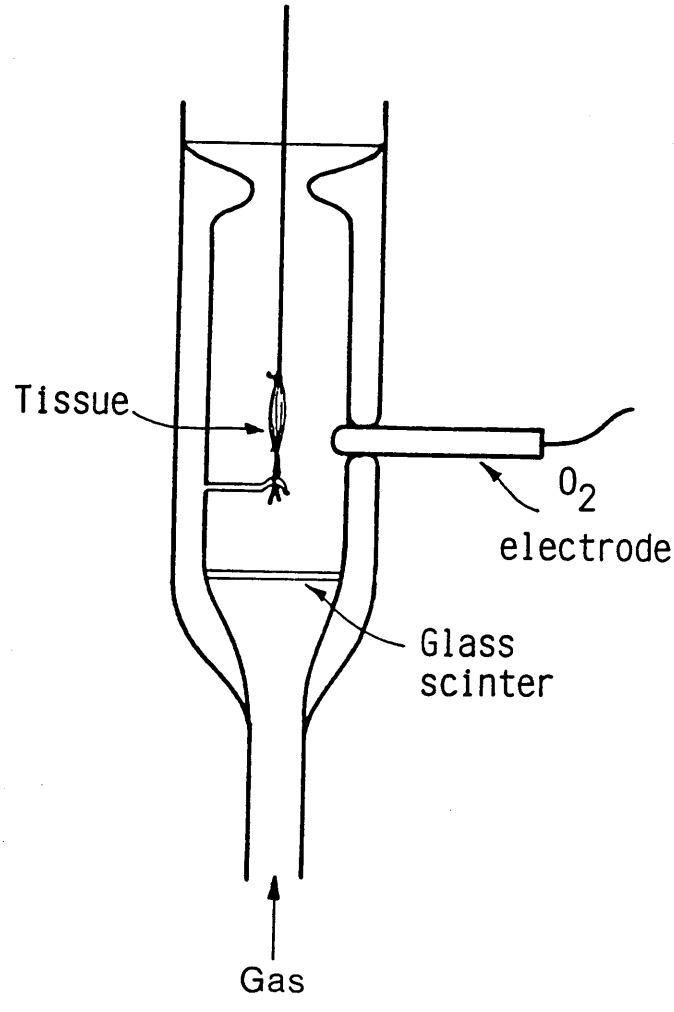


Figure 1 A diagram showing the organ bath which was designed for this study to incorporate an oxygen electrode. This allowed continuous monitoring of the oxygen tension of the saline. A rubber O-ring was used to make the seal between the electrode and organ bath. The neck of the organ bath was made as small as was practicle to reduce as far as possible the natural tendency of atmospheric oxygen to equilibrate with the saline. As gas passed through the glass scinter it was dissociated into a fine stream of bubbles, thus enabling a fast equilibration between the partial pressures of the gas mixture with those in solution.

allowed continuous monitoring of the oxygen tension (Po_2). It was established that the Po_2 measured in one bath was within ±3mmHg of the Po_2 found for the Krebs solution in any of the three other baths used (when aerated with the same gas mixture, and at a similar rate), throughout the range tested - 0 to 600 mmHg. The Po_2 was displayed on an oxygen meter (Strathkelvin Instruments , model 781). An analogue signal was taken from the output of the oxygen meter to the Grass Polygraph to obtain a "hard copy" of the continuous reading on one channel of the Grass Polygraph.

3 Analysis of responses to agonists and antagonists.

Agonists and antagonists were studied at three levels of Po_2: (i) ~15mmHg (2.5% O_2), (ii) ~120mmHg (17% O_2), (iii) ~500mmHg (92% O_2). The values in parenthesis are the % O_2 composition of the gas mixtures used to aerate the Krebs solution. The remaining gas was composed of 8% CO_2/balance N_2.

The gas mixtures were made up in Douglas bags using the rotameters from an anaesthetics trolley. The Krebs solution was gassed with these mixtures by a small aquarium pump.

Agonists

Concentration-response curves (CRC's) to agonists were constructed as follows: all agonists were added cumulatively in steps which increased the bathing solution concentration by ~threefold, i.e. at 0.5 log_{10} increments. Higher concentrations were added when the preceding response had reached a plateau - this was at approximately 3 minute intervals for all the agonists. Response (% of maximum response, grams tension or % of a prior 50mM KCl contraction) was plotted against log(agonist concentration). pD_2 values were calculated as $-log(EC_{50})$, where EC_{50} is the concentration of agonist which gives 50% of the

maximum response.

The potency of each test agonist was compared to that of 5-HT, or adrenaline, in paired or non-paired preparations.

Paired preparations: a CRC to 5-HT or adrenaline was constructed followed by one for the test agonist in the same preparation. The potency of the test agonist, relative to that of 5-HT or adrenaline was calculated as:

$$\frac{EC_x \text{ (test agonist)}}{EC_x \text{ (5-HT or adrenaline)}}$$

where x is the level of response, e.g. EC_{50}, EC_{75}, as given in the text. The relative potencies are given as the geometric mean and 95% confidence limits. Where the potency of 5-CT relative to 5-HT was examined the calculated potency was corrected for the change in sensitivity of the tissue with time, by division by the concentration-ratio of a control preparation in which two successive CRC's to 5-HT were constructed.

Non-paired preparations: The potency of test agonists, relative to that of 5-HT or adrenaline found in separate experiments was calculated as:

$$\frac{EC_{50} \text{ (test agonist)}}{EC_{50} \text{ (5-HT or Adrenaline)}} \quad \frac{\text{(geometric mean)}}{\text{(geometric mean)}}$$

This analysis therefore gives the relative potency as a single value.

Antagonists

5-HT and 5-CT were the only agonists of the isolated HUA which did not show tachyphylaxis. For this reason antagonists of the response to 5-HT and 5-CT were quantitatively assessed by a different method to that used to study antagonists of the response to adrenaline, LSD and methysergide, which showed tachyphylaxis.

(i) Antagonists of 5-HT/5-CT

(a) Po_2 ~15mmHg

Four preparations from the one artery were used. In each

preparation CRC's to 5-HT or 5-CT were constructed as already described. After washout of the drug (60 minutes) different concentrations of antagonist were added to three preparations and allowed to equilibrate for 30 minutes before again constructing a CRC to 5-HT (or 5-CT). One preparation therefore acted as a control to assess the change in sensitivity (with time), which was approximately twofold. Response was calculated as the % of the 1st curves' maximum response and plotted against log(5-HT concentration), (log[5-HT]). For each preparation the concentration-ratio (CR) was calculated as:

$$\frac{EC_{50}\ 5\text{-HT (+antagonist)}}{EC_{50}\ 5\text{-HT (control)}}$$

This was corrected for change in sensitivity by division by the CR of the control preparation. Using the CR's, Schild plots were constructed: log(CR-1) (ordinate) was plotted against log(antagonist concentration) (abscissa), (Arunlakshana and Schild, 1959). A line of best-fit was found for the points by linear regression (least squares) which gave the slope and an estimate of the pA_2 as the intercept of the regression line with the line, log(CR-1)=0. In each estimate of a pA_2, 4 to 6 preparations were used. The mean values of the slope of the regression line and estimated pA_2 are reported together with their respective 95% confidence limits.

Non-competitive antagonists

Cyproheptadine gave rise to a non-surmountable antagonism. In this case a pD'_2 was estimated (Van Rossum, 1963). In each preparation the concentration of antagonist causing a 50% reduction of the control maximum response was estimated: At each antagonist concentration the ratio (x) of the the maximum response elicited by the agonist in antagonist treated tissue divided by that in untreated (control) tissue was calculated. X (ordinate) was plotted against log(antagonist

concentration) (abscissa), and regression analysis used to determine the -log(antagonist concentration) where x=0.5 (pD'_2). The mean values for pD'_2 and 95% confidence limits, from 4-6 estimates, are reported.

b) Antagonists of 5-HT (Po_2 ~120mmHg)

In any one preparation a single concentration of antagonist was studied at higher than physiological Po_2 (~120mmHg) as follows: A CRC to 5-HT was constructed at Po_2 ~15mmHg. After washout (60 minutes) the Po_2 was increased to ~120mmHg which sometimes caused a contraction, but which was always transient. The antagonist was added to the bath for 30 minutes before constructing a 2nd CRC to 5-HT. A third CRC to 5-HT was constructed in the presence of the same antagonist concentration plus indomethacin (1uM, 30mins.). Response (% of 1st (low Po_2) maximum response) was plotted against log[5-HT]. Estimates of pK_B (-logarithm of the antagonist dissociation constant, K_B) were made from the following equation:

$$pK_B = \log(CR-1) - \log B$$

(Arunlakshana and Schild, 1959). B is the antagonist concentration The concentration-ratio (CR) was calculated as:

$$\frac{EC_x \text{ 5-HT (+antagonist)}}{EC_x \text{ 5-HT (control)}}$$

where x is the level of response, as stated in the text. Estimates of pK_B were made for the antagonist in the presence and absence of indomethacin.

(ii) Antagonists of Adrenaline, LSD and Methysergide

Since the above mentioned agonists showed tachyphylaxis two successive CRC's to these agonists (before and in the presence of the antagonist) could not be constructed. Antagonists were studied as follows: One strip acted as a control and antagonist(s) were added to

other strips from the same artery. CRC's to the agonist were constructed simultaneously on all strips. (Experiments had shown that CRC's to these agonists in separate strips from the same artery, constructed simultaneously, were not significantly different). Response was plotted against log(agonist concentration). Response was calculated as (a) % of maximum response and (b) % of a prior 50mM KCl contraction - the latter calculation to assess any change in the maximum response caused by the antagonist. Estimates of pK_B of the antagonist were made as already stated.

4 Analysis of responses to O_2

CRC's to oxygen were constructed non-cumulatively since responses to stepped increments in Po_2 were not always maintained. 5 mins exposure to each Po_2 was allowed with return to the original mixture for 25 min intervals upon which the tissue relaxed to baseline tension. The initial increments in Po_2 were kept small in order to accurately establish the threshold to oxygen, i. e. , in order to obtain points on the concentration-response curve close to threshold and minimise the extrapolation required to estimate the threshold to oxygen. The Po_2 was increased with sequential exposures. In each experiment 8 different tensions within the range 30-450 mmHg were employed. Response was plotted against $\log\{Po_2\}$. Response was calculated in two ways:

(i) as a % of the maximum response. In each experiment the relationship between % of maximum response and $\log\{Po_2\}$ was approximately linear. The Po_2's producing 0, 10, 30, 50, 70, 85 and 100 % of the maximum were interpolated from the curve for each tissue: $\log\{Po_2\}$-response curves were plotted from the geometric mean $\pm$ s.e.m. of these. The threshold to contraction (0% of maximum) for each tissue was estimated by extrapolation of its $\log\{Po_2\}$-response curve to zero response, the

assumption being made that the relationship between response and $\log\{Po_2\}$ remained linear to zero response. The mean (geometric) Po_2 producing 0, 10, 30, 50, 70, 85 and 100% of the maximum was calculated as the antilog of the mean $\log\{Po_2\}$.

(ii) as grams/gram of (wet) tissue weight. At each Po_2 to which the tissues were exposed the mean± s.e.m. of the response was plotted against the $\log\{Po_2\}$.

In the experiments where evoked contractions were expressed relative to the tissue weight, the wet weight of the tissues was recorded after blotting on filter paper for 1 min, at the end of the experiment.

5 Saline and Drugs

The physiological salt solution used in this study was Krebs-bicarbonate saline of composition, (mM): NaCl 119, KCl 4.7, $MgSO_4$ 1.2, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25.0, glucose 11.1.

The following drugs were used:	Source
5-hydroxytryptamine creatinine sulphate	Sigma
(-)Adrenaline bitartrate	Sigma
Bradykinin triacetate	Sigma
Indomethacin	Sigma
l-Phenylephrine hydrochloride	Sigma
Acetylsalicylic acid	Sigma
Methysergide bimaleate	Sandoz
d-lysergic acid diethylamide	Sandoz
(±)Pindolol	Sandoz
Phentolamine mesylate	Ciba
Xylazine hydrochloride	Bayer
Nifedipine	Bayer
Bay K 8644	Bayer
Prazosin hydrochloride	Pzifer
UK 14,304 tartrate	Pzifer
Compound X*	—
Ketanserin tartrate	Janssen
5-carboxamidotryptamine	Glaxo
Cyproheptadine hydrochloride	Merck, Sharpe and Dohme
Flurbiprofen	Boots
Sodium nitroprusside	Roche
Buspirone hydrochloride	Bristol-Meyers
Wyeth 26703 hydrochloride	Wyeth

* This compound is a novel drug still undergoing research within the company. At such time when the drug comes under a patent this thesis shall be updated to include the available data.

6 Histology

Lengths of artery were fixed in a 10% formalin solution. Transverse sections were cut at 10uM thickness and stained with haematoxylin and eosin (H & E).

7 Statistics

Statistical comparisons of the means of groups of data was made using the Students t-test for paired or unpaired data, where appropriate. A level of probability of $P<0.05$ was taken to indicate statistical significance. This is indicated on the figures as an asterisk (*) beside values which are significantly different from, say, control responses. The means of several groups of data were first compared by a 1-way analysis of variance.

Chapter 1: A comparison of the effects of Oxygen, 5-HT, Bradykinin and Adrenaline in the Human Umbilical Artery assessed by isometric and isotonic contraction and perfusion pressure techniques

Introduction

The great majority of the studies which have examined the effects of vasoactive drugs in the human umbilical artery (HUA) have used perfused preparations but these have employed different techniques. Thus, some groups have perfused the artery and placenta (von Euler, 1938; Eliasson and Astrom, 1955; Astrom and Samelius, 1957). Others have perfused the artery while within the cord (Gokhale et al, 1966) or have perfused the isolated artery alone (Panigel, 1962; Davignon et al, 1965; Lewis, 1968). Either constant flow, or constant pressure, perfusion systems were used.

In the earlist studies the main point of interest was the gross effect of various drugs on the umbilical vasculature, i.e. would they constrict or dilate the vessel, and yet there is little agreement between groups. For example, the effects of adrenaline and acetylcholine have been described by several groups. Adrenaline has been reported to cause vasoconstriction only (von Euler, 1938) and vasoconstriction or vasodilation (Eliasson and Astrom, 1955, Gokhale et al, 1966). Sometimes adrenaline was reported to have an effect on all preparations , and in other studies the effect was seen only in some. Similarly, acetylcholine had a vasoconstrictor effect alone (Eliasson and Astrom, 1955), or vasoconstriction or vasodilation (von Euler, 1938; Gokhale et al, 1966). Sometimes these effects were seen in all the preparations and in other studies were observed in only a few preparations. It is a matter of speculation that these differences were due to the different experimental approaches.

A quantitative assessment of the potency of the drugs examined in these studies is impossible because (i) the drugs were added as a bolus to the perfusing solution and (ii) the flow rates used in the experiments varied considerably between the different groups.

Qualitatively, 5-HT was always found to be the most potent agonist. Other papers which have described the effects of drugs on the isolated HUA have employed isotonic contraction (Somlyo et al, 1965; Eltherington et al, 1968) or isometric contraction (Altura et al, 1972; Roach, 1972). Here again there is little agreement between groups. Eltherington et al (1968) also employed perfused arteries and found that bradykinin was ~15 fold more potent than 5-HT and adrenaline was almost equipotent to 5-HT, while Altura et al (1972), using strips of HUA, found that 5-HT was slightly more potent than bradykinin, recorded by isometric contraction, and adrenaline was a very weak agonist. One factor which could explain these differences (besides the obviously different experimental techniques) is that different oxygen tensions were employed in the two studies, which, as already described, can modify the response to various agonists on smooth muscle. Indeed such a modification was found by Eltherington et al (1968) who found that the artery was almost insensitive to all the agonists tested on reducing the oxygen tension from ~120mmHg to the physiological level (~20mmHg).

Since I wish to devise *in vitro* preparations to predict the sensitivity of the umbilical vessels *in utero*, to blood gases and drugs, it was necessary to try to resolve these differences and to decide how they might relate to the *in vivo* situation. This problem has been approached by simultaneously examining paired tissues using (i) isometric and isotonic contractions or (ii) isometric contraction and constant flow perfusion pressure techniques.

Results

Isometric/isotonic

Figure 1 shows that contraction of the human umbilical arterial smooth muscle, evoked by oxygen, was concentration-dependent when recorded by either isometric or isotonic contraction. The concentration-response curves (CRC's) to oxygen are parallel, the isometric curve generally lying to the left of the isotonic curve in the physiological oxygen tension (Po_2) range (15–100mmHg) but there was no significant difference ($P>0.05$) between the two curves at any point except at the maximum response. The thresholds, predicted by extrapolation, for the isometric and isotonic curves were 20mmHg (geometric mean, 95% confidence limits 8–51mmHg) and 28mmHg (13–59mmHg) respectively which were not significantly different from the initial level (14±2mmHg) from which the Po_2 was increased. The maximum responses were at 234mmHg (190–302mmHg) and 199mmHg (144–275mmHg) respectively and were 0.76±0.18g (mean±s.e.mean) and 2.05±0.42mm respectively. At higher Po_2 the responses were submaximal (not shown).

The potencies of 5-HT (1nM-3μM), bradykinin (1nM-1μM) and adrenaline (10nM-10μM) were assessed by cumulative additions of each agonist. Preparations from six cords were examined. All were contracted by 5-HT at low (~20mmHg) and high (~120mmHg) Po_2. At low Po_2 adrenaline only weakly contracted one tissue. At high Po_2 only one preparation was not contracted by adrenaline. One tissue was not contracted by bradykinin at either low or high Po_2. The isometric and isotonic cumulative concentration-response curves are shown in figures 2a and 2b respectively. The sensitivities to all the agonists were potentiated by the higher Po_2 in both isometric and isotonic preparations. At the EC_{30} the sensitivity to 5-HT was significantly increased by 6.6 fold (2.1–20.9), (isometric) and by 6.2 fold (1.3–26.3), (isotonic), which

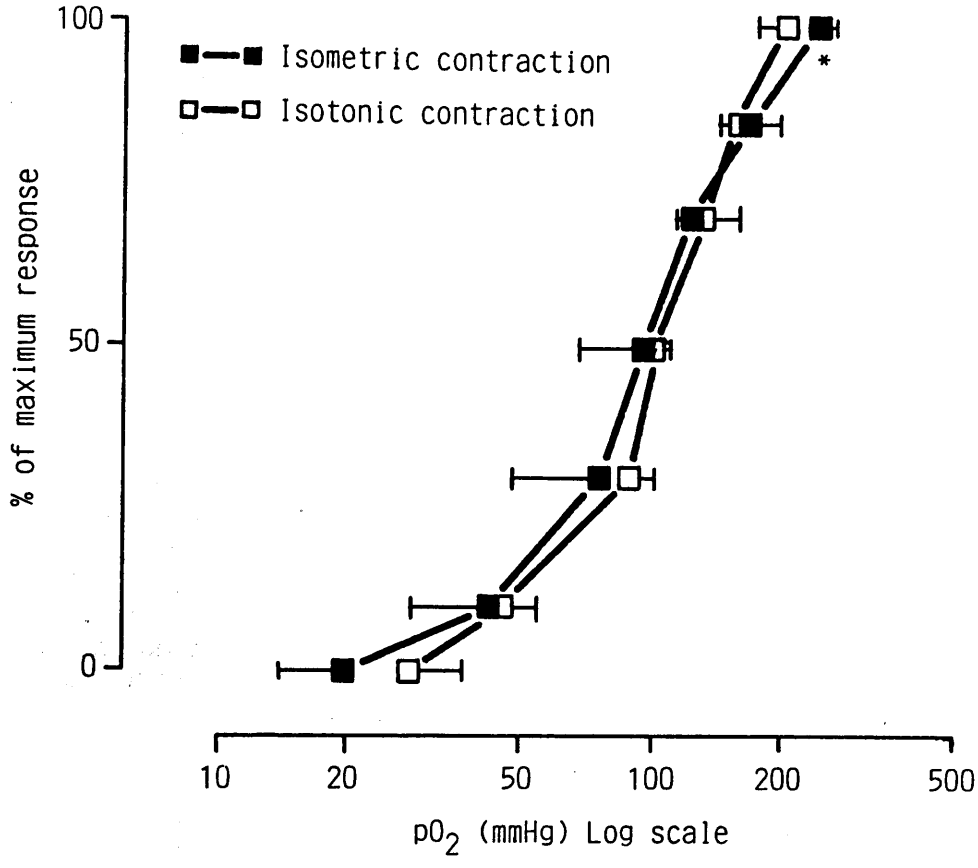
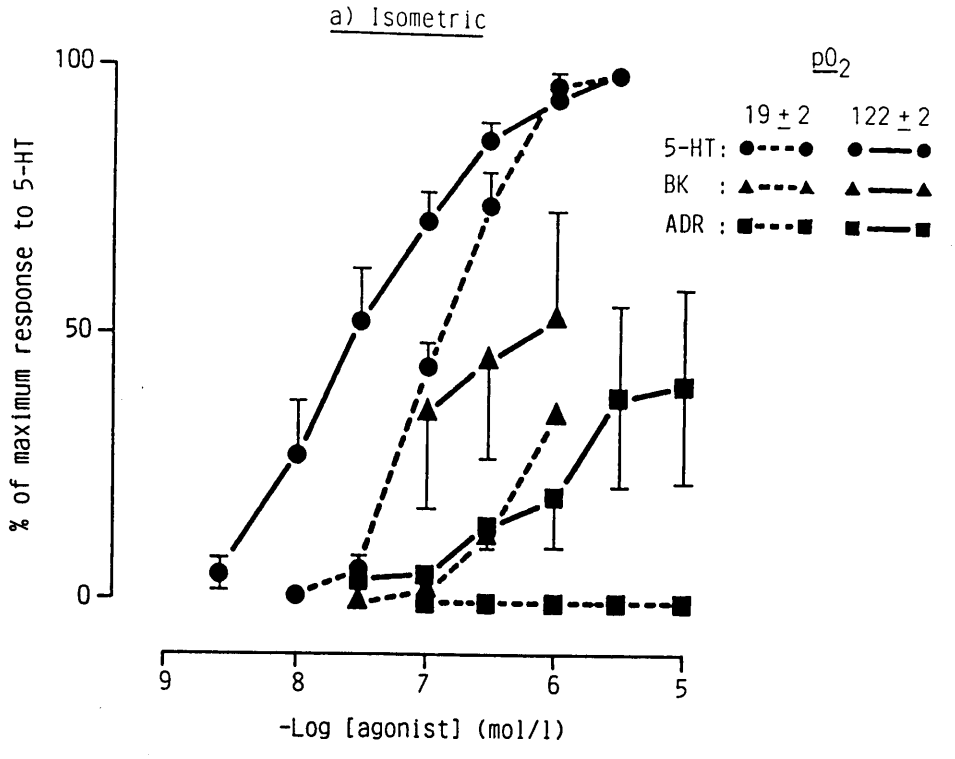


Figure 1 Concentration-response curves to oxygen in longitudinal strips of human umbilical artery, recorded by isometric and isotonic contraction. Paired preparations, n=6. Horizontal bars represent geometric means±s.e.mean. Asterisk denotes significant difference between the curves ($0.01<P<0.05$).

Figure 2 Log concentration-response curves to 5-HT, bradykinin and adrenaline in longitudinal strips of umbilical artery at low (19±2mmHg) and high (122±2mmHg) Po_2, recorded by (a) isometric and (b) isotonic contraction. Paired preparations, n=6. Response (ordinate) to each agonist is expressed as a % of the maximum response to 5-HT, at the same Po_2. At concentrations where more than 3 of the 6 preparations did not contract the values are not shown. For clarity, error bars (mean±s.e.mean) are omitted at some points.


a) Isometric
100
50
0
% of maximum response to 5-HT
9
8
7
6
5
-Log [agonist] (mol/l)
pO2
19 ± 2
122 ± 2
5-HT:
BK :
ADR :

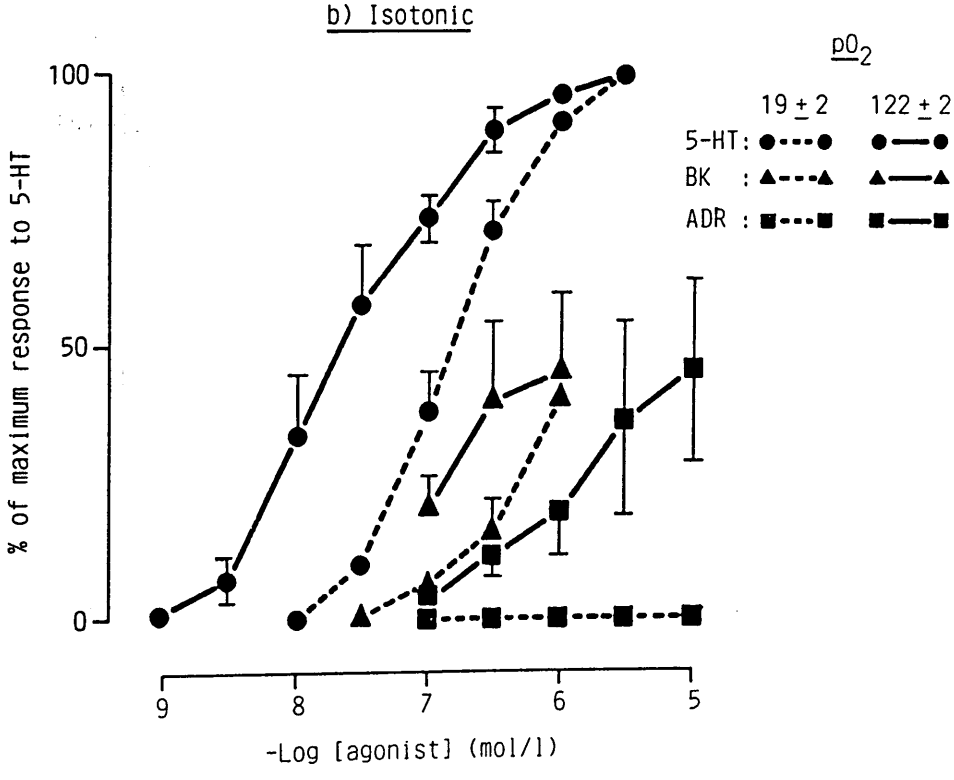

b) Isotonic
100
50
0
% of maximum response to 5-HT
9
8
7
6
5
-Log [agonist] (mol/l)
pO2
19 ± 2
122 ± 2
5-HT:
BK :
ADR :

Table 1 Comparison of the potencies (in terms of the EC_{30}) of bradykinin (BK) and adrenaline (ADR), relative to 5-HT, in preparations of human umbilical artery at Po_2 ~20mmHg and ~120mmHg, recorded by isometric and isotonic contraction.

Preparation	Po_2 (mmHg)	EC_{30} 5-HT (nM)	$\frac{EC_{30}\ test}{EC_{30}\ 5\text{-HT}}$ BK	ADR
Isometric	19±2	69.2 (47.9–100)	11.7 (2.5–54.9)	—
	122±2	10.5 (4.8–22.9)	4.2 (0.3–66.1)	112 (8.9–1412)
Isotonic	19±2	72.4 (42.6–123)	7.2 (1.3–39.8)	—
	122±2	12.0 (3.9–37.2)	11.0 (0.4–316)	129 (10.0–1659)

Values are the geometric means (95% confidence limits) of 4–6 estimates.

were not statistically different.

Adrenaline contracted only one of six tissues at low Po_2 in both the isometric and isotonic preparations (from the same vessel) while at high Po_2 five of six were contracted. It is therefore clear that the higher Po_2 potentiated the response to adrenaline.

The responses to 5-HT, bradykinin and adrenaline were compared at the EC_{30} in individual tissues (where this was possible), the EC_{30} being the concentration of agonist giving 30% of the maximum response to 5-HT. Table 1 shows the mean responses to the agonists. In both the isometric and isotonic preparations it was found that at low Po_2 5-HT was significantly more potent than bradykinin while responses to adrenaline were negligible. At high Po_2 5-HT was not significantly more potent than bradykinin but was more than 100 fold more potent than adrenaline. Comparing the isometric and isotonic preparations, there was no significant difference in the potency of each agonist using the different techniques. This applied at either Po_2.

Isometric/Perfused

At the start of the perfusion the artery was very constricted: for a flow rate of only 5ml/min the inflow pressure was usually in excess of 150mmHg. With time the resistance fell (the inflow pressure decreased) and so the flow was increased by stepped increments to 25ml/min. At each increase of the flow rate the inflow pressure increased rapidly but slowly fell (figure 3). The time taken to reach a flow rate of 25ml/min was 2 hours at which time the inflow pressure was 29±7mmHg (n=15).

In these experiments the responses to 5-HT (1nM-50nM), bradykinin (1nM-50nM) and adrenaline (10nM-10μM) were investigated. In the initial experiments there was no longitudinal tension on the artery.

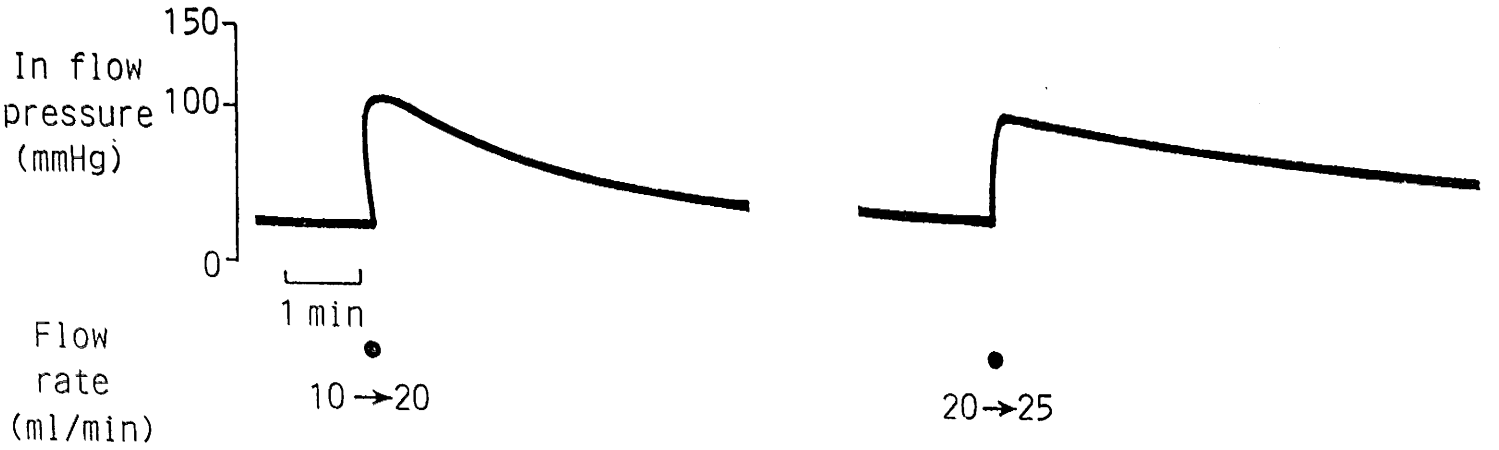


Figure 3 A tracing showing the perfusion pressure in an isolated human umbilical artery. At the start of the perfusion the resistance to flow was very great: the flow rate was increased by stepped increments to 25ml/min over the course of 2 hours prior to any experimentation. Each time the flow rate was increased the perfussion pressure increased sharply but which decayed as the artery relaxed. In this example the flow rate was increased from 10 to 20ml/min, and then from 20 to 25ml/min. At a flow rate of 25ml/min the perfusion pressure eventually acquired a stable level, and which in different preparations was 29±7mmHg.

At low Po_2 a cumulative increase of the perfusate agonist concentration evoked concentration-related pressor responses within the range tested (figure 4). In some preparations the responses to lower concentrations were better maintained than in others. At higher concentrations (i.e. greater contractions) , responses were not maintained. In contrast to its action in paired isometric strips, adrenaline contracted the perfused artery at low Po_2. In the perfused artery bradykinin was equipotent to 5-HT at low Po_2 (figure 5a). No quantitative assessment of the potencies was attempted as only a narrow concentration range was tested. The reason for this was that to make a comparison of the 3 agonists at the 2 levels of Po_2 constituted a very long experiment , even with the limited concentration range.

At high Po_2 however, the agonist-evoked contractions were not so concentration-related. In some tissues the pressure response of the perfused artery to these agonists was a sharp transient rise followed by relaxation almost to baseline pressure. Perfusion of higher concentrations of agonist either produced no constriction at all or a constriction which did not emulate the initial response (figure 6). In most cases it was the first, threshold concentration which caused the massive vasoconstriction. In three of six preparations the threshold to 5-HT was $5X10^{-9}M$. The threshold for contraction to bradykinin in these same tissues was $1X10^{-8}M$. In the other arteries the threshold to contraction to 5-HT was variable but the threshold for bradykinin was always lower. Thus at high Po_2 bradykinin was less potent than 5-HT in perfused arteries. The order of potency was 5-HT>bradykinin>>adrenaline.

In perfused arteries the threshold for contraction to 5-HT at high Po_2 was always lower than at low Po_2. For the other two agonists, bradykinin and adrenaline, potentiation of the responses at the higher Po_2 was not always seen, although the nature of the response (as

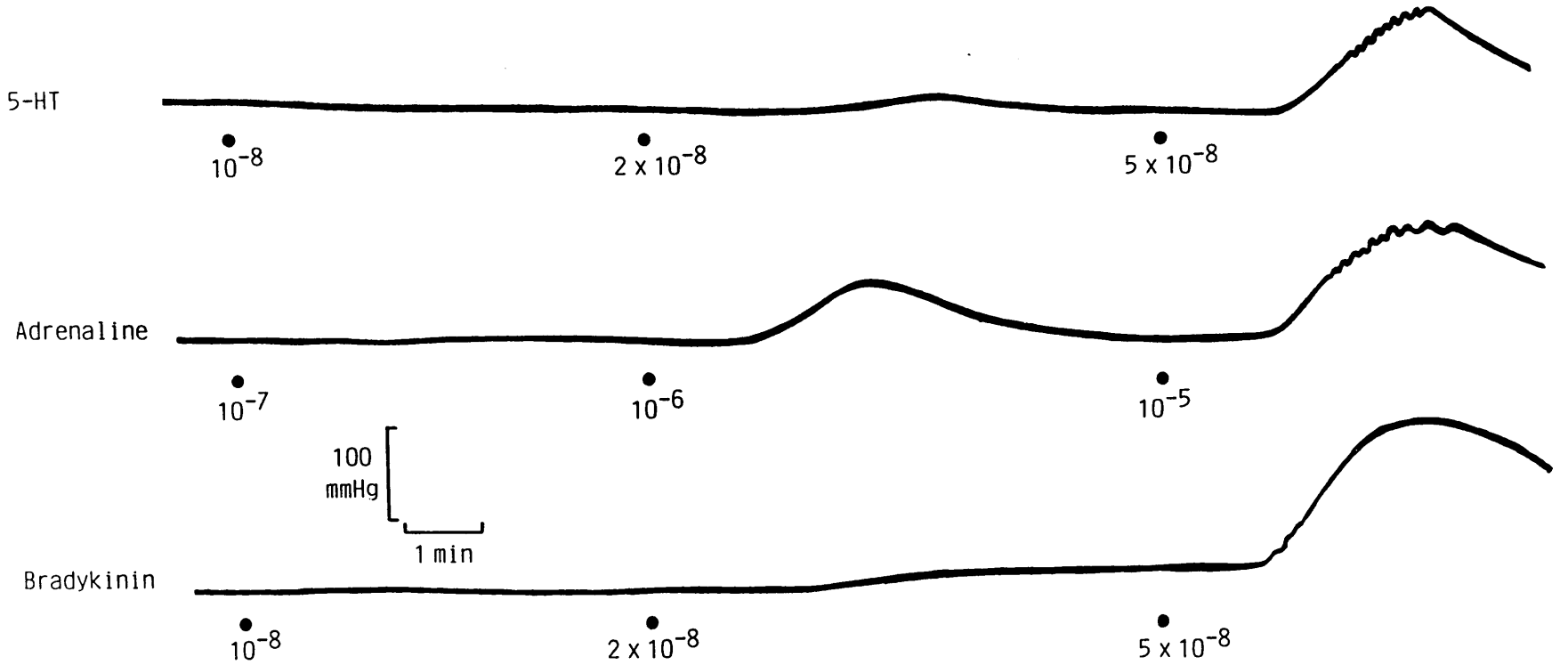
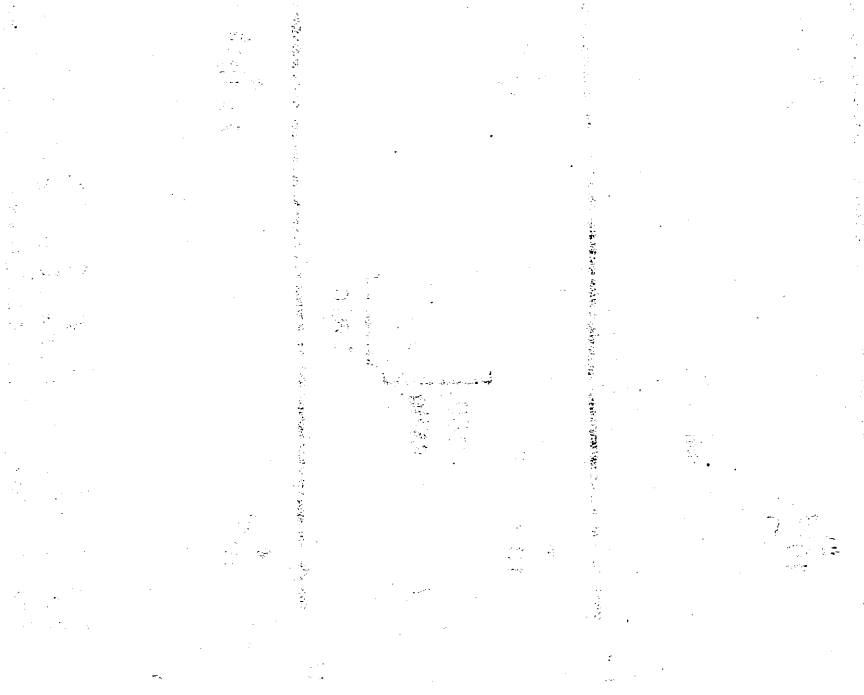
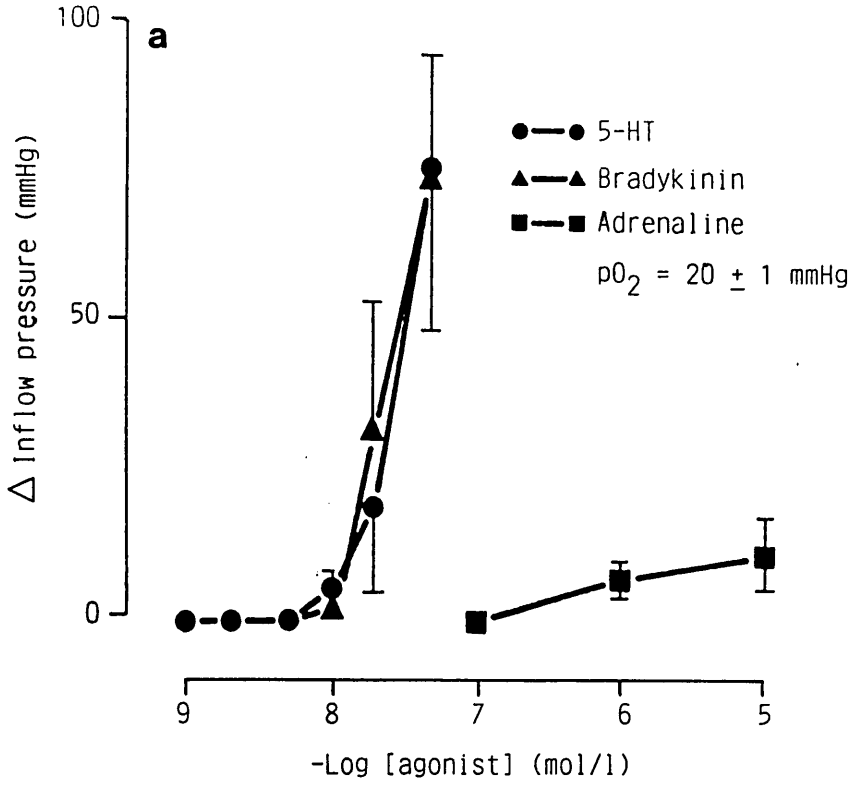
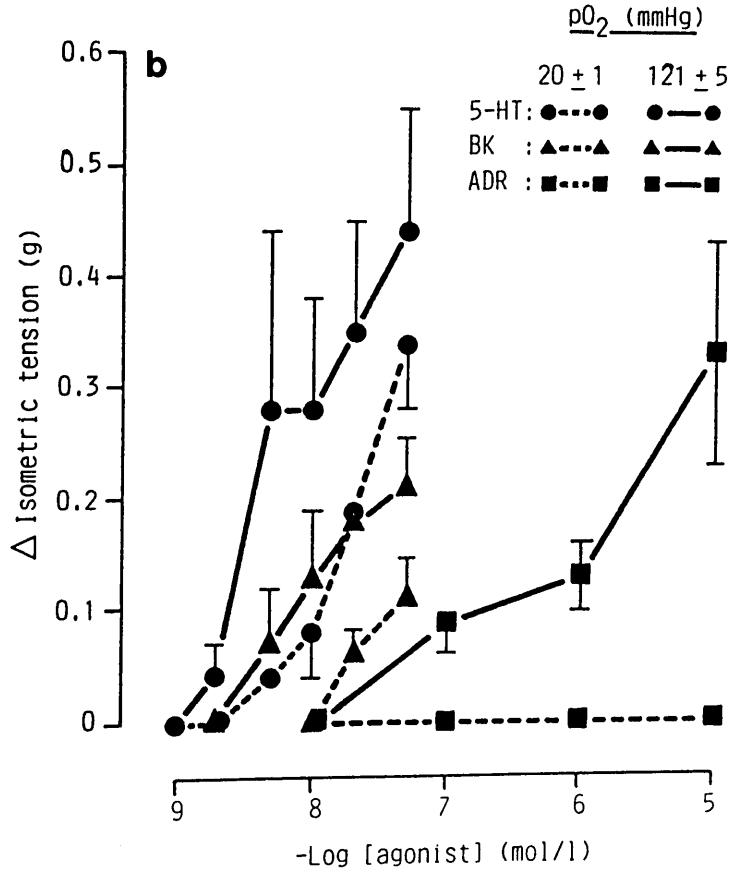


Figure 4 Tracings of representative recordings of perfusion pressure responses to 5-HT, adrenaline and bradykinin in the umbilical artery at low Po_2 (~20mmHg). The perfusate agonist concentration was increased in a cumulative manner.

Figure 5 Log concentration-response curves to 5-HT, bradykinin and adrenaline in the umbilical artery recorded by (a) perfusion pressure (low Po_2 only) and (b) isometric contraction of longitudinal strips of HUA at both low and high Po_2. Paired preparations, n=6. Concentration range tested was for 5-HT: 1nM–50nM; bradykinin: 1nM–50nM; adrenaline: 10nM– 1μM. For clarity, error bars (mean±s.e.mean) are omitted at some points.




a
100
50
0
Δ Inflow pressure (mmHg)
5-HT
Bradykinin
Adrenaline
pO2 = 20 ± 1 mmHg
9
8
7
6
5
-Log [agonist] (mol/l)


pO2 (mmHg)
20 ± 1
121 ± 5
5-HT:
BK :
ADR :
b
0.6
0.5
0.4
0.3
0.2
0.1
0
Δ Isometric tension (g)
9
8
7
6
5
-Log [agonist] (mol/l)

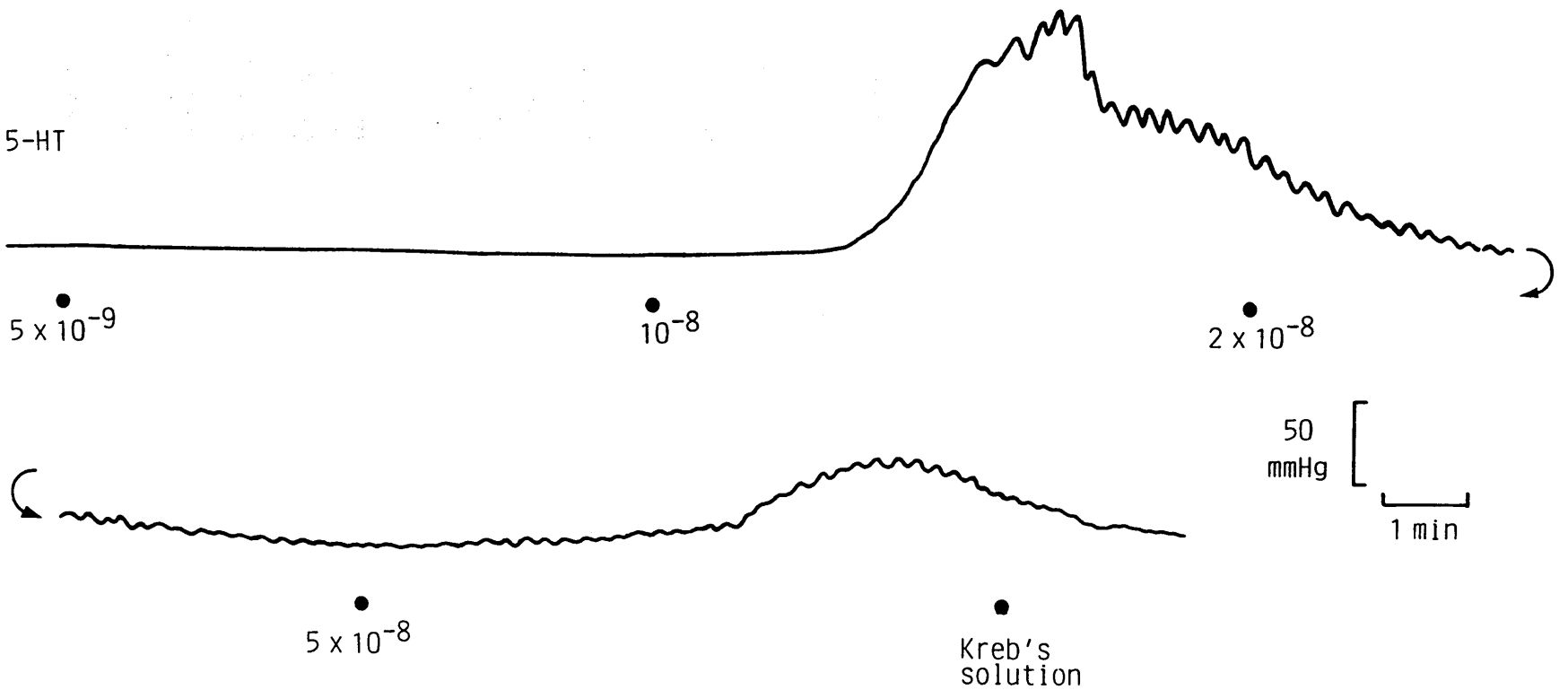


Figure 6 An example of the perfussion pressure response to agonists in the umbilical artey at high Po_2 (~120mmHg). Shown here is the response to a cumulative increase of the perfusate concentration of 5-HT. The threshold concentration to cause contraction often induced the maximal response: higher concentrations either did not cause a further contraction, or induced a contraction which did not emulate the initial threshold response.

already described) was noted. Figure 5b shows the responses to these agonists in paired isometric preparations at both low and high Po_2, and in which the agonists caused concentration-related contractions. It is evident that the higher Po_2 caused a potentiation of the responses to the agonists, and the rank order of potency was that already found in isometric preparations: 5-HT>bradykinin>>adrenaline.

The irregular nature of the response to the agonists in the perfused preparations required further investigation. It was possible that a cumulative increase of the agonist concentration was not the best method to adopt in this preparation. The preparations seem to acquire a super-sensitivity at the higher Po_2 and it is in this situation that the agonist responses are often not concentration related. A non-cumulative addition of 5-HT gave concentration-related pressor responses in all the preparations at low Po_2 (as already found by a cumulative increase of the agonist concentration) and in three of the six preparations at high Po_2. In these three preparations the response to 5-HT was clearly potentiated by the high Po_2 (figure 7).

A significant finding was that the stepped increase in Po_2, from approximately 20 to 120 mmHg, evoked only small contractions (~20mmHg) in two of the twelve perfused preparations while in the paired isometric tissues an increase in tension was always recorded. Visual inspection of the vessel revealed that the degree of spiral of the artery increased on raising the Po_2. The results of a simultaneous comparison of isometric and perfused techniques, in which the perfused vessel was held under longitudinal tension, are shown in figures 8 and 9.

Figure 8 shows that when the perfused vessel was held under longitudinal tension then it contracted in a concentration-related manner to stepped increments in Po_2 which is in marked contrast to its

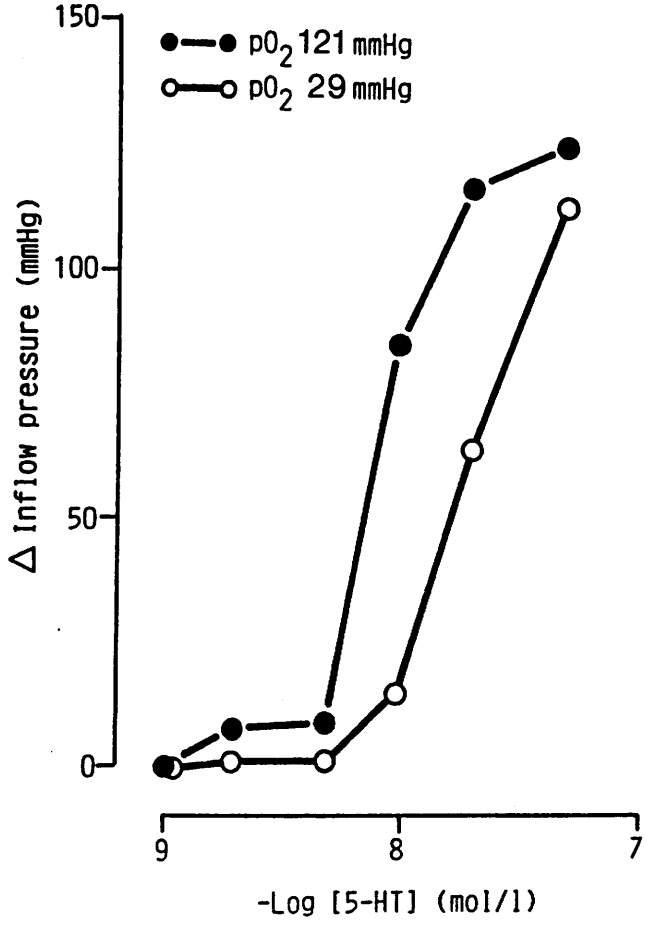


Figure 7 Log concentration-response curves to 5-HT in those perfused arteries which gave concentration-related responses at both low (29mmHg) and high (121mmHg) oxygen tensions. This was found in only 3 of 6 arteries which were tested, therefore no error bars are shown. The perfusate concentration of 5-HT was increased in a non-cumulative manner.

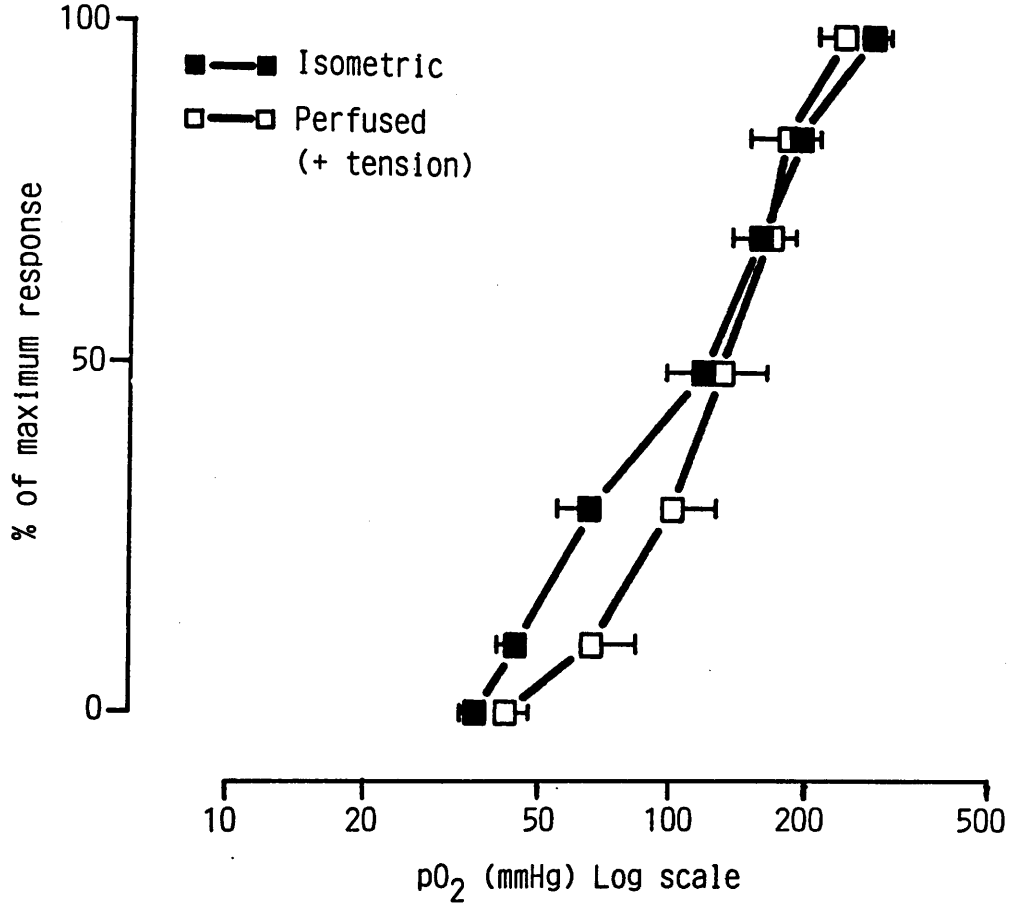


Figure 8 Concentration-response curves to oxygen in the human umbilical artery, recorded in isomteric longitudinal strips, and in perfused preparations held under tension. Paired preparations, n=5. Horizontal bars are the geometric means±s.e.mean.

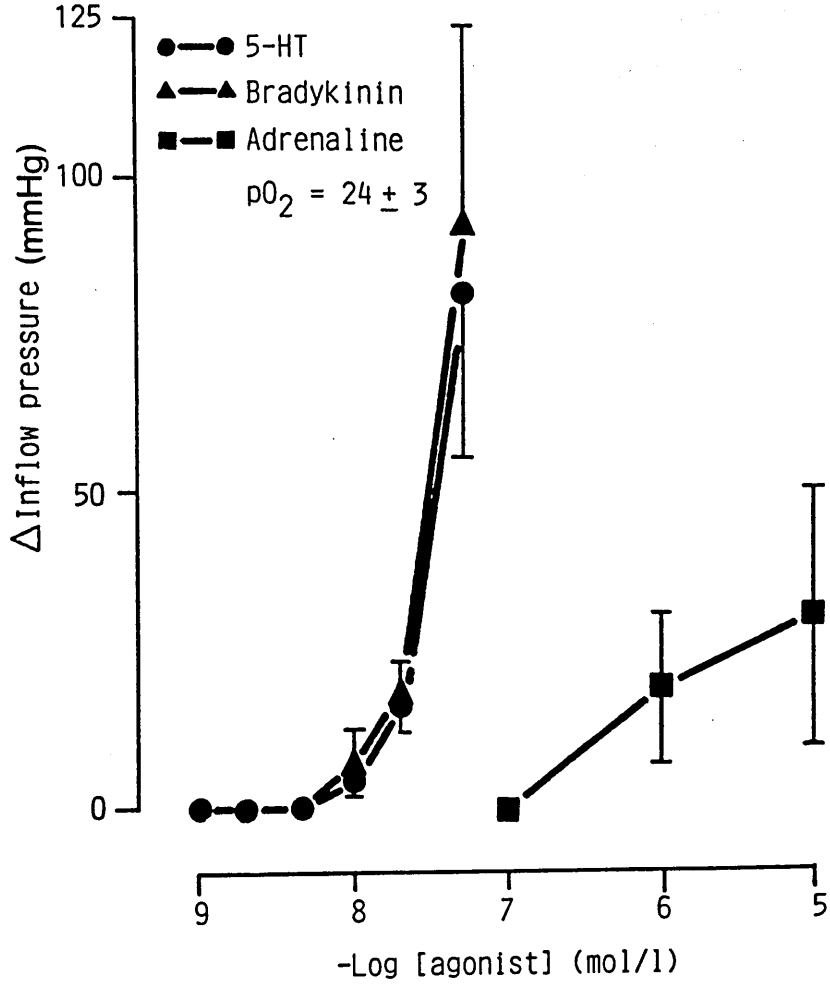


Figure 9 Log concentration-response curves to 5-HT, bradykinin and adrenaline in perfused umbilical arteries held under longitudinal tension. Low Po_2 (24±3mmHg). In each tissue (n=6) the response to each agonist was examined by a cumulative increase of the agonist concentration. Vertical bars are the mean±s.e.mean.

lack of response when not held under tension. The predicted threshold to oxygen in either experimental technique was not significantly different. In isometric strips this was 35mmHg (geometric mean, 95% confidence limits, 30–40mmHg) and in the perfused artery 42mmHg (31–54mmHg). There was no significant difference between the curves at any level. The maximum response to oxygen in the isometric preparations was 1.03±0.21g (mean±s.e.mean) and in the perfused preparations 172±42mmHg.

With the perfused vessel held under tension the perfusion pressure concentration-relationships for the agonists 5-HT, bradykinin and adrenaline at low Po_2 were the same as found in the perfused artery when not held under tension (figure 9; cf. figure 5a). 5-HT and bradykinin were approximately equipotent while adrenaline was substantially less potent. In paired isometric preparations the rank order of potency of the agonists was as already found: 5-HT>bradykinin>>adrenaline. At high Po_2 the pressor response to the agonists showed a similar "threshold phenomenon" as previously described: at the concentration of agonist which was threshold for contraction the response was often maximal with either no further contraction at higher concentrations, or a contraction which did not emulate the initial response. Nevertheless it was clear that 5-HT was always either equipotent with, or more potent than, bradykinin whereas adrenaline was somewhat less potent.

Spontaneous Activity

Longitudinal strips of artery were always quiescent and showed no spontaneous contractions when initially equilibrated at low (~20mmHg) or at high (~120mmHg) Po_2 (more than 250 in the whole study). Spontaneous activity has however been recorded in helical strips (4 of 24) and in perfused vessels (14 of 31) at low Po_2 (figure 10). Figure

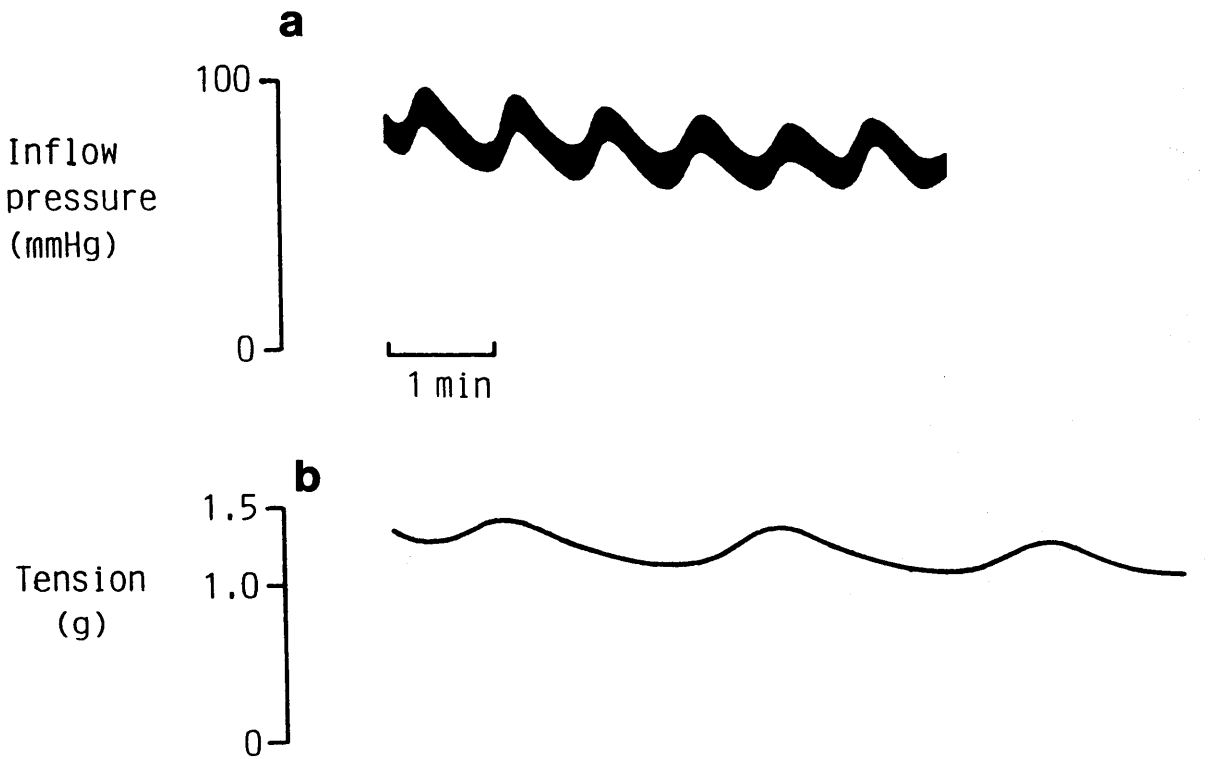


Figure 10 Tracings of spontaneous rhythmical contractions of the umbilical arterial smooth muscle at low Po_2 (~15mmHg) in (a) perfused preparations and (b) helical strips.

10 shows the maximum amplitude of these rhythmic contractions which were 14mmHg and 0.25g respectively. The frequency of contraction varied between 1 and 2 mins in perfused arteries, and between 1 and 3 mins in helical strips.

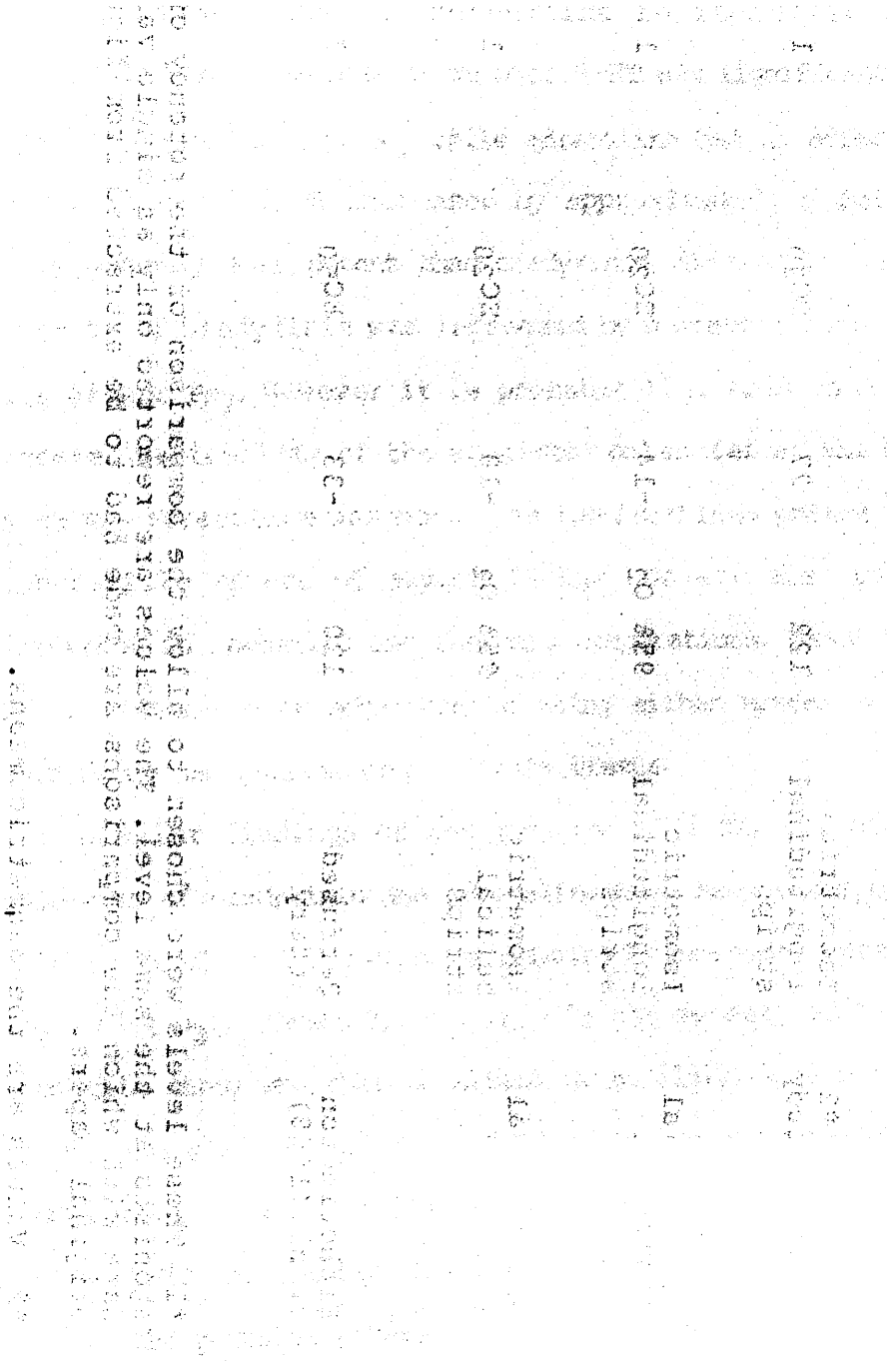


Table 2 Equipotent molar ratios of the agonists bradykinin (BK), adrenaline (ADR) and 5-HT in the human umbilical artery, 5-HT=1: a comparison of the results from this study and from two other studies.

	Preparation (arterial)	Po_2 (mmHg)	EC_{50} 5-HT (nM)	Level at*1 which compared	Potency, relative to 5-HT		
					5-HT	BK	ADR
Present study *2	Isometric longitudinal strips	122	27	EC_{30}	1	4.2	112
Altura et al (1972)	Isometric longitudinal strips	95% O_2	~14	EC_{20}	1	4	64
Altura et al (1972)	Isometric helical strips	95% O_2	~10	EC_{20}	1	4	not tested
Eltherington et al (1968)	Perfused artery	120	~33	EC_{50}	1	0.07	1.4

*1 These levels were chosen to allow the comparison of the potency of the three agonists at the same level. The values are reported only as single values as the data with which the comparisons are made had to be extracted from figures in the original papers.

*2 Values are the geometric means.

Discussion

In the HUA the isometric or isotonic contractions of longitudinal strips to 5-HT, bradykinin, adrenaline or oxygen showed similar concentration-response characteristics. However, in the perfused artery their relative effects were different.

There was no significant difference between the relative potencies of 5-HT, bradykinin and adrenaline in isometric and isotonic preparations. In either technique 5-HT was significantly more potent than bradykinin at low Po_2 while adrenaline had no effect. At high Po_2 the potency of 5-HT increased by approximately 6 fold and was not significantly more potent than bradykinin. This might suggest that the potency of bradykinin was increased by a greater extent than 5-HT at the higher Po_2. However it is probable that this is due more to the greater variability of the agonists' potencies at the higher Po_2. At high Po_2 adrenaline was more than 100 fold less potent than 5-HT. The contractile effect of oxygen in the HUA was also found not to be different in isometric and isotonic preparations. Hence there seems to be no difference or advantage in using either system for studying the physiology and pharmacology of this tissue.

Similar findings of the potency of 5-HT, and of the relative potencies of bradykinin and adrenaline have been found by other groups (Altura et al, 1972) who have examined the vessel under much higher Po_2's (95% O_2), (Table 2). Not only is the potency of 5-HT similar in both this study and that of Altura et al (1972) in longitudinal strips (EC_{50}'s are similar) but the relative potencies of bradykinin (~4 fold less potent) and adrenaline (~2 orders of magnitude less potent) to that of 5-HT are similar in these studies.

In the perfused artery at low Po_2 bradykinin was equipotent to 5-HT. Adrenaline contracted the artery but was substantially less potent

than the other two agonists. Adrenaline did not contract longitudinal strips of artery at low Po_2. This difference could be explained in terms of an action of adrenaline on specific muscle bundles in the artery, i.e. on the circular muscle alone. The other main muscle bundle in the HUA is a longitudinal one and it has been shown that these two main orientations of muscle react differently to a number of stimuli including adrenaline, indeed it was found that adrenaline contracted the circular but not the longitudinal muscle (Roach, 1972).

At high Po_2 the perfused artery acquired a supersensitivity to the agonists which were tested which often resulted in a maximal contraction at the threshold concentration for each agonist. However, it was still clear that bradykinin was never greater in potency than 5-HT and that adrenaline was ~2 orders of magnitude less potent than 5-HT. Our results in the HUA indicate that in the three standard physiological techniques for measuring smooth muscle tone i. e. isometric, isotonic and perfusion pressure, the rank order of potency of the agonists is 5-HT$\geq$ bradykinin>>adrenaline , at either Po_2. Table 2 shows that the potency of the agonists tested here is in agreement with that found by Altura et al (1972), in both longitudinal strips and in helical strips which primarily examines the circular musculature.

The results of Eltherington et al (1968) are however substantially different. They found that in perfused preparations bradykinin (EC_{50} ~2nM) was 15 fold more potent than 5-HT and that adrenaline was only slightly less potent than 5-HT. No explanation is readily apparent to reconcile these latter results with those of my study or with those of Altura et al (1972). Another difference exists between my results and those of Eltherington et al who found that the responses to 5-HT and bradykinin were negligible at low Po_2 (~15mmHg). In this study increasing the Po_2 certainly facilitated the responses but at low Po_2 the responses were large and consistent, certainly not negligible as

they found.

As already described, the potencies of the agonists 5-HT, bradykinin and adrenaline were found to be similar in preparations examined by constant flow perfusion pressure or by longitudinal isometric tension techniques. In the perfused preparation the response to stimuli is a summation of effects on circular and longitudinal muscle of which the HUA contains one distinct layer of each type (Roach, 1972). (In addition there are two small assymmetric bundles). An increase in inflow pressure will be brought about only if there is a decrease in the cross-sectional area of the vessel lumen, a significant change in length being unlikely. Our results for 5-HT, bradykinin and adrenaline show the same rank order of potency in the perfused vessel and in paired preparations recorded isometrically and also in perfused vessels under isometric tension. For each agonist our results show similar relative potencies in each experimental set-up. This suggests that the various muscle layers respond in a similar manner to these stimuli.

The effects of oxygen in the different preparations are somewhat different however. In the isometric, isotonic and the "perfused tensioned" vessel oxygen could produce a response. However it could not constrict the "perfused untensioned" vessel. This variable effect of O_2 has been described in earlier separate studies although the requirement of the artery to be under tension was not noted as an explanation for the difference of the effect of O_2. Thus, in perfused arteries not held under tension oxygen was a poor stimulus for contraction (Lewis, 1968) while in perfused-tensioned arteries oxygen caused concentration-related contractions (Eltherington et al, 1968).

This difference can be explained if oxygen acts only on the longitudinal muscle. In the perfused untensioned vessel the isotonic

contraction of the longitudinal smooth muscle may not affect the lumen diameter but simply alter the overall spiral of the vessel, as was usually observed. Thus, 5-HT, bradykinin and adrenaline can act on each muscle layer but oxygen can act only on the longitudinal muscle.

The response to the agonists at high Po_2 -a maximal or near maximal contraction at the threshold concentration was rather perplexing and one which defied further attempts to find an explanation. This effect was not due to tachyphylaxis as higher concentrations would sometimes evoke an equal or greater response. These perfusions were made by a cumulative increase of the agonist concentration. A non-cumulative perfusion of 5-HT at high Po_2 gave concentration-related contractions in only three of six arteries tested and so did not resolve the problem. A few other experiments in which the flow was varied, or where the tension on the perfused vessel was varied, did not provide any clearer picture.

Variablity of the effects of agonists within any one perfused preparation have been noted by some authors (Eliasson and Astrom, 1955; Astrom and Samelius, 1957) but not by others who reported that agonists evoked reproducible responses (von Euler, 1938; Gokhale et al, 1966). There seems to be no reasonable, simple explanation to account for these differences.

For two reasons I feel that this effect of the agonists in the perfused artery is entirely related to the high Po_2 (~120mmHg): (i) The first reason is very simple in that agonist-induced responses at <u>low</u> Po_2 were concentration-related. (ii) In other experiments on longitudinal strips low concentrations of the 5-HT analogue, 5-carboxamidotryptamine, induced similar "all or nothing" contractions in some preparations and which required prostaglandins (see chapter 3). At low Po_2 this was not seen, presumably due to the requirement of the prostaglandin synthesising enzyme (cyclo-oxygenase) for oxygen. The

reason why this agonist effect should be manifested in the perfused artery to the extent that in some preparations higher concentrations of agonist were unable to induce a contraction is not apparent. Possibly there is some long lasting prostaglandin-induced desensitisation of the receptors or of the excitation-contraction coupling process. However if this were so it would appear to occur only in the perfused preparation. This explanation seems hard to accept.

The highly constricted nature of the perfused artery which was found at the start of the perfusion has been commented on in the past by a number of groups (von Euler, 1938; Lewis, 1968; Eltherington et al, 1968). This has generally been attributed to cooling of the umbilical cord after birth as cooling of the perfusate during the experiment would cause vasospasm (von Euler, 1938; Gokhale, 1966).

In the human umbilical vein vasoconstriction caused by cooling of the perfusate was mediated via prostaglandins as incubation with indomethacin significantly reduced the subsequent constriction caused by reducing the perfusate temperature (Boura et al, 1979). It is therefore possible that vasoconstriction of the HUA is due to prostaglandins whose production is stimulated by cold and/or oxygen since this study has shown that the oxygen-induced contraction of the HUA is mediated by prostaglandins (see chapter 2).

The concentration of bradykinin found in the umbilical arterial blood at term (4.5ng/ml, Melmon et al, 1968) would only be a very weak stimulus at either Po_2 studied here, in any of the experimental techniques used (isometric, isotonic and perfused preparations).

Stacey (1966) has estimated the concentration of 5-HT in the umbilical arterial blood at birth. This was 43ng/ml of blood. Even if only 1% of this was free then it would seem that 5-HT could be a potential physiological vasoconstrictor of the HUA. If 5-HT were

released into the fetal circulation, by a drug or, perhaps, by an anaphylactic reaction, it would reduce or cut off the blood supply leading to growth retardation or abortion. Similarly, any drug with agonism at these receptors and which reached the fetal circulation would have similar consequences, as has been postulated previously for LSD (Gant and Dyer, 1971). On the other hand 5-HT antagonists, if there was a 5-HT involvement in spontaneous abortion, might lead to its suppression and hence to the continued developement of a fetus which might otherwise have been rejected.

The plasma concentrations of the catecholamines adrenaline and noradrenaline are elevated at term (10^{-9}–10^{-7}M, Inglis et al, 1981) but are still below the threshold necessary for contraction of this tissue (>10^{-7}M, this study). It is therefore unlikely that adrenaline and noradrenaline may play a physiological constrictor role in the umbilical circulation.

