ADRENERGIC CONTROL OF POTASSIUM AND MAGNESIUM:

INTERACTION WITH DRUG THERAPY.

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Thesis submitted for the degree of Doctor of Medicine

to the

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PREFACE

This thesis describes research undertaken during my appointment as Registrar in the University Department of Materia Medica, Stobhill General Hospital, Glasgow. Some of the work which it contains has been published or presented to learned societies. Reprints, where available are submitted with the thesis. I have been fortunate in having the cooperation and collaboration of a number of colleagues and friends. They are formally acknowledged. Except where stated, the work of this thesis has been personally carried out by me. The writing of this thesis is entirely my own work.

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ACKNOWLEDGEMENTS.

During the period of research leading to the submission of this thesis, I have been fortunate in having the encouragement, advice and colloboration of a number of my colleagues. I am indebted to them all.

I am grateful to Professor J.L. Reid for making the facilities of the University Department of Materia Medica available to me, and for his support and encouragement during this work. He has always been generous in his time and advice, both critical and practical. I thank Dr. G.J. Addis for encouraging and help planning these studies and for providing the time in which I carried out much of this work.

The practical support of the research nurses and assistants in the Clinical Pharmacological Laboratory who helped carry out these studies is gratefully acknowledged. The cheerful, friendly help of Mr. Robert Whitesmith of the Department of Biochemistry with the analysis of large numbers of samples was also invaluable.

I am also grateful for the patient help of Ms. Lorna Gilbert of the Department of Pharmacy who supplied and supervised the blinding of the studies and who went to considerable lengths to provide identical placebos. Many other members of the department of Materia Medica also helped during these studies and their contribution is much appreciated. I would like to particularly thank

Dr. Andrew Kelman and Dr. David Summner who were always happy to help with statistical advice and comment and who wrote the statistical programmes used for the analysis.

Finally I would like to warmly thank my collaborator, Dr. Colin Reid who helped to complete some of the studies in my absence and his contribution is duly acknowledged in the appropriate chapter.

SUMMARY

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Hypokalaemia is potentially fatal (**Chapter 2**). The internal regulation of potassium, i.e. the movement of potassium between body compartments, has not been extensively investigated (**Chapter 1**). Rapid movements of potassium can occur across cell membranes, e.g. in diabetic ketoacidosis the hyperkalaemia can be rapidly reversed by insulin administration, and the existence of a specific membrane enzyme controlling movement of potassium and sodium between the intracellular and extracellular compartments, Na^+/K^+ ATPase, has been known for 30 years (**Chapter 1.5**).

Some acutely ill patients have hypokalaemia on admission to hospital which resolves without treatment. This observation led to the hypothesis that increased sympathetic activity, raising circulating adrenaline levels, stimulates a Na^+/K^+ ATPase linked to a $beta_2$-adrenoreceptor on cell membranes pumping potassium into cells. Animal work by Clausen supported this theory and several studies in humans, some carried out in the Department of Materia Medica, were also supportive, demonstrating that infusing adrenaline resulted in hypokalaemia (**Chapter 1**).

In the studies presented in this thesis both the mechanism and the clinical relevance of adrenaline induced hypokalaemia, with particular emphasis on the effects of a number of widely used drugs, have been

studied. Many drugs have been designed to specifically act on receptors in the sympathetic nervous system, either as agonists or antagonists, e.g. beta-blockers and $beta_2$-agonists, and they are frequently administered to patients with cardiovascular disease who are at increased risk of dysrhythmias should hypokalaemia occur. Such patients are at increased risk of suffering acute stress, such as myocardial infarction, which increases circulating adrenaline levels.

An infusion regimen of (-)-adrenaline which would safely raise circulating adrenaline to concentrations similar to those seen in acute severe illness was developed (**Chapter** 3). This regimen consistently raised adrenaline levels seen in normal subjects during supine rest by 10 fold or more. During the infusions adrenaline levels did fluctuate, but they remained in the pathophysiological range. The regimen involved stepwise increases in the rate of adrenaline infusion and proved safe despite the adrenaline infusion being combined with other drugs with sympathomimetic activity.

The mechanism of adrenaline induced hypokalaemia in man is unproven. However, the possibility that adrenaline induced hypokalaemia could be the result of β-agonist induced changes in plamsa insulin was excluded (**Chapter 4.2**). Both plasma insulin and potassium concentrations fell during the adrenaline infusion. Attenuation of adrenaline induced hypokalaemia by beta-adrenoceptor

antagonists with varying degrees of cardioselectivity (β_1) was studied and demonstrated that adrenaline induced hypokalaemia was mediated via the β_2 adrenoceptor (**Chapter 4.3 & 4.4**). Whether the fact that cardioselective beta-antagonists will be less effective in protecting patients from adrenaline induced hypokalaemia during the acute stress of severe illness is of any clinical significance remains unknown.

Salbutamol, a selective beta$_2$-agonist, was also shown to cause hypokalaemia when given intravenously (**Chapter 4.2**). It is administered in high doses in acute attacks of asthma, where it might be expected that circulating adrenaline levels are raised. An additive hypokalaemic effect of exogenous adrenaline and salbutamol was demonstrated (**Chapter 4.2**).

Hypokalaemia is a relatively common adverse effect of many diuretics and such hypokalaemia could increase the severity of hypokalaemia during acute stress. No synergistic action on potassium levels was demonstrated (**Chapter 5**) between adrenaline and any diuretic. However, both frusemide and bendrofulazide lowered plasma potassium and, therefore, during the adrenaline infusion more profound hypokalaemia was observed because baseline potassium was lower.

Theophylline, widely used as a bronchodilator, has been reported to increase circulating catecholamine

levels and to interact with sympathomimetics (**Chapter 6.1 & 6.2**). Chronic theophylline therapy increased adrenaline induced hypokalaemia (**Chapter 6.4**). The mechanism of this interaction was not demonstrated, though the possibility that it was the result of theophylline increasing circulating adrenaline levels was excluded (**Chapter 6.3**).

Theophylline and sympathomimetics, such as salbutamol, are frequently given in combination, both as chronic therapy and in acute bronchospasm. The safety of this combination therapy has been questioned. Chronic oral theophylline therapy significantly increased the hypokalaemic effect of intravenous salbutamol (**Chapter 6.5**). The clinical relevance of this observation is uncertain as patients are frequently on chronic β_2-agonist therapy which may cause down regulation of receptors.

Hypomagnesaemia can occur in situations in which circulating adrenaline levels are known to be raised, such as acute myocardial infarction (**Chapter 7.1**). The control of internal regulation of magnesium is not understood (**Chapter 1.7**). The role of adrenaline in the control of magnesium levels was studied, using the same adrenaline infusion regimen, and a small but significant fall in plasma magnesium was observed (**Chapter 7.2**). This was unaltered by pretreatment with diuretics (**Chapter 7.3**). The mechanisms and clinical relevance of adrenaline

induced hypomagnesaemia require further study but these have not yet been attempted.

CHAPTER 1

PHYSIOLOGICAL CONTROL OF PLASMA POTASSIUM AND MAGNESIUM

1. BACKGROUND AND SCOPE OF THESIS.

1.1 Background.

This thesis describes a series of investigations into the phenomenon of adrenaline induced hypokalaemia. Though originally described over 50 years ago (D'Silva, 1934), adrenaline induced hypokalemia had been forgotten and it is only very recently that it has again been recognised and its clinical significance questioned.

The increasing use of auto-analysers in departments of biochemistry has revealed frequent cases of unexplained hypokalaemia and the observation that a significant proportion of these patient's plasma potassium concentrations spontaneously recovered without the need for potassium supplementation therapy (Morgan & Young, 1982) aroused renewed interest in the causes of hypokalaemia, the homeostatic mechanisms controlling plasma potassium concentrations and the clinical importance of hypokalaemia.

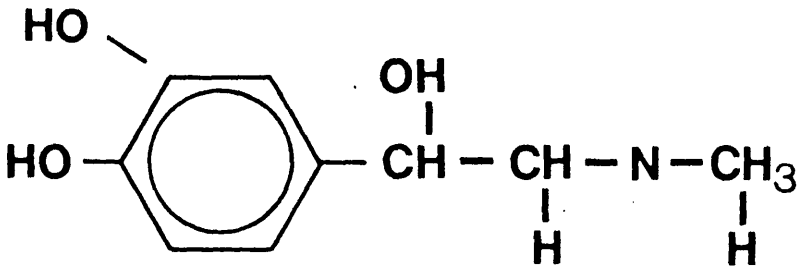
Following the development of sensitive and reliable catecholamine assays it is now possible to measure circulating levels of catecholamines in many physiological situations ranging from rest to the stress of physical activity and illness (Engelman, Portnoy & Lovenberc, 1968; Passon & Peuler 1973; Da Prada & Zurcher 1975). The observation that many of the patients with

unexplained hypokalaemia had acute illnesses which were associated with rises in circulating adrenaline led to the hypothesis that the hypokalaemia was adrenaline induced. This hypothesis was supported by studies showing that raising circulating adrenaline levels by exogenous infusion of adrenaline resulted in hypokalaemia.

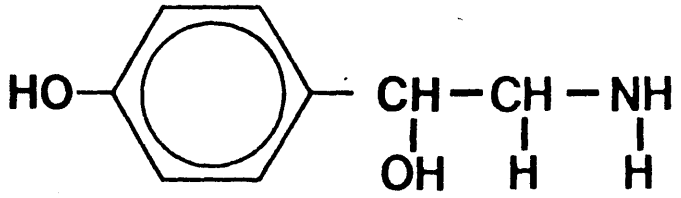
This thesis describes the results of studies of adrenaline induced hypokalaemia which had the aim of determining the mechanism of adrenaline induced hypokalaemia and of examining the interactions with adrenaline, of a number of drugs used in general and specialist practice which may alter adrenaline induced hypokalaemia.

1.2 The sympathetic nervous system and adrenal medulla.

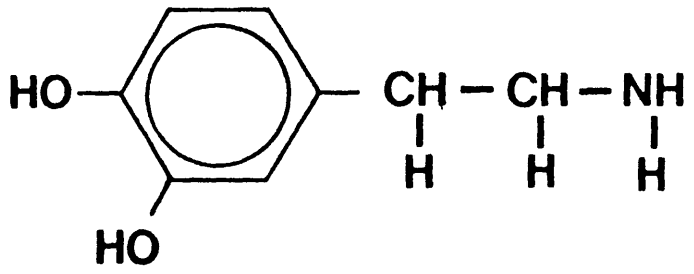
In man the sympathetic nervous system and the adrenal medulla regulates both metabolic and haemodynamic actions. In plasma three circulating catecholamines can be detected, noradrenaline, adrenaline and dopamine (Figure 1.2.1). Dopamine is a precursor of adrenaline and noradrenaline though specific dopamine receptors have been described in the renal circulation. There is still controversy about the physiological or pathological role of circulating dopamine (Weiner, 1985). Noradrenaline is primarily a neurotransmitter and, in contrast, adrenaline is a hormone in the traditional sense. From the classical work of Ahlquhist (Ahlquist, 1948) and later Lands (Lands



ADRENALINE



NORADRENALINE



DOPAMINE

Fig.1.2.1. : STRUCTURE OF THE CIRCULATING CATECHOLAMINES

et al.,1967a; Lands, Luduena & Buzzo, 1967b) it is known that the sympathetic nervous system exerts its effects on a variety of target tissues via receptors. These receptors can be divided into alpha (α) and beta (β) subtypes which themselves can be further subdivided into α_1 and α_2 subtypes and β_1 and β_2 subtypes respectively. α_1 receptors are predominantly post-synaptic and their effect on smooth muscle is predominantly excitatory. α_2 receptors are mostly pre-synaptic and act as a feedback system to modulate further noradrenaline release from sympathetic nerve ending's storage vesicles. β_1 receptors predominate in cardiac muscle and are excitatory whereas β_2 receptors are present on smooth muscle and glandular tissue. Many tissues have mixtures of receptors, an excellent example is the lung which has both α_1 as well as β_1 receptors in addition to the well recognised β_2 receptors on airway smooth muscle (Barnes, Karliner & Dollery, 1980).

Current evidence suggests that in man at rest the majority, if not all, the circulating noradrenaline is the result of spill-over of noradrenaline from post ganglionic sympathetic nerve endings in which noradrenaline is the sympathetic neurotransmitter. Sympathetic stimulation results in prompt exocytotic release of stored noradrenaline into the synaptic cleft. It has been shown that the adrenal medulla does release

noradrenaline and, under certain conditions, is a major source of noradrenaline (Silverberg et al., 1978) and achieves concentrations that produce biological effects. Noradrenaline is a potent α1-agonist and is equipotent with adrenaline at β_1-receptors but has little effect on β_2-receptors. 10-20% of the catecholamine content of the adrenal medulla is noradrenaline. Noradrenaline levels increase on exercise and the relative contributions to this rise from increased spill-over from post-ganglionic sympathetic nerves and from adrenal medullary release is uncertain (Cryer, 1980).

All circulating adrenaline appears to originate from the adrenal medulla and with acute severe physical stress there is a large increase in adrenaline concentrations as a result of direct sympathetic stimulation of the adrenal medulla. Massive elevation, several hundred times resting values, has been reported following cardiac arrest (Wortsman Frank & Cryer, 1984) and insulin induced hypoglycaemia has been shown to increase levels several fold (Cryer, 1980) as does myocardial infarction. Adrenaline is approximately 10 times more potent than noradrenaline in producing metabolic effects, though the profile of actions of adrenaline and noradrenaline are not identical (Clutter et al.,1980), due to their differing affinities for α_1, α_2, β_1 and β_2 receptors.

At rest physiological concentrations of adrenaline are

small and, whilst the effects of supraphysiological doses of adrenaline have been well documented (Weiner, 1985), it is only with the relatively recent development of highly sensitive assay techniques that it has become possible to examine the physiological role of circulating adrenaline. It remains unclear if basal resting adrenaline levels have any physiological role (Cori & Buchwald,1930; Clutter et al.,1980; Fellows, Bennett & McDonald, 1985; Freyschuss et al.,1986) and many authors have assumed that detectable adrenaline is the result of leakage from the adrenal medullae. It is now clear that low concentrations of adrenaline in the physiological range, two to three times the basal concentration, such levels are seen with mild exercise and smoking, have detectable metabolic and haemodynamic actions, with increases in heart rate and lipolysis (Clutter et al.,1980; Freyschuss et al.,1986).

Do pathophysiological concentrations of adrenaline cause significant hypokalaemia? As noted previously the observation of hypokalaemia on admission to hospital in a significant proportion of patients with acute severe illnesses, such as myocardial infarction, in whom circulating adrenaline levels are known to be raised, and that in some patients the plasma potassium levels spontaneously recovered has led to investigation of the role of catecholamines in the acute control of extracellular potassium.

1:3 The physiological role of potassium.

Potassium is the chief intracellular cation and is present in cells in a concentration of approximately 160 mmol l^{-1} and 98%, 3,500mEq, of total body potassium is intracellular. Extracellular potassium concentration is low, 3.5-5.0 mmol l^{-1}, only 60-70 mEq in total (DeFonzo & Bia, 1985). There is thus a high concentration gradient across cell membranes which contributes critically to the membrane potential of excitable tissues such as skeletal muscle, smooth muscle and the nervous system. Conversely intracellular sodium concentrations are low and extracellular concentration high. Work is therefore required to overcome both a chemical and an electrical gradient to move these ions across cell membranes. Movement of these ions is carried out by a specific, enzyme dependent, cell membrane bound, active transport mechanism, the Na^+/K^+ ATPase.

High intracellular concentrations of potassium are required by many metabolic processes, such as reabsorption of solutes by the kidney and uptake of nutrients by the intestine whilst active extrusion of sodium is required to maintain osmotic equilibrium across the membrane.