The spontaneous rhythmic contractions of the HUA seem unlikely to be due to the toxic effects of high partial pressures of oxygen, as suggested by Roach (1972), since it was found in this study that the rhythmic activity occurred at physiological Po_2, i. e. approximately 20mmHg. I suggest, rather, that this activity is due to rhythmic contractions of the circular muscle layer since I have never seen spontaneous contractions of longitudinal strips at low Po_2 (>250). I also consider that these contractions, if they occur <u>in utero</u>, would be of no great benefit in the propulsion of blood in the fetal circulation as suggested by Altura et al, (1972). Although the spontaneous contractions were quite large (up to 14mmHg at a base line pressure of 60mmHg) the greatest frequency was 1 per min. This possible contribution to the flow rate is surely negligible in comparison to the flow rate recorded in the artery of ~120ml/min (Stembera et al, 1965).

Roach (1972) has shown from theoretical considerations and

experimental findings that it is the longitudinal muscle layer which is probably involved in the complete closure of the vessel at birth, the circular muscle being capable only of narrowing the vessel lumen. Since I wished to investigate the effects of oxygen and drugs on the umbilical artery, *in vitro*, and to predict their actions *in utero*, then preparations which examined the longitudinal muscle were necessary. This initial study has concluded that the effects of oxygen and of drugs on the longitudinal smooth muscle of the HUA were similar whether recorded by isometric or isotonic contraction or by perfusion pressure techniques. In chapters 2 and 3 isometric contraction was employed to investigate respectively (a) the contractile effect of oxygen on the HUA and (b) the modifying influence of oxygen on the action of drugs, and in particular 5-HT, on the HUA. Isometric contraction was chosen in preference to the two other methods which have been described in this section for no better reason than for its technical simplicity.

Chapter 2: The Oxygen-induced contraction of the Human Umbilical Artery

Introduction

In 1982 McGrath and Stuart-Smith demonstrated that elevating the oxygen tension (Po_2) of the saline bathing isolated strips of human umbilical artery (HUA) evoked a contraction which was inhibited by indomethacin. This confirmed earlier work (Lewis, 1968; Bor and Guntheroth, 1970; Oberhansli-Weiss et al, 1972) as to the effect of O_2 but also suggested that the effect was mediated via prostaglandins. Since this current study closely examines the effect of physiological changes of Po_2 (→100mmHg), (which these earlier studies did not do) and the role of prostaglandins it seems pertinent to introduce this family of biologically active compounds and their role in the fetal cardiovascular system.

The acidic, lipid-soluble, pharmacologically active extract of human seminal fluid was first described by von Euler (1936) who coined the term prostaglandin in the belief that it was secreted from the prostate gland. He found that intravenous administration of a crude extract of the prostaglandin gave persistent lowering of the blood pressure in several species and increased the activity of intestinal and uterine smooth muscle. Later work showed that the prostaglandin was synthesised in the seminal vesicles and vesicular gland (Eliasson, 1959).

In the 30 years after von Euler's discovery, research on the prostaglandins was fairly stagnant: by 1964 only 30 papers or so, concerned with prostaglandins, were published in that year. Since then, research in this field has probably undergone one of the greatest upsurges in scientific interest and which saw publications rise almost exponentially to ~7500 in 1974 (Karim and Rao, 1974). In the intervening years it had been shown that prostaglandins had profound effects in virtually all organ and cellular systems, e.g. the cardiovascular, respiratory, gastro-intestinal, central and peripheral

nervous systems and the eye. Indeed the capability of all (and other) of these systems to synthesise prostaglandins has been found (reviewed by Hansen, 1974).

The prostaglandins are 20-carbon fatty acids which are synthesised from 3 polyunsatturated fatty acids: 8,11,14-Eicosatrienoic acid (dihomo γ-linoleic acid); 5,8,11,14-Eicosatetraenoic acid (arachidonic acid) and 5,8,11,14,17-Eicosapentaneoic acid, which are themselves formed from the essential fatty acid linoleic acid. The conversion of these fatty acids to the prostaglandins is catalysed by the cyclooxygenase (prostaglandin synthetase) enzyme system. The prostaglandins formed from these fatty acids are given the subscripts 1,2 or 3 respectively as this reflects the degree of unsaturation of the compound (the number of double bonds). Since the most abundant precursor in animal tissues is arachidonic acid, the principal prostaglandins are of the 2 series. The prostaglandins are also designated letters e.g. PGF, PGA, PGB, PGE. Of these, PGE and PGF are known as the primary prostaglandins since the other prostaglandins (with the exception of PGI_2 and thromboxane A_2) are formed from them. There is some debate whether prostaglandins of the A and B series which have been detected in some tissues, are true enzymatic products of PGE since they are easily formed by treatment of PGE with acid or base respectively, and thus may simply be formed during extraction and isolation procedures (Flower, 1974). Other evidence suggests that they may be true enzymatic products (Atallah et al, 1974).

The following notes represent the principal steps in the elucidation of the cascade of arachidonic acid metabolism.

1 Conversion of arachidonic acid into PGE_2 demonstrated in homogenates of sheep vesicular glands (van Dorp et al, 1964; Bergstrom et al, 1964). Formation of F series prostaglandins shown later.

2 The oxygen atoms incorporated in the synthesis of prostagladins were shown to be derived from molecular O_2 (Nugteren and van Dorp, 1965; Samuelsson, 1965). The latter work also suggested the concept of an unstable endoperoxide intermediate in prostaglandin biosynthesis.

3 A highly unstable product was found to be released into the effluent following anaphylactic shock in guinea-pig lungs. Because of the bioassay which was used this was called "rabbit aorta contracting substance" (RCS). Its release was inhibited by aspirin-like drugs (Piper and Vane, 1969). It was later suggested that its activity was due to the unstable endoperoxide (Gryglewski and Vane, 1972).

4 The unstable endoperoxide intermediate of prostaglandin biosynthesis was isolated and shown to consist of two products, both of which were endoperoxides. These were termed PGG_2 and PGH_2 (Nugteren and Hazelhof, 1973, Hamberg et al, 1974).

5 With pure forms of PGG_2 and PGH_2 available it was shown that the activity of RCS could not be due to the endoperoxides as their half lives were so different (~5 mins and ~30secs respectively), (Svensson et al, 1975). The main activity of RCS was subsequently shown to be due to thromboxane A_2 (TxA_2), a highly unstable product formed from the cyclic endoperoxides, while some of the activity was due to PGG_2 and PGH_2 (Hamberg et al, 1975).

6 Prostacyclin (PGI_2) was discovered by Vane and co-workers (Moncada et al, 1976; Bunting et al, 1976). This unstable product formed from the endoperoxides was shown to be a potent vasodilator and inhibitor of platelet aggregation. It was first known as PGX but its structure was soon elucidated (Johnson et al, 1976).

The possible physiological role of the prostagladins has been investigated in three main ways (1) By examining the effects of exogenous prostaglandins (2) By using specific inhibitors of prostaglandin biosynthesis (3) By using specific antagonists of the

receptors for prostaglandins. These points shall be discussed in relation to vascular smooth muscle.

Vascular smooth muscle and prostaglandins

The actions of the prostaglandins (before the discovery of the unstable endoperoxides, and of TxA_2 and PGI_2) on blood pressure, blood flow and on isolated tissues has been comprehensively reviewed (Malik and McGiff, 1976). Prostaglandins of the E, A, B and C series when given systemically lower arterial blood pressure and increase blood flow by their dilator action on resistance vessels (arterioles, metaarterioles and precapilary sphincters). Of these, prostaglandins of the E series are the most potent. Prostaglandins of the F series increse arterial pressure via constriction of capacitance vessels. These are the general findings and, as with most drugs, there is some species differences as to their action and potency.

The effects on isolated vascular smooth muscle are somewhat different since in many arterial preparations both PGE_2 and PGF_2 cause contraction. This includes an effect on rabbit aorta and dog coronary artery (Strong and Bohr, 1967) and human umbilical artery (HUA), (Hillier and Karim, 1968).

Since the discovery of PGI_2 and TxA_2, the physiological role of these other prostaglandins has been questioned since in many isolated vascular tissues PGI_2 has been found to be the main metabolite of arachidonic acid (Moncada and Vane, 1980). This has led to an intense study to reassess the effects of arachidonic acid and its metabolites on vascular tissue and the cardiovascular system.

The enzymatic transformation of PGG_2 and PGH_2 into TxA_2 was first demonstrated in platelets (Hamberg et al, 1975) but has since been shown in lung tissue, spleen, kidney, brain and in vascular smooth muscle. Both the unstable endoperoxides and TxA_2 are potent stimulants

of platelet aggregation (Hamberg et al, 1974; 1975) and potently contract vascular smooth muscle of the rabbit aorta (Hamberg et al, 1975), pig coronary artery (Svensson and Hamberg, 1976) and HUA (Tuvemo et al, 1976). In the HUA PGE_2 was ~60 fold less potent than the endoperoxides while PGH_2 was 9 to 60 times less potent than TxA_2. Quantitatively similar results of these relative potencies have been found in other smooth muscle preparations.

PGI_2 is 20 to 30 times more potent than PGE_1 as an inhibitor of human platelet aggregation. Moreover, PGI_2 is able to disaggregate platelets after aggregation has been induced (Moncada et al, 1976). In vivo, PGI_2 causes a fall in blood pressure in dog, rat and rabbit with a greater potency than PGE_2 (Armstrong et al, 1977). In vitro, PGI_2 induces relaxation of most vascular strips (see Moncada and Vane, 1976 for references) but there are some exceptions in which it causes contraction, e.g. pig coronary artery (Dusting et al, 1977a).

In general then, PGI_2 and TxA_2 have opposing actions. This led Vane and co-workers (Moncada and Vane, 1976; Bunting et al, 1976; Dusting et al, 1977b) to propose an interesting hemostatic hypothesis. They proposed that (1) the basal formation of PGI_2 by the vascular endothelium may be important in the maintenance of the normal integrity of vessel walls by inhibiting the adherance of platelets (which is promoted by TxA_2); (2) PGI_2 may normally limit thrombus formation; (3) when a vessel wall is damaged, the formation of a normal hemostatic plug may be assisted by diminished PGI_2 production.

Prostaglandin synthesis inhibition

This has been reviewed by Flower (1974). Since the cyclo-oxygenase enzyme system catalyses the first step of the conversion of arachidonic acid to the cyclic endoperoxides, not only will formation of the primary prostaglandins be inhibited by synthesis inhibitors but so to

will formation of the endoperoxides, as well as PGI_2 and TxA_2.

Three main classes of compounds have been shown to inhibit prostaglandin biosynthesis.

1 Structural analogues of the precursor, arachidonic acid, and other fatty acids have been prepared and tested mainly against the enzyme system in vitro (see Table 1, Flower, 1974). Many of the substrate analogues possess good inhibitory potency (in the micromolar range) but other fatty acids which inhibit the enzyme system seem to do so in a non-specific manner and only in high concentrations, e.g. oleic, linoleic and linolenic acids inhibited the enzyme from sheep seminal vesicles and in acetone powders of rat stomach, but the concentrations required for inhibition were high (1.8–5.0mM), (Pace-Asiak and Wolfe, 1968). The nature of the inhibition by this class of compounds is either by a reversible, competitive interaction at the substrate site alone, or a combination of a competitive inhibition and a time-dependent inactivation (destruction) of the enzyme (Lands, 1985).

2 The "aspirin-like" drugs were first shown to inhibit prostaglandin synthesis by Vane and co-workers (Vane, 1971; Smith and Willis, 1971; Ferreira et al, 1971) in cell-free homogenates of guinea-pig lungs, in human platelets and in dog spleen respectively. Indeed they proposed that the therapeutic benefits of the antiinflammatory agents they used (aspirin, indomethacin and sodium salicylate) were due to their inhibition of prostaglandin synthesis. Inhibition of prostaglandin synthesis has since been demonstrated in almost all laboratory species and in many biologic$_{\wedge}^{al}$ preparations, ranging from cell-free microsomal preparations to whole animals including man (see Tables 4,5 and 6, Flower, 1974). The mode of action of the aspirin-like drugs indomethacin, flurbiprofen, and aspirin itself, is by a competitive interaction at the sustrate site in addition to a time-dependent,

irreversible inactivation of the enzyme (Lands, 1985). Aspirin forms a covalent acetylated derivative of the enzyme which is the basis of the irreversible inactivation (Roth and Majerus, 1975). Such an explanation is not readily available, however, for the other irreversible inhibitory agents (Lands, 1981).

3 The third group of inhibitors includes such diverse agents as metal ions, anti-oxidants and nucleotides. However, the concentrations necessary to achieve inhibition are often high and no degree of specificity can be claimed (Flower, 1974).

Receptors for Prostaglandins

Substances which inhibit the actions of prostaglandins were discoverd before it was found that aspirin-like drugs inhibited the synthesis of prostaglandins. However the first prostaglandin antagonists on which research was centred (analogues of 7-oxaprostaglandin; SC19220 and poly phloretin phosphate (PPP)) have not gained the prominence of the cyclo-oxygenase inhibitors in prostaglandin research for the following reasons (Sanner and Eakins, 1976). 1 They do not inhibit all the actions of prostaglandins. 2 Different antagonists inhibit different prostaglandin activities. 3 There are species and tissue differences. 4 PPP and 7-OPyA (the latter an analogue of 7-oxaprostaglandin) inhibit various enzymes. 5 They are not highly potent. 6 Little is known about their therapeutic potential. Nevertheless the evidence gained using these antagonists in many isolated tissues, and in vivo, overwhelmingly suggested that prostaglandins exerted their effects via distinct receptors.

The first classifications of receptors for prostaglandins were made by Anderson and Ramwell (1974) and Jones (1976). These classifications have since been criticised as they relied on the comparison of the rank order of potency of agonists alone (Coleman et

al, 1984). The latter group (Kennedy et al, 1982; Coleman et al, 1984) have proposed a classification for receptors on smooth muscle and platelets which is based not only on the potency of agonists but is supported by evidence of the selective antagonists SC19220 and AH19437. They propose that there are distinct receptors for each of the natural prostaglandins (E_2, F_2, D_2 and I_2) and for TxA_2. At each receptor one of the four prostaglandins and TxA_2 was found to be most potent while the others were considerably weaker. SC19220 antagonised some but not all PGE_2-sensitive receptors, but was without effect at all other receptors. AH19437 blocked TxA_2-sensitive receptors but not any of the prostaglandin-sensitive receptors.

Prostaglandins and the Fetus

Investigation of the action of prostaglandins in the fetus has largely been confined to a study of their possible role in specialised vessels whose constriction or dilation at birth cause dramatic cardiovascular changes.

In utero, the ductus arteriosus allows blood in the pulmonary artery to bypass the functionally inactive lungs, to the aorta. The current evidence strongly suggests that patency of the vessel, in utero, is maintained by the influence of vasodilating prostaglandins (Coceani and Olley, 1980; Coceani et al, 1980). Foremost amongst this evidence is that both in vitro (Clyman, 1978) and in vivo (Friedman et al, 1978), (see also Coceani et al, (1980) for references) indomethacin constricts the ductus. This effect has been seen in several species including lamb, rabbit and rat. The prostaglandin which causes relaxation is probably PGE_2, since, under hypoxic conditions (the normal physiological environment) PGE_2 is the most potent vasodilator formed by the ductus arteriosus (EC_{50} ~$1X10^{-12}$–$1X10^{-10}$M) while the endoperoxides and PGI_2, which also cause relaxation, are 2 and 4 orders

of magnitude less potent than PGE_2 respectively (Coceani and Olley, 1980).

Although it is considered that the postnatal increase in arterial blood Po_2 is crucial to closure of the ductus at birth (Heymann and Rudolph, 1975) the mechanism underlying this is unclear. Increasing the Po_2, in vitro, contracts the ductus arteriosus (Kovlavich, 1963) but which is not inhibited by indomethacin (Coceani et al, 1976). This argues against the idea of a contractile prostaglandin ($PGF_{2\alpha}$) being the mediator of the O_2 effect (Starling and Elliott, 1974). The most recent suggestion is that the loss of sensitivity of ductal tissue to PGE_2 which occurs towards term (Clyman, 1980) in combination with the almost complete loss of relaxant effect of PGE_2 at high Po_2's (Coceani et al, 1980) leads to the O_2-induced contraction of the vessel by some, as yet unknown, mechanism.

Similar evidence has been presented which suggests that the ductus venosus (which allows well oxygenated umbilical venous blood to bypass the liver) is under the influence of a vasodilator prostaglandin in utero (Adeagbo et al, 1982). In vitro, PGI_2 and PGE_2 were almost equipotent as vasodilators, so that either PGI_2 or PGE_2 may be the endogenous vasodilator (a stable endoperoxide and $PGF_{2\alpha}$ were vasoconstrictors). Although O_2 contracted the ductus venosus it was not demonstrated that this was not prostaglandin mediated (i.e. indomethacin was not tested against O_2-induced contractions). Therefore O_2 may contract the ductus venosus at birth by inducing the synthesis of a vasoconstrictor prostaglandin, although this is only my own speculation.

Other evidence also implicates prostaglandins as mediators of the pulmonary vascular relaxation which is coincident with the initiation of breathing at birth (Leffler et al, 1978). From evidence of the relative potencies of vasodilators it is considered that PGI_2 may be

the likely vasodilator prostaglandin which is formed at birth when the Po_2 increases (Coceani et al, 1980). It is not known whether the vasoconstriction of the pulmonary system, in utero, is prostaglandin mediated.

Prostaglandins have also been suggested as mediators of the closure of the umbilical vessels at birth (Dawes, 1978; Tuvemo, 1980), although the evidence on which these claims have been based may be regarded as somewhat circumstantial. Karim (1967) identified the smooth muscle stimulating substance in human umbilical cords as a prostaglandin and later showed that PGE_2 and $PGF_{2\alpha}$ caused contraction of the isolated cord vessels while PGE_1 caused relaxation (Hillier and Karim, 1968). Other studies have subsequently shown that the endoperoxides and TxA_2 are more potent constrictors of the isolated artery (Tuvemo et al, 1976; Svensson et al, 1977) while the principal products of arachidonic acid metabolism in the artery are PGI_2 and PGE_2 (Bjoro et al 1986).

This line of evidence has never been considered in association with other studies which have shown that O_2 (but only at high Po_2's) causes vasoconstriction of the HUA. Bor and Guntheroth (1970), Roach (1972) and Oberhansli-Weiss et al (1972) found that an increase of Po_2 from "hypoxic" (which is normal for umbilical cord blood) to "hyperoxic" conditions contracted the arterial smooth muscle. These studies could not answer the question whether the increase of Po_2 of umbilical cord blood from fetal (15mmHg) to neonatal levels following delivery, could be an important stimulus to bring about vessel closure at birth, since the appropriate range was not investigated.

However, if contraction of the vessel smooth muscle does occur at the appropriate levels of O_2 it may represent a vital physiological mechanism promoting the change from fetal to neonatal circulation. An

investigation of the effect of O_2 on the isolated human umbilical artery is therefore the subject of this chapter, together with a study of the possible role of prostaglandins.

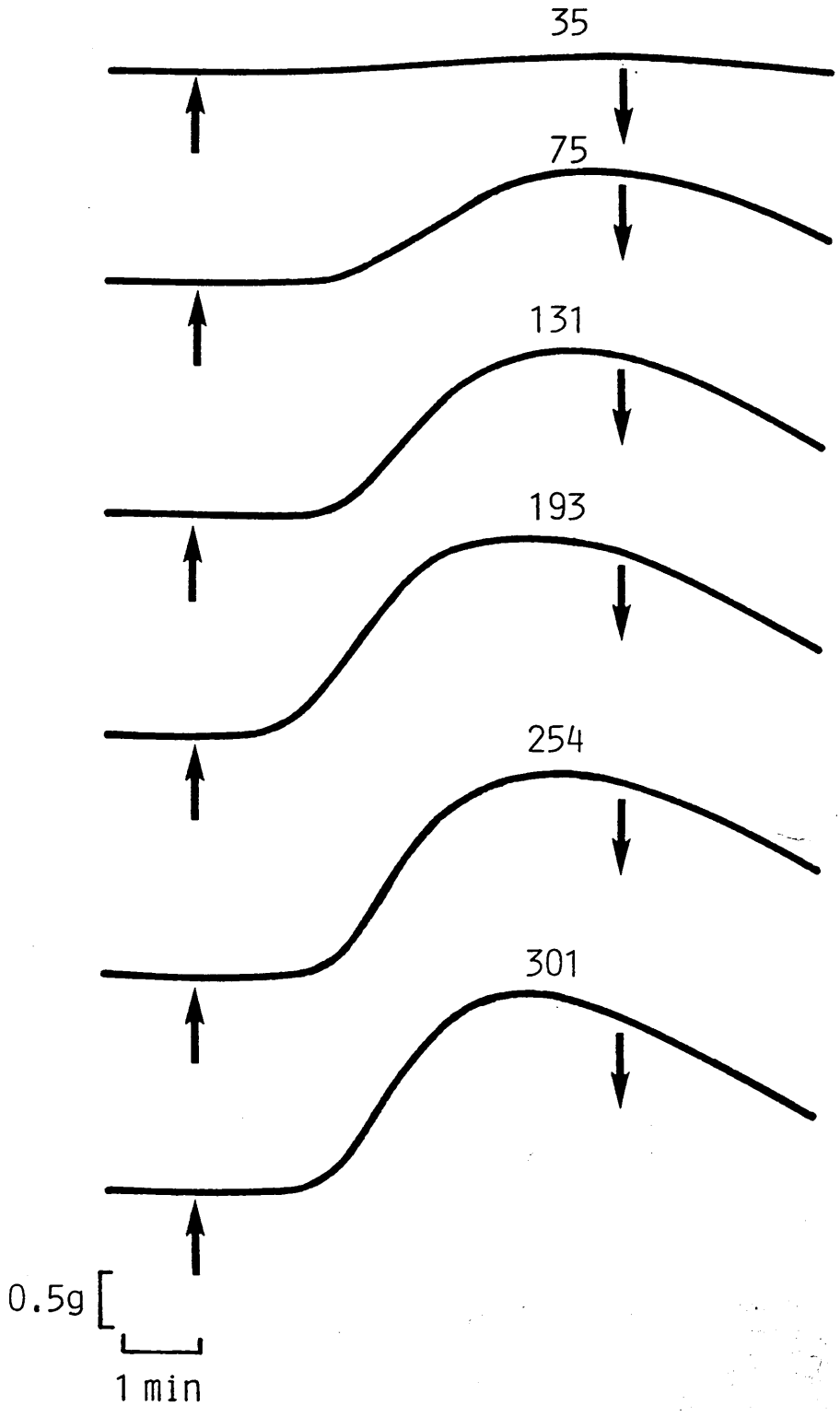
Results

After setting up longitudinal strips of HUA in saline they were allowed to stabilise for 2 hours under physiological gas tensions before increasing the Po_2. The physiological gas tensions were: Po_2 ~15mmHg; Pco_2 ~45mmHg and pH ~7.28. This was acheived by aerating the saline with a gas mixture of composition 2.5% O_2; 8% CO_2; balance N_2. Higher Po_2's were achieved by increasing the % O_2 composition while keeping constant the CO_2 content.

Increasing the Po_2 (for 5 mins) evoked "concentration"-related contractions (figure 1a). Contractions induced at lower Po_2's (i.e. smaller contractions) were usually better maintained than contractions induced at higher Po_2's: Larger contractions were not maintained and fell towards baseline tension before the Po_2 was reduced. Since the contractions were not maintained cumulative concentration-response curves (CRC's) could not be constructed. Reproducibility of CRC's to oxygen was not investigated. The effects of drugs on the response to oxygen were tested by adding them to separate strips from the same artery, a separate strip acted as a control. CRC's to oxygen were then constructed in parallel on all the strips.

The apparent lag time between changing the Po_2 and the consequent contraction (as shown in figure 1a) is due to the time taken for the new gas mixture to reach the saline. Figure 1b shows that the initial rise of the O_2-induced contraction is virtually co-incident with the rise in Po_2 of the saline

As already described, the contraction of human umbilical arterial smooth muscle by oxygen was concentration-dependent and was related linearly to the $\log(Po_2)$, (figure 2). The threshold to oxygen was found to be influenced by pH. The sensitivity to O_2 at the different pH values was examined in three separate groups of preparations. At


a
35
75
131
193
254
301
0.5g
1 min

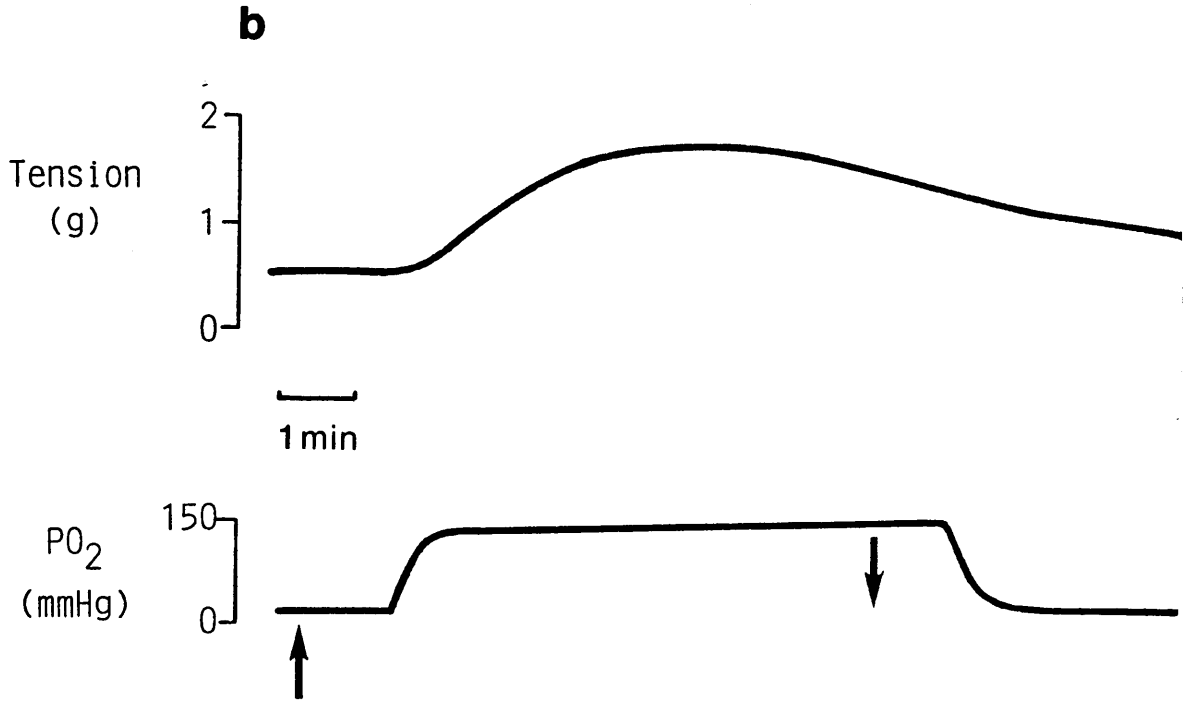


Figure 1 (a) These tracings are representative recordings of oxygen-induced contractions in one longitudinal strip of human umbilical artery. The oxygen content of gas mixture aerating the saline was increased (↑) for 5mins and then decreased (↓) to the original value. The Po_2 of the saline increased in each case from 16mmHg to the values shown. Smaller contractions were better maintained than larger contractions: larger contractions were generally not maintained and decayed even before the Po_2 declined. (b) This tracing shows a contraction induced by oxygen and the simultaneous recording of the Po_2 of the saline. The oxygen content of the gas mixture was increased and decreased at the arrows. This shows that the initiation of the contraction is coincident with the rise in Po_2 of the saline. The apparent lag after increasing the oxygen content of the gas mixture, before the onset of the contraction, is due to the time taken after changing the gas mixture for the new gas mixture to reach the saline.

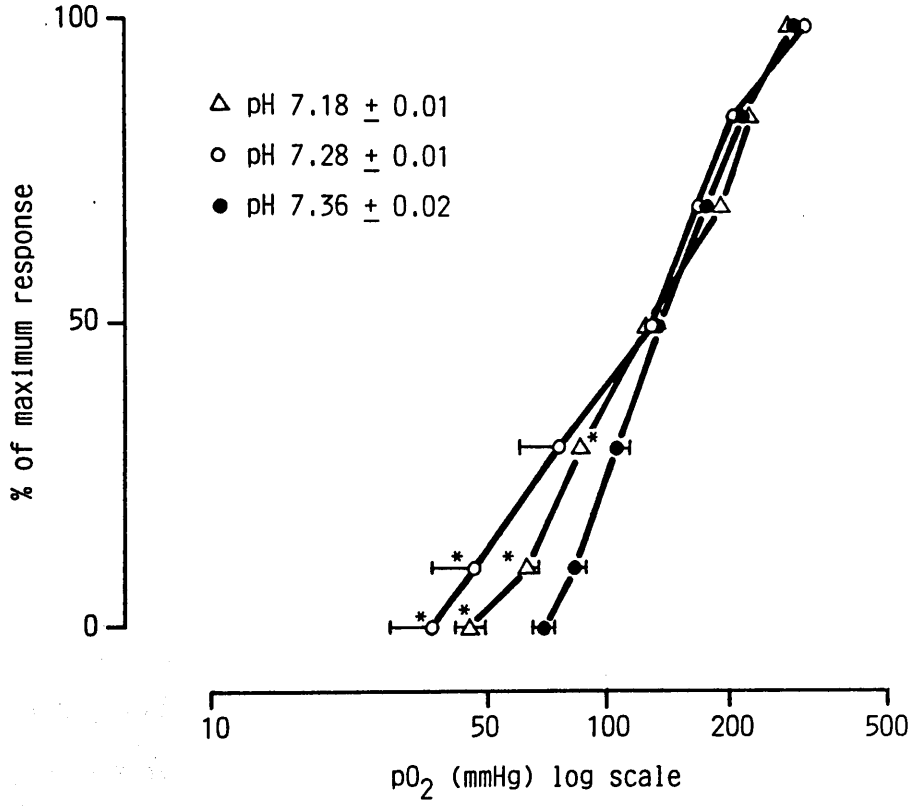


Figure 2 Concentration-response relationship for contraction of human umbilical artery by oxygen at pH=7.18; pH=7.28 and at pH=7.36 (n=6 each group). Asterisks denote significant differences between the curves at pH 7.18 or 7.28 and the curve at pH 7.36. For clarity, error bars are shown only at some points. For each individual tissue the response (% of maximum response) was plotted against log{Po_2}. The Po_2 producing 0, 10, 30, 50, 70, 85 and 100% of the maximum response was interpolated from each curve and the geometric means±s.e.m. calculated. Log{Po_2}-response curves of the geometric means±s.e.m. were drawn.

pH=7.36 (Pco_2 =31±1mmHg) the threshold was 69mmHg (geometric mean, 95% confidence limits 60–79mmHg); at pH=7.28 it was 36mmHg (19–69mmHg) and at pH 7.18 ($[HCO_3^-]$=17mM) it was 45mmHg (37–54mmHg). The threshold to O_2 at pH 7.36 was significantly greater ($P<0.05$) than at pH 7.18 or 7.28. There was no statistically significant difference between the concentration-response curves at pH 7.18 and 7.28. Figure 2 shows the lower sensitivity to oxygen at pH 7.36.

Maximum contractions were induced at (i) Po_2= 282mmHg (geometric mean, 95% confidence limits 203–390mmHg), (pH=7.36) ; (ii) 297mmHg (259–341mmHg), (pH=7.28) and (iii) 276mmHg (254–300mmHg), (pH 7.18). There was no significant difference between the mean size of the maximum contractions or the Po_2 at which they occurred, at the different pH's. At pH 7.28 the maximum contraction was 0.96±0.12g. At higher Po_2's the responses were submaximal: at Po_2 460 mmHg (pH 7.28) the contraction induced was 70±9% of the maximum. In all further experiments pH=7.28 was used.

In these initial experiments there was a large variation between the maximum contractions to oxygen even in preparations from the same artery, under identical experimental conditions. In an attempt to limit this variation the (wet) weight of tissue was recorded and contractions expressed relative to this.

Effect of cyclo-oxygenase inhibitors

In preparations incubated with indomethacin ($1X10^{-6}M$ or $1X10^{-7}M$) oxygen did not induce concentration-dependent contractions in individual tissues. This precluded the construction of Po_2-response curves as previously described. In this case the contraction induced by O_2 (g/gram of tissue) was plotted against $\log\{Po_2\}$ for each tissue and the mean±s.e.m. calculated (figure 3). These concentrations of indomethacin ($1X10^{-6}M$ or $1X10^{-7}M$) caused neither a relaxation nor

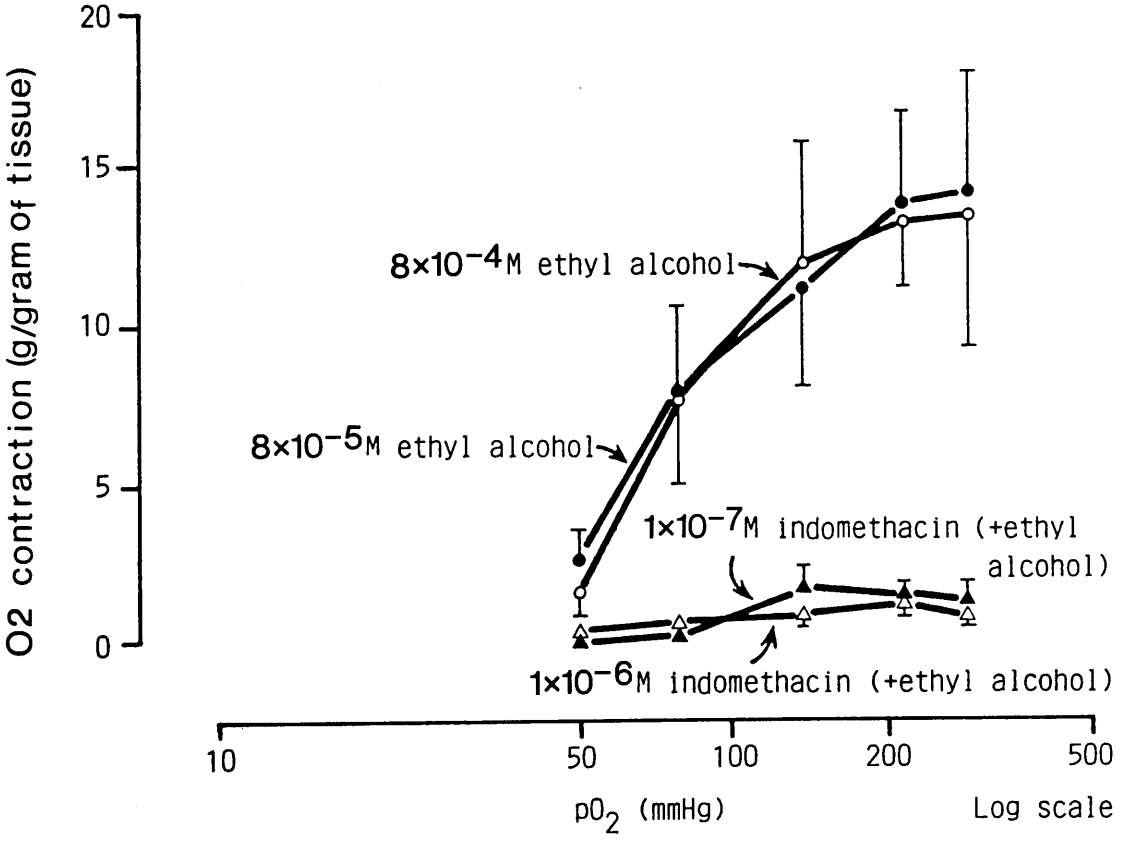


Figure 3 Indomethacin, $1X10^{-6}M$ or $1X10^{-7}M$ significantly reduced the oxygen-induced contractions of human umbilical artery. In paired preparations the vehicle for indomethacin, ethyl alcohol, ($8X10^{-4}M$ or $8X10^{-5}M$ respectively), did not prevent the response to oxygen. (n=6 each group). Vertical bars are the mean±s.e.m.

contraction of the tissue.

Indomethacin ($1X10^{-6}M$ or $1X10^{-7}M$) significantly reduced the contractions at all Po_2's when compared to control preparations which were exposed to the indomethacin vehicle, ethyl alcohol ($P<0.02$). Ethyl alcohol ($8X10^{-4}M$ or $8X10^{-5}M$ respectively) alone did not significantly alter the concentration-response curve to O_2 (figure 4).

Indomethacin significantly reduced the maximal O_2-induced contractions by 89±4% ($1X10^{-6}M$) and by 87±5% ($1X10^{-7}M$) when compared to control preparations exposed to ethyl alcohol. Ethyl alcohol alone did not significantly change the maximal contractions to O_2 or to 5-HT of the preparations, which in the controls were 14.9±3.2 g/gram of tissue (mean±s.e.m.) and 24.4±3.2 g/gram of tissue respectively. There was no statistically significant difference of the mean sizes of the maximal contractions induced by 5-HT between the groups of preparations, whether in the presence of indomethacin (+ ethyl alcohol) or ethyl alcohol alone, when compared to control (non-paired) tissues (figure 5). The concentration of ethyl alcohol in the saline with $1X10^{-6}M$ indomethacin was $8X10^{-4}M$. In separate experiments ethyl alcohol ($1.5X10^{-5}$–$4.5X10^{-2}M$) did not contract (or relax) the HUA (n=3). These tissues were shown to be viable by contraction to KCl ($5X10^{-2}M$), O_2 (35–350mmHg) or 5-HT ($3X10^{-6}M$).

An initial comparison of the potencies of three cyclo-oxygenase inhibitors (COI's), at inhibiting the O_2-induced contraction of the HUA, was made in paired preparations (figure 6). $1X10^{-8}M$ indomethacin, $1X10^{-8}M$ flurbiprofen or $1X10^{-6}M$ acetylsalicylic acid (aspirin) was added to different strips from the same artery. $1X10^{-8}M$ indomethacin significantly reduced the O_2-induced contraction of HUA at all Po_2's. $1X10^{-8}M$ flurbiprofen significantly reduced the response but at higher Po_2's only (>140mmHg). $1X10^{-6}M$ aspirin did not inhibit the contractions at any Po_2. The maximum response (irrespective of the Po_2 at which the

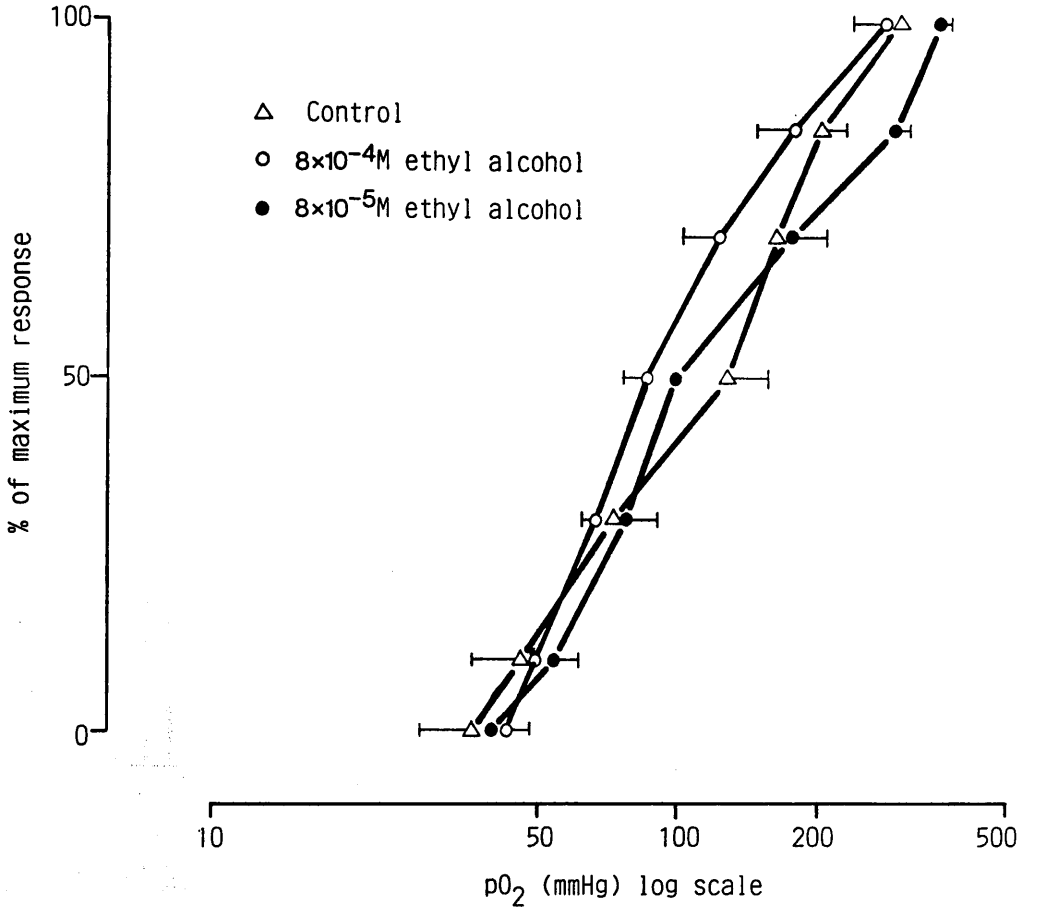
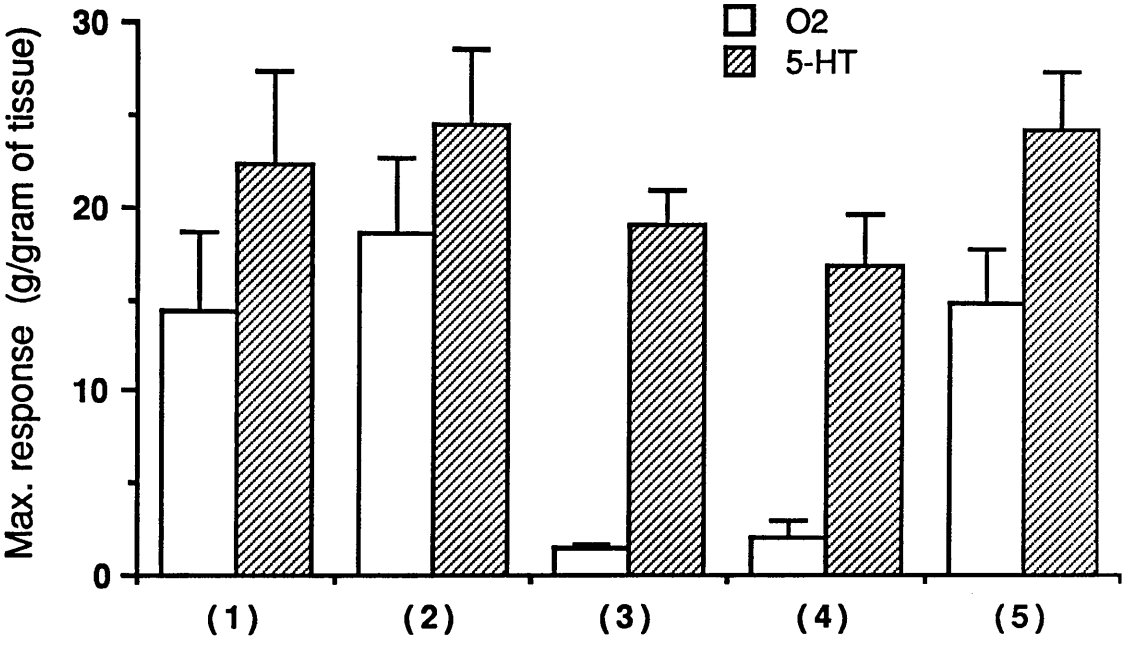


Figure 4 Ethyl alcohol, $8X10^{-5}M$ or $8X10^{-4}M$ did not significantly alter the concentration-response relationship for O_2 on human umbilical artery from that already found in separate experiments at pH 7.28 (from figure 2). (n=6 each group). Horizontal bars are the geometric mean±s.e.mean.



	(1)	(2)	(3)	(4)	(5)
E.A. ($\times 10^{-3}$M)	0.8	0.08	0.8	0.08	0
Indomethacin ($\times 10^{-6}$M)	0	0	1	0.1	0

Figure 5 Maximal induced contractions to 5-HT and oxygen in human umbilical artery in 5 groups of tissues, each group in the presence of different drugs. Groups 1-4 are paired preparations. Group 5 comprises a different set of tissues. Group1: $8X10^{-4}$M ethyl alcohol (E.A.); group 2: $8X10^{-5}$M E.A.; group 3: $1X10^{-6}$M indomethacin (+$8X10^{-4}$M E.A.); group4: $1X10^{-7}$M indomethacin (+$8X10^{-5}$M E.A.); group 5: control (no drugs). There was no significant difference between groups of values for 5-HT. Values for O_2 are significantly lower for indomethacin treated groups, 3 and 4. (n=6 each group).

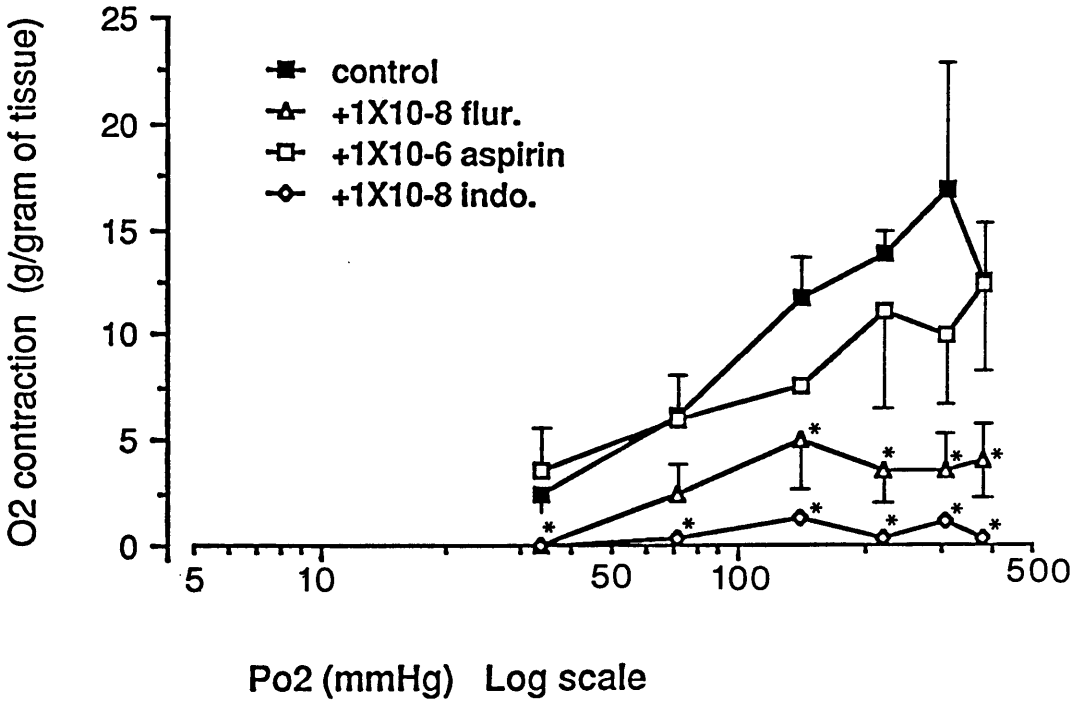


Figure 6 Concentration-response curves to oxygen in longitudinal strips of umbilical artery in the presence of cyclo-oxygenase inhibitors (COI): $1X10^{-8}M$ flurbiprofen; $1X10^{-6}M$ aspirin; $1X10^{-8}M$ indomethacin. The different COI's were added to separate strips from the same artery. A fourth strip acted as a control. For clarity error bars (mean±s.e.mean) are omitted at some points. n=6. Asterisks indicate responses which are significanly different from those of control preparations ($0.01<P<0.05$).

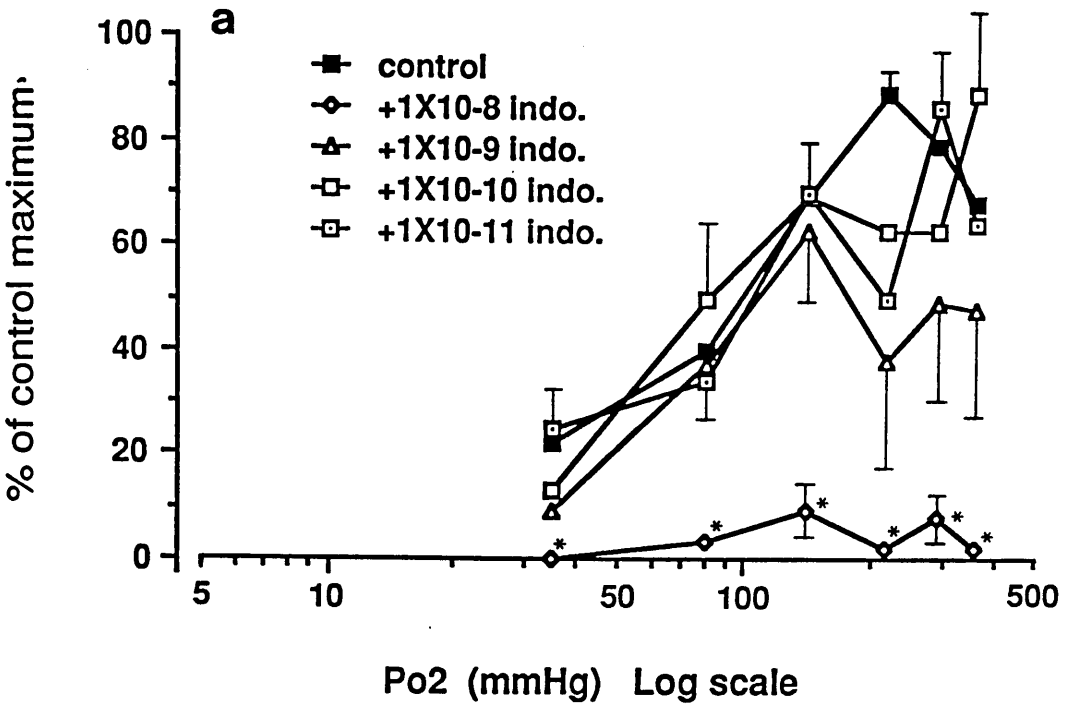
maximum occurred) was reduced by: (i) 90±5% ($1X10^{-8}$M indomethacin); (ii) 58±14% ($1X10^{-8}$M flurbiprofen); (iii) 16±22% ($1X10^{-6}$M aspirin). This suggested that indomethacin was marginally more potent than flurbiprofen, while a 100 fold greater concentration of aspirin did not inhibit the O_2-induced contraction of HUA. The contraction induced by 5-HT ($3X10^{-6}$M) was not significantly inhibited by these COI's, which in the controls was 29.7±7.8g/gram of tissue.

Other concentrations of these COI's were each investigated further in separate experiments. Concentrations of one COI (appart from the concentration already investigated, see above) were added to 3 separate strips of artery, while a fourth acted as a control. The response to oxygen (g/gram of tissue) at each Po_2 was calculated as a % of the maximum response to oxygen (irrespective of the Po_2 at which the maximum occurred) in the control strip.

Indomethacin. $1X10^{-11}$–$1X10^{-9}$M indomethacin was studied against the response to O_2. At each of these concentrations of indomethacin, O_2 failed to induce concentration-related contractions: at lower Po_2's (35–140mmHg) contractions were concentration-related but in each preparation (n=6) there was a "dip" in the concentration-response curve at Po_2 ~210mmHg (figure 7a). This was also noted in the previous experiments in the presence of $1X10^{-8}$M indomethacin. The responses in the presence of the COI, at Po_2 ~210mmHg, were significantly lower than the control response only in the presence of $1X10^{-8}$M indomethacin however. Indeed the maximum response, irrespective of the Po_2 at which it occurred, was significantly reduced only by $1X10^{-8}$M indomethacin (figure 7b). The IC_{50} for indomethacin (the concentration of indomethacin reducing the maximum response by 50%) was interpolated from figure 7b: this was $2.1X10^{-9}$M.

Flurbiprofen. The concentrations of flurbiprofen studied were $1X10^{-10}$M,

Figure 7 (a) Concentration-response curves to oxygen in longitudinal strips of umbilical artery in the presence of the cyclo-oxygenase inhibitor, indomethacin. Each concentration of indomethacin was added to a separate strip from the same artery (n=6). A separate strip was used as a control. Response (ordinate) is expressed as a % of the maximum response of the control. Each curve was constructed by a non-cumulative increase of the Po_2 from 10±1mmHg to the values shown. In each experiment the responses to the different levels of oxygen were assessed at the same Po_2's, but between experiments on different days there was always some small variation: at each Po_2 the s.e.mean of the Po_2 was, in ascending order (mmHg): 2; 3; 6; 6; 8; 8. For clarity, vertical error bars (mean±s.e.mean) are omitted at some points. Asterisks indicate values which are significantly smaller than those of control preparations ($0.01<P<0.05$).
(b) Maximum oxygen-induced contractions in the presence of indomethacin, from figure (a). The maximum response (% of the control maximum response) is the maximum contraction in each tissue, irrespective of the Po_2 at which the maximum occurred.


a
control
+1X10-8 indo.
+1X10-9 indo.
+1X10-10 indo.
+1X10-11 indo.
% of control maximum
100
80
60
40
20
0
5
10
50
100
500
Po2 (mmHg) Log scale

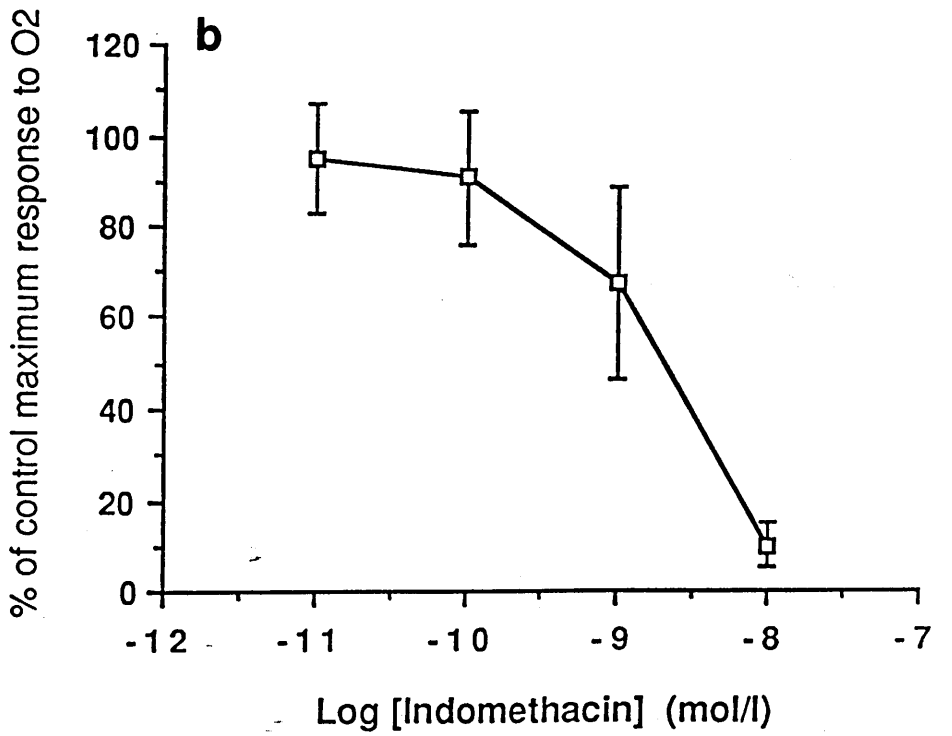

b
% of control maximum response to O2
120
100
80
60
40
20
0
-12
-11
-10
-9
-8
-7
Log [Indomethacin] (mol/l)

Table 3 Potency of cyclo-oxygenase inhibitors (COI) to inhibit the oxygen-induced contraction of the human umbilical artery.

COI	Inhibitiory Potency IC_{50} (M)	Molar Ratio*
Indomethacin	$2.1X10^{-9}$	1
Flurbiprofen	$7.6X10^{-9}$	3.6
Acetylsalicylic acid (Aspirin)	$2.5X10^{-5}$	12000

*The molar ratio was calculated as the ratio of the IC_{50}'s, indomethacin = 1.

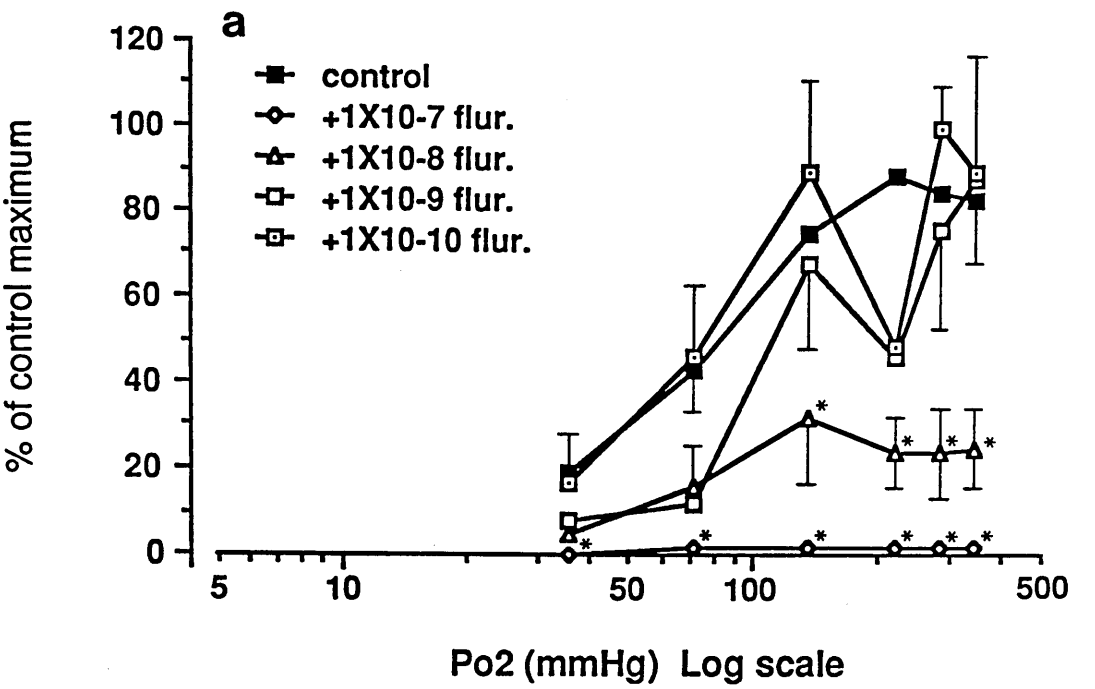
$1X10^{-9}M$ and $1X10^{-7}M$. The CRC's to O_2 in the presence of $1X10^{-10}M$ and $1X10^{-9}M$ flurbiprofen were not concentration-related: as found for indomethacin there was a dip in the CRC at Po_2 ~210mmHg. The responses in the presence of flurbiprofen at this Po_2 were not significantly lower than the control responses however (figure 8a). The maximum response, irrespective of the Po_2 at which it occurred, was significantly reduced only at concentrations of flurbiprofen greater than or equal to $1X10^{-8}M$ (figure 8b). There was no significant difference between the curves at any other Po_2. $1X10^{-7}M$ flurbiprofen virtually abolished the response to O_2. The IC_{50} for flurbiprofen was interpolated from figure 8b: this was $7.6X10^{-9}M$.

Aspirin. The concentrations of aspirin studied were $1X10^{-7}M$, $1X10^{-5}M$ and $1X10^{-4}M$. $1X10^{-5}M$ aspirin prevented O_2-induced, concentration-related contractions: at Po_2 ~210mmHg there was a dip in the concentration-response curve (figure 9a). The response at Po_2 ~210mmHg in the presence of $1X10^{-5}$ aspirin was not significantly lower than in the controls however. The maximum response, irrespective of the Po_2 at which it occurred, was significantly reduced in the presence of $1X10^{-4}M$ aspirin only (figure 9b). The IC_{50} for aspirin was interpolated from figure 9b: this was $2.5X10^{-5}M$.

The potencies of indomethacin, flurbiprofen and aspirin as inhibitors of the O_2-induced contraction of the HUA are summarised in Table 3. This suggests that indomethacin is ~4 fold more potent than flurbiprofen while both indomethacin and flurbiprofen are 3 to 4 orders of magnitude more potent than aspirin.

These COI's were found to relax strips of HUA, at the physiological Po_2 of ~15mmHg, when added to the saline. As already stated, a single concentration of COI was added to each strip from the same artery. For flurbiprofen and aspirin this relaxation was found to be concentration-dependent (figures 10a and 10b respectively). At the

Figure 8 (a) Concentration-response curves to oxygen in longitudinal strips of umbilical artery in the presence of the cyclo-oxygenase inhibitor, flurbiprofen. Each concentration of flurbiprofen was added to a separate strip from the same artery (n=6). A separate strip was used as a control. Response (ordinate) is expressed as a % of the maximum response of the control. Each curve was constructed by a non-cumulative increase of the Po_2 from 12±1mmHg to the values shown. In each experiment the responses to the different levels of oxygen were assessed at the same Po_2's, but between experiments on different days there was always some small variation: at each Po_2 the s.e.mean of the Po_2 was, in ascending order (mmHg): 2; 3; 5; 8; 10; 10. For clarity, vertical error bars (mean±s.e.mean) are omitted at some points. Asterisks indicate values which are significantly smaller than those of control preparations ($0.01<P<0.05$).
(b) Maximum oxygen-induced contractions in the presence of flurbiprofen, from figure (a). The maximum response (% of the control maximum response) is the maximum contraction in each tissue, irrespective of the Po_2 at which the maximum occurred.


a
control
+1X10-7 flur.
+1X10-8 flur.
+1X10-9 flur.
+1X10-10 flur.
% of control maximum
120
100
80
60
40
20
0
5
10
50
100
500
Po2 (mmHg) Log scale

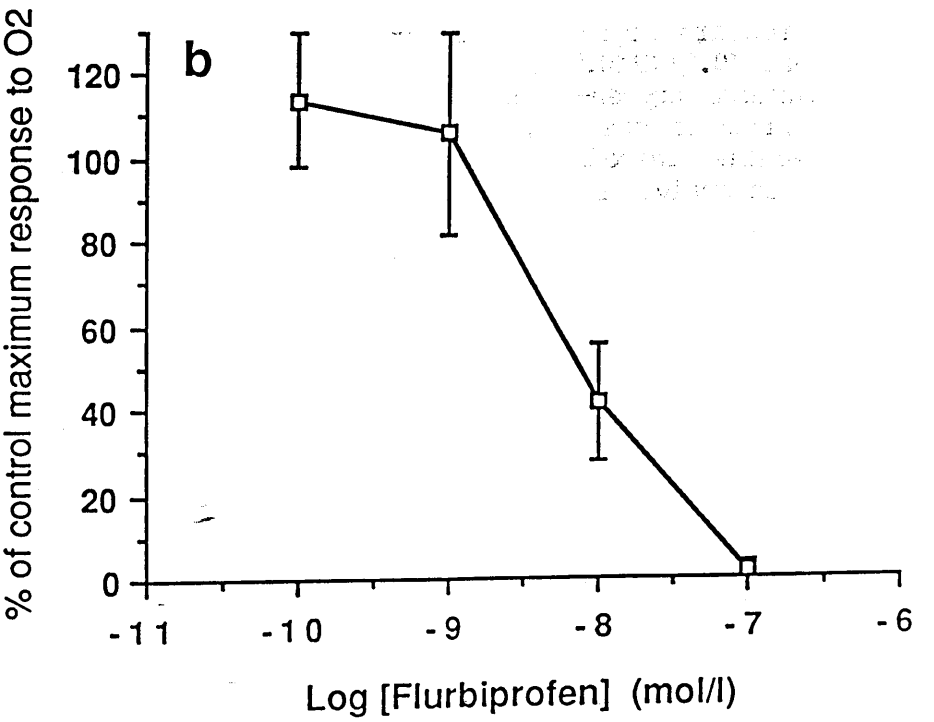
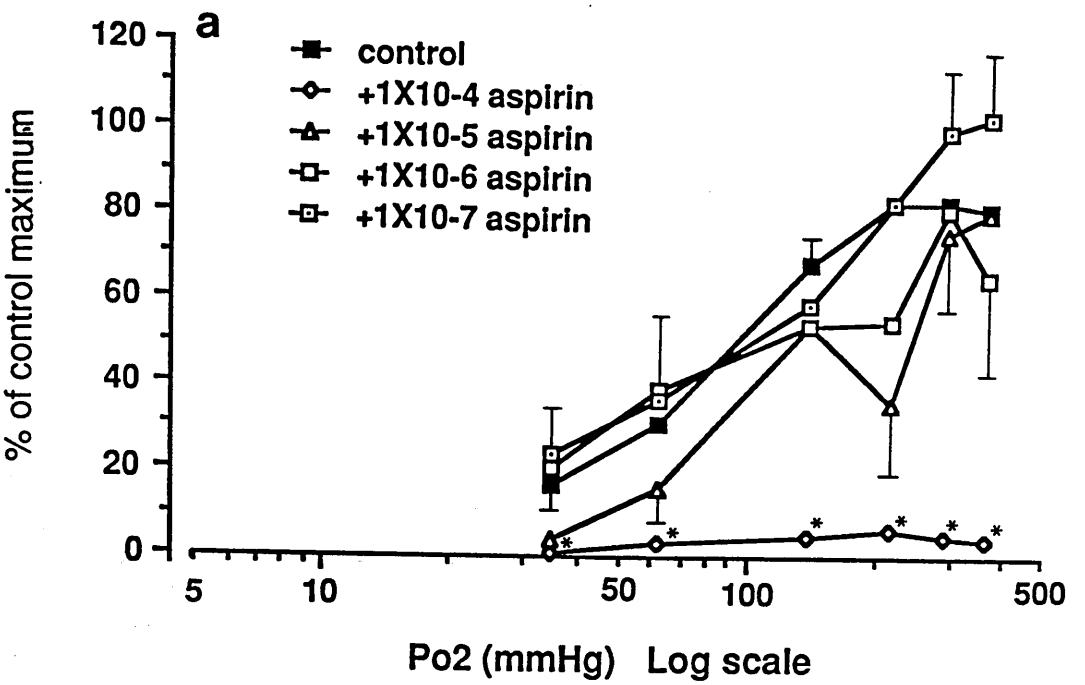
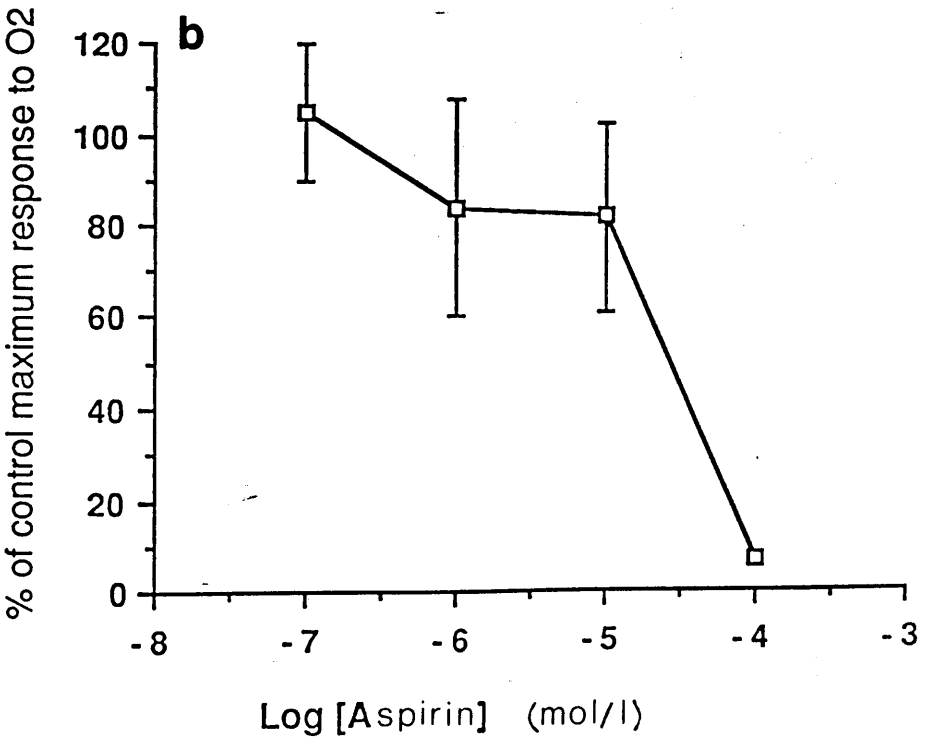

b
% of control maximum response to O2
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
Log [Flurbiprofen] (mol/l)

Figure 9 (a) Concentration-response curves to oxygen in longitudinal strips of umbilical artery in the presence of the cyclo-oxygenase inhibitor, aspirin. Each concentration of aspirin was added to a separate strip from the same artery (n=6). A separate strip was used as a control. Response (ordinate) is expressed as a % of the maximum response of the control. Each curve was constructed by a non-cumulative increase of the Po_2 from 8±2mmHg to the values shown. In each experiment the responses to the different levels of oxygen were assessed at the same Po_2's, but between experiments on different days there was always some small variation: at each Po_2 the s.e.mean of the Po_2 was, in ascending order (mmHg): 1; 2; 4; 8; 12; 13. For clarity, vertical error bars (mean±s.e.mean) are omitted at some points. Asterisks indicate values which are significantly smaller than those of control preparations ($0.01<P<0.05$).
(b) The maximum oxygen-induced contraction in the presence of aspirin, from figure (a). The maximum response (% of the control maximum response) is the maximum contraction in each tissue, irrespective of the Po_2 at which the maximum occurred.


a
control
+1X10-4 aspirin
+1X10-5 aspirin
+1X10-6 aspirin
+1X10-7 aspirin
% of control maximum
120
100
80
60
40
20
0
5
10
50
100
500
Po2 (mmHg) Log scale


b
% of control maximum response to O2
120
100
80
60
40
20
0
-8
-7
-6
-5
-4
-3
Log [Aspirin] (mol/l)

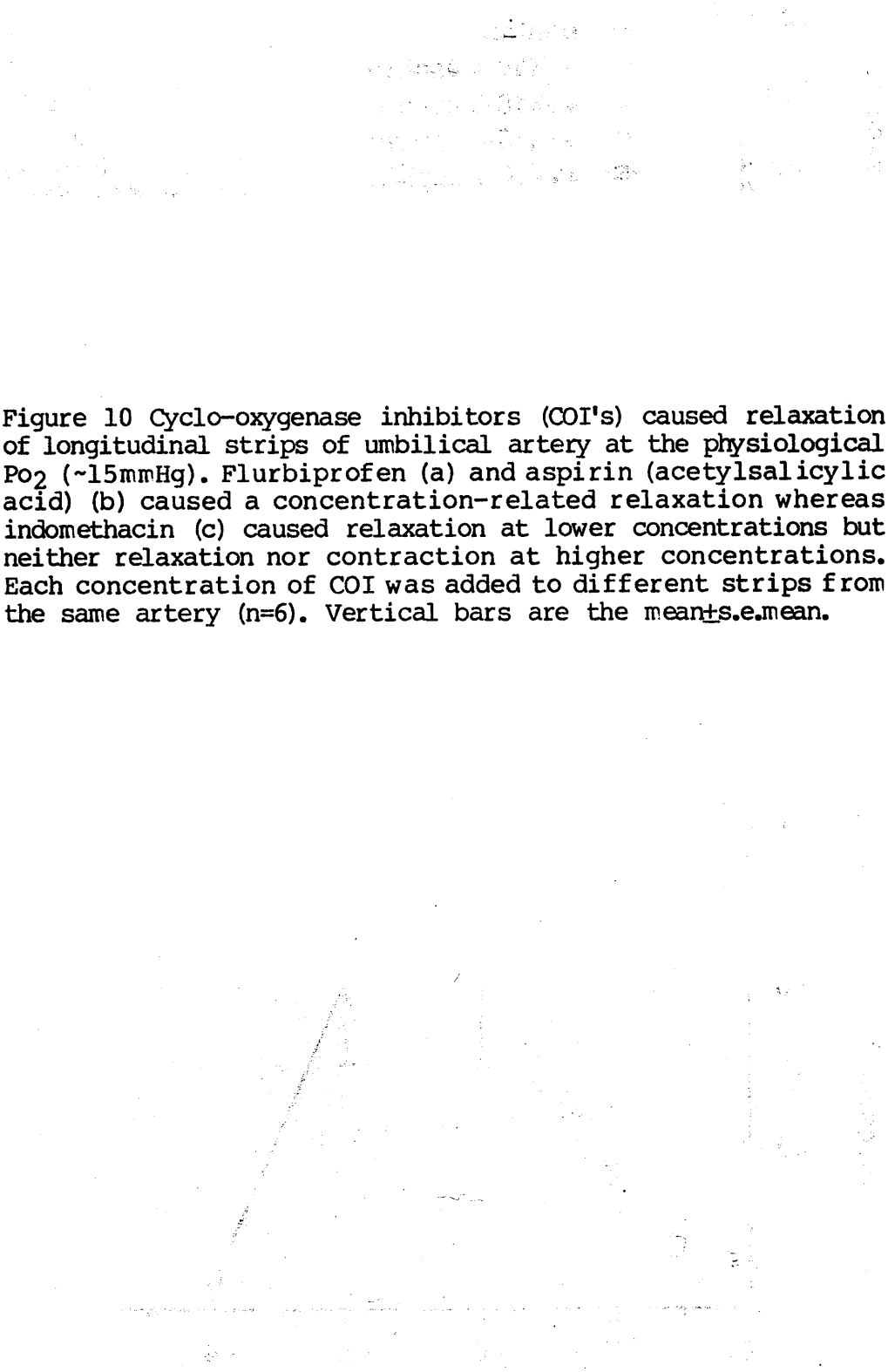
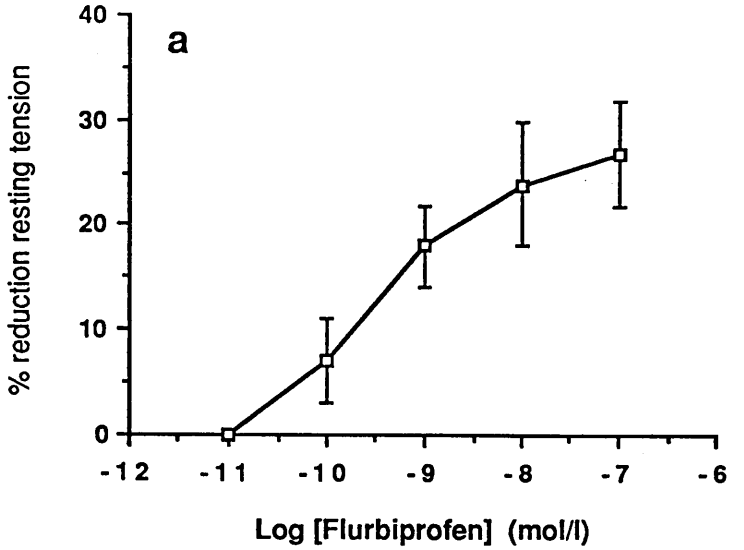
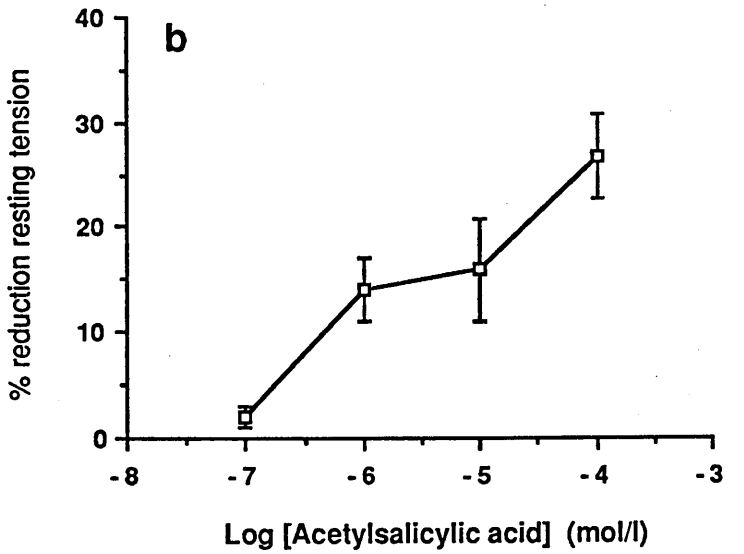
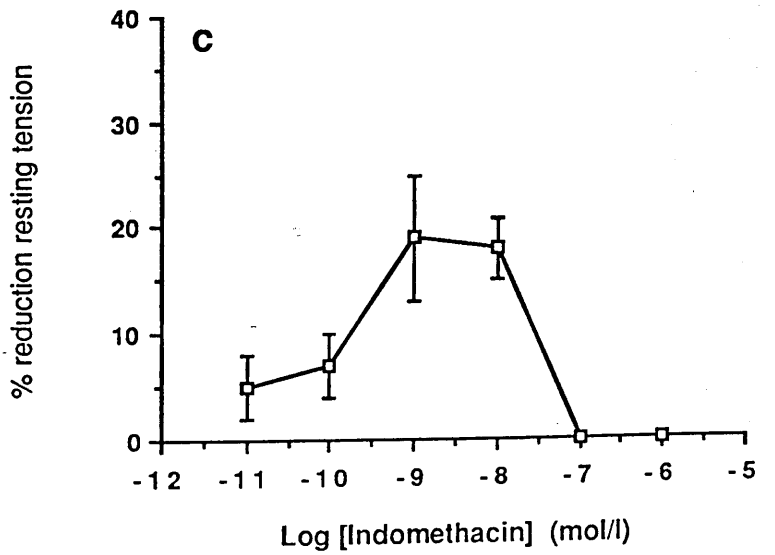


Figure 10 Cyclo-oxygenase inhibitors (COI's) caused relaxation of longitudinal strips of umbilical artery at the physiological Po_2 (~15mmHg). Flurbiprofen (a) and aspirin (acetylsalicylic acid) (b) caused a concentration-related relaxation whereas indomethacin (c) caused relaxation at lower concentrations but neither relaxation nor contraction at higher concentrations. Each concentration of COI was added to different strips from the same artery (n=6). Vertical bars are the mean$\pm$s.e.mean.


a
% reduction resting tension
40
30
20
10
0
-12
-11
-10
-9
-8
-7
-6
Log [Flurbiprofen] (mol/l)


b
% reduction resting tension
40
30
20
10
0
-8
-7
-6
-5
-4
-3
Log [Acetylsalicylic acid] (mol/l)


c
% reduction resting tension
40
30
20
10
0
-12
-11
-10
-9
-8
-7
-6
-5
Log [Indomethacin] (mol/l)

highest concentrations tested this reduction was by 27±5% ($1X10^{-7}M$ flurbiprofen) and by 27±4% ($1X10^{-4}M$ aspirin) of the initial resting tension. The resting tension of the strips of artery to which the COI's were added was not significantly different from the resting tension in control preparations which was 0.67±0.16g. (The tension put on the tissues when first setting them up was 1g which decayed to the given value of 0.67±0.16g). Indomethacin did not evoke a relaxation which was so directly related to concentration (figure 10c). $1X10^{-11}$–$1X10^{-9}M$ indomethacin relaxed the HUA which was by 19±6% of the initial resting tension, at $1X10^{-9}M$. At higher concentrations the induced relaxations were smaller and at $1X10^{-7}M$ and $1X10^{-6}M$ there was no change in tone (no relaxation nor contraction), as already stated.