Failure to maintain potassium homeostasis will alter the function of many organs but the most dramatic effect is on the two highly specialised tissues which have the

property of excitability, thus allowing the transmission of signals along the surface of the cell and between cells. Normal function of these tissues is dependent on the maintenance of an electrical potential across the membrane of the cell at a specific voltage, nerve and muscle.

Voltages across cell membranes are measured relative to the outside of the cell. The contribution of an individual ion is determined by the permeability of the membrane for that ion and the concentration gradient across the membrane. The prime ions in mammalian cells are sodium and potassium with a membrane permeability of 1:30 and this ratio results in the resting membrane potential of -70mV. The excitability of such cells is dependent on the resting potential, -70mV, which is in turn dependent on the relative concentrations of potassium and sodium ions across the membrane. Hypokalaemia and hyperkalaemia will therefore alter the relative concentrations of potassium across the cell membrane and thus alter the resting potential and hence excitability.

1.4 The control of plasma potassium.

Plasma potassium concentration depends on intake and excretion, external balance, which determines whole body potassium stores, and the distribution between

extracellular and intracellular compartments, the internal balance.

External Balance:

The daily estimated intake of potassium is between 50-150mEq (3.7-11.0g of potassium chloride) in a normal diet (Fregly, 1981). Potassium is absorbed from the intestine by diffusion but absorptive processes may be involved. Thus a single meal may contain as much potassium, 3 grams, as is present in the total extracellular space.

External potassium balance is primarily regulated by the kidney with, in health, only a small contribution from the gastrointestinal tract (Fig. 1.4.1). Excretion of potassium by the kidney is largely determined by the quantity of potassium in the diet, renal excretion rising in response to high potassium diets (Adam and Dawborn, 1972).

Most filtered potassium is reabsorbed in the proximal tubule and by the ascending loop of Henle and the major site for control of potassium excretion is the distal nephron (Berliner, 1961). In recent years it has become clear that renal tubular handling of potassium is more complex than originally thought and the exact mechanisms remain uncertain. In the early distal tubule active transport systems move potassium into cells on both the peritubular and luminal surfaces. Movement of potassium into tubular urine is passive as it is dependent on

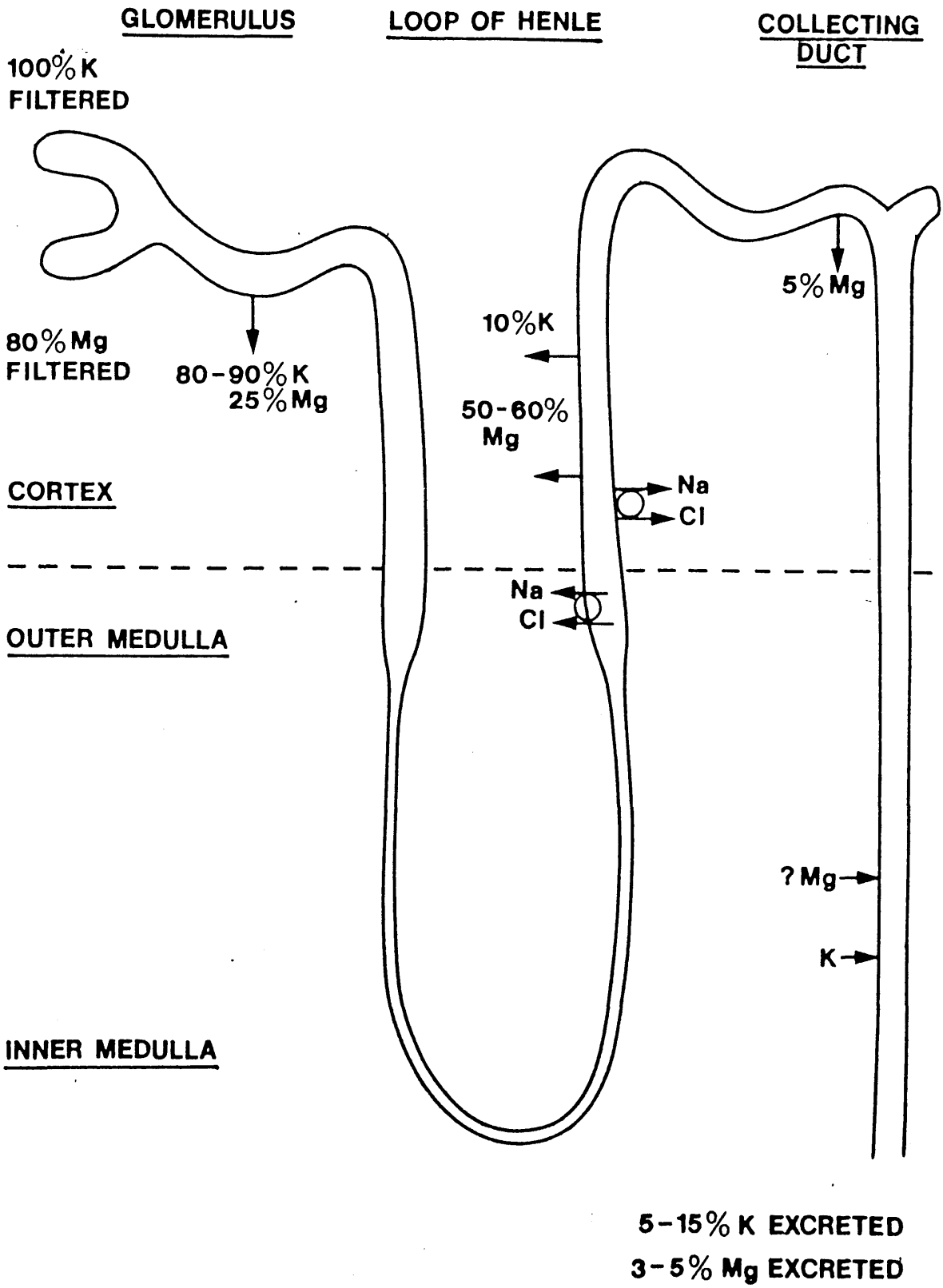


Fig. 1.4.1. : RENAL HANDLING OF POTASSIUM (K) AND MAGNESIUM (Mg)

electrical and chemical gradients. The luminal concentration of sodium and its accompanying anion are crucial, though poorly understood, controlling factors. More recent studies have suggested that under some circumstances the flow rate in the distal tubule is a further controlling factor (Wright, 1987). In the cortical collecting ducts potassium transport into tubular fluid is active and is again indirectly linked to intraluminal sodium concentrations and rate of flow of tubular fluid. Finally under conditions of metabolic alkalosis, solute diuresis or potassium loading further secretion of potassium may occur in the medullary collecting ducts. Thus potassium load, sodium delivery to the distal nephron and distal flow rates may all have a direct effect on renal potassium handling though the exact mechanisms are unknown.

Aldosterone is the principal mineralocorticoid and aldosterone levels increase in response to an increase in plasma potassium levels resulting in enhanced sodium reabsorption and potassium secretion in the distal tubules, probably by increasing the permeability of the luminal cell membranes and by increasing active transport of potassium across the peritubular membranes into the distal tubular cells (Cox, Sterns & Singer, 1978).

pH changes affect internal potassium regulation but, by altering renal tubular cell potassium content, also

indirectly alter distal tubular potassium loss.

In renal failure the colon can become a route for significant potassium excretion but is not thought to be of significance in either health or non-renal diseases.

Internal Balance:

As stated above a single meal may contain as much potassium as the extracellular compartment and thus control mechanisms must be able to rapidly handle acute potassium loads. Previously it was believed that the actions of insulin, pH and aldosterone were the principal mechanisms recruited to maintain homeostasis.

Insulin levels rise following meals and it is also known that an increase in plasma potassium stimulates insulin release resulting in an intracellular shift of potassium (Bia & Defronzo, 1981). Experimentally supra-physiological doses of insulin results in plasma potassium uptake by the liver and muscle (DeFronzo, 1987). It has not yet been convincingly shown that physiological doses of insulin have a significant effect on plasma potassium. Though it may be that the relatively high portal vein levels of insulin are sufficient to promote hepatic potassium uptake. Tolerance of potassium loads in insulin deficient diabetics and in normal subjects infused with somatostatin, which causes insulinopenia, is reduced which suggests that insulin does have a role in potassium homeostasis in health

though it may not be major (DeFronzo et al., 1978).

Aldosterone's principal mode of action on plasma potassium is renal, as discussed above, however it may influence the handling of acute potassium loads though the evidence is controversial. Adrenalectomized high potassium load adapted animals are unable to handle acute potassium loads (Bia, Tyler & Defronzo, 1981).

pH and plasma bicarbonate separately influence internal potassium balance with an increase in either pH or bicarbonate increasing intracellular potassium (Fraley & Adler, 1977). The hyperkalaemia of acidosis has long been attributed to an exchange of hydrogen ions for potassium in cells but hyperkalaemia is not a feature of post-ictal lactic acidosis, nor does an infusion of organic as opposed to a mineral acid in dogs induce hyperkalaemia (Ledingham, 1983). The explanation for these discrepancies is not known.

1.5 Adrenaline and plasma potassium.

Over fifty years ago the first evidence that the adrenergic nervous system may influence plasma potassium levels emerged and there were several further reports suggesting an interaction subsequently. Despite this evidence the possibility that physiological concentrations of catecholamines may have a regulatory action, and be of clinical significance, had been ignored

until this decade.

In 1934 D'Silva reported that the intravenous injection of 0.05mg of adrenaline in cats resulted in a very rapid and large rise in serum potassium but that this rise was short lived and was followed by a fall to levels below normal for 15-30 minutes (D'Silva, 1934). This worker then went on to demonstrate that the rise in serum potassium was the result of potassium release from the liver (D'Silva, 1936) and later workers demonstrated that the rise was mediated principally by alpha adrenergic receptors (Todd & Vick, 1971). In 1940 it was shown that adrenaline infusions had an identical effect on serum potassium as bolus injection (Larson, 1940). In these early experiments plasma catecholamine levels were unavailable thus making it impossible to relate observed effects to physiological doses and the mechanism of the hypokalaemia remained unknown.

In 1941 Dean proposed the existence of a sodium 'pump' in mammalian muscle cells to explain the observation that as the skeletal muscle cell membrane was permeable to sodium then there must be an active sodium excreting system to maintain intracellular sodium levels (Dean, 1941). The next development was the description of adenosine triphosphate dependent fluxes of sodium and potassium across red cell membranes (Schatzmann, 1953) and in 1956 these fluxes were shown to be dependent, in

the nervous system, on a Na^+/K^+ ATPase which could be inhibited by cardiac glycosides (Skou, 1956). Initially it was not thought that this 'pump' directly affected the cell membrane potential (Hodgkin & Horowicz, 1959). However, it was soon demonstrated that the sodium ' pump' could be electrogenic in many tissues (Thomas, 1972). In the 1970's membrane physiologists realised that the Na^+/K^+ ATPase could be directly stimulated by adrenergic amines with beta-adrenergic activity (Hays et al., 1974). In an elegant series of experiments since 1977 Clausen has shown in frogs the existence of a $beta_2$-adrenergic receptor on skeletal muscle membranes linked to Na^+/K^+ ATPase (Clausen & Flatman,1977; Clausen & Flatman, 1980, Clausen, 1983).

In man the importance of catecholamines in potassium homeostasis was first suggested by a report in 1974 that the tolerance to an intravenous potassium load was impaired by adrenalectomy (Lockwood & Lum, 1974) and in 1980 it was shown that $beta_2$-adrenergic blockade reduced the hypokalaemic effect of adrenaline as well as blocking the protective effect of adrenaline on potassium loading (Rosa et al., 1980).

Further reports followed rapidly implicating $beta_2$-adrenergic linked membrane bound Na^+/K^+ ATPase; chemical sympathectomy reduced potassium tolerance and adrenaline partially restored tolerance only in the absence of beta-

blockade (Silva & Spokes, 1981) yet there was no change in the number of skeletal muscle cell pumps (Clausen, Hansen & Larrson, 1981); pre-treatment with the $beta_2$-agonist reduced the effects of endogenous catecholamines on Na^+-K^+ distribution (Buur et al., 1982).

Thus, there was increasing evidence from animal work and, to a lesser extent, from human work to implicate adrenaline in potassium homeostasis in man. The major questions that remained were whether adrenaline at physiological doses, that is those seen at rest or in stress, could influence potassium homeostasis in man and whether such effects were mediated through beta-adrenergic receptors which would have serious implications for many modern drugs which influence such receptors directly or indirectly.

1.6 The physiological role of magnesium.

Magnesium is the second most abundant intracellular cation and is present in plasma in relatively small amounts (<1%). Magnesium's primary role is as an essential co-factor for many intracellular enzyme systems including several which are involved in key steps in intermediatory metabolism and phosphorylation both within the cell and at the cell membrane (Levine & Coburn, 1984). Thus magnesium is essential for the hydrolysis of adenosine triphosphate, a central component of the cellular pumps which maintain the homeostasis of sodium,

potassium and calcium. Magnesium is also involved in protein and DNA synthesis and may have a crucial role in the regulation of mitochondria (Anast & Gardner, 1981).

Total body magnesium stores are approximately 1000 mmoles in man and magnesium is distributed in three compartments, 65% in the mineral phase of the skeleton, 34% in the intracellular space and only 1% in the extracellular fluid. In plasma 75-80% of magnesium is in the ionic and complexed form and 20-25% is protein bound. Extracellular magnesium concentration is approximately 0.8 mmol l^{-1}. The intracellular concentration is 10 mmol l^{-1}, but most is bound to cellular constituents, such as nucleic acid and protein. It is likely that the free intracellular magnesium concentration is between 0.1 and 1.0 mmol l^{-1} (Levine & Coburn, 1984). Intracellular concentrations are carefully maintained despite changes in extracellular concentrations which implies the existence of specialized magnesium transport systems since magnesium can penetrate membranes, though slowly, and magnesium concentration is kept well below electrochemical equlibrium. Magnesium ions are small and highly polarized and have a large hydrated size in solution. They will thus pass with difficulty through small water-filled channels. These physical characteristics would explain the low membrane permeability of magnesium (Flatman, 1984).

1.7 The control of plasma magnesium.

Regulation of magnesium balance is poorly understood. Magnesium is absorbed from the gut. Though studies of absorption are few in man, they have suggested both that absorption is sub-optimal, 35-45% (Johansson et al., 1980), may be influenced by the composition of the diet (Lindeman, 1980) and that the magnesium salt present alters the extent of absorption (Hodgkinson & Heaton, 1965). Average recommended daily magnesium requirement is 350 milligrams, approximately 30mEq. (Dupin, 1981). However, there is evidence that magnesium requirements are altered by the composition of the diet, requirements being higher with high protein diets (Caddell & Goddard, 1967). Absorption sites are predominantly located in the small bowel.

60-70% of ingested magnesium is detected in faeces but there is no evidence of intestinal secretion of magnesium into the bowel lumen. The regulation of external magnesium balance is primarily via the kidneys and the filtered magnesium quantity is dependent on plasma magnesium concentration, only 3-5% of filtered magnesium is excreted (Massry, 1977). Excess magnesium results in decreased proximal tubular reabsorption. Renal conservation is very efficient during periods of depletion (Levine & Coburn, 1984). Many diverse factors, including a variety of hormones, alter urinary magnesium

excretion. These include extracellular volume expansion, hypercalcaemia, diuretics, alcohol ingestion, phosphate depletion, decreased parathyroid hormone activity, calcitonin, adrenocortical steroids, thyroxine and vitamin D (Dirks & Quamme, 1983). The relative importance of these various factors in magnesium homeostasis in man remains unknown and many of the reported effects may be indirect and of little physiological significance.