On adding these COI's to the saline there was a noticeable delay of onset of the relaxation. At the concentrations of COI which caused the greatest relaxation this lag time (T_L, secs.) and time for the relaxation to plateau (T_P, mins) were (i) flurbiprofen ($1X10^{-7}M$): T_L=101±20; T_P=17.5±2.0 (ii) aspirin ($1X10^{-4}M$): T_L=117±12; T_P=18.5±3.0 (iii) indomethacin ($1X10^{-9}M$): T_L=210±52; T_P=19.0±3.0. The lag time before the onset of the indomethacin-induced relaxation was not significantly greater than for flurbiprofen or aspirin.

Role of the endothelium

The possible involvement of the endothelium in the O_2-induced contraction of the HUA was investigated by comparison of paired preparations, one of which had the endothelium removed by mechanical abrasion (using the edge of forceps). In rubbed preparations O_2 induced greater contractions than in unrubbed preparations, but this was only significant at higher Po_2's (figure 11). Contractions induced by 5-HT ($3X10^{-6}M$) were not significantly different in rubbed (31.4±5.2g/gram of tissue) or unrubbed (29.3±1.6g/gram of tissue) preparations (n=4).

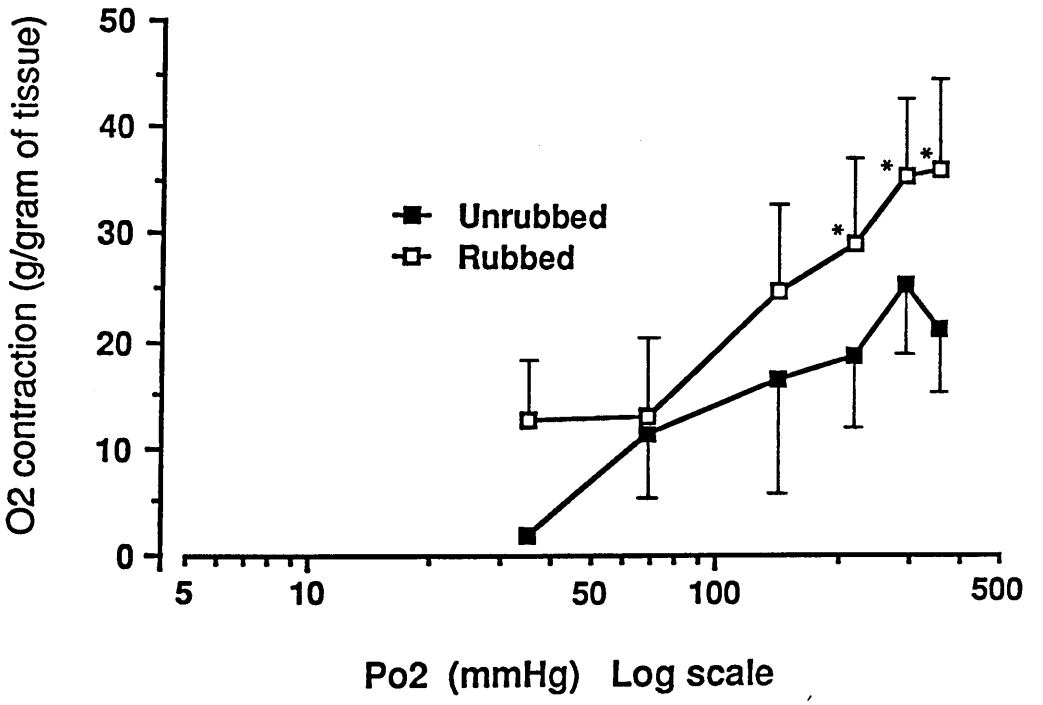


Figure 11 Concentration-response curves to oxygen in longitudinal strips of umbilical artery. In paired preparations (n=4) one strip had the endothelium removed (by mechanical abrasion), (rubbed) while in the other strip the endothelium was left intact (unrubbed). Vertical bars are the mean±s.e.mean. Asterisks denote significant differences between the curves ($0.01<P<0.05$).

Successful removal of the endothelium was confirmed in histological sections of the longitudinal strips. Figure 12 shows a transverse section of an artery under low and high magnification. A single layer of endothelial cells is clear. Other features of note are the clear division between the inner longitudinal muscle and outer circular layers and the small asymmetric longitudinal bundle on the outer surface which is thought to be responsible for the coiling of the umbilical cord (Roach, 1972). In unrubbed longitudinal strips of artery the endothelium was still largely present while in rubbed preparations the endothelium was found to be almost completely removed (figure 13).

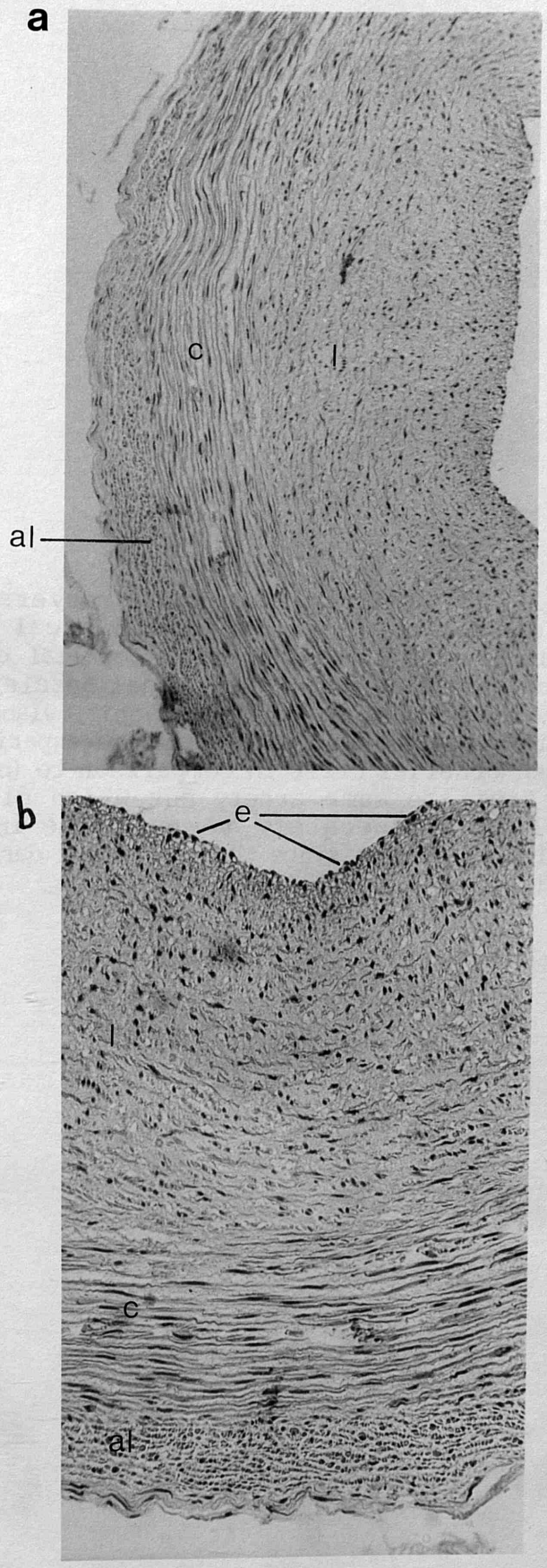
The effect of anoxia and of drugs

To test whether there was an inherent O_2-induced tone in the HUA at physiological Po_2, the effect of reducing the Po_2 from ~15mmHg to 0mmHg was investigated. In addition the effects of various drugs on the tone of the preparations at Po_2= 0mmHg were studied. The O_2 electrode was always routinely calibrated at 0mmHg. Cross-checking several methods of calibrating the electrode showed that a reading of 0mmHg in the saline was accurate.

On reducing the Po_2 from ~15mmHg to 0mmHg (anoxia) there followed a slow relaxation which was coincident with the fall in Po_2 of the saline (figure 14), i.e. there was an intrinsic, oxygen-induced tone in the preparations. In control preparations the decrease in tone due to the reduction in Po_2 was by 20±6% of the initial tension (at Po_2 ~15mmHg) which was 17.4±2.3g/gram of tissue. This decrease in tone, due to the reduction in Po_2, was found to be significantly correlated ($P<0.01$) to the initial resting tension (figure 15).

The resting tension (tone), and the consequent reduction in tone on decreasing the Po_2, was not significantly different between the group of preparations to be treated with drugs, and the control group

Figure 12 Photomicrographs of transverse sections of a human umbilical artery under low (a) and higher (b) magnification. These show the inner longitudinal muscle (l), the circular muscle (c) and the assymmetric longitudinal muscle (al) muscle bundle. This latter bundle is thought to cause the coiling of the umbilical cord. The dark-stained nuclei of the endothelial cells (e) are also clear in (b).


a
c
l
al
b
e
l
c
al

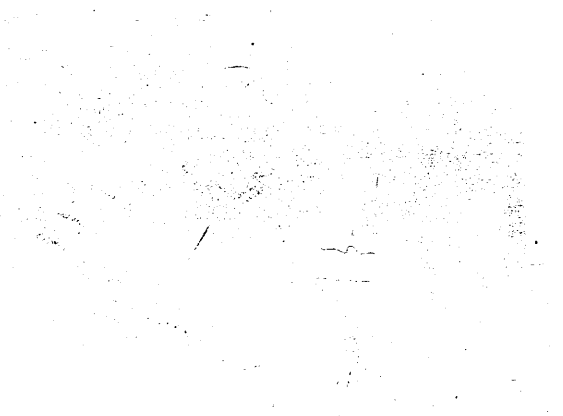
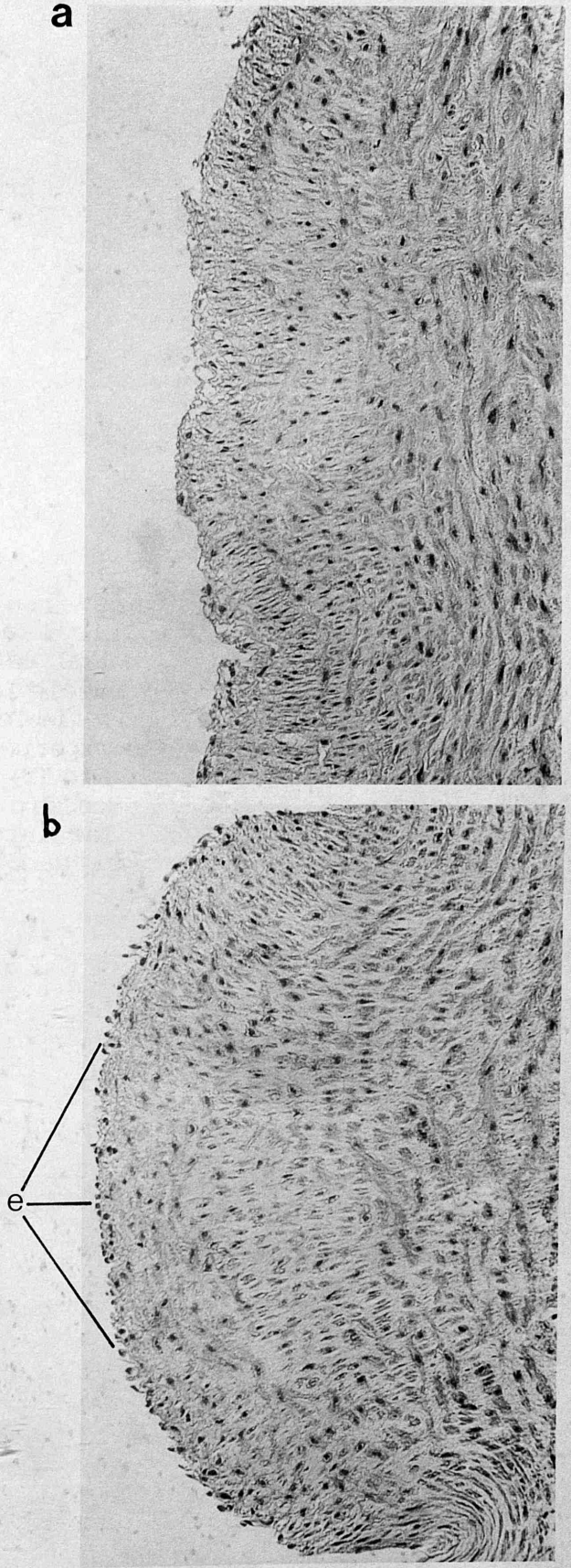


Figure 13 Photomicrographs of transverse sections of longitudinal strips of human umbilical artery. (a) A longitudinal strip which had the endothelial cells removed by mechanical abrasion. The longitudinal muscle layer (l) shows some damage but the preparation was still viable as judged by contraction to different stimuli in the experiments. Note the lack of endothelial cells in comparison to (b) which shows a section from the same artery but which did not have the endothelium deliberately removed. The integrity of the endothelium is evident since there are many dark-stained nuclei of the endothelial cells (e).


a
b
e

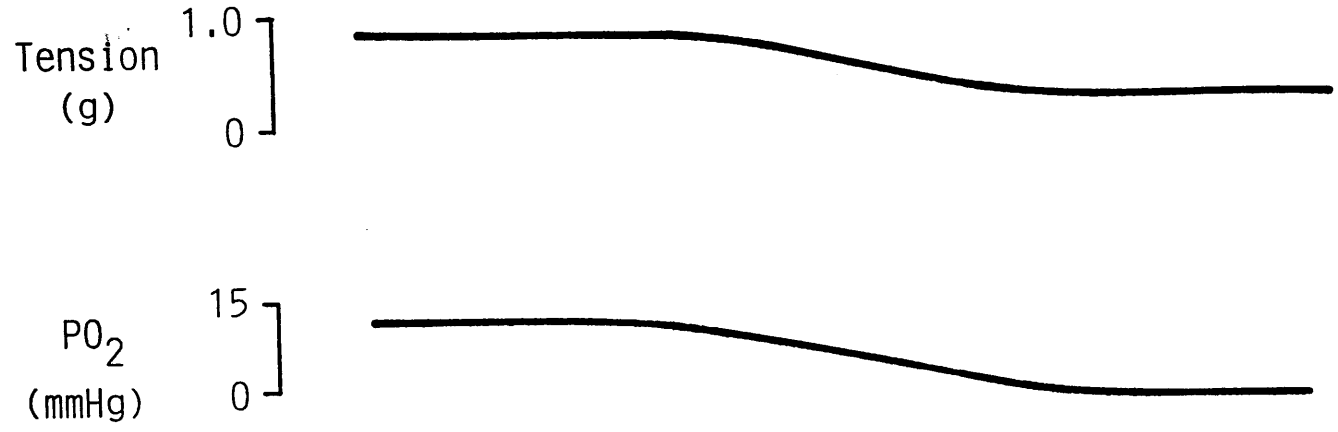


Figure 14 This figure shows the coincidental fall in tone (resting tension) of a longitudinal strip of umbilical artery on reducing the Po_2 from 12mmHg to 0mmHg.

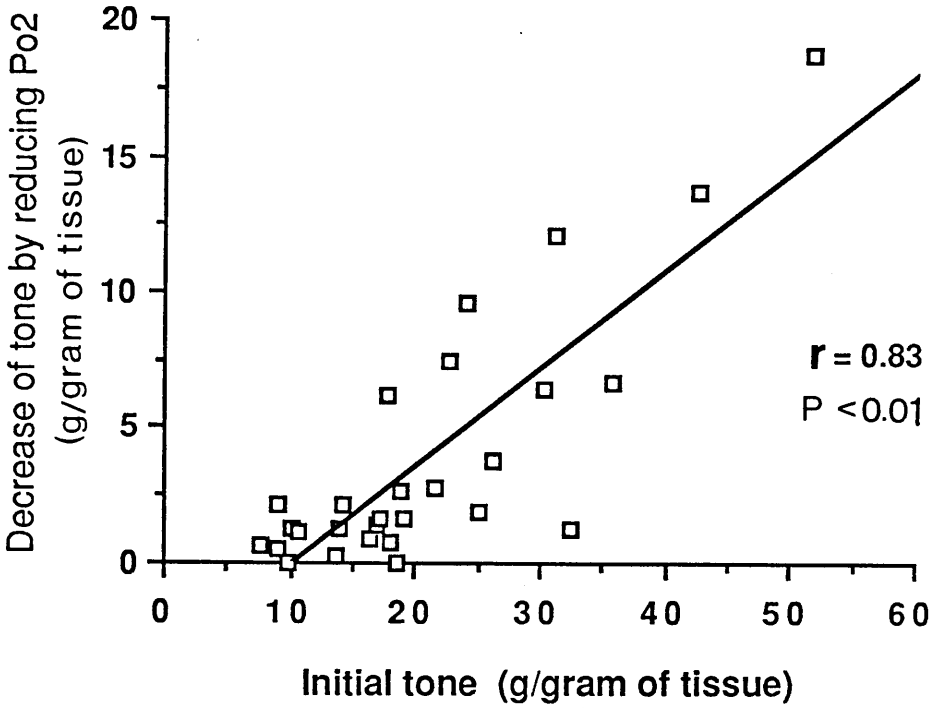


Figure 15 A highly significant correlation (r=0.83; P<0.01) was found of the relationship between the decrease in tone of strips of umbilical artery, on reducing the Po_2 from ~15mmHg to 0mmHg, and the initial tone at Po_2 ~15mmHg. Each point (30) represents the response in one strip of artery. Five longitudinal strips from each of six arteries were examined.

of preparations. This was also the case in preparations set-up in saline where Ca^{++} was omitted from the saline. In this group the tone was 25.9±5.4g /gram of tissue and the reduction of the tone on decreasing the Po_2 was by 8±3%. Although this reduction of tone was less than in control preparations, the difference was not significant due to the wide variation between tissues.

After 30mins at Po_2 0mmHg the following drugs were added to different strips from the same artery: $1X10^{-7}M$ Bayer K 8644; $1X10^{-6}M$ nifedipine; $1X10^{-6}M$ nitroprusside and $1X10^{-7}M$ flurbiprofen. The effects of these drugs on the tone at Po_2 0mmHg are summarised in figure 16.

Bayer K 8644. In 2 of 6 preparations $1X10^{-7}M$ Bayer K 8644 had no effect. In the others it caused large contractions which varied in magnitude between preparations. These contractions were not maintained and decayed to the resting tension (at Po_2=0mmHg) within 30mins.

Nifedipine. $1X10^{-6}M$ nifedipine caused a small further reduction in tone which was 6±3% (of the tone at Po_2=15mmHg). This reduction in tone was maintained.

Flurbiprofen. $1X10^{-7}M$ flurbiprofen caused a small further reduction in tone of 7±1%. The combined reduction in tone caused by decreasing the Po_2 and of $1X10^{-7}M$ flurbiprofen was 25±9% (of the tone at Po_2=15mmHg), i.e. equivalent to the reduction in tone caused by $1X10^{-7}M$ flurbiprofen alone at Po_2=15mmHg (see figure 10a). This reduction in tone was maintained.

Nitroprusside. $1X10^{-6}M$ nitroprusside caused a large reduction in tone of 26±8% (of the tone at Po_2=15mmHg). The combined effect of reducing the Po_2 from 15 to 0mmHg and of $1X10^{-6}M$ nitroprusside was a decrease in tone of 37±11%. This reduction in tone was maintained.

The effects of these drugs and of omitting Ca^{++} from the saline, on the response to O_2 was investigated (figure 17). The Po_2 was

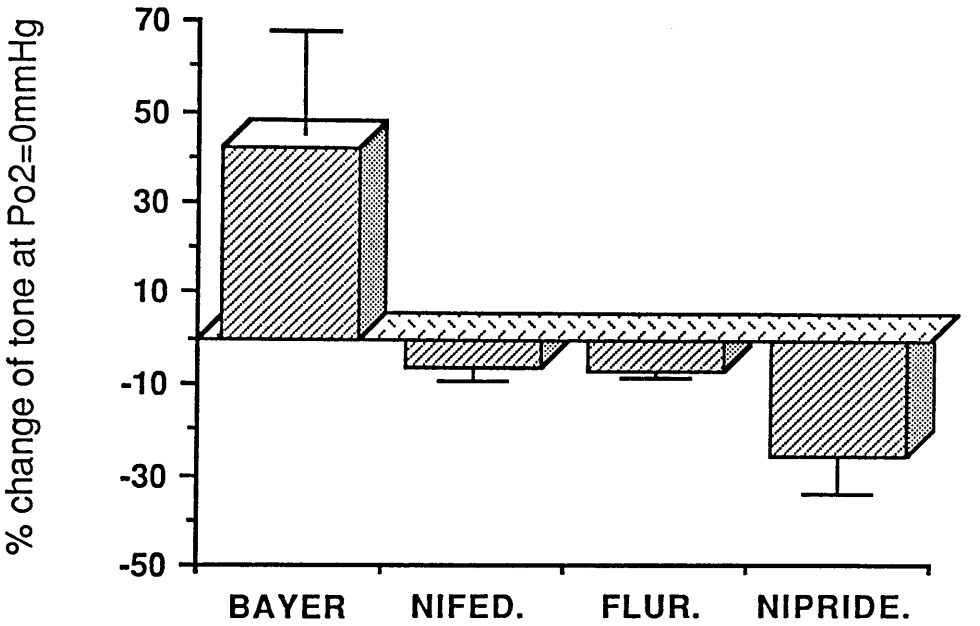


Figure 16 The histograms represent the % change of tone of strips of artery at Po_2=0mmHg, on addition of: Bayer K8644 ($1X10^{-7}M$); nifedipine ($1X10^{-6}M$); flurbiprofen ($1X10^{-7}M$); nipride ($1X10^{-6}M$), (sodium nitroprusside). $n=6$.

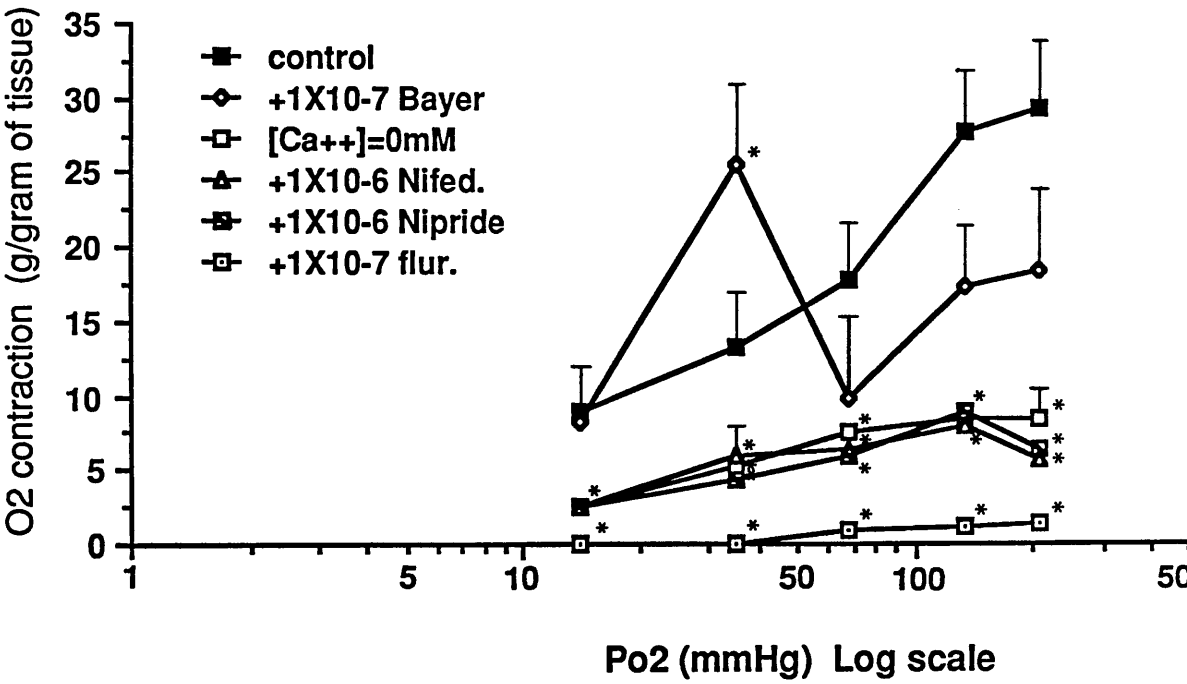


Figure 17 Concentration-response curves to oxygen in longitudinal strips of artery (n=6) in the presence of: Bayer K8644 ($1X10^{-7}M$); nifedipine ($1X10^{-6}M$); nipride ($1X10^{-6}M$), (sodium nitroprusside); flurbiprofen ($1X10^{-7}M$), and in strips of artery set-up in saline where Ca^{++} was omitted ($[Ca^{++}]=0mM$). Each curve was constructed by a non-cumulative increase of the Po_2 from 0mmHg to the values shown. At each Po_2 the s.e.mean of the Po_2 was less than 5mmHg. For clarity vertical error bars (mean±s.e.mean) are omitted at some points. Asterisks indicate responses which are significantly different from those of control preparations ($0.01<P<0.05$).

increased non-cumulatively from 0mmHg.

In preparations set-up in saline where Ca^{++} was omitted, O_2 caused only weak contractions which were significantly smaller than in control preparations, at all Po_2's. The maximum response was 39±7% of that in the controls. As already found, flurbiprofen ($1X10^{-7}M$), virtually abolished O_2-induced contractions at all Po_2's. Nifedipine ($1X10^{-6}M$) and nitroprusside ($1X10^{-6}M$) significantly reduced the responses at all Po_2's. Bayer K 8644 ($1X10^{-7}M$) significantly increased O_2-induced contractions but only at one low Po_2 (35±2mmHg). These large contractions in the presence of Bayer K 8644 were well maintained and decayed only slowly, even after the Po_2 had been reduced to 0mmHg. Thereafter contractions induced by oxygen in the presence of Bayer K 8644 were not concentration-related and were smaller than in the controls, although the difference was not significant.

The effects of these drugs on the response to a single concentration of 5-HT ($3X10^{-6}M$) were somewhat different however. Only in preparations where Ca^{++} was omitted from the saline were 5-HT-induced contractions significantly smaller than those in control preparations: the maximum response was 40±12% of the control maximum response (figure 18). Although the contractions to 5-HT in drug-treated groups were in each group smaller than the control responses, the differences between the mean responses were not significant.

Higher concentrations of Bayer K 8644 ($1X10^{-6}M$) and nifedipine ($1X10^{-5}M$) facilitated and depressed, respectively, cumulative CRC's to 5-HT, but the significance of this was not estimated as only 2 preparations were tested (figure 19).

Influence of gestational age

Firstly it was important to determine whether the type of birth could influence the response to the contractile agents which were to be

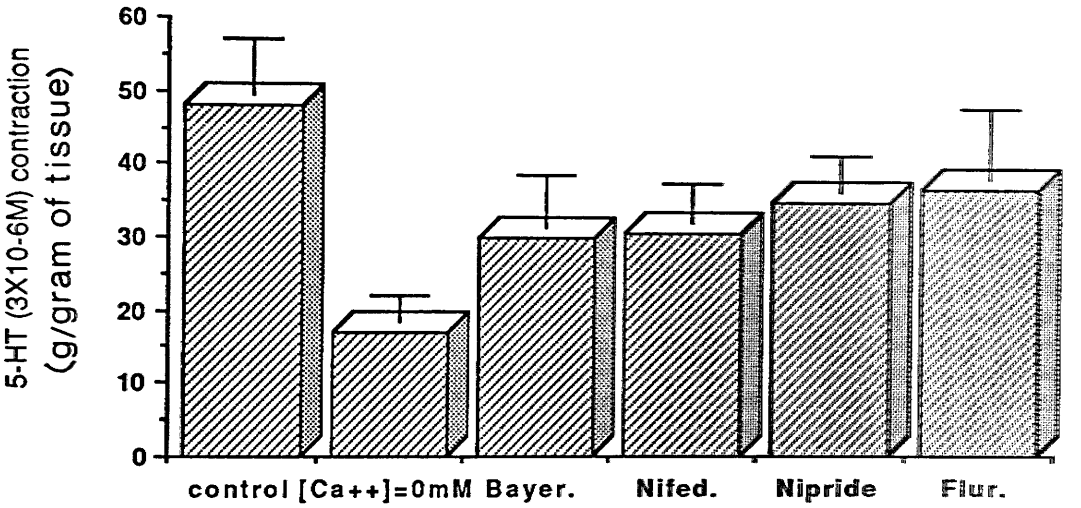


Figure 18 Histograms represent the maximum contraction to 5-HT ($3X10^{-6}M$) in longitudinal strips of artery (n=6) in the presence of: Bayer K8644 ($1X10^{-7}M$); nifedipine ($1X10^{-6}M$); nipride ($1X10^{-6}M$), (sodium nitroprusside); flurbiprofen ($1X10^{-7}M$), and in strips of artery set-up in saline where Ca^{++} was omitted ($[Ca^{++}]=0mM$). The only significant difference between the groups, of the maximum contraction, was between the control group and the group where $[Ca^{++}]=0mM$. $Po_2 = 14 \pm 2$ mm Hg.

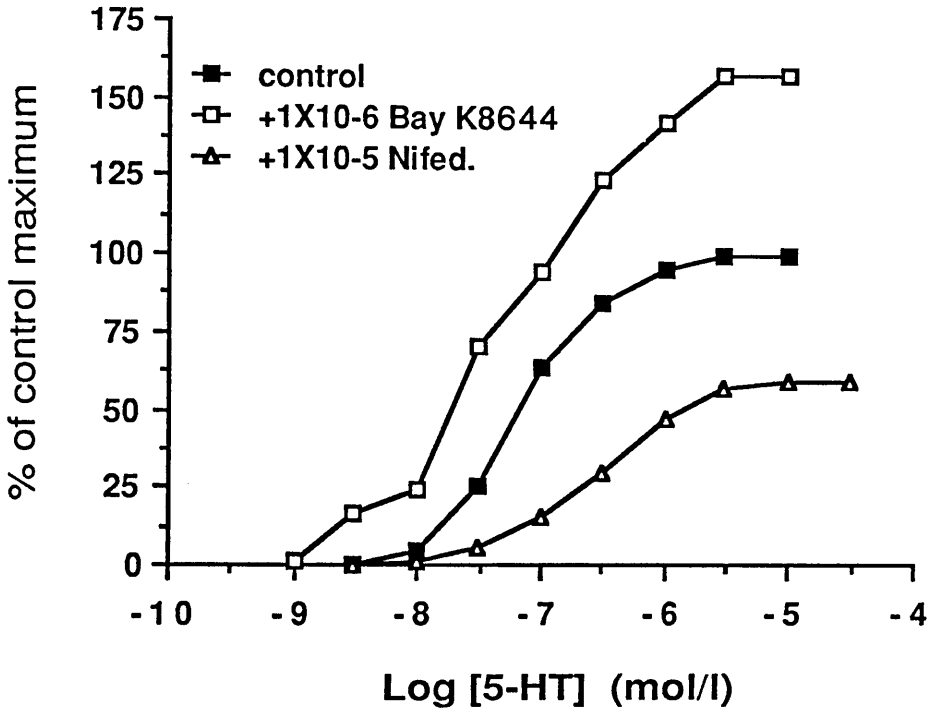


Figure 19 Log concentration-response curves to 5-HT in longitudinal strips of umbilical artery at low Po_2 (16mmHg) in the presence of Bayer K8644 ($1X10^{-6}M$) and nifedipine ($1X10^{-5}M$). The response was calculated as a % of the control maximum response in the same tissue. Only 2 preparations were tested. The mean responses are shown without error bars.

tested, since if this were found it would introduce an extra complicating factor to the analysis of the results from births of different gestational ages. There are three main categories of birth: (i) spontaneous vaginal delivery (SVD); (ii) births which are induced (by oxytocin only in this study); (iii) elective caesarian section (ECS). These different categories of birth were found not to significantly affect the sensitivity to O_2 of the preparations, at any level, (figure 20) or to influence the maximal contractions to O_2 or 5-HT (figure 21).

An attempt was made to find if any correlation existed between the experimental results on the isolated preparations and clinical observations of the health of the neonate, or complications at delivery. While some of the neonates with particular complications (e.g intra-uterine growth retardation; patent ductus arteriosus) were found to be associated with experimental findings in which O_2 induced large contractions in strips of HUA (from the same birth), there were other cases of the same complication which were associated with only weak O_2-induced contractions or which were not contracted by O_2 at all. Similarly there was no clear correlation between clinical complications and any difference from the mean response to 5-HT or adrenaline. There was therefore no reason to exclude data from births of any particular complication from the overall analysis.

A total of 99 cords were collected from births whose gestational age ranged from 26 to 41 weeks. In the hospital from which the cords were collected, births which are considered "at term" are greater than or equal to 37 weeks gestation. Non-cumulative CRC's to O_2 were constructed in longitudinal strips of artery from each birth. CRC's to 5-HT were constructed at Po_2 ~120mmHg in arteries from pre-term births. A single concentration of 5-HT ($3X10^{-6}M$) was added to strips of artery from term births. CRC's to adrenaline were constructed in "pre-term

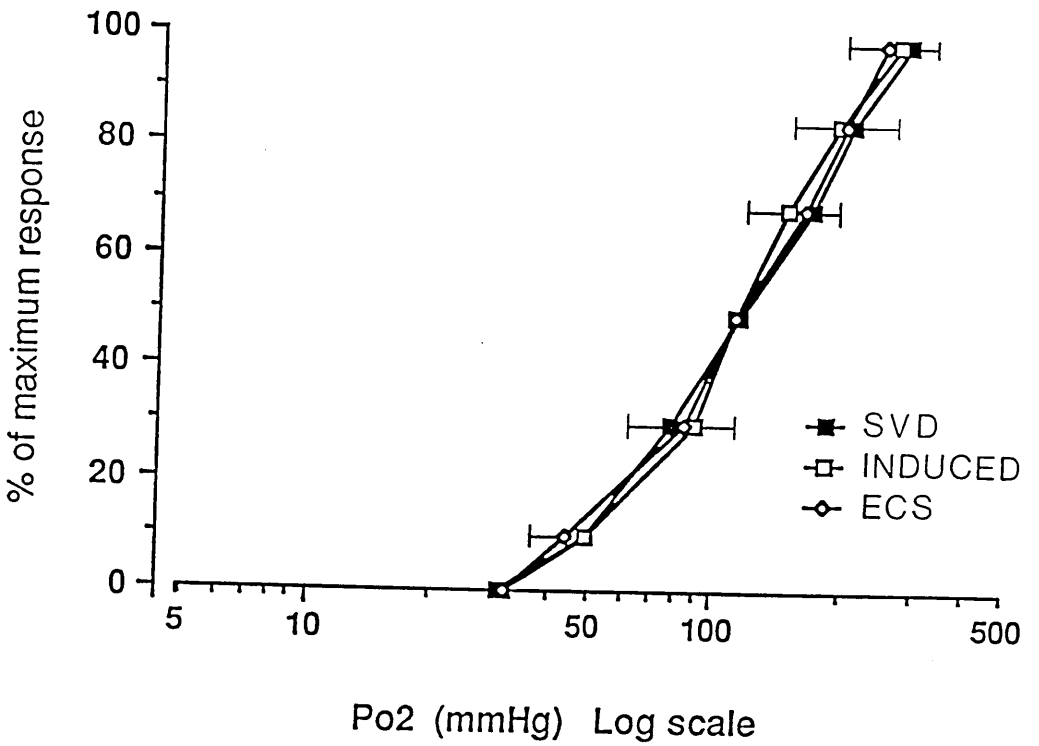


Figure 20 Concentration-response curves to oxygen in longitudinal strips of umbilical artery from infants delivered by different methods: (a) spontaneous vaginal delivery (SVD), n=13; (b) by induction, n=11, and (c) by elective caesarian section (ECS), n=7. For clarity, error bars (geometric mean±s.e.mean) are omitted at some points.

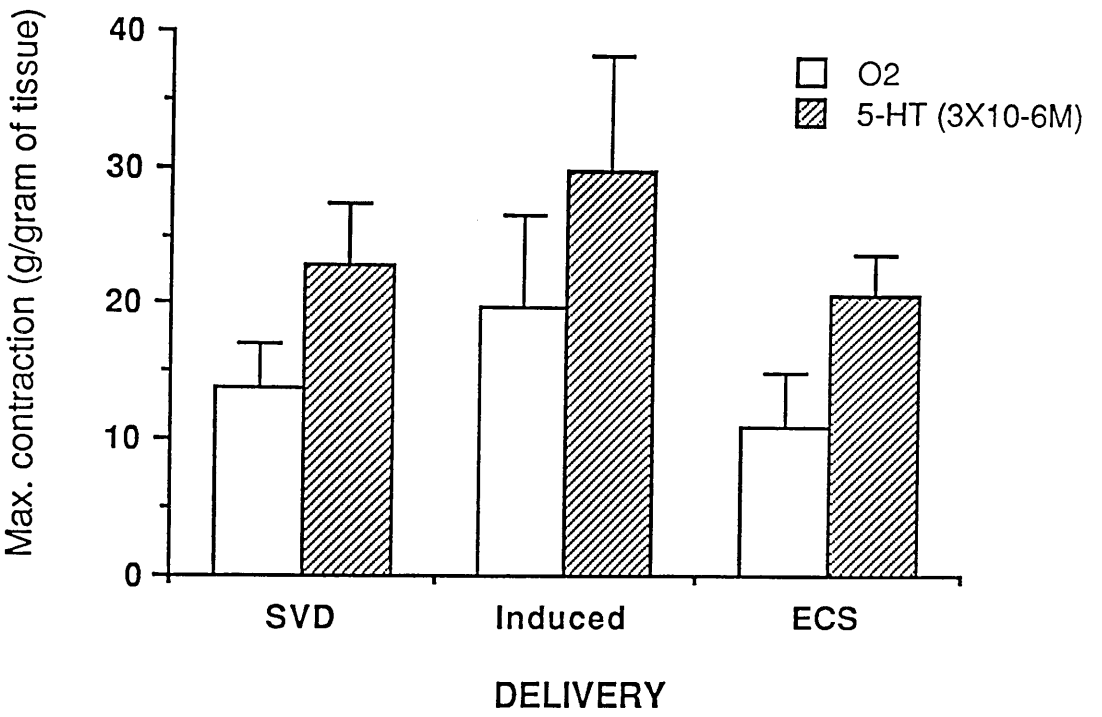


Figure 21 Histograms represent the maximum contractions induced by oxygen and 5-HT in longitudinal strips of umbilical artery from infants delivered by: (a) spontaneous vaginal delivery (SVD), n=13; (b) by induction, n=11, and (c) by elective caesarian section (ECS), n=7.

strips" at Po_2 ~15mmHg and ~120mmHg.

Response to O_2

Arteries of gestation up to 31 weeks fell into two distinct groups: (1) preparations were either not contracted by O_2, or only weakly contracted, (2) in 3 preparations O_2-induced contractions were very large. This is shown in figure 22. This shows the contractions induced by O_2 at Po_2=100mmHg, in each preparation, as a function of gestational age. The contractions at Po_2=100mmHg were interpolated from the individual CRC's. The maximal O_2-induced contractions in preparations of greater gestational ages were more uniform, when considered at any one gestational age (week), with the exception of some large contractions (1 at 38 weeks; 2 at 39 weeks; 1 at 41 weeks) which are separated from the other data of the same gestational age by a large margin. The maximal contractions to O_2 of each of these seven pieces of data (3 from pre-term births and four from term births) which seem very large were each compared statistically to the mean response of all the data and in each case found to be highly significantly different ($P<0.01$) from the mean response to O_2. However, the response to 5-HT ($3X10^{-6}M$) in each of these seven preparations was also found to be significantly greater than the mean response to 5-HT. There therefore seems to be no sound reason for excluding these data from an overall examination of the results.

For another analysis of the experimental data from the gestational-age study the results were split into groups of 2-weeks gestation (e.g 40-41 weeks) except for the data from the earliest gestational ages of which relatively few umbilical cords were collected. Here the data were collected into a group of preparations of gestation up to, and including, 31 weeks. This allowed a one-way analysis of variance of sets of data which were normally distributed.

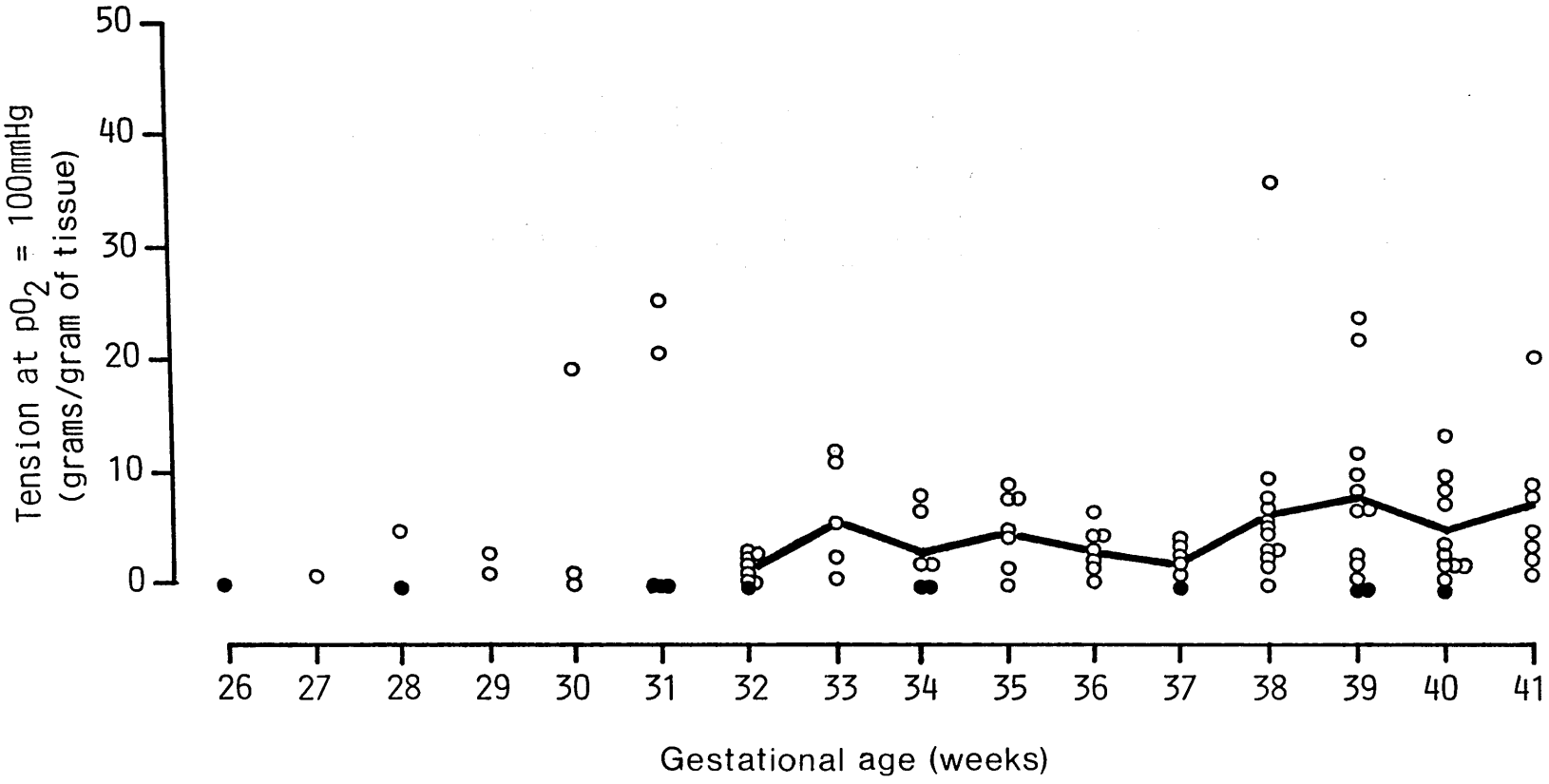


Figure 22 A scatter diagram showing the contraction (g/gram of tissue) induced by oxygen in longitudinal strips of human umbilical artery, for each preparation used in the investigation of gestational age. The contraction induced by oxygen at Po_2=100mmHg was interpolated from the individual concentration-response curves to oxygen. This value was chosen since 100mmHg is the maximum Po_2 to which umbilical arterial blood would rise in utero. Filled circles represent preparations which were not contracted by oxygen. The line is the curve through the mean responses at each gestational age, from 32 weeks onwards only.

A significant difference between the groups with respect to the maximal O_2-induced contractions was found only between the groups of gestation 36–37 weeks and 38–39 weeks (figure 23a). The maximal induced contractions to 5-HT ($3X10^{-6}M$) were found to be significantly greater in preparations of gestation 30–31 weeks than those in preparations of any other gestational age (figure 23b).

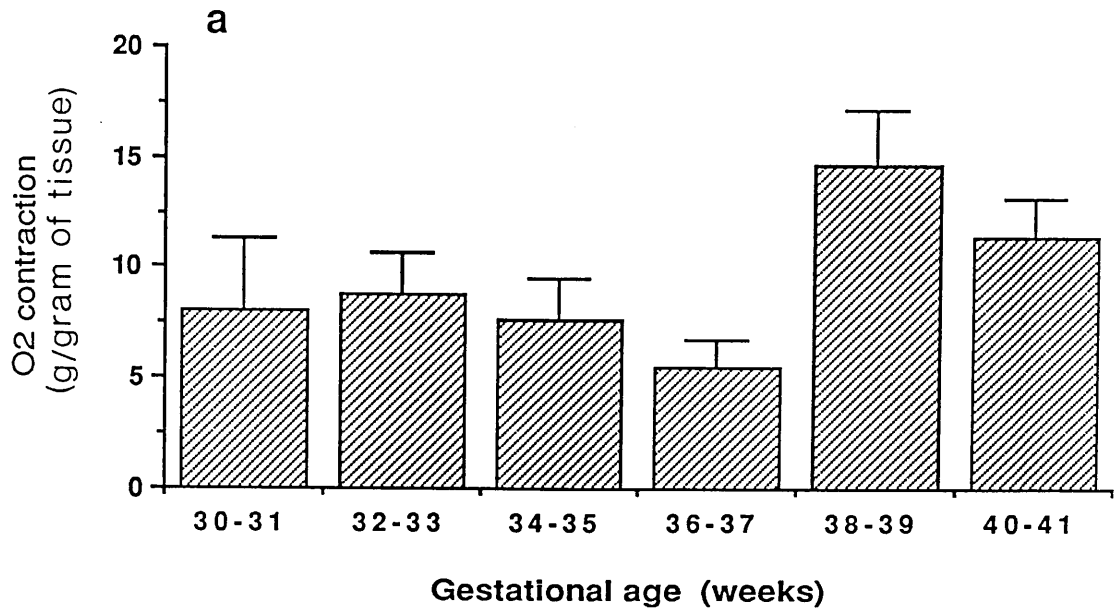
There was no correlation between gestational age and sensitivity to O_2 assessed as (i) the threshold for contraction to O_2 $\{EC_0\}$ or (ii) the Po_2 giving 50% of the maximal response $\{EC_{50}\}$.

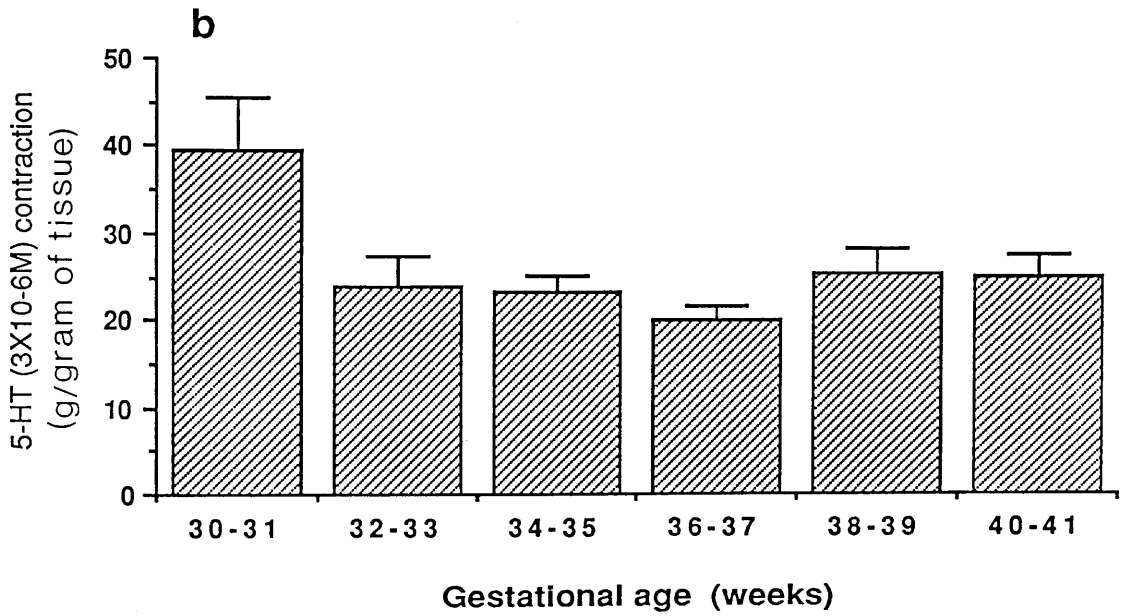
There was no significant difference of the sensitivity to 5-HT (at Po_2 ~120mmHg) of the groups of preparations from pre-term births (<37 weeks gestation) and that of preparations from term births ($pD_2=7.73\pm0.07$) (which were of different gestational ages), (figure 24a). The group which were least sensitive were those of gestation 36 weeks ($pD_2=7.27\pm0.16$) (in which preparations O_2 induced the smallest contractions) whilst the group which was most sensitive to 5-HT were of gestation 33 weeks ($pD_2=7.72\pm0.07$). Adrenaline did not contract preparations of any gestational age at low Po_2 (~15mmHg). At Po_2 ~120mmHg adrenaline did not contract preparations of gestation less than 30 weeks and was found to be significantly less potent in arteries of gestation 33–34 weeks than in preparations of any other gestation (figure 24b).

As previously described there were 7 preparations in which O_2 (and 5-HT) induced significantly greater contractions than the mean response of all other preparations. 3 of these 7 observations were in the group of gestation 30–31 weeks. If these 7 preparations are omitted from the analysis (and as already discussed there seems to be no sound reason to do so) a comparison of the preparations of greatest and least gestational age showed that O_2-induced contractions were significantly smaller in preparations of gestation 30–31 weeks than in preparations

of gestation 40–41 weeks, at all Po_2's (figure 25). However there was no correlation between gestational age and the maximal O_2 (or 5-HT) - induced contractions. The proportion of preparations which were not contracted by O_2 were 5 of 14 at 30–31 weeks and only 1 of 19 at 40–41 weeks. All of the preparations (99 in total) were contracted by 5-HT.

Figure 23 Maximal contractions to (a) oxygen and (b) 5-HT in longitudinal strips of umbilical artery from infants of different gestational ages. The maximal responses to oxygen were significantly different only between groups of gestational age 36-37 weeks and 38-39 weeks. The maximal responses to 5-HT were significantly greater in preparations of 30-31 weeks than in preparations from any other gestational age group.





n =	14	15	13	14	24	19

Figure 24 Log concentration-response curves (CRC's) to (a) 5-HT and (b) adrenaline at high Po_2 (~120mmHg) in longitudinal strips of umbilical artery from infants of different gestational ages. In (a) the curves which are shown are from those groups of preparations which were most sensitive to 5-HT (33 weeks gestation) and least sensitive to 5-HT (36 weeks gestation). The curve from TERM preparations (>37 weeks gestation) is of an arbitrary group (from figure 12a, chapter 3). The CRC's to adrenaline are for each group of preparations from pre-term preparations (<37 weeks gestation). The curve from TERM preparations is of an arbitrary group (from figure 37a, chapter 3). The figures in parenthesis are the number of preparations in each group.

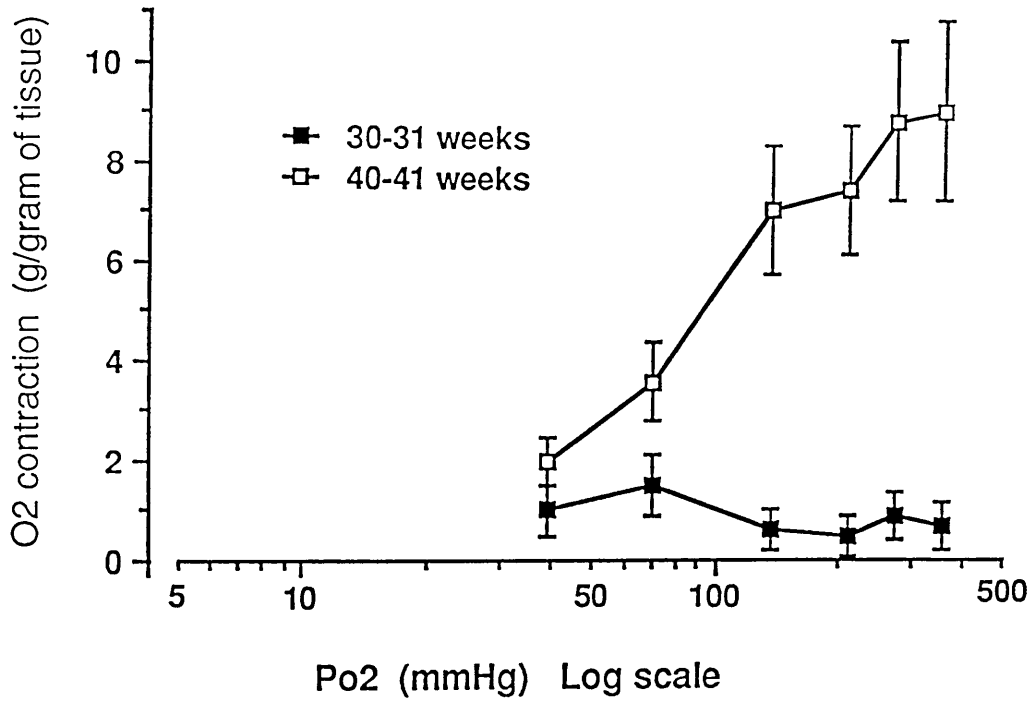


Figure 25 Concentration-response curves to oxygen in longitudinal strips of umbilical artery from infants of different gestational ages. These curves were constructed, however, with the omission of certain pieces of data (see text for details). Error bars are the mean±s.e.m.

Discussion

In this study I have confirmed that the human umbilical artery (HUA) is contracted by oxygen (O_2), and I have extended this to show that oxygen tensions (Po_2's) within the physiological range found in the arterial blood of the human umbilical cord (0–100mmHg), (Modanlou et al, 1973) can evoke profound contractions. It is well documented that O_2 can contract the isolated HUA but until now partial pressures outwith the physiological range have mainly been tested. Lewis (1968) found that an increase in Po_2 from 30 to 120mmHg contracted only a small proportion of perfused preparations. This failure may be explained by the fact that his perfused preparations were not under any longitudinal tension, which, from experiments in this study (see chapter 1) seems to be a requirement in the perfused HUA for contraction to O_2. In other reports an increase in Po_2 from ~30 to ~500mmHg (Bor and Guntheroth, 1970; Oberhansli-Weiss et al, 1972) contracted isolated strips of HUA, but this of course failed to demonstrate an effect of physiological Po_2's on the vessel.

The threshold for contraction to O_2 was found to be 36mmHg at pH=7.28 i.e. at the pH of umbilical blood *in utero* (Pearson, 1976). This threshold lies between the normal fetal (15-25mmHg) and neonatal levels (100mmHg) and thus potentially represents a major physiological mechanism capable of initiating the change from fetal to neonatal circulations at birth. (It will be discussed later that the concept of a threshold for contraction may not exist as further experiments showed that increasing the Po_2 from 0mmHg to 15mmHg caused contraction).

The sensitivity of the smooth muscle to O_2 at pH 7.18 was not found to be different from that at pH 7.28. This would suggest that the fetal metabolic acidosis which occurs at delivery (Modanlou et al, 1973) would not affect the artery's ability to constrict. At pH=7.36

the sensitivity to O_2 was significantly lower than at either 7.18 or 7.28. Although the significance of this is unclear this effect of pH could be as a result of a lower cyclo-oxygenase activity. Cyclo-oxygenase is the enzyme which catalyses prostaglandin synthesis and, as will be discussed, prostaglandins mediate the contraction to O_2. It is of course well known that all enzymes have an optimum pH at which their activity is maximal. This also applies to the cyclo-oxygenase system (Flower and Vane, 1974).

Other investigations on the possible role of O_2 in the closure of the umbilical artery at birth have generally concluded that the post-natal rise of the umbilical arterial Po_2 would be an inadequate stimulus for closure. This increase in Po_2 is from ~20mmHg to ~70mmHg within 16 minutes of delivery, (Modanlou et al, 1973) and is due to the initiation of neonatal respiration. It is worth discussing these other studies and to suggest why their arguments are invalid.

Roach (1972) suggested that the post-natal increase of arterial blood Po_2 would not be an adequate stimulus to cause vessel closure. However, the change in arterial gas tensions at birth was "mimicked" in her study by a change in gas mixtures from 10% O_2/5% CO_2, balance N_2, to 20% O_2/5% CO_2, balance N_2. In our experiments this would represent a change in Po_2 from 80 mmHg to 145 mmHg, at pH 7.36 which does not mimic the physiological changes at birth.

McReavy and McAllum (1974) found that the diameter of the artery lumen decreased by ~30% within 2 mins of delivery while the Po_2 of arterial blood did not significantly increase during the same period. However, this shows that the vessel would still be patent as this reduction was from 3.5mm to 2.5mm. The Po_2 of the blood only increases from the basal value of ~20mmHg <u>after</u> 2mins of delivery but rises steeply to ~60mmHg within the next 3mins (Modanlou et al, 1973) at which time the artery is generally closed, as judged by the cessation

of the pulsatile blood flow (Walsh et al, 1974).

Dawes (1968) considered that the vasoconstrictor effect of O_2 *in vitro* was simply an artefact of the high Po_2's used (~700mmHg) in the studies which he was reviewing, and therefore concluded that O_2 could have no role *in vivo*. This argument now has to be considered in the light of the evidence gained in this study which has shown that physiological Po_2's contract the HUA.

In this study longitudinal strips of HUA were used so that contractions are as a result of an effect on the longitudinal muscle. I have therefore shown that O_2 acts on that particular muscle bundle which a theoretical and biophysical examination has shown to be most likely to be involved in the closure of the artery at birth (Roach, 1972), i.e. contraction of the longitudinal muscle will cause the greatest decrease of the cross-sectional area of the vessel lumen. This contraction is segmental in nature (Roach, 1972; Walsh et al, 1974).

It has been shown here that O_2 contracts the HUA via prostaglandins since the non-steroid, antiinflammatory, "aspirin-like" drugs, indomethacin and flurbiprofen, inhibited the response to O_2 with high potency (IC_{50}'s were $2X10^{-9}M$ and $7.6X10^{-9}M$ respectively) while aspirin was three to four orders of magnitude less potent. The action of the aspirin-like drugs is by an inhibition of the cyclo-oxygenase enzyme which catalyses the synthesis of prostaglandins. This discovery was made by Vane (1971) and co-workers (Smith and Willis, 1971; Ferreira et al, 1971) in cell-free homogenates and in isolated tissues. Inhibition of the cyclo-oxygenase enzyme system by these aspirin-like drugs has been the focus of a multitude of research in homogenates of cells, in isolated tissues, and in whole animals including man (see Flower, 1974; Lands and Rome, 1976).

A comparison of the potencies of the drugs used here, with that

found in other studies is complicated since here the potencies were measured as the abliity to inhibit the biological response to the prostaglandin(s), whereas in nearly all other studies cyclo-oxygenase inhibition has been assessed as a direct function of the concentration of prostaglandin synthesised (in tissues it is the amount released which is measured but as cells do not store prostaglandins release is equated to synthesis -Piper and Vane, 1971). Since the relationship between prostaglandin concentration and the evoked contraction is unlikely to be linear, a direct comparison of the actual potencies of each drug would not be strictly accurate. However the ratios of the equimolar ratios of the inhibitory potencies of the compounds can be used in a comparison.

Table 4 compares the relative potencies of the prostaglandin-synthesis inhibiting drugs indomethacin and aspirin. The examples which are given are representative of the extremes to be found in the literature regarding the relative potencies of these drugs. Aspirin varies from being almost equipotent to indomethacin to being 20,000 fold less potent. The HUA therefore represents a tissue in which the cyclo-oxygenase is inhibited by indomethacin and aspirin with widely different potencies.

An interesting theory has been put forward by Vane (1972) to explain the wide variation of the potency of the aspirin-like drugs in different tissues, even from the same species. His suggestion was that the prostaglandin generating system exists in multiple molecular forms within the organism, the synthetase enzymes from each tissue type having a different pharmacological profile to those of any other tissue. Such a property has also been observed with inhibitors of phosphodiesterase (Horovitz et al, 1972) and monoamine oxidase (Fuller, 1972).

The examples given in Table 4 may suggest that the reason aspirin

is several orders of magnitude less potent than indomethacin is due to the extremely high potency of indomethacin. This does not apply generally since there are examples (e.g. bovine seminal vesicles, Tomlinson et al, 1972) in which the aspirin/indomethacin molar ratio is 2,000 yet this reflects the rather low potency of aspirin (IC_{50}= $1.5X10^{-2}M$) rather than a high potency of indomethacin ($IC_{50}=7X10^{-6}M$).

Flurbiprofen has not been investigated as extensively as either aspirin or indomethacin but where published data exists it has been shown to be exactly equipotent to indomethacin as an inhibitor of platelet synthetase activity (Cockbill et al, 1979), and as an inhibitor of the cyclo-oxygenase system in bovine seminal vesicles (Egan et al, 1978). In this study flurbiprofen was ~4 fold less potent than indomethacin which is in agreement with this other data.

The discovery made here that O_2 contracts the HUA via prostaglandins suggests that this vessel can be considered as being similar to the ductus arteriosus as one in which the closure of the vessel at birth is linked to a prostaglandin mechanism. A role for prostaglandins in the closure of the umbilical artery has been tentatively suggested by others (Dawes, 1978; Tuvemo, 1980) but without the knowledge of how prostaglandin synthesis is stimulated at birth. The evidence for such a mechanism is as follows:

(1) Following delivery the Po_2 of umbilical arterial blood rises (Modanlou et al, 1973).

(2) The physiological increase in Po_2 at birth (~15mmHg to ~70mmHg) contracts the isolated HUA via prostaglandins (this study).

(3) Molecular O_2 is a requirement of the cyclo-oxygenase system (Nugteren and van Dorp, 1965; Samuelsson, 1965).

(4) Following delivery the concentrations of PGE_2 and $PGF_{2\alpha}$ in the umbilical cord plasma rise significantly (Mitchell et al, 1978).

(5) The vascular wall of the umbilical artery can generate PGE_2 (Tuvemo et al, 1976; Jonsson et al, 1976), $PGF_{2\alpha}$ (Bjoro et al, 1986) and small amounts of TxA_2 (Mitchell et al, 1980; Bjoro et al, 1986). Each of these prostaglandins (TxA_2 shall be considered here as a prostaglandin) has been shown to be a potent constrictor of the isolated HUA (Hillier and Karim, 1968; Tuvemo, 1978).

A problem with this theory is that in vivo a constrictor action of O_2 on the umbilical artery has not been successfully demonstrated. Increasing the Po_2 of fetal lamb blood (to ~100mmHg) did not cause constriction of the umbilical vasculature (Campbell et al, 1966). Further investigation is obviously necessary before an action of O_2, in utero, can be considered of physiological importance. Additionally there may be a species difference between man and sheep as to the reaction to O_2. However no comparison of in vitro experiments is possible since there are no published data on the effect of O_2 on isolated sheep umbilical vessels.

The actual prostaglandin involved in the closure would therefore appear to be one, or a mixture of, PGE_2, $PGF_{2\alpha}$ and TxA_2, or possibly the endoperoxides PGG_2 and PGH_2 which are potent constrictors of the isolated HUA (Tuvemo, 1978). It is significant to note that the generation of these prostaglandins has been shown only in conditions of high Po_2 (95% O_2). It is therefore necessary to determine if these various prostaglandins could be synthesised under physiological Po_2's, although the results from this study suggest that this is likely.

The HUA is also capable of synthesising prostacyclin (PGI_2), but PGI_2 has almost universally been found to be a vasodilator (Moncada and Vane, 1980). This includes an effect of low concentrations of PGI_2 on the HUA (Hamberg et al, 1979). Higher concentrations caused vasoconstriction but as this was at concentrations greater than $1X10^{-6}M$ the physiological relevance of this is perhaps doubtful. Since the

levels of PGI_2 do not rise at birth (Mitchell et al, 1980) there would not seem to be a role for PGI_2 in the closure of the HUA at birth.

In a parallel study in my laboratory, O_2 failed to contract the isolated umbilical vein. These preparations were shown to be viable as they were of similar sensitivity to 5-HT as the artery. According to my evidence from the effect of COI's, O_2-induced contractions of the umbilical artery are mediated by prostaglandins. The umbilical vein is able to synthesise prostaglandins since Boura et al (1979) found that contraction of the perfused umbilical vein , induced by a reduction in perfusate temperature, was significantly reduced by prior incubation with indomethacin, thus showing that the venous smooth muscle can synthesise constrictor prostaglandins. Bjoro et al, (1986) have confirmed using radioimmunoassay that the vein can synthesise PGE_2, $PGF_{2\alpha}$, PGI_2 and TxA_2. Therefore O_2 does not fail to contract the vein because of an inability of the venous smooth muscle to synthesise constrictor prostaglandins. Presumably the failure is due, rather, to the lack of the mechanism by which O_2 induces prostaglandin synthesis or release.

Since O_2 contracts the artery but not the vein, the selective closure of the artery when the Po_2 rises after birth could allow transfusion to the fetus at birth, of the considerable amount of blood contained in the placenta, a well-known observation in the newborn when the cord is not ligated (Windle, 1940; Dawes, 1968). This will not occur if the cord is quickly clamped.

The experiments carried out in this study also show that isolated strips of HUA have an inherent O_2-induced tone, even at the physiological Po_2 of ~15mmHg. The evidence is conclusive since both reducing the Po_2 from 15mmHg to 0mmHg, and addition of the cyclo-oxygenase inhibitors flurbiprofen, indomethacin and aspirin caused

relaxation. All of the preparations were set-up under a tension of 1g. On reducing the Po_2 the preparations relaxed but to a different degree in different preparations. This relaxation, on reducing the Po_2, was found to be highly significantly correlated to the initial tension at Po_2=15mmHg.

Flurbiprofen and aspirin caused concentration-dependent relaxations which at the highest concentrations studied ($1X10^{-7}M$ and $1X10^{-4}M$ respectively) were by ~25%. Indomethacin, however, caused relaxation at the lower concentrations only. At higher concentrations ($1X10^{-7}M$ and $1X10^{-6}M$) neither relaxation nor contraction was seen. This effect could not be attributed to a possible contractile action of the vehicle ethyl alcohol, since a 50 fold greater concentration ($4X10^{-2}M$) than that added with indomethacin ($8X10^{-4}M$) had no effect on its own. Altura et al (1983) have shown that ethyl alcohol contracts the dog coronary artery by a mechanism which did not involve alpha-receptors, 5-HT receptors etc. The threshold for contraction was however ~10mM, a 10 fold higher concentration than was used here.

Northover (1973) has shown that indomethacin may act as a Ca^{++} antagonist, by inhibiting the uptake of Ca^{++} by membranous fractions of arterial cells from the HUA. This effect was only seen in millimolar concentrations, whereas micromolar concentrations (or less) were used here. Nevertheless it is hard to equate smooth muscle relaxation with a requirement for Ca^{++}. Indeed in this present study it was found that relaxation, induced by a reduction of the Po_2, was independent of extracellular Ca^{++}: relaxation was seen in preparations in saline where Ca^{++} was omitted. This relaxation was small when compared with that in controls ($[Ca^{++}]_e$=2.5mM), but the difference was not significant.

Even in anoxia (Po_2=0mmHg) there was an inherent tone since drugs which affect the mobilisation of Ca^{++}, Bayer K 8644, nifedipine and nitroprusside modified the tone under this condition. Bayer K 8644

contracted the tissue while nifedipine and nitroprusside caused a relaxation. This suggests that both intra- and extra-cellular Ca^{++} is involved in the cellular mechanism responsible for the inherent tone since Bayer K 8644 and nifedipine are thought to modify extracellular Ca^{++} mobilisation only whereas nitroprusside modifies intracellular Ca^{++} mobilisation only via an increase in cGMP accumulation (Murad et al, 1985).

These results extend the proposals put forward by Tuvemo et al, (1976); Tuvemo (1978), that the HUA has an inherent prostaglandin-induced tone, to show that this phenomenon exists at the physiological Po_2, since in these other studies the effects of indomethacin (and the prostaglandin antagonist polyphloretin phosphate) were examined at a Po_2 of 100mmHg. At this Po_2 prostaglandin synthesis would be quite profound and could therefore not reflect the physiological situation.

Since there is prostaglandin formation at Po_2=15mmHg (and possibly even lower since an increase from 0mmHg to 15mmHg evoked prostaglandin-mediated contractions) the concept of an absolute threshold for contraction to O_2, suggested from the results of the initial experiments (figure 2) would now appear to be invalid. These latter experiments suggest, rather, that prostaglandin synthesis is possible at very low Po_2's, i.e. probably at any Po_2 greater than 0mmHg. One implication of this is a possible role in autoregulation of the fetal Po_2. If, for example, fetal oxygenation were to fall (by a reduction in placental gas exchange say) then umbilical resistance would fall and placental perfusion increase to compensate for the reduction in gas exchange.

An O_2-induced tone is not peculiar to the HUA and has been noted in many smooth muscle preparations. As early as 1928 Garry described the effect of "O_2-lack" on smooth muscle and found that changing the

gassing from one of 100% O_2 to 100% N_2 caused relaxation of the uterus and intestinal preparations from many species. More recently Smith and Vane (1966) investigated the effect of Po_2's which were more physiological (~100mmHg) and found that the resting tension of both vascular and non-vascular smooth muscle varied with the Po_2 of the bathing solution. These studies could not investigate the role of prostaglandins although the latter investigators suggested that the effect of O_2 was on the formation or release of substances within the smooth muscle.

Since the upsurge in research (c. 1965) the possible role of prostaglandins on the tone of isolated preparations has been investigated using both synthesis inhibitors and receptor antagonists and has been shown to induce the inherent tone in many isolated tissues including guinea-pig trachea (Farmer et al, 1974; Orehek et al, 1975), bovine coronary artery (Kalsner, 1976) and intestinal smooth muscle (Eckenfels and Vane, 1972). Indeed most of these studies have shown that prostaglandins may induce tone in isolated preparations at physiological Po_2's.

The endothelial lining of the HUA also plays a role in the vasoconstrictor response to O_2 as, after its removal, O_2 induced significantly greater responses. This is interpreted as suggesting that an inhibitory factor is produced by the endothelium, and that in unrubbed strips O_2 stimulates the synthesis of both an inhibitory factor and the vasoconstrictor prostaglandin, with the net effect being the balance of the production of the two. It would therefore be of interest to find if the degree to which the endothelium is intact, in the longitudinal strips which were used, is a contributing factor to the wide variation of the responsiveness of the HUA to O_2 which was found here. I tentatively suggest that this inhibitory factor is PGI_2. Two lines of evidence support this view. (1) PGI_2 is produced by the

HUA (Hamberg et al, 1979; Mitchell et al, 1980) and in the HUA is a vasodilator (Hamberg et al, 1979) (2) PGI_2 is the principal prostanoid product of the vascular endothelium (Smith, 1986). This theory could be easily tested using selective inhibitors of the enzyme necessary for PGI_2 production, PGI_2 synthetase (Fried et al, 1980).

The effects of Bayer K 8644 ($1X10^{-7}M$) and nifedipine ($1X10^{-6}M$) give an insight as to the possible mobilisation of intra- and extracellular Ca^{++} in O_2 and 5-HT-induced responses. These drugs were found to significantly potentiate and depress O_2-induced contractions. Bayer K 8644 potentiated the response to O_2 at one low Po_2 (~35mmHg) only. Thereafter the Ca^{++} channels would appear to have been somehow desensitised since at higher Po_2's the responses were smaller than those in controls, although this was not significant. The effects of these same concentrations of Bayer K 8644 and nifedipine on 5-HT-induced contractions were not significant however, and in the presence of Bayer K 8644 the responses were actually smaller in the controls, although this was not significant. O_2 and 5-HT -induced contractions seem to rely on extracellular Ca^{++} to a similar extent as exclusion of Ca^{++} from the saline reduced the maximal responses to either agent to ~40% of that in controls ($[Ca^{++}]_e$=2.5mM). This implies that both 5-HT and O_2-induced responses utilise extracellular Ca^{++} but through different channels- those involved in 5-HT-induced contractions being less sensitive to Bayer K 8644 and nifedipine than those involved in O_2-induced contractions. Ten fold higher concentrations of Bayer K 8644 and nifedipine potentiated and depressed 5-HT-induced responses respectively but as only 2 preparations were tested the significance of this could not be evaluated.

The effect of gestational age on the responsiveness (assessed as sensitivity to vasoconstrictors, or maximal responses to

vasoconstrictors) of the HUA to different stimuli was tested. The sensitivity to O_2 or to 5-HT did not vary with gestational age. Sensitivity to adrenaline was found to be significantly lower during the period 33–34 weeks (term is 37 weeks) than at any other gestational age. The relevance of this finding is unclear but does not reflect any decrease in prostaglandin synthesis ability as adrenaline acts at a specific $alpha_1$-receptor (at term at least) which is not linked to prostaglandin synthesis (see chapter 3). Perhaps this implies some down-regulation of these receptors during this particular period of gestation.

Significant differences were found between the gestational age groups as to the size of the maximal induced response to O_2 and 5-HT. The interpretation of these results is complicated by two factors (1) The gestational age of the fetus (and hence umbilical arterial tissue) was determined by ultrasound whose accuracy is to within one week only if ultrasound scaning has been performed in the 20th week of pregnancy (Chervenak et al, 1983). This means that the data may not necessarily be in the correct group (the data were split into groups, each group comprised of experimental data from arteries within a 2 week gestational period); (2) In a few of the individual preparations (7 of 99) O_2 evoked significantly greater contractions than the mean response of the other data. The problem is in deciding whether these pieces of data represent "odd-points" which should be discarded before analysing the other data. This point was discussed in the results section and the conclusion reached was that they could not be.

The only significant difference found between the groups of different gestational age, as to the maximal O_2-induced contractions was between the groups of gestation 36–37 weeks and 38–39 weeks and which were greater in the latter group. No special significance is attached to this finding due to the possible errors involved in

establishing the gestational age. The conclusion that I have reached is that the HUA can synthesise constrictor prostaglandins from at least 27 weeks gestation since this was the earliest age at which O_2 evoked contractions (one preparation of 26 weeks gestation was not contracted). This is supported by other evidence: (1) The high potency of 5-HT at Po_2 ~120mmHg did not vary with gestational age. This high sensitivity to 5-HT at Po_2=120mmHg is due to prostaglandins since in the presence of indomethacin, or at low Po_2 (~15mmHg), 5-HT is ~4 fold less potent than at Po_2=120mmHg (see chapter 3), (2) Fetal tissue (lung, brain and vascular smooth muscle) from lamb and calf are able to synthesise (and catabolise) prostaglandins from an early gestational age. In the case of lamb this is from 30 days (term is 140 days), (Pace-Asciak, 1978) and for calf from at least 100 days (term is 265 days), (Powell and Solomon, 1978). Although this is rather indirect evidence it does show the capability of fetal tissue to synthesise prostaglandins from an early gestational age.

If one does choose to believe that some of the data (as discussed above) should be omitted from the analysis, then a comparison of the O_2-induced contractions in preparations of greatest and least gestational ages, 30–31 weeks and 40–41, weeks suggests a relative inability to synthesise prostaglandins during early gestation. As the proportion of tissues which were not contracted by O_2 was much greater in the earlier gestational group, this might be taken as supporting this view.

The overall conclusion I have reached is that constrictor prostaglandins can be synthesised by the vascular wall of the HUA from at least 27 weeks gestation. No relationship between sensitivity to O_2, or magnitude of contraction (as a measure of the capacity to synthesise prostaglandins) and gestational age has been conclusively established.

This conclusion suggests that from at least 27 weeks gestation any fluctuations in Po_2 or the presence of drugs or physiological agents which sensitise the process (e.g. stimulating constrictor prostaglandin synthesis) could cause vasoconstriction and so reduce fetal blood flow.

It has of course to be considered that the difference between preparations as to their responsiveness to O_2 may be related to the prostaglandin receptor population and/or the establishment of the link between receptor excitation and coupling. These points could be easily investigated by a comprehensive study in preparations challenged by O_2, exogenous prostaglandins and in binding studies. Specific receptors for prostaglandins in the HUA have been demonstrated by Park and Dyer (1973) but without any investigation of their gestational developmental. Hillier and Karim (1968) found that arteries from early gestation (16–24 weeks) were not contracted by prostaglandins. However, as their preparations were not contracted by 5-HT either, this can not act as evidence to suggest an absence of prostaglandin receptors in early gestation as their preparations might not have been viable.

Some of the umbilical arteries came from infants who displayed clinical symptoms of a patent ductus arteriosus (PDA), intra-uterine growth retardation (IUGR) and other complications. None of the clinical observations could be correlated to experimental results such as the sensitivity of the umbilical arterial preparations to O_2, 5-HT, adrenaline etc. Although one preparation from an infant who had symptoms of a PDA was not contracted by O_2, another preparation from another infant of similar disposition was contracted by O_2. Therefore failure of the HUA to contract to O_2 is not a reflection of a general failure to contract to prostaglandins.

Similarly, neither a failure to contract to O_2, nor large O_2-induced contractions in some preparations could be correlated to clinical observations of, say, IUGR. One may have liked to suggest that

IUGR was possibly due to a restricted umbilical blood flow as a result of a high sensitivity to changes in Po_2, but this link could not be established. The question as to whether there is a correlation between the infants' health and experimental findings in isolated samples of the umbilical artery has therefore not been established. A much larger study of particular cases involving complications would have to be undertaken to try to answer this point with any certainty.

Chapter 3: Characterization of the receptors for Serotonin (5-HT) and Adrenaline

Introduction

Classification of receptors for 5-hydroxytryptamine

Receptors for serotonin (5-HT) were first classified into "D" and "M" categories in 1957 (Gaddum and Picarelli, 1957). In the following 20 years or so this proposal remained unchanged despite criticisms of the basis of the classification. Since 1979 however, with the knowledge of data from radiolabelled ligand binding studies in brain tissue and the advent of selective, high affinity antagonists and agonists the most recent proposals now categorise 5-HT receptors as "5-HT_1-like", 5-HT_2 and 5-HT_3 (Bradley et al, 1986a). Ligand binding studies suggest that the 5-HT_1 recognition site is probably composed of three sub-groups (5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C}) and as functional correlates of these sites have recently been proposed they may be regarded as receptors. There may also be sub-groups of 5-HT_3 receptors but this is based on functional studies only as no equivalent recognition sites have been found in binding studies of brain tissue. The reasons behind the need for this new classification shall now be set out.

Independently, but working at the same time, 2 groups found that 5-HT contracted the isolated guinea-pig ileum by two mechanisms, one was indirect by stimulating cholinergic intramural nerves and the other by a direct effect on the smooth muscle (Rocha e Silva et al, 1953; Gaddum and Hameed, 1954). One representative from each group published a paper together which described the first classification of 5-HT receptors (Gaddum and Picarelli, 1957). The receptor on the parasympathetic nerve endings was designated "M" as it was blocked by morphine while the "D" receptor was on the smooth muscle and was blocked by dibenzyline (phenoxybenzamine). This paper has been much criticised because of the lack of selectivity of the antagonists used. Phenoxybenzamine also blocks alpha-adrenoceptors (Bickerton, 1963) and

the direct response of histamine and acetylcholine on the smooth muscle of the guinea-pig ileum and also blocks the M receptors (Day and Vane, 1963). The action of morphine was subsequently shown not to be as a receptor antagonist but inhibited the release of acetylcholine by a local anaesthetic action (Lewis, 1960). However the overall conclusions they reached that there are 2 receptors for 5-HT in the guinea-pig ileum have not been invalidated (see Feniuk, 1984; Fozard, 1984; Humphrey, 1984). Gaddum and Picarelli did however substantiate their results using LSD and dihydroergotamine (LSD was a particularly potent antagonist of the D receptor) and cocaine which selectively antagonised the M receptor. This latter finding has since been confirmed (Fozard et al, 1979).

Thus the term "D receptor" has been used to describe receptors where lysergic acid derivatives (e.g. LSD, 2-bromo LSD, methysergide) antagonised the response to 5-HT (Trendelenburg, 1960; Cerletti and Doepfner, 1958; Rose and Lazaro, 1958; see also Gyermek (1966) for references).

The antiserotonin activity of antihistamines has been studied and where the potencies of these latter compounds have been simultaneously compared to that of lysergic acid derivatives they have generally been found to be somewhat less potent (Doepfner and Cerletti, 1957) but with one notable exception -cyproheptadine, which in different test systems (rat uterus-contraction; anaesthetised dog-increase of arterial blood pressure) was as effective as LSD as an antagonist of the response to serotonin (Stone et al, 1961). Subsequently, methysergide and cyproheptadine have been used to characterize receptors for serotonin and where they have been found to be selective and competitive antagonists the receptors have been described as "D receptors" and have been found in many smooth muscle preparations including the rabbit aorta (Apperley et al, 1976), dog femoral artery (Apperley et al, 1980)

and rat caudal artery (Bradley et al, 1983).

Various experimental evidence from functional studies began to accumulate and cast doubt on whether the D and M nomenclature could encompass the receptor types which were found in some tissues and, accompanied by the findings from ligand binding studies, has ultimately led to the present classification of 5-HT receptors.

In some tissues the receptors mediating the response to 5-HT were conclusively shown to be neither D nor M. In the dog saphenous vein methysergide and cyproheptadine were weak antagonists of both the pre-junctional receptor (mediating inhibition of neurotransmitter release), (Feniuk et al, 1979; Watts et al, 1981) and the post-junctional receptor (mediating contraction), (Apperley et al, 1980). Indeed, at both receptors methysergide was a partial agonist. These receptors could therefore not be described as D receptors and neither were they M receptors as the post-junctional receptor was resistant to metoclopramide and phenylbiguanide (Apperley UNPUBLISHED OBSERVATION et al, 1980) and the neuronal receptor was resistant to morphine, i.e. resistant to M receptor antagonists. Similarly the receptor mediating relaxation of the pre-contracted cat saphenous vein and guinea-pig ileum could not be described as either D or M on the basis of antagonist data and also since the agonist α-methyl 5-HT , an agonist at both D and M receptors in the guinea-pig ileum (Gaddum and Picarelli, 1957; Fozard and Mobarok Ali, 1978a) was a very weak agonist (Feniuk et al, 1983).

In ligand binding studies using membranes from rat cortex two distinct recognition sites for 5-HT were found (Peroutka and Snyder, 1979). At both sites [^{3}H]LSD had high affinity and they were distinguished between since at one site [^{3}H]5-HT had high affinity (labelled 5-HT_1) and at the other site (labelled 5-HT_2) [^{3}H]spiperone

had high affinity. In the subsequent study of these binding sites many ligands were found to lack selectivity (see Leysen et al, 1984). However the discovery of the piperidine derivative, ketanserin, which had high affinity for the 5-HT_2 recognition site and negligible affinity for the 5-HT_1 site (Leysen et al, 1981) has been an invaluable tool in characterizing 5-HT receptors in both binding and functional studies.

Various groups pointed out the similarity of the 5-HT_2 binding site and the vascular D receptor in rabbit aorta (Humphrey et al, 1982; Maayani et al, 1984), in guinea-pig ileum (Engel et al, 1984, 1985) and in other vascular tissue from the rat (Bradley et al, 1983; Cohen et al, 1981) as in these tissues the affinity of a number of 5-HT antagonists, including ketanserin, was similar at both the binding site and at the receptor. Since ketanserin differentiated between the two 5-HT recognition sites it has been widely used as a probe in 5-HT receptor characterization and the vascular D (or 5-HT_2) receptor has been identified in many tissues (see Table 4, Humphrey, 1984).

Ligand binding studies began to cloud the D and M classification of 5-HT receptors, not only because it introduced a new classification, 5-HT_1 and 5-HT_2, but for two other reasons. (1) The 5-HT_2 receptor antagonist ketanserin was ineffective at the D receptor in guinea-pig ileum (Van Neuten et al, 1983) on which the original classification of D amd M was based and (2) the D and M receptor antagonists phenoxybenzamine and morphine were weakly active, and inactive respectively, in the binding models of 5-HT_2 and 5-HT_1 receptors (Leysen et al, 1981).

The doubt raised by the first point has since been overcome as subsequent studies have suggested for two reasons that the 5-HT_2 and D receptors in the smooth muscle of the guinea-pig ileum may be the same. (1) A highly significant correlation was found for the potency of a

series of antagonists to inhibit the contraction of the guinea-pig ileum and for their affinity at the 5-HT_2 binding site (Engel et al, 1984, 1985). (2) The rank order of potency of a series of agonists for causing contraction of guinea-pig ileum and rabbit aorta was the same (see Table 2, Humphrey, 1984), and the receptor in the rabbit aorta is undoubtedly 5-HT_2 (Humphrey et al, 1982; Maayani et al, 1984; Feniuk et al, 1985).

Thus the classification of 5-HT receptors was becoming rather confused mainly due to ligand binding data although the "D receptor" classification had been broadly replaced by the "5-HT_2 receptor" terminology.

Functional correlates of the 5-HT_1 binding site have been more difficult to determine as the 5-HT_1 site is comprised of subsites for which selective antagonists have not yet been identified (see Bradley et al, 1986a) although metergoline, metetipine and methysergide have high affinity for the 5-HT_1 site but without selectivity (Leysen et al, 1981). However, receptor characterization is also based on the relative potencies of agonists. The carboxamide analogue of 5-HT, 5-carboxamidotryptamine (5-CT) (1) was found to have a marginally greater affinity for the 5-HT_1 binding site than the 5-HT_2 site (Engel et al, 1983), (2) in functional studies was found to mimick with equal or greater potency, the response to 5-HT in tissues where the receptor did not fit either the D or M classification (Feniuk et al, 1981, 1985), (3) was somewhat less potent at 5-HT_2 receptors (Feniuk et al, 1985), and at M receptors (see Table 2, Humphrey, 1984). Hence the terminology "5-HT_1-like" has come into use.

As already mentioned the 5-HT_1 binding site may be comprised of 3 sub-sites (or more, see Pazos et al, 1984a) and evidence of functional correlates for these sites has been presented. It is however considered

"that developements have not progressed to the point where it would be profitable to consider these (subtypes of the 5-HT_1 binding site) in a classification of 5-HT receptors" (Bradley et al, 1986a). A brief mention of these sites will be made here.

On the basis that spiperone biphasically inhibited the binding of [^{3}H]5-HT it was proposed that the 5-HT_1 site was comprised of two populations of 5-HT receptors: 5-HT_{1A}, where binding of [^{3}H]5-HT was inhibited by low concentrations of spiperone, and 5-HT_{1B} where binding was unaffected by low, but inhibited by high concentrations of spiperone (Pedigo et al, 1981). Evidence for a functional role for these two subtypes has been recently presented. In the CNS the selective 5-HT_{1A} receptor agonist 8-OH-DPAT (Middlemiss and Fozard, 1983; Hamon et al, 1984) potently stimulated adenylate cyclase in guinea-pig hippocampus and was antagonised by spiperone but not by ketanserin (5-HT_2) or by MDL 72222 (an M receptor antagonist, see later), (Shenker et al, 1985). In the periphery the receptor mediating inhibition of transmitter release on the cholinergic nerve endings in the guinea-pig ileum may be of the 5-HT_{1A} type as 8-OH-DPAT inhibited the stimulation-evoked release of transmitter and was antagonised by spiperone and buspirone (Fozard and Kilbinger, 1986), the latter shown to selectively displace 8-OH-DPAT from the 5-HT_{1A} binding site (Gozlan et al, 1983).

The nerve terminal 5-HT autoreceptor in the rat frontal cortex is almost certainly of the 5-HT_{1B} type. Various authors proposed that the autoreceptor was of the 5-HT_1 class, although the subtype was not known (Gothert, 1982; Martin and Sanders Bush, 1982; Engel et al, 1983). Subsequently it was suggested that the autoreceptor was of the 5-HT_{1B} type on the basis that (-)propranolol displaced [^{3}H]5-HT from the 5-HT_{1B} binding site in rat frontal cortex with a similar potency and stereoselectivity to its activity at the autoreceptor (Middlemiss, 1984) and at which the selective 5-HT_{1A} agonist 8-OH-DPAT had little or

no agonist (or antagonist) activity (Middlemiss and Fozard, 1983; Hamon et al, 1984). Other studies have shown that other receptor antagonists including pindolol and cyanopindolol were stereoselective antagonists of the central autoreceptor, of which (±)cyanopindolol is the most potent (pA_2 8.32), (Middlemiss, 1986). Subsequent studies utilising more powerful and selective agonists and antagonists (30 in total) of the central 5-HT recognition sites strongly indicated that the 5-HT autoreceptor belonged to the 5-HT_{1B} subtype (Engel et al, 1986).

In the pig choroid plexus [^{3}H]mesulergine displayed high affinity binding while other ligands, selective for 5-HT_{1A} (8-OH-DPAT), 5-HT_{1B} (RU 24969) and 5-HT_2 (ketanserin) binding sites, did not show this high affinity. On this basis the site in pig choroid plexus (and pig frontal cortex) was termed 5-HT_{1C} (Pazos et al, 1984b). A 5-HT_{1C} receptor possibly mediates a component of the 5-HT-induced contraction of rat stomach fundus but the authors themselves pointed out possible complications when using non-selective agonists, as they had e.g 5-HT, 5-carboxamidotryptamine (Buchheit et al, 1986).

The term "M receptor" has almost been synonymous with "peripheral neuronal receptor" no matter the anatomical location or the functional response which it mediates (see Wallis, 1981). This is despite criticisms of the basis of the classification and no doubts reflects in part the lack of identification of a selective antagonist of these neuronal receptors since the introduction of the D and M nomenclature.

Neuronal receptors have been reviewed from two main standpoints: from functional studies (see Fozard, 1984a) and from electrophysiological studies (see Wallis, 1981). Each has suggested a tentative classification. Since this study deals with functional responses this brief introduction shall therefore look at M receptors from this standpoint.

Having said that M receptors and peripheral neuronal receptors had become almost synonymous I would now like to qualify this. Even before the introduction of a selective M receptor antagonist (c. 1983) at least one group of receptors, on postganglionic neurones of the sympathetic nervous system mediating inhibition of neurotransmitter release, had convincingly been shown to be neither D nor M on the basis of both agonist and antagonist data. Such receptors have been most extensively studied in the dog saphenous vein (Feniuk et al, 1979, 1980; Watts et al, 1981; Muller-Schweinitzer, 1981).

Two preparations have been the focus of attention in the study of peripheral neuronal receptors for 5-HT which mediate functional responses. In the guinea-pig ileum the 5-HT-induced contraction is mainly indirect and due to the release of acetylcholine from pre-junctional neurones and was conclusively shown to be mediated by specific receptors since (1) many antagonists of other types of receptors could not selectively antagonise the response to 5-HT (e.g. hyoscine, procaine) and the response to 5-HT was not antagonised by D receptor antagonists (LSD, methysergide), (Brownlee and Johnson, 1963; Day and Vane, 1963) and (2) the receptors could be rapidly and selectively desensitised by 5-HT (Gaddum, 1953; Rocha e Silva et al, 1953; Brownlee and Johnson, 1963). These receptors were designated as M receptors (Gaddum and Picarelli, 1957). This receptor in guinea-pig ileum was insensitive to blockade by phenylbiguanide which was effective as an antagonist at neuronal receptors for 5-HT in the mouse duodenum (Drakontides and Gershon, 1968) and which first indicated a difference between these M receptors.

The indirect +ve inotropic effect of 5-HT on the isolated rabbit atrium was antagonised by morphine and cocaine, and abolished by prior reserpination of the animal. The receptor mediating this response via release of neurotransmitter (noradrenaline) from sympathetic neurones

was described as M (Trendelenburg, 1960).

However, it has been demonstrated that there is a difference between the M receptors in guinea-pig ileum and rabbit heart in respect of their susceptibility to blockade by morphine. In the Langendorff perfused rabbit heart the +ve chronotropic effect of 5-HT was not selectively antagonised by morphine (Fozard and Mobarok Ali, 1978a) and a concentration of morphine which gave a non-selective inhibition (10ug/ml) was 100 times greater than the concentration of morphine which blocked 5-HT-induced transmitter release from cholinergic neurones in the guinea-pig ileum (Day and Vane, 1963).

It has now been demonstrated that (-)cocaine (and some derivatives) selectively block M receptors of the cholinergic nerve endings of the guinea-pig ileum and of the sympathetic nerve endings in the rabbit heart by a simple competitive antagonism of the receptors (Fozard et al, 1979).

Just as has been shown for the characterization of D receptors, great advances were made in characterizing M receptors with the identification of a selective antagonist, viz. MDL 72222, which in nanomolar concentrations blocked the 5-HT-induced release of transmitter from sympathetic neurones of the rabbit heart (Fozard, 1984b). Several other M receptors were identified on post-ganglionic sympathetic and parasympathetic neurones and on afferent neurones all of which were susceptible to blockade by MDL 72222, and which were without exception excitatory (see Fozard, 1984a). MDL 72222 also confirmed earlier findings that the M receptor in the guinea-pig ileum was different to the other excitatory M receptors, since in the guinea-pig ileum it had negligble affinity (Fozard, 1984b). Metoclopramide was a surmountable antagonist at excitatory 5-HT receptors on post-ganglionic sympathetic nerves and on afferent neurones (Fozard and

Mobarok Ali, 1978b; Fozard and Host, 1982; see also Fozard 1984a) but is a partial agonist at M receptors in the ileum (Kilbinger et al, 1982). The selective agonist 5-methoxytryptamine similarly shows a difference between the M receptor in guinea-pig ileum and the other excitatory neuronal receptors: in the rabbit heart 5-methoxytryptamine is inactive while in the ileum it is a partial agonist (Fozard and Mobarok Ali, 1978a).

In a separate but almost parallel study selective agonists and antagonists were identified which pointed to subtypes of M receptors in different M receptor systems (Richardson et al, 1985). The agonist 2-methyl-5-HT was equipotent at M receptors in the rabbit heart, guinea-pig ileum and rabbit vagus nerve (although approximately 2 fold less potent than 5-HT) while α-methyl-5-HT was less potent than 2-methyl-5-HT, but to a different degree, in each preparation.

A series of highly potent and selective M receptor antagonists confirmed that the M receptors in the three systems were different. Compounds I and II had significantly different affinities for each of the receptors in guinea-pig ileum, rabbit heart and rabbit vagus nerve while compound III (ICS 205-930) differentiated only between the receptors in rabbit vagus nerve (pA_2 10.2), rabbit heart (10.6) and in the guinea-pig ileum (7.8). In ligand binding studies ICS 205-930 was shown to be highly selective as it lacked affinity for all the receptor types tested: 5-HT, alpha, beta, dopamine, histamine, muscarinic.

These excitatory M receptors which are blocked by either MDL 72222 or ICS 205-930 now make up the category of receptors to be known as 5-HT_3 receptors but which do not have an equivalent binding site in brain tissue (Bradley et al, 1986a).

The criteria which have been set down for a response to be mediated by 5-HT_1-like, 5-HT_2 and 5-HT_3 receptors (Bradley et al, 1986a) have been indirectly discussed above. In summary (from Bradley

et al, 1986a) the following criteria have to be met.

(1) 5-HT_1-like receptors: the response to 5-HT should be

a) antagonised by metitepin or methysergide (which may be a partial agonist) with a potency somewhat less than at 5-HT_2 receptors
b) resistant to 5-HT_3 antagonists
c) mimicked by 5-CT with equal or greater potency as that to 5-HT.

5-HT_2 receptors: the response to 5-HT should be

a) antagonised by ketanserin and methysergide with high potency
b) resistant to 5-HT_3 antagonists
c) no selective agonist is available.

5-HT_3 receptors: the response to 5-HT should be

a) antagonised by (-)cocaine, MDL 72222 or ICS 205-930
b) resistant to 5-HT_2 and 5-HT_1-like (metitipine) antagonists
c) mimicked by 2-methyl-5-HT.

The importance of relating binding sites to functional correlates before describing them as receptors has been much stressed (Leysen et al, 1984; Fozard, 1984a; Humphrey, 1984; Bradley et al, 1986a) since inherent in the definition of a receptor is that binding of a substance to a receptor site is inseparably linked to eliciting or antagonising a pharmacological effect (Langley, 1878). Many of the functional responses mediated by 5-HT_1-like and 5-HT_2 receptors (whose nomenclature was prompted by binding studies) and 5-HT_3 receptors have been described. In-depth coverage of this is given in a recent review (Bradley et al, 1986a).

Receptors for 5-HT in the human umbilical artery (HUA)

To date there has been no quantitative study of the receptors in the HUA mediating responses to various known vasoactive substances, and this includes 5-HT. While various drugs including histamine, acetylcholine, adrenaline, noradrenaline, bradykinin and 5-HT have been examined in the isolated HUA, it is mainly the relative potency of the drugs which has been of interest (Eliasson and Astrom, 1955; Gokhale et al, 1966; Lewis, 1968; Altura et al, 1972). Thus it has been found that

of these drugs, all of which generally contract the HUA, 5-HT was the most potent though the data is qualitative rather than quantitative i.e. one drug was simply reported as being "more potent" than another drug.

In those studies which have employed antagonists, the response to 5-HT has been shown to be susceptible to blockade by cyproheptadine (Gokhale et al, 1966), phentolamine and yohimbine (Astrom and Samelius, 1957) and LSD (Astrom and Samelius, 1957), but without demonstrating specific receptors for 5-HT. Again these experiments yielded only qualitative results, e.g. the response to 5-HT was described as being "blocked" or "antagonised".

In other umbilical vasculature, e.g sheep umbilical arteries and human umbilical veins, specific receptors for 5-HT have been demonstrated using a) selective antagonists e.g. cinanserin (Dyer and Gant, 1973) and b) in "protection experiments" where prior exposure to 5-HT protected the receptor for 5-HT, but not the receptor for noradrenaline or acetylcholine, from irreversible blockade by phenoxybenzamine. Similarly, the receptor for 5-HT was not protected from irreversible blockade by histamine, acetylcholine, noradrenaline or angiotensin (Dyer, 1974).

In all of these studies the actions of drugs were examined in oxygen tensions greater than that found physiologically (~15mmHg -Wulf, 1964). The normal gas mixture used was 95%O_2/5% CO_2 which gives a Po_2 in the region of 400–600mmHg. In only two studies have physiological gas conditions been employed but they did not undertake any analysis of the response to 5-HT using antagonists (Lewis, 1968; Eltherington et al, 1968). In these studies it was concluded that at physiological oxygen tensions the HUA was rather insensitive to 5-HT. This was not borne out in a preliminary study in our laboratory (McGrath and Stuart-Smith, 1982). Since I wished to characterize the receptor for 5-HT

which mediates its vasoconstrictor effect, under physiological conditions, and predict its action *in utero*, then appropriate physiological blood-gas conditions should be employed *in vitro*. This was undertaken in the first part of this study. In latter experiments higher oxygen tensions were employed (~120mmHg). This higher level however, may still be within the physiological range.

Results

Part 1: Receptors for 5-HT

Characterization of the receptors which may mediate the response to 5-HT in the isolated HUA was made using antagonists and other agonists shown to have affinity for 5-HT receptors.

(i) Antagonists

Antagonists were studied at two different levels of oxygen - "low Po_2" (~15mmHg) and "high Po_2" (~120mmHg).

a) Low Po_2 (~15mmHg)

Cumulative additions of 5-HT ($1X10^{-8}$–$1X10^{-5}$M) caused concentration-related contractions of the isolated HUA (figures 1 and 2), which were well maintained at each response. The pD_2 for 5-HT was 7.22±0.09 (mean±s.e.mean, n=11). The maximum contraction was 1.62±0.12g (range, 1.12g–2.14g) which represents a considerable difference between tissues.

With washout of the drug (3 times, at 10 minute intervals) there was a slow relaxation to baseline tension which was complete within 45 minutes (of the 1st wash). After a further 30 minutes 5-HT was re-added. The 2nd CRC was similar to the 1st (figure 2): The maximum response was 1.72±0.16g, which, although greater than the 1st CRC, the increase (6±2%) was not significant ($P>0.05$). The potency of 5-HT was slightly but significantly decreased ($P<0.05$) on the 2nd addition by 1.7±0.2 fold. Successive CRC's to 5-HT in the HUA could therefore be reproduced with only a small and consistent shift, i.e., there was no tachyphylaxis to 5-HT if 60-75 minutes was allowed between CRC's.

Indomethacin

Indomethacin ($1X10^{-6}$M) did not antagonise the response to 5-HT (figure 3a): in the presence of indomethacin the CRC to 5-HT was not

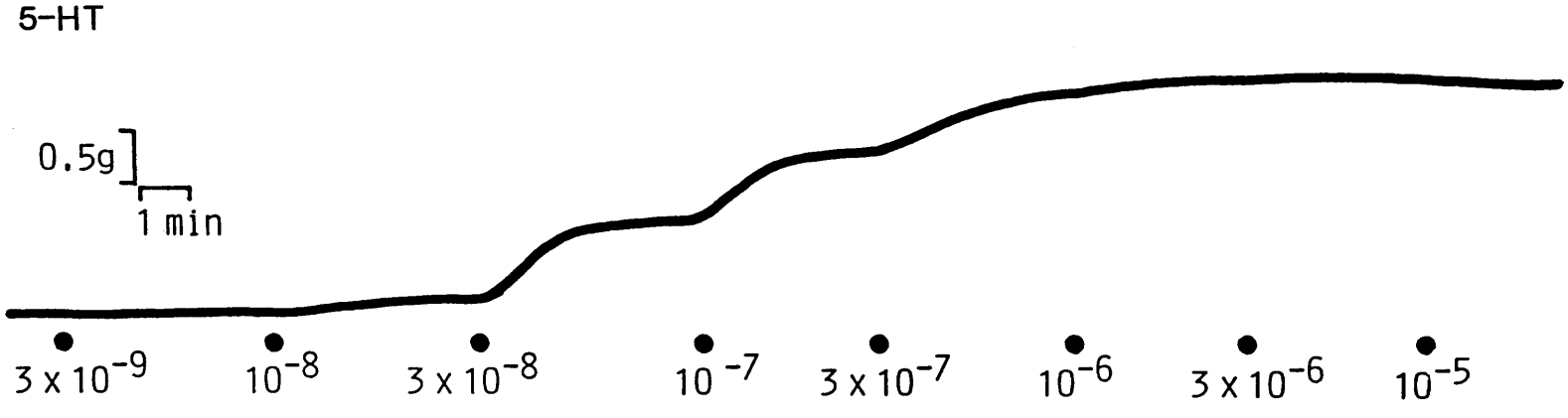


Figure 1 A tracing of a representative recording of a cumulative concentration-response curve to 5-HT in a longitudinal strip of human umbilical artery at low Po_2 (~15mmHg). Responses are well maintained and do not generally decay.

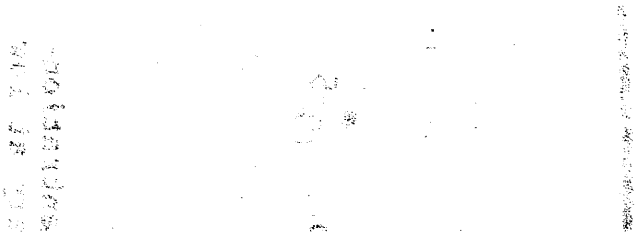
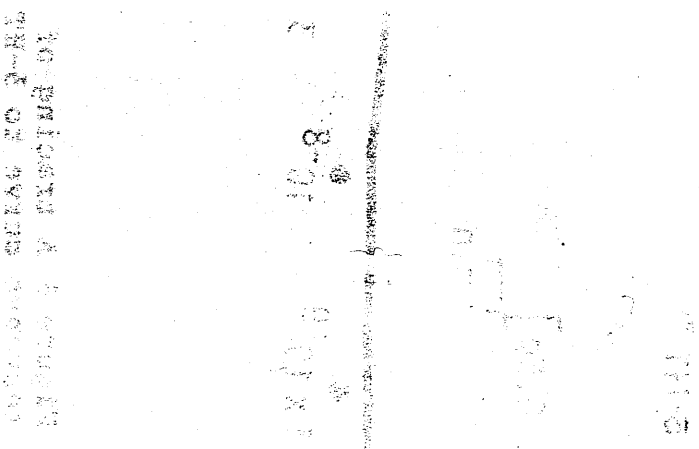
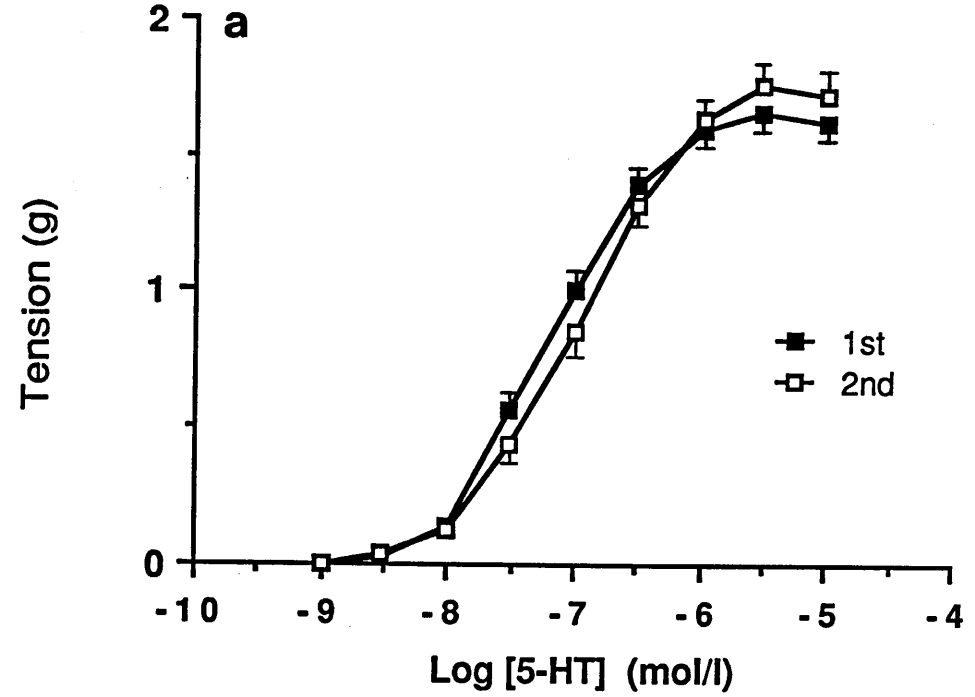


Figure 2 Log concentration-response curves (CRC's) to 5-HT at low Po_2 (13±1mmHg) in longitudinal strips of umbilical artery. CRC's were constructed twice in each preparation (n=11). Response (ordinate) is expressed in two ways: (a) grams tension (b) % of the maximum response of the 1st curve. There was no significant change of the maximum response on the 2nd addition of 5-HT. There was a small but significant decrease of the sensitivity to 5-HT on the 2nd addition by 1.7±0.2 fold. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.




a
2
1
0
Tension (g)
1st
2nd
-10
-9
-8
-7
-6
-5
-4
Log [5-HT] (mol/l)

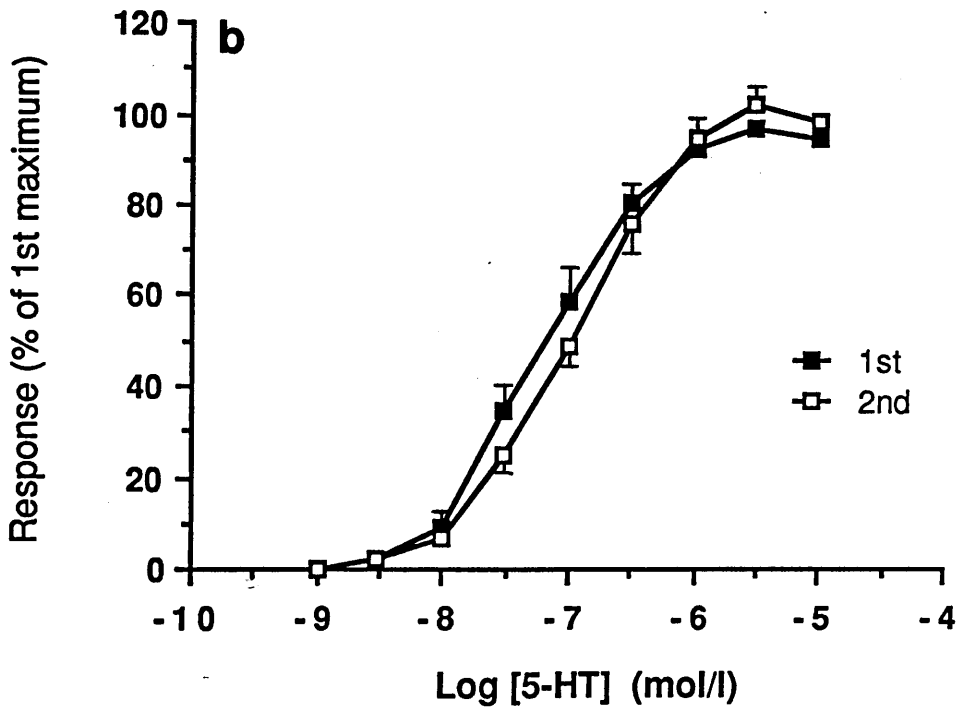
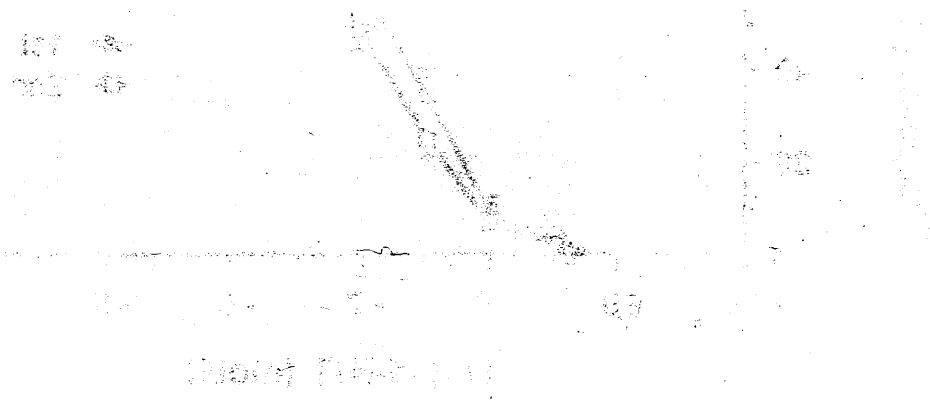
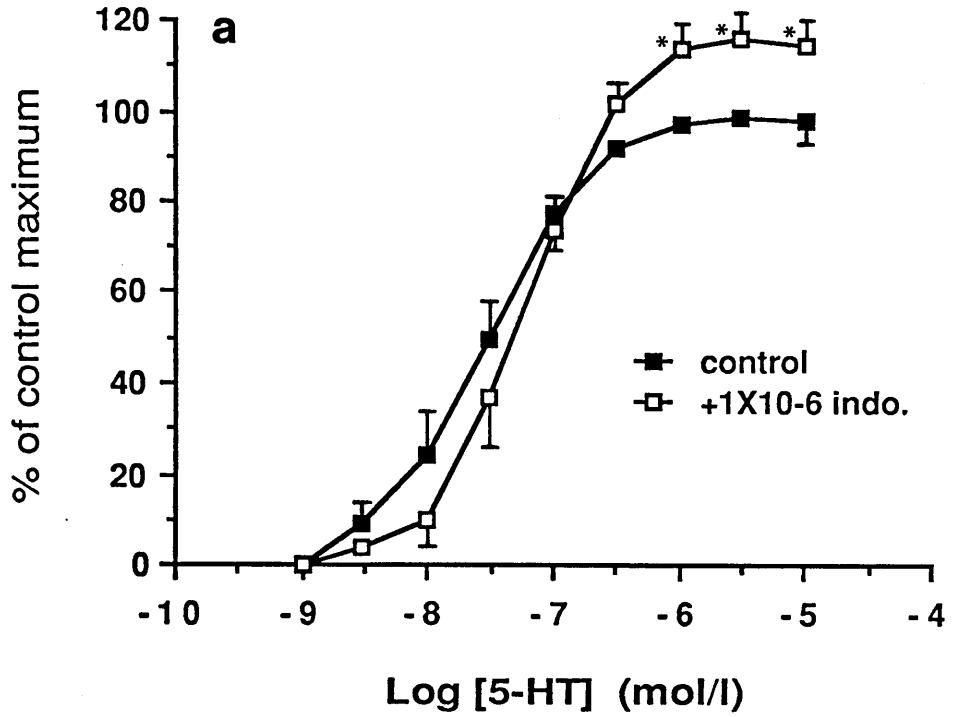

b
120
100
80
60
40
20
0
Response (% of 1st maximum)
1st
2nd
-10
-9
-8
-7
-6
-5
-4
Log [5-HT] (mol/l)

Figure 3 Log concentration-response curves (CRC's) to 5-HT in longitudinal strips of umbilical artery (n=6) at low Po_2 (16 ± 2mmHg) in the presence and absence of indomethacin ($1X10^{-6}$M). (a) the 1st curve is the control and the 2nd curve is in the presence of indomethacin (b) in paired preparations two consecutive CRC's to 5-HT were constructed in the same preparation. Vertical bars (mean$\pm$s.e.mean) are shown where these are greater than the height of the symbols. Asterisks indicate values which are significantly greater for the 2nd curve than for the 1st curve.




a
120
100
80
60
40
20
0
% of control maximum
control
+1X10-6 indo.
*
*
*
-10
-9
-8
-7
-6
-5
-4
Log [5-HT] (mol/l)

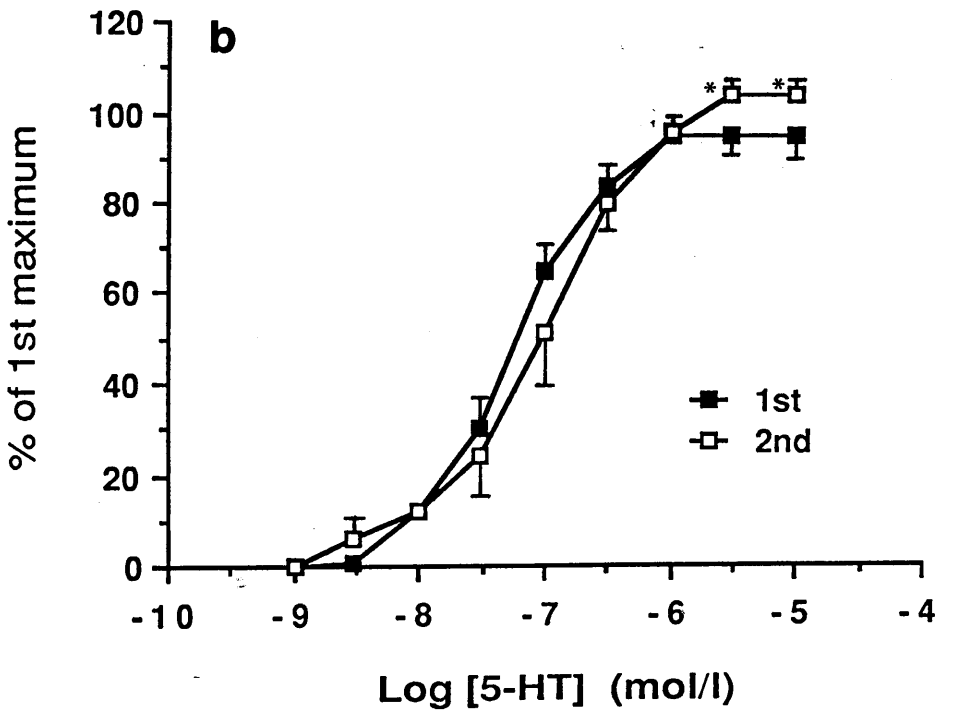

b
120
100
80
60
40
20
0
% of 1st maximum
1st
2nd
*
*
-10
-9
-8
-7
-6
-5
-4
Log [5-HT] (mol/l)

Table 5. Parameters of the Schild plot for the interaction of antagonists with the receptor for 5-HT in the isolated human umbilical artery at low Po_2 (~15mmHg).

	pA_2	Slope	n	Relevant figure
Antagonist				
Methysergide	8.52 (8.32–8.72)	0.94 (0.76–1.12)	6	5
Phentolamine	6.37 (5.88–6.86)	1.04 (0.82–1.26)	6	6
Ketanserin	8.92 (8.70–9.14)	0.91 (0.60–1.22)	6	7
Ketanserin (+1uM indomethacin)	8.94 (8.22–9.66)	0.99 (0.66–1.32)	4	8
Compound X	8.86 (8.54–9.18)	0.97 (0.63–1.31)	6	9
Cyproheptadine	Non-surmountable antagonist	—	6	10
LSD	Partial agonist	—	5	11

Values are the mean (95% confidence limits) of n estimates.

significantly shifted (P>0.05), either to the left or to the right. At higher concentrations of 5-HT (>1X10^{-7}M), the maximum response to 5-HT was significantly increased by 18±4% in the presence of indomethacin. However, paired control tissues not exposed to indomethacin, (figure 3b) showed a smaller but significant increase of the maximum of 4±3%. Indomethacin (1X10^{-6}M) caused no change of the resting baseline tension.

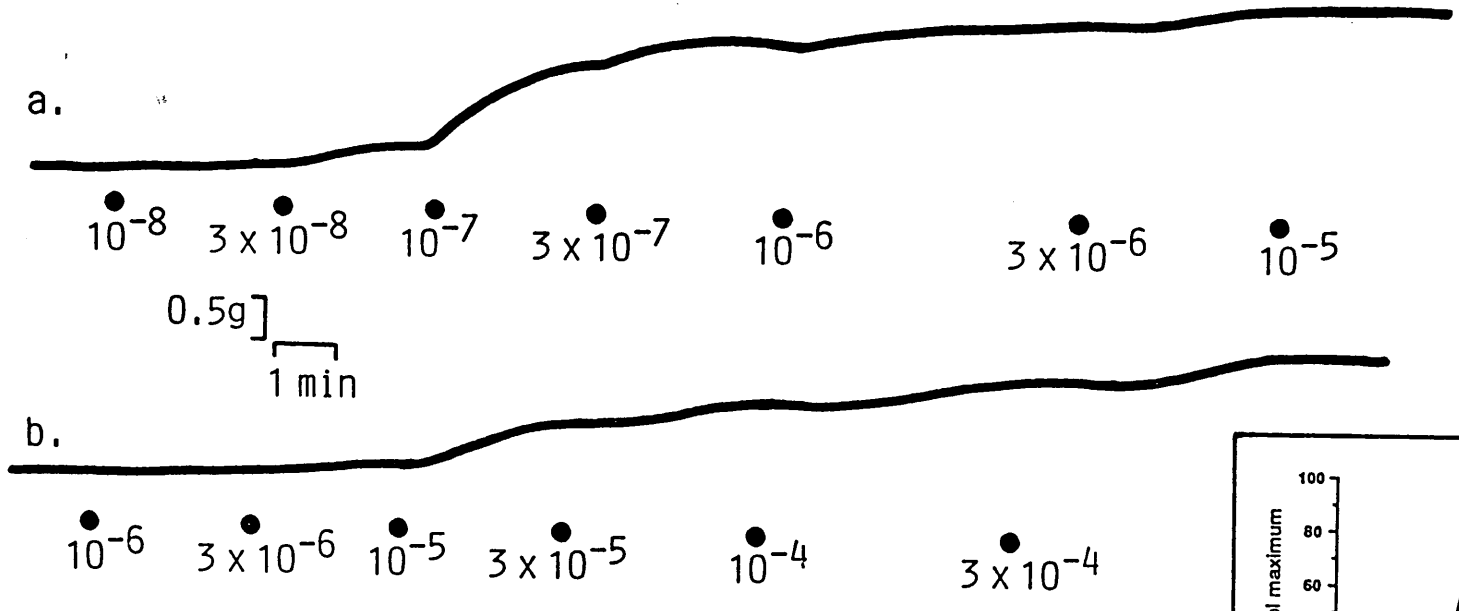
The results of the interaction of the antagonists methysergide, phentolamine, ketanserin, compound X, cyproheptadine and LSD are summarised in Table 5. All of these antagonists, with the exception of cyproheptadine and LSD (which were found to be a non-surmountable antagonist and a partial agonist respectively), were found to be competitive antagonists of the response to 5-HT in HUA at low Po_2.

Methysergide

Methysergide was a potent surmountable antagonist of 5-HT (figure 4). CRC's to 5-HT in the presence of methysergide (1X10^{-8}– 1X10^{-6}M) were parallel (figures 4 and 5a). The change in sensitivity to 5-HT with time, of the control preparations was 2.0±0.7 fold. The pA_2, and the slope of the regression line of the Schild plot (figure 5b), (which was not significantly different from 1) were 8.52 (mean) and 0.94 (mean) respectively.

Phentolamine

Phentolamine (1X10^{-6}–1X10^{-4}M) caused a parallel rightward displacement of the 5-HT CRC (figure 6a). The sensitivity of control preparations decreased by 1.8±0.5 fold with time. At the highest concentration of phentolamine (1X10^{-4}M) the displacement of the 5-HT CRC appeared visually to be greater at the higher concentrations of 5-HT than at the lower concentrations of 5-HT. However, estimates of pK_B



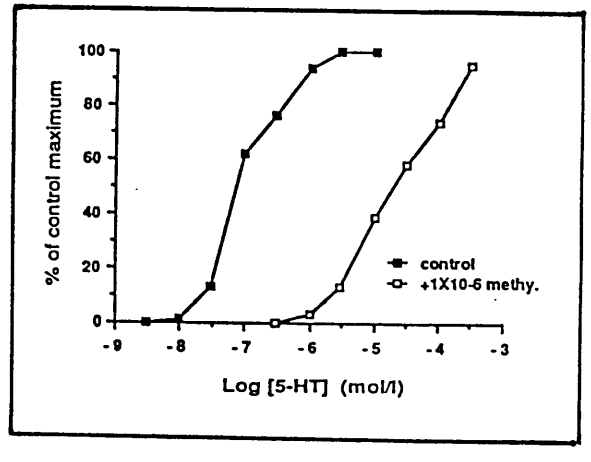


Figure 4 Tracings of concentration-response curves to 5-HT in a longitudinal strip of human umbilical artery at low Po_2 (~15mmHg). (a) control; (b) in the presence of $1X10^{-6}M$ methysergide. The insert shows the log(concentration)-response curves (CRC) to 5-HT in the presence and absence of methysergide, for this preparation. Methysergide caused a parallel shift to the right of the CRC to 5-HT without a reduction of the maximum response.

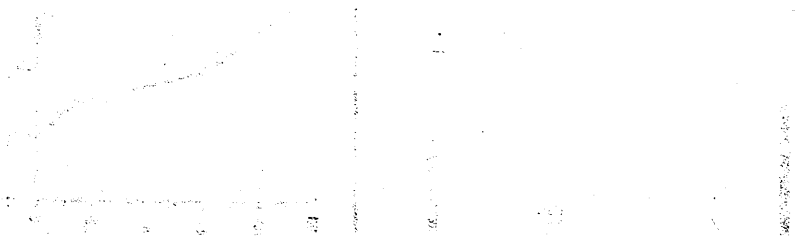
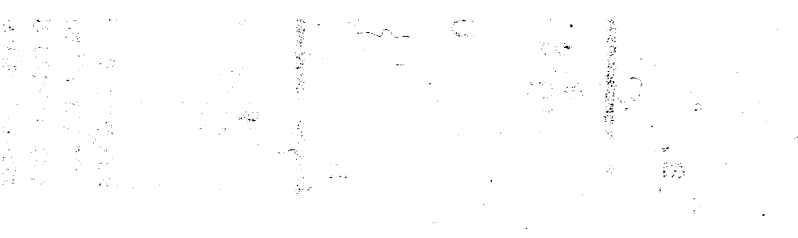
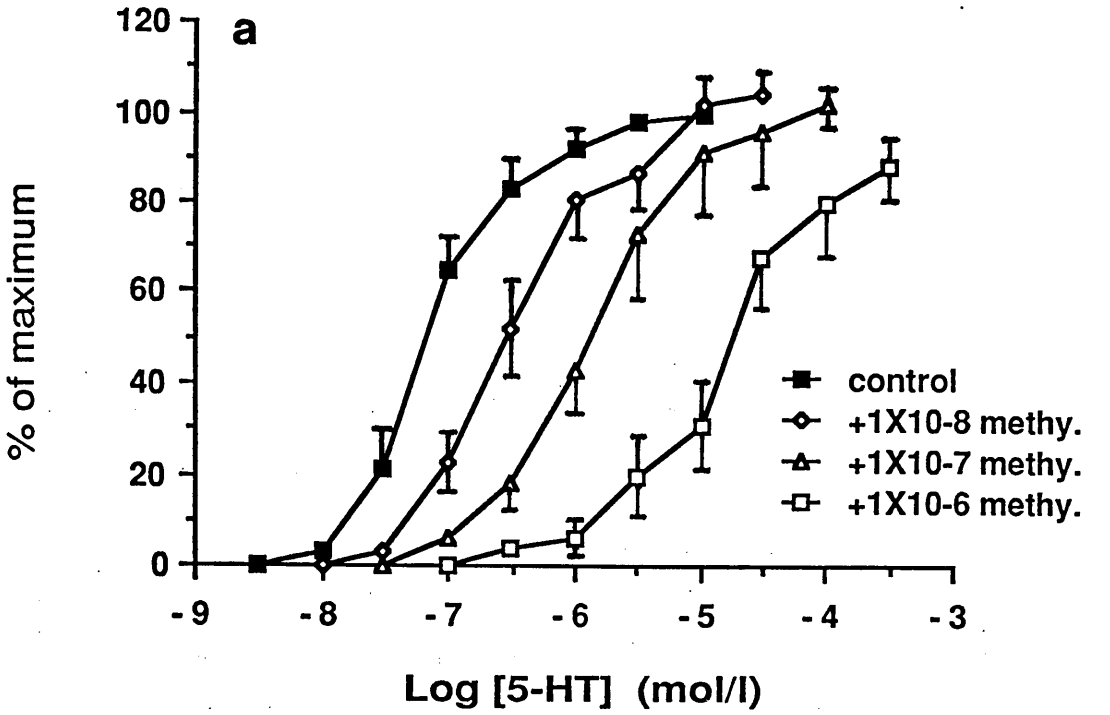
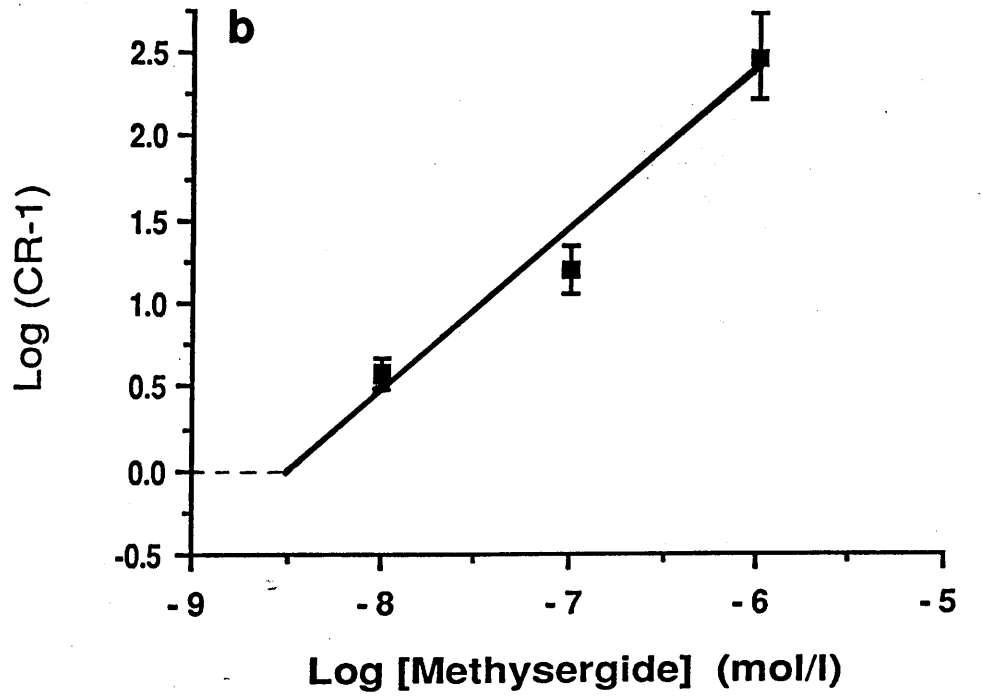


Figure 5 (a) Log concentration-response curves (CRC's) to 5-HT in the presence of methysergide (methy) at low Po_2 (11±2mmHg) in longitudinal strips of umbilical artery (n=6). CRC's to 5-HT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of methysergide. In the fourth (control) preparation the 1st (■) and second CRC's were constructed without antagonist in order to assess the change in sensitivity to 5-HT with time, which was 2.0±0.7 fold. Vertical bars are the mean±s.e.mean.

(b) Schild plot for the interaction of methysergide with 5-HT. For each preparation an individual Schild plot was constructed. This figure shows points representing the mean±s.e.mean at each concentration of methysergide and the line is based on the mean pA_2 (8.52) and mean slope (0.94) from the group of experiments. The pA_2 value and slope of individual plots were calculated by linear regression (least squares). The intercept of the linear regression line with the line, $\log(CR-1)=0$, (broken line) gave an estimate of the pA_2.




a
120
100
80
60
40
20
0
% of maximum
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)
control
+1X10-8 methy.
+1X10-7 methy.
+1X10-6 methy.


b
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Log (CR-1)
-9
-8
-7
-6
-5
Log [Methysergide] (mol/l)

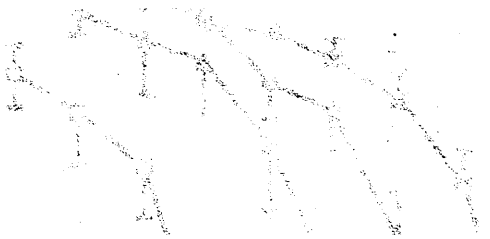
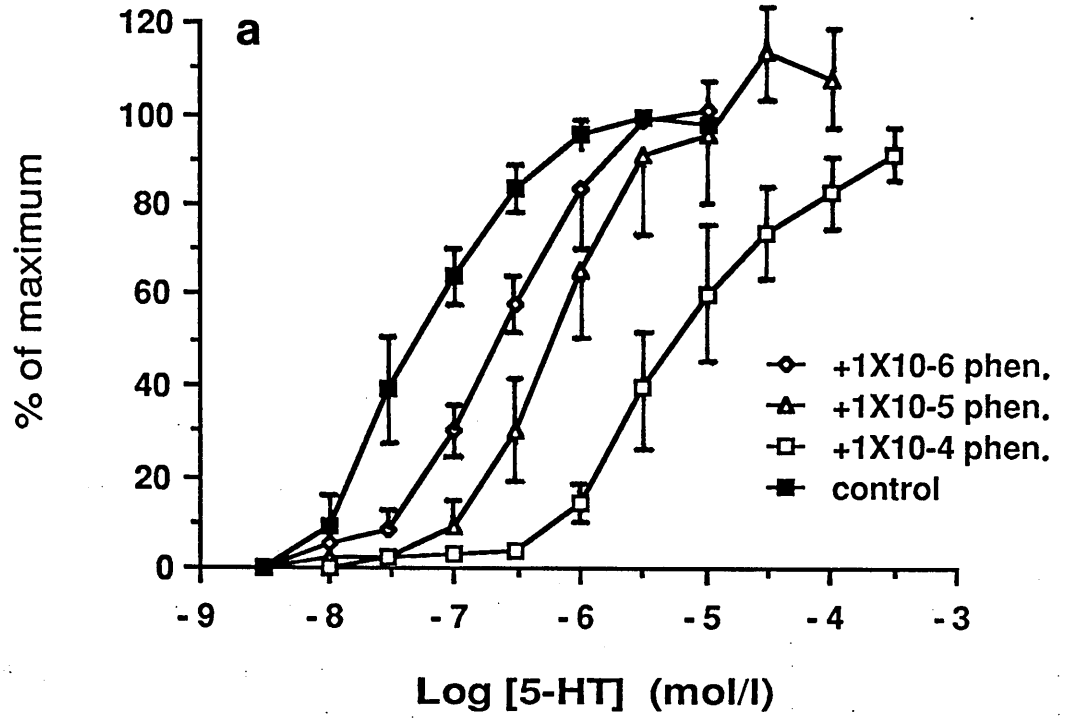
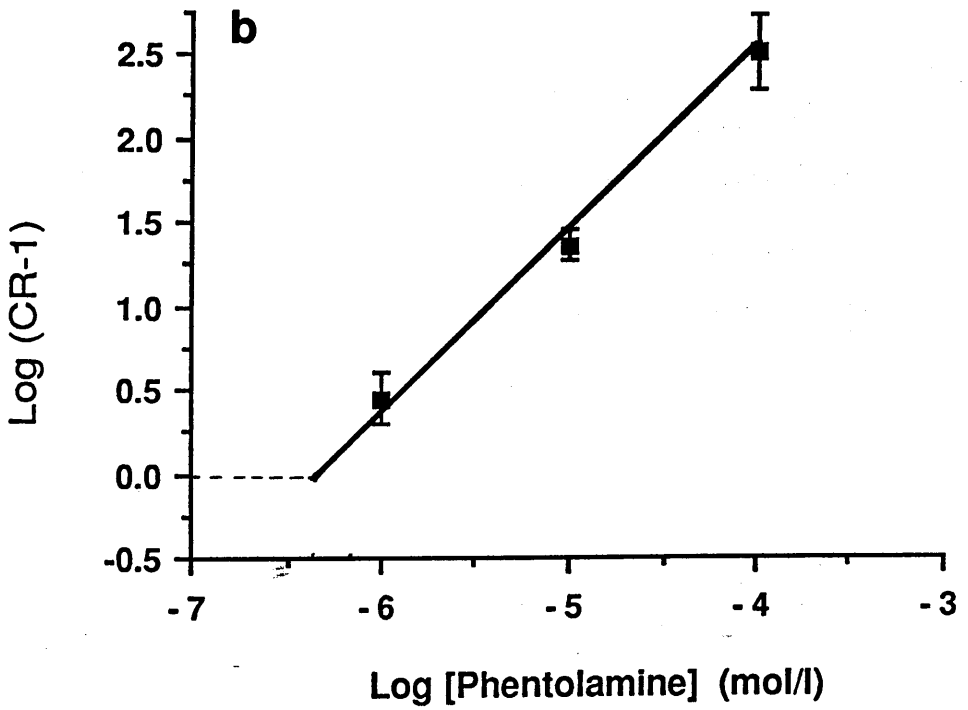


Figure 6 (a) Log concentration-response curves (CRC's) to 5-HT in the presence of phentolamine (phen) at low Po_2 (16±2mmHg) in longitudinal strips of umbilical artery (n=6). CRC's to 5-HT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of phentolamine. In the fourth (control) preparation the 1st (■) and second CRC's were constructed without antagonist in order to assess the change in sensitivity to 5-HT with time, which was 1.8±0.5 fold. Vertical bars are the mean±s.e.mean.

(b) Schild plot for the interaction of phentolamine with 5-HT. For each preparation an individual Schild plot was constructed. This figure shows points representing the mean±s.e.mean at each concentration of phentolamine and the line is based on the mean pA_2 (6.37) and mean slope (1.04) from the group of experiments. The pA_2 value and slope of individual plots were calculated by linear regression (least squares). The intercept of the linear regression line with the line, $\log(CR-1)=0$, (broken line) gave an estimate of the pA_2.


a
120
100
80
60
40
20
0
% of maximum
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)
+1X10-6 phen.
+1X10-5 phen.
+1X10-4 phen.
control


b
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Log (CR-1)
-7
-6
-5
-4
-3
Log [Phentolamine] (mol/l)

at the EC_{25} and EC_{75} (from the curves in the presence of $1X10^{-4}M$ phentolamine) were 6.41 (mean, 95% C.L.'s 5.77–7.05) and 6.50 (5.78–7.22) respectively, which were not significantly different and thus indicates that the shift in the presence of $1X10^{-4}M$ phentolamine was parallel. The pA_2, estimated from the Schild analysis (figure 6b) was 6.37 (mean), and the slope was 1.04 (not significantly different from 1). This shows that phentolamine is a weak but competitive antagonist of 5-HT in the HUA.

Ketanserin

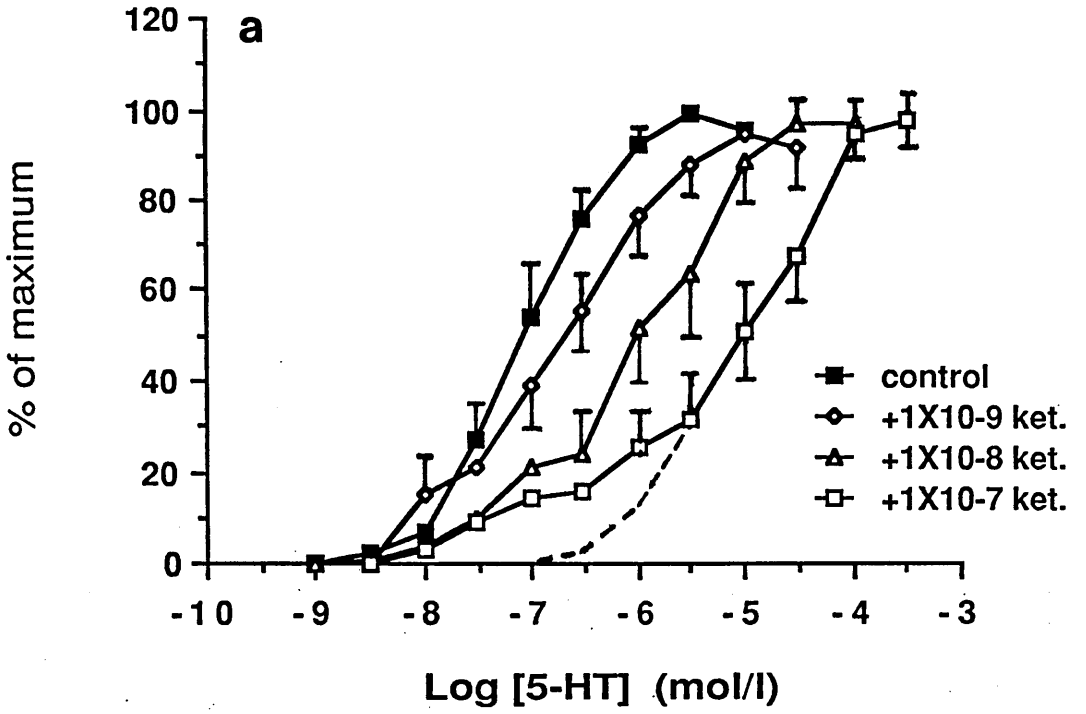
The antagonism of the response to 5-HT by ketanserin ($1X10^{-9}$–$1X10^{-7}M$) was investigated in the presence and absence of indomethacin ($1X10^{-6}M$), (figures 7 and 8 respectively).

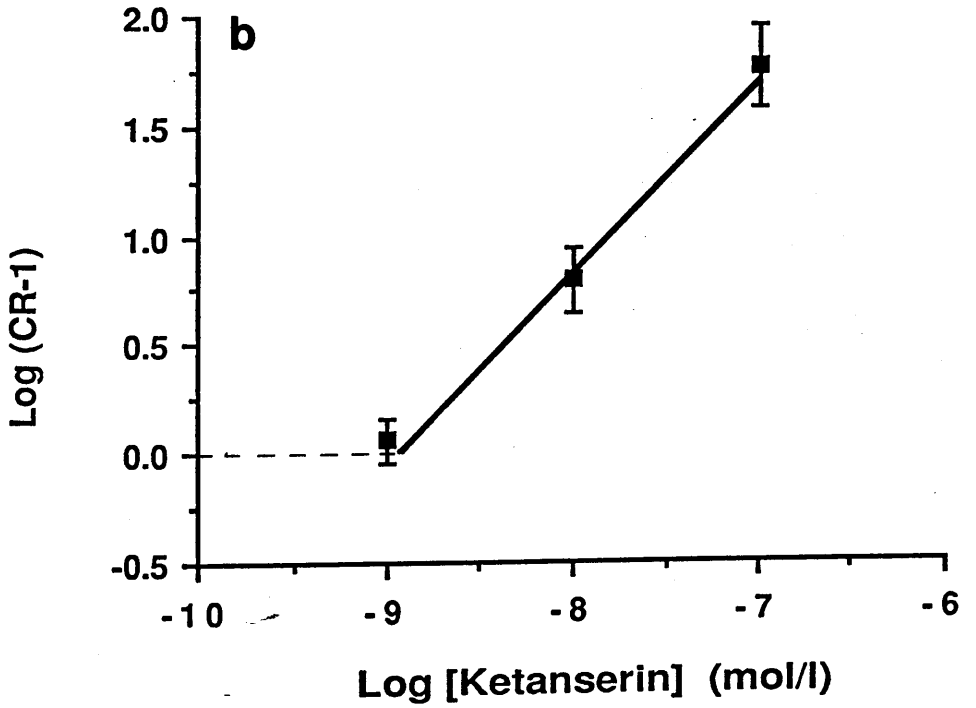
Without indomethacin present ketanserin caused a rightward shift of the 5-HT CRC which was parallel only at concentrations (of 5-HT) which produced greater than 30% of the maximum response (approximately), (figure 7a). i.e. at low Po_2 there was a small component of the response to 5-HT which was resistant to ketanserin. pK_B values were calculated from $1X10^{-7}M$ ketanserin. At the EC_{25} the pK_B (8.23, geometric mean, 95% C.L's 7.18–9.28) was smaller than at either the EC_{50} (8.77, 8.31–9.23) or EC_{75} (8.88, 8.30–9.46) but this was not significant. Nevertheless the Schild plot (figure 7b), which is based on concentration-ratios calculated at the EC_{50}, indicated that ketanserin was a competitive antagonist of 5-HT at low Po_2 as the slope of the regression line (0.91, mean) was not significantly different from 1. The estimated pA_2 for ketanserin was 8.92 (mean). Control preparations showed a decrease in sensitivity to 5-HT of 1.8 ± 0.3 fold with time.

In the presence of indomethacin ($1X10^{-6}M$), ketanserin displaced the 5-HT CRC in a parallel manner at all concentrations (figure 8a).

Figure 7 (a) Log concentration-response curves (CRC's) to 5-HT in the presence of ketanserin (ket) at low Po_2 (14±2mmHg) in longitudinal strips of umbilical artery (n=6). CRC's to 5-HT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of ketanserin. In the fourth (control) preparation the 1st (■) and second CRC's were constructed without antagonist in order to assess the change in sensitivity to 5-HT with time, which was 1.8±0.3 fold. The broken line is the CRC to 5-HT in the presence of $1X10^{-7}M$ ketanserin plus $1X10^{-6}M$ indomethacin (from figure 8a). Vertical bars are the mean±s.e.mean.

(b) Schild plot for the interaction of ketanserin with 5-HT. For each preparation an individual Schild plot was constructed. This figure shows points representing the mean±s.e.mean at each concentration of ketanserin and the line is based on the mean pA_2 (8.92) and mean slope (0.91) from the group of experiments. The pA_2 value and slope of individual plots were calculated by linear regression (least squares). The intercept of the linear regression line with the line, $\log(CR-1)=0$, (broken line) gave an estimate of the pA_2.


a
120
100
80
60
40
20
0
% of maximum
-10
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)
control
+1X10-9 ket.
+1X10-8 ket.
+1X10-7 ket.


b
2.0
1.5
1.0
0.5
0.0
-0.5
Log (CR-1)
-10
-9
-8
-7
-6
Log [Ketanserin] (mol/l)

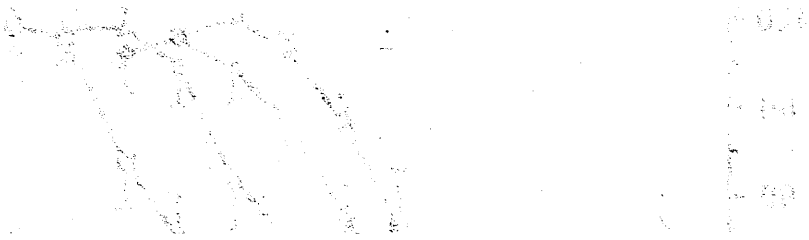
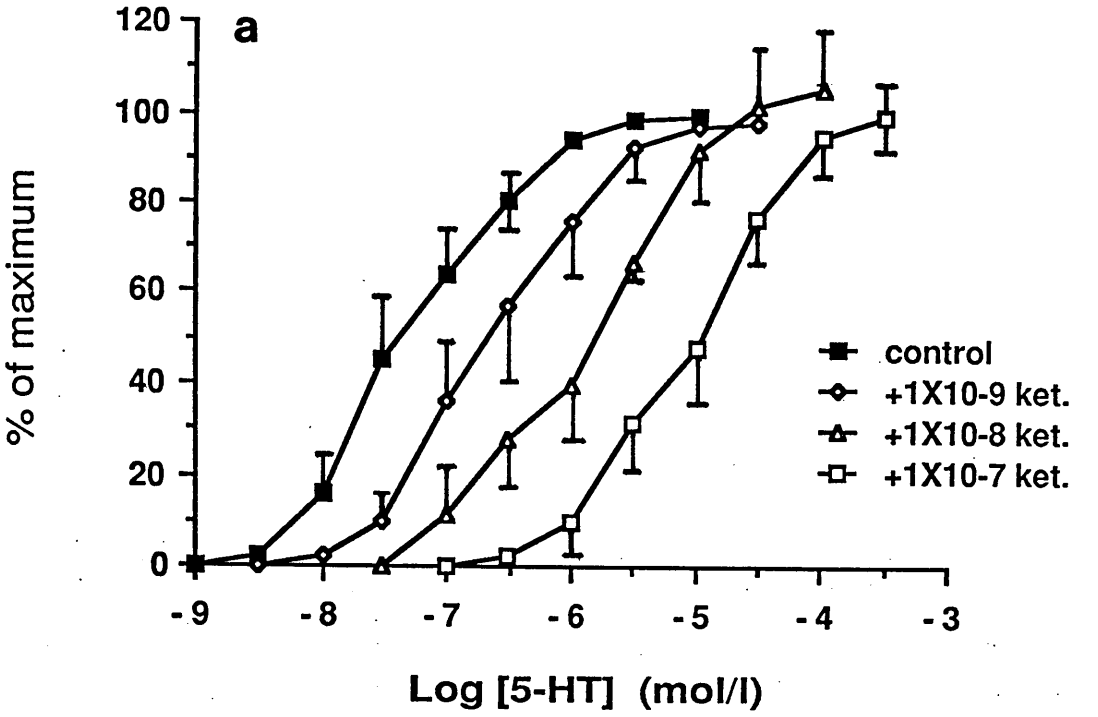
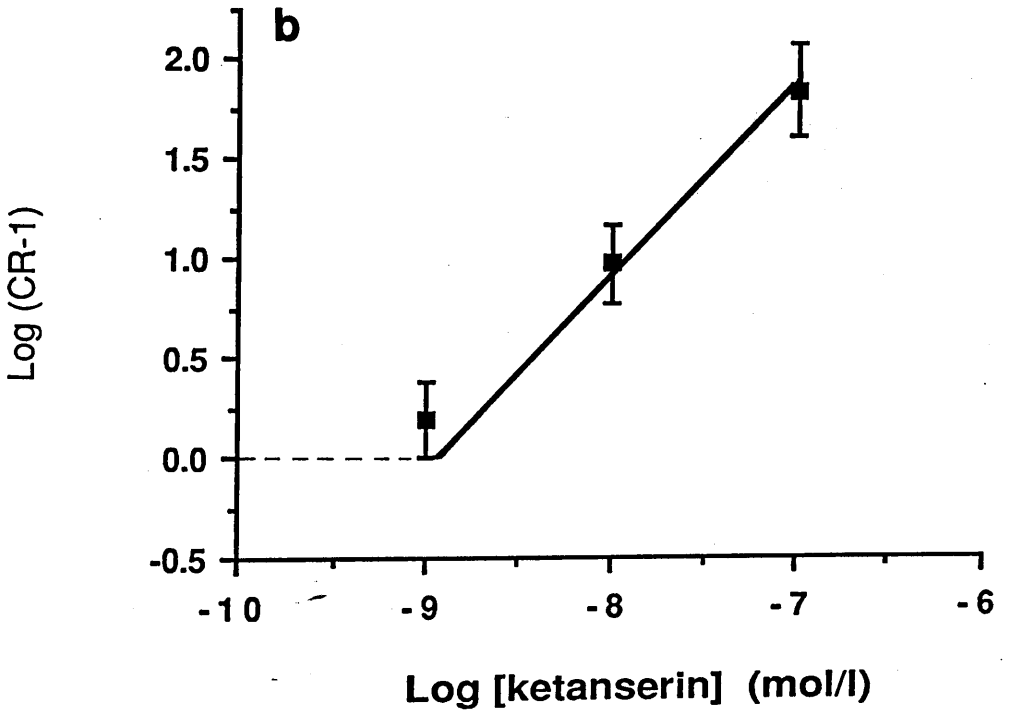


Figure 8 (a) Log concentration-response curves (CRC's) to 5-HT in the presence of ketanserin (ket) plus indomethacin ($1X10^{-6}M$) at low Po_2 (14 ± 2mmHg) in longitudinal strips of umbilical artery (n=4). CRC's to 5-HT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of ketanserin (plus indomethacin). In the fourth (control) preparation the 1st (■) and second CRC's were constructed without antagonist (but with indomethacin) in order to assess the change in sensitivity to 5-HT with time, which was 1.5 ± 0.5 fold. Vertical bars are the mean$\pm$s.e.mean.