Internal regulation, the movement of magnesium between the intracellular and extracellular compartments, has received little attention though it is known that intracellular concentrations are maintained within a narrow range and that this homeostatic control is capable of adapting to wide variations in extracellular concentrations (Levine & Coburn, 1984). The mechanisms of this regulation are unknown (Ferment & Touitou, 1985).

In humans it has been reported that salbutamol, a β_2-agonist produces a small fall in magnesium concentrations (Philips et al., 1980). Additional evidence of a role for the adrenergic system in internal magnesium balance is suggested by the observations that in situations of acute stress with increased circulating catecholamine levels, such as myocardial infarction (Dyckner, 1980), cardiac surgery (Holden, Ionescu & Wooler, 1972; Scheinmann, Sullivan & Hyatt, 1969) and insulin-induced hypoglycaemia (Lindsay, 1976) hypomagnesaemia may occur.

1.8 The scope of this thesis.

Thus there is evidence that the adrenergic neuroendocrine system may be involved in the internal regulation of potassium and magnesium. With the recent development of a number of drugs whose principal mechanism of action is either via adrenergic receptors or which are known to alter body cation stores, it is important to examine the potential of such drugs to interfere with potassium and magnesium homeostasis. The work presented in this thesis had the following aims in examining the effect of adrenergic stimulation on plasma potassium and magnesium.

(1) To determine a safe adrenaline infusion regimen capable of producing circulating adrenaline concentrations similar to concentrations reported in acute stress;

(2) Using such an adrenaline infusion regimen to determine if physiological adrenaline concentrations alter plasma concentrations of potassium and magnesium.

(3) To determine the subtype of beta-adrenoceptor responsible for the effect of adrenaline on plasma potassium by administering appropriate agonists and antagonists.

(4) Having established that physiological circulating

concentrations of adrenaline induce significant hypokalaemia to demonstrate if there is any interaction with diuretic therapy.

(5) To investigate if there is any interaction between methyl-xanthines and circulating adrenaline on plasma potassium concentrations.

(6) To investigate if physiological circulating concentrations of adrenaline alter plasma magnesium concentrations and to examine there is any interaction with diuretic therapy.

CHAPTER 2

PATHOLOGICAL CONSEQUENCES AND IMPLICATIONS OF HYPOKALAEMIA AND HYPOMAGNESAEMIA

2. PATHOLOGICAL CONSEQUENCES AND IMPLICATIONS OF HYPOKALAEMIA AND HYPOMAGNESAEMIA

2.1 The maintenance of cell membrane potential in excitable tissues.

Though all mammalian cells have a voltage potential across their resting cell membranes it is the two highly specialised cells, neurones and muscle cells, that have the property of excitability, that is when their cell membrane potential falls to a specific threshold then the cell depolarizes. Depolarization may be the result of transmission of an impulse down the cell, from stimulation of receptors on the cell membrane or by the transmission from adjacent cells, as in neuronal axons, skeletal muscle and cardiac muscle respectively. Finally in the case of the cardiac conducting system the cells may spontaneously depolarise at a predetermined rate; that is show automaticity.

Alterations in intracellular and extracellular cation concentrations in these tissues will affect neuronal transmission in the case of the nervous system, contractility in skeletal muscle, and pacemaker functions and impulse conduction in cardiac conducting tissue, and rates of spontaneous depolarization in cardiac muscle.

Membrane potentials are a direct result of the ratio of potassium concentration across the cell membrane. Surprisingly the exact intracellular concentration of

potassium remains uncertain and quoted figures have varied from 140-160 mmol l^{-1}. Though physical chemical measurements suggest that the bulk of intracellular potassium has chemical activity and the potassium is completely exchangeable under physiological conditions it is also uncertain if there is non-uniform distribution or binding of a certain fraction of potassium within the cell (Kushmerick & Podolsky, 1969; Sperelakis & Ler, 1979).

Since Bernstein it has been appreciated that the non-uniform distribution of inorganic ions across cell membranes must result in electrical potential differences across the membrane (Bernstein, 1902) and his original theory that the membrane was impermeable to all ions except potassium was modified when Boyle and Conway demonstrated that the frog sartorious cell membrane was also permeable to chloride and bicarbonate (Boyle & Conway, 1941) and the Nernst equation was thought to explain the relationship:-

$$E = \frac{R\,T}{F} \ln \frac{[Cl]i}{[Cl]o} = \frac{R\,T}{F} \ln \frac{[K]i}{[K]o} \ldots\text{etc.}$$

where E is the membrane potential, R is the gas constant, T is absolute temperature, F is the Faraday constant, and $[K]_i$ is the intracellular potassium concentration and $[K]_o$ is the extracellular potassium concentration.

This equation therefore predicts a direct linear

relationship between resting membrane potential and potassium concentrations across the membrane. There is, however, evidence to suggest that the situation is more complex.

In general resting cell membranes do have some degree of permeability to sodium ions which, combined with the fact the action potential involves influx of sodium, requires a mechanism of active sodium transport to remove sodium from cells thus achieving a steady state in which active transport out of the cell balances diffusion into the cell. The pump is considered to be nonelectrogenic, i.e. no net charge transfer across the membrane occurs with active transport. The passive diffusion of sodium will alter the relationship between potassium concentrations and membrane potential according to the following equation:-

$$E = \frac{R}{F} \frac{T}{} \ln \frac{[K]o + b[Na]o}{[K]i + b[Na]i}$$

where b is the ratio of the membrane permeability for Na and K, P_{Na}/P_K and b is thought to be about 0.01 for muscle and nerve. Therefore $b[Na]_i$ is small compared to $[K]_i$, and at high $[K]_o$ and low $b[Na]_o$, $b[Na]_o$ can be neglected and the equation reduces to the form of the Nernst Equation so that E is a linear function of $[K]_o$. However at low $[K]_o$ in the physiological range, $b[Na]_o$

becomes a significant fraction of the sum $[K]_o + b[Na]_o$ and the linear relationship between E and $[K]_o$ no longer exists (Sperelakis & Lee 1979), Figure 2.1.1.

The inward current, represented by the spike of the action potential, is thought to be carried by sodium ions which enter the cell because of a sudden increase in membrane sodium permeability associated with excitation. Repolarization involves the movement of ionic charge in the opposite direction. The cell has thus lost potassium and gained sodium and active transport is required to restore the situation.

The effects of alterations in the concentrations of extracellular potassium on excitability are the result of changes in resting membrane potential. The relationship between cardiac resting membrane potential and extracellular potassium concentration is linear across the physiological range but deviates from linearity at the extremes of levels reported in man (Figure 2.1.1) In order to achieve propogated depolarization it is necessary to decrease the resting membrane potential to a given value, the threshold value. Not all tissues respond identically to changes in potassium concentrations, for example, the cells of the sinus node are particularly insensitive to changes in potassium concentration. The automaticity of the sinus node increases with increasing extracellular potassium concentration up to approximately

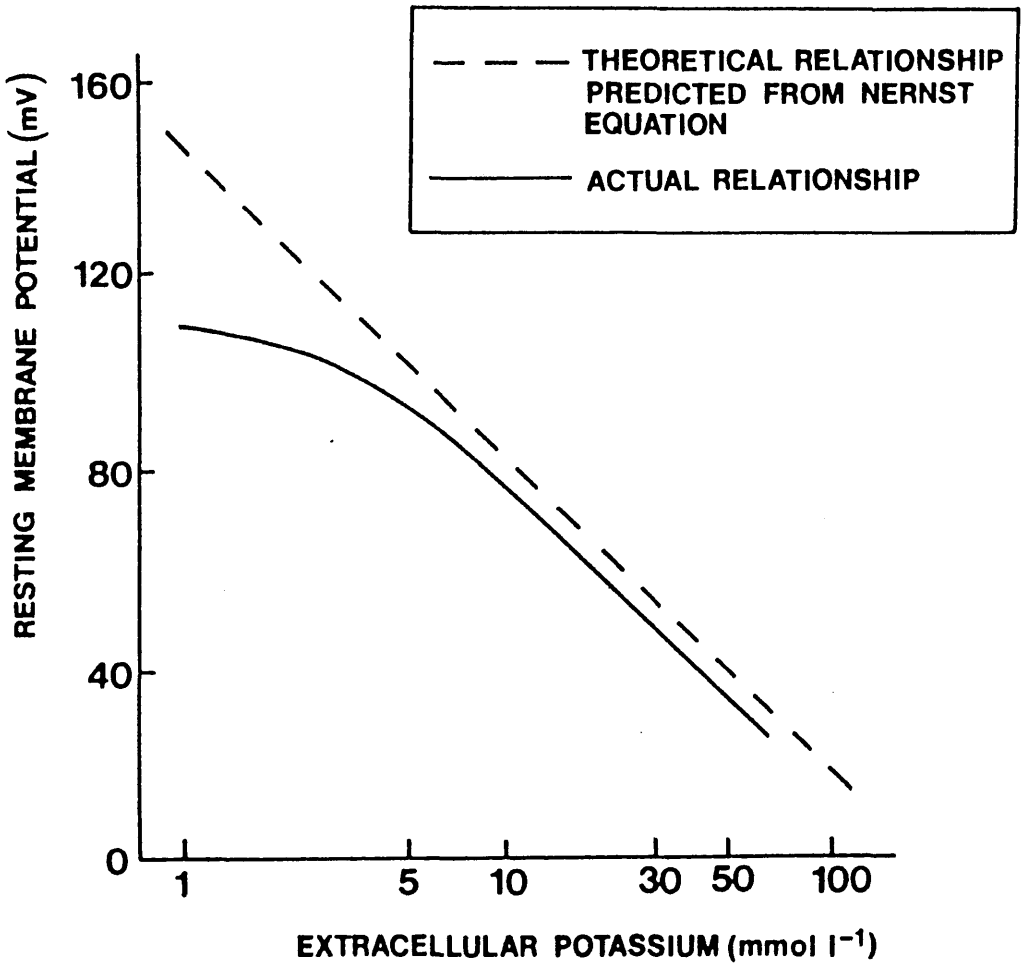


Fig. 2.1.1.: RELATIONSHIP BETWEEN RESTING MEMBRANE POTENTIAL AND EXTRACELLULAR POTASSIUM CONCENTRATION

6.0 mmol l^{-1} and decreases when potassium concentrations exceed 6.0-8.0 mmol l^{-1}. The automaticity of latent pacemaker cells is inhibited much earlier than the sinus node ((Antoni, Herkel & Fleckenstein, 1963; Vasalle et al., 1964). Thus hyperkalaemia decreases the incidence of both supraventricular and ventricular dysrhythmias (Fisch, 1973). Depolarizations to less than the threshold value results in small local changes but not to propogation. If the threshold potential is reached then an action potential occurs and the rate of rise of the action potential, which is thought to be due to the rate of inward movement of sodium current, can be shown to be dependent on the resting membrane potential. As resting membrane potential falls below -60mV the inward sodium current decreases to zero and therefore no action potential can occur.

Thus, any increase in extracellular potassium concentration leads to a decrease in resting membrane potential. The initial effect is to narrow the fall in membrane potential required to reach the threshold potential and, therefore, there is an increase in excitability with moderate hyperkalaemia. Spontaneous rhythmicity initially increases as a result of rises in extracellular potassium. However, as resting membrane potential falls further with severe hyperkalaemia, >8.0 mmol l^{-1}, then conduction velocity decreases due to the

slower rise of the action potential and finally excitability falls as the inward sodium current which triggers the change in potential disappears. This has been demonstrated in atrium, AV node, Purkinje fibres and ventricular muscle (Swain & Weidner, 1957; Dominguez & Fozzard, 1970; Paes de Carvalho & Langer, 1963; Mendez, Mueller & Urguiaga, 1970).

Hypokalaemia increases resting membrane potential and thus prolongs depolarisation (Surawics, 1967) and may decrease autorhythmicity of myocardial cells but there is evidence it increases automaticity of Purkinje fibres (Vasalle et al., 1964; Vasalle & Hoffman, 1965; Dominguez & Fozzard, 1970). These changes lead to an increased incidence of escape rhythms, both supraventricular and ventricular (Fisch, 1973). Hypokalaemia can result in A-V conduction delay or even block and may depress intraventricular conduction by an unknown mechanism (Fisch, 1973).

In cardiac muscle changes in extracellular potassium concentrations are not thought to alter cardiac muscle contractility and the same probably holds true for skeletal muscle, though some doubt persists.

It has been demonstrated that Na^+/K^+ ATPase is a vectorial enzyme and extends across the cell membrane bridging both the inner and outer layers. It is activated by both Na^+ and K^+, different sites are involved, the K^+-

sensitive sites being located on the outer surface whilst the Na^+sensitive sites are on the cytosolic face of the membrane. The preferred substrate is ATP complexed with $Mg2^+$, and the complex is required at the cytosolic surface. Heart muscle contains 6 x 10^6 molecules of ATPase per gram, and each ATPase transports 3 Na^+ out and 2 K^+ in per cycle (Bentfield et al., 1977). Provided that sufficient Mg-ATP substrate is available the normal myocardium has a large reserve of Na^+/K^+ ATPase activity (Nayler, 1981).

2.2 Hypokalaemia: Risks in ischaemic heart disease and hypertension.

Hypokalaemia is a relatively common finding in hospitalized patients, Lawson reporting significant hypokalaemia in 1% of 58,000 hospitalised patients and the hypokalaemic group had a fourfold greater mortality compared to their age and sex matched normokalaemic controls (Lawson et al., 1979). An interesting point to emerge from this study was that diuretic therapy rarely caused profound hypokalaemia (<2.0mmol l^{-1}).

A wide variety of conditions have been reported to cause potassium deficiency and hypokalaemia. These range from inadequate dietary intake, increased gastrointestinal losses and a variety of renal abnormalities, leading to increased renal excretion ranging from hormonal influences to drug therapy. A full list of causes is given in Table 2.1.1. Drugs may

alter plasma potassium, either by altering total body potassium stores or by altering the distribution of potassium between the intracellular and extracellular compartments and these are listed in Table 2.1.2.

Clinically hypokalaemia is often asymptomatic but, if symptomatic, the pattern of symptoms may be as dependent on the rate of fall of plasma potassium as well as to the absolute level of plasma potassium and may be unrelated to the extent of total body potassium loss. Hypokalaemia secondary to potassium loss, for example due to diuretic therapy, reflects a depletion of whole body potassium, both intracellular and extracellular. There has to be large potassium loss, usually 200mEq or more before significant hypokalaemia develops. The fall in plasma potassium is slow as adaptive mechanisms attempt to decrease potassium losses.

Eventually homeostatic mechanisms are exhausted and then plasma potassium falls rapidly. Initially intracellular potassium levels are maintained to avoid osmotic pressure damage and intracellular enzyme systems failing. In skeletal muscles the concentration difference increases as plasma potassium levels fall resulting in hyperpolarization as predicted by the Nernst equation (Bilbrey et al., 1973). As potassium deficiency increases further skeletal muscle plasma membrane integrity apparently decomposes so that membrane potential falls to

Table 2.1.1 Causes of hypokalaemia.

1: Intracellular shifts-

High dose insulin	Alkalosis
Periodic paralysis	

2: Gastrointestinal Loss-

Pyloric stenosis	Diarrhoea
Bulimia nervosa	Villous adenoma
Anorexia nervosa	Purgative abuse
Ileostomy	Fistulas
Ureterosigmoidostomy.	

3: Increased renal losses-

a) Primary renal disorders:

Renal tubular acidosis	Chronic renal failure
Acute tubular necrosis	

b) Solute diuresis:

Glucose	Urea
Mannitol	Saline infusion
Carbenicillin	Penicillin

c) Endocrine disease:

Aldosteronism (Primary or Secondary)	Renin secreting tumours
Cushing's disease	

4: Secondary to pharmacological agents: see Table 2.1.2.

Table 2.1.2 Pharmacological agents which modify potassium balance.

1: Drugs causing potassium depletion.

Potassium losing diuretics - Thiazides
- Loop diuretics
- Mercurial diuretics
- Carbonic anhydrase inhibitors

Corticosteroids with mineralocorticoid activity

Excess Liquorice

Nephrotoxic antibiotics - Aminoglycosides
- Outdated tetracycline
- Amphotericin B

Penicillin and semisynthetic penicillins (high dose)

Laxative abuse

2: Drugs causing potassium retention.

Potassium sparing diuretics - Spironolactone
- Triamterene
- Amiloride

Converting enzyme inhibitors- Captopril

Non-steroidal anti-inflammatory drugs.

3: Drugs increasing cellular uptake of potassium.

Insulin

Salbutamol

4: Drugs decreasing cellular uptake of potassium.

Phenylephrine

Digitalis intoxication

Chemotherapy lysis

Succinylcholine

Arginine infusion

abnormally low levels and, at this point, muscle enzymes appear in the plasma (Knochel & Carter, 1976). It is known that skeletal muscle is the primary site of potassium deficiency and it seems that skeletal muscle gives up its potassium content so that other vital tissues are less affected (Knochel & Schlein, 1972).

It is probably inappropriate to assume that myocardial muscle cells respond identically. There are no studies of myocardial potassium concentrations in living, potassium-deficient humans. If, however as seems likely, there is an increase in the relative concentrations of potassium across the myocardial cell membrane then hyperpolarization will occur. This will result in the cell requiring a larger impulse to trigger depolarization. If the impulse is not sufficient to invoke depolarization then the excitation-contraction sequence does not occur and ventricular escape activity is more likely (Knochel, 1984). Acute hypokalaemia secondary to stimulation of the active transport system, as seen with metabolic alkalosis, results in a very rapid change in the relative concentrations of potassium across the membrane as potassium is pumped into the cells and extracellular concentrations fall. Maintenance of the resting potential is impossible, hyperpolarization results and the electrical stability of the cell is compromised.

The life-threatening effects of hypokalaemia result from changes in resting membrane potential in cardiac conducting tissue and myocardial cells which increase the risk of cardiac dysrhythmias. Though severe hypokalaemia may by asymptomatic, reported symptoms include muscle weakness, varying from mild to profound, postural hypotension, decreased gatrointestinal motility, both due to decreased smooth muscle tone (Biglieri and McIlroy, 1966), rhabdomyolysis (Knochel & Schlein, 1972), confusional states and, as a result of renal tubular effects, polyuria, metabolic alkalosis and sodium retention occur.

Severe hypokalaemia presents a risk even to otherwise healthy humans, however mild to moderate hypokalaemia is much commoner. Which particular groups of patients could be at increased risk from mild to moderate degrees of potassium deficiency?

Patients with myocardial ischaemia secondary to coronary heart disease have an increased risk of clinically significant or fatal dysrhythmias (Braunwald, 1984). Co-existent hypokalaemia increases this risk further (Dyckner, Helmers & Wester, 1984). Ischaemic heart disease is frequently complicated by ventricular failure requiring diuretic therapy, commonly loop diuretics, such as frusemide, or in milder cases thiazide diuretics. Both groups of diuretics cause a degree of potassium loss and

may thus result in hypokalaemia. On commencing thiazide diuretics the average fall in serum potassium is 0.5 mmol l^{-1} (Morgan & Davidson, 1980). In the Veteran's Administration Study of treatment of hypertension 23% of patients receiving thiazide diuretics developed serum potassium levels between 2.5 and 3.4 mmol l^{-1} and these remained low throughout the two year study period (VA Cooperative Study, 1970) and 3% of these patients had persistent potassium levels below 3.0 mmol l^{-1}. Other reports have suggested that up to 50% of hypertensives receiving thiazide diuretics have a resting serum potassium <3.5 mmol l^{-1} (Morgan & Davidson, 1980; MRC Working Party, 1981) and the incidence of ventricular ectopic beats has been shown to be inversely related to serum potassium level (MRC Working Party, 1983).

Recently there have been more and more reports of hypokalaemia occuring in a proportion of patients admitted to hospital with an acute myocardial infarction (Dyckner et al., 1975, Solomon & Cole, 1980; Donnelly et al., 1980; Dyckner et al., 1984; Thomas & Hicks, 1981). These hypokalaemic infarct patients have an increased incidence of dysrhythmias and a higher morbidity and mortality (Dyckner et al., 1975). The only identifiable predisposing cause in many cases is diuretic therapy. However, much of this evidence is controversial, these studies are observational, often retrospective and

uncontrolled and some authorities doubt that chronic mild to moderate hypokalaemia secondary to diuretics is clinically important.

In addition, there are a significant number of patients who have no known predisposing cause for hypokalaemia. Circulating adrenaline and noradrenaline levels rise in acute myocardial infarction though the extent of this rise is very variable, varying from normal levels to a twenty-five fold increase. This rise is not simply explained by the extent of the infarct or the presence of complications. Patients with cardiogenic shock have high levels but equally patients with clinically uncomplicated infarction may also have raised levels (Christenson & Videbaek, 1974; Cryer, 1980; Karlsberg, Cryer & Roberts, 1981; Vetter et al., 1974). The chronotropic action of high catecholamine levels will increase the risk of cardiac dysrhythmias and, in addition, the increased catecholamine levels may be a cause of hypokalaemia.

Thus patients with ischaemic heart disease are at increased risk if they develop hypokalaemia, whether the hypokalaemia is the result of diuretic therapy or of acute stress, such as myocardial infarction.

A similar situation exists in the treatment of hypertensive patients, who will often have co-existent ischaemic heart disease and are at risk from myocardial infarction and cardiac dysrhythmias. The cornerstone of

many anti-hypertensive treatment regimens is thiazide therapy, either alone or in combination with other anti-hypertensive drugs. The Veterans Administration Cooperative study and the MRFIT hypertension study suggested that thiazide diuretic therapy may carry a risk of significant complications including hypokalaemia and dysrhythmias (MRFIT Research Group, 1982; Veterans Administration Cooperative Study Group, 1982), though these interpretations remain controversial (Moser, Black & Stair, 1986). Therefore, if a hypertensive patient with diuretic induced hypokalaemia, suffers an acute ischaemic event, then the risk of fatal dysrhythmias will increase if adrenaline does indeed lower plasma potassium further.

2.3 Hypokalaemia: Consequences in acute asthma and chronic airflow limitation.

Hypokalaemia is not a recognised problem of standard bronchodilator drug regimens routinely used in asthma and chronic airflow limitation (chronic bronchitis and emphysema). Hypokalaemia has been observed during the treatment of acute exacerbations of both asthma and chronic airflow limitation but has received little attention.

The treatment of acute asthmatic attacks has been closely scrutinised recently because the death rate from asthma is increasing in many countries despite the

introduction of effective and specific anti-asthmatic therapy (Burney, 1986; British Thoracic Association, 1982; Stableforth, 1983; Johnson et al., 1984; Wilson, 1984) and in New Zealand this increase has reached 'epidemic' proportions since the start of this decade (Jackson et al., 1982). There is now increasing evidence of a rise in asthmatic deaths in the U.S.A. (Coleman, Paulozzi & Buist, 1985; Robin, 1988).

This reawakening of interest in asthma deaths mirrors an identical response in the 1960's when certain countries, U.K., New Zealand and Australia reported a dramatic increase in asthmatic death rates following the introduction of the first metered dose aerosol inhaler containing a high dose of a non-selective β-adrenergic agonist, isoprenaline (Spiezer, Doll & Heaf, 1968; Speizer et al 1968; Inman & Adelstein, 1969; Anonymous (Editorial), 1969). No epidemic was seen in countries which did not introduce this device (Fraser & Doll,1971; Stolley, 1972). One country, Australia, did, however, fail to demonstrate a simple relationship between the sales of isoprenaline and asthma deaths (Gandevia, 1973). Though many theories were proposed to explain this epidemic none was ever proven (Speizer et al., 1968b).

The major theories were: i) that because isoprenaline is non-selective patients were, during attacks of acute asthma, taking excessive doses which triggered fatal

cardiac dysrhythmias either by direct myocardial stimulation or by increasing hypoxia secondary to alterations in ventilation-perfusion induced by isoprenaline effects on pulmonary vessels (Palmer & Diament, 1969; Collins et al., 1969; Knudsen & Constatine, 1967); ii) that excess use of the inhaler meant increased inhalation of the propellant, freon, which is known to be cardiotoxic and arrhythmogenic in high doses; iii) patients delayed seeking medical advice because they believed that with the advent of a new powerful bronchodilator, which they could administer themselves, they could control attacks. None of these theories has been totally disproven though the role of freon was widely investigated and it was demonstrated that it was virtually impossible to achieve cardiotoxic levels of freon from an aerosol inhaler (Clarke & Tinston, 1972; Dollery et al., 1974; Anonymous (Editorial), 1975). Following warnings in the medical press the sale of isoprenaline inhalers fell, the high dose isoprenaline inhaler, Isoforte, was withdrawn, the use of corticosteroids increased, as did the admission rate for asthma and the asthma death rate fell back towards previous figures (Committee on Safety of Drugs, 1967). Inhaled sympathomimetic agents were, however left under a cloud for many years thereafter, though today sales of sympathomimetic inhalers now exceed the mid-

sixties figures several fold (Stableforth, 1983) and all subsequent studies examining the circumstances of death in asthma have emphasised undertreatment as a major cause rather than overtreatment (McDonald, Seaton & Williams, 1976, Fraser et al., 1971; B.T.A., 1982).

The current increase in asthmatic death rates is equally difficult to explain. The epidemic in New Zealand does seem to be waning, though death rates for asthma remain higher than in other comparable countries, and it has been suggested that the cause may have been the excessive self-administration of β_2-agonists via home nebulisers, widely used in New Zealand since just prior to the start of this recent increase in the death rate. The widespread use of nebulisers leading to patients delaying seeking medical advice until it was to late (Grant, 1983). The risks of nebulised beta-agonists in asthma or chronic airflow limitation are unclear from the current literature. Sudden death has been associated with nebuliser use (Anonymous, 1984; Cochrane, Prior & Rees 1985; Sears et al, 1987). One group has reported no increase in cardiac dysrhythmias following nebulisation on oxygen (Ebden et al, 1987) this contrasts with the observations of an increase in cardiac dysrhythmias by others in an elderly group of patients using air driven nebulisers (Higgins et al, 1987).

The alternative explanation for the New Zealand

"epidemic" offered has been that combination therapy with β_2-agonists and methyl-xanthines is cardiotoxic or increases the incidence of dysrhythmias during attacks of asthma (Sutherland & Wilson, 1981; F.D.A., 1981; Wilson, Sutherland & Thomas, 1981). It has been demonstrated in animals that cardiac dysrhythmias are commoner in animals treated with this combination (Joseph et al., 1981; Nicklas, Whitehurst & Donohue,1982) and there are reports in the literature that patients receiving this combination have an increased incidence of extrasystoles when continous cardiac monitoring is carried out (Banner et al., 1979; Pierson et al., 1980; Josephson, 1982; Billing et al., 1982; Al-Hillawi, Hayward & Johnson, 1984). The clinical significance of these findings from uncontrolled studies is uncertain. There has been one report suggesting that cardiac dysrhythmias were increasingly complicating severe and fatal attacks of asthma predominately in older subjects (Coleman et al., 1985).

Prior to the introduction of specific β-agonists, when oral ephedrine was widely used in combination with oral theophylline, it was recognised that there was an interaction between these drugs leading to an increase in the frequency of side-effects (Weinberger & Bronsky, 1975).

How might either β_2-agonists or theophylline alone or

in combination put asthmatic and bronchitic patients at risk? Hypoxia is present even in relatively mild attacks of asthma and respiratory acidosis will develop in severe attacks (Crofton & Douglas, 1981). Both these metabolic derangements are known to increase the risks of cardiac dysrhythmias and it has been demonstrated that hypoxia increases the cardio-toxicity of isoprenaline (Collins et al., 1969). As previously discussed it is possible that β_2-adrenergic stimulation may cause hypokalaemia. Thus specific β_2-agonist bronchodilators, such as salbutamol, though they will possess less chronotropic activity on the heart via the β_1-adrenoceptor, may precipitate significant hypokalaemia which, accompanied by hypoxia and acidosis, may trigger life-threatening dysrhythmias. Combination with theophylline, which by itself has been reported to cause hypokalaemia in overdosage (Helliwell & Berry, 1979; Buckley, Brathwaite & Vale, 1983; Kearney et al., 1985) and which previous evidence has suggested increases the sympathomimetic action of adrenergic agonists may further compound the situation. Thus hypokalaemia could be a common pathway by which both these bronchodilators would place patients at increased risk when combined with the other acute metabolic derangements present during the stress of acute attacks of bronchospasm.

One final area of dispute is the possible role of

circulating catecholamines in acute attacks of asthma and bronchitis. Severe asthma is both frightening and stressful and would be expected to cause a sympathoadrenal response and a rise in circulating adrenaline. A possible additional factor may be hypoxia which has been shown to increase circulating catecholamines, at least during the stress of exercise (Escourrou, Johnson & Rowell, 1984). The original evidence, using less sensitive assay methods suggested that even simple exercise induced asthma resulted in increases in circulating adrenaline (Zielinski et al., 1980; Griffiths et al., 1972; Chryssanthouplous et al., 1978). However a more recent similar study found no change (Barnes et al., 1981) and a study of patients during acute severe attacks of asthma could only demonstrate a rise in noradrenaline levels but not adrenaline (Ind et al., 1985). In a personal communication these authors did report that the subjects who were so severely ill as to require intensive therapy did have raised adrenaline levels. It would seem logical from our knowledge of the sympathoadrenal system that the chronic stress of a prolonged asthmatic attack leads to increased neuromuscular activity, and hence increased spillover of noradrenaline into the blood stream, but no rise in adrenaline. However when the attack becomes so severe that significant hypoxia and respiratory acidosis

supervene then possibly the adrenal medulla responds by releasing adrenaline which could further stimulate β_2-adrenoceptors linked to the Na^+/K^+ ATPase and could further increase any fall in plasma potassium already caused by bronchodilator therapy.

Therefore, in conclusion, hypokalaemia secondary to bronchodilator therapy and, possibly to increased circulating adrenaline, may occur in acute severe attacks of either asthma or bronchitis.

2.4 Hypomagnesaemia: Clinical consequences.

The incidence and importance of magnesium deficiency in clinical practice has only recently been investigated. An increasing number of causes of magnesium deficiency are being recognised and are listed in Table 2.4.1. World wide the most important causes are malnutrition and alcoholism. In western medical practice the two aetiologies which have received most attention have been magnesium deficiency secondary to total parenteral nutrition and magnesium deficiency complicating diuretic therapy with loop diuretics and thiazide diuretics (Wester & Dyckner, 1981).

Assessing the relevance of magnesium deficiency is difficult as serum magnesium levels, unlike potassium levels, show no clear relationship with muscle magnesium levels (Heaton & Martindale, 1965), though some studies have demonstrated decreased muscle magnesium in magnesium

deficiency states (Ryan, 1986).

Not all patients receiving these diuretics will develop magnesium deficiency and even of those that do the vast majority will be asymptomatic (Sheenan & White, 1982; Swales, 1982). 6.9 to 11% of hospitalized patients have been reported to be hypomagnesaemic (Whang et al., 1980; Wong et al., 1983), 42% of patients with hypokalaemia have coexistent hypomagnesaemia ((Whang et al., 1984) and 37% of patients with congestive cardiac failure who are receiving diuretic therapy will have low serum magnesium levels (Wester & Dyckner, 1985). Thus, though relatively common, the severity and clinical importance of diuretic induced hypomagnesaemia remains uncertain. Dyckner and Wester have reported that coexistent magnesium deficiency increased potassium depletion (Wester & Dyckner, 1981), these findings would support the view that A.T.P. does require intracellular magnesium as an essential cofactor. A further interaction between these two cations is that membrane permeability to potassium is altered by magnesium depletion (Skou, 1965). Magnesium deficiency can be fatal even to otherwise healthy subjects, as shown by a number of sudden deaths which occurred in the 1970's in patients on liquid protein preparations for the treatment of gross obesity. A large magnesium loss occured which, combined with

Table 2.4.1 Causes of magnesium deficiency.