(b) Schild plot for the interaction of ketanserin (plus indomethacin) with 5-HT. For each preparation an individual Schild plot was constructed. This figure shows points representing the mean$\pm$s.e.mean at each concentration of ketanserin and the line is based on the mean pA_2 (8.94) and mean slope (0.99) from the group of experiments. The pA_2 value and slope of individual plots were calculated by linear regression (least squares). The intercept of the linear regression line with the line, $\log(CR-1)=0$, (broken line) gave an estimate of the pA_2.


a
120
100
80
60
40
20
0
% of maximum
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)
control
+1X10-9 ket.
+1X10-8 ket.
+1X10-7 ket.


b
2.0
1.5
1.0
0.5
0.0
-0.5
Log (CR-1)
-10
-9
-8
-7
-6
Log [ketanserin] (mol/l)

The Schild plot (figure 8b) had a slope of 0.99 (mean) and the estimated pA_2 for ketanserin against 5-HT was 8.94 which was not significantly different from the pA_2 against 5-HT, without indomethacin present, which was 8.92. The broken line of figure 7a (which is the CRC to 5-HT in the presence of $1X10^{-7}M$ ketanserin plus $1X10^{-6}M$ indomethacin - from figure 8a) highlights the small component of the response to 5-HT which was resistant to $1X10^{-7}M$ ketanserin but which was sensitive to indomethacin.

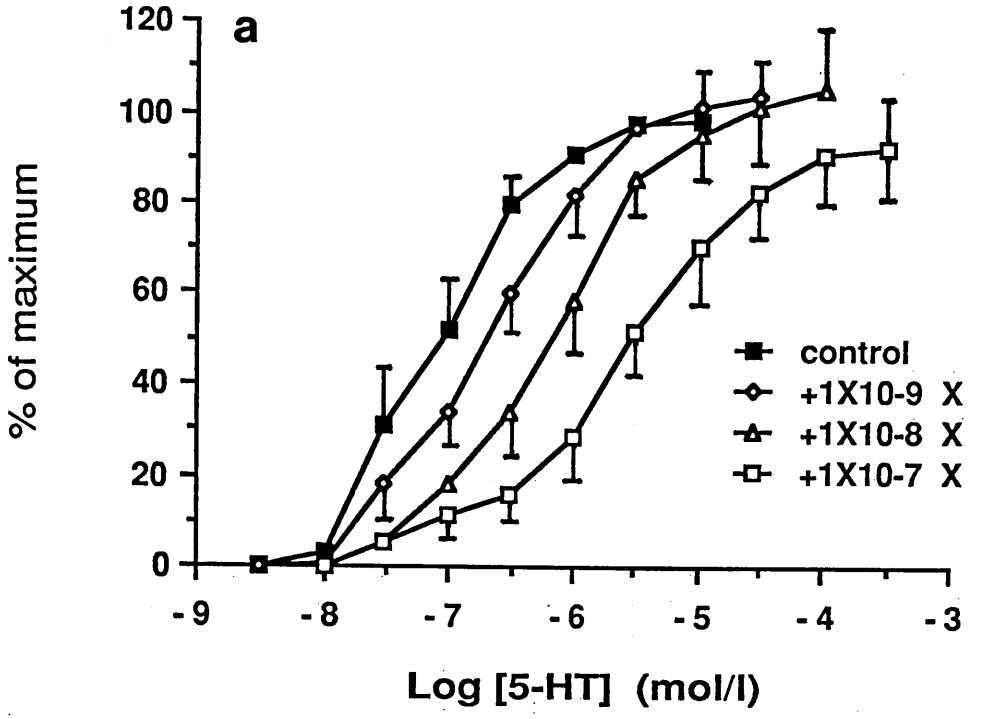
Compound X

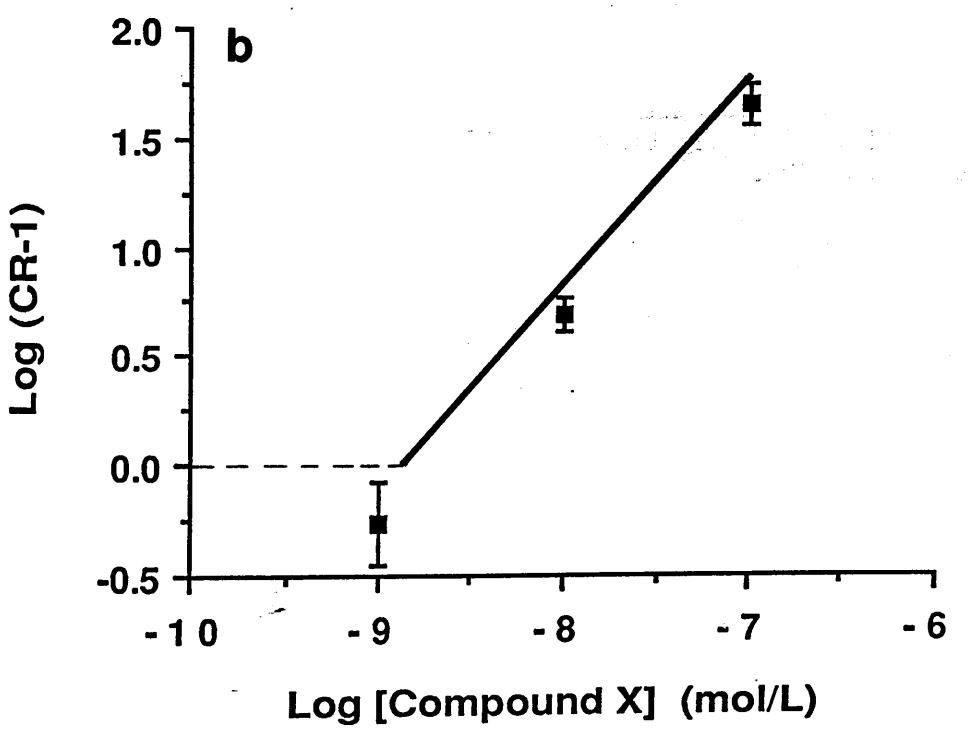
The antagonism of the response to 5-HT by compound X ($1X10^{-9}$–$1X10^{-7}M$) was, like that for ketanserin, slightly biphasic (figure 9a). The shift at lower concentrations was not parallel. pK_B values were calculated from $1X10^{-7}M$ compound X. At the EC_{25} the pK_B (8.29, mean, 95% C.L's 8.12–8.46) was significantly smaller than at the EC_{50} (8.68, 8.47–8.89) or at the EC_{75} (8.79, 8.29–9.29). At concentrations of 5-HT producing greater than 30% of the maximum response the curves in the presence of compound X were parallel. From the Schild plot the pA_2 was estimated to be 8.86 (mean) and the slope (which was not significantly different from 1) was 0.97 (mean), (figure 9b).

Cyproheptadine

Cyproheptadine was found to be a non-competitive antagonist of 5-HT in HUA. At all concentrations ($1X10^{-9}$–$1X10^{-7}M$) it caused a rightward shift of the 5-HT CRC but which was non-surmountable (figure 10a). At $1X10^{-9}M$ cyproheptadine the maximum response was reduced by 18±13%. This was not significant. The pK_B calculated from $1X10^{-9}M$ was 8.86 (mean, 95% C.L.'s 7.72–10.0). At concentrations greater than $1X10^{-9}M$ the maximum response was significantly reduced and at $1X10^{-7}M$ this was 49±7% of the control maximum response. In individual preparations the reduction in the maximum response was concentration-related -a plot of

Figure 9 (a) Log concentration-response curves (CRC's) to 5-HT in the presence of compound X (X) at low Po_2 (13±4mmHg) in longitudinal strips of umbilical artery (n=6). CRC's to 5-HT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of compound X. In the fourth (control) preparation the 1st (■) and second CRC's were constructed without antagonist in order to assess the change in sensitivity to 5-HT with time, which was 1.8±0.5 fold. Vertical bars are the mean±s.e.mean.
(b) Schild plot for the interaction of compound X with 5-HT. For each preparation an individual Schild plot was constructed. This figure shows points representing the mean±s.e.mean at each concentration of ketanserin and the line is based on the mean pA_2 (8.86) and mean slope (0.97) from the group of experiments. The pA_2 value and slope of individual plots were calculated by linear regression (least squares). The intercept of the linear regression line with the line, $\log(CR-1)=0$, (broken line) gave an estimate of the pA_2.


a
120
100
80
60
40
20
0
% of maximum
control
+1X10-9 X
+1X10-8 X
+1X10-7 X
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)


b
2.0
1.5
1.0
0.5
0.0
-0.5
Log (CR-1)
-10
-9
-8
-7
-6
Log [Compound X] (mol/L)

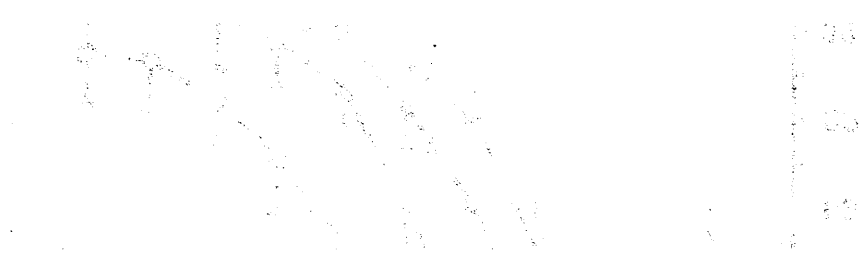
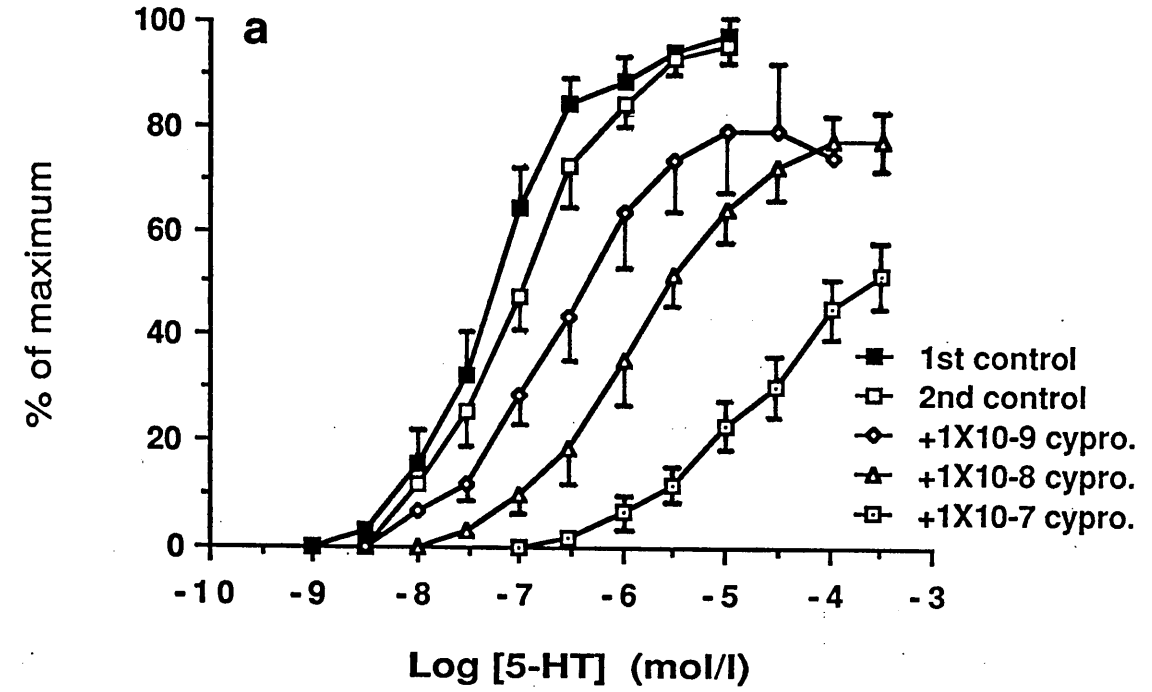
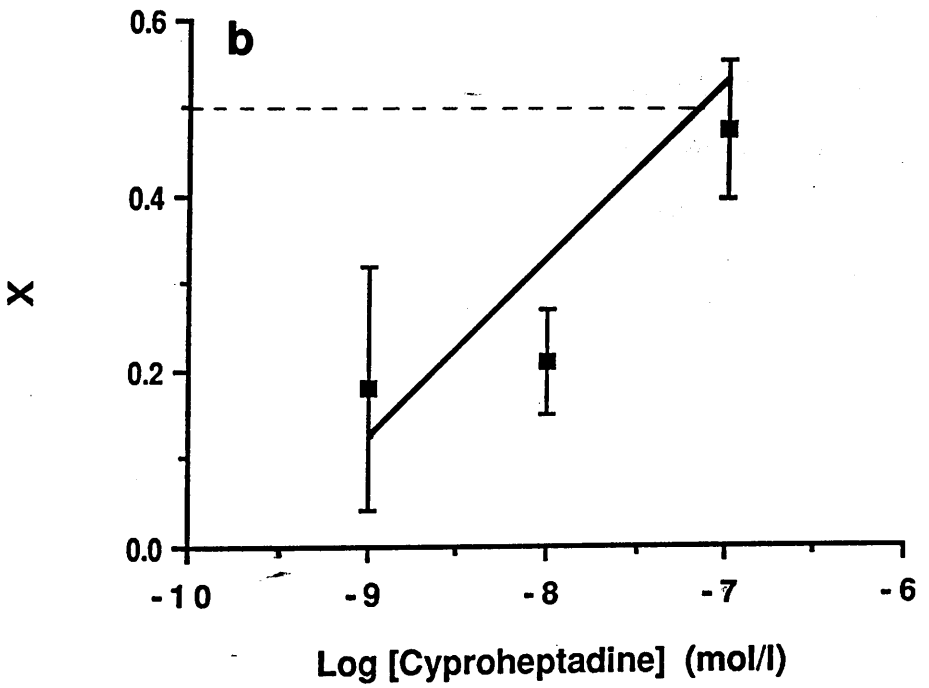


Figure 10 (a) Log concentration-response curves (CRC's) to 5-HT in the presence of cyproheptadine (cypro) at low Po_2 (15±2mmHg) in longitudinal strips of umbilical artery (n=6). CRC's to 5-HT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of cyproheptadine. In the fourth (control) preparation the 1st (■) and second (□) CRC's were constructed without antagonist. Vertical bars are the mean±s.e.mean.
(b) This figure shows the reduction (X) of the maximum response to 5-HT at each concentration of cyproheptadine and the line is based on the mean pD'_2 (7.19) and slope of the individual plots. The pD'_2 value of individual plots was calculated by linear regression (least squares). The intercept of the linear regression line with the line, X=0.5, (broken line) gave an estimate of the pD'_2.


a
100
80
60
40
20
0
% of maximum
-10
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)
1st control
2nd control
+1X10-9 cypro.
+1X10-8 cypro.
+1X10-7 cypro.


b
0.6
0.4
0.2
0.0
x
-10
-9
-8
-7
-6
Log [Cyproheptadine] (mol/l)

X (the reduction in the maximum response) against cyproheptadine concentration (figure 10b) yielded a significant correlation coefficient of 0.85. The estimated pD'_2 was 7.19 (mean, 95% C.L.'s 5.65–8.73).

LSD

LSD, added as single concentrations of $1X10^{-9}M$, $1X10^{-8}M$ and $1X10^{-7}M$, to study the antagonism of the response to 5-HT, contracted the HUA but this was not concentration-related (figure 11a). The antagonism caused by LSD was non-surmountable and the reduction of the maximum response to 5-HT was not concentration-related: at $1X10^{-7}M$ the maximum reduction was 75±5% and at $1X10^{-9}M$ was 68±6%, which was not significantly smaller. In the presence of LSD the response to 5-HT was rather rhythmic, and contractions were not well maintained. In a separate group of tissues LSD ($1X10^{-12}$–$1X10^{-10}M$), had no contractile action and only the highest concentration antagonised the response to 5-HT. This was characterized by a significant reduction of the maximum response (36±13%) and a significant rightward shift of 9.1 fold (geometric mean, 95% C.L.'s 1.3–61.6), assessed at the EC_{30}. $1X10^{-11}M$ and $1X10^{-12}M$ LSD did not significantly shift the CRC or reduce the maximum response (figure 11b). (In other experiments (figure 30), where LSD was added cumulatively, $1X10^{-10}M$ was found to have a small contractile action).

b) High Po_2 (~120mmHg)

The effect, *per se*, of increasing the Po_2 from low to high Po_2, on the response to 5-HT was investigated. CRC's to 5-HT were constructed at Po_2=8±3mmHg (mean±s.e.mean). Increasing the Po_2 to 117±3mmHg caused a significant leftward shift of 4.3 fold (mean, 95% C.L.'s, 0.6–8.0), calculated at the EC_{50} (figure 12a). In paired preparations incubated

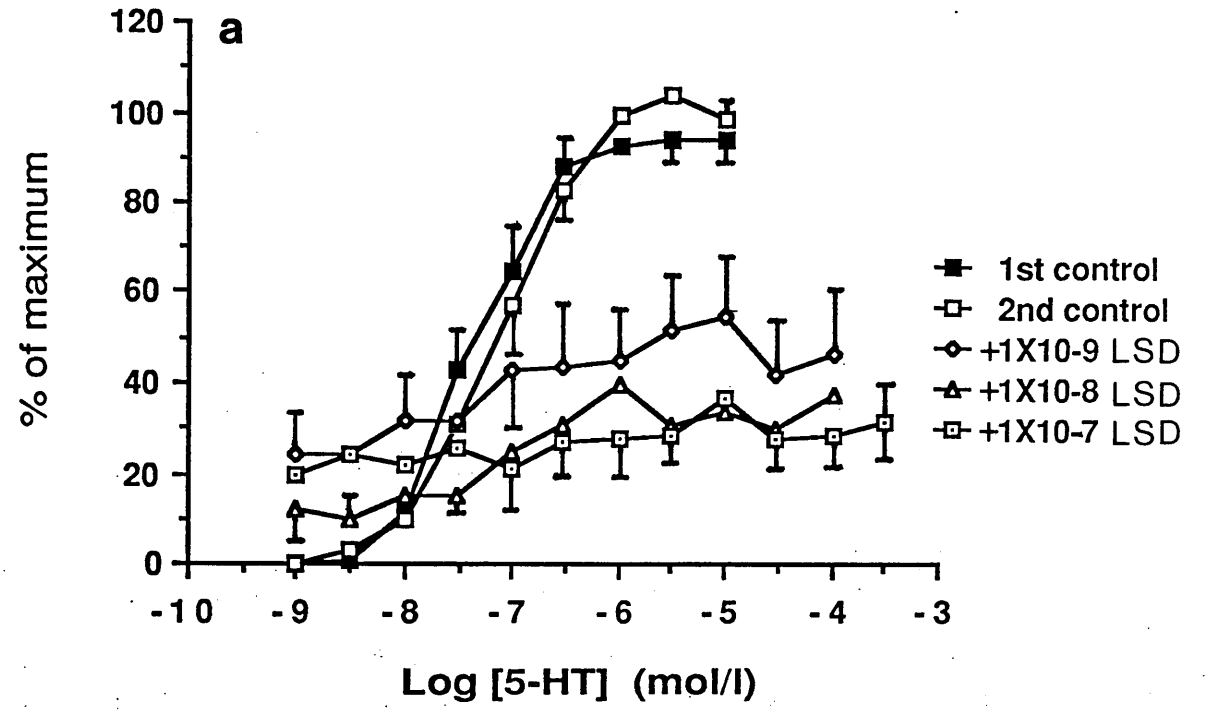
with indomethacin ($1X10^{-6}M$), increasing the Po_2 did not significantly increase (or decrease) the potency of 5-HT (figure 12b). In the absence of indomethacin increasing the Po_2 contracted the HUA. This contraction was 35±10% of the maximum contraction to 5-HT but was not maintained and the change in baseline tension before constructing a 2nd CRC was +4±3% (of the resting tension before increasing the Po_2), which was not significant. Raising the Po_2 failed to induce a contraction in the presence of indomethacin.

pK_B values of antagonists were calculated at the EC_{25} and EC_{75} from CRC's in the presence and absence of the antagonist. These gave estimates of the potency of the antagonists against the two components of the response to 5-HT at high Po_2 which emerged during the analysis.

Ketanserin

At high Po_2, ketanserin ($1X10^{-9}$–$1X10^{-7}M$) caused a distinctly biphasic antagonism of the response to 5-HT. An inflection appeared in the 5-HT CRC after responses reached ~50% of the maximum (figure 13a). $1X10^{-9}M$ and $1X10^{-8}M$ ketanserin did not shift the CRC at lower concentrations of 5-HT while $1X10^{-7}M$ caused a small rightward shift: the pK_B for ketanserin against this phase of the response (calculated at the EC_{25}) was 7.70 (geometric mean, 95% C.L.'s 7.11–8.29). The shifts at higher concentrations were parallel. A Schild plot was constructed from concentration-ratios calculated at the EC_{75}: the slope for this was 1.03 (mean, 95% C.L.'s 0.41–1.65) and the estimated pA_2 was 8.91 (7.88–9.94), (figure 13b). The concentration-ratios at the EC_{25} and EC_{75}, calculated from the curve in the presence of $1X10^{-7}M$ ketanserin, show that ketanserin is 33 fold (geometric mean, 95% C.L's 4.6–240) more potent against 5-HT at the level of the EC_{25} than at the EC_{75}. Thus, at low concentrations of 5-HT there is a "ketanserin-resistant" 1st phase and at higher concentrations there is a

Figure 11 Log concentration-response curves (CRC's) to 5-HT in the presence of lysergic acid diethylamide (LSD) at low Po_2 (15±2mmHg) in longitudinal strips of umbilical artery (n=5). (a) [LSD]=$1x10^{-9}$–$1X10^{-7}$M, which caused contraction; (b) [LSD]=$1X10^{-12}$–$1X10^{-10}$M, which did not cause contraction. CRC's to 5-HT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of LSD. In the fourth (control) preparation the 1st (■) and second (□) CRC's were constructed without antagonist. Vertical bars are the mean±s.e.mean.


a
120
100
80
60
40
20
0
% of maximum
-10
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)
1st control
2nd control
+1X10-9 LSD
+1X10-8 LSD
+1X10-7 LSD

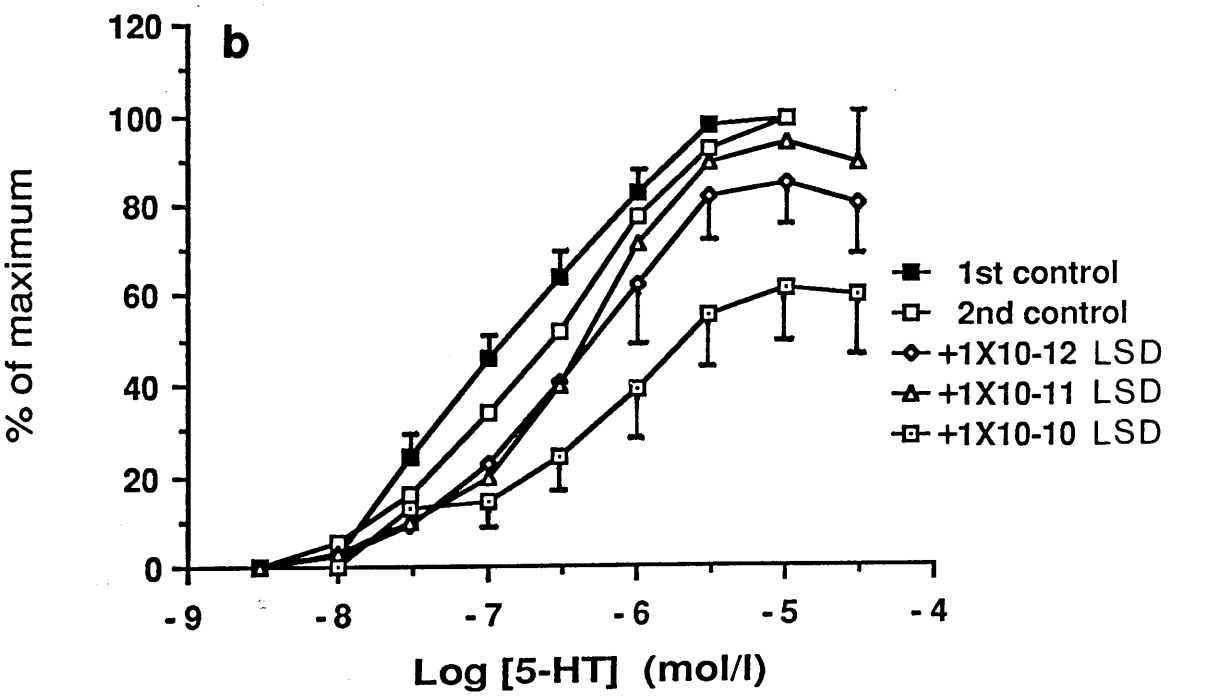
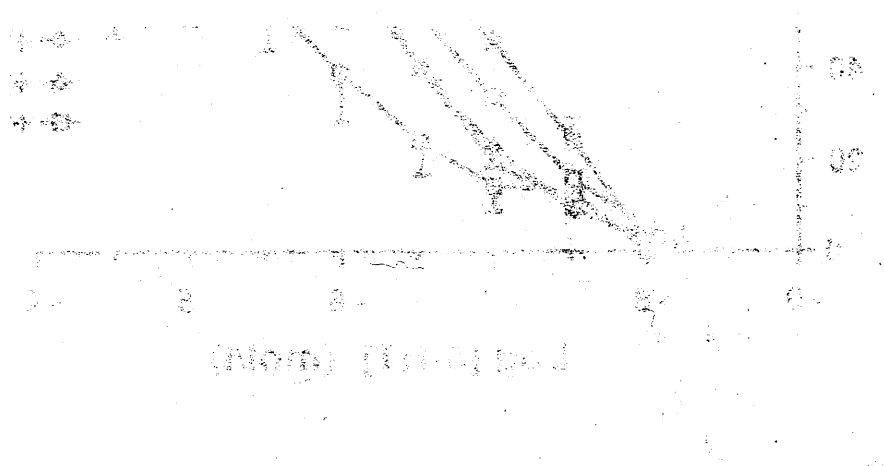
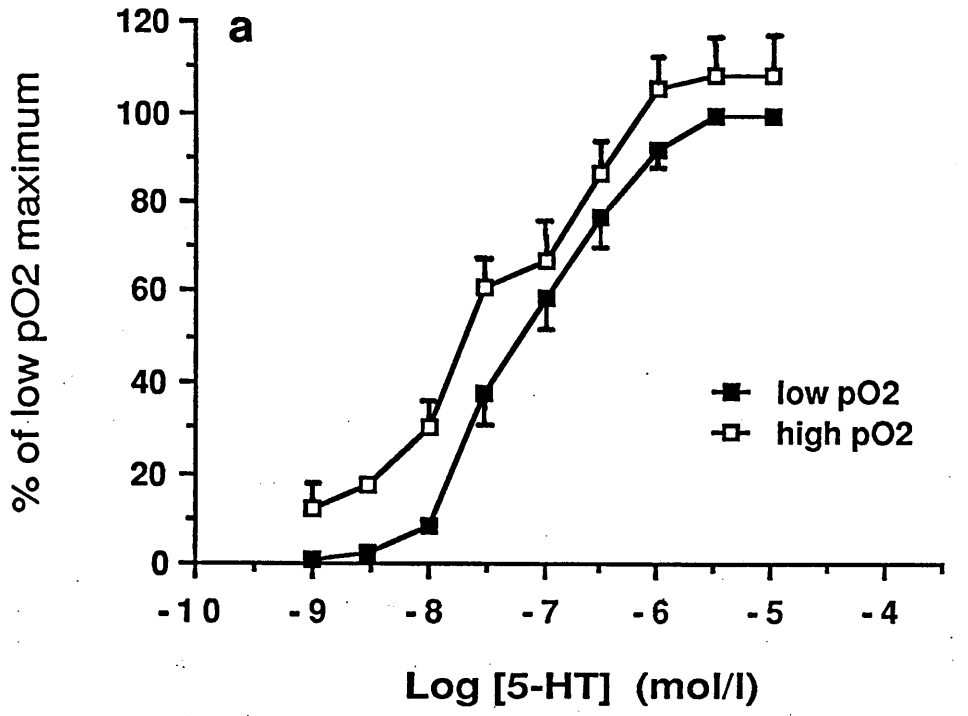

b
120
100
80
60
40
20
0
% of maximum
-9
-8
-7
-6
-5
-4
Log [5-HT] (mol/l)
1st control
2nd control
+1X10-12 LSD
+1X10-11 LSD
+1X10-10 LSD

Figure 12 Log concentration-response curves (CRC's) to 5-HT in longitudinal strips of umbilical artery (n=7), at low Po_2 (8±3mmHg), and at high Po_2 (118±3mmHg) in the presence and absence of indomethacin ($1X10^{-6}M$). (a) The protocol was to construct a CRC to 5-HT at low Po_2. Following washout the Po_2 was increased and a 2nd CRC constructed. (b) Protocol as for (a) except that indomethacin (indo), was added 30mins before increasing the Po_2. Response (ordinate) is expressed as a % of the maximum response to 5-HT at low Po_2. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.




a
120
100
80
60
40
20
0
% of low pO2 maximum
low pO2
high pO2
-10
-9
-8
-7
-6
-5
-4
Log [5-HT] (mol/l)

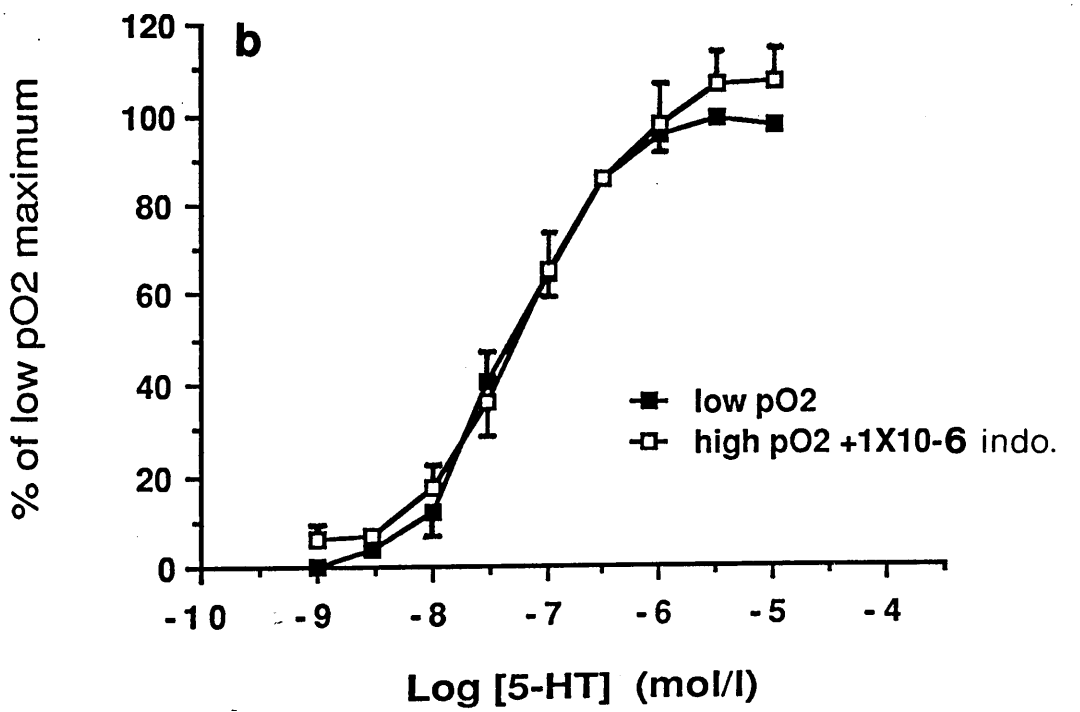
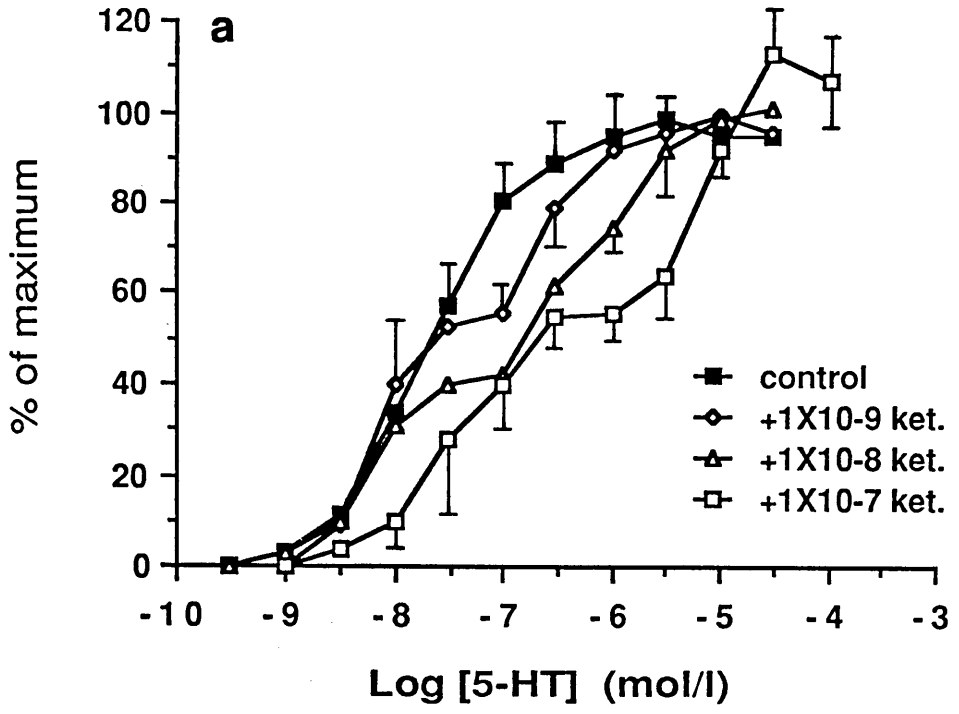

b
120
100
80
60
40
20
0
% of low pO2 maximum
low pO2
high pO2 +1X10-6 indo.
-10
-9
-8
-7
-6
-5
-4
Log [5-HT] (mol/l)

Figure 13 (a) Log concentration-response curves (CRC's) to 5-HT in the presence of ketanserin (ket) at high Po_2 (123±2mmHg) in longitudinal strips of umbilical artery (n=5). CRC's to 5-HT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of ketanserin. In the fourth (control) preparation the 1st (■) and second CRC's were constructed without antagonist. For clarity, error bars (mean±s.e.mean) are omitted at some points.
(b) Schild plot for the interaction of ketanserin with 5-HT at high Po_2. For each preparation an individual Schild plot was constructed from concentration-ratios calculated at the EC_{75}. (All other Schild plots were constructed from concentration-ratios calculated at the EC_{50}). This figure shows points representing the mean±s.e.mean at each concentration of ketanserin and the line is based on the mean pA_2 (9.12) and mean slope (1.06) from the group of experiments. The pA_2 value and slope of individual plots were calculated by linear regression (least squares). The intercept of the linear regression line with the line, log(CR-1)=0, (broken line) gave an estimate of the pA_2.


a
120
100
80
60
40
20
0
% of maximum
control
+1X10-9 ket.
+1X10-8 ket.
+1X10-7 ket.
-10
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)

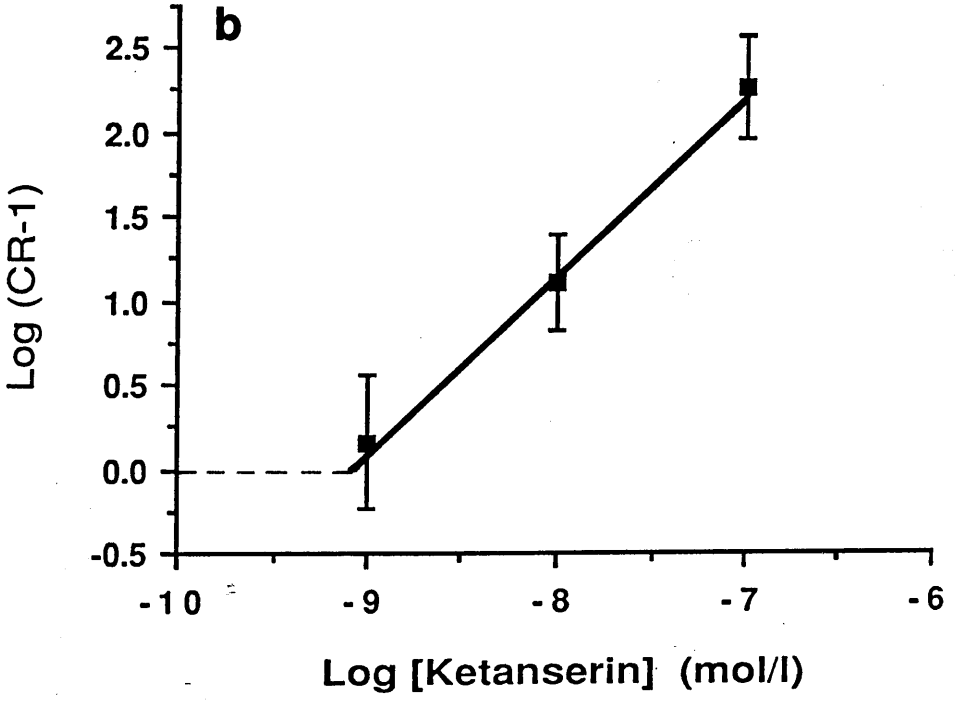

b
2.5
2.0
1.5
1.0
0.5
0.0
-0.5
Log (CR-1)
-10
-9
-8
-7
-6
Log [Ketanserin] (mol/l)

Table 6 Estimates of pK_B (the -logarithm of the dissociation constant, K_B) for ketanserin at high Po_2 (~120mmHg) calculated at the EC_{25} and EC_{75}: a) with and b) without cyclo-oxygenase inhibitor (COI) present.

	a) pK_B (+COI)			b) pK_B (-COI)		
	COI	EC_{25}	EC_{75}	EC_{25}	EC_{75}	n
[Ketanserin]						
$1X10^{-8}$	Indomethacin ($1X10^{-6}M$)	8.64 (8.33–8.95)	9.28 (8.68–9.88)	8.20* (7.84–8.56)	8.50 (8.03–8.97)	5
$1X10^{-7}$	Indomethacin ($1X10^{-6}M$)	9.12 (8.80–9.44)	8.97 (8.73–9.21)	7.40* (6.84–7.96)	8.66 (8.20–9.12)	8
$1X10^{-7}$	Flurbiprofen ($3X10^{-7}M$)	8.95 (8.57–9.33)	9.21 (8.72–9.70)	7.51* (6.14–8.88)	8.57 (7.30–9.84)	4
$3X10^{-7}$	Indomethacin ($1X10^{-7}M$)	8.81 (8.43–9.19)	9.12 (8.45–9.79)	8.23 (7.40–9.06)	8.56 (8.07–9.05)	8

pK_B values are the mean (95% confidence limits) of n estimates. $pK_B = \log(CR-1) - \log B$. B is the concentration of antagonist. The concentration-ratio (CR) is the shift of the CRC to 5-HT in the presence of the antagonist (see methods section for details on the calculation).
a) In each estimate of the pK_B there was an individual control curve to 5-HT. This was the CRC to 5-HT at low Po_2. The concentration-ratios at the EC_{25} and EC_{75} for each experiment were calculated with reference to its own control curve.
b) In each estimate of the pK_B the control curve to 5-HT was from a separate experiment. This was the CRC to 5-HT at high Po_2, from figure 12a. The concentration-ratios at the EC_{25} and EC_{75} for each experiment were calculated with reference to the geometric mean value of the EC_{25} and EC_{75} of this control curve.
* Values which are significantly different from the pA_2 for ketanserin (8.92) against 5-HT at low Po_2.

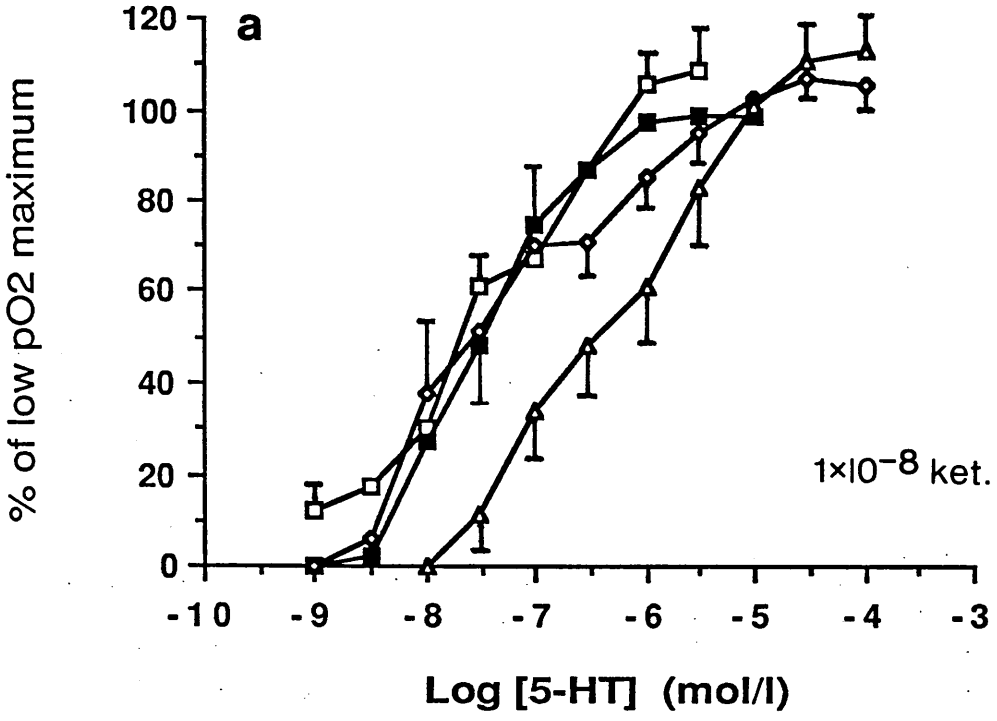
"ketanserin-sensitive" 2nd phase within the 5-HT CRC.

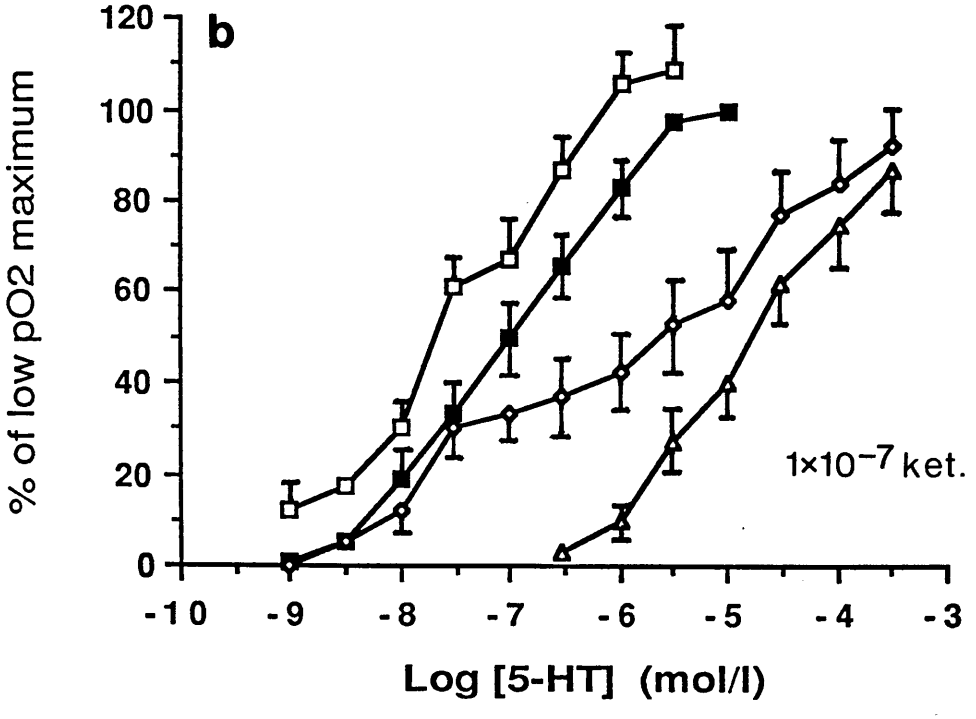
The CRC to 5-HT at high Po_2 was further shown to be comprised of two components in the presence of other antagonists which were examined in this study: lower concentrations of 5-HT were relatively resistant to antagonist blockade (the antagonist resistant (AR) component), while higher concentrations of 5-HT were relatively more susceptible to blockade (the antagonist sensitive (AS) component).

The antagonism by ketanserin, with and without indomethacin ($1X10^{-6}M$) present, was investigated at high Po_2 by using single concentrations of the antagonist in different preparations. pK_B values were calculated at the EC_{25} and EC_{75} which were chosen as parameters of the AR and AS components of the response to 5-HT respectively. Concentration-ratios were calculated in the presence and absence of indomethacin with reference to the following control CRC's: without indomethacin present the control curve was that for 5-HT at high Po_2 (non-paired tissues, from figure 12a); with indomethacin present the control curve was that to 5-HT at low Po_2 (same tissue), the rationale for this was that CRC's to 5-HT at low Po_2 and at high Po_2 plus indomethacin were not significantly different (figure 12b).

Ketanserin, $1x10^{-8}M$, $1X10^{-7}M$ and $3X10^{-7}M$ caused a biphasic antagonism at high Po_2 (figures 14a, b and c respectively). At the highest concentration ($3X10^{-7}M$) the antagonism was not so clearly biphasic - a ketanserin resistant component was less evident than at lower ketanserin concentrations, but still present as can be seen by comparison with the curve in the presence of indomethacin. In the presence of $1X10^{-6}M$ indomethacin the shifts were parallel i.e. the "ketanserin-resistant" component was "indomethacin-sensitive". The pK_B values calculated from these concentrations of ketanserin are given in Table 6.

At each concentration of ketanserin, the pK_B values, calculated at


a
120
100
80
60
40
20
0
% of low pO2 maximum
-10
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)
1×10−8 ket.


b
120
100
80
60
40
20
0
% of low pO2 maximum
-10
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)
1×10−7 ket.

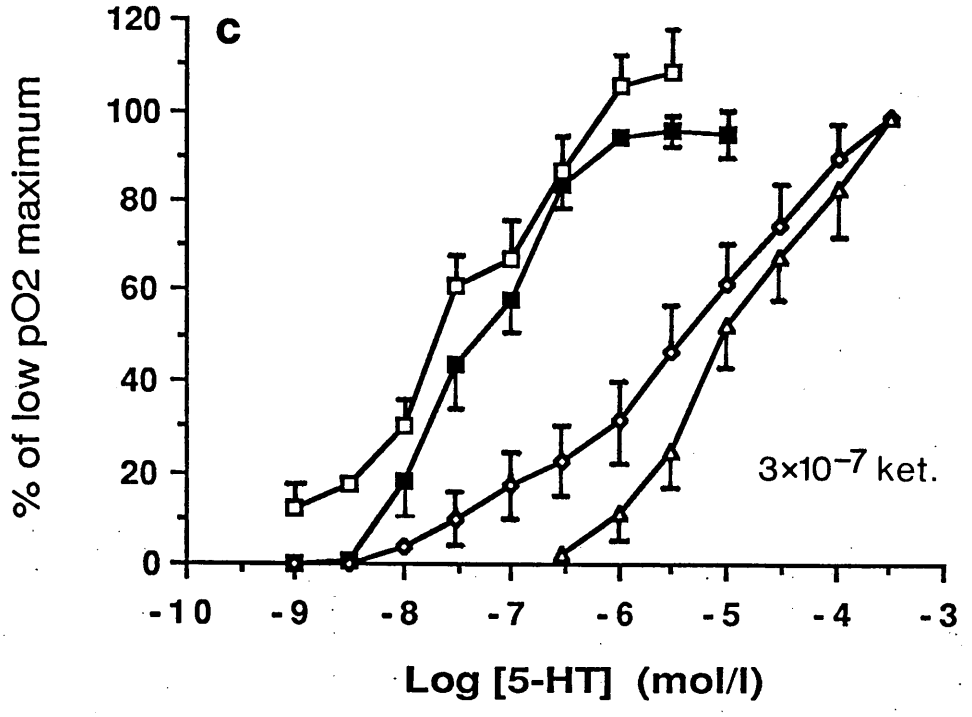


Figure 14 Log concentration-response curves (CRC) to 5-HT in longitudinal strips of umbilical artery in the presence of ketanserin at high Po_2. In each experiment (figures a, b and c) the protocol was to first construct a CRC to 5-HT at low Po_2 (~15mmHg), (■). Following washout the Po_2 was then increased (to ~120mmHg), ketanserin was added and a CRC to 5-HT repeated (◇). A third CRC to 5-HT was then constructed in the presence of ketanserin plus indomethacin ($1X10^{-6}M$) at the high Po_2 (△). A control CRC to 5-HT at high Po_2 (□), (from non-paired experiments, figure 12) is shown in each figure. Responses were calculated as a % of the maximum response to 5-HT at low Po_2. The difference between figures a-c is the concentration of ketanserin:

(a) [ketanserin]=$1X10^{-8}M$; low Po_2=11±2mmHg; high Po_2=121±3mmHg; n=5.
(b) [ketanserin]=$1X10^{-7}M$; low Po_2=13±2mmHg; high Po_2=117±1mmHg; n=8.
(c) [ketanserin]=$3X10^{-7}M$; low Po_2=14±2mmHg; high Po_2=114±3mmHg; n=8.
For clarity, error bars (mean±s.e.mean) are omitted at some points.

the EC_{25} and EC_{75} in the presence of indomethacin, were not significantly different ($P>0.05$), which indicates a parallel shift, and were not significantly different from the pA_2 (8.92) estimated for ketanserin at low Po_2 from a Schild analysis. In the absence of indomethacin the pK_B values were significantly smaller at the EC_{25}, but not at the EC_{75}, than the corresponding values calculated for ketanserin alone, at both $1X10^{-7}M$ and $3X10^{-7}M$. Without indomethacin present pK_B values calculated from $1X10^{-8}M$ ketanserin were significantly smaller at the EC_{25} and EC_{75}, from the pA_2 for ketanserin at low Po_2.

At one concentration of ketanserin ($1X10^{-7}M$) the average CRC's were calculated by a different empirical method. Elsewhere CRC's have been averaged at each concentration of 5-HT and the mean±s.e.mean of the response (% of maximum response) plotted against the -log (5-HT concentration). For this one experiment the concentrations of 5-HT producing 10, 30, 40, 50, 60, 70, and 90% of the maximum response were interpolated from the log(concentration)-response curve for each tissue and the mean (geometric) at each level of response calculated (figure 15). This analysis also showed that ketanserin biphasically inhibited the response to 5-HT at high Po_2, and that the AR component was blocked by indomethacin ($1X10^{-6}M$).

The antagonism of the response to 5-HT at high Po_2 by ketanserin ($1X10^{-7}M$) was assessed in the presence and absence of flurbiprofen ($3X10^{-7}M$). As already shown, ketanserin caused a biphasic antagonism of the response to 5-HT (figure 16). This was changed to a monophasic, parallel shift in the presence of flurbiprofen. pK_B values were calculated at the EC_{25} and EC_{75}, in the presence and absence of flurbiprofen (Table 6). At the EC_{75} the pK_B values were not significantly different, whether in the presence or absence of

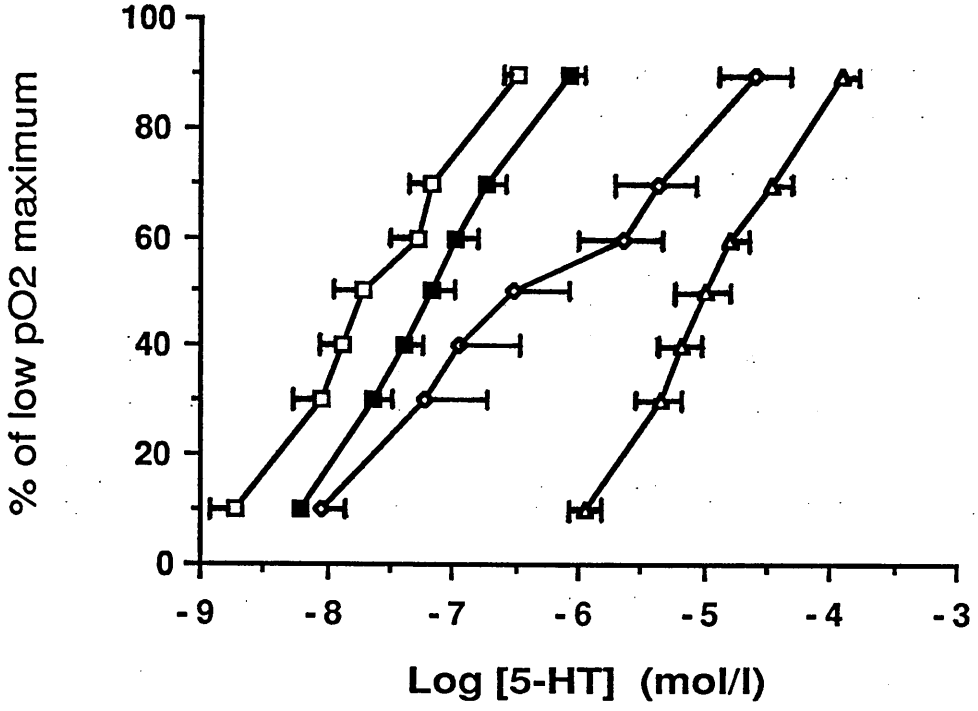


Figure 15 Log concentration-response curves (CRC's) to 5-HT. The data was taken from figure 14b ($+1X10^{-7}$M ketanserin) and the average CRC's calculated by a different method. Here the concentration of 5-HT producing 10, 30, 40, 50, 60, 70, and 90% of the maximum response was interpolated from the log(concentration)-response curve for each tissue and the geometric mean at each level of response calculated. Symbols are the same as in figure 14. Horizontal error bars are the geometric mean±s.e.mean.

Table 7 Estimates of pK_B (the -logarithm of the dissociation constant K_B) of the antagonists compound X, methysergide and phentolamine at high Po_2 (~120mmHg): a) with and b) without the cyclo-oxygenase inhibitor, indomethacin ($1X10^{-6}M$), present. pK_B values were calculated at the EC_{25} and EC_{75}.

	a) pK_B (+COI)		b) pK_B (-COI)		
	EC_{25}	EC_{75}	EC_{25}	EC_{75}	n
[Antagonist]					
Compound X ($1X10^{-7}M$)	9.21 (8.49–9.93)	9.31 (8.53–10.09)	<7.00	8.65 (6.82–10.48)	4
Methysergide ($1X10^{-7}M$)	8.59 (7.65–9.53)	8.90 (8.42–9.38)	8.23 (7.55–8.92)	8.57 (7.80–9.34)	5
Phentolamine ($1X10^{-5}M$)	5.77 (4.92–6.62)	5.89 (5.26–6.52)	6.20 (5.95–6.45)	5.75 (5.13–6.37)	5
Phentolamine ($1X10^{-4}M$)	6.57 (5.47–7.67)	6.57 (5.65–7.49)	6.69 (6.39–6.99)	6.57 (5.85–7.29)	4

pK_B values are the mean (95% confidence limits) of n estimates.
$pK_B = \log(CR-1) - \log B$. B is the concentration of antagonist. The concentration-ratio (CR) is the shift of the CRC to 5-HT in the presence of the antagonist (see methods section for details on the calculation).
a) In each estimate of the pK_B there was an individual control curve to 5-HT. This was the CRC to 5-HT at low Po_2. The concentration-ratios at the EC_{25} and EC_{75} for each experiment were calculated with reference to its own control curve.
b) In each estimate of the pK_B the control curve to 5-HT was from a separate experiment. This was the CRC to 5-HT at high Po_2, from figure 12a. The concentration-ratios at the EC_{25} and EC_{75} for each experiment were calculated with rereference to the geometric mean value of the EC_{25} and EC_{75} of this control curve.
None of the values were significantly different from the pA_2 (of the same antagonist) calculated from a Schild analysis at low Po_2.

flurbiprofen. Neither value was significantly different from the pA_2 (8.92) estimated for ketanserin, at low Po_2, from a Schild analysis. In the absence of flurbiprofen the pK_B at the EC_{25}, but not at the EC_{75}, was significantly lower than the estimated pA_2 for ketanserin.

In three experiments $1X10^{-7}M$ ketanserin had been studied at high Po_2. Comparing the pKB values at the EC_{25} and EC_{75} gave an estimate of the relative potency of ketanserin against the AR and AS components. In these three experiments ketanserin was found to be 11.7, 17.3 and 33.0 fold less potent (mean, 20.1) against the AR component than against the AS component.

The antagonists compound X, methysergide and phentolamine were studied at high Po_2, in the presence and absence of indomethacin. pK_B values are given in Table 7.

Compound X

At high Po_2 the antagonism caused by compound X ($1X10^{-7}M$) was distinctly biphasic (figure 17). Low concentrations of 5-HT were not antagonised: at the EC_{25} there was no significant shift of the CRC (and hence a pK_B was not calculated). The inflection in the CRC was at concentrations of 5-HT producing ~40-50% of the maximum. In the presence of indomethacin ($1X10^{-6}M$) the shift was parallel. pK_B values for compound X, at the EC_{75} whether in the presence or absence of indomethacin, and at the EC_{25} in the presence of indomethacin, were not significantly different from the pA_2 for compound X (8.86) at low Po_2, estimated from a Schild analysis.

Methysergide

In high Po_2 (but not in low Po_2) methysergide ($1X10^{-6}M$) caused rhythmic contractions of HUA which varied in both frequency and amplitude (figure 18). The maximum amplitude was 35±11% of the maximum response to 5-HT. These large methysergide-induced contractions

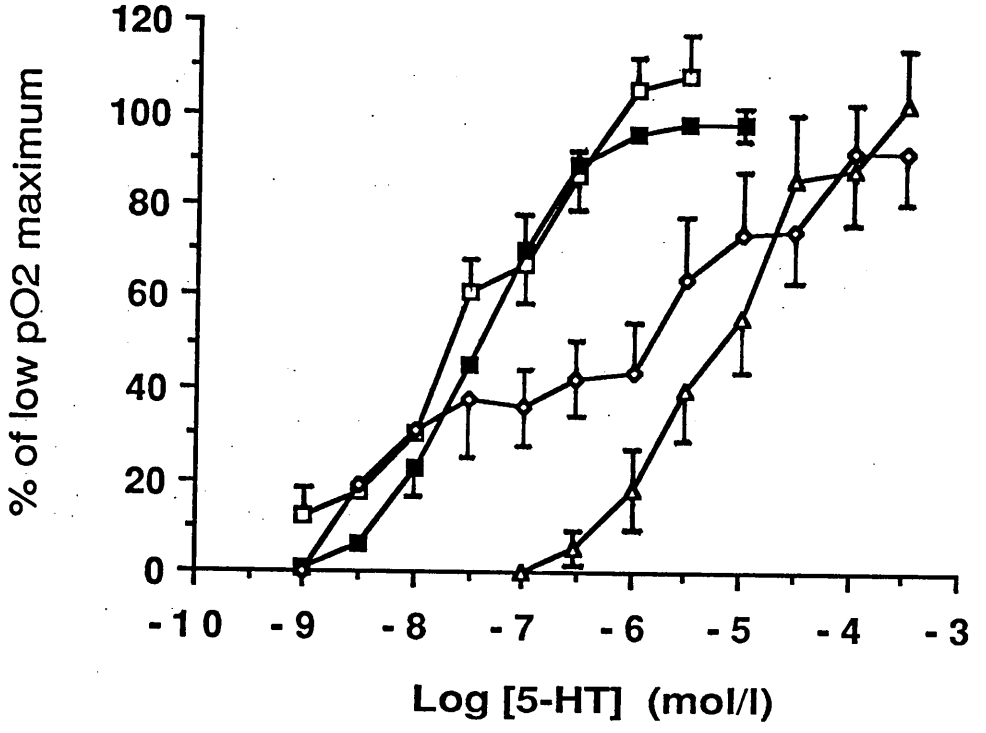


Figure 17 Log concentration-response curves to 5-HT in longitudinal strips of umbilical arteryin the presence of compound X ($1X10^{-7}M$) at high Po_2 (n=6). The protocol was to first construct a CRC to 5-HT at low Po_2 (15±1mmHg), (■). Following washout the Po_2 was increased (to 117±2mmHg), compound X ($1X10^{-7}M$) was added and the CRC to 5-HT repeated (◇). A third CRC to 5-HT was then constructed in the presence of compound X plus indomethacin ($1X10^{-6}M$) at the high Po_2 (△). A control CRC to 5-HT at high Po_2 (□), (from non-paired experiments, figure 12) is shown. Responses were calculated as a percentage of the maximum response to 5-HT at low Po_2. For clarity, error bars (mean±s.e.mean) are omitted at some points.

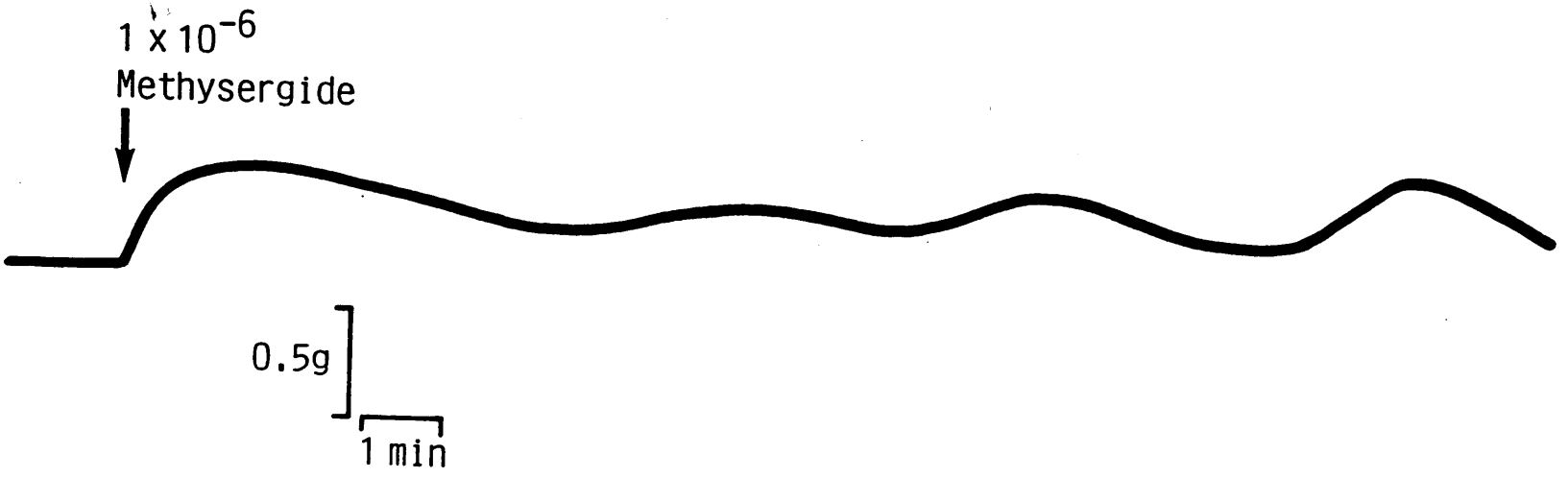


Figure 18 Methysergide ($1X10^{-6}M$) caused large rhythmical contractions in longitudinal strips of human umbilical artery at high Po_2 (~120mmHg). These rhythmical contractions varied in both amplitude and frequency.

precluded any meaningful analysis of CRC's to 5-HT.

Lower concentrations of methysergide ($1X10^{-7}M$) still evoked rhythmical contractions (16±8% of the 5-HT maximum) but which were significantly smaller than at $1X10^{-6}M$. In the presence of methysergide ($1X10^{-7}M$) the CRC to 5-HT was shifted to the right in a biphasic manner (figure 19). At lower concentrations of 5-HT the shift was parallel with an inflection in the curve at concentrations producing ~50% of the maximum response (the inflection is at ~70% of the maximum response if the methysergide-induced tone is included, as in figure 19). pK_B's calculated at the EC_{25}(8.23) and EC_{75} (8.57) suggest that methysergide was 2.2 fold less potent against the AR component than against the AS component. After incubation with indomethacin ($1X10^{-6}M$) methysergide ($1X10^{-7}M$) did not contract the HUA and the displacement of the 5-HT CRC was parallel at all concentrations: pK_B values calculated at the EC_{25} (8.59, geometric mean) and EC_{75} (8.90) were not significantly different.

Phentolamine

$1X10^{-5}M$ and $1X10^{-4}M$ phentolamine displaced the CRC to 5-HT in a parallel manner (figures 20a and 20b respectively), whether in the presence or absence of indomethacin. pK_B values, calculated at the EC_{25} or EC_{75}, in the presence or absence of indomethacin, were not significantly different from the pA_2 (6.37) for phentolamine at low Po_2, estimated from a Schild analysis (see Table 7).

Phentolamine was investigated for possible cyclo-oxygenase inhibitory activity. CRC's to O_2 were constructed in the presence of $1X10^{-5}M$ and $1X10^{-4}M$ phentolamine. O_2-induced contractions of the HUA (which are mediated via prostaglandins -see chapter 2) in the presence of either $1X10^{-5}M$ or $1X10^{-4}M$ phentolamine were not significantly different from that in control preparations, at any Po_2 (figure 21).

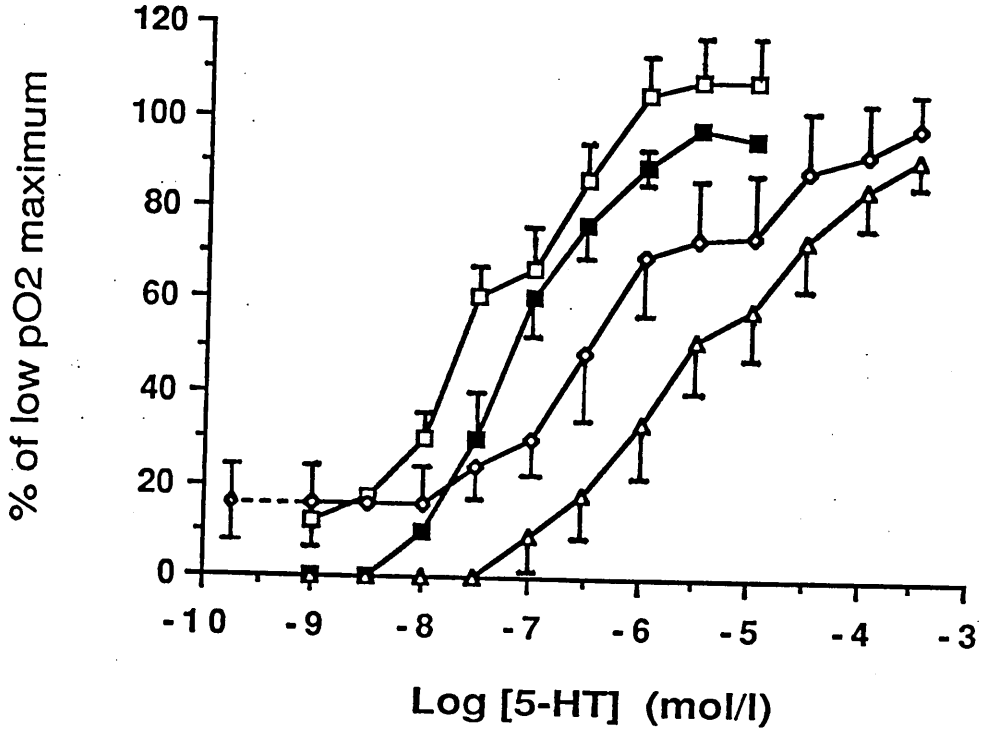
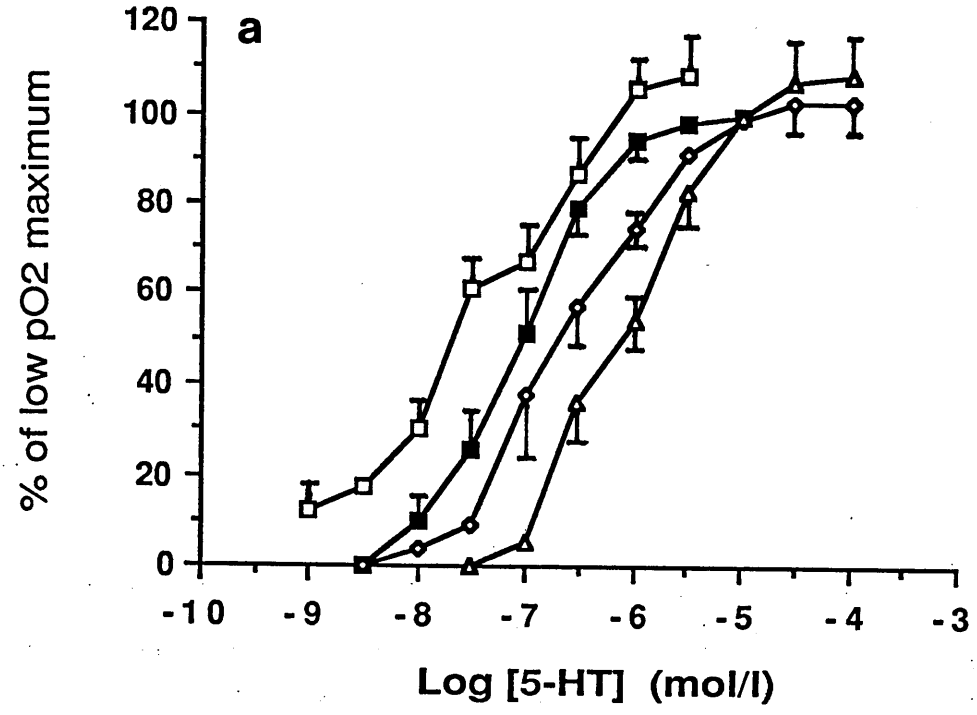


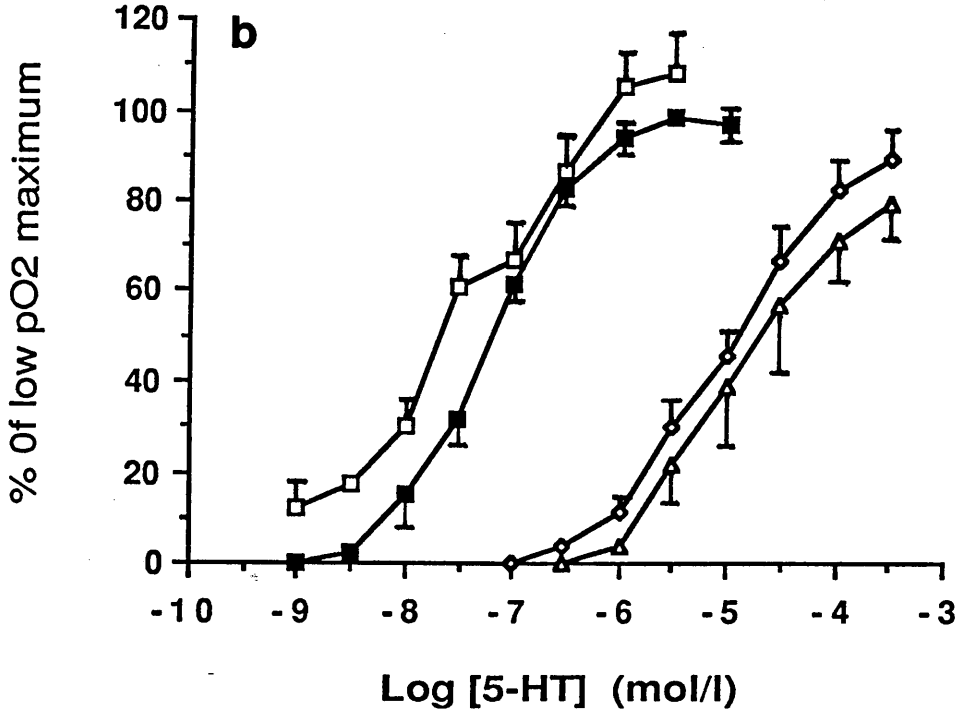
Figure 19 Log concentration-response curves to 5-HT in longitudinal strips of umbilical artery in the presence of methysergide ($1X10^{-7}M$) at high Po_2 (n=6). The protocol was to first construct a CRC to 5-HT at low Po_2 (14 ± 2mmHg), (■). Following washout the Po_2 was increased (to 123 ± 3mmHg), methysergide ($1X10^{-7}M$) was added and the CRC to 5-HT repeated (◇). A third CRC to 5-HT was then constructed in the presence of methysergide plus indomethacin ($1X10^{-6}M$) at the high Po_2 (△). A control CRC to 5-HT at high Po_2 (□), (from non-paired experiments, figure 12) is shown. Responses were calculated as a percentage of the maximum response to 5-HT at low Po_2. For clarity, error bars (mean±s.e.mean) are omitted at some points.