I - Nutritional Causes:

- Prolonged parenteral feeding.
- Starvation with acidosis.
- Protein calorie malnutrition.
- Diabetic ketoacidosis.
- Alcoholism

II - Intestinal Causes:

- Chronic diarrhoea - any aetiology.
- Intestinal Malabsorption.

III- Renal Disease:

- Renal tubular acidosis.
- Acute tubular necrosis-diuretic phase.
- Chronic glomerulonephritis.
- Familial and sporadic renal magnesium loss.

IV - Drug Therapy:

Diuretics:-

- Thiazide diuretics.
- "Loop" diuretics.

Antibiotic induced tubular dysfunction:-

- Gentamicin.
- Amphotericin.
- Carbenicillin.
- Tircacillin.

Antineoplastic therapy - Cisplatin.

V - Endocrine Causes:

- Primary and secondary hyperaldosteronism.
- Malignant hypercalcaemia.
- Primary hyperparathyroidism.
- Hyperthyroidism.
- Excessive lactation.
- Pregnancy.

lipolysis, which releases long chain free fatty acids which bind magnesium, resulted in acute and severe hypomagnesaemia (Lantigua et al., 1980) leading to cardiac dysrhythmias.

Though patients with severe magnesium deficiency may have no symptoms or clinical signs, the signs, symptoms and clinical complications of magnesium deficiency are well recognised and are listed in Table 2.4.2. It is of interest to note the similarity of many symptoms to those of hypocalcaemia.

The variability of the clinical manifestations of hypomagnesaemia may be the result of the effects of magnesium on cell calcium handling. Magnesium may bind competitively to the same sites as calcium, producing the same physiologic response; it may compete with calcium for a binding site but not exert an effect; or it may alter the distribution of calcium by changing the flux of calcium across the cell membrane or by displacing it from intracellular binding sites with a rise in intracellular calcium concentrations. Profound magnesium deficiency primarily affects excitable tissues producing neuromuscular irritability and tetany in the nervous system and in the cardiovascular system, cardiac dysrhythmias and increased vascular tone and reactivity. This increase in vascular tone and reactivity is the result of increased intracellular calcium concentrations

Table 2.4.2 Clinical manifestations of magnesium deficiency.

I - Neuromuscular Hyperactivity:

Limb and facial muscle tremor.	Myoclonic jerks.
Convulsions.	Ataxia.
Chvostek sign.	Trousseau sign.
Spontaneous carpopedal spasm.	Nystagmus.
Dysophagia and gut hypomotility.	

II - Psychiatric Disturbances:

Apathy.	Delirium.
Coma.	

III - Cardiovascular Effects:

Ventricular dysrhythmias - Sudden death.

- premature ventricular beats.
- ventricular tachycardia.
- ventricular fibrillation.

secondary to magnesium deficiency (Altura & Altura, 1978) and magnesium deficiency may cause coronary artery spasm (Turlapaty & Altura, 1980). In the presence of magnesium deficiency Na^+/K^+ ATPase is impaired and this results in a fall in resting membrane potential, partial depolarisation of the cell membrane and hyperirritability of excitable tissues. A recent report showed that magnesium deficiency increased the cardiotoxic effects of supraphysiological doses of adrenaline in rats (Vormann et al., 1983), though the mechanism of this interaction is unclear.

Undoubtedly the most serious and increasingly recognised complication is cardiac dysrhythmias and these may appear suddenly and be unresponsive to conventional anti-dysrhythmic drug therapy.

Assessing the significance of magnesium deficiency in the genesis of dysrhythmias in these patients is complicated by the frequent co-existent potassium deficiency. Often the dysrhythmia is attributed to the potassium deficiency. Further complicating accurate assessment of aetiological factors is the observation that in some magnesium deficient patients potassium replacement is ineffective until magnesium is also replaced (Chadda, Lichenstein & Gupta, 1973). In animals severe magnesium deficiency has been shown to result in myocardial fibrosis, necrosis and calcification

(Heggtveitt, Herman & Mishra, 1964). Digitalis toxicity is enhanced by hypomagnesaemia (Kim, Freed & Bures, 1961). There are well documented cases of magnesium deficient patients with life-threatening dysrhythmias being successfully treated with magnesium supplements (Loeb et al., 1968, Chadda et al., 1973; Iseri, Freed & Bures, 1975). However, there are no controlled series and the incidence of severe magnesium related tachydysrhythmias is not known. An increasing number of reports have shown that patients admitted with acute myocardial infarction have lower serum magnesium levels than matched controls (Abraham et al., 1977; Dyckner, 1980), even when preceding diuretic therapy is taken into account (Petersen, Christiansen & Transbol, 1978). In two studies of patients with myocardial infarction a higher incidence of atrial fibrillation and supraventricular tachycardia was found in the hypomagnesaemic patients (Dyckner, 1980; Bigg & Chia, 1981). A recent observation in a placebo controlled randomised study of patients with acute myocardial infarction who received intravenous magnesium on admission to hospital has been a lower incidence of complicating cardiac dysrhythmias and a lower mortality at four weeks in those given magnesium (Rasmussen et al., 1986). This interesting study requires confirmation but emphasises the possibility that magnesium deficiency may be important in these patients.

In asthma diuretics are not a standard form of therapy, however, they are widely used in patients with advanced chronic airflow limitation when complicated by cor pulmonale. Patients with severe chronic airflow limitation are frequently malnourished (Wilson, Rogers & Hoffman, 1985) and this is an additional risk factor for hypomagnesaemia. There have been no published studies of magnesium balance and status in these patients.

CHAPTER 3

METHODS

3. METHODS

3.1 Physiological and pathophysiological catecholamine levels.

The sympathoadrenal system exists to provide a rapid response to situations of 'fight or flight'. There is evidence that not only physical stress but also psychological stress can result in release of adrenaline from the adrenal medulla (Anfilogoff et al, 1987).

Catecholamines are cleared from extracellular fluid rapidly by uptake into sympathetic postganglionic neurons ($uptake_1$), where they are stored, and into extraneuronal cells ($uptake_2$) where they are metabolized (Iverson,1975). $Uptake_1$, a relatively high affinity, low capacity system has a higher affinity for noradrenaline, whereas, in contrast, $uptake_2$ is a low affinity, high capacity system with a higher affinity for adrenaline. There is now evidence that both noradrenaline and adrenaline clearance systems are in turn modulated by beta-adrenergic receptors (Cryer et al, 1980). Adrenaline is rapidly cleared from the blood to metanephrine following methylation by catecholamine-o-methyl transferase (C.O.M.T.) and to a lesser extent by deamination by monoamineoxidase (M.A.O.). The majority of an injected dose of adrenaline appearing in the urine as metabolites, principally 4-OH, 3 methoxymandelic acid (V.M.A.), though in health trace amounts of adrenaline

are detectable in urine.

Plasma concentrations of adrenaline vary widely during day to day activities, varying from 0.19 to 0.22 nmol l^{-1} (35-40 pg ml^{-1}) whilst resting supine to 0.30-0.41 nmol l^{-1} (55-75 pg ml^{-1}) on standing (Cryer, Santiago & Shah, 1974) and to 1.9 to 2.9 nmol l^{-1} (350-540 pg ml^{-1}) on severe physical exercise (Galbo, Holst & Christensen, 1975).

Acute stress results in release of adrenaline, cortisol, growth hormone and prolactin (Rose & Sachar, 1981). Adrenaline is claimed to be the most sensitive and consistent index of the response to stress (Wortsmann, et al, 1984). Adrenaline rises with psychological stress when the rise of the other hormones is only modest (Anfilogoff et al, 1987). Stress in illness obviously differs depending on the individual situation and involves a mixture of acute and chronic physical stress and psychological stress. In animals the rise in plasma adrenaline is related, following coronary artery ligation, to the fall in arterial pressure temporally, but the magnitude of the rise is related to the size of the infarct (Karlsberg et al, 1979). In man the most widely used experimental tool to study adrenomedullary stress has been insulin induced hypoglycaemia, an acute, life-threatening form of stress, leading to large rises in adrenaline levels. Adrenaline levels have also been

found to rise in a variety of illnesses, the highest levels of circulating adrenaline, increases of 30 to 300 fold, have been reported following cardiac arrest.

The sympathetic response may be further modified by the individual patient's psychological response to their illness. In a group of patients in an intensive care unit plasma adrenaline levels were approximately twice normal, whereas in patients admitted during an acute myocardial infarction levels may be elevated up to twenty-five fold (Christensen & Videbaek,1974; Karlsberg et al,1981). Elevation of catecholamine levels has been correlated with the presence of left ventricular failure, shock and life threatening ventricular dysrhythmias (McAlpine & Cobbe,1988), nevertheless in these studies there were wide differences between individual patient's adrenaline levels despite similar severity of illness.

As stated previously (see Chapter 1.1), it is unclear if the plasma adrenaline levels found on supine rest have any biological effects. For example, debate continues regarding the relationship between diurnal variation of plasma adrenaline and nocturnal bronchoconstriction in asthma (Barnes et al, 1980). There is now convincing evidence that in the lower part of the physiological range adrenaline has detectable physiological effects. Increases in heart rate, peripheral blood flow and metabolic rate, along with falls in diastolic blood pressure, were seen with low dose infusions of adrenaline

(10 ng min-1 kg-1) which raised circulating levels from 0.15 nmol l^{-1} (21 pg ml^{-1}) to 0.73 nmol l^{-1} (135 pg ml^{-1}) (Fellows et al, 1985). Venous plasma catecholamine measurements have the limitation that peripheral tissues, principally skeletal muscle, remove adrenaline resulting in a fall across the peripheral circulation of 50% from arterial levels (Best & Halter, 1982). Freyschuss and co-workers infused low doses of adrenaline into normal subjects whilst measuring arterial adrenaline levels and demonstrated significant metabolic and haemodynamic effects at arterial concentrations of 1.3 nmol l^{-1} (235 pg ml^{-1}) (Freyschuss et al, 1986).

As all previous studies in patients examining the pathophysiological role of circulating catecholamine levels have related their observations to venous adrenaline concentrations it is therefore logical to examine the effects of similar venous concentrations of adrenaline when investigating adrenaline induced hypokalaemia and, therefore, venous blood has been used to measure circulating adrenaline concentrations throughout in these studies.

3.2 Catecholamine assays.

Fluorimetry

Until the early nineteen seventies the only method available for measuring adrenaline and noradrenaline levels in biological materials and plasma was by

fluorimetry (Lund, 1949). The lower limit of detection using the fluorometric method was 38 nmol l^{-1} (700 pg ml^{-1}) for adrenaline and 10 nmol l^{-1} (200 pg ml^{-1}) for noradrenaline (Griffiths & Leung, 1970). The detection limit for adrenaline was, therefore, well above the concentrations found at rest in normal human subjects (Anton & Sayre, 1962). The introduction of semi-automated methods made this technique less time-consuming and less expensive but it remained a research tool (Merrils, 1963). Principally because of its high lower limit of detection, the only clinical application was in the investigation of suspected phaeochromocytoma, a condition in which circulating catecholamines are greatly increased.

Gas Liquid Chromatography

In the early nineteen seventies more sensitive gas liquid chromatography methods were developed. Again the cost of the equipment, the complexity of sample cleanup and derivatization made it expensive and limited its use for both clinical and research applications (Jacob & Vogt, 1977).

Radioimmunoassay

At approximately the same time a cheaper method of measuring low plasma and tissue concentrations of catecholamines was developed using a specific and sensitive radioimmunoassay technique [R.I.A.] (Passon & Peuler, 1973; DaPrada & Zurcher, 1975). This method has

proven accurate and sensitive but does involve multiple sample handling, enzyme preparations and labelled compounds. The required materials are available as kits allowing widespread use of this technique though the cost per sample assayed does remain relatively high, approximately £30.00/sample at commercial assay rates. Nonetheless this method allows accurate measurement of adrenaline concentrations into the femtomole range from plasma samples (DaPrada & Zurcher, 1975). Thus, for the first time it was possible to investigate the physiological role of the low concentrations of adrenaline present at rest and during light exercise. For much of the work in this thesis this was the assay method used to measure circulating adrenaline and noradrenaline concentrations.

Briefly, the two catecholamines are converted to their O-methyltransferase analogues by catechol-O-methyltansferase in the presence of S-adenosyl-methionine-3H thus labelling O-methylated products. These are transformed to less polar complexes by adding sodium tetraphenylborate and extracted into diethyl ether and the products of the reaction separated by thin layer chromatography. Metanephrine, the product of methylation of adrenaline, and normetanephrine, the product of methylation of noradrenaline, are further oxidised to vanililin and the radioactivity counted after extraction. Internal and external standards are also run and the samples are counted in a liquid scintillation

spectrometer and radioactivity in both the samples and the standards is corrected for the counts per minutes in the blanks. The concentration of the amines for each sample is then calculated based upon the internal standard. Though it has been claimed that this method can be used to measure catecholamine levels in the femtomole range, it is certainly accurate with good linearity over the picogram and nanogram ranges. The intra-assay variability is acceptable with a coefficient of variation of approximately 12% in human plasma and a inter-assay coefficient of variation of 18% (Da Prada & Zurcher, 1975) Thus the method is accurate but, though little sophisticated equipment is needed, it is complex and relies on the purity of the catechol-O-methyltransferase enzyme and careful extraction procedures by laboratory staff. A single technician can only analyse, approximately, 40-50 samples in two days. This relative expense limited the number of plasma catecholamine samples which could be assayed in these studies.

High Performance Liquid Chromatography

Since the beginning of this decade increasingly sophisticated assays based on high performance liquid chromatography (H.P.L.C.) have been used to measure plasma catecholamines and the recent application of electrochemical detection methods to H.P.L.C. has, for the first time, allowed accurate detection of relatively

low plasma concentrations of catecholamines (Mefford et al.,1981; Watson, 1981; Kissinger, Bruntlett & Shoup, 1981).

The technique is based on a direct conversion of chemical information to an electrical signal without the need for intermediate optical or magnetic carriers. All catechol derivatives can be readily oxidised at a graphite electrode to generate the corresponding othroquinone, two protons and two electrons. Thus if the the anodic current, i.e. the rate at which electrons are transferred across the electrode-solution interface, is measured then the instantaneous current is directly proportional to the number of molecules coming into contact with the interface per unit time and this can be used to determine the concentration of the reactant in the neighbouring solution. The problem faced in assaying catecholamines in both tissues and plasma was that all catechol derivatives react similarly and cannot be distinguished. The development of modern reversed phase or ion-exchange liquid chromatography, using microparticle columns capable of rapidly separating closely related compounds, has now made this technique applicable to the measurement of catecholamines in plasma. The instrumentation and reagents are relatively inexpensive and the number of sample manipulations are fewer than with earlier methods.

The method used was developed by Howes and co-workers

and has a coefficient of variation of 5-10% and a limit of detection of 0.1 nmol l^{-1} (20 pg ml^{-1}) for both adrenaline and noradrenaline. The system was a paired ion reverse phase HPLC system with an electrochemical detector with a mobile phase containing phosphate buffer and octyl sulphonic acid as the paired ion reagent. A 5 micron ODS column was employed with 3,4 dihydroxy benzolamine hydrobromide as the internal standard (Howes, Miller & Reid, 1985).

The major disadvantage of this method is that the reliability of the instrumentation and columns is not perfect and, thus, the degree of sensitivity is less than with the radio-enzymatic assay method.