Figure 20 Log concentration-response curves (CRC's) to 5-HT in longitudinal strips of umbilical artery at high Po_2 in the presence of phentolamine. In each experiment (figures a and b) the protocol was to first construct a CRC to 5-HT at low Po_2 (~15mmHg), (■). Following washout the Po_2 was then increased (to ~120mmHg), phentolamine was added and a CRC to 5-HT repeated (◇). A third CRC to 5-HT was then constructed in the presence of phentolamine plus indomethacin ($1X10^{-6}M$) at the high Po_2 (△). A control CRC to 5-HT at high Po_2 (□), (from non-paired experiments, figure 12a) is shown in each figure. Response (ordinate) is expressed as a % of the maximum response to 5-HT at low Po_2. The difference between figures a and b is the concentration of phentolamine:

(a) [phentolamine]=$1X10^{-5}M$; low Po_2=15±3mmHg; high Po_2=123±4mmHg; n=5.
(b) [phentolamine]=$1X10^{-4}M$;low Po_2=14±2mmHg; high Po_2=127±2mmHg; n=4.

Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.


a
120
100
80
60
40
20
0
% of low pO2 maximum
-10
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)


b
120
100
80
60
40
20
0
% 0f low pO2 maximum
-10
-9
-8
-7
-6
-5
-4
-3
Log [5-HT] (mol/l)

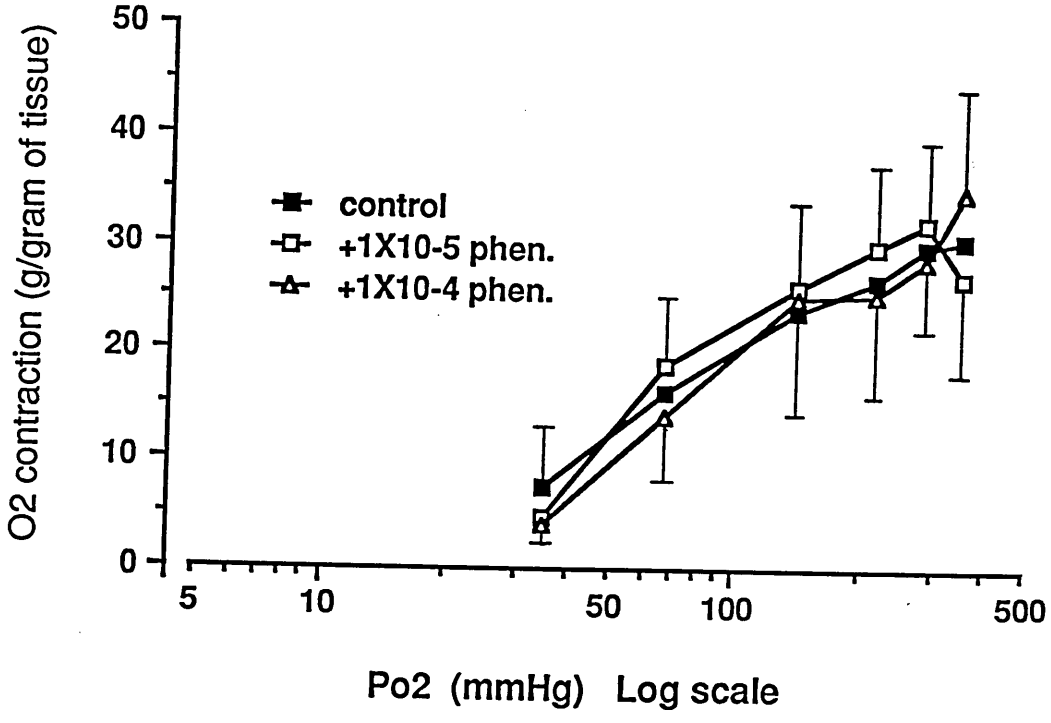


Figure 21 Concentration-response curves to oxygen in longitudinal strips of umbilical artery in the presence of phentolamine. Three strips from each artery (n=4) were investigated in parallel: one strip acted as a control while $1X10^{-5}M$ and $1X10^{-4}M$ were added to two other strips. For clarity, error bars (mean±s.e.mean) are omitted at some points.

Table 8 The potency of 5-carboxamidotryptamine (5-CT) relative to 5-HT, at low (~15mmHg) and high (~120mmHg) Po_2, calculated at the EC_{25} and EC_{75}.

	5-CT CRC			5-HT CRC		$\frac{EC_X 5\text{-CT}}{EC_X 5\text{-HT}}$		
	EC_{25} (nM)	EC_{75} (μM)	% of 5-HT maximum	EC_{25} (nM)	EC_{75} (μM)	EC_{25}	EC75	n
Po_2 (mmHg)								
13±1	13.2 (1.5–115)	1.1 (0.26–4.4)	81±10	17.8 (12.6–25.1)	0.22 (0.09–0.51)	0.64 (0.04–9.1)	5.4 (1.3–21.4)	5
123±1	0.25 (0.06–1.0)	0.58 (0.1–2.6)	88±8	3.3 (0.9–12.3)	0.1 (0.05–0.19)	0.15 (0.02–1.4)	3.5 (1.6–7.4)	6

Values are the geometric mean (95% confidence limits) or mean±s.e.mean, of n estimates.

(ii) Agonists

Other agonists of 5-HT receptors, 5-carboxamidotryptamine (5-CT), methysergide and LSD were examined at both low (~15mmHg) and high (~120mmHg) Po_2.

5-CT

The potency of 5-CT, relative to 5-HT was investigated at both low and high Po_2. At low Po_2 the response to 5-CT was sometimes slightly biphasic (figure 22a) while at high Po_2 the response was distinctly biphasic (figure 22b). At high Po_2 the 1st phase lay between $1X10^{-10}M$ and $3X10^{-8}M$, and the 2nd phase between $3X10^{-8}M$ and $1X10^{-5}M$. The maximum responses to 5-CT were 81±10% and 88±8% of that to 5-HT at low and high Po_2 respectively. The potency of 5-CT, relative to 5-HT was calculated at the EC_{25} and EC_{75}, which were taken as parameters of the 1st and 2nd phases respectively (Table 8). The potency of 5-CT was quite variable between tissues e.g. at low Po_2, calculated at the EC_{75}, the potency ranged from being equipotent with 5-HT, to 36 fold less potent. The mean response showed that (i) at low Po_2 5-CT was equipotent to 5-HT at the EC_{25} and 5 fold less potent at the EC_{75}, and (ii) at high Po_2 5-CT was 7 fold more potent at the EC_{25} and 4 fold less potent at the EC_{75} than 5-HT. At high Po_2 the 1st phase of the response to 5-CT was very variable and in different preparations this was either a transient or maintained contraction (figure 23).

The effects of indomethacin and ketanserin against the response to 5-CT were tested at both low and high Po_2. In addition buspirone and (±)pindolol were tested at high Po_2.

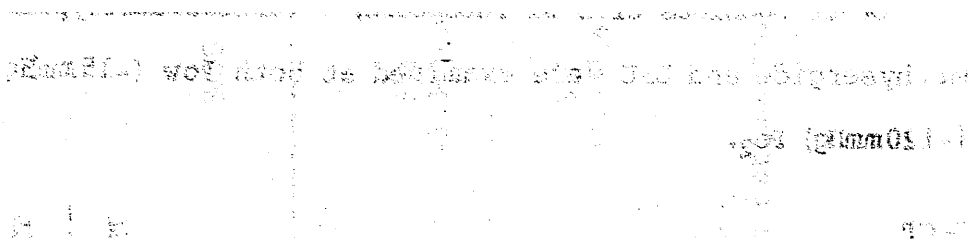
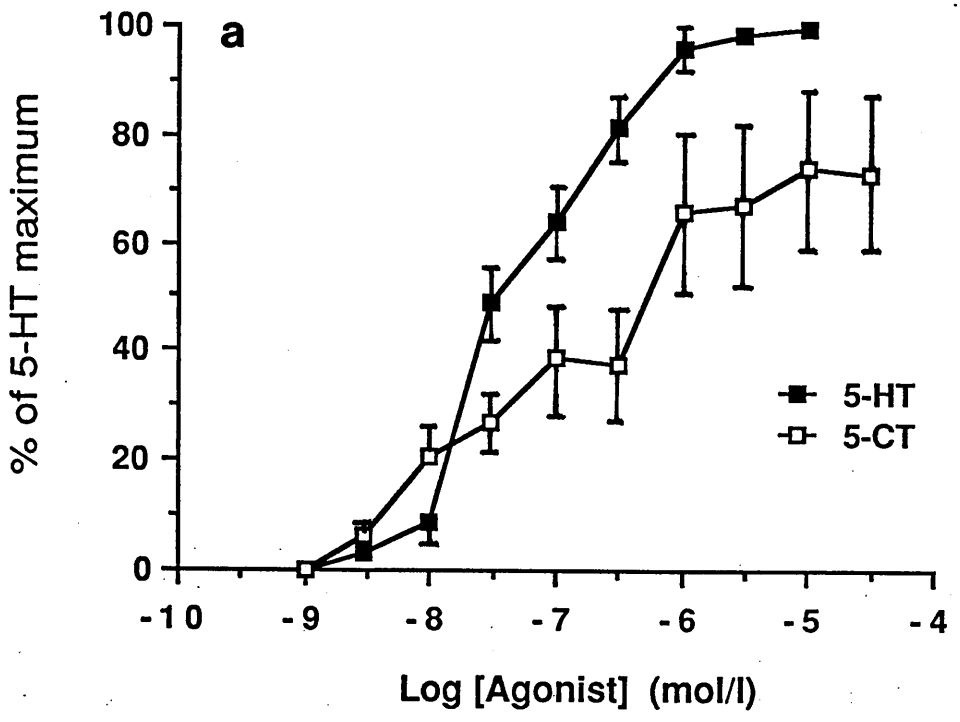
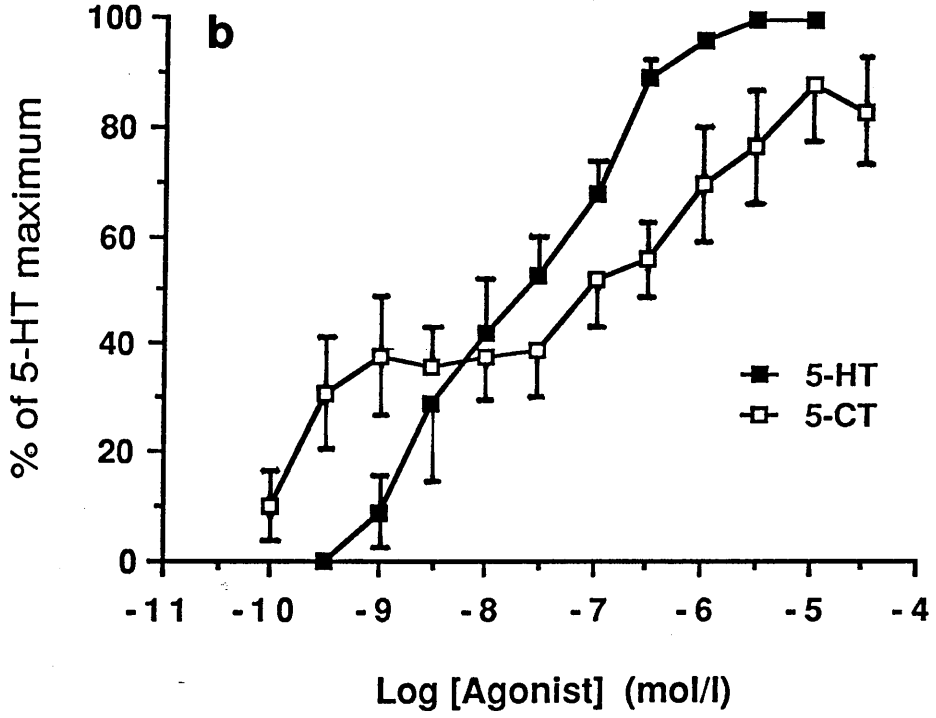


Figure 22 Log concentration-response curves (CRC's) to 5-HT and 5-CT in longitudinal strips of umbilical artery at (a) low Po_2 (13±1mmHg), n=5; (b) high Po_2 (123±1mmHg), n=6. The protocol was to construct a CRC to 5-HT followed by one to 5-CT in each preparation. Response (ordinate) is expressed as a % of the maximum response to 5-HT. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.


a
100
80
60
40
20
0
% of 5-HT maximum
-10
-9
-8
-7
-6
-5
-4
Log [Agonist] (mol/l)
5-HT
5-CT


b
100
80
60
40
20
0
% of 5-HT maximum
-11
-10
-9
-8
-7
-6
-5
-4
Log [Agonist] (mol/l)
5-HT
5-CT

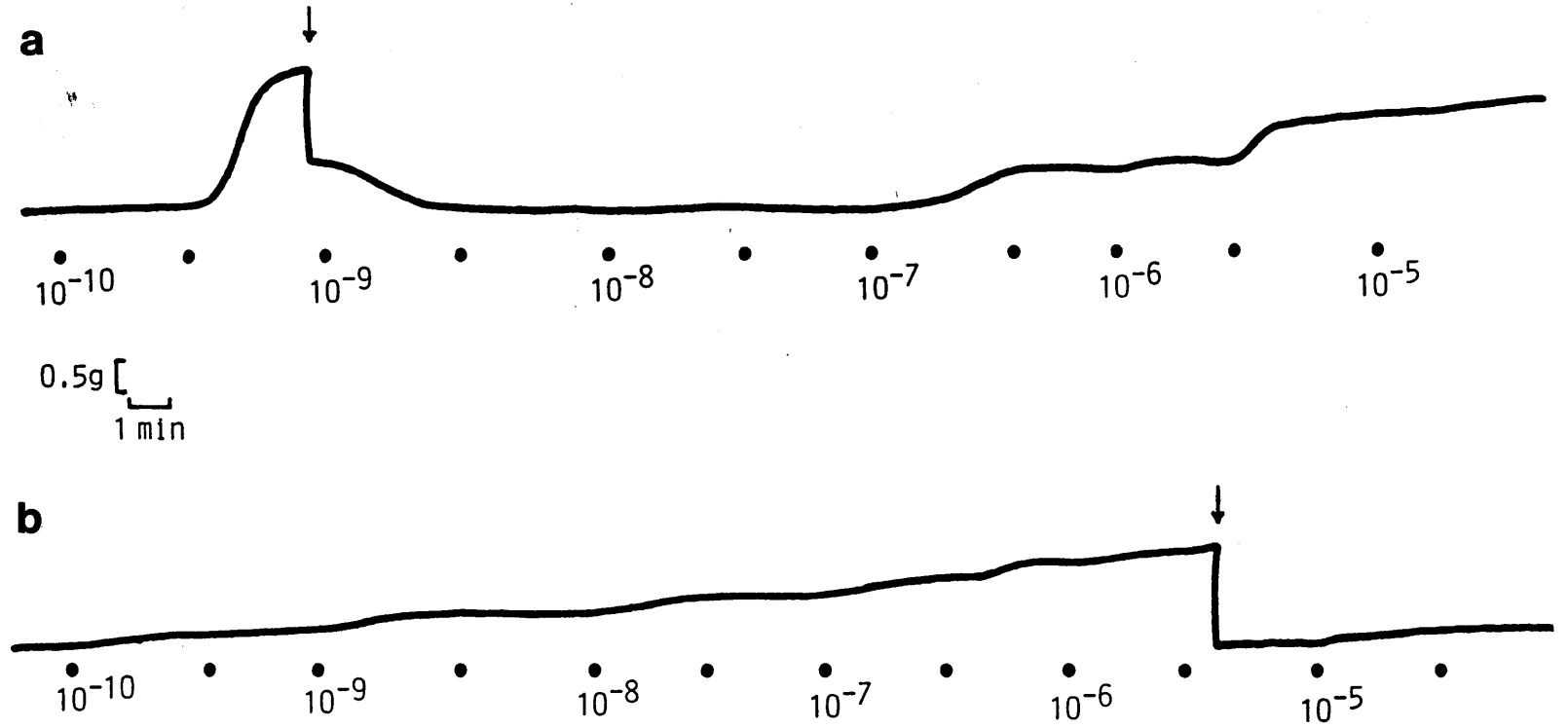


Figure 23 Examples of concentration-response curves to 5-carboxamidotryptamine (5-CT) in longitudinal strips of human umbilical artery at high Po_2 (~120mmHg). The responses to low concentrations of 5-CT could be either (a) very large and not maintained or (b) small but maintained, or the response could be between these two extremes. Higher concentrations of 5-CT evoked stepwise contractions which were maintained. In each of these recordings the amplification of the recording had to be halved (↓).

Table 9 Parameters of the Schild plot for ketanserin against 5-carboxamidotryptamine at low Po_2 (~15mmHg), with and without indomethacin present.

	pA_2	Slope	n
Antagonist			
Ketanserin	8.77 (8.39–9.15)	1.19 (0.68–1.70)	5
Ketanserin ($+1X10^{-6}M$ indomethacin)	9.05 (8.58–9.52)	1.00 (0.75–1.25)	4

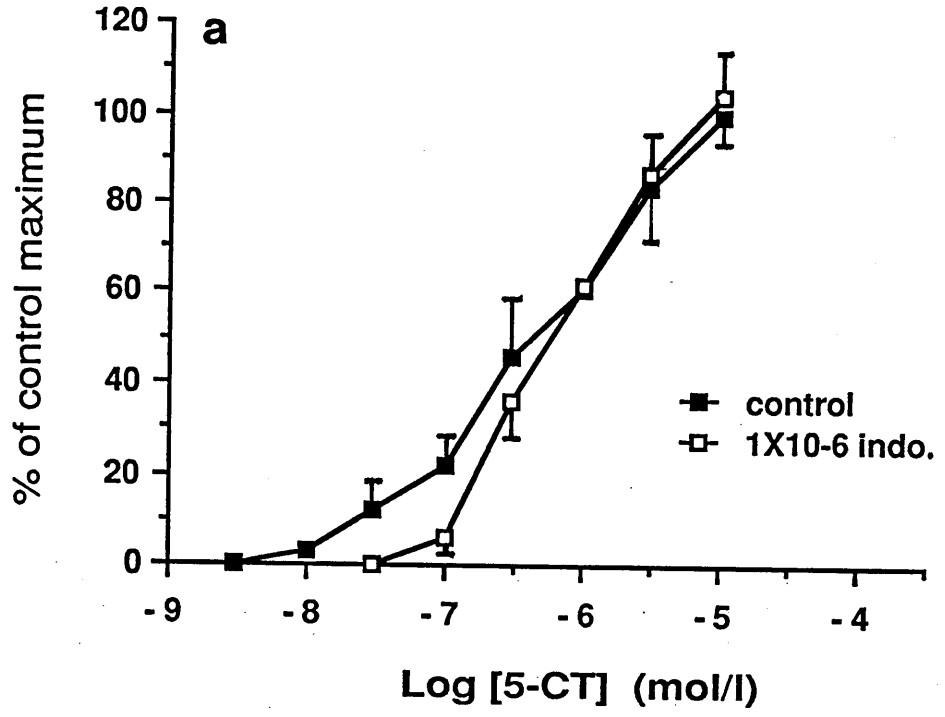
Values are the geometric means (95% confidence limits) of n estimates.

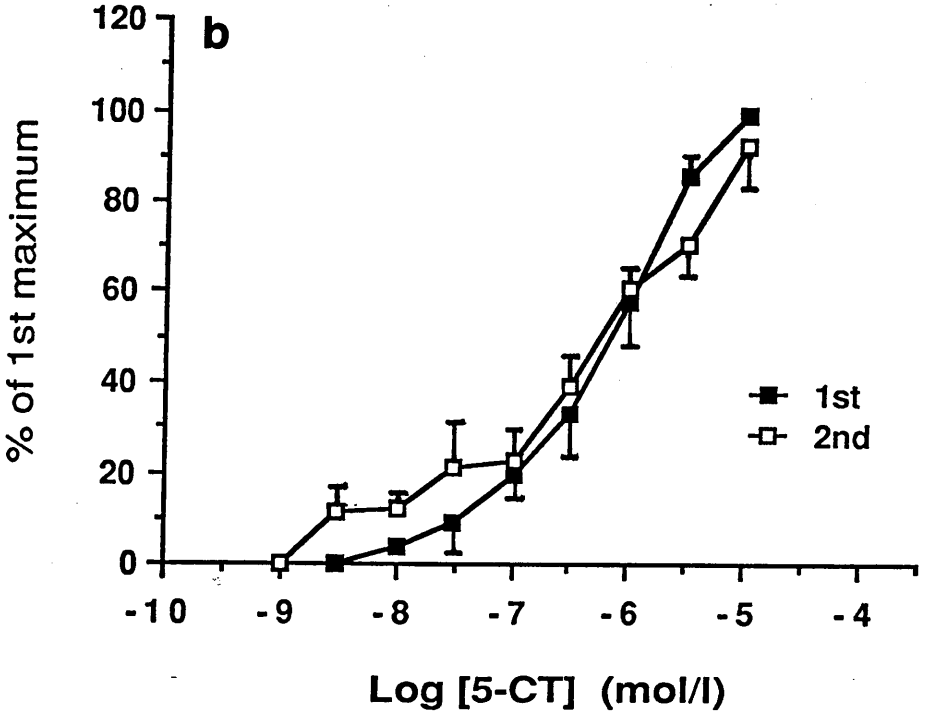
a) Low Po_2

At low Po_2 indomethacin caused a small rightward shift of the CRC to 5-CT at low concentrations ($<1X10^{-7}M$) but at the EC_{25} this shift was not significant (figure 24a). In paired control tissues where two successive CRC's to 5-CT were constructed, the 2nd CRC showed a small leftward shift at low concentrations ($<1X10^{-7}M$), (figure 24b).

Ketanserin ($3X10^{-9}$–$3X10^{-8}M$) antagonised the response to 5-CT. This interaction, with and without indomethacin ($1X10^{-6}M$) present, is summarised in Table 9. Without indomethacin present there was a small component at lower concentrations which was resistant to ketanserin (figure 25a), and the CRC was displaced in a parallel manner only at concentrations producing greater than (~)30% of the maximum response. Nevertheless a Schild plot (based on concentration-ratios at the EC_{50}), (figure 25b) had a slope not significantly different from one, suggesting competitive antagonism. The slopes of the regression lines of the Schild plots were, in two out of five preparations, much greater than unity (1.57 and 1.78). pK_B values calculated from the lowest and highest concentrations of ketanserin ($3X10^{-9}M$ and $3X10^{-8}M$) were 8.0 and 9.07, and 7.96 and 9.12 for the two preparations respectively. In the presence of indomethacin ($1X10^{-6}M$), (figure 26a) this ketanserin-resistant component was abolished and the CRC's were displaced in a parallel manner at all concentrations. The slopes of the individual Schild plots ranged from 0.85 to 1.24 and the mean (1.00), (figure 26b) was not significantly different from one. The estimated pA_2's for ketanserin against 5-CT were not significantly different in the presence (9.05) (mean) or absence (8.77) of indomethacin. These pA_2's with 5-CT as the agonist were not significantly different from their respective values (8.94 and 8.92) in the presence and absence of indomethacin, with 5-HT as the agonist.

Figure 24 Log concentration-response curves (CRC's) to 5-CT in longitudinal strips of umbilical artery (n=5), at low Po_2 (13±2mmHg) in the presence and absence of indomethacin ($1X10^{-6}M$). (a) the 1st curve is the control and the 2nd curve is in the presence of indomethacin, (b) in paired preparations two consecutive CRC's to 5-CT were constructed in the same preparation. Response (ordinate) is expressed as a % of the maximum response to the 1st curve. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.


a
120
100
80
60
40
20
0
% of control maximum
control
1X10-6 indo.
-9
-8
-7
-6
-5
-4
Log [5-CT] (mol/l)


b
120
100
80
60
40
20
0
% of 1st maximum
1st
2nd
-10
-9
-8
-7
-6
-5
-4
Log [5-CT] (mol/l)

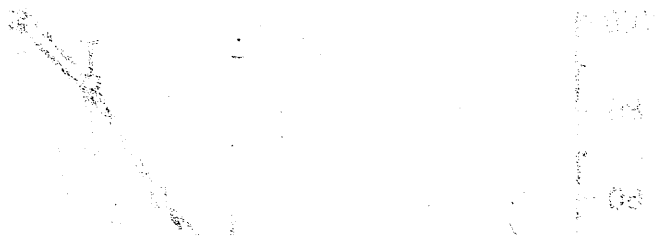
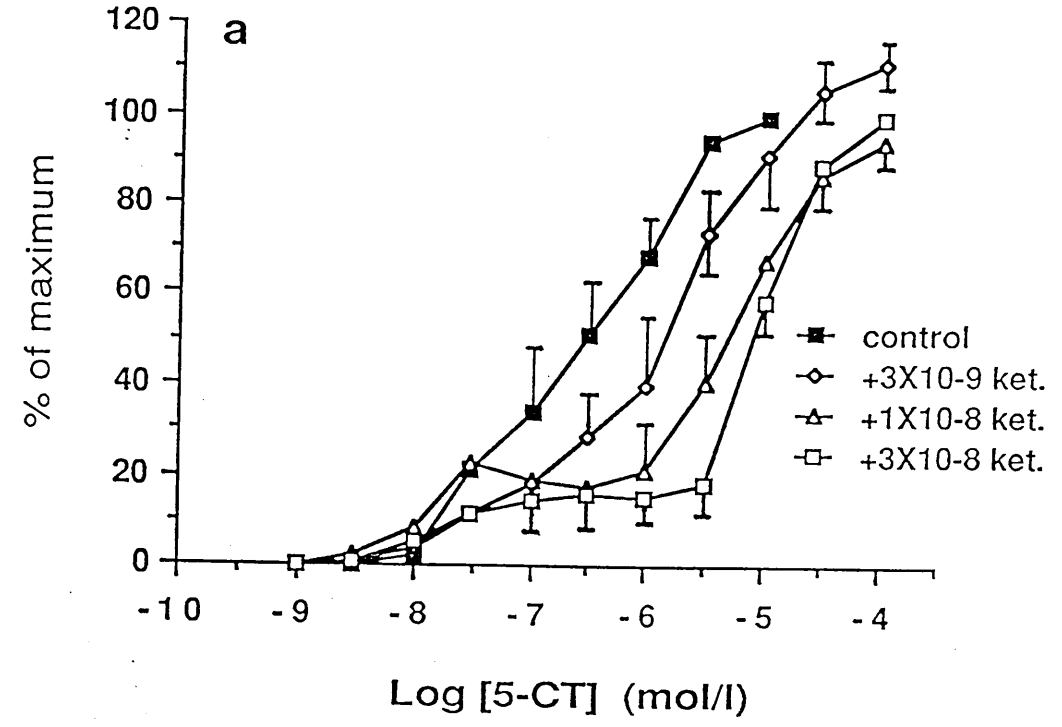
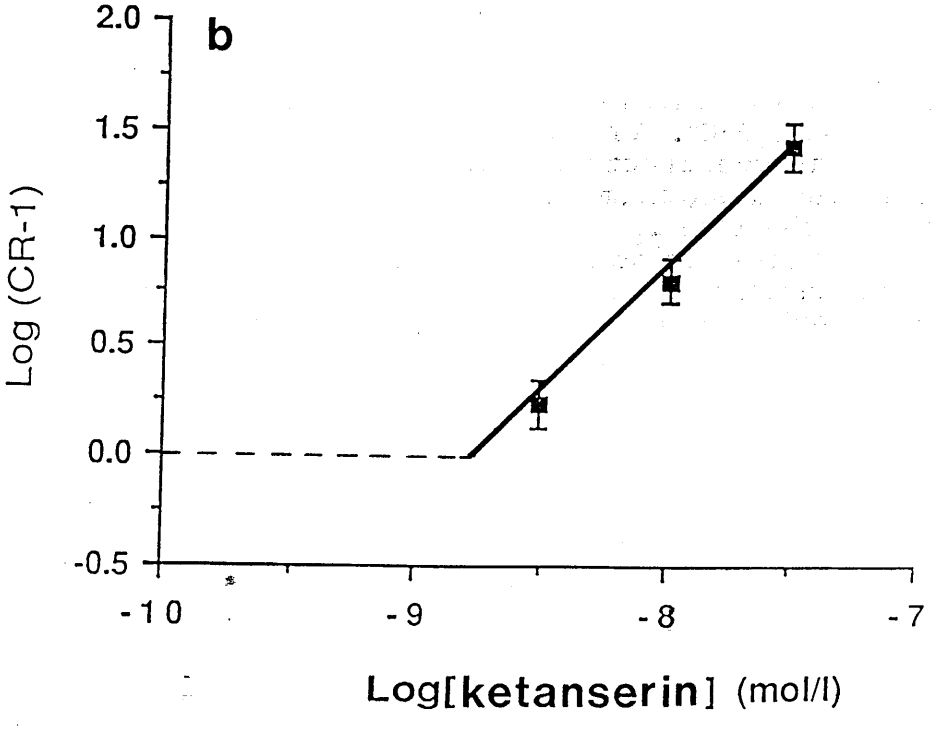


Figure 25 (a) Log concentration-response curves (CRC's) to 5-CT in the presence of ketanserin (ket) at low Po_2 (14±2mmHg) in longitudinal strips of umbilical artery (n=5). CRC's to 5-CT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum response to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of ketanserin. In the fourth (control) preparation the 1st (■) and second CRC's were constructed without antagonist in order to assess the change in sensitivity to 5-CT with time, which was 2.5±0.3 fold. For clarity, error bars (mean±s.e.mean) are omitted at some points.
(b) Schild plot for the interaction of ketanserin with 5-CT. For each preparation an individual Schild plot was constructed. This figure shows points representing the mean±s.e.mean at each concentration of ketanserin and the line is based on the mean pA_2 (8.77) and mean slope (1.19) from the group of experiments. The pA_2 value and slope of individual plots were calculated by linear regression (least squares). The intercept of the linear regression line with the line, $\log(CR-1)=0$, (broken line) gave an estimate of the pA_2.


a
120
100
80
60
40
20
0
% of maximum
-10
-9
-8
-7
-6
-5
-4
Log [5-CT] (mol/l)
control
+3X10-9 ket.
+1X10-8 ket.
+3X10-8 ket.


b
2.0
1.5
1.0
0.5
0.0
-0.5
Log (CR-1)
-10
-9
-8
-7
Log[ketanserin] (mol/l)

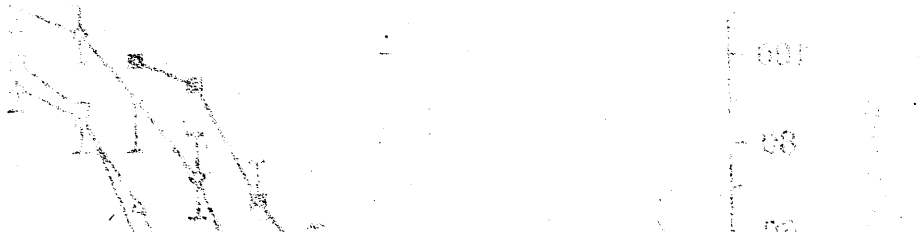
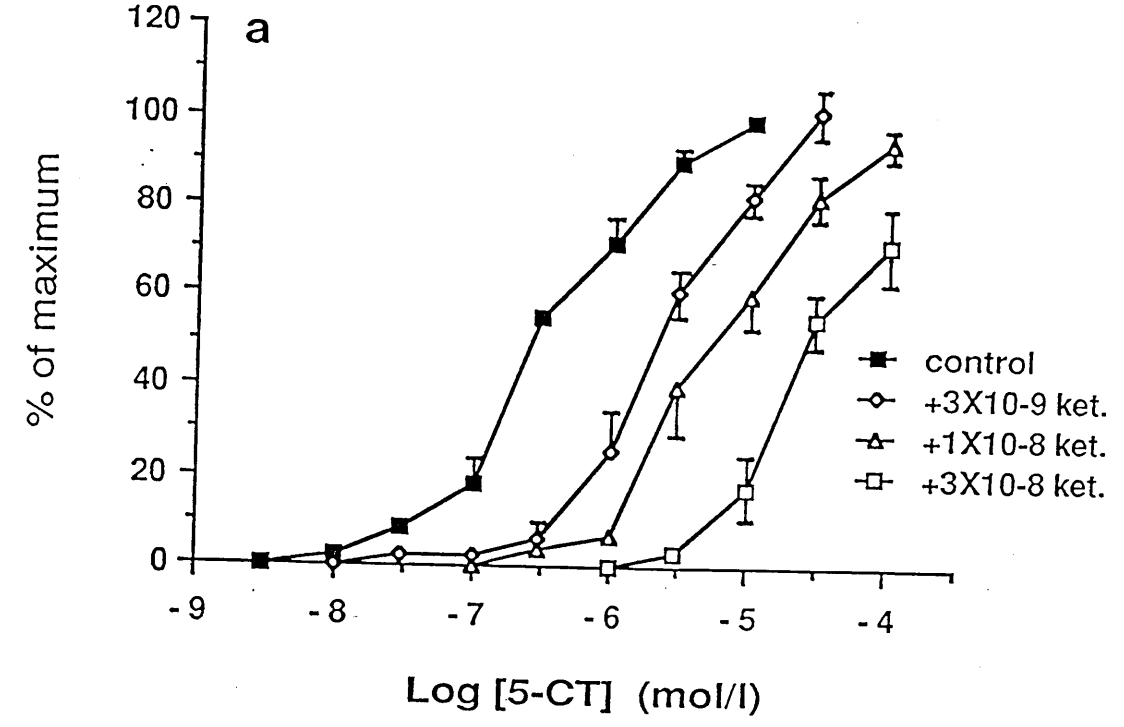
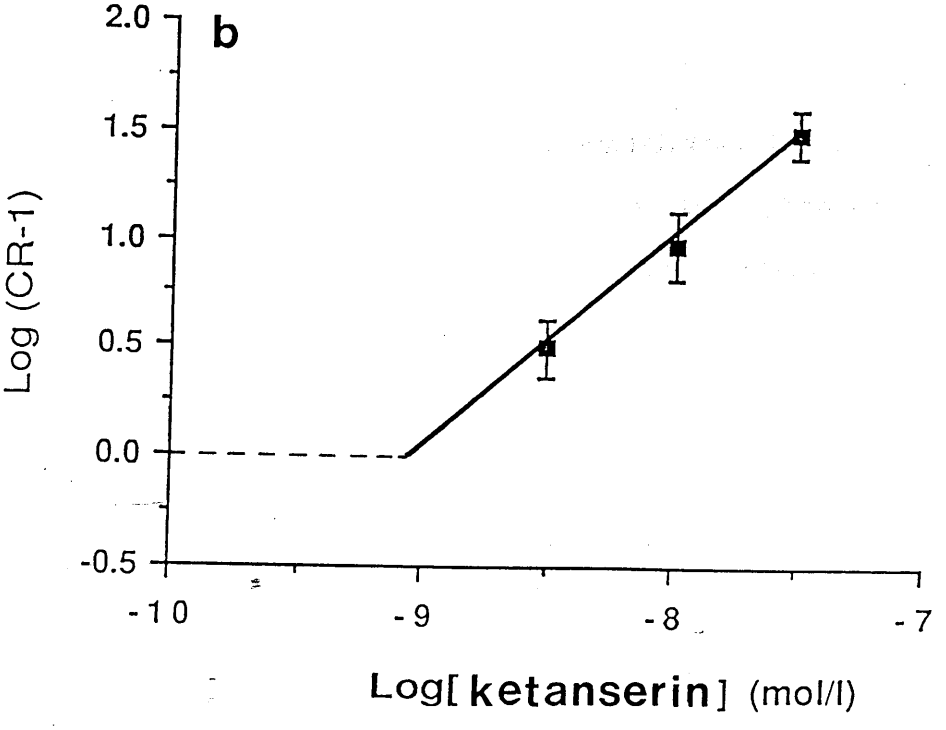


Figure 26 (a) Log concentration-response curves (CRC's) to 5-CT in the presence of ketanserin (ket) plus indomethacin ($1X10^{-6}M$) at low Po_2 (10 ± 2mmHg) in longitudinal strips of umbilical artery (n=4). CRC's to 5-CT were constructed twice in each of four preparations from the same artery. Response (ordinate) was calculated as a % of the maximum to 5-HT of the 1st curve. In three of the four preparations the second CRC was repeated in the presence of one concentration of ketanserin (plus indomethacin). In the fourth (control) preparation the 1st (■) and second CRC's were constructed without antagonist (but with indomethacin) in order to assess the change in sensitivity to 5-CT with time, which was 3.0 ± 0.4 fold. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.
(b) Schild plot for the interaction of ketanserin (plus indomethacin) with 5-CT. For each preparation an individual Schild plot was constructed. This figure shows points representing the mean±s.e.mean at each concentration of ketanserin and the line is based on the mean pA_2 (9.05) and mean slope (1.00) from the group of experiments. The pA_2 value and slope of individual plots were calculated by linear regression (least squares). The intercept of the linear regression line with the line, $\log(CR-1)=0$, (broken line) gave an estimate of the pA_2.


a
120
100
80
60
40
20
0
% of maximum
-9
-8
-7
-6
-5
-4
Log [5-CT] (mol/l)
control
+3X10-9 ket.
+1X10-8 ket.
+3X10-8 ket.


b
2.0
1.5
1.0
0.5
0.0
-0.5
Log (CR-1)
-10
-9
-8
-7
Log[ketanserin] (mol/l)

b) High Po_2

CRC's to 5-CT were first constructed at low Po_2. On increasing the Po_2 to ~120mmHg, the response to 5-CT became distinctly biphasic as already found (figure 27a). The variability of the 1st phase was especially marked in this series of experiments: the maximum of the 1st phase ranged from 43–97% of the contraction produced by $1X10^{-5}M$ 5-CT. In paired tissues (figure 27b) which were incubated with indomethacin, the 1st phase of the response to 5-CT at high Po_2 was abolished, without antagonising the 2nd phase.

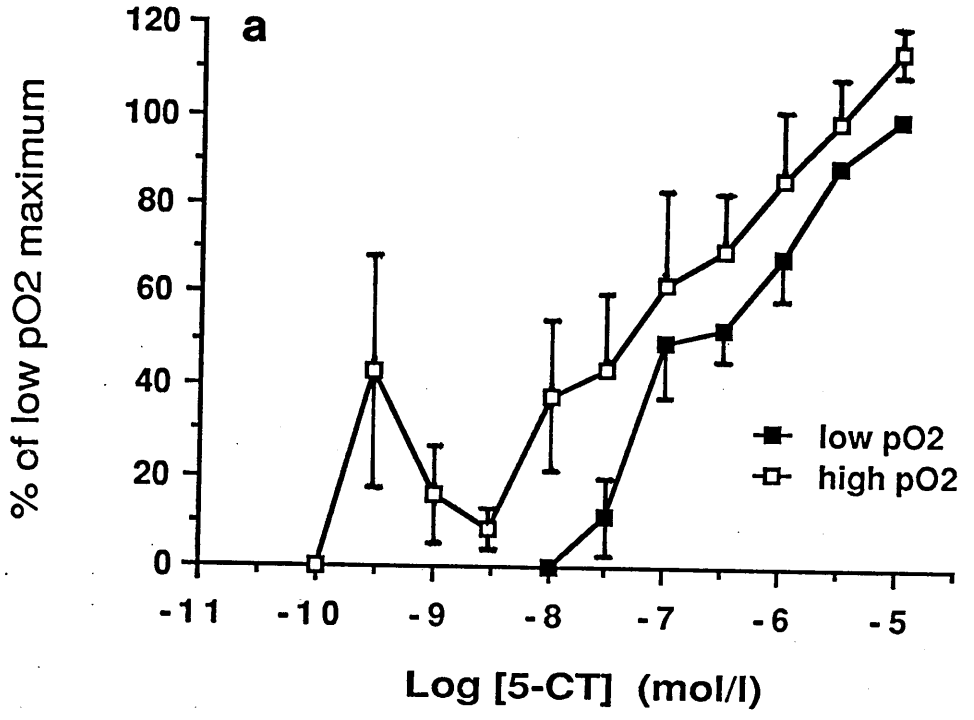
Ketanserin ($1X10^{-7}M$) antagonised the 2nd phase, but not the 1st phase , of the 5-CT CRC (figure 28a). The estimated pK_B for ketanserin was 9.30. (This was calculated from the concentration-ratio at the EC_{75}, but, as not all the tissues produced 75% of the control maximum response in the presence of ketanserin, the concentration-ratio was simply calculated from the ratio of the geometric means at the EC_{75}).

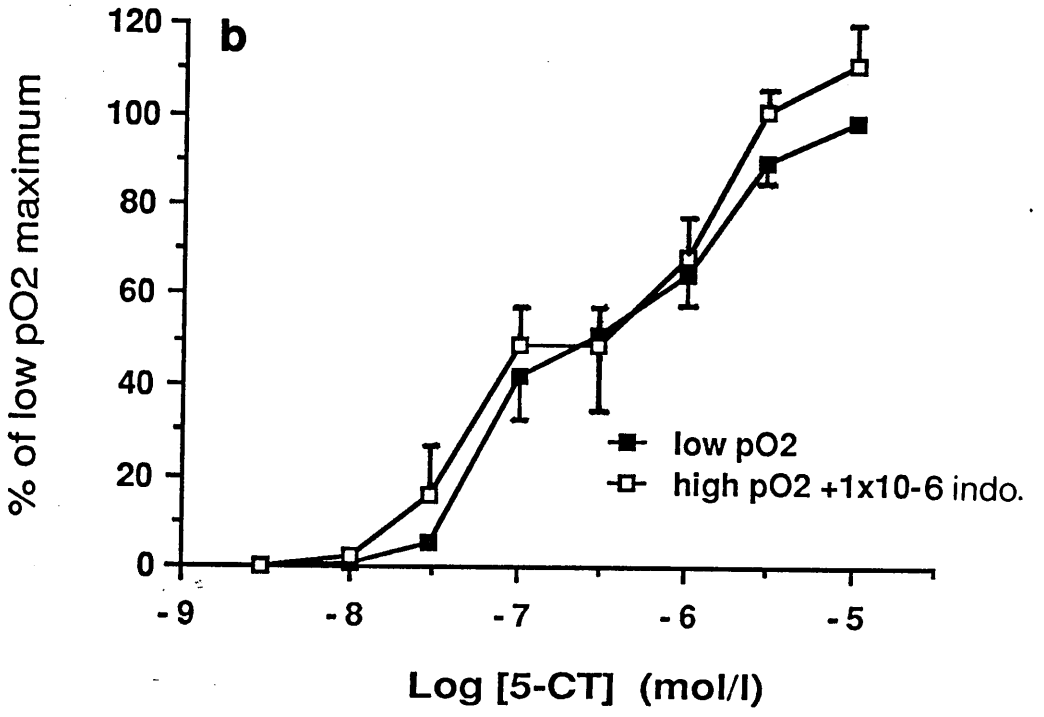
Neither buspirone ($1X10^{-7}M$), (figure 28b) nor (±)pindolol ($3X10^{-6}M$), (figure 28c) antagonised either the 1st or 2nd phases of the 5-CT CRC. In the former series of experiments the 1st and 2nd phases of the CRC were not distinct from each other when the responses were averaged to plot the graph. They were however still visible in individual tissues.

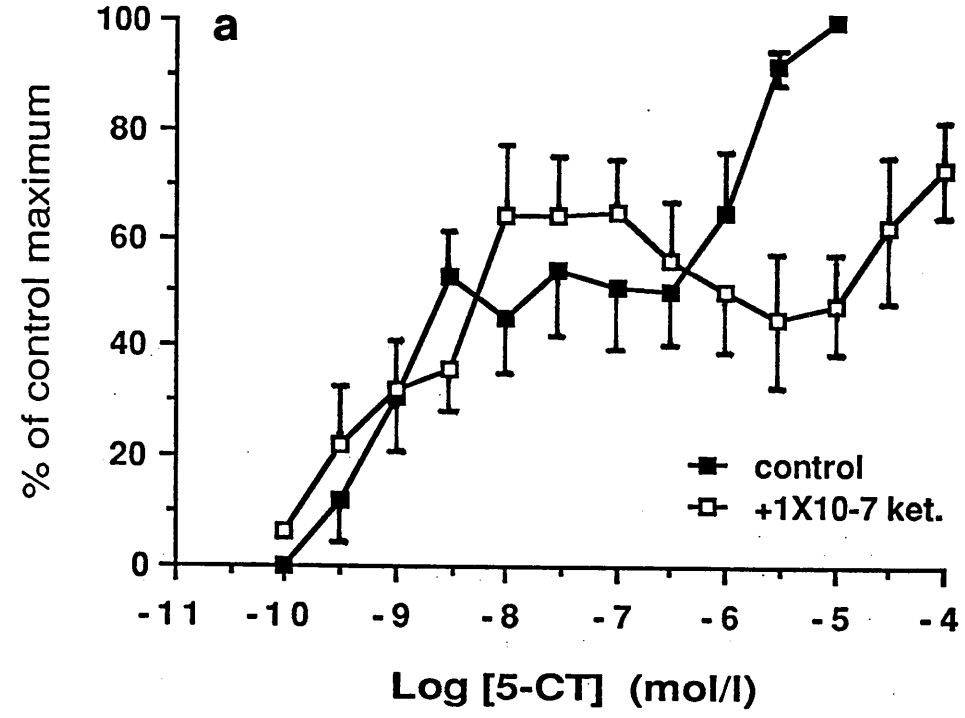
Methysergide

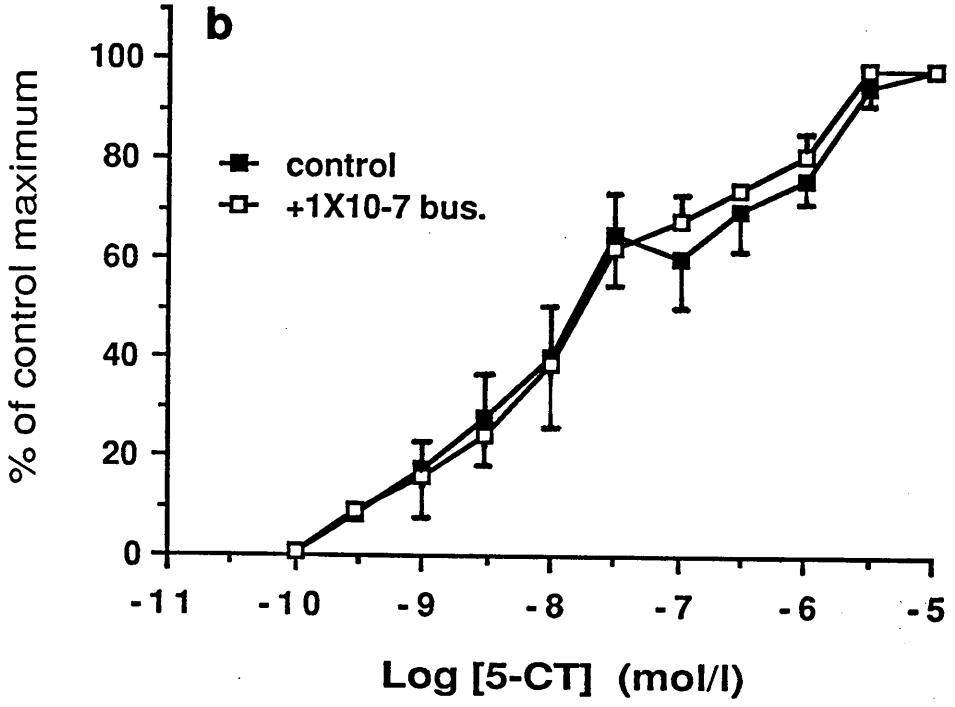
At low Po_2 methysergide showed no agonism ($1X10^{-8}$–$1X10^{-6}M$) (n=6). At high Po_2 methysergide contracted the HUA between $1X10^{-8}M$ and $1X10^{-6}M$, the EC_{50} was 0.15uM (geometric mean, 95% C.L.'s 0.06–0.36uM). The EC_{50} for 5-HT at high Po_2 was 9.8nM (2.3–40.7nM) (non-paired tissues, from figure 22b). Thus 5-HT was approximately 15 fold more potent than methysergide. The maximum response to methysergide was 64±18% of a 50mM KCl contraction. In contrast the 5-HT maximum

Figure 27 Log concentration-response curves (CRC's) to 5-CT in longitudinal strips of umbilical artery (n=5) at low Po_2 (12±3mmHg), and at high Po_2 (119±4mmHg) in the presence and absence of indomethacin ($1X10^{-6}M$). (a) The protocol was to construct a CRC to 5-CT at low Po_2. Following washout the Po_2 was increased and a 2nd CRC constructed. (b) Protocol as for (a) except that indomethacin was added 30mins before increasing the Po_2. Response (ordinate) is expressed as a % of the maximum response to 5-CT at low Po_2. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.


a
120
100
80
60
40
20
0
% of low pO2 maximum
-11
-10
-9
-8
-7
-6
-5
Log [5-CT] (mol/l)
low pO2
high pO2


b
120
100
80
60
40
20
0
% of low pO2 maximum
-9
-8
-7
-6
-5
Log [5-CT] (mol/l)
low pO2
high pO2 +1x10-6 indo.


a
100
80
60
40
20
0
% of control maximum
control
+1X10-7 ket.
-11
-10
-9
-8
-7
-6
-5
-4
Log [5-CT] (mol/l)


b
100
80
60
40
20
0
% of control maximum
control
+1X10-7 bus.
-11
-10
-9
-8
-7
-6
-5
Log [5-CT] (mol/l)

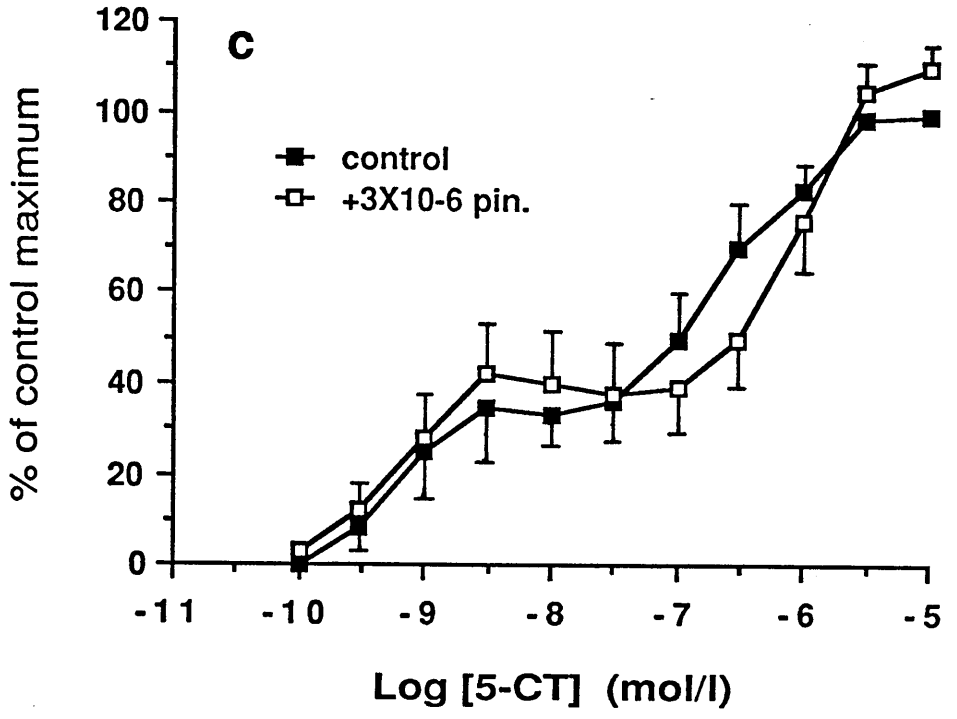


Figure 28 Log concentration-response curves to 5-CT in longitudinal strips of umbilical artery at high Po_2 in the presence of (a) $1X10^{-7}M$ ketanserin (Po_2=118±4mmHg; n=5), (b) $1X10^{-7}M$ buspirone (Po_2=118±1mmHg; n=5), (c) $3X10^{-6}M$ (±)pindolol (Po_2=121±2mmHg; n=5). Error bars are the mean±s.e.mean.

(relative to a 50mM KCl contraction) was 222±26% (non-paired tissues), (figure 29). Neither prazosin ($3X10^{-8}M$) nor ketanserin ($3X10^{-8}M$) shifted the methysergide CRC or reduced the maximum response. Indomethacin ($1X10^{-6}M$) did not significantly shift the methysergide CRC but significantly reduced the maximum response to 43±11% (relative to a 50mM KCl contraction), (figures 30a and b).

LSD

a) Low Po_2

LSD was a potent agonist of the HUA (figure 31). The EC_{50} was 0.35nM (geometric mean, 95% C.L.'s 0.17–0.71nM). The EC_{50} for 5-HT at low Po_2 was 40nM (16.9–93.3nM), (non-paired tissues, from figure 22a). Thus LSD was approximately 114 fold more potent than 5-HT at low Po_2. The maximum contraction to LSD was 78±10% of a 50mM KCl contraction. In contrast the maximum response to 5-HT was 174±6% of a 50mM KCl contraction (non-paired tissues).

Ketanserin ($3X10^{-8}M$) caused a parallel rightward displacement of the LSD CRC (figure 32). The estimated pK_B was 8.90 (geometric mean, 95% C.L.'s 8.15–9.65), calculated from concentration-ratios at the EC_{50}. Indomethacin did not antagonise the response to LSD (figure 31). Neither ketanserin nor indomethacin reduced the maximum response, measured relative to a 50mM KCl contraction. Prazosin ($3X10^{-8}M$) did not antagonise the response to LSD nor reduce the maximum response.

b) High Po_2

Increasing the Po_2 from ~15mmHg to ~120mmHg increased the potency of LSD (figure 33a): at low Po_2 the EC_{50} was 1.2 nM (geometric mean, 95% C.L.'s 0.63–2.5nM) and at high Po_2 was 0.2 nM (0.10–0.50nM). This represents a significant increase in potency , at the higher Po_2, of 5.8 fold (geometric mean, 95% C.L.'s 2.3–14.4). This increase in

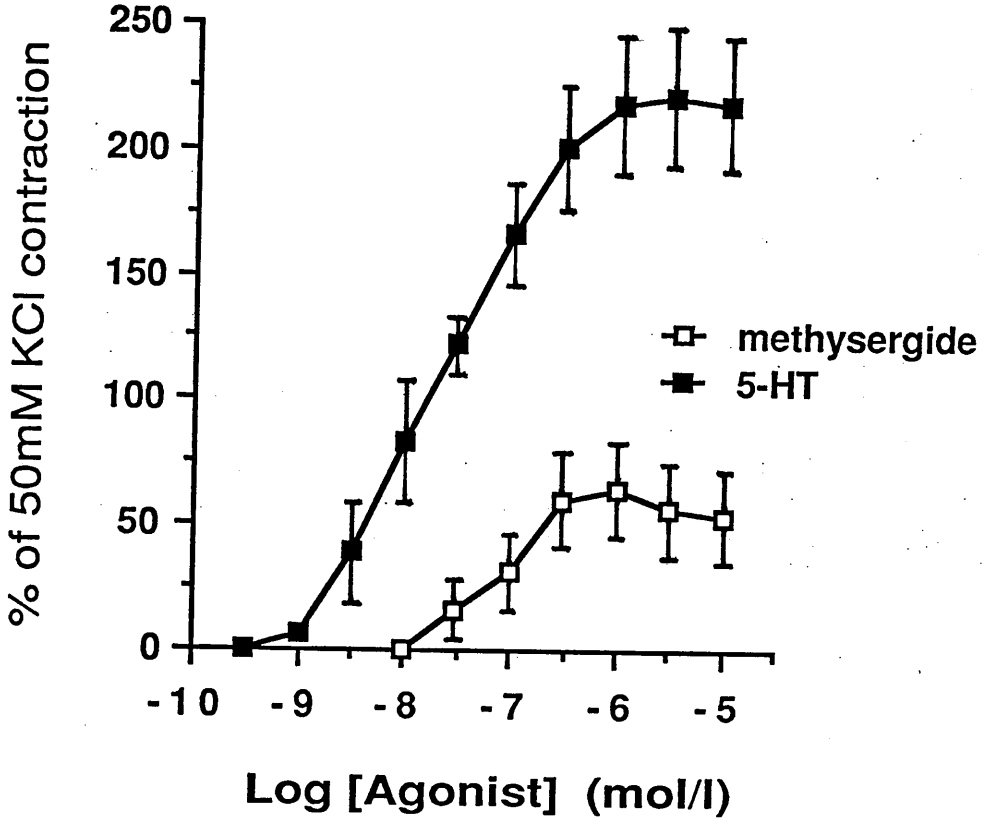
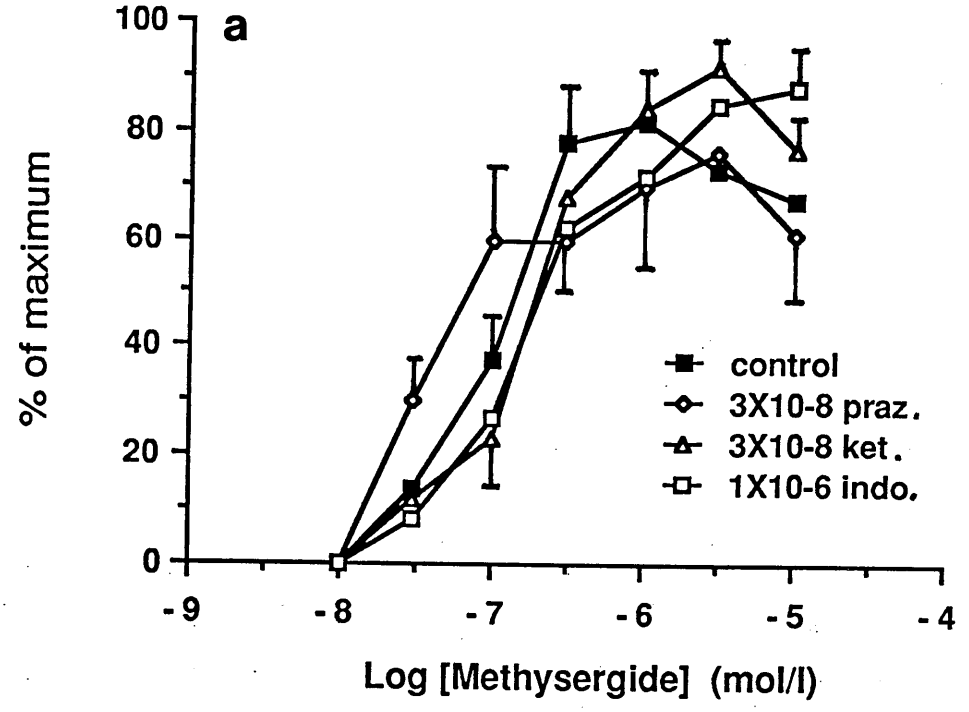
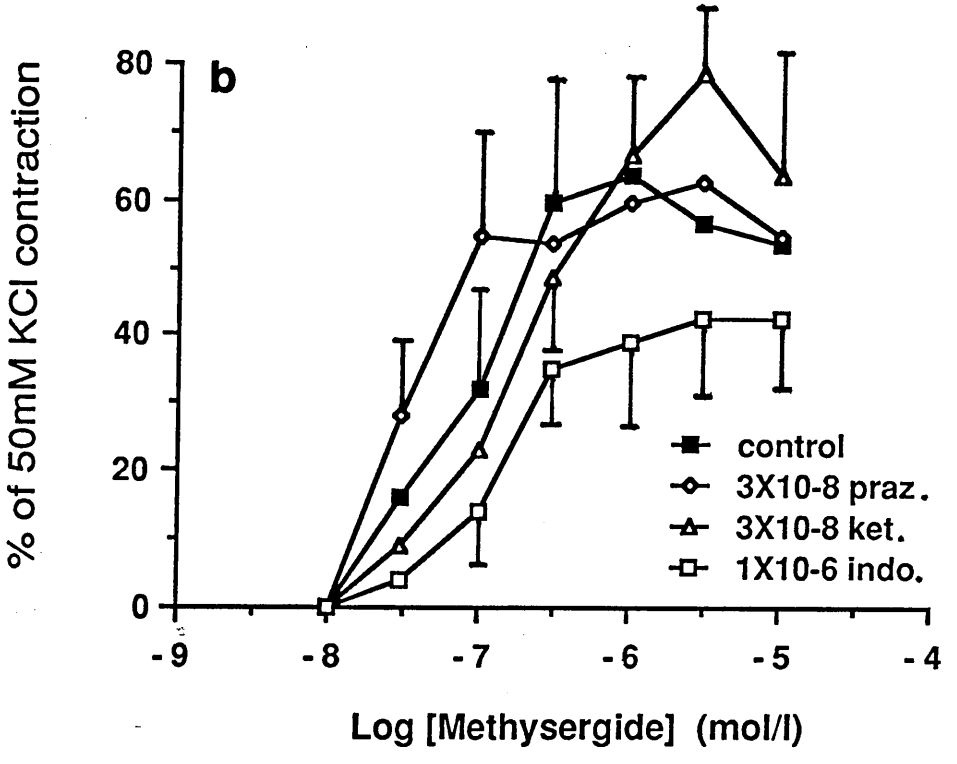


Figure 29 Log concentration-response curves to 5-HT (n=6) and methysergide (n=7) in longitudinal strips of umbilical artery at high Po_2 (~120mmHg), non-paired tissues. Responses were calculated as a % of a 50mM KCl contraction. Error bars are the mean±s.e.mean.

Figure 30 Log concentration-response curves (CRC's) to methysergide in longitudinal strips of umbilical artery (n=7) at high Po_2 (124±4mmHg). Since CRC's to methysergide were not reproducible four strips from each artery were investigated in parallel: one strip acted as a control while the following drugs were added to three other strips: prazosin ($3X10^{-8}M$); ketanserin ($3X10^{-8}M$) and indomethacin ($1X10^{-6}M$). CRC's to methysergide were then constructed in parallel in all four strips. Response (ordinate) was calculated in two ways: (a) as a % of the maximum response in the same strip and (b) as a % of a 50mM KCl contraction in the same strip. For clarity, error bars (mean±s.e.mean) are omitted at some points.


a
100
80
60
40
20
0
% of maximum
-9
-8
-7
-6
-5
-4
Log [Methysergide] (mol/l)
control
3X10-8 praz.
3X10-8 ket.
1X10-6 indo.


b
80
60
40
20
0
% of 50mM KCl contraction
-9
-8
-7
-6
-5
-4
Log [Methysergide] (mol/l)
control
3X10-8 praz.
3X10-8 ket.
1X10-6 indo.

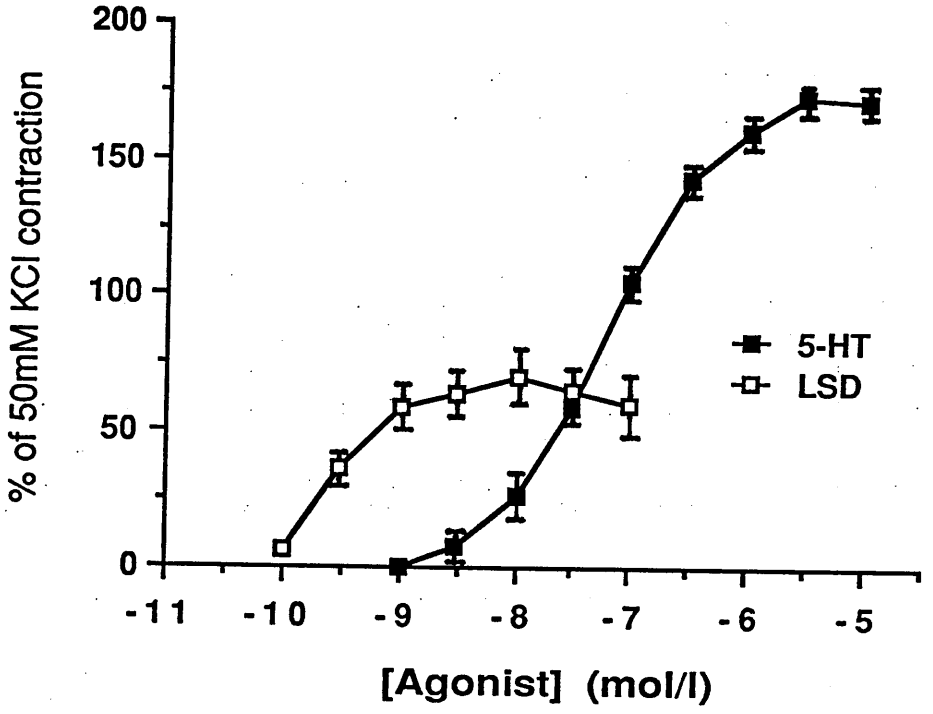


Figure 31 Log concentration-response curves to 5-HT and LSD in longitudinal strips of umbilical artery at low Po_2 (10±1mmHg), paired tissues (n=5). Responses were calculated as a % of a 50mM KCl contraction. Error bars are the mean±s.e.mean.

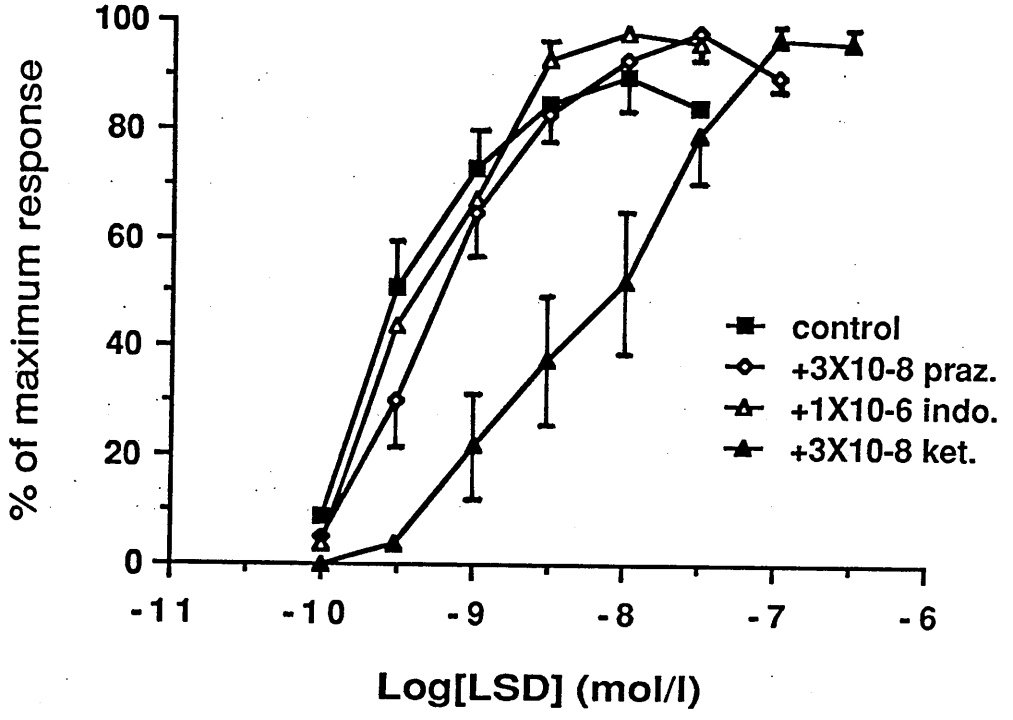
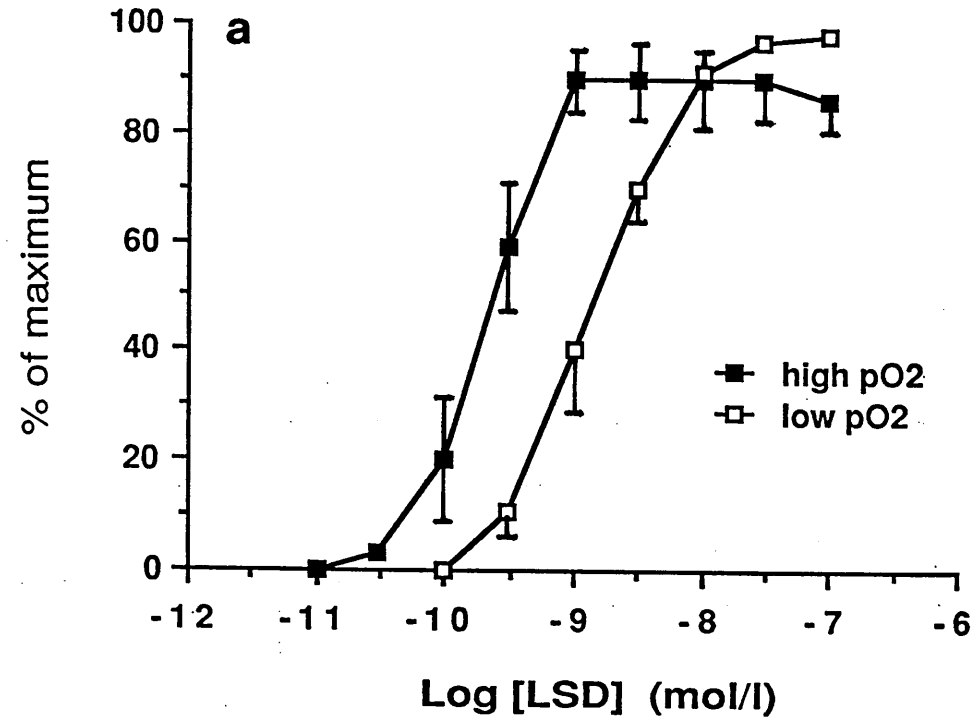
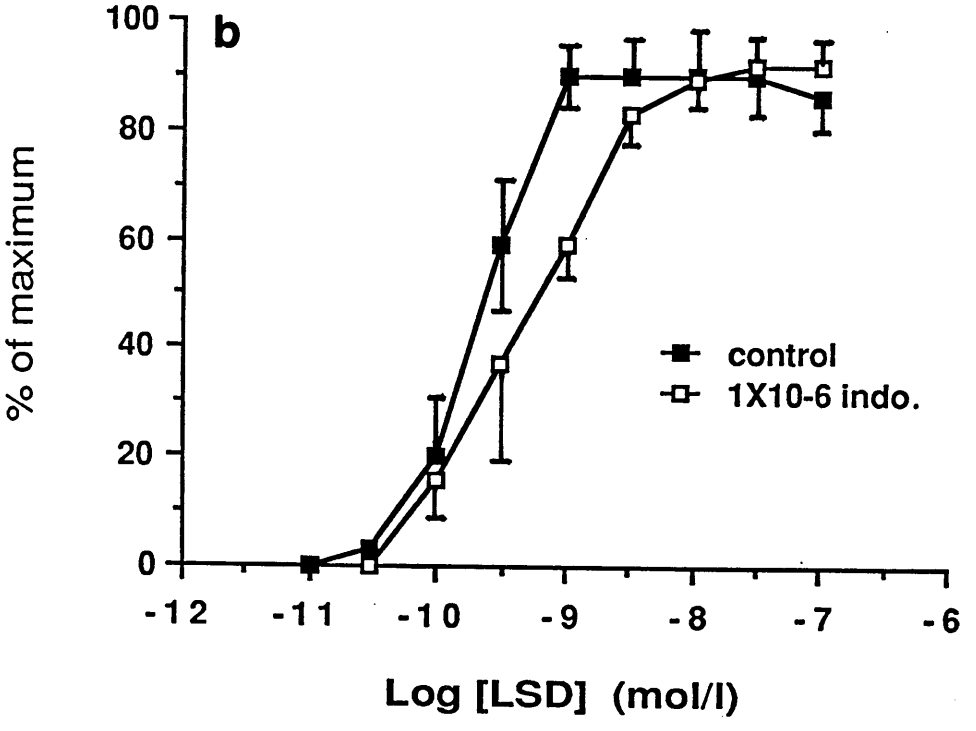


Figure 32 Log concentration-response curves (CRC) to LSD in longitudinal strips of umbilical artery at low Po_2 (17±4mmHg). Since CRC's to LSD were not reproducible four strips from the one artery (n=5) were investigated in parallel: one strip acted as a control while the following drugs were added to the other three strips: prazosin ($3X10^{-8}M$), indomethacin ($1X10^{-6}M$) and ketanserin ($3X10^{-8}M$). For clarity error bars (mean±s.e.mean) are omitted at some points.


a
100
80
60
40
20
0
% of maximum
high pO2
low pO2
-12
-11
-10
-9
-8
-7
-6
Log [LSD] (mol/l)


b
100
80
60
40
20
0
% of maximum
control
1X10-6 indo.
-12
-11
-10
-9
-8
-7
-6
Log [LSD] (mol/l)

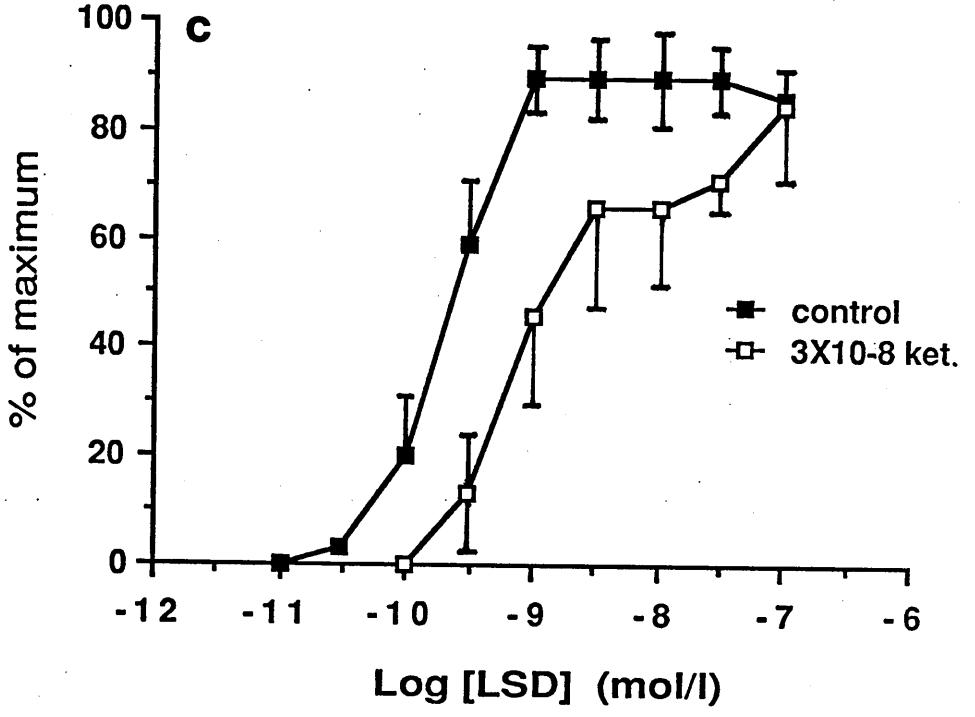


Figure 33 Log concentration-response curves (CRC) to LSD in longitudinal strips of umbilical artery: (a) at low Po_2 (16±3mmHg) and at high Po_2 (128±2mmHg); (b) at high Po_2 (128±2mmHg) in the presence of indomethacin ($1X10^{-6}M$); (c) at high Po_2 in the presence of ketanserin ($3X10^{-8}M$). Because CRC's to LSD were not reproducible, four strips from each artery were investigated in parallel under the different experimental conditions as described, i.e., at low Po_2; high Po_2; high Po_2 plus indomethacin; high Po_2 plus ketanserin. Response was calculated as a % of the maximum. Error bars are the mean±s.e.mean.

potency was only partially reversed by indomethacin ($1X10^{-6}M$): the shift at the EC_{50} was only 2.2 fold (1.2–4.2), (figure 33b).