Goldstein and coworkers directly compared the accuracy of the liquid chromatography method, using electrochemical detection, with the radio-enzymatic assay in measuring noradrenaline and adrenaline levels in normal resting healthy subjects. In a series of 25 samples the correlation between the two methods was 0.99. However at concentrations below 0.55 nmol l^{-1} (100 pg ml^{-1}) the coefficient of variation of the H.P.L.C. methods with E.C.D. was higher (26-46% for adrenaline concentrations less than 0.55 nmol l^{-1} (100 pg ml^{-1}) compared to a C.V. of 7-11% above this level) and these authors conclude that at very low plasma adrenaline and noradrenaline concentrations then the H.P.L.C. method is less reliable (Goldstein et al., 1981). As resting plasma

adrenaline concentrations are usually in the range 0.19-0.41 nmol l^{-1} (35-75 pg ml^{-1}) then this method is not applicable to situations where adrenaline levels are known to be this low. It is, however, accurate at the levels of adrenaline which result from the adrenaline infusion protocols used in these studies.

3.3 Adrenaline infusion regimen.

Infusion of high doses of adrenaline into subjects, normal or ill, can be hazardous with a variety of possible adverse effects occurring, ranging from acute chronotropic effects on the heart, triggering cardiac arrhythmias, to myocardial necrotic lesions and renal glomerular damage with chronic infusions (Horak et al, 1983; Mandal et al, 1977). Early studies of the effects of adrenaline in man used intravenous bolus injections and more recent studies used short infusions of relatively large doses of adrenaline. These studies failed to achieve steady-state adrenaline concentrations and, thus, make observation of concentration-effect relationships impossible (Duff & Swan, 1951; Swan 1951; Massara, Tripodina & Rotunno, 1971).

An infusion regimen which was safe, which could be continued for 120 minutes and which would achieve circulating adrenaline level in the physiological range with levels similar to those seen in acute severe illness, between 1.0-5.0 nmol l^{-1}, had to be developed.

An additional complicating factor was the wide inter-individual variation in catecholamine clearance (Fitzgerald et al, 1979) and, therefore, any infusion regimen chosen would result in a wide range of adrenaline concentrations in different individuals (Clutter et al., 1980). For safety a step infusion regimen was designed, starting with a very low dose of adrenaline, and increasing the dose once steady state had been achieved with each dose, if there were no untoward effects. Previously published data suggested that steady state would be achieved for a given dose after 5-8 minutes and the infused dose was therefore increased at 10 minute intervals, providing the heart rate had not increased by 20 beats min^{-1} or the systolic blood pressure had not increased by 30mm Hg.

The starting dose of adrenaline was 0.01µg kg^{-1} min^{-1}. This dose was increased stepwise at 10 minute intervals to 0.02µgkg^{-1} min^{-1}, then to 0.04µgkg^{-1} min^{-1} and finally to 0.06µg kg^{-1} min^{-1}. This dose was then continued for the period of the adrenaline infusion, a further 90 minutes. In 4 subjects plasma adrenaline concentrations were sampled both during and following the adrenaline infusion to confirm the plasma adrenaline levels achieved by this regimen were in the desired range and to document the time course for adrenaline levels to return to baseline after the infusion. Such a sampling protocol was not possible in every subject in these studies due to

considerations of cost, as the more expensive radio-enzymatic assay was required to measure the low adrenaline levels seen after the infusions.

The data in the 4 subjects in whom the time adrenaline concentration profile was examined is given in Table 3.3.1 and indivdual concentration time curves are given in Appendix 3.

During the stepwise increase in the infused dose the effect of an infusion rate of 0.02μg kg^{-1} min^{-1} was unpredictable, the adrenaline levels rising in one subject, falling in one and being unaltered in a third. A dose of 0.04μg kg^{-1} min^{-1} increased adrenaline levels in all subjects to a very variable degree, one to six fold times basal levels. The maximum dose, 0.06μg kg^{-1} min^{-1}, increased adrenaline levels in all subjects five to ten fold within 5 minutes of commencing this dose (95').

During the period of the infusion of this dose, 0.06μg kg^{-1} min^{-1} adrenaline levels did fluctuate. The infusion pump used, the Braun IV is a highly accurate volume infusion device and it is unlikely that it is the cause of this variablity. The most likely explanation being variation in the subject's arm position. The arm was not splinted, so as to allow subjects to read, eat and drink. Possibly, flexion of the arm led to partial venous obstruction and a fall in the delivery of adrenaline to the central circulation due to the resultant venous pooling. Equally when the arm is straightened then a

Table 3.3.1 Time-Plasma adrenaline concentration curves during and after the adrenaline infusion regimen in 4 normal subjects.

Time	Adr. Dose	Subject Number			
		1	2	3	4
(mins)	($\mu g\ kg^{-1}\ min^{-1}$)				
30	**0**	0.11	0.24	0.27	0.55
80	**0.02**	0.13	0.58	0.09	-
90	**0.04**	0.88	2.00	2.90	0.39
95	**0.06**	2.60	2.60	2.70	1.90
100	**0.06**	1.40	2.10	2.90	3.80
110	**0.06**	1.00	3.50	3.60	3.80
120	**0.06**	1.70	2.40	4.50	3.20
150	**0.06**	2.70	-	2.60	2.60
180	**0.06**	2.20	3.80	3.50	1.50
182	**0**	0.49	4.20	1.10	1.70
185	**0**	0.83	1.60	0.54	0.78
210	**0**	0.33	0.94	0.21	0.20

bolus of adrenaline would be delivered to the central circulation.

The range of plasma adrenaline concentrations observed during the infusion was wide, ranging from 1.0 to 5.0 nmol l^{-1}. In all subjects the average plasma adrenaline concentration lay between 2.0 and 4.0 nmol l^{-1} and this was at least 20 times basal plasma adrenaline concentrations in all subjects. At no time during the infusions were plasma adrenaline concentrations less than 10 times the basal concentrations. During the 90 minute period of a constant rate of infusion of adrenaline (0.06μg kg^{-1} min^{-1}) the adrenaline levels fluctuated randomly in three of the subjects, but in the fourth the adrenaline levels fell progressively during the period of the infusion. The reason for this progressive fall is unclear, though one possible explanation is that the patient was inadvertently given a large bolus of adrenaline whilst the adrenaline infusion was being altered and the levels fell thereafter.

The infusion of adrenaline was stopped at 180 minutes and an infusion of 5% (+)-glucose continued, thereafter, through the same intravenous cannula at the same rate, 12 mls hr-1. Sampling at 180' and 182' revealed that in two subjects the plasma adrenaline concentration had risen above the 180' level at 182', two minutes after the adrenaline infusion had ceased. The most likely explanation for this finding is that small volumes of the

adrenaline containing solution were in the cannula and were infused when the (+)-glucose infusion was commenced. However, in both these subjects the adrenaline concentration had fallen when measured after a further 3 minutes at 185'. Paradoxically in one subject (No. 1) the adrenaline concentration which had fallen steeply between 180' and 182' rose slightly between 182' and 185' minutes, possibly because of delay in restarting the infusion pump with the 5% (+)-glucose solution. As a result of this transient rise in adrenaline concentration after the end of the adrenaline infusion it is difficult to accurately calculate the half-life of the infused adrenaline. In these four subjects the half life varied between 3 and 13 minutes but it is important to emphasise these are only estimates and there may be a relatively large error in some subjects for the reasons discussed above. At 210 minutes, 30 minutes after the cessation of the adrenaline infusion, adrenaline levels had returned to basal levels in all but one of the subjects in whom it remained elevated at approximately four times the previous basal level (subject 2).

In summary, the results suggest that, with this infusion regimen, circulating plasma adrenaline levels were increased by at least 10 fold. During the period of continous adrenaline infusion marked fluctuations in adrenaline levels did occur. Thirty minutes after the end of the adrenaline infusion plasma adrenaline levels will

usually have returned to basal levels.

3.4 Standard Measurements and Assays.

Sample handling and analysis.

Plasma catecholamine and plasma potassium samples were collected and handled in a similar fashion for all studies reported in this thesis. In separate studies other assays were used, e.g. plasma insulin (4.2) and details of these assays are given in the appropriate chapter.

Briefly, catecholamine samples were collected into heparinised tubes, kept on ice, centrifuged at $4^{o}C$ and stored at $-70^{o}C$ until assayed by one of two methods as outlined above (3.2), either by the radioenzymatic method (Da Prada & Zurcher, 1975) or by H.P.L.C. (Howes et al, 1985).

Plasma potassium samples were collected into glass tubes at room temperature and centrifuged within 20 minutes and separated. They were subsequently analysed on an automated autoanlayser using standard flame photometry methods (SMA-C, Technicon Instruments Corporation, New York, U.S.A.).

Haemodynamic Measurements.

In all studies heart rate and blood pressure were closely monitored during the adrenaline infusions. Heart rate and blood pressure were measured, in duplicate, at all appropriate time points by the use of a semi-

automated sphygmomanometer, Sentron (Bard Biomedical Division, Lombard, Illinois, U.S.A.). Electrocardiograms were recorded with praecordial electrodes and displayed continously on an oscilloscope linked to a polygraph (Grass Model 7D).

3.5 Statistical Analysis.

As the number of subjects studied in each of these experiments was relatively small, and as all studies involved multiple comparision of treatments with placebo, the most appropriate form of analysis was analysis of variance as applied to repeated measures (Winer, 1971). If there were significant differences between baseline then allowance for such baseline differences was made in the analysis of variance program used (Bryce, 1981). Where appropriate paired student's t-test was used to examine differences before and after an intervention, if repeated measurements were not involved, e.g. adrenaline clearance on placebo and theophylline (Chapter 6.3).

3.6 Ethical Permission.

The protocols of all the studies which are described in this thesis were submitted to, and approved by, the Research and Ethical Committee of the Northern District of Greater Glasgow Health Board. All subjects gave fully informed, written consent before participating in these studies.

CHAPTER 4

BETA ADRENOCEPTORS IN THE CONTROL OF PLASMA POTASSIUM

4. BETA ADRENOCEPTORS IN THE CONTROL OF PLASMA POTASSIUM.

4.1 Introduction.

As discussed above (Chapter 1.5), a number of separate pieces of evidence have accumulated, since D'Silva's original experiment in 1934 (D'Silva, 1934), particulary the work of Clausen, to suggest that a $beta_2$-adrenoceptor was central to the phenomenon of adrenaline induced hypokalaemia.

The aims of the three studies described in this chapter were to examine the mechanism of adrenaline induced hypokalaemia further by studying:-

(1) the effect of adrenaline infusions on insulin secretion, as increased insulin levels would result in hypokalaemia by increasing transport of potassium into cells.

(2) the effect of stimulation of the $beta_2$-adrenoceptor, using the highly selective $beta_2$-adrenoceptor agonist, salbutamol, to exclude any possible contribution to adrenaline induced hypokalaemia by the activity of adrenaline at the $beta_1$-adrenoceptor or alpha-receptor.

(3) the effect of selective and non-selective antagonists of the beta-adrenoceptor on adrenaline induced hypokalaemia. In two similar studies the effect on adrenaline induced hypokalaemia of five beta-adrenoceptor agonists, each with different pharmacological profiles

was examined. In addition, the action of the beta-antagonists on the haemodynamic effects of adrenaline, i.e. its chronotropic action, a predominately $beta_1$-adrenoceptor action, and the fall in diastolic blood pressure, a $beta_2$-adrenoceptor action, were also examined.

4.2 Non-selective and selective beta-adrenergic stimulation - the effect on plasma potassium.

4.2.1 Introduction.

Insulin promotes intracellular shift of potassium, a fact long applied in clinical practice in the treatment of life-threatening hyperkalaemia. Control of insulin release is complex and involves many factors, including the sympathetic nervous system (Porte et al, 1966). Insulin release, stimulated by adrenaline, is a possible mechanism for adrenaline induced hypokalaemia. The effect of the adrenaline infusion regimen on plasma insulin was therefore studied.

In animal skeletal muscle previous work has suggested that the effect of adrenaline on plasma potassium is mediated via a membrane bound Na^+/K^+ ATPase linked to a $beta_2$-adrenoceptor (Clausen & Flatman, 1977). By adding another limb to this study the effect of a highly selective $beta_2$-adrenoceptor, salbutamol, on plasma potassium, insulin and glucose could be compared to the effects of adrenaline.

The two principal aims were therefore to exclude insulin release as the mechanism of either adrenaline induced hypokalaemia or salbutamol induced hypokalaemia. Secondly to examine if adrenaline induced hypokalaemia is the result of $beta_2$-adrenoceptor stimulation by comparing adrenaline's effects with salbutamol's.

Modifying the design of the study allowed study of a further question, do salbutamol and adrenaline have an additive effect in lowering plasma potassium? Salbutamol is a widely used bronchodilator, frequently given to patients both as a maintenance treatment and during the treatment of life-threatening attacks of dyspnoea when adrenaline levels may be raised (2.3).

In summary in this single blind, crossover study salbutamol or placebo was administered to subjects in whom adrenaline or placebo had been infused sufficient to achieve pathophysiological circulating adrenaline concentrations (Struthers & Reid, 1984). The effects of these combinations of infusions on cardiac rhythm, blood pressure, plasma potassium, glucose and insulin were studied.

4.2.2 Methods.

Eight healthy subjects, 4 males, 4 females, age range 22-37 years, weight range 52-85kg., were studied on four separate occasions at least one week apart, receiving one of four treatments in random order in a single blind,

crossover design. They had normal electrocardiograms, serum biochemistry and haematology before study. They were receiving no drug therapy.

The four treatments consisted of:

- 1) An (-)-adrenaline infusion intravenously and a control vehicle salbutamol infusion;

- 2) A control vehicle adrenaline infusion and an active salbutamol infusion;

- 3) Both an active (-)-adrenaline and an active salbutamol infusion;

- 4) Two control vehicle infusions.

Randomisation was by means of a balanced Latin square design. Study days were identical in all respects other than the treatments administered.

Subjects were studied supine after a standard light breakfast and no alcohol, caffeine or cigarettes for at least 12 hours. Cannulae were inserted, one into each forearm. The adrenaline infusion regimen used was a modified protocol from that outlined in Chapter 3. After 30 minutes supine rest, a 5% (+)-glucose infusion containing ascorbic acid, 1 mg ml^{-1}, was given, at a rate of 12 ml $hour^{-1}$ for 60 minutes. This was followed by an (-)-adrenaline infusion in 5% (+)-glucose containing ascorbic acid, 1mg ml^{-1}, at a rate of 0.03µg kg^{-1} min^{-1} for 10 minutes or 5% (+)-glucose also containing ascorbic acid was continued as a control. If there were no symptoms or adverse effects then the infusion rate was

increased to 0.06μg kg^{-1} min^{-1} and continued for a total of 110 minutes.

During the second hour an intravenous infusion of salbutamol (Ventolin, Allen & Hanburys Ltd., Greenford , Middlesex, U.K.) or an identical (+)-glucose vehicle salbutamol infusion was administered according to the randomised design. The salbutamol infusion was commenced at a dose of 40 ng kg^{-1} min^{-1} increasing stepwise at 15 minutes intervals to a dose of 80 ng kg^{-1} min^{-1} and then to 120 ng kg^{-1} min^{-1} if no adverse effects occurred. This final dose of salbutamol was continued for 30 minutes, a total duration of salbutamol of 60 minutes, average rate of delivery over the hour being 90 ng kg^{-1} min^{-1}. Following the salbutamol and adrenaline infusions or identical vehicle controls a 5% (+)-glucose infusion was continued for a further one hour. Exact times of the various measurements and treatments are shown in Fig 4.2.1.

Blood pressure and heart rate were measured by a semi-automated sphygmomanometer (Chapter 3.4).

Samples for plasma potassium and plasma catecholamines were handled as described previously (Chapter 3.4). Plasma catecholamine levels were assayed by the radioimmunoassay method (Chapter 3.2).

Plasma insulin samples were collected into heparinised tubes, kept on ice, and centrifuged at 4°C and stored at -70°C for subsequent analysis using a conventional

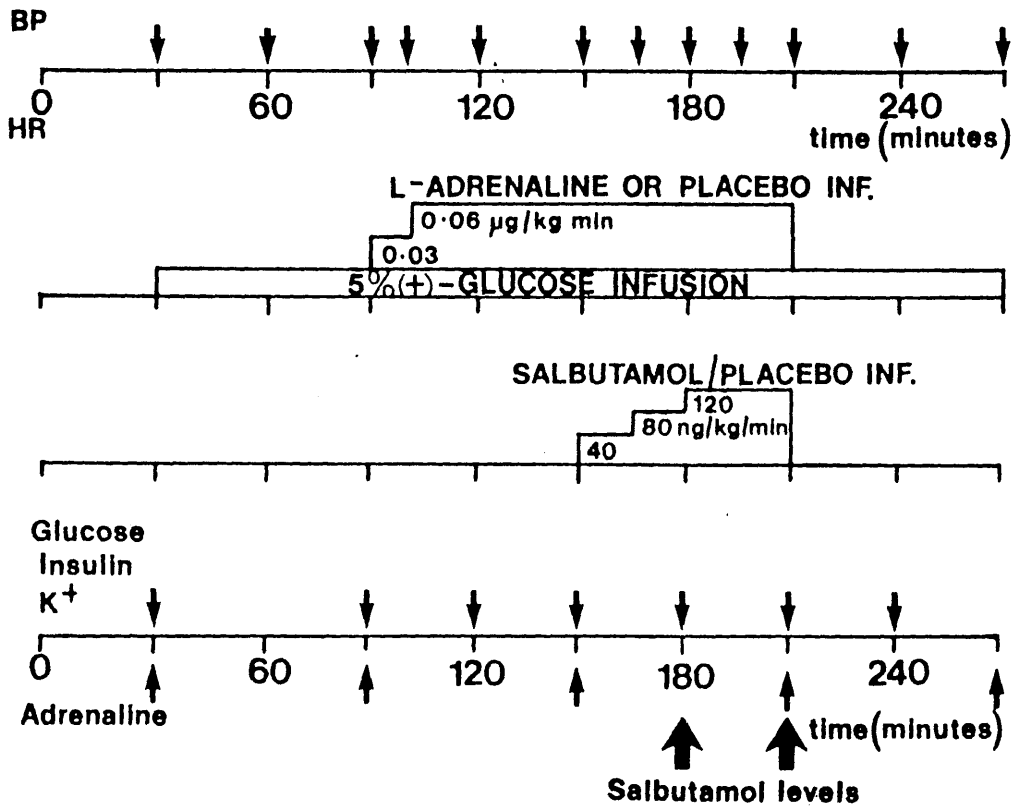


Fig. 4.2.1: OUTLINE OF STUDY DAY

double antibody radioimmunoassay based on guinea-pig anti-insulin serum (Wellcome). I_{125}-insulin (Amersham International) and a rabbit anti-guinea pig second antibody covalently linked to Sepharose C1 4B. Glucose samples were measured by a Beckman glucose analyser using the glucose oxidase method. Salbutamol levels were measured at the start of the 120 ng kg^{-1} min^{-1} dose and at the end of the infusion by high pressure liquid chromatography.

Results are expressed as mean $\pm$ standard deviation. The four treatment days were compared by repeated measures analysis of variance and significance values are quoted, corrected for variations in baseline (Chapter 3.5).

4.2.3 Results.

All subjects tolerated the infusions of both adrenaline and salbutamol alone and together with no serious adverse effects though all were aware, on direct questioning, of tremor of the upper limbs with salbutamol but not with adrenaline.

Basal resting plasma adrenaline levels, prior to the infusions, were not significantly different on any of the four study periods (Table 4.2.1).

Salbutamol was only identified in plasma following active salbutamol infusions. Salbutamol concentrations are shown in Table 4.2.1 at the start and at the end of the highest dose of salbutamol infused (120 ng kg^{-1} min^{-1}). As would be predicted from salbutamol's long half-life,

Table 4.2.1:- Salbutamol and adrenaline concentrations.

Plasma adrenaline (lower limit of detection 0.1 nmol l^{-1}) before and at the end of the adrenaline infusion period and salbutamol concentrations after 30 and 60 minutes of the active or control vehicle infusions (mean±S.D.; n=8).
(A=adrenaline; S=salbutamol; U.D.=undetectable.).

Treatment	**Salbutamol Conc.** ($ng\ ml^{-1}$)		**Adrenaline Conc.** ($nmol\ l^{-1}$)	
	180'	**210'**	**30'**	**210'**
Vehicle A				
+	U.D.	U.D.	<0.1	<0.1
Vehicle S				
Adrenaline				
+	U.D.	U.D.	0.16±0.16	2.33±1.62
Vehicle S				
Vehicle A				
+	2.6±0.8	4.6±0.8	0.12±0.03	<0.1
Salbutamol				
Adrenaline				
+	1.9±0.5	4.4±0.3	<0.1	3.17±1.89
Salbutamol				

salbutamol levels were still rising at the end of the infusion.

Heart rate before the infusion was not significantly different on any of the treatments (Table 4.2.2). Heart rate did not alter significantly during the study in which both vehicle infusions were given. It did alter significantly on all three of the other treatments (Table 4.2.2). the greatest increase in heart rate was seen on the combined adrenaline and salbutamol treatment (99±19 beats min^{-1} compared to 65±11,$p<0.001$, ANOVA). There were smaller significant rises in heart rate on salbutamol alone and on adrenaline alone (Table 4.2.2).

Systolic blood pressure rose significantly with salbutamol alone but did not rise significantly on the other study days compared to the control study day (Table 4.2.3). Diastolic blood pressure showed a small but significant fall on adrenaline alone (Table 4.2.4).

On the control study day potassium levels rose slightly, but not significantly, from the baseline of 3.8±0.3 mmol l^{-1} to 4.0±0.3 mmol l^{-1} at the end of the 5% (+)-glucose infusions (Fig 4.2.2). Potassium levels fell and were significantly different from placebo on all three active treatments, by 0.45mmol l^{-1} on adrenaline alone ($p<0.01$), by 0.48 mmol l^{-1} on salbutamol alone ($p<0.01$) and by 0.94 mmol l^{-1} with the combination of adrenaline and salbutamol ($p<0.001$, Figure 4.2.2, Table 4.2.5). The fall in plasma potassium with the combination

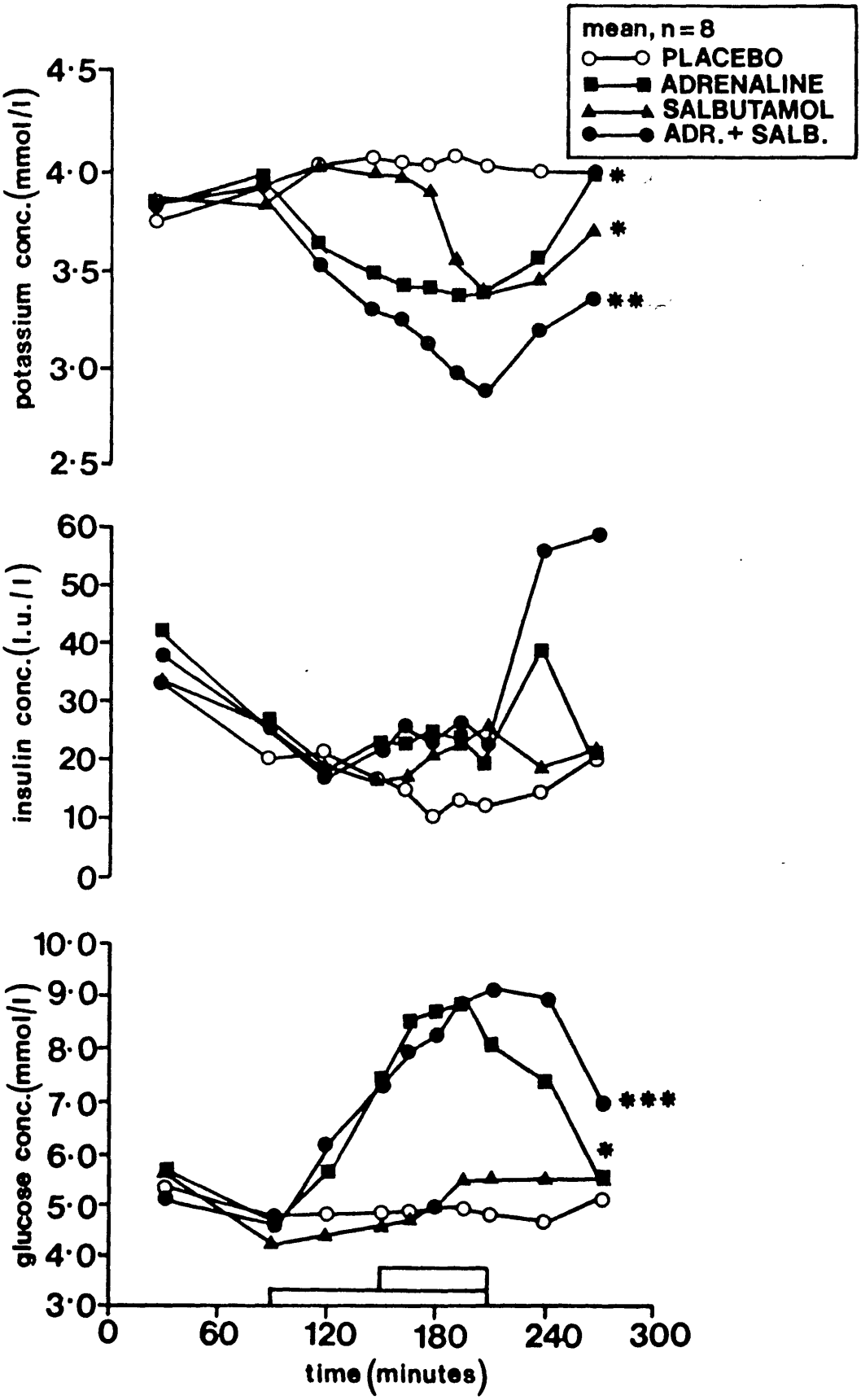


Fig.4.2.2: EFFECTS OF THE INFUSIONS ON PLASMA POTASSIUM, INSULIN AND GLUCOSE

(* - $p < 0.01$; ** - $p < 0.005$; *** - $p < 0.001$)

Table 4.2.2:- Mean heart rate at each time point on each treatment.

(beats min^{-1}; n=8, mean±S.D.).

Time	Placebo	Adrenaline	Salbutamol	Adren. + Salb.
30	67 ± 9	71 ± 9	67 ± 9	68 ± 7
60	64 ± 9	70 ± 7	67 ± 8	65 ± 8
90	64 ± 9	68 ± 8	65 ± 10	64 ± 6
100	61 ± 10	70 ± 9	65 ± 7	71 ± 11
110	62 ± 11	74 ± 11	64 ± 9	71 ± 10
120	62 ± 11	73 ± 11	66 ± 7	71 ± 9
150	63 ± 10	77 ± 9	64 ± 10	77 ± 13
165	62 10	76 ± 12	68 ± 14	82 ± 14
180	64 ± 10	75 ± 12	74 ± 12	90 ± 16
195	62 ± 7	78 ± 12	78 ± 18	98 ± 19
210	65 ± 11	79 ± 14	87 ± 19	99 ± 19
240	63 ± 9	75 ± 10	82 ± 14	88 ± 14
270	66 ± 10	76 ± 13	75 ± 15	87 ± 12
		p<0.01	p<0.05	p<0.001

Table 4.2.3:- Mean systolic blood pressure at each time point on each treatment.

(mm Hg; n=8; mean±S.D.).

Time (mins)	Placebo	Adrenaline	Salbutamol	Adren. + Salb.
30	119 ± 12	116 ± 9	115 ± 9	116 ± 12
60	114 ± 8	117 ± 13	111 ± 9	114 ± 12
90	112 ± 8	111 ± 14	110 ± 12	113 ± 12
100	111 ± 8	111 ± 14	108 ± 8	113 ± 10
110	110 ± 10	116 ± 11	108 ± 11	118 ± 12
120	109 ± 12	120 ± 17	110 ± 11	119 ± 14
150	108 ± 11	122 ± 12	106 ± 7	120 ± 13
165	108 ± 9	120 ± 16	112 ± 7	121 ± 16
180	110 ± 9	119 ± 12	118 ± 9	123 ± 12
195	113 ± 9	117 ± 15	122 ± 14	131 ± 11
210	111 ± 8	118 ± 17	123 ± 12	121 ± 17
240	113 ± 7	114 ± 10	115 ± 16	115 ± 10
270	113 ± 9	114 ± 11	113 ± 12	121 ± 10
		$p>0.05$	$p<0.05$	$p>0.05$

Table 4.2.4:- Mean diastolic blood pressure at each time point on each treatment.

(mm Hg; n=8; mean±S.D.)

Time (mins)	Placebo	Adrenaline	Salbutamol	Adren. + Salb.
30	63 ± 6	64 ± 3	63 ± 6	63 ± 4
60	62 ± 7	59 ± 3	59 ± 6	62 ± 4
90	61 ± 3	62 ± 3	60 ± 5	59 ± 5
100	61 ± 5	60 ± 6	60 ± 4	58 ± 7
110	59 ± 5	60 ± 5	60 ± 6	57 ± 3
120	62 ± 7	60 ± 8	61 ± 6	56 ± 4
150	60 ± 8	60 ± 6	57 ± 5	58 ± 3
165	62 ± 4	57 ± 10	57 ± 5	58 ± 7
180	61 ± 6	55 ± 8	58 ± 2	61 ± 5
195	63 ± 5	56 ± 9	58 ± 4	56 ± 5
210	62 ± 5	57 ± 5	60 ± 4	56 ± 5
240	60 ± 8	61 ± 8	62 ± 8	62 ± 8
270	62 ± 4	64 ± 3	60 ± 4	61 ± 7
		$p<0.05$	$p>0.05$	$p>0.05$

Table 4.2.5:- Mean plasma potassium at each time point on each treatment.

(mmol l^{-1}; n=8; mean±S.D.).

Time (mins)	Placebo	Adrenaline	Salbutamol	Adren. + Salb.
30	3.8 ± 0.3	3.8 ± 0.5	3.9 ± 0.3	3.8 ± 0.2
90	3.9 ± 0.2	4.0 ± 0.3	3.8 ± 0.2	3.9 ± 0.2
120	4.1 ± 0.3	3.6 ± 0.6	4.0 ± 0.2	3.5 ± 0.3
150	4.1 ± 0.2	3.5 ± 0.6	4.0 ± 0.2	3.3 ± 0.3
165	4.1 ± 0.2	3.4 ± 0.5	4.0 ± 0.2	3.3 ± 0.3
180	4.1 ± 0.2	3.4 ± 0.4	3.9 ± 0.4	3.1 ± 0.4
195	4.1 ± 0.2	3.4 ± 0.4	3.6 ± 0.3	3.0 ± 0.3
210	4.0 ± 0.3	3.4 ± 0.5	3.4 ± 0.2	2.9 ± 0.3
240	4.0 ± 0.2	3.5 ± 0.4	3.5 ± 0.3	3.2 ± 0.2
270	4.0 ± 0.3	4.0 ± 0.4	3.7 ± 0.4	3.4 ± 0.2
		$p<0.01$	$p<0.01$	$p<0.001$

of adrenaline and salbutamol was 0.94 mmol l^{-1} and is similar to the sum (0.93 mmol l^{-1}) of the individual falls observed with adrenaline (0.45 mmol l^{-1}) and salbutamol (0.48 mmol l^{-1}) alone.