In the presence of ketanserin ($3X10^{-8}M$) the response to LSD was very variable e.g. the EC_{50} range was 0.02–41 nM. There was no significant shift at either the EC_{25} or EC_{75}, in the presence of ketanserin (figure 33c).

Role of the endothelium

The possible involvement of the endothelium in the response to 5-HT was investigated. CRC's to 5-HT were constructed at high Po_2 in paired preparations, one preparation of each pair having had the endothelium removed by mechanical abrasion (using the edge of forceps). The sensitivity to 5-HT in rubbed and unrubbed preparations was not significantly different: in rubbed preparations the pD_2 was 7.63 ± 0.27 and in unrubbed preparations was 7.69 ± 0.27 (figure 34). Neither value was significantly different from that already found at high Po_2 of 7.75 ± 0.08 (see figure 12a).

In rubbed preparations ketanserin ($1X10^{-7}M$) caused a biphasic antagonism of the response to 5-HT at high Po_2 (figure 35). Lower concentrations of 5-HT were weakly antagonised and there was an inflection in the curve at concentrations of 5-HT causing ~50% of the maximum response. This biphasic antagonism was changed to a monophasic parallel shift in the presence of indomethacin ($1X10^{-6}M$). This is similar to the effect of ketanserin in unrubbed preparations (see figure 14b). The presence and absence of the endothelium, in unrubbed and rubbed preparations respectively, was confirmed in histological sections of the tissue (see chapter 2).

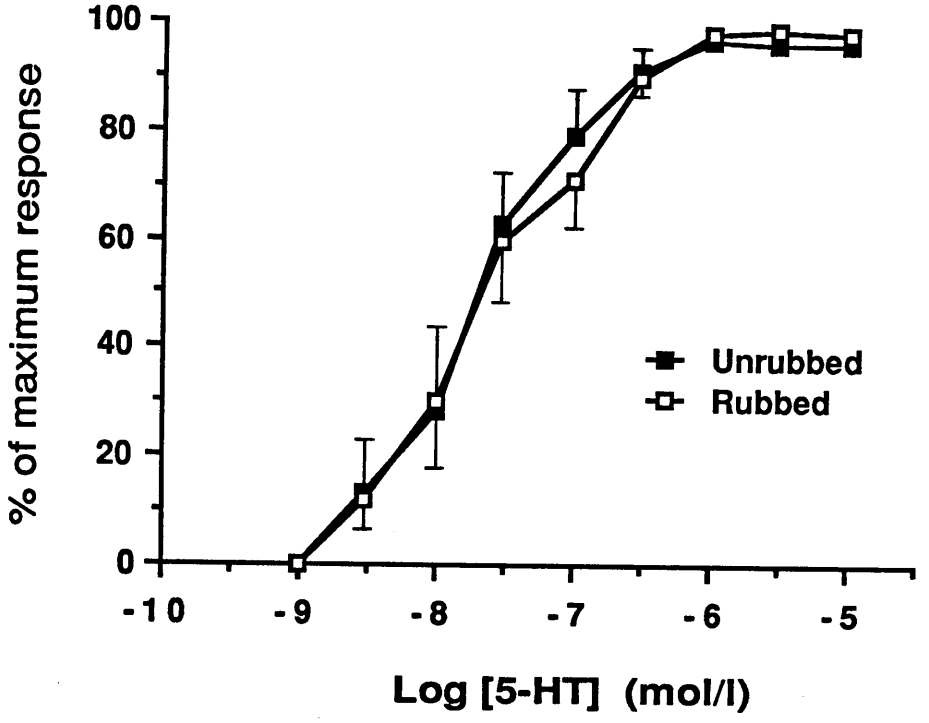


Figure 34 Log concentration-response curves to 5-HT at high Po_2 (123±4mmHg) in paired strips of umbilical artery (n=5). One strip of each pair had the endothelium removed by mechanical abrasion (rubbed) while in the other strip the endothelium was left intact (unrubbed). Error bars are the mean±s.e.mean.

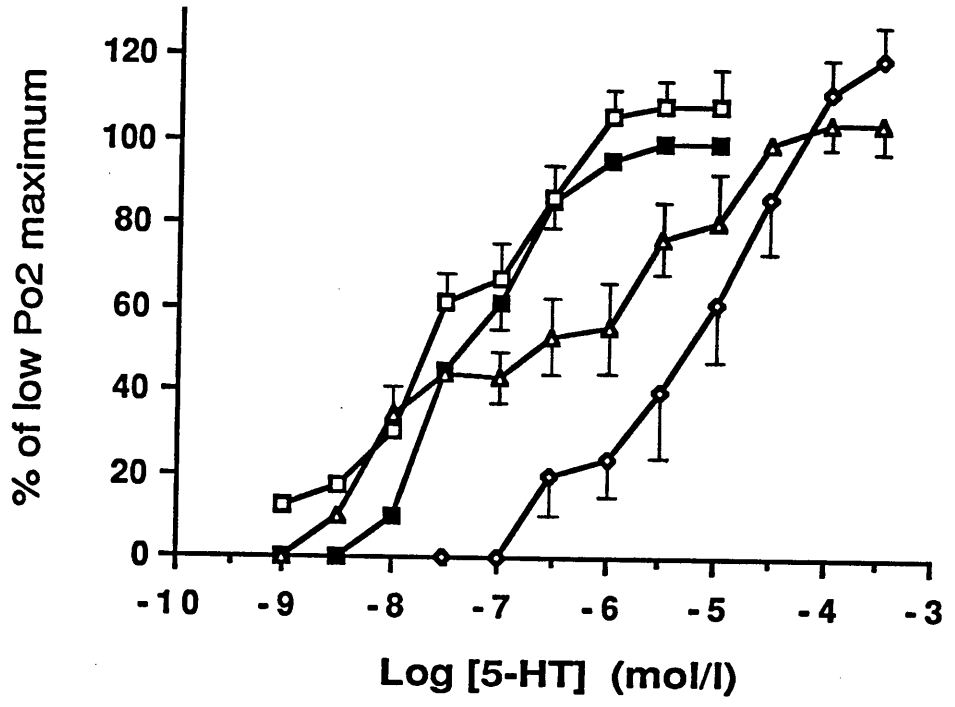


Figure 35 Log concentration-response curves to 5-HT in the presence of ketanserin ($1X10^{-7}M$) at high Po_2 in longitudinal strips of umbilical artery in which the endothelium was removed (by mechanical abrasion). (n=5). The protocol was to first construct a CRC to 5-HT at low Po_2 (12±2mmHg), (■). Following washout the Po_2 was then increased (to 121±1mmHg), ketanserin ($1X10^{-7}M$) was added and the CRC to 5-HT repeated (△). A third CRC to 5-HT was then constructed in the presence of ketanserin ($1X10^{-7}M$) plus indomethacin ($1X10^{-6}M$) at the high Po_2 (◇). A control CRC to 5-HT at high Po_2 (□), (from non-paired experiments, figure 12) is shown. For clarity, error bars (mean±s.e.mean) are omitted at some points.

PART 2: Receptors for adrenaline

The possible involvement of alpha-adrenoceptors in contraction of the human umbilical artery (HUA) was examined by cumulative additions of adrenaline, and of synthetic alpha-agonists, at three oxygen tensions (Po_2): (i) ~15mmHg (2.5% O_2), (ii) ~120mmHg (17% O_2), (iii) ~500mmHg (92% O_2). The figures in parenthesis are the O_2 compositions of the gas mixtures used to aerate the saline. The pH was held constant at 7.28 by maintaining the CO_2 content of the gas mixtures at 8%.

(i) At the physiological Po_2 (~15mmHg) adrenaline did not contract the HUA (n=18). Other alpha-agonists were not tested.

(ii) At Po_2 121±2mmHg (mean±s.e.mean) adrenaline was a weak agonist and in initial experiments contracted 10 of 15 preparations. In further experiments the proportion of tissues which were contracted by adrenaline was similar. Those preparations not contracted by adrenaline were shown to be viable by contraction to KCl and 5-HT.

In tissues which were contracted, adrenaline induced stepwise concentration-related contractions but which were not maintained at the highest concentrations (figure 36). Adrenaline was less potent than 5-HT and the maximum response was significantly smaller: The pD_2 for adrenaline was 6.13±0.11 and in the same preparations the pD_2 for 5-HT was 7.68±0.13. Thus 5-HT was 45 fold (geometric mean, 95% confidence limits, 10–158) more potent than adrenaline. The maximum response to adrenaline was 37±9% of the maximum response to 5-HT (figure 37a).

(iii) Increasing the Po_2 to 500±10mmHg did not increase the proportion of preparations contracted by adrenaline which was 7 of 15. The potency of adrenaline was actually lower than at Po_2 ~120mmHg: the pD_2 was 5.89±0.32 (although this was not significantly smaller than at Po_2=120mmHg). The potency of 5-HT was greater than at Po_2 ~120mmHg: the pD_2 was 7.84±0.16 (figure 37b). Thus 5-HT was 112 fold (9.5–1320) more

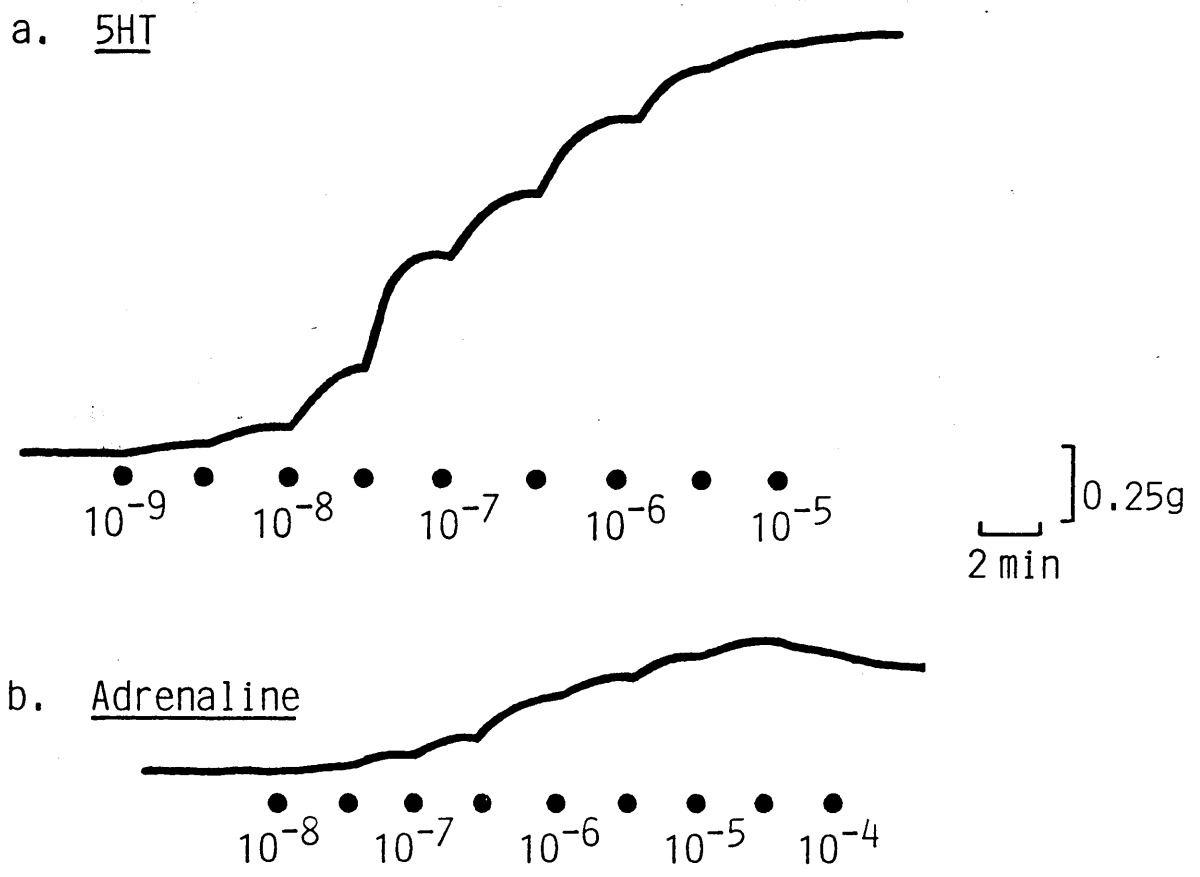
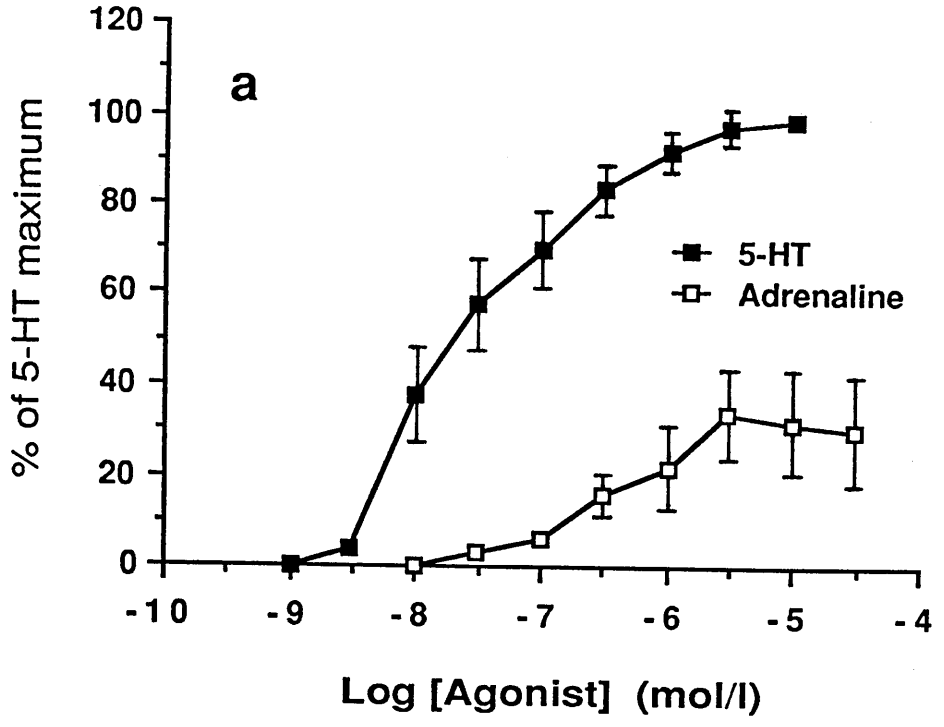
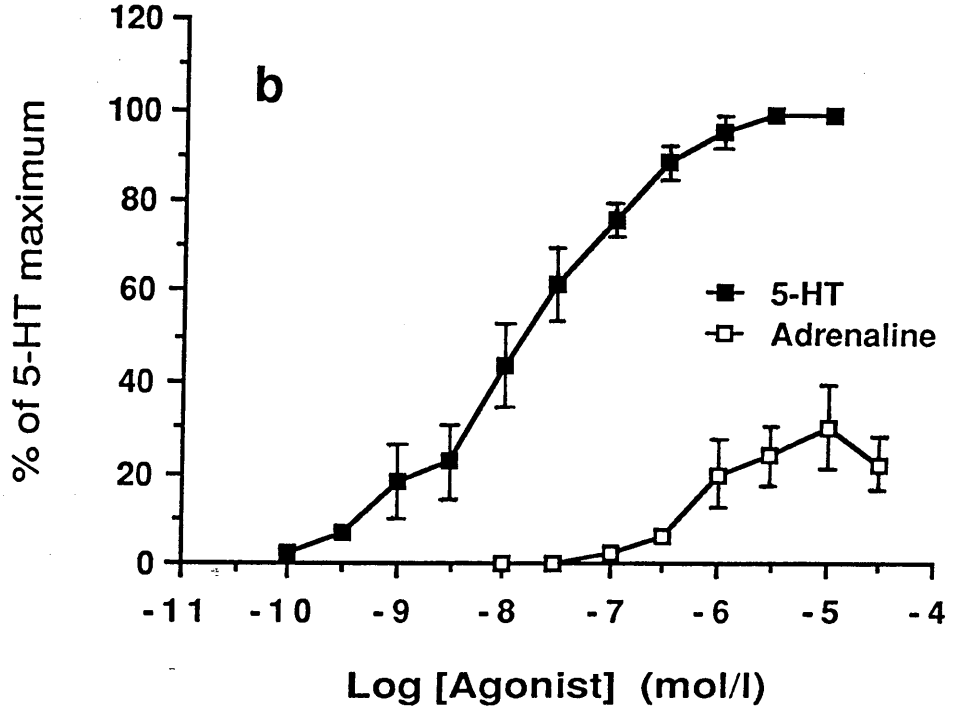


Figure 36 Tracings of representative recordings of concentration-response curves to (a) 5-HT and (b) adrenaline in one longitudinal strip of human umbilical artery at high Po_2 (~120mmHg). Responses to 5-HT were well maintained at all concentrations. Responses to adrenaline were generally not maintained at the higher concentrations.

Figure 37 Log concentration-response curves to 5-HT and adrenaline in paired preparations of longitudinal strips of umbilical artery. (a) at Po_2=121±1mmHg, n=10; (b) at Po_2=500±10mmHg, n=6. Response (ordinate) is expressed as a % of the maximum response to 5-HT. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.


a
% of 5-HT maximum
120
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
5-HT
Adrenaline
Log [Agonist] (mol/l)


b
% of 5-HT maximum
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
-4
5-HT
Adrenaline
Log [Agonist] (mol/l)

potent than adrenaline. The maximum response was 39±8% of the maximum response to 5-HT, which was not significanly different from the maximal response at Po_2 ~120mmHg.

Effect of prazosin

At Po_2 ~500mmHg the effect of the synthetic $alpha_1$ antagonist, prazosin, was tested against the response to adrenaline. Prazosin ($3X10^{-8}M$) caused a parallel rightward shift of the adrenaline CRC (figure 38a). From concentration-ratios calculated at the EC_{50} the pK_B was estimated to be 8.72 (7.30–10.04). Prazosin ($3X10^{-8}M$) did not antagonise the response to 5-HT (figure 38b). If anything there was a slight leftward shift of the CRC, but this was not significant.

As it was found that raising the Po_2 from ~120mmHg to hyperoxic levels (~500mmHg) did not impart any advantage in studying the response to adrenaline, in terms of potency or maximal size of response, the former, more physiological Po_2, was employed in all other experiments.

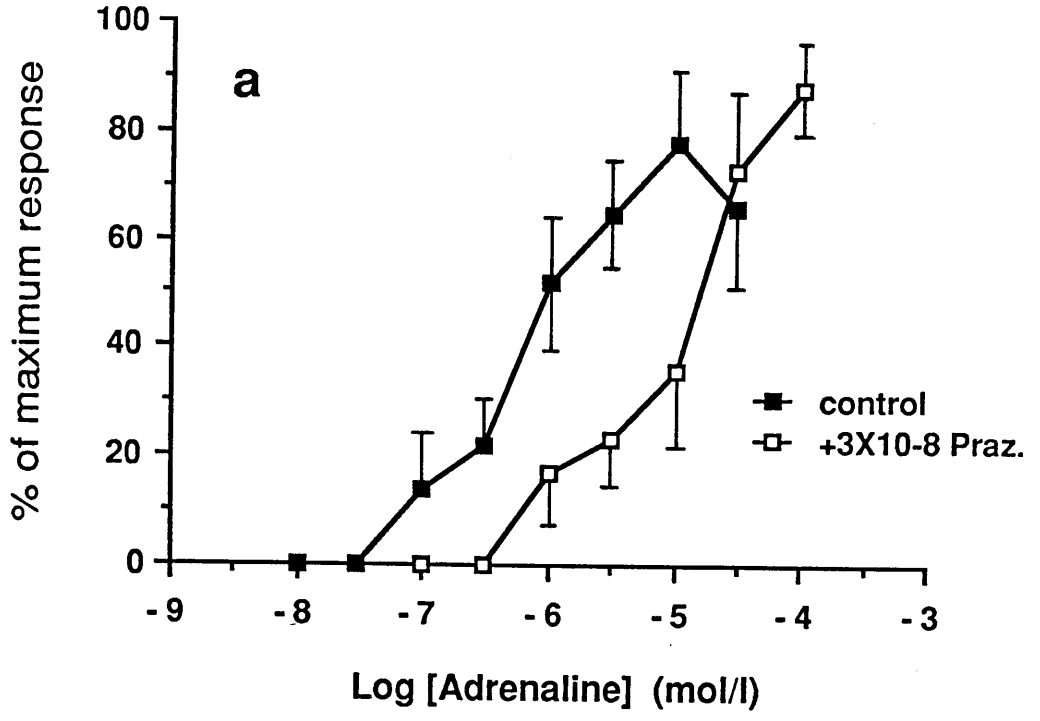
Effect of Wyeth 26703 (WY 26703)

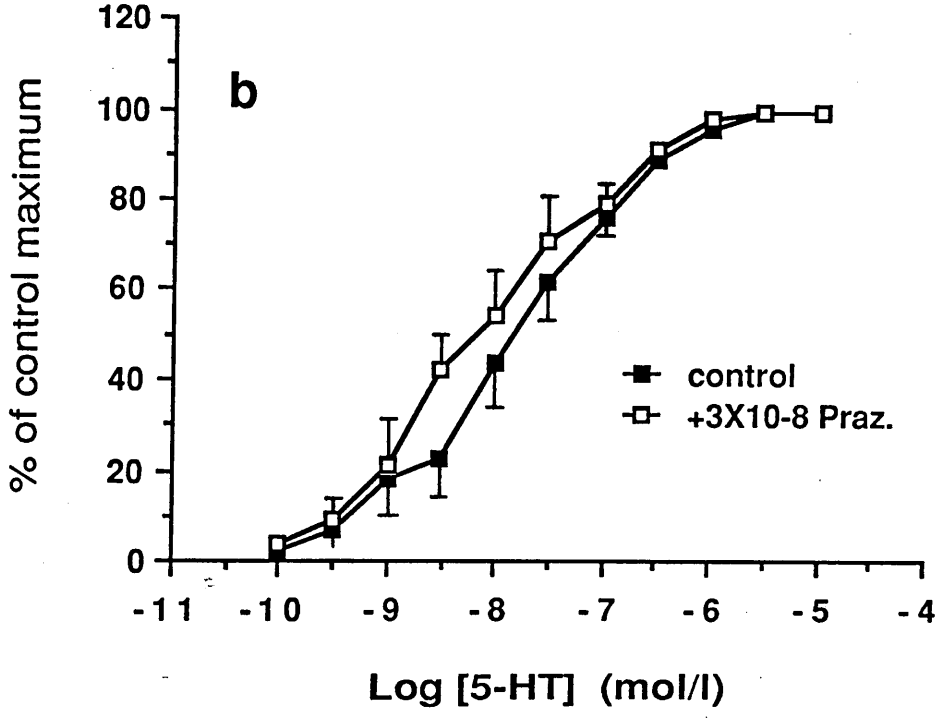
The selective $alpha_2$-antagonist WY 26703 ($3X10^{-8}M$) did not antagonise the response to adrenaline (figure 39a) or 5-HT (figure 39b). There was a small rightward shift of the adrenaline CRC but this was not significant. WY 26703 ($1X10^{-5}M$) caused a parallel shift of the CRC to 5-HT: pK_B values were calculated at the EC_{25} and EC_{75} as measures of the potency against the two components of the response to 5-HT at high Po_2 (~120mmHg), (see part 1). At the EC_{25} the pK_B was 7.15 (6.19–8.11) and at the EC_{75} was 6.94 (5.60–8.28) which was not significantly different.

Compound X and indomethacin

Neither compound X ($3X10^{-8}M$), a selective $5\text{-}HT_2$ antagonist nor indomethacin ($1X10^{-6}M$), a cyclo-oxygenase inhibitor, antagonised the

Figure 38 Log concentration-response curves (CRC's) to (a) adrenaline and (b) 5-HT in longitudinal strips of umbilical artery at Po_2=500±10mmHg in the presence of prazosin ($3X10^{-8}M$). Since CRC's to adrenaline were not reproducible, they were constructed in parallel in different strips from the same artery. One strip acted as a control and prazosin was added to another strip before constructing CRC's to adrenaline. Response (ordinate) was calculated as a % of the maximum response to adrenaline in each strip. CRC's to 5-HT were constructed in the presence and absence (control) of prazosin in the same strip. Response was calculated as a % of the control maximum response. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.


a
% of maximum response
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
-3
control
+3X10-8 Praz.
Log [Adrenaline] (mol/l)


b
% of control maximum
120
100
80
60
40
20
0
-11
-10
-9
-8
-7
-6
-5
-4
control
+3X10-8 Praz.
Log [5-HT] (mol/l)

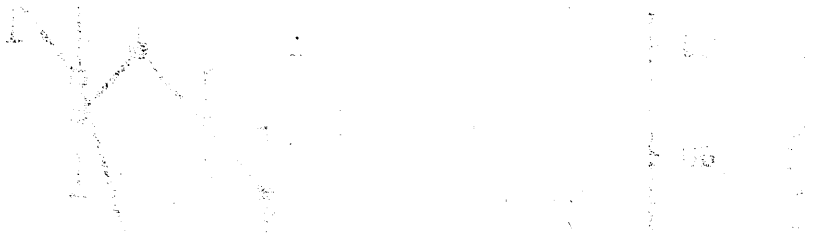
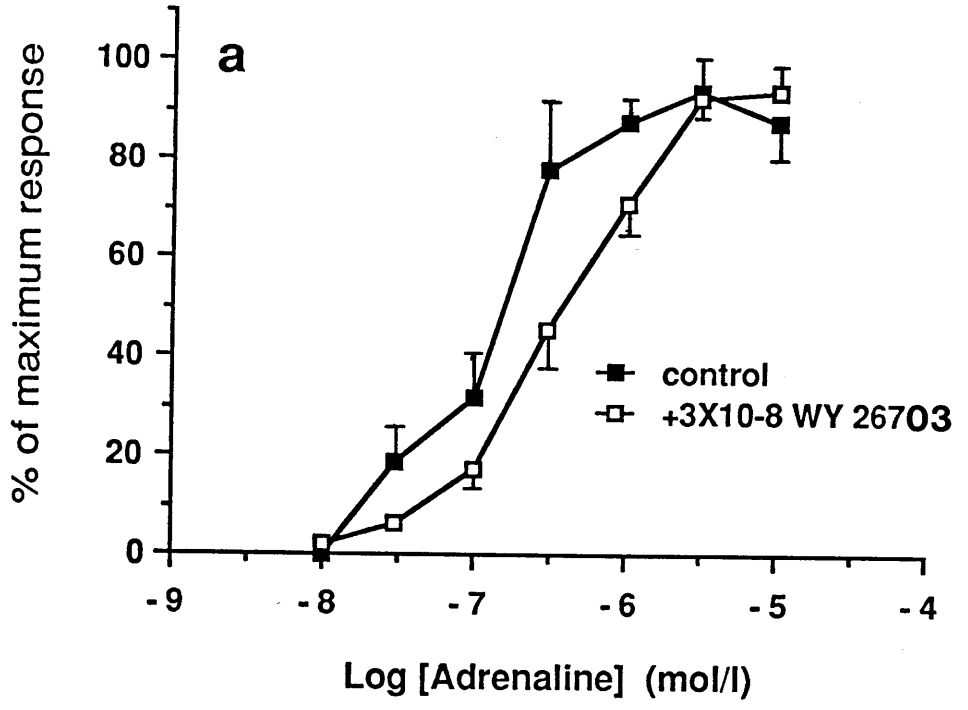
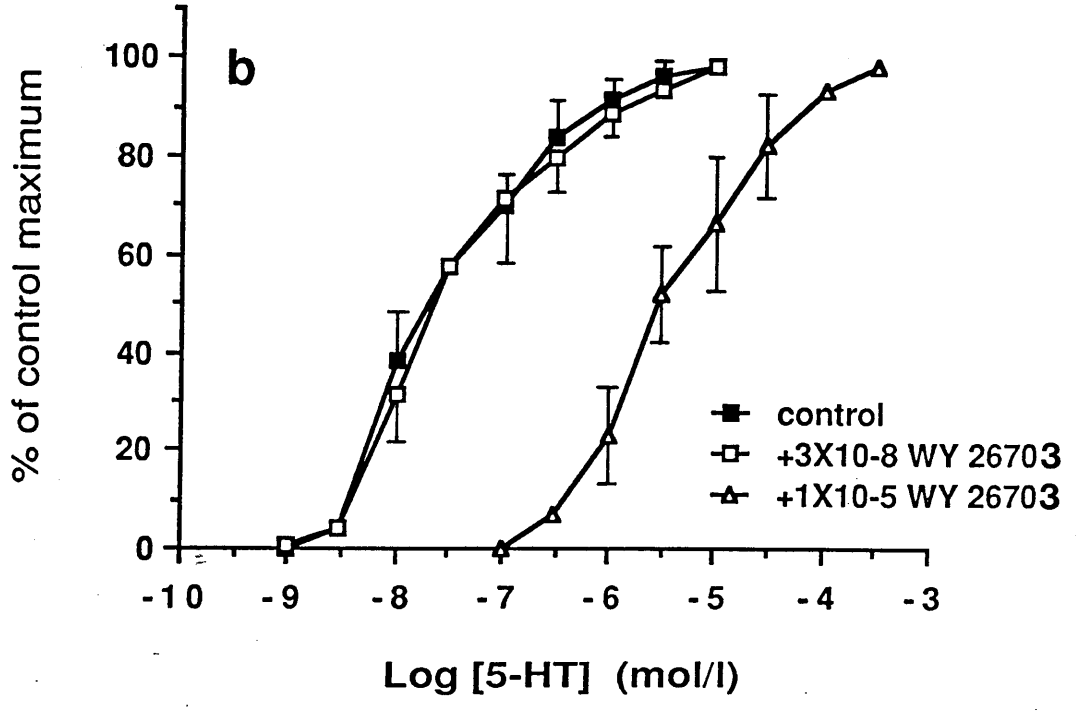


Figure 39 Log concentration-response curves (CRC's) to (a) adrenaline and (b) 5-HT, in longitudinal strips of umbilical artery (n=4) at high Po_2 (119±1mmHg) in the presence of Wyeth 26703. Since CRC's to adrenaline were not reproducible, they were constructed in parallel in different strips from the same artery. One strip acted as a control and Wyeth 26703 was added to another strip before constructing CRC's to adrenaline. Response (ordinate) was calculated as a % of the maximum response to adrenaline in each strip. CRC's to 5-HT were constructed in the presence and absence (control) of Wyeth 26703 in the same strip. Response was calculated as a % of the control maximum response. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.


a
% of maximum response
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
control
+3X10-8 WY 26703
Log [Adrenaline] (mol/l)


b
% of control maximum
100
80
60
40
20
0
-10
-9
-8
-7
-6
-5
-4
-3
control
+3X10-8 WY 26703
+1X10-5 WY 26703
Log [5-HT] (mol/l)

response to adrenaline (figures 40a and 40b respectively).

Synthetic alpha-agonists

The potencies of the selective alpha-adrenoceptor agonists phenylephrine ($alpha_1$), xylazine ($alpha_2$) and UK 14304 ($alpha_2$) were compared to that of adrenaline. CRC's to each agonist were constructed in separate preparations from the same artery. In arteries contracted by adrenaline, phenylephrine was 32.3 (4.3–204) fold less potent than adrenaline while xylazine and UK 14304 did not contract the HUA (figure 41). Preparations not contracted by adrenaline were not contracted by these other synthetic alpha-adrenoceptor agonists.

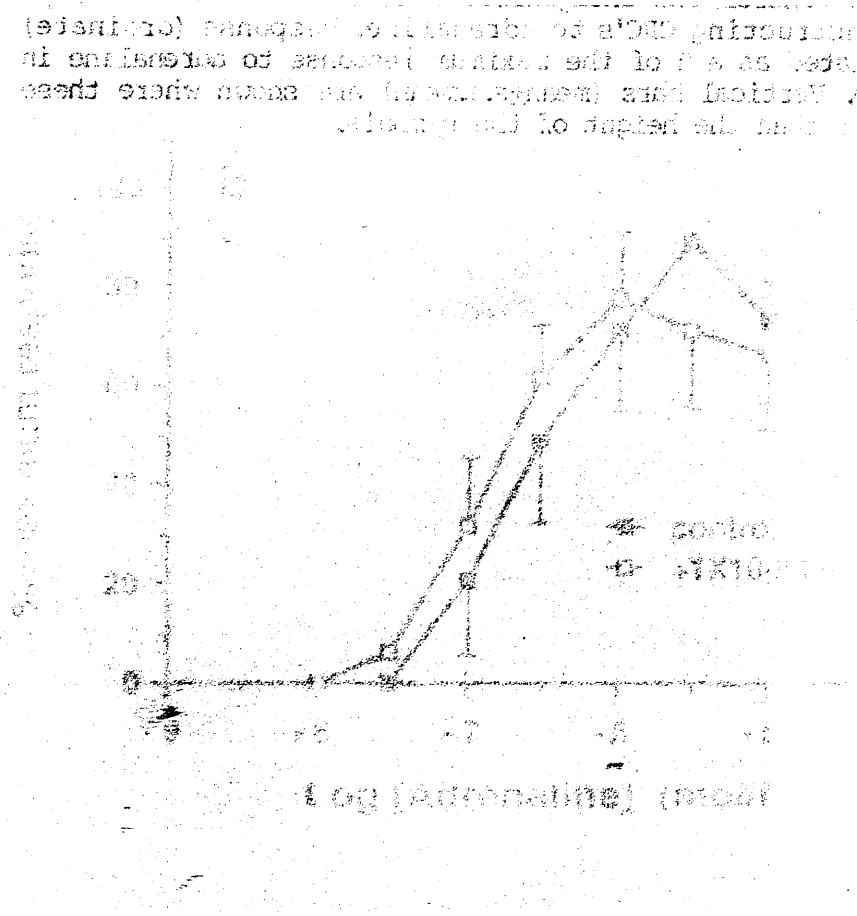
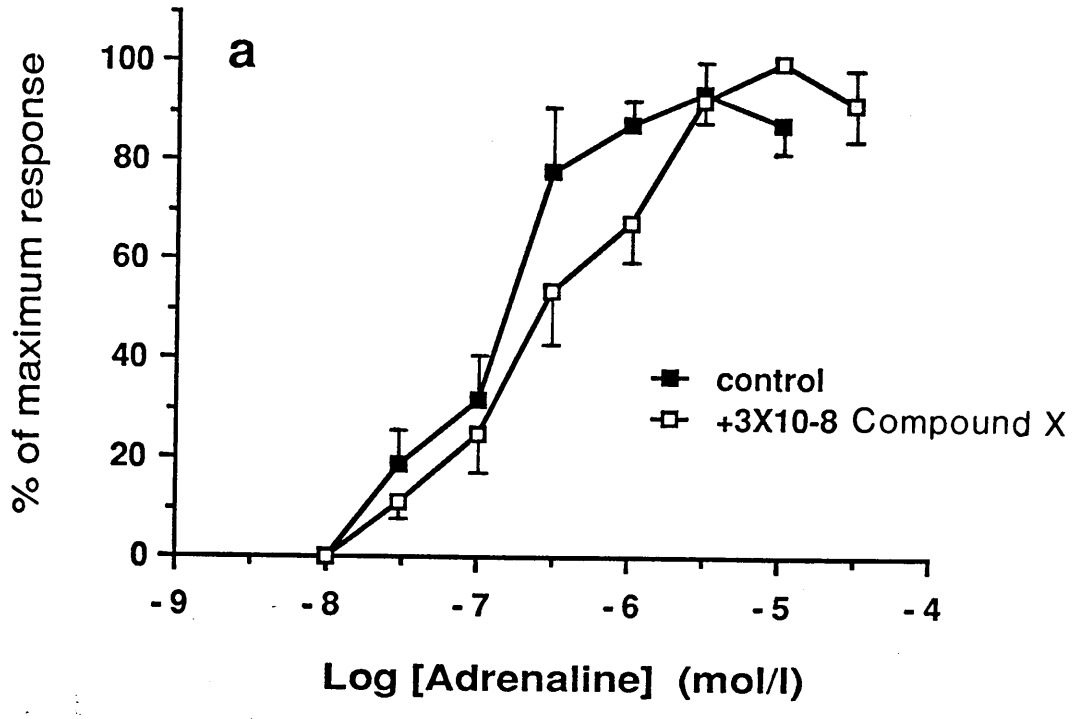


Figure 40 Log concentration-response curves (CRC's) to adrenaline in longitudinal strips of umbilical artery at high Po_2 in the presence of (a) compound X (Po_2=121±4mmHg, n=4) and (b) indomethacin (Po_2=117±4mmHg, n=6). Since CRC's to adrenaline were not reproducible, they were constructed in parallel in different strips from the same artery. One strip acted as a control and antagonists were added to other strips before constructing CRC's to adrenaline. Response (ordinate) was calculated as a % of the maximum response to adrenaline in each strip. Vertical bars (mean±s.e.mean) are shown where these are greater than the height of the symbols.


a
% of maximum response
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
Log [Adrenaline] (mol/l)
control
+3X10-8 Compound X

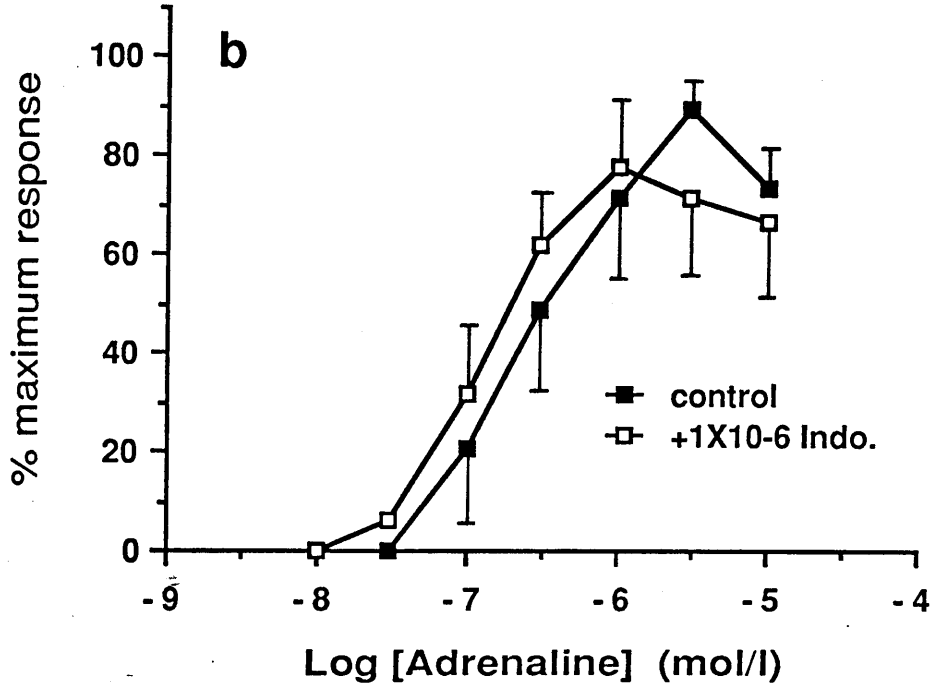
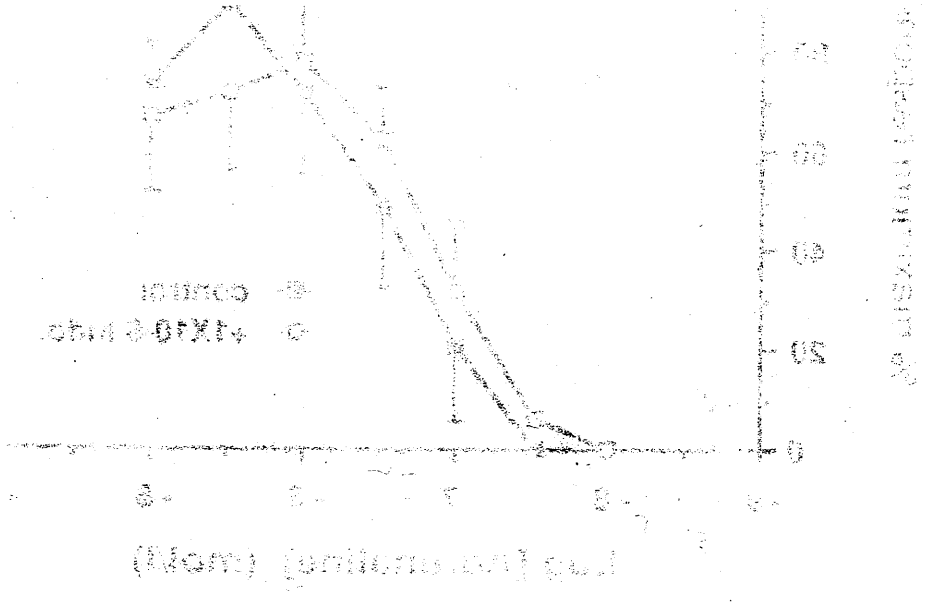
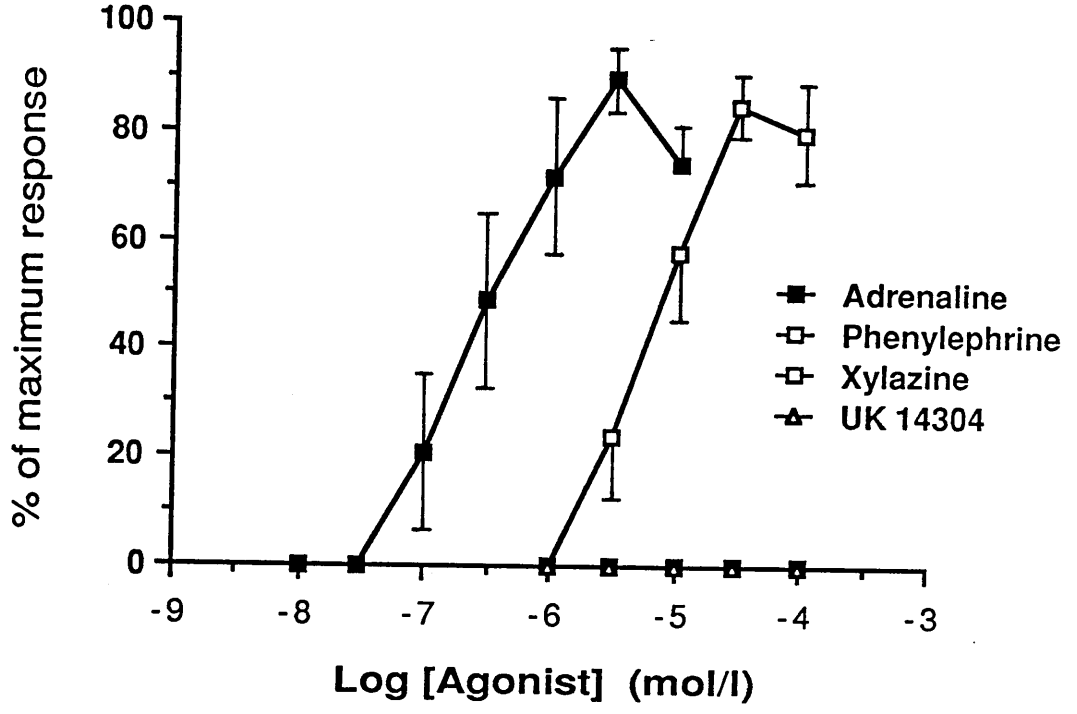

b
% maximum response
100
80
60
40
20
0
-9
-8
-7
-6
-5
-4
Log [Adrenaline] (mol/l)
control
+1X10-6 Indo.

Figure 41 Log concentration-response curves (CRC's) to adrenaline (n=6); phenylephrine (n=6); xylazine (n=6), and UK 14304 (n=4) at high Po_2 (121±3mmHg) in longitudinal strips of umbilical artery. CRC's to each agonist were constructed in separate strips from the same artery. Response (ordinate) was calculated as a % of the maximum response to each agonist. Vertical bars are the mean±s.e.mean.




100
80
60
40
20
0
% of maximum response
-9
-8
-7
-6
-5
-4
-3
Log [Agonist] (mol/l)
Adrenaline
Phenylephrine
Xylazine
UK 14304

Discussion

In this study raising the oxygen tension (Po_2) from ~15mmHg (low Po_2) to ~120mmHg (high Po_2) had a pronounced facilitatory effect on the potency of drugs found to be agonists of the isolated human umbilical artery (HUA), and on the interaction of various antagonists with these agonists. Increasing the Po_2 without exception led to an increase in potency of agonists (5-HT, 5-CT, methysergide, LSD and adrenaline) and antagonists, which at low Po_2 were shown to be simple competitive antagonists, at high Po_2 were found to have complex interactions with the agonist. The receptor(s) which may mediate the response to these agonists at the two different levels of oxygen will be discussed separately.

The receptor for 5-HT at low Po_2

The procedures for the characterization of adrenoceptors (Furchgott, 1972) can equally well be used as a guideline for the characterization of 5-HT receptors in isolated tissue (see Humphrey, 1984). The procedures are (1) to establish the potency of competitively acting antagonists in blocking the response to an agonist and (2) a comparison of a rank order order of potency of a series of agonists. In this study the characterization of the receptor relies mainly on the former method as the potency of only two 5-HT receptor agonists (5-HT itself and 5-CT) were rigourously compared. As will be discussed below an attempt has been made to satisfy the desired optimal conditions, as outlined in these preceding papers, for the characterization of receptors.

Antagonists

Six antagonists were studied here: methysergide, phentolamine, ketanserin, compound X, cyproheptadine and LSD. Of these the first four were found to be silent competitive antagonists of the response to 5-HT

Table 10 Comparison of the affinities of competitively acting antagonists, in terms of their estimated pA_2's, at receptors for 5-HT in the rabbit aorta (Apperley et al, 1976; Fenuik et al, 1985), and in the human umbilical artery (HUA) at low Po_2 (~15mmHg).

Antagonist	Rabbit Aorta (pA_2)	HUA (pA_2)
Ketanserin	8.67 (8.38–8.95)	8.92 (8.70–9.14)
Methysergide	8.49 (7.85–9.14)	8.52 (8.32–8.72)
Phentolamine	6.21 (5.52–6.92)	6.37 (5.69–7.05)

pA_2 values are the means (95% confidence limits).

in the HUA. Cyproheptadine was a non-surmountable antagonist and LSD was a partial agonist and therefore cannot be considered in a classification of the receptor. A discussion of their effects will be made later.

The criteria which were met in order to classify these antagonists as competitive were (1) CRC's to 5-HT were displaced in a parallel manner without any significant depression of the maximum response and (2) a Schild plot (log{CR-1} vs log [antagonist]) had a slope not significantly different from unity.

Of these four competitively acting antagonists, compound X is a novel 5-HT antagonist and is still under research within the drug company. There is therefore no available published data with which to make a comparison of results. A comparison of the receptors in the HUA and other tissues relies then on a comparison of the potencies of ketanserin, methysergide and phentolamine.

Ketanserin and methysergide have been studied in many tissues but all three of the above mentioned antagonists have been studied together only in a few tissues. One such tissue is the isolated rabbit aorta (Apperley et al, 1976; Feniuk et al, 1985). The pA_2 values for these antagonists in the HUA and rabbit aorta are compared in Table 10. The values for each antagonist are similar and show that in both these preparations ketanserin and methysergide are potent antagonists while phentolamine is more than 100 times less potent against the response to 5-HT. From the pA_2 values for these antagonists it is concluded that the receptors for 5-HT in the HUA, at low Po_2, and in the rabbit aorta are the same.

However if characterization of 5-HT receptors is to depend entirely on the use of specific competitively-acting blocking drugs (Humphrey, 1984) then phentolamine should not be considered since in

Table 11 Comparison of the affinities of the antagonists ketanserin and methysergide for the 5-HT_2 binding site in brain tissue (rat prefrontal cortex) and the receptor for 5-HT in the human umbilical artery (HUA).

	Binding[1] Site	Receptor[2] in HUA
Antagonist		
Ketanserin	9.04	8.92 (8.70–9.14)
Methysergide	8.48	8.52 (8.32–8.72)

1 Values are $-\text{Log}K_i$ (mean), calculated from Leysen et al, 1982.
2 pA_2 values estimated from a Schild analysis and are the mean (95% confidence limits).

tissues where it has been studied against responses to 5-HT and noradrenaline, such as the rabbit aorta (Apperley et al, 1976) and dog coronary artery (Muller-Schweinitzer, 1980) phentolamine has been shown to be about 50 fold more potent at alpha-adrenoceptors than at 5-HT receptors.

A comparison of the affinities of the antagonists ketanserin and methysergide at the 5-HT_2 recognition site in radiolabelled ligand binding studies (Leysen et al, 1982) and the pA_2 values from the present study in HUA are given in Table 11. It is clear that there is a marked similarity of the affinities of the antagonists for the 5-HT_2 recognition site in brain tissue and for the receptor in the HUA and suggests therefore that they are "one and the same". Hence the receptor for 5-HT in the HUA at low Po_2 can be classified as a 5-HT_2 type receptor.

Other studies (Humphrey et al, 1982; Maayani et al, 1984) which have compared the affinities of a series of antagonists for the 5-HT_2 recognition site and the 5-HT receptor in rabbit aorta showed the similarity of these two sites and hence give support to the idea that the receptor in HUA can be called 5-HT_2 since, on the basis of the affinity of three antagonists, the receptors in HUA and rabbit aorta appear identical.

There is also evidence of a good correlation between the 5-HT_2 binding site and receptors mediating responses to 5-HT in the guinea-pig ileum (mediating contraction), (Engel et al, 1984, 1985) and in cat and human platelets (mediating aggregation), (De Clerck et al, 1984a, b). Many other functional responses have been reported to be mediated via 5-HT_2 receptors which include *in vitro*, contraction of vascular and non-vascular smooth muscle, and various other responses *in vivo*, both in the CNS and in the periphery (see Bradley et al, 1986a; Van Neuten et al, 1981), although in the latter study spurious claims were made to

explain the action of 5-HT in some isolated preparations, e.g. the blockade of the 5-HT-induced contraction of the dog saphenous vein by ketanserin was attribted to an effect at alpha receptors but this is evidently not the case as 5-HT acts at a specific "5-HT_1-like" receptor in this tissue (Apperley et al, 1976; Feniuk et al, 1985).

The importance of distinguishing between the effects of 5-HT which are mediated via specific 5-HT receptors and those mediated via alpha-adrenoceptors has been discussed elsewhere (Feniuk, 1984) since some 5-HT and alpha-adrenoceptor antagonists have appeciable affinity for alpha receptors and 5-HT receptors respectively (Apperely et al, 1976; Van Neuten et al, 1981). It has also been shown that alpha-agonists may act at 5-HT receptors (Vane, 1960) and that 5-HT agonists may act at alpha-adrenoceptors (Apperley et al, 1976).

In the HUA it can be considered that the action of 5-HT and that the action of the antagonists methysergide, ketanserin and phentolamine are solely due to their action at the 5-HT_2 receptor for the following reasons: (1) In the HUA it is concluded that there is only a very small population of functional alpha_1-adrenoceptors whose expression depends on a higher than physiological Po_2 and which are not activated by 5-HT. This conclusion was reached since prazosin antagonised the response to adrenaline (pK_B 8.72) while $3X10^{-8}M$ prazosin did not antagonise the response to 5-HT. In addition the selective alpha_2 agonists xylazine and UK 14304 were inactive. (2) The antagonists methysergide (whose affinity is 200 times greater for the 5-HT_2 binding site than for the alpha_1 binding site -Leysen et al, 1981) and ketanserin (which is extremely selective for the 5-HT_2 binding site as opposed to the 5-HT_1 binding site but has a high affinity for alpha_1-adrenoceptors -Leysen et al, 1981) had affinities for the vascular receptor in the HUA which were very similar to their affinities at the 5-HT_2 binding site (see

earlier) and therefore suggests no involvement of alpha-adrenoceptors.

Another consideration is that 5-HT can act as an indirect sympathomimetic (Innes, 1962; Humphrey, 1978; Humphrey et al, 1983) and can release endogenous compounds e.g. prostaglandins (Alabaster and Bakhle, 1976). The general consensus of opinion is that the HUA is not innervated (Spivack, 1943; Roach, 1972) so that the possibility that 5-HT could release endogenous noradrenaline is discounted, although the possibility was not pharmacologically examined.

At low Po_2 indomethacin did not antagonise the response to 5-HT but there was a small ketanserin-resistant, indomethacin-sensitive component which suggested a small 5-HT-stimulated release of prostaglandins. This would not seem to be via the $5\text{-}HT_2$ receptor since it was resistant to ketanserin, although an antagonist resistant component was not evident in the presence of either methysergide or phentolamine (CRC's in the presence of these antagonists were parallel). The only receptor (to my knowledge) which has been characterized as $5\text{-}HT_2$ and shown to stimulate prostaglandin release is in cultured bovine aortic smooth muscle cells (Coughlin et al, 1984). This receptor was characterized as $5\text{-}HT_2$ using a series of antagonists and the prostaglandin whose synthesis was mediated by this receptor was prostacyclin (PGI_2) which is almost invariably a vasodilator (Moncada and Vane, 1980). In the HUA however, the 5-HT-stimulated prostaglandin is a vasoconstrictor. It will be discussed in more detail later that the 5-HT-stimulated prostaglandin release is via a "$5\text{-}HT_1$-like" receptor.

The presence of this non-$5\text{-}HT_2$, prostaglandin-linked receptor did not significantly alter the Schild analysis for ketanserin however, since the pA_2 values determined in the presence and absence of indomethacin (8.94 and 8.92 respectively) were not significantly different.

The antagonist compound X provides supporting evidence for the conclusion that the receptor for 5-HT at low Po_2 can be described as 5-HT_2. In ligand binding studies compound X has been shown to be a selective ligand of high affinity (-logKi=8.55) for the 5-HT_2 recognition site while its affinity at 5-HT_1 and alpha-adrenoceptors is more than 1,000 fold lower (personal communication). This is similar to its affinity for the receptor in the HUA (pA_2=8.86) found in this study.

Cyproheptadine and LSD

Cyproheptadine was found to be a non-competitive antagonist in the HUA: in addition to causing a rightward shift of the CRC to 5-HT (pK_B 8.86) it caused a non-surmountable blockade (pD'_2 7.19). This was surprising since in the rabbit aorta and dog femoral artery (Apperley et al, 1976, 1980) the receptors for 5-HT have been described as 5-HT_2 (Humphrey, 1984) yet at which cyproheptadine acts as a competitive blocking drug. Cyproheptadine has been shown to possess Ca^{++} channel antagonist properties although this effect has as yet been demonstrated only against voltage-operated Ca^{++} channels (Lowe et al, 1981). As well as being a 5-HT receptor antagonist, cyproheptadine could also be an antagonist of receptor-operated Ca^{++} channels in the HUA. If this were the case it would point to a difference between the 5-HT_2 receptors in the rabbit aorta and HUA, with regard to the coupling between receptor occupancy and Ca^{++} influx.

In the HUA it was shown that at low Po_2 the response to 5-HT was partly dependent on extracellular Ca^{++} since the calcium channel facilitator Bay K 8644 and the calcium channel blocker, nifedipine, potentiated and antagonised the response to 5-HT respectively (see chapter 2). Since the response to 5-HT was not abolished when the extracellular Ca^{++} concentration ($[Ca^{++}]_e$) was reduced to zero, a

mobilization of intracellular Ca^{++} may also be involved. (The $[Ca^{++}]_e$ was reduced to "zero" by omitting Ca^{++} from the saline so reducing the $[Ca^{++}]_e$ to the contamination level of ~$2X10^{-5}M$, Miller and Smith, 1984). The response to 5-HT in the isolated rabbit aorta is also dependent on extracellular Ca^{++} since when the $[Ca^{++}]_e$ was reduced to zero (by omitting Ca^{++} from the saline) the response to 5-HT ($1X10^{-5}M$) was significantly reduced but not abolished (Ratz and Flaim, 1984). If cyproheptadine is a receptor-operated Ca^{++} channel blocker in the HUA this would suggest a difference of the susceptibility to blockade of the Ca^{++} channels in HUA and rabbit aorta, as in the latter preparation cyproheptadine was a simple competitive receptor antagonist. Such differences between tissues as to their susceptibility to blockade by Ca^{++} antagonists has been established (Van Neuten and Vanhoutte, 1981). In the absence of such an additional effect it would have to be concluded that the receptor in the HUA is different with respect to cyproheptadine from the $5\text{-}HT_2$ receptor in rabbit aorta and dog femoral artery.

LSD was found to be a potent but partial agonist (judged by its maximum response with respect to 5-HT) in the HUA: LSD was more than 100 fold more potent than 5-HT while the maximum response was ~30% of that to 5-HT. LSD was also found to be an antagonist of the response to 5-HT but only at concentrations which caused a contraction (except at $1X10^{-10}M$ which caused a small antagonism. In another series of experiments $1X10^{-10}M$ LSD caused a very small contraction). Clearly $1X10^{-10}M$ is at the very threshold for contraction of HUA. Therefore from classical drug-receptor theory (Ariens, 1964) LSD would be described as a partial agonist at the $5\text{-}HT_2$ receptor. This conclusion is supported by the finding that the dissociation constant for ketanserin (or rather the pK_B), with LSD as the agonist was 8.90, thus suggesting that the response to LSD was mediated by the $5\text{-}HT_2$ receptor.

Based on a simple comparison of the EC_{50}'s of LSD and 5-HT, LSD was 114 fold more potent than 5-HT. However EC_{50} values are unreliable estimates of affinity since (1) in the case of 5-HT, which is assumed to be a full agonist, there is a receptor reserve and the dissociation constant will be somewhat less than the EC_{50}. (2) in the case of a partial agonist (LSD), where the EC_{50} might approximate to the dissociation constant (no receptor reserve), it cannot be assumed that a linear relationship between receptor occupancy and response exists and hence the EC_{50} is not a totally reliable estimate of a drugs affinity. Nevertheless, LSD clearly has an affinity many fold greater than 5-HT for the 5-HT_2 receptor in the HUA.

Parallel findings in relation to the affinity and efficacy of LSD and 5-HT in the HUA have been found for naphazoline and phenylephrine in the rat aorta (Ruffolo et al, 1979). The affinity of naphazoline for alpha adrenoceptors was found to be 60 fold greater than that of phenylephrine, yet the intrinsic activity of naphazoline was 0.5 of that of phenylephrine. This demonstrates that affinity and efficacy are independent of each other as previously proposed (Stephenson, 1956).

In the human umbilical vein it has also been shown that LSD is more potent than 5-HT in the lower concentration range (Gant and Dyer, 1971). The maximum response effected by LSD was approximately 90% of the response to 5-HT which is rather greater than that found here in the HUA. Other studies have concluded that in both sheep umbilical arteries and human umbilical veins 5-HT and LSD act at a common receptor (1) Using selective antagonists (e.g. cinanserin), (Gant and Dyer, 1973) it was concluded that 5-HT and LSD contract the umbilical vasculature via a common serotonergic mechanism, although this evidence was qualitative rather than quantitative. (2) Phenoxybenzamine antagonised the response to both 5-HT and LSD (Gant and Dyer, 1971) and

occupation of the receptors in sheep umbilical arteries by either 5-HT or LSD prior to exposure to phenoxybenzamine protected the receptors from irreversible blockade as after washout of the drugs the response to 5-HT or LSD was not antagonised (Dyer, 1974). In both sheep and human vasculature there is therefore strong evidence to suggest that 5-HT and LSD act a common serotonergic receptor, and that in the HUA this is 5-HT_2. In the rabbit aorta, it has been found that LSD is a weak partial agonist and acts at a common receptor as 5-HT (Black et al, 1983), presumably this is the 5-HT_2 receptor.

In the HUA responses to LSD were not antagonised by prazosin and it is concluded that in this tissue at low Po_2 LSD does not act at the $alpha_1$ receptor at low Po_2 (under which physiological conditions this receptor was found to be functionally inactive anyway). LSD was a partial agonist at the 5-HT_2 receptor. In other tissues such as cat or rat anococcygeus it has also been suggested that LSD is a partial agonist at the 5-HT receptor (Gillespie and McGrath, 1974, 1975). However in these tissues due to the fact that noradrenaline was released by LSD (Gillespie and McGrath, 1975; McGrath and Olverman, 1977) or by 5-HT (McGrath, 1973), phentolamine and other alpha-adrenoceptor blockers always produced some inhibition of the response, thus rendering "absolute" separation of the receptors impossible (McGrath, 1978).

5-CT

At low Po_2 a measurable component of the response to 5-CT was attributable to prostaglandin release since (1) responses to low concentrations of 5-CT were antagonised by indomethacin and (2) a component of the response to 5-CT, which was resistant to ketanserin, was abolished by indomethacin.

Ketanserin, either in the presence or absence of indomethacin antagonised the response to 5-CT with apparent pA_2's of 9.05 and 8.77

respectively, which were not significantly different. On this basis it is concluded that 5-CT acts at the 5-HT_2 receptor. In each case the mean slope of the Schild plots was not significantly different from one. Without indomethacin present however, the slopes of two of the five individual regression lines had slopes much greater than unity- 1.57 and 1.78. These high slopes could theoretically be due to rather low concentration-ratios (CR's) at the smallest concentration of antagonist or rather high CR's at the greatest antagonist concentration. pK_B values calculated from these experiments with the anomolous slopes, at the highest and lowest antagonist concentration, suggested that it was the low CR's calculated from the lowest concentration of antagonist which gives rise to the steep slopes of the Schild plots. This indicates that the small release of prostaglandins, which is ketanserin-resistant, leads to the anomolous slopes. Nevertheless the slope of the Schild plot (the mean) was not significantly different from one, suggesting competitive antagonism with 5-CT at the 5-HT_2 receptor.

The influence of removal processes (uptake and enzymatic degradation) on the estimated potency of competitive antagonists has been discussed by Furchgott (1972). The operation of a saturable removal process for an agonist would give rise to Schild plots (log{CR-1} vs log[antagonist]) with slopes somewhat less than unity and thereby overestimate the pA_2. With 5-CT as the agonist the erroneous slopes where ketanserin was the antagonist were rather greater than unity so that removal processes do not explain these results. It would also appear that for 5-HT and 5-CT there is no <u>selective</u> degradation as this would give estimated pA_2 values for one rather greater than for the other. Since the estimates of pA_2 for the antagonist ketanserin against 5-HT and 5-CT were not significantly different there would not

Table 12 Comparison of the activities of 5-HT and 5-CT in the rabbit aorta (Feniuk et al, 1985), and in the human umbilical artery (HUA) at low Po_2 (~15mmHg).

	Rabbit Aorta		HUA	
	EC_{50} (μM)	Intrinsic Activity	EC_{50} (μM)	Intrinsic Activity
5-HT	0.45 (0.20–0.97)	1	0.04 (0.02–0.09)	1
5-CT	14.9 (2.6–85)	0.57	0.13 (0.02–1.2)	0.81

The intrinsic activity is the ratio of the maximal response (mean) of 5-CT to 5-HT. EC_{50} values are the geometric mean (95% confidence limits).

appear to be a selective removal process for either agonist.

The potencies of 5-CT and 5-HT were compared. At low Po_2 5-CT was approximately 5 fold less potent than 5-HT and the maximum response was approximately 80% of that to 5-HT. Qualitatively similar results have been found in the rabbit aorta (Feniuk et al, 1985): 5-CT was 26 fold less potent than 5-HT and the maximum response was ~60% of that to 5-HT. If one merely took the EC_{50} values for 5-HT and 5-CT and the ratio of the maximum responses (intrinsic activity) as measures of affinity and efficacy of the two drugs (Table 12) it could be suggested that the 5-HT receptors in the HUA and rabbit aorta were different due to the quite different effects of 5-HT and 5-CT in the two tissues. However this is incorrect for two reasons (1) The EC_{50} of a drug will be somewhat lower than its affinity, the difference being an indication of its receptor reserve which will be different for two drugs and (2) intrinsic activity cannot be taken as a measure of a drug's efficacy since the former term assumes that a) the response of a drug is linearly related to receptor occupation and b) a maximal effect could be produced only when 100% of the receptors are occupied. These latter points are demonstrably not the case (Stephenson, 1956; Furchgott, 1955; Nickerson, 1956).

Efficacy is itself not a parameter of the drug alone and depends on the characteristics of the tissue in question, i.e. receptor number and reserve, coupling between the receptor occupancy and response. The term intrinsic efficacy (Furchgott, 1966) is a true parameter of the drug and is related to its efficacy (efficacy being the product of intrinsic efficacy and the concentration of active receptors in a given tissue). More commonly the relative efficacy is calculated for a series of agonists which relates equieffective responses in terms of the % receptor occupancy.

Thus it is clear that relative potencies (in terms of EC_{50}'s) and

intrinsic activities may not necessarily reflect receptor differences. However, when antagonist potencies in different tissues suggest receptor differences and the rank order of a series of agonists would seem to confirm this (e.g. between rabbit aorta and dog saphenous vein, Feniuk et al, 1985) then it would seem appropriate to use the latter evidence as confirmation of the antagonist data.

In considering these points then, the qualitatively similar findings for the relative potency of 5-CT in the HUA and rabbit aorta is taken as supporting evidence for a common 5-HT_2 receptor in these tissues, based on similar antagonist potencies.

These arguments have been put forward since it has recently been suggested that antagonists which bear no structural similarity to the natural agonist may not be reliable receptor probes (Leff and Martin, 1986). In this study it was found that in three vascular preparations (which had previously been claimed to contain 5-HT_2 receptors) only trazodone and spiperone were simple competitive antagonists while ketanserin and methysergide were non-surmountable antagonists in some of the preparations. Furthermore, the estimated affinity of trazodone varied by more than an order of magnitude between the preparations. In a subsequent study simple tryptamine analogues, either agonists or antagonists, did not differentiate between the receptors and it was considered that tryptamine analogues would be more reliable receptor probes (Leff et al, 1986). However in this study in the HUA the antagonists which were used (with the exception of cyproheptadine) did not differentiate between the receptors in the HUA and in the rabbit aorta and which appear similar to the 5-HT_2 binding site. From the results outlined in this study I am confident that most of the antagonists which were used were reliable receptor probes. Cyproheptadine would seem to be an example of an unreliable receptor

probe since in the two preparations compared here, the HUA and rabbit aorta, the nature of cyproheptadine's antagonism is different at receptors which otherwise appear identical.

Summary

At the physiological Po_2 the receptor for 5-HT has been characterized as 5-HT_2 due to the similarity of the affinity of methysergide and ketanserin for the receptor in HUA and the 5-HT_2 recognition site in ligand binding studies. The response to 5-HT is not mediated by alpha-adrenoceptors. This largely meets the criteria recently put forward, for classifying the receptors which mediate functional responses (Bradley et al, 1986a), to describe the receptor as 5-HT_2. The receptor was not shown to be resistant to 5-HT_3 antagonists but as 5-HT_3 receptors have as yet been identified only on peripheral neurones (see Bradley et al, 1986a) the possibility of 5-HT_3 receptors in the HUA is discounted as the HUA is a non-innervated tissue.

The qualitatively similar potency of 5-CT to 5-HT in the HUA and rabbit aorta confirm the evidence provided by the three antagonists phentolamine, methysergide and ketanserin that the 5-HT receptors in the HUA and rabbit aorta are the same and are 5-HT_2.

Receptors for 5-HT at high Po_2

At high Po_2 (~120mmHg) the potency of three agonists, 5-HT, 5-CT and LSD was greater than that found at low Po_2, while a fourth, methysergide, which at low Po_2 had no agonism was found to be a weak partial agonist. It will be argued that these additional effects at high Po_2 are due to an action at a receptor which is not 5-HT_2, and which may be described as "5-HT_1-like".

The 5-HT_2 antagonists ketanserin, methysergide and compound X revealed a component of the response to low concentrations of 5-HT

which, at high Po_2, was relatively resistant to blockade by these antagonists, the first two of which are recognised as "classical" 5-HT_2 antagonists: in the presence of these antagonists CRC's to 5-HT at high Po_2 were biphasic. These antagonist-resistant and antagonist-sensitive components shall be termed AR and AS components respectively.

Waud (1975) has pointed out that in descriptive terms such biphasic curves may be a function of the empirical analysis. If two log CRC's which lie considerably apart are averaged at each concentration then the resulting curve will be biphasic. If the CRC's are averaged horizontally (i.e at different levels of response, as in figure 15) the average curve will be monophasic and will truly represent the mean response. Since it was found here that the CRC in the presence of ketanserin at high Po_2, averaged horizontally, was still biphasic then this does represent a response which is comprised of two components. It was conceded anyway that the "wiggle" would probly be lost in the random variation when several curves were averaged at each concentration (Waud, 1975).

The AS component

This component is without doubt due to an action at the 5-HT_2 receptor. pK_B values calculated at the EC_{75}, which was taken as a parameter of the AS component and at which level of response the curves were parallel, for a series of concentrations of ketanserin ($1X10^{-8}$–$3X10^{-7}M$), invariably gave values which were not significantly different from the pA_2 (8.92) for ketanserin against 5-HT at low Po_2, and as already described this receptor has been classified as 5-HT_2. pK_B values for the other antagonists methysergide ($1X10^{-7}M$), compound X ($1X10^{-7}M$) and phentolamine ($1X10^{-5}M$ and $1X10^{-4}M$) also gave values not sigificantly different from their respective pA_2 values estimated at low Po_2. This evidence unequivically shows that the AS component of the response to 5-HT is mediated via the 5-HT_2 receptor.

The AR component

What then is the mechanism which subserves the AR component? The evidence suggests that the AR component is receptor mediated since (1) if 5-HT was able to release prostaglandins by some indirect action then the AR component would have been resistant to all antagonist concentrations yet ketanserin (greater than $1X10^{-8}M$) and phentolamine antagonised this component and (2) if 5-HT was taken up and caused prostaglandin release then this would suggest that 5-CT was preferentially taken up as it was found to be 7 fold more potent than 5-HT in the concentration range which is "antagonist-resistant". This 2nd point has been discussed and discounted in a preceding section.

At what receptor then does 5-HT act to cause prostaglandin release? At high Po_2 a functional alpha-adrenoceptor was present which was antagonised by prazosin (pK_B 8.72) but not by indomethacin. However, prazosin did not antagonise either component of the response to 5-HT, and since the AR component of the response to 5-HT was abolished by indomethacin the possibility that the AR component was mediated via alpha-adrenoceptors is rejected.

Features of the AR component suggest that the receptor may be called "5-HT_1-like" (see Bradley et al, 1986a). This AR component was resistant to ketanserin at concentrations up to $1X10^{-8}M$. $1X10^{-7}M$ ketanserin caused a small shift and the calculated pK_B values at the EC_{25} (taken as a parameter of the AR component) were 7.4, 7.51 and 7.7 (3 estimates from different experiments). A comparison of the pK_B values calculated at the EC_{25} and EC_{75} (the latter taken as a parameter of the response mediated by the 5-HT_2 receptor) suggests that the potency of ketanserin at the EC_{25}, relative to its potency at the 5-HT_2 receptor, was in different experiments 11.7, 17.3 and 33 fold (mean 20.1) less potent. Similarly Ketanserin has relatively low affinity for the 5-HT_1 compared with the

5-HT_2 recognition site in ligand binding studies: at the former recognition site ketanserin is virtually devoid of activity (Leysen et al, 1981).

In the dog saphenous vein the receptor for 5-HT mediating contraction has been described as "5-HT_1-like" (Feniuk et al, 1985). In two studies (Van Neuten et al, 1981; Feniuk et al, 1985) the contractile response to 5-HT was resistant to ketanserin up to $1X10^{-8}M$. $1X10^{-5}M$ ketanserin reduced the maximum response by 50% in the former study while in the latter study $1X10^{-6}M$ ketanserin reduced the maximum response by 10%. Thus the potency of ketanserin is qualitatively similar for the 5-HT receptor in the dog saphenous vein, and for its potency against the AR component in the HUA, and which is similar to its affinity (or rather its lack of affinity) for the 5-HT_1 recognition site in ligand binding studies and allows the receptor which mediates these responses to be called "5-HT_1-like".

Inherent in the equation used here to calculate the dissociation constant for the antagonists (see methods) is the assumption that the antagonist acts in a competitive manner with the agonist for the receptor. At high Po_2 the response to 5-HT has been shown to be mediated by 5-HT_2 and 5-HT_1-like receptors so rendering this assumption invalid and the calculated dissociation constant (or pK_B) as inaccurate. Nevertheless, if this limitation is borne in mind then the dissociation constant can still give useful information on the drugs' affinity, allbeit a rough estimate.

The potency of compound X against the 5-HT_1-like mediated response could not be estimated since at $1X10^{-7}M$ there was no significant shift of the CRC at the EC_{25}. This would imply however that compound X had a lower affinity than ketanserin at this receptor since the same concentration of ketanserin caused an ~10 fold shift of the 5-HT CRC at the EC_{25}. Thus compound X separates the two components of 5-HT's

response even more clearly than ketanserin.

In ligand binding studies methysergide has a lower affinity at the 5-HT_1 than at the 5-HT_2 recognition site but the discrimination between the two sites is relatively poor (~10 fold less active at the 5-HT_1 site) in comparison to ketanserin which has negligible affinity for the 5-HT_1 site (Leysen et al, 1981). In the HUA methysergide was approximately 2 fold less potent at the 5-HT_1-like receptor than at the 5-HT_2 receptor based on a comparison of pK_B values at the EC_{25} (8.23) and EC_{75} (8.57) respectively.

In the dog saphenous vein methysergide is a weak antagonist of the post-junctional 5-HT receptor which mediates contraction (Apperley et al, 1980), and of the pre-junctional 5-HT receptor which inhibits electrically stimulated transmitter release (Watts et al, 1981) and at both these receptors higher concentrations of methysergide had a marked partial agonism. A further similarity between these receptors exists in the potency of a series of tryptamine analogues (Feniuk et al, 1980; Muller-Schweintzer, 1981). In the HUA methysergide was an agonist at high Po_2 but was 15 fold less potent than 5-HT. This is similar to its potency at the receptors in dog saphenous vein where it was 10 fold less potent than 5-HT at the post-junctional receptor and 30 fold less potent at the pre-junctional receptor (Apperley et al, 1980; Watts et al, 1981). In the HUA the maximum response was significantly reduced by indomethacin but was not antagonised by either prazosin or ketanserin, suggesting that the response is mediated via the 5-HT_1-like receptor as in the dog saphenous vein where both the pre and post-junctional receptors have been described as 5-HT_1-like (Bradley et al, 1986a; Feniuk et al, 1985).

In other isolated vascular tissues methysergide has been found to be a partial agonist at 5-HT receptors. In some cases this receptor is

clearly not 5-HT_2, e.g. rabbit basilar artery (Bradley et al, 1986b) and human basilar artery (Forster and Whalley, 1982) and so methysergide would appear to be a partial agonist at these non-5-HT_2 receptors. In rabbit basilar artery the criteria have largely been met to allow the receptor to be called 5-HT_1-like (Bradley et al, 1986a). In other tissues which may contain a heterogeneous population of 5-HT_2 and 5-HT_1-like receptors (see later) e.g. dog coronary (Brazenor and Angus, 1981, 1982) and basilar arteries (Muller-Schweinitzer, 1980), where methysergide is a weak agonist, it has not been clearly shown (if at all) at which receptor it exerts its agonism through.

Further evidence for describing the receptor which mediates the response to low concentrations of 5-HT, at high Po_2, as 5-HT_1-like, is the relative potency of 5-CT to 5-HT in this concentration range. The response to 5-CT at high Po_2 was clearly biphasic, the 1st phase was resistant to ketanserin but was abolished by indomethacin, while the 2nd phase was antagonised by ketanserin (pK_B 9.30) but resistant to indomethacin suggesting that the 2nd phase was mediated via the 5-HT_2 receptor. At the level of the EC_{25} (a parameter of the 1st phase) 5-CT was ~7 fold more potent than 5-HT which is rather greater (but qualitatively similar) to the relative affinity at the 5-HT_1 binding site at which 5-CT has a marginally greater affinity than 5-HT (Engel et al, 1983). However, as already discussed, comparisons of EC values are of no great value in terms of evaluating differences of affinity. In summary: The potency of 5-HT is increased by increasing the Po_2. This increase in potency, which requires prostaglandins, is mediated by receptors which are not 5-HT_2. These receptors have been shown to have the following characteristics: (1) the response to 5-HT was resistant to antagonism by ketanserin; (2) 5-CT was more potent than 5-HT and (3) the response to 5-HT was antagonised by methysergide which was a partial agonist. These features of the response to 5-HT largely meet

the criteria which have recently been suggested, to allow it to be classified as "5-HT_1-like" (Bradley et al, 1986a). Although a possible 5-HT_3 receptor involvement was not investigated it is discounted for the reasons already given, i.e the HUA is not innervated and, to date, 5-HT_3 receptors have been identified only on peripheral neurons.

Buspirone has a high affinity for the 5-HT_1 recognition site in calf hippocampus (Glaser and Traber, 1983) and is an antagonist (pA_2 7.39) at the pre-synaptic receptor in the guinea-pig ileum mediating the inhibition of Ach release. This receptor is probably similar to the 5-HT_{1A} subtype of the 5-HT_1 recognition site since low concentrations of 8-OH-DPAT, a highly selective ligand for the 5-HT_{1A} binding site (Middlemiss and Fozard, 1983), inhibited the electrically stimulated transmitter overflow (Fozard and Kilbinger, 1986). Pindolol is one of a number of β-blockers which display stereoselective antagonism at the 5-HT_{1B} subsite: (-)pindolol (pKi=7.19) is almost 100 fold more potent than (+)pindolol (pKi=5.29), (Engel et al, 1986).

Neither buspirone nor (±)pindolol antagonised the response to 5-CT at concentrations which saturate more than half of the 5-HT_1 recognition sites in rat brain tissue (Gozlan et al, 1983; Middlemiss et al, 1977). This does not necessarily shed doubt on the suggestion of a 5-HT_1-like receptor for two reasons: (1) the 5-HT_1 recognition site may have a third subtype besides 5-HT_{1A} and 5-HT_{1B}, which is a 5-HT_{1C} site at which [^{3}H]mesulergine has high affinity (Pazos et al, 1984b) and which possibly has a functional correlate in the rat stomach fundus which mediates contraction (Buchheit et al, 1986); (2) the reason why "5-HT_1-like" receptors have been classified as such is because functional responses mediated via these receptors have in some cases been shown to be resistant to blockade by 5-HT_1 ligands e.g. in the dog saphenous vein the pre-junctional receptor has been characterized as

5-HT_1-like (and meets the criteria to be called as such) yet was resistant to propranolol (Feniuk et al, 1979; Watts et al, 1981). The functionally similar receptor in the perfused rat kidney was resistant to cyanopindolol, pindolol and propranolol (Charlton et al, 1986). The characterization of these receptors awaits selective potent antagonists (Bradley et al, 1986a).

Numerous other examples of receptors which appear 5-HT_1-like exist in the literature, some of which have been referred to. Others mediate smooth muscle relaxation (vascular and non-vascular) tachycardia in cats, hypotension in cat and rat and some CNS effects (see Bradley et al, 1986a for references).

Indomethacin abolished the increased potency of 5-HT and 5-CT, induced by high Po_2, and also abolished the AR phase of the response to 5-HT which has been concluded to be due to an action at 5-HT_1-like receptors, i.e. the 5-HT_1-like receptor in the HUA requires prostaglandins for its functional expression. It is not however known whether (1) the prostaglandin(s) are released by 5-HT from the cells and act on prostanoid receptors to ultimately cause an increase in free intracellular calcium and hence contraction or (2) are present due to high Po_2 and facilitate the expression of the response mediated directly on smooth muscle by 5-HT acting at 5-HT_1 receptors.

The possibility that indomethacin was acting as a receptor antagonist rather than as a cyclo-oxygenase inhibitor (COI) was considered since indomethacin is an indole derivative, some of which compounds have 5-HT receptor antagonist properties (Gymermek, 1966). However flurbiprofen, a COI which does not contain a indole moiety also abolished the ketanserin-resistant component, confirming the conclusion that the response mediated by the 5-HT_1-like receptor required prostaglandin(s).

The ability of agonists, and in particular various amines, to

stimulate prostaglandin formation is well known although the receptors which mediate the response have, in the main, not been characterized pharmacologically using antagonists, but have mainly been studied biochemically, e.g. astothe second messengersystems(Berridge, 1981).

5-HT has been shown to cause liberation of prostaglandin-like substances from the perfused rat lung (Alabaster and Bakhle, 1976) and to increase the activity of cyclo-oxygenase in ram seminal vesicles (Takeguchi et al, 1971) but the nature of the receptors was not identified. However in cultured bovine aortic smooth muscle cells the receptor mediating prostacyclin synthesis has been characterized using selective antagonists and appears to belong to the 5-HT_2 subtype (Coughlin et al, 1984)

In the HUA the receptor mediating prostaglandin synthesis has been characterized as being 5-HT_1-like. However, current evidence suggests that receptors which stimulate prostaglandin formation are in general linked to the PI response (hydrolysis of phosphatidylinositol) and Ca^{++} influx, and not to adenylate cyclase (Berridge, 1981) while 5-HT_1 receptors in brain tissue appear to stimulate adenylate cyclase (Peroutka, 1984). While neither of these generalsations need hold in human vascular tissue it will be interesting to find whether in the HUA the 5-HT_1-like receptor is linked to the PI response. Of course, if the role of the prostaglandins is facilitatory to the 5-HT_1-mediated contraction then the second messenger utilised by the 5-HT_1 receptor would have no connection with prostaglandin formation. In any case, stimulation of adenylate cyclase usually causes relaxation of smooth muscle.

In other preparations where the response to 5-HT has been shown to be mediated by 5-HT_1-like receptors an indirect action via synthesis of prostaglandins has been ruled out. The contraction of the dog saphenous

vein (Apperley et al, 1980) and rabbit basilar artery (Bradley et al, 1986b) did not involve prostaglandins as the response was resistant to indomethacin. Similarly, the receptor mediating relaxation of cat saphenous vein and guinea-pig ileum has no prostaglandin involvement (Feniuk et al, 1983). 5-HT_1-like receptor mediated prostaglandin synthesis is therefore not a common feature of these receptors.

Although the potency of both methysergide and LSD was greater at the higher Po_2 than at the physiological Po_2 this was in each case only partially reversed by indomethacin. A possible involvement of 5-HT_2 receptors and $alpha_1$-adrenoceptors was ruled out as the agonist response was resistant to ketanserin and prazosin. The responses were partially sensitive to indomethacin and would thus suggest an action at the 5-HT_1-like receptor. A possible action at ergot receptors might explain this tolerance to indomethacin (P.P.A Humphrey, personal communication). Since the $alpha_1$ receptor in this tissue requires high Po_2 for its functional expression yet is resistant to indomethacin, it must remain a possibility that some further action of high Po_2 can facilitate the responses to methysergide and LSD.

It was very interesting to find that phentolamine and the selective $alpha_2$-adrenoceptor antagonist WY 26703 (Lattimer et al, 1984) antagonised both the 5-HT_2 and 5-HT_1-like receptor mediated responses. pK_B values for phentolamine at these receptors were 6·57 and 6·69 respectively (from $1X10^{-4}M$) and for WY 26703 ($1X10^{-5}M$) were 6·94 and 7·15, thus suggesting that these $alpha_2$ antagonists were slightly more potent at the 5-HT_1-like receptor than at the 5-HT_2 receptor although the difference in potency was not significant.

The cross-reactivity of alpha-adrenoceptor antagonists and 5-HT_2 receptors is well known (Apperley et al, 1976; Kaumann, 1983) but it has only recently been suggested that these $alpha_2$-adrenergic antagonists are possibly 5-HT_1 receptor antagonists. The results

described here represent novel supporting evidence. In two previous studies it has been demonstrated that the response to 5-HT in the dog saphenous vein was competitively antagonised by phentolamine (pA_2 6.11 and 6.05), (Humphrey, 1978; Curro et al, 1978) although these findings were not particularly remarked upon. The receptor in this tissue has since been described as 5-HT_1-like.

$Alpha_2$ antagonists (but not the $alpha_2$ agonist clonidine) are able to displace 8-OH-DPAT from the 5-HT_{1A} binding site (D.N. Middlemiss, personal communication). There would therefore seem to be some similarity between the $alpha_2$ receptor and the 5-HT_{1A} recognition site. Other studies have also suggested that $alpha_2$ antagonists may act as agonists at 5-HT autoreceptors in the rabbit hippocampus (Feuerstein et al, 1985) or at post-junctional 5-HT receptors in rat vas deferens (Kapur and Mottram, 1979), and as antagonists at 5-HT receptors in the rat ileum (McAdams and Rhodes, 1983) and human saphenous vein (Docherty and Hyland, 1986). In these studies the 5-HT receptors were not characterized but it is clear that there is much evidence of a similarity between $alpha_2$ and 5-HT receptors.

In the latter study in human saphenous vein a component of the response to 5-HT was not mediated via 5-HT_2 receptors, as it was resistant to ketanserin, but was competitively antagonised by yohimbine (pA_2 5.48). This ketanserin-resistant component, which may or may not be mediated by 5-HT_1-like receptors, was not abolished by indomethacin suggesting that in this tissue prostaglandin synthesis is not required for expression of the response (J.R. Docherty, personal communication).

A further link between $alpha_2$ and 5-HT_1-like receptors may be a common 2nd messenger system as activation of these receptors may be linked to adenylate cyclase (see McGrath, 1983; Bradley et al, 1986a).

Both phentolamine and the selective $alpha_2$-antagonist, WY 26703,

antagonised the 5-HT_1-mediated response in addition to the 5-HT_2-mediated response. Phentolamine was shown to be acting as a 5-HT_1 receptor antagonist and not simply as a cyclo-oxygenase inhibitor: the O_2-induced contraction of the HUA which is mediated by prostaglandins was not blocked by either $1X10^{-5}M$ or $1X10^{-4}M$ phentolamine which abolished the AR component of the response to 5-HT at high Po_2. No other evidence exists to my knowledge to suggest that phentolamine or WY 26703 are cyclo-oxygenase inhibitors.

Other vascular smooth muscle tissues, in addition to the HUA, may contain a heterogeneous population of 5-HT receptors, two of which (coronary and basilar arteries of dog) have been subject to controversy as to their classification. In dog coronary artery ketanserin was found to be an insurmountable antagonist of the response to 5-HT (Brazenor and Angus, 1982) and methysergide was a partial agonist (Brazenor and Angus, 1981, 1982). However, this insurmountable antagonism by ketanserin is surmountable if high enough concentrations of 5-HT are used (Frenken and Kaumann, 1985), i.e there is a component of the response to 5-HT resistant to ketanserin (up to $1X10^{-7}M$) and a component sensitive to ketanserin and mediated via 5-HT_2 receptors (pK_B 9.3). The ketanserin resistant component has not been characterized but it could be suggested that it was mediated by 5-HT_1-like receptors since methysergide was a partial agonist.

A similar picture exists for 5-HT receptors in dog basilar artery. In two independent studies ketanserin caused an insurmountable antagonism of the contractile response to 5-HT (Peroutka et al, 1983; Muller-Schweinitzer and Engel, 1983). However these studies came to different conclusions based on correlations between agonist and antagonist affinities in the dog basilar artery (which were based on EC_{50} and IC_{50}'s respectively) and 5-HT_1 and 5-HT_2 recognition sites in binding studies. These correlations are subject to criticism however

since EC_{50}'s are unreliable estimates of affinity, for the reasons already discussed and IC_{50}'s are dependent on the agonist concentration. Again, it has been shown that provided that the concentration of 5-HT is high enough, the ketanserin blockade can be surmounted and the component antagonised by ketanserin could be attributed to 5-HT_2 receptor activation (Frenken and Kaumann, 1986). Again it may be suggested that the component of the response to low concentrations of 5-HT, which was resistant to ketanserin, is due to 5-HT_1-like receptor activation as phentolamine, which has been shown to be an antagonist at other 5-HT_1-like receptors, competitively antagonised the response to 5-HT (pA_2 6.83) (Muller-Schweinitzer, 1980).

Thus in several tissues it would appear that two receptor populations may exist. In saphenous veins and umbilical arteries from humans there are both 5-HT_2 and 5-HT_1-like receptors (although these have not been fully characterized as such in saphenous veins) and in coronary and basilar arteries from dogs (5-HT_2 receptors certainly; possible 5-HT_1-like receptor classification awaits their characterization). In each of these tissues a ketanserin-resistant component at lower concentrations of 5-HT has been identified, and in each case this ketanserin-resistant component (which may or may not be mediated via 5-HT_1-like receptors) has been shown to be antagonised by phentolamine (phentolamine against 5-HT in the dog coronary artery had a pA_2 of 6.12, Muller-Schweinitzer, 1980). Whether $alpha_2$ antagonists may be useful tools in the study of 5-HT_1-like receptors awaits further investigation but from the results which have been discussed here this outlook seems promising.

General Discussion

Two of the principal findings of this study have been: (1) O_2 contracts the human umbilical artery via prostaglandins, thus implying O_2 stimulates the endogenous synthesis of constrictor prostaglandins (2) The receptor for 5-HT, described as "5-HT_1-like", required prostaglandins for its functional expression. Consideration of these two points leads me to re-examine a question which was briefly touched upon earlier: Does (a) 5-HT, acting at the 5-HT_1-like receptor, stimulate the synthesis of constrictor prostaglandins, or (b) do prostaglandins, present due to a high Po_2 (which was necessary for 5-HT_1-like receptor expression) facilitate the expression of a response mediated directly on smooth muscle by 5-HT acting at 5-HT_1-like receptors? These two alternative proposals for the mechanism subserving the expression of the 5-HT_1 receptor have not been investigated here.

If proposal (b) was the mechanism then the following observations might be expected: (1) 5-HT_1 receptor expression might be seen at low Po_2 in the presence of sub-threshold concentrations of exogenous prostaglandins, or (2) at high Po_2 in the presence of indomethacin plus exogenous prostaglandins. This 2nd point has been briefly examined in one experiment: $1X10^{-8}M$ and $3X10^{-8}M$ $PGF_{2\alpha}$ did not cause contraction of longitudinal strips of umbilical artery but did not facilitate 5-HT_1 receptor expression. Higher concentrations of $PGF_{2\alpha}$ ($1X10^{-6}M$) caused contraction but 5-HT_1 receptor expression was not observed. This preliminary evidence argues against proposal (b) as the mechanism subserving the 5-HT_1-like receptor. This does not rule out other prostaglandins or TxA_2 as possibly facilitating 5-HT_1 receptor mediated responses.

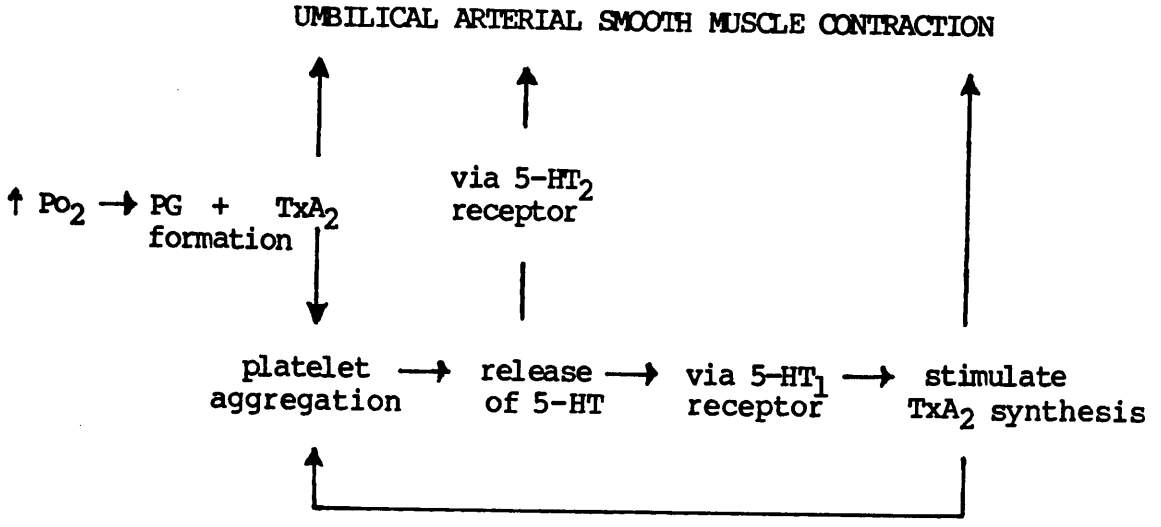
Whichever mechanism holds, the nature of the prostaglandin(s) remains unknown, although any of the endogenously synthesised prostaglandins have to be considered. PGI_2 is the major prostanoid product of the human umbilical artery while smaller amounts of PGE_2,

$PGF_{2\alpha}$ and TxA_2 are also continuously formed (Mitchell et al, 1980; Bjoro et al, 1986). The major synthesis of PGI_2 occurs in the vascular endothelium (Smith, 1986). In this study it was shown that denuding the umbilical artery of the endothelium did not alter the 5-HT_1 receptor mediated response: The first phase of the CRC to 5-HT at high Po_2, which is mediated by the 5-HT_1 receptor, was similarly resistant to ketanserin in denudded preparations and in preparations with an intact endothelium. This argues against a role for PGI_2. However the possibility of PGI_2 derived from smooth muscle cells cannot be discounted.

It has recently been shown that in the perfused human umbilical artery, 5-HT stimulates the synthesis of TxA_2 but not PGI_2 (Bjoro, 1986). Since this effect was blocked by indomethacin, 5-HT did not simply stimulate TxA_2 release. This evidence not only suggests that PGI_2 is not involved in the transduction of the 5-HT_1 receptor-mediated contraction, but it supports proposal (a) that 5-HT_1 receptor activation stimulates constrictor prostaglandin synthesis since TxA_2 is a potent agonist in the human umbilical artery (Svensson et al, 1977). It is significant that the 5-HT-stimulated formation of TxA_2 was demonstrated only in a high Po_2 environment (95% O_2). Further evidence would be gained for the hypothesis that 5-HT_1 receptor activation stimulates TxA_2 formation if it was demonstrated that 5-HT-stimulated TxA_2 formation was resistant to ketanserin at high Po_2, and that at low Po_2 5-HT did not stimulate TxA_2 synthesis.

In chapter 2 it was discussed how the increase in Po_2 of the umbilical arterial blood at birth might lead to closure of the umbilical arteries via prostaglandins. The preceding points suggest other steps by which closure of the arteries might occur. Bearing in mind that TxA_2 causes platelet aggregation which in turn causes the

release of 5-HT, the following scenario could be envisaged at birth, on the increase of the Po_2:



Each of these mechanisms suggested for smooth muscle contraction has been established by in vitro experiments. A possible doubt is whether the free concentration of 5-HT increases at birth. Stacey (1966) has estimated the concentration of 5-HT in the umbilical artery at birth. This was 43ng/ml of blood, i.e. ~$1X10^{-7}$M. If this represented the free concentration of 5-HT it would cause profound vasospasm. However, most of this probably represents platelet-derived 5-HT and therefore the free concentration of 5-HT is not known. If only 1% of this concentration were free it would lead to contraction via the 5-HT_1-like receptor. This in turn would lead to further 5-HT release from aggregating platelets and hence activation of 5-HT_2 receptors. As ever, the reservations of extrapolating from in vitro experiments to the in vivo (in utero!) situation are recognised.

It is clear from these proposals that fluctuations of the Po_2 could trigger the various mechanisms to cause vasoconstriction and so reduce fetal blood flow, while cyclo-oxygenase inhibitors (e.g. indomethacin) would effectively render this mechanism for vessel closure inoperative. However, modern obstetric practise of clamping the

umbilical cord quickly after birth obviates the need for such a mechanism.

Speculation on 5-HT receptors and prostaglandins

As already discussed the response to 5-HT in several tissues has been suggested as being mediated via 5-HT_1-like receptors. In several of these tissues prostaglandins do not seem to be involved in the response. These tissues include the dog saphenous vein (Apperley et al, 1980), rabbit basilar artery (Bradley et al, 1986b), cat saphenous vein and guinea-pig-ileum (Feniuk et al, 1983). This in itself might suggest that varying the Po_2 might not affect agonist-antagonist interactions via changes in prostaglandin synthesis, as was found here in the HUA.

Other tissues such as the dog basilar and coronary arteries may have a heterogeneous population of receptors for 5-HT: concentration-response curves to 5-HT in the presence of ketanserin were biphasic, the first phase at low concentrations was resistant to ketanserin while the second phase was sensitive to ketanserin thus suggesting the effect was mediated via 5-HT_2 receptors (Frenken and Kaumann, 1985; Kaumann and Frenken, 1986). It is interesting to speculate to what extent prostaglandins may be involved in the ketanserin-resistant component, which may or may not be mediated via 5-HT_1 receptors since it has not been characterized. This ketanserin-resistant component was described in experiments in which the Po_2 was very high (95% O_2). In other species (e.g. bovine) coronary artery production of prostaglandins has been noted to vary with the prevailing Po_2 (Kalsner, 1976). This leads me to suggest that in the dog coronary artery, prostaglandin production may be stimulated by 5-HT via receptors which are not 5-HT_2. To my knowledge inhibition of the response to 5-HT in these tissues, by cyclo-oxygenase inhibitors, has never been tested (for references see discussion, chapter 3).

A role for 5-HT in the pathophysiology of migraine has been established since Sicuteri et al (1961) found that during migraine attacks some patients excreted increased amounts of 5-hydroxyindole acetic acid, the principle metabolite of 5-HT. The latest theory assigns a key role for the "5-HT-innervation" of the cerebral vasculature in the aetiology of migraine (Fozard, 1985). The principal causes of migraine appear to be constriction of cerebral arteries, and a subsequent cerebral ischaemia, during the preheadache phase, and vasodilation during the headache phase (Saper, 1978). An additional factor is the "sterile" inflammation around the dilated blood vessels which may add to the pain associated with migraine. Since 5-HT_2 receptors have been shown to stimulate prostaglandin synthesis (Coughlin et al, 1984) and since prostaglandin synthesis inhibitors (e.g. indomethacin) are effective in some cases of migraine (Mathew, 1981) it has been suggested that 5-HT_2 receptors may mediate the sterile inflammatory response (Fozard, 1985). However, 5-HT_1 receptors may be involved since (1) this present study has shown that 5-HT, acting at 5-HT_1 receptors, stimulated prostaglandin synthesis (probably). (2) Many of the arteries which are likely to be involved in the disturbance of cerebral blood flow during migraine attacks either do not appear to contain 5-HT_2 receptors (e.g. human basilar artery, Forster and Whalley, 1982; intracranial arteries of cat and dog, Lamar and Edvinsson, 1980) or appear to contain a heterogeneous population of 5-HT_2 receptors and other receptors for 5-HT which may be 5-HT_1 (e.g. dog basilar artery, Frenken and Kaumann, 1985). Whether 5-HT_1 receptor-mediated prostaglandin synthesis occurs in these vessels, and which is involved in migraine, awaits to be investigated.

Since the human umbilical artery but not the vein is contracted by O_2, and such contractions are mediated by prostaglandins, it is

interesting to speculate whether the vein may lack functional, prostaglandin-linked, 5-HT_1 receptors, as have been described here in the umbilical artery. However the vein is able to synthesise constrictor prostaglandins (Boura et al, 1979) so that the vein may indeed contain a heterogenous population of both 5-HT_2 and prostaglandin-linked, 5-HT_1 receptors.

The lack of functional alpha-adrenoceptors and preponderance of 5-HT receptors at physiological oxygen tensions make this an interesting and useful preparation for the study of human vascular 5-HT_2 receptors. Indeed the response to 5-HT can be ascribed to an action at 5-HT_2 receptors under these conditions without the need for a fully quantitative investigation involving an analysis of the response to 5-HT mediated via alpha-receptors. Such a quantitative assessment is necessary in most other tissues due to the cross-reactivity of alpha-adrenoceptor and 5-HT receptor antagonists, for 5-HT receptors and alpha-adrenoceptors respectively (Feniuk, 1984).

References

Adeagbo, A.S.O., Coceani, F. and Olley, P.M. (1982). The response of the lamb ductus venosus to prostaglandins and inhibitors of prostaglandin and thromboxane synthesis. Circ. Res., **51**, 580–586.

Alabaster, V.A. and Bakhle, Y.S. (1976). Release of smooth muscle contracting substances from isolated perfused lungs. Eur. J. Pharmac., **35**, 349–360.

Altura, B.M., Malaviya, D., Reich, C.F. and Orkin, L.R. (1972). Effects of vasoactive agents on isolated human umbilical arteries and veins. Am. J. Physiol., **222**, 345-355.

Altura, B.M., Altura, B.T. and Carella, A. (1983). Ethanol produces coronary vasospasm: evidence for a direct action of ethanol on vascular muscle. Br. J. Pharmac., **78**, 260–262.

Anderson, N.H. and Ramwell, P.W. (1974). Biological aspects of prostaglandins. Arch. Intern. Med., **133**, 30–50.

Apperley, E., Humphrey, P.P.A. and Levy, G.P. (1976). Receptors for 5-hydroxytryptamine and noradrenaline in rabbit isolated ear artery and aorta. Br. J. Pharmac., **58**, 211-221.

Apperley, E., Fenuik, W., Humphrey, P.P.A. and Levy, G.P. (1980). Evidence for two types of excitatory receptor for 5-hydroxytryptamine in dog isolated vasculature. Br. J. Pharmac., **68**, 215–224.

Ariens, E.J. (1964). In, Molecular Pharmacology: The mode of action of biologically active compounds. Academic Press. New York.

Armstrong, J.M., Chapple, D., Dusting, G.J., Hughes, R., Moncada, S. and Vane, J.R. (1977). Cardiovascular actions of prostacyclin (PGI_2) in chloralose anaesthetised dogs. Br. J. Pharmac., **61**, 136P.

Arunlakshana, O. and Schild, H.O. (1959). Some quantitative uses of drug antagonists. Br. J. Pharmac., **14**, 48–58.

Astrom, A. and Samelius, U. (1957). The action of 5-hydroxytryptamine and some of its antagonists on the umbilical vessels of the human placenta. Br. J. Pharmac., **12**, 410–414.

Atallah, A., Payakkapan, W., Lee, J., Carr, A. and Brazelton, E. (1974). PGA: Fact, not artifact. Prostaglandins, **5**, 69–72.

Bergstrom, S., Danielsson, H. and Samuelsson, B. (1964). The enzymatic formation of prostaglandin E_2 from arachidonic acid. Prostaglandins and related factors, **32**, Biochim. Biophys. Acta, **90**, 207–210.

Berridge, M.J. (1981). Phosphatidylinositol hydrolysis: A multifunctional transducing mechanism. Mollec. Cellular Endocrinol., **24**, 115–140.

Black, J.W., Brazenor, R.M., Gerkowitch, V.P. and Leff, P. (1983). The problem of insurmountable antagonism in 5-hydroxytryptamine receptor classification. Br. J. Pharmac., **80**, 607P.

Bjoro, K. (1986). Prostacyclin and thromboxane formation in human umbilical arteries following stimulation with vasoactive autocoids. Prostaglandins, **31**, 699–714.

Bjoro, K., Hovig, T., Stokke, K.T. and Stray-Pederson, S. (1986). Formation of prostanoids in human umbilical vessels perfused *in vitro*. Prostaglandins, **31**, 683–697.

Bor, I. and Guntheroth, W.G. (1970). In vitro responses to oxygen of human umbilical arteries and of animal ductus arteriosus. Can. J. Physiol. Pharmac., **48**, 500-502.

Boura, A.L.A., Boyle, L., Sinnathuray, T.A. & Walters, W.A.W. (1979). Release of prostaglandins during contraction of the human umbilical vein on reduction of temperature. Br. J. Pharmac., **65**, 360-362.

Bradley, P.B., Engel, G., Feniuk, W., Fozard, J.R., Humphrey, P.P.A., Middlemiss, D.N., Mylecharane, E.J., Richardson, B.P. and Saxena, S.R. (1986a). Proposals for the classification of functional receptors of 5-hydroxytryptamine Br. J. Pharmac., **25**, 563–575.

Bradley, P.B., Humphrey, P.P.A. and Williams, R.H. (1983). Are vascular "D" and "5-HT_2" receptors for 5-hydroxytryptamine the same? Br. J. Pharmac., **79**, 295P.

Bradley, P.B., Humphrey, P.P.A. and Williams, R.H. (1986b). Evidence for the existence of 5-hydroxytryptamine receptors, which are not of the 5-HT_2 type, mediating contraction of rabbit isolated basilar artery. Br. J. Pharmac., **87**, 3–4.

Brazenor, R.M. and Angus, J.A. (1981). Ergometrine contracts isolated canine coronary arteries by a serotonergic mechanism: no role for alpha receptors. J. Pharmac. exp. Ther., **218**, 530–536.

Brazenor, R.M. and Angus, J.A. (1982). Actions of serotonin antagonists on dog coronary artery. Eur. J. Pharmac., **81** 569–576.

Brownlee, G. and Johnson, E.S. (1963). The site of the 5-hydroxytryptamine receptor on the intramural nervous plexus of the guinea-pig isolated ileum. Br. J. Pharmac. Chemother., **21**, 306–322.

Buchheit, K.H., Engel,G., Hoyer, D., Kalkman, K.O. and Seiler, M.P. (1986). The rat isolated stomach fundus strip, a model for 5-HT_{1C} receptors. Br. J. Pharmac., **88**, 367P.

Bunting, S., Gryglewski, R., Moncada, S. aand Vane, J.R. (1976). Arterial walls generate from prostaglandin endoperoxides a substance (prostaglandin X) which relaxes strips of mesenteric and coeliac arteries and inhibits platelet aggregation. Prostaglandins, **12**, 897–914.

Campbell, A.G.M., Dawes, G.S., Fishman, A.P., Hyman, A.I. and James, G.B. (1966). The oxygen consumption of the placenta and foetal membranes in the sheep. J. Physiol., **182**, 439–464.

Cerletti, A. and Doepfner, W. (1958). Comparitive study on the serotonin antagonism of hydrazides of lysergic acid and of ergot alkaloids. J. Pharmac., **122**, 124–136.

Chang, A.E. and Detar, R. (1980). Oxygen and vascular smooth muscle revisited. Am. J. Physiol., **238**, H716–728.

Charlton, K.G., Bond, R.A. and Clarke, D.E. (1986). An inhibitory prejunctional 5-HT_1-like receptor in the isolated perfused rat kidney. Apparent distinction from the 5-HT_{1A}, 5-HT_{1B} and 5-HT_{1C} subtypes. Naunyn-Schmiedeberg's Arch. Pharmac., **332**, 8–15.

Clyman, R.I. (1980). Ductus arteriosus: Developmental response to endogenous prostaglandins, oxygen, and indomethacin. In, Advances in Prostaglandin and Thromboxane Research, **7**. Eds. Samuelsson, B., Ramwell, P.W. and Paoletti, R. Raven Press, New York, 887–890.

Clyman, R.I., Mauray, F., Heymann, M.A. and Rudolph, R.M. (1978). Ductus arteriosus: developmental response to oxygen and indomethacin. Prostaglandins, **15**, 993–998.

Coburn, R.F., Grubb, B. and Aronson, R.D. (1979). Effect of cyanide on oxygen tension-dependent mechanical tension in rabbit aorta. Circ. Res., **44**, 368–378.

Coceani, F. and Olley, P.M. (1980). Considerations on the role of prostaglandins in the ductus arteriosus. In, Advances in Prostaglandin and Thromboxane Research, **7**. Eds. Samuelsson, B., Ramwell, P.W. and Paoletti, R. Raven Press, New York, 871–878.

Coceani, F., Olley, P.M. and Bodach, E. (1976). Prostaglandins: A possible regulator of muscle tone in the ductus arteriosus. Ibid., **1**. Eds. Samuelsson, B. and Paoletti, R. Raven Press, New York, 417–424.

Coceani, F., Olley, P.M. & Lock, J.E. (1980). Prostaglandins, ductus arteriosus, pulmonary circulation: current concepts and clinical potential. Eur. J. Clin. Pharmac., **18**, 75-81.

Cockbill, S.R., Heptinstall, S. and Taylor, P.M. (1979). A comparison of the abilities of acetylsalicylic acid, flurbiprofen and indomethacin to inhibit the release reaction and prostaglandin synthesis in human blood platelets. Br. J. Pharmac., **67**, 73–78.

Coleman, R.A., Humphrey, P.P.A., Kennedy, I. and Lumley, P. (1984). Prostanoid receptors -the development of a working classification. In, Receptors, Again. Eds. Lamble, J.W. and Abbott, A.C. Elsevier Science Publishers, Amsterdam, 263–269.

Coughlin, S.R., Moskowitz, M.A., Antoniades, H.N. and Levine, L. (1981). Serotonin receptor-mediated stimulation of bovine smooth muscle cell prostacyclin synthesis and its modulation by platelet-derived growth factor. Proc. Natl. Acad. Sci. USA, **78**, 7134–7138.

Coughlin, S.R., Moskowitz, M.A. and Levine, L. (1984). Identification of a serotonin type 2 receptor linked to prostacyclin synthesis in vascular smooth muscle cells. Biochem. Pharmac., **33**, 692–695.

Davignon, J., Lorenz, R.R. and Shepherd, J.T. (1965). Response of human umbilical artery to changes in transmural pressure. Am. J. Physiol., **209**, 51–59.

Dawes, G.S. (1968). In, Foetal and neonatal physiology. Year Book Medical Publishers Inc., Chicago.

Dawes, G.S. (1978). Conclusion. In, Advances in Prostaglandin and Thromboxane Research, **4**. Eds. Coceani, F. and Olley, P.M. Raven Press, New York, 387–393.

De Clerck, F., Xhonneux, B., Leysen, J. and Janssen, P.A.J. (1984a). The involvement of 5-HT_2-receptor sites in the activation of cat platelets. Thromb. Res., **33**, 305–321.

De Clerck, F., Xhonneux, B., Leysen, J. and Janssen, P.A.J. (1984b). Evidence for functional 5-HT_2 receptor sites on human blood platelets. Biochem. Pharmac., **33**, 2807–2811.

Detar, R. (1980). Mechanism of physiological hypoxia-induced depression of vascular smooth muscle contraction. Am. J. Physiol., **238**, H761–769.

Detar, R. and Bohr, D.F. (1968). Oxygen and vascular smooth muscle contraction. Am. J. Physiol., **214**, 241–244.

Docherty, J.R. and Hyland, L. (1986). An examination of 5-hydroxytryptamine receptors in human saphenous vein. Br. J. Pharmac., **89**, 77–81.

Doempfner, W. and Cerletti, A. (1958). Comparison of lysergic acid derivatives and antihistamines as inhibitors of the oedema provoked in the rat's paw by serotonin. Int. Arch. Allergy, **12**, 89–97.

Drakontides, A.B. and Gershon, M.D. (1968). 5-hydroxytryptamine receptors in the mouse duodenum. Br. J. Pharmac. Chemother., **33**, 480–492.

Dusting, D.J., Moncada, S. and Vane, J.R. (1977a). Prostacyclin is a weak contractor of coronary arteries of the pig. Eur. J. Pharmac., **45**, 301–304.

Dusting, D.J., Moncada, S. and Vane, J.R. (1977b). Prostacyclin (PGX) is the endogenous metabolite for relaxation of coronary arteries induced by arachidonic acid. Prostaglandins, **13**, 3–15.

Dyer, D.C. (1974). Evidence for the action of d-Lysergic acid diethylamide, mescaline and bufotenine on 5-hydroxytryptamine receptors in umbilical vasculature. J. Pharm. exp. Ther., **188**, 336–341.

Dyer, D.C. and Gant, D.W. (1973). Vasoconstriction produced by hallucinogens on isolated human and sheep umbilical vasculature. J. Pharm. exp. Ther., **184**, 366–375.

Eckenfels, A. and Vane, J.R. (1972). Prostaglandins, oxygen tension and smooth muscle tone. Br. J. Pharmac., **45**, 451–462.

Egan, R.W., Humes, J.L. and Kuehl Jnr., F.A. (1978). Differential effects of prostaglandin synthetase stimulators on inhibition of cyclo-oxygenase. Biochemistry, **17**, 2230–2234.

Eliasson, R. (1959). Studies on prostaglandin. Occurrence, formation and biological actions. Acta Physiol. Scand., **46**, **Suppl. 158**, 1–73.

Eliasson, R. and Astrom, A. (1955). Pharmacological studies on the perfused human placenta. Acta Pharmac. Toxicol., **11**, 254–264.

Eltherington, L.G., Stoff, J., Hughes, T. and Melmon, K.L. (1968). Constriction of human umbilical arteries. Interaction between oxygen and bradykinin. Circ. Res., **22**., 747-752.

Engel, G., Gothert, M., Muller-Schweinitzer, E., Schlicker, E., Sistonen, L. and Stadler, P.A. (1983). Evidence for common pharmacological properties of ^{3}H 5-hydroxytryptamine binding sites, presynaptic 5-hydroxytryptamine autoreceptors in CNS and inhibitory presynaptic 5-hydroxytryptamine receptors on sympathetic nerves. Naunyn-Schmiedeberg's Arch. Pharmac., **324**, 116–124.

Engel, G., Hoyer, D., Kalkman, H.O. and Wick, M.B. (1984). Identification of 5-HT_2-receptors on longitudinal muscle of the guinea-pig ileum. J. Recep. Res., **4**, 113–126.

Engel, G., Hoyer , D., Kalkman, H.O. and Wick, M.B. (1984). Pharmacological similarity between the 5-HT D-receptor on the guinea-pig ileum and the 5-HT_2 binding site. Br. J. Pharmac., **84**, 106P.

Engel, G., Gothert, M., Hoyer, D., Schlicker, E. and Hillenbrand, K. (1986). Identity of presynaptic 5-hydroxytryptamine (5-HT) autoreceptors in the rat brain cortex with 5-HT_{1B} binding sites.
Naunyn-Schmiedeberg's Arch. Pharmac., **332**, 1–7.

Farmer, J.B., Farrar, D.G. and Wilson, J. (1974). Antagonism of tone and prostaglandin-mediated responses in a tracheal preparation by indomethacin and SC19220. Br. J. Pharmac., **52**, 559–565.

Fasehun, O.A., Jennett, S.M., McGrath, J.C. and Ugwu, A.C. (1985). What is a suitable O_2 tension for smooth muscle in vitro? Br. J. Pharmac., **86**, 753P.

Feniuk, W. (1984). An analysis of 5-hydroxytryptamine receptors mediating contraction of isolated smooth muscle. Neuropharmacology, **23**, 1467–1472.

Feniuk, W., Humphrey, P.P.A. and Watts, A.D. (1980). Further characterization of pre- and post-junctional receptors for 5-hydroxytryptamine in isolated vasculature. Br. J. Pharmac., **73**, 191–192P.

Feniuk, W., Humphrey, P.P.A. and Watts, A.D. (1983). 5-hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle. Eur. J. Pharmac., **96**, 71–78.

Feniuk, W., Humphrey, P.P.A., Perren, M.J. and Watts, A.D. (1985). A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein: evidence for different receptor types obtained by use of selective agonists and antagonists. Br. J. Pharmac., **86**, 697–704.

Ferreira, S.H., Moncada, S. and Vane, J.R. (1971). Indomethacin and aspirin abolish prostaglandin release from the spleen. Nature (New Biol.), **231**, 237–239.

Feuerstein, T.J., Hertting, G. and Jackisch, R. (1985). Endogenous noradrenaline as modulator of hippocampal serotonin (5-HT)-release. Naunyn-Schmiedeberg's Arch. Pharmac., **329**, 216–221.

Flower, R.J. (1974). Drugs which inhibit prostaglandin biosynthesis. Pharmacol. Rev., **26**, 33–67.

Flower, R.J. and Vane, J.R. (1974). Some pharmacologic and biochemical aspects of prostaglandin biosynthesis and its inhibition. In, Prostaglandin Synthetase Inhibitors. Eds. Robinson, H.J. and Vane, J.R. Raven Press, New York, 9–18.

Forster, C. and Whalley, E.T. (1982). Analysis of the 5-hydroxytryptamine induced contraction of the human basilar arterial strip compared with the rat aortic strip in vitro. Naunyn-Schmiedeberg's Arch. Pharmac., **319**, 12–17.

Fozard, J.R. (1984a). Neuronal receptors in the periphery. Neuropharmacology, **23**, 1473–1486.

Fozard, J.R. (1984b). MDL 72222: a potent and highly selective antagonist at neuronal 5-hydroxytryptamine receptors. Naunyn-Schmiedeberg's Arch. Pharmac., **326**, 36–44.

Fozard, J.R. (1985). 5-hydroxytryptamine in the pathophysiology of migraine. In, Vascular Neuroeffector Mechanisms. Ed. Bevan, J.A. Elsevier Science Publishers, Amsterdam, 321–328.

Fozard, J.R. and Mobarok Ali, A.T.M. (1978a). Receptors for 5-hydroxytryptamine on the sympathetic nerves of the rabbit heart. Naunyn-Schmiedeberg's Arch. Pharmac., **301**, 223–235.

Fozard, J.R. and Mobarok Ali, A.T.M. (1978b). Blockade of neuronal tryptamine receptors by metoclopramide. Eur. J. Pharmac., **49**, 109–112.

Fozard, J.R., Mobarok Ali, A.T.M. and Newgrosh, G. (1979). Blockade of serotonin receptors on autonomic neurones by (-)cocaine and some related compounds. Eur. J. Pharmac., **59**, 195–210.

Fozard, J.R. and Host, M. (1982). Selective inhibition of the Bezold-Jarisch effect of 5-HT in the rat by antagonists at neuronal 5-HT receptors. Br. J Pharmac., **77**, 520P.

Fozard, J.R. and Kilbinger, H. (1986). 8-OH-DPAT inhibits transmitter release from guinea-pig enteric cholinergic neurones by activating 5-HT_{1A} receptors. Br. J. Pharmac., **86**, 601P.

Frenken, M. and Kaumann, A.J. (1985). Ketanserin causes surmountable antagonism of 5-hydroxytryptamine contractions of large coronary arteries of dog. Naunyn-Schmiedeberg's Arch. Pharmac., **328**, 301–303.

Fried, J., Barton, J., Kittisopikul, S., Needleman, P. and Wyche, A. (1980). Synthesis and biological properties of selective inhibitors of the prostaglandin cascade. In, Advances in Prostaglandin and Thromboxane Research, **6**. Eds. Samuelsson, B., Ramwell, P.W. and Paoletti, R. Raven Press, New York, 427–436.

Friedman, W.F., Printz, M.P. and Kirkpatrick, S.E. (1978). Blockers of prostaglandin synthesis: A novel therapy in the management of the premature human infant with patent ductus arteriosus. Ibid., **4**, Eds. Coceani, F. and Olley, P.M. Raven Press, New York, 373–385.

Fuller, R.W. (1972). In, Monoamine Oxidase-New Vistas. Eds. Costa, E. and Sandler, M. Raven Press, New York, 339–354.

Furchgott, R.F. (1955). The pharmacology of vascular smooth muscle. Pharmac. Rev., **7**, 183–265.

Furchgott, R.F. (1966). The use of β-haloalkylamines in the differentiation of receptors and in determination of dissociation constants of receptor-agonist complexes. In: Advances in Drug Research, **3**. Eds. Harper, N.J. and Simmonds, A.D. Academic Press, London, 21–35.

Furchgott, R.F. (1972). The classification of adrenoceptors (adrenergic receptors). An evaluation from the standpoint of receptor theory. In: Handbook of Experimental Pharmacology (Catecholamines), **33**. Eds. Blaschko, H. and Muscholl, E. Springer-Verlag, Berlin, 283–335.

Gaddum, J.H. (1953). Tryptamine receptors. J. Physiol., **119**, 363–368.

Gaddum, J.H. and Hameed, K.A. (1954). Drugs which antagonize 5-hydroxytryptamine. Br. J. Pharmac., **9**. 240–248.

Gaddum, J.H. and Picerelli, Z.P. (1957). Two kinds of tryptamine receptor. Br. J. Pharmac. Chemother., **12**, 323–328.

Gant, D.W. and Dyer, D.C. (1971). d-Lysergic acid diethylamide (LSD_{25}): A constrictor of human umbilical artery. Life Sci., **10**, 235–240.

Garry, R.C. (1928). The effect of oxygen lack on surviving smooth muscle. J. Physiol., **66**,235–248.

Gillespie, J.S. and McGrath, J.C. (1974). The response of the cat anococcygeus muscle to nerve and drug stimulation and a comparison with the rat anococcygeus. Br. J. Pharmac., **50**, 109–118.

Gillespie, J.S. and McGrath, J.C. (1975). The effects of lysergic acid diethylamide on the response to field stimulation of the rat vas deferens and the rat and cat anococcygeus muscles. Br. J. Pharmac., **54**, 481–488.

Glaser, T. and Traber, J. (1983). Buspirone: action on serotonin receptors in calf hippocampus. Eur. J. Pharmac., **88**, 137–138.

Gokhale, S.D., Gulati, O.D., Kelkar, L.V. and Kelkar, K.K. (1966). Effect of some drugs on human umbilical artery in vitro. Br. J. Pharmac. Chemother., **27**, 332–346.

Gothert, M. (1982). Modulation of serotonin release in the brain via pre-synaptic receptors. Trends Pharmac. Sci., **3**, 437–440.

Gozlan, H., El Mestikawy, S., Pichat, L., Glowinski, J. and Hamon, M. (1983). Identification of presynaptic serotonin autoreceptors using a new ligand: ^{3}H-PAT. Nature, **305**, 140–142.

Gryglewski, R. and Vane, J.R. (1972). The release of prostaglandins and rabbit aorta contracting substance (RCS) from rabbit spleen and its antagonism by anti-inflammatory drugs. Br. J. Pharmac., **45**, 37–47.

Gyermek, L. (1966). Drugs which antagonise 5-hydroxytryptamine and related indolalkylamines. In: Handbook of Experimental Pharmacology, **19**. Ed. Erspamer V. Springer-Verlag, Berlin, 471–528.

Hamberg, M., Svensson, J., Wakabayashi, T. and Samuelsson, B. (1974). Isolation and structure of two prostaglandin endoperoxides that cause platelet aggregation. Proc. Natl. Acad. Sci. USA, **71**, 345–349.

Hamberg, M., Svensson, J. and Samuelsson, B. (1975). Thromboxanes: A new group of biologically active compounds derived from prostaglandin endoperoxides. Proc. Natl. Acad. Sci. USA, **72**, 2994–2998.

Hamberg, M., Tuvemo, T., Svensson, S. and Jonsson, C.E. (1979). Formation and action of prostacyclin in the isolated human umbilical artery. Acta. Physiol. Scand., **106**, 289–292.

Hamon, M., Bourgoin, S., Gozlan, H., Hall, M.D., Goetz, C. Artaud, F. and Horn, A.S. (1984). Biochemical evidence for the 5-HT agonist properties of PAT [8-hydroxy-2-(di-n-propylamino)tetralin] in the rat brain. Eur. J. Pharmac., **100**, 263–276.

Hansen, H.S. (1974). Inhibition by indomethacin and aspirin of 15-hydroxyprostaglandin dehydrogenase *in vitro*. Prostaglandins, **8**, 95–105.

Heymann, M.A. and Rudolph, A.M. (1975). Control of the ductus arteriosus. Physiol. Rev., **55**, 62–78.

Hill, A.V. (1928a). The role of oxidation in maintaining the dynamic equilibrium of the muscle cell. Proc. Roy. Soc., **B103**, 138–162.

Hill, A.V. (1928b). The diffusion of oxygen and lactic acid through tissues. Proc. Roy. Soc., **B104**, 39–96.

Hillier, K. and Karim, S.M.M. (1968). Effects of prostaglandins E_1, E_2, $F_{1\alpha}$, $F_{2\alpha}$ on isolated human umbilical and placental blood vessels. Br. J. Obstet. Gynaec., **75**, 667-673.

Horovitz, Z.P., Beer, B., Clody, D.E., Vogel, J.R. and Chasin, M. (1972). Cyclic AMP and anxiety. Psychosomatics, **13**, 85–92.

Humphrey, P.P.A. (1978). The effects of α-adrenoceptor antagonists on contractile responses to 5-hydroxytryptamine in dog saphenous vein. Br. J. Pharmac., **63**, 671–675.

Humphrey, P.P.A. (1984). Peripheral 5-hydroxytryptamine receptors and their classification. Neuropharmacology, **23**, 1503–1510.

Humphrey, P.P.A., Feniuk, W. and Watts, A.D. (1982). Ketanserin–a novel antihypertensive? J. Pharm. Pharmacol., **34**, 541.

Humphrey, P.P.A., Feniuk, W. and Watts, A.D. (1983). Prejunctional effects of 5-hydroxytryptamine on noradrenergic nerves in the cardiovascular system. Fedn. Proc. Fedn. Am. Socs. exp. Biol., **42**, 218–222.

Inglis, G.C., Whittle, M.J., Wilson, A.I. and Bull, S.G. (1981). Plasma catecholamine concentrations in mother and infant at term. Clin. Sci., **60**, P25.

Kennedy, I., Coleman, R.A., Humphrey, P.P.A., Levy, G.P. and Lumley, P. (1982). Studies on the characterisation of prostanoid receptors: a proposed classification. Prostaglandins, **24**, 667–689.

Kilbinger, H., Kuel, R., Pfeuffer-Friederich, I. and Wessler, I. (1982). The effects of metoclopramide on acetylcholine release and on smooth muscle response in the isolated guinea-pig ileum. Nauyn-Schmiedebergs Arch Pharmac., **319**, 231–238.

Jones, R.L. (1976). Cardiovascular actions of prostaglandins D and E in the sheep: evidence for two distinct receptors. In, Advances in Prostaglandin and Thromboxane Research, **1**, 221–230.

Johnson, R.A., Morton, D.R., Kinner, J.H., Gorman, R.R., McGuire, J.C., Sun, F.F., Whittaker, N., Bunting, S., Salmon, J., Moncada, S. and Vane, J.R. (1976). The chemical structure of prostaglandin X (prostacyclin). Prostaglandins, **12**, 915–928.

Jonsson, C.E., Tuvemo, T. and Hamberg, M. (1976). Prostaglandin biosynthesis in the human umbilical cord. Biol. Neonate, **29**, 162–170.

Kalsner, S. (1976). The effect of hypoxia on prostaglandin output and on tone in isolated coronary arteries. Can. J. Physiol. Pharmacol., **55**, 882–887.

Kapur, H. and Mottram, D.M. (1979). The interaction of WB4101, and other α-adrenoceptor antagonists, on 5-hydroxytryptamine receptors. J. Pharm. Pharmacol., **31**, 337–338.

Karim, S.M.M. (1967). The identification of prostaglandins in human umbilical cord. Br. J. Pharmac., **29**, 230–237.

Karim, S.M.M. and Rao, B. (1975). General introduction and comments. In, Prostaglandins and Reproduction. Ed. Karim, S.M.M. MTP Press, Lancaster. 1–21.

Kaumann, A.J. (1983). Yohimibine and rauwolscine inhibit 5-hydroxytryptamine induced contraction of large coronary arteries of calf through blockade of 5-HT_2-receptors. Naunyn-Schmiedeberg's Arch. Pharmac., **323**, 149–154.

Lamar, J.C. and Edvinsson, L. (1980). 5-hydroxytryptamine receptors. Contractile activity and mode of inhibition by methysergide in mammalian intracranial and extracranial vessels. Arch. int. Pharmacodyn., **243**, 245–254.

Lands, W.E.M. (1981). Actions of anti-inflammatory drugs. Trends Pharmac. Sci., **2**, 78–80.

Lands, W.E.M. (1985). Mechanisms of action of antiinflammatory drugs. Adv. Drug Res., **14**, 147–164.

Lands, W.E.M. and Rome, L.H. (1976). Inhibition of prostaglandin biosynthesis. In, Prostaglandins: Chemical and Biochemical Aspects. Ed. Karim, S.M.M. MTP Press, Lancaster. 87–137.

Langley, J.N. (1878). On the physiology of the salivary secretion. J. Physiol., **1**, 339–369.

Lattimer, N., McAdams, R.P., Rhodes, K.F., Sharma, S., Turner. S.J. and Turner, J.F. (1984). Alpha$_2$-adrenoceptor antagonism and other pharmacological antagonist properties of some substituted benzoquinolizines and yohimbine in vitro. Naunyn-Schmiedeberg's Arch. Pharmac., **327**, 312–318.

Leff, P. and Martin, G.R. (1986). Peripheral 5-HT$_2$-like receptors. Can they be classified with the available antagonists? Br. J. Pharmac., **88**, 585–593.

Leff, P., Martin, G.R. and Morse, J.M. (1986). The classification of peripheral 5-HT$_2$-like receptors using tryptamine agonist and antagonist analogues. Br.J. Pharmac., **88**, 585–593.

Leffler, C.W., Tyler, T.L. and Cassin, S. (1978). Effect of indomethacin on pulmonary vascular response to ventilation of fetal goats. Am. J. Physiol., **234**, H346–351.

Lewis, B.V. (1968). The response of isolated sheep and human umbilical arteries to oxygen and drugs. J. Obstet. Gynaec. Br. Cwlth., **75**, 87–91.

Lewis, G.P. (1960). The inhibition of morphine on the action of smooth muscle stimulants on the guinea-pig intestine. Br. J. Pharmac., **15**, 425–431.

Leysen, J.E., Awouters, F., Kennis, L., Laudron, P.M., Vandenberk, J. and Janssen, P.A.J. (1981). Receptor binding profile of R 41 468, a novel antagonist at 5-HT$_2$ receptors. Life Sci., **28**, 1015–1022.

Leysen, J.E., Niemegeers, C.J.E., Tollenaere, J.P. and Laudron, P.M. (1982). [^{3}H]Ketanserin (R 41 468), a novel selective ^{3}H-ligand for serotonin$_2$ receptor binding sites. Molec. Pharmac., **21**, 301–314.

Leysen, J.E., De Chaffoy De Courcelles, D., De Clerck, F., Niemegeers, C.J.E. and Van Neuten, J.M. (1984). Serotonin-S$_2$ receptor binding sites and functional correlates. Neuropharmacology, **23**, 1493–1501.

Lowe, D.A., Mathews, E.K. and Richardson, B.P. (1981). The calcium antagonistic effects of cyproheptadine on contraction, membrane electrical events and calcium influx in the guinea-pig taenia coli. Br. J. Pharmac., **74**, 651–663.

Maayani, S., Wilkinson, C.W. and Stollak, J.S. (1984). 5-hydroxytryptamine receptor in rabbit aorta: Characterization by butyrophenone analogs. J. Pharm. exp. Ther., **229**, 346–350.

Malik, K.U. and McGiff, J.C. (1976). Cardiovascular actions of prostaglandins. In, Prostaglandins: Physiological, Pharmacological and Pathological Aspects. Ed. Karim, S.M.M. MTP Press, Lancaster. 103–200.

Martin, L.L. and Sanders-Bush. (1982). Comparison of the pharmacological characteristics of 5-HT_1 and 5-HT_2 binding sites with those of serotonin autoreceptors which modulate serotonin release. Naunyn-Schmiedeberg's Arch. Pharmac., **321**, 165–170.

Mathew, N.T. (1981). Indomethacin responsive headache syndromes. Headache, **12**, 147–150.

McAdams, R.P. and Rhodes, K.F. (1983). Antagonist actions of some novel benzoquinolizines at 5-hydroxytryptamine receptors and α_2-adrenoceptors. Br. J. Pharmac., **79**, 308P.

McAreavy, D.R.M. and McCallum, W.D. (1975). Changes in oxygen tension and vessel diameter in the human umbilical cord after delivery. J. Physiol., **244**, 67–68P.

McGrath, J.C. (1973). In: The Inhibitory and Motor Innervation of the Annococcygeus Muscle, Ph.D. Thesis, University of Glasgow.

McGrath, J.C. (1978). Modification of release by Lysergic Acid Diethylamide. In: The release of catecholamines from adrenergic neurons. (Ed. Paton, D.M.). Pergamon Press, Oxford.

McGrath, J.C. (1983). The variety of vascular α-adrenoceptors. Trends Pharmac. Sci., **4**, 14–18.

McGrath, J.C. and Olverman, H.J. (1977). Release of [^{3}H]-noradrenaline by field stimulation and by drugs from the anococcygeus muscle. Br. J. Pharmac., **60**, 305–306P.

McGrath, J.C. and Stuart-Smith, K. (1982). Human umbilical artery is contracted by oxygen, KCl or 5-HT but not by α-adrenoceptor agonists. Br. J. Pharmac., **76**, 258P.

Melmon, K.L., Cline, M.J., Hughes, T. and Nies, A.S. (1968). Kinins: possible mediators of neonatal circulatory changes in man. J. Clin. Invest., **47**, 1295–1302.

Middlemiss, D.N. (1983). Lack of effect of the putative central 5-HT agonist, 8-hydroxy-2-(di-n-propylamino) tetralin (8-OH-DPAT) on the 5-HT autoreceptor. Naunyn-Schmiedeberg's Arch. Pharmac., **76**, 324S.

Middlemiss, D.N. (1984). Stereoselective blockade at [^{3}H]5-HT binding sites and at the 5-HT autoreceptor by propranolol. Eur. J. Pharmac., **101**, 289–293.

Middlemiss, D.N. (1986). Blockade of the central 5-HT autoreceptor by β-adrenoceptor antagonists. Eur. J. Pharmac., **120**, 51–56.

Middlemiss, D.N., Blakeborough, L. and Leather, S.R. (1977). Direct evidence for an interaction of β-adrenergic blockers with the 5-HT receptor. Nature, **267**, 289–290.

Middlemiss, D.N. and Fozard, J.R. (1983). 8-hydroxy-2-(di-n-propylamino)-tetralin discriminates between subtypes of the 5-HT_1 recognition site Eur. J. Pharmac., **90**, 151–153.

Miller, D.J. and Smith, G.L. (1984). EGTA purity and the buffering of calcium ions in physiological solutions. Am. J. Physiol., **246**, C160–166.

Mitchell, M.D., Brunt, J., Bibby, J., Flint, A.P.F., Anderson, A.B.M. and Turnbull, A.C. (1978). Prostaglandins in the human umbilical circulation at birth. Br. J. Obstet. Gynaec., **85**, 114–118.

Mitchell, M.D., Jamieson, D.R.S., Sellers, S.M. and Turnbull, A.C. (1980). 6-keto-$PGF_{1\alpha}$: concentrations in human umbilical plasma and production by umbilical vessels. In Advances in Prostaglandin and Thromboxane Research. **7**. Eds. Samuelsson, B., Ramwell, P.W. and Paoletti, R. Raven press, New York, 891–896.

Moncada, S., Gryglewski, R., Bunting, S. and Vane, J.R. (1976). An enzyme isolated from arteries transforms prostaglandin endoperoxides to an unstable substance that inhibits platelet aggregation. Nature, **263**, 663–665.

Moncada, S. and Vane, J.R. (1980). Prostacyclin in the cardiovascular system. In, Advances in Prostaglandin and Thromboxane Research, **6**. Eds. Samuelsson, B., Ramwell, P.W. and Paoletti, R. Raven Press, New York, 43–60.

Modanlou, H., Yeh, S., Hon, E.H. & Forsythe, A. (1973). Fetal and neonatal biochemistry and Apgar scores. Am. J. Obstet. Gynaecol., **117**, 942-951.

Muller-Schweinitzer, E. (1980). The mechanism of ergometrine-induced coronary arterial spasm: <u>In vitro</u> studies on canine arteries. J. Cardiovasc. Pharmacol., **2**, 645–655.

Muller-Schweinitzer, E. (1981). Agonist potencies of tryptamine derivatives at pre- and post-junctional receptors in canine saphenous vein. Postgraduate Med. J., **57**, suppl. 1, 36–44.

Muller-Schweinitzer, E. and Engel, G. (1983). Evidence for mediation by 5-HT_2 receptors of 5-hydroxytryptamine contraction of canine basilar artery. Naunyn-Schmiedeberg's Arch. Pharmac., **324**, 287–292.

Murad, F., Rapoport, R.M. and Fiscus, R. (1985). Role of cyclic-GMP in relaxations of vascular smooth muscle. J. Cardiovasc. Pharmac., **7** (suppl. 3), S111–118.

Nahorski, S.R. and Willcocks. (1983). Interactions of β-adrenoceptor antagonists with 5-hydroxytryptamine receptor subtypes in rat cerebral cortex. Br. J. Pharmac., **78**, 107P.

Nickerson, M. (1956). Receptor occupancy and tissue response. Nature, **178**, 697–698.

Nugteren, D.H. and Van Dorp, D.A. (1965). The participation of molecular oxygen in the biosynthesis of prostaglandins. Biochim. Biophys. Acta., **98**, 652–654.

Nugteren, D.H. and Hazelhof, E. (1973). Isolation and properties of intermediates in prostaglandin biosynthesis. Biochim. Biophys. Acta., **326**, 448–461.

Oberhansli-Weiss, I., Heymann, M.A., Rudolph, A.M. and Melmon, K.L. (1972). The pattern and mechanisms of response to oxygen by the ductus arteriosus and umbilical artery. Ped. Res., **6**, 693–700.

Orehek, J., Douglas, J.S. and Bouhuys, A. (1975). Contractile responses of the guinea-pig trachea in vitro: modification by prostaglandin synthesis-inhibiting drugs. J. Pharmac. exp. Ther., **194**, 554–564.

Pace-Asciak, C.R. (1978). Prostaglandin biosynthesis and catabolism in several organs of developing fetal and neonatal animals. In, Advances in Prostaglandin and Thromboxane Research, **4**. Eds. Coceani, F. and Olley, P.M. Raven Press, New York, 45–59.

Pace-Asciak, C. and Wolfe, L.S. (1968). Inhibition of prostaglandin synthesis by oleic, linoleic and linolenic acids. Biochim. Biophys. Acta., **152**, 784–787.

Panigel, M. (1962). Placental perfusion experiments. Am. J. Obstet. Gynaec., **84**, 1664–1683.

Park, M.K. and Dyer, D.C. (1973). Effect of polyphloretin phosphate and 7-oxa-13-prostynoic acid on vasoactive actions of prostaglandin E_2 and 5-hydroxytryptamine on isolated human umbilical arteries. Prostaglandins, **3**, 913–920.

Paul, R.J. (1980). Chemical energetics of vascular smooth muscle. In, Handbook of Physiology, Section 2: The Cardiovascular System, Vol. 2 Eds. Bohr, D.F., Somlyo, A.P. and Sparks Jnr., H.V. American Physiological Society, Washington, 201–235.

Paul, R.J., Bauer, M. and Pease, W. (1979). Vascular smooth muscle; Aerobic glycolysis linked to sodium and potassium transport processes. Science, **206**, 1414–1416.

Pazos, A., Hoyer, D. and Palacois, J.M. (1984a). Multiple serotonin receptors in the mammalian brain: II, binding studies. IUPHAR 9th int. congress, 161P.

Pazos, A., Hoyer, D. and Palacois, J.M. (1984b). The binding of serotonergic ligands to the porcine choroid plexus: Characterization of a new type of serotonin recognition site. Eur. J. Pharmac. **106**, 539–546.

Pearson, J.F. (1976). Maternal and fetal acid-base balance. In Fetal physiology and medicine. Eds. Beard, R.W. and Nathanielsz, P.W. London, WB Saunders, 492-509.

Pedigo, N.W., Yamamura, H.I. and Nelson, D.L. (1981). Discrimination of multiple ^{3}H 5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. J. Neurochem., **36**, 220–226.

Peroutka, S.J. (1984). 5-HT_1 receptor sites and functional correlates. Neuropharmacology, **23**, 1487–1492.

Peroutka, S.J. and Snyder, S.H. (1979). Multiple serotonin receptors: Differential binding of [^{3}]-5-hydroxytryptamine. [^{3}H]-lysergic acid diethylamide and [^{3}H]-spiroperidol. Molec. Pharmacol., **16**, 687–699.

Peroutka, S.J., Noguchi, M., Tolner, D.J. and Allen, G.S. (1983). Serotonin-induced contraction of canine basilar artery: mediation by 5-HT_1 receptors. Brain Res., **259**, 327–330.

Piper, P.J. and Vane, J.R. (1969). Release of additional factors in anaphylaxis and its antagonism by anti-inflammatory drugs. Nature, **233**, 29–35.

Piper, P.J. & Vane, J.R. (1971). The release of prostaglandins from lung and other tissues. Ann. New York Acad. Sci., **180**, 363-385.

Pittman, R.N. (1981). Influence of oxygen lack on vascular smooth muscle contraction. In, Vasodilation. Eds. Vanhoutte, P.M. and Leusen, I. Raven Press, New York, 181–191.

Pittman, R.N. and Duling, B.R. (1973). Oxygen sensitivity of vascular smooth muscle. I. In vitro studies. Microvasc. Res., **6**, 202–211.

Pittman, R.N. and Quinn, J.V. (1979). A physicochemical model of adenosine's role in hypoxic relaxation of isolated vascular smooth muscle. Microvasc. Res., **17**, S37.

Powell, W.S. and Solomon, S. (1978). Biosynthesis of prostaglandins and thromboxanes in fetal tissues. In, Advances in Prostaglandin and Thromboxane Research, **4**. Eds. Coceani, F. and Olley, P.M. Raven Press, New York, 61–74.

Ratz, P.H. and Flaim, S.F. (1984). Mechanism of 5-HT contraction in isolated bovine ventricular coronary arteries. Evidence for transient receptor-operated calcium influx channels. Circ. Res., **54**, 135–143.

Richardson, B.P., Engel, G., Donatch, P. and Stadler, P.A. (1985). Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drugs. Nature, **316**, 126–131.

Roach, M.R. (1972). A biophysical look at the relationship of structure and function in the umbilical artery. In Proceedings of the Barcroft Symposium. Cambridge, Cambridge University Press, 141-163.

Rocha e Silva, M., Valle, J.R. and Picarelli, Z.P. (1953). A pharmacological analysis of the mode of action of serotonin (5-hydroxytryptamine) upon the guinea-pig ileum. Br. J. Pharmac., **8** 378–388.

Roth, G.J. and Majerus, P.W. (1975). The mechanism of the effect of aspirin on human platelets. J. Clin. Invest., **56**, 624–632.

Rose, J.C. and Lazaro, E.J. (1958). Pulmonary vascular responses to serotonin and effects of certain serotonin antagonists. Circ. Res., **6**, 283–288.

Ruffolo, R.R. Jr., Rosing, E.L. and Waddell, J.E. (1979). Receptor interactions of imidazolines. I. Affinity and efficasy for alpha-adrenergic receptors in rat aorta. J. Pharmac. exp. Ther., **209**, 429–436.

Samuelsson, B. (1965). On the incorporation of oxygen in the conversion of 8,11,14-eicosatrienoic acid to prostaglandin E_1. J. Am. Chem. Soc., **87**, 3011–3013.

Sanner, J.H. and Eakins, K.E. (1976). Prostaglandin antagonists. In, Prostaglandins: Chemical and Biochemical Aspects. Ed. Karim, S.M.M. MTP Press, Lancaster, 139–189.

Saper, J.R. (1978). Migraine. I. Classification and pathogenesis. J. Amer. Med. Assoc., **239(22)**, 2380–2383.

Schlicker, E., Gothert, M. and Hillenbrand, K. (1985). Cyanopindolol is a highly potent and selective antagonist at the presynaptic serotonin autoreceptor in the rat brain cortex. Naunyn-Schmiedeberg's Arch. Pharmac., **331**, 398–401.

Sicuteri, F., Testi, A. and Anselmi, B. (1961). Biochemical investigation in headache: Increase in hydroxyindoleacetic acid excretion during migraine attacks. Int. Arch. Allergy Appl. Immunol., **19**, 55–58.

Smith, W.L. (1986). Prostaglandin biosynthesis and its compartmentation in vascular smooth muscle and endothelial cells. Ann. Rev. Physiol., **48**, 251–262.

Smith, D.J. and Vane, J.R. (1966). Effects of oxygen tension on vascular and other smooth muscle. J. Physiol., **186**, 284–294.

Smith, J.B. and Willis, A.L. (1971). Aspirin selectively inhibits prostaglandin production in human platelets. Nature (New Biol.), **231**, 235–237.

Somlyo, A.V., Woo, C. and Somlyo, A.P. (1965). Responses of nerve-free vessels to vasoactive amines and polypeptides. Am. J. Physiol., **208**, 748–753.

Sparks Jnr., H.V. and Belloni, F.L. (1978). The peripheral circulation: local regulation. Ann. Rev. Physiol., **40**, 67–92.

Spivack, M. (1946). The anatomical peculiarities of the human umbilical cord and their clinical significance. Am. J. Obstet. and Gynaecol., **52**, 387-401.

Stacey, R.S. (1966). Clinical aspects of cerebral and extracerebral 5-hydroxytryptamine. In, Handbook of Experimental Pharmacology, **19**. Ed. Erspamer, V. Springer-Verlag, Berlin, 744–786.

Starling, M.B. and Elliott, R.B. (1974). The effects of prostaglandins, prostaglandin inhibitors, and oxygen on the closure of the ductus arteriosus, pulmonary arteries and umbilical vessels in vitro. Prostaglandins, **8**, 187–203.

Stembera, Z.K., Hodr, J. and Janda, J. (1965). Umbilical blood flow in healthy newborn infants during the first minutes after birth. Am. J. Obstet. Gynaecol., **91**, 568–574.

Stephenson, R.P. (1956). A modification of receptor theory. Br. J. Pharmac., **11**, 379–393.

Stone, C.A., Wenger, H.C., Ludden, C.T., Stavorski, J.M. and Ross, A.C. (1961). Antiserotonin-antihistiminic properties of cyproheptadine. J. Pharmac., **131**, 73–84.

Strong, C.G. and Bohr, D.F. (1967). Effects of prostaglandins E_1, E_2, A_1 and $F_{1\alpha}$ on isolated vascular smooth muscle. Am. J. Physiol., **213**, 725.

Svensson, J. and Hamberg, M. (1976). Thromboxane A_2 and prostaglandin H_2: Potent stimulators of the swine coronary artery. Prostaglandins, **12**, 943–950.

Svensson, J., Hamberg, M. and Samuelsson, B. (1975). Prostaglandin endoperoxides. IX. Characterization of rabbit aorta contracting substance (RCS) from guinea-pig lung and human platelets. Acta Physiol. Scand., **94**, 222–228.

Takeguchi, T.C., Kohno, E. and Sih, C.J. (1971). Mechanism of prostaglandin biosynthesis. I. Characterisation and assay of bovine prostaglandin synthetase. Biochemistry, **10**, 2372–2376.

Tomlinson, R.V., Ringold, H.J., Qureshi, M.C. and Forchielli, E. (1972). Relationship between inhibition of prostaglandin synthesis and drug efficasy: support for the current theory on mode of action of aspirin-like drugs. Biochim. Biophys. Res. Commun., **46**, 552–559.

Trendelenburg, U. (1960). The action of histamine and 5-hydroxytryptamine on isolated mammalian atria. J. Pharmac., **130**, 450–460.

Tuvemo, T. (1978). Action of prostaglandins and blockers of prostaglaglandin synthesis on the isolated human umbilical artery. In, Advances in Prostaglandin and Thromboxane Research, **4**. Eds. Coceani, F. and Olley, P.M. Raven Press, New York, 271–274.

Tuvemo, T, (1980). Role of prostaglandins, prostacyclin, and thromboxanes in the control of the umbilical-placental circulation. Semin. Perinatol., **4**, 91–95.

Tuvemo, T., Strandberg, K., Hamberg, M. and Samuelsson, B. (1976). Maintenance of the tone of the human umbilical artery by prostaglandin and thromboxane formation. In, Advances in Prostaglandin and Thromboxane research, **1**. Eds. Samuelsson, B. and Paoletti, R. Raven Press, New York, 425–428.

van Dorp, D.A., Beerthuis, R.K., Nugteren, D.H. and Vonkeman, H. (1964). The biosynthesis of prostaglandins. Biochim. Biophys. Acta, **90**, 204–207.

Van Harn, G.L., Rubio, R. and Berne, R.M. (1977). Formation of adenosine nucleotide derivatives in isolated hog carotid artery strips. Am. J. Physiol., **233**, H299–304.

Van Neuten, J.M., Janssen, P.A.J., Van Beek, J., Xhonneux, R., Verbeuren, T.J. and Vanhoutte, P.M. (1981). Vascular effects of ketanserin (R 41 468), a novel antagonist of 5-HT_2 serotonergic receptors. J. Pharmac. exp. Ther., **218**, 217–230.

Van Neuten, J.M. and Vanhoutte, P.M. ((1981). Selectivity of calcium antagonists and serotonin antagonism with respect to venous and arterial tissues. Angiology, **32**, 476–484.

Van Rossum, J.M. (1963). Cumulative dose-response curves. II. Technique for the making of dose-response curves in isolated organs and the evaluation of drug parameters. Arch. Int. Pharmacodyn. Ther., **143**, 299–330.

Vane, J.R. (1960). The actions of sympathomimetic amines on tryptamine receptors. In, Adrenergic Mechanisms. Eds. Vane, J.R., Wolstenholme, A.E.W. and O'Conner, M. Churchill, London, 356–372.

Vane, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action for aspirin-like drugs. Nature (New Biol.), **231**, 232–235.

Vane, J.R. (1972). Prostaglandins and the aspirin-like drugs. Hosp. Pract., **7**, 61–71.

von Euler, U.S. (1936). On the specific vasodilating and plain muscle stimulating substances from accessory glands in man and certain animals (prostaglandin and vesiglandin). J. Physiol., **84**, 21P.

von Euler, U.S. (1938). Action of adrenaline, acetylcholine and other substances on nerve-free vessels (human placenta). J. Physiol., **93**, 129–143.

Walsh, S.Z., Meyer, W.W. and Lind, J. (1974). In, The Human Fetal and Neonatal Circulation. Charles C. Thomas, Illinois.

Watts, A.D., Feniuk, W. and Humphrey, P.P.A. (1981). A pre-junctional action of 5-hydroxytryptamine and methysergide on noradrenergic nerves in dog isolated saphenous vein. J. Pharm. Pharmac., **33**, 515–520.

Waud, D.R. (1975). Analysis of dose-response curves. In, Methods in Pharmacology. Eds. Daniel, E.E. and Paton, D.M. Plenum Press, New York, 471–506.

Welburn, P.J. and Jones, R.L. (1978). A comparison of prostaglandin F_2 and three 16-aryloxy analogues on the isolated rabbit jejunum. Prostaglandins, **15**, 287–296.

Windle, W. (1940). In, Physiology of the Fetus. WB Saunders, Philadelphia and London,

Wulf, H. (1964). The oxygen and carbon dioxide tension gradients in the human placenta at term. Am. J. Obstet. Gynaec., **88**, 38-44.