Insulin levels fell during the control study period (Fig 4.2.2, Table 4.2.6). During the salbutamol alone infusion there was an insignificant rise in insulin levels, from 16±6 i.u. l^{-1} to 26±18 i.u. l^{-1}. Insulin levels were unchanged during the combined salbutamol and adrenaline and on the adrenaline alone study periods (Figure 4.2.2). Insulin levels rose sharply following the cessation of the adrenaline infusion, from 19±6 i.u. l^{-1} at the end of the adrenaline infusion to 39±23 on the adrenaline alone day and from 22±5 i.u. l^{-1} to 56±23 i.u. l^{-1} on the adrenaline plus salbutamol day compared to a rise from 12±8 to 14±12 i.u. l^{-1} on the vehicle control plus vehicle control study day.

Glucose concentrations remained unchanged during the vehicle control infusion and on salbutamol alone study period (Figure 4.2.2, Table 4.2.7). On the other two days there was a significant rise in glucose levels during the infusion period (adrenaline alone $p<0.01$; adrenaline and salbutamol $p<0.001$). There was a dramatic fall in glucose levels following the end of the adrenaline infusions (Figure 4.2.2, Table 4.2.7).

4.2.4 Discussion.

During the period of the adrenaline infusion plasma

Table 4.2.6:- Mean plasma insulin at each time point on each treatment.

(i.u. l^{-1}, n=8, mean ± S.D.)

Time (mins)	Placebo	Adrenaline	Salbutamol	Adren. + Salb.
30	33 ± 22	42 ± 27	33 ± 19	38 ± 28
90	20 ± 5	24 ± 9	27 ± 8	26 ± 12
120	21 ± 8	18 ± 6	20 ± 10	17 ± 6
150	17 ± 6	22 ± 9	16 ± 6	21 ± 10
165	15 ± 5	23 ± 8	17 ± 6	25 ± 11
180	10 ± 4	24 ± 12	21 ± 7	23 ± 6
195	13 ± 5	24 ± 10	22 ± 13	27 ± 8
210	12 ± 8	19 ± 6	26 ± 18	22 ± 5
240	14 ± 12	39 ± 23	18 ± 12	56 ± 23
270	20 ± 21	19 ± 13	21 ± 32	58 ± 30
		p>0.05	p>0.05	p>0.05

Table 4.2.7:- Mean plasma glucose at each time point on each treatment.

(mmol l^{-1}, n=8; mean ± S.D.).

Time (mins)	Placebo	Adrenaline	Salbutamol	Adren. + Salb.
30	5.4 ± 0.8	5.6 ± 1.3	5.6 ± 0.8	5.1 ± 0.8
90	4.7 ± 1.1	4.6 ± 0.8	4.2 ± 1.0	4.6 ± 0.9
120	4.8 ± 0.5	5.6 ± 0.8	4.3 ± 0.5	6.1 ± 1.5
150	4.8 ± 0.8	7.4 ± 1.4	4.5 ± 0.5	7.3 ± 1.6
165	4.8 ± 0.8	8.5 ± 1.6	4.7 ± 0.6	7.9 ± 1.7
180	4.9 ± 0.9	8.7 ± 1.9	4.9 ± 0.9	8.2 ± 1.9
195	4.8 ± 0.3	8.8 ± 1.7	5.5 ± 1.0	8.8 ± 2.0
210	4.7 ± 0.4	8.0 ± 1.6	5.5 ± 1.2	9.1 ± 1.8
240	4.6 ± 0.4	7.4 ± 1.9	5.4 ± 0.9	8.9 ± 2.1
270	5.1 ± 0.5	5.5 ± 1.0	5.5 ± 0.7	7.0 ± 1.6
		$p<0.01$	$p>0.05$	$p<0.001$

insulin levels did not rise thus excluding insulin release as a mechanism of adrenaline induced hypokalaemia. Porte and co-workers demonstrated that insulin release is inhibited by alpha$_1$-stimulation and increased by beta$_2$-stimulation (Porte et al, 1966; Imsura et al, 1971). A further study also suggested that the inhibitory alpha action is dominant at these levels of circulating adrenaline (Williams-Olson et al, 1979). In this study plasma glucose rose during the adrenaline infusion but insulin failed to rise in response to this relative hyperglycaemia until after the adrenaline infusion was stopped. During the period of increasing insulin levels, following the end of the adrenaline infusion, plasma potassium levels actually increased providing further evidence that rises in circulating insulin are not the mechanism of adrenaline induced hypokalaemia.

Salbutamol is a highly selective beta$_2$-receptor agonist and has no alpha-agonist properties. Thus during the salbutamol infusion insulin levels rose whilst plasma potassium levels fell. It has been previously suggested that salbutamol induced hypokalaemia, an increasingly frequently recognised phenomenon, is the result of stimulation of insulin release (Leitch et al, 1976). Though these modest changes in plasma insulin cannot be definitely excluded as a cause of salbutamol induced hypokalaemia, careful analysis of the data reveals that the rise in plasma insulin was greater following the

light standard breakfast, a period when plasma potassium levels were unaltered, than during the salbutamol infusion. Further evidence against insulin being implicated is provided by changes in plasma insulin and plasma potassium when both adrenaline and salbutamol were administered. Plasma insulin levels did not rise yet the fall in plasma potassium was increased when the salbutamol infusion was added to the adrenaline infusion, again suggesting that salbutamol was lowering plasma potassium by a mechanism other than increasing plasma insulin levels.

The second aim was to examine if adrenaline induced hypokalaemia could be the result of $beta_2$-adrenoceptor stimulation by comparing adrenaline and salbutamol. Salbutamol is a highly selective $beta_2$-receptor agonist which has been noted to cause hypokalaemia. Original reports of hypokalaemia occurring followed intravenous doses (Korda, Lyneham & Jones, 1974; Leitch et al, 1976; Neville et al, 1977; Philips et al, 1980; Moravec & Hurlebert, 1980; Smith & Thompson, 1977) or self-poisoning with salbutamol (O'Brien et al, 1981). Recently reports have appeared of hypokalaemia with lower doses of salbutamol and other similar selective $beta_2$-agonists administered subcutaneously (Kung, White & Burki, 1984) and by inhalation (Smith & Kendall, 1984; Haalboom, Deenstra & Struyvenberg, 1985). As salbutamol has a long

half-life (4-6 hours), salbutamol levels rose throughout the infusion period and the maximum effect of this dose (120 ng kg^{-1} min^{-1}), which is equivalent to a dose of 8.4µg min^{-1} (33.6nmol min^{-1}) in a 70kg. man, was not observed. Nevertheless, allowing for the differences in the infusion regimens used for adrenaline and salbutamol the pattern of fall in plasma potassium is very similar, though recovery is delayed following salbutamol presumably due to its lower rate of clearance.

Finally, combining therapeutic doses of intravenous salbutamol and pathophysiological levels of circulating adrenaline demonstrated the effects of the combination of adrenaline and salbutamol were additive. Clearly neither adrenaline nor salbutamol, in the doses given in this study, were maximally stimulating the movement of potassium from the extracellular compartment. Combining the drugs in these doses resulted in profound hypokalaemia.

A significant chronotropic action was observed with both active agents and this effect was increased by combined therapy. Hypokalaemia predisposes to cardiac dysrhythmias, however, none were observed in these young healthy subjects. As previously discussed in asthmatic or bronchitic patients there is conflicting evidence as to circulating adrenaline levels (Chapter 2.3). However, the use of beta-agonists and other sympathomimetic agents as bronchodilators during acute episodes of severe

bronchospasm, with associated acidosis and hypoxaemia, could result in hypokalaemia and increase the risk of cardiac dysrhythmias.

In conclusion, this study excludes insulin release as a cause of adrenaline induced hypokalaemia and strongly suggests, but does not prove, that salbutamol induced hypokalaemia is also not the result of insulin release and may be due to stimulation of a $beta_2$-receptor, most probably a membrane-bound $beta_2$-linked Na^+/K^+ ATPase on skeletal muscle causing potassium influx into cells.

4.3 Adrenaline induced hypokalaemia: the effect of non-selective and selective beta adrenergic antagonists.

4.3.1 Introduction.

If indeed adrenaline induced hypokalaemia is the result of stimulation of a membrane bound Na^+/K^+ ATPase then it should be possible to further characterise the beta-receptor involved by examining the effects of beta-adrenergic antagonists with differing selectivity for $beta_1$- and $beta_2$-adrenoceptors. Beta antagonists may, in addition, to selectivity, have a number of other pharmacological properties including intrinsic sympathomimetic activity (ISA), i.e. the drug has a partial agonist effect when competitively bound to the receptor.

The effects of chronic treatment with three beta-antagonists with different pharmacological profiles on

the hypokalaemia and haemodynamic changes induced by adrenaline were studied. The beta-antagonists studied were propranolol (non-selective with no ISA); oxprenolol (non-selective with modest ISA); and atenolol (β_1-selective with no ISA).

4.3.2 Methods.

Eight healthy volunteers were studied in a double-blind, placebo controlled crossover design. Individual details are given in Table 4.3.1. Treatments were randomly allocated by a balanced Latin square design. All treatments were given in a disguised formulation and had an identical appearance.

Volunteers were on no drug therapy and all subjects received all the treatments, each for one week, and there was a minimum 2 week washout period between treatments. Two alternative doses of each treatment were available, atenolol 100mg or 200 mg; propranolol 160mg or 320mg; oxprenolol 250mg or 500mg. The dose of the beta-antagonist was increased after 48 hours if there was not a 20% reduction in exercise induced heart rate compared to an identical exercise test on no treatment at the start of the study. In all subjects the lower dose was used.

Subjects were studied on the seventh day of each treatment period having fasted for at least eight hours and having abstained from tobacco, alcohol and caffeine

Table 4.3.1:-Individual volunteer data.

Volunteer No.	Sex	Age (yrs)	Weight (kg)	Biochemistry. (N=normal)
1	Female	25	51	N
2	Female	21	54	N
3	Female	29	73	N
4	Male	36	65	N
5	Male	34	84	N
6	Male	36	76	N
7	Male	31	62	γ-GT 96i.u. l^{-1}
8	Male	23	58	N

containing beverages for 10 hours. Intravenous cannulae were inserted into each antecubital fossa and the subjects given a light standard breakfast. The subjects were studied supine throughout the study period. Following one hour of rest baseline measurements of heart rate, blood pressure and plasma potassium were made and a 5% (+)-glucose (dextrose) infusion with ascorbic acid (1mg ml^{-1}) was commenced at an infusion rate of 12 ml $hour^{-1}$. After one hour of the vehicle solution the baseline measurements were repeated. The adrenaline infusion was then started and increased stepwise at 10 minute intervals in the standard infusion protocol as detailed in Chapter 3.3. Adrenaline was given for a total period of 120 minutes and throughout this period heart rate, blood pressure and plasma potassium were measured regularly as shown in Fig.4.3.1. Following the administration of the adrenaline the vehicle was continued for a further two hours.

Plasma concentrations of each of the beta-blockers were measured by high performance liquid chromatography from a 5ml. lithium heparin venous sample.

Samples for plasma catecholamines were collected as outlined in Chapter 3.4 and analysed at a later date by the radioenzymatic assay method for the samples prior to infusion and by the H.P.L.C. method with electrochemical detection for the samples taken during the adrenaline infusion (see Chapter 3.2).

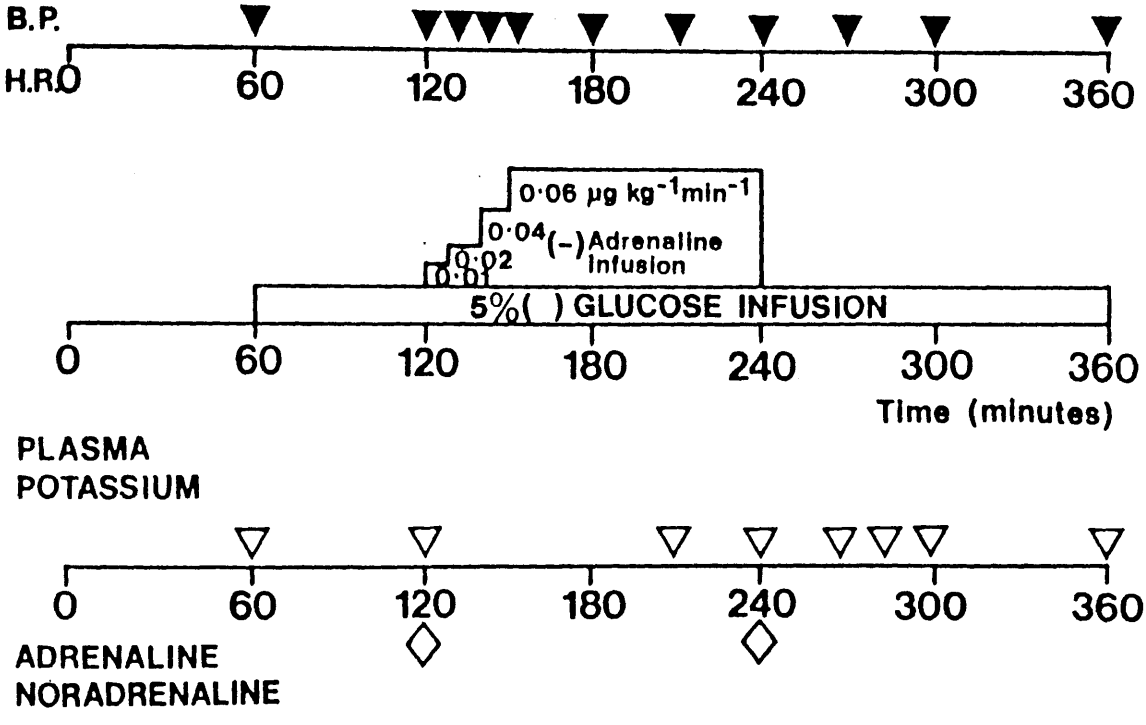


Fig. 4.3.1.: OUTLINE OF STUDY DAY

Results are quoted as mean ± standard deviation and treatment periods were compared with the placebo study day by repeated measures analysis of variance and significance values are quoted corrected for baseline differences (see Chapter 3.5).

4.3.3 Results.

No volunteer reported any adverse effects from either the beta-antagonists or the adrenaline infusions. The plasma concentrations of the beta-antagonists are shown in Table 4.3.2, a wide range being seen with all three agents. The baseline plasma adrenaline before the adrenaline infusion was significantly different on placebo compared with the non-selective beta-adrenoceptor antagonists, propranolol and oxprenolol, but not with atenolol. Plasma adrenaline increased during the adrenaline infusion (Table 4.3.3). There were no significant differences in adrenaline concentrations during the adrenaline infusion between the placebo study day and the three active treatment study days, suggesting that the beta-antagonists did not influence adrenaline clearance.

Haemodynamic Results:

The resting heart rate was significantly reduced ($p<0.001$) by all three active treatments compared with placebo. The reduction in resting heart rate following oxprenolol, was less than that seen with either

Table 4.3.2:- Plasma beta-antagonist concentrations on each treatment.

Volunteer No.	Oxprenolol ($ng\ ml^{-1}$)	Propranolol ($ng\ ml^{-1}$)	Atenolol ($ng\ ml^{-1}$)
1	504	122	172
2	289	46	103
3	142	8	41
4	393	35	143
5	213	37	88
6	116	6	32
7	289	103	98
8	299	132	159
Mean	281	62	102
S.D.	128	51	53

Table 4.3.3:- Mean adrenaline concentrations during the study period on each treatment.

(nmol l^{-1}, n=8; mean ± S.D.)

Time (mins)	Placebo	Propranolol	Oxprenolol	Atenolol
120	0.2 ± 0.1	0.4 ± 0.3	0.5 ± 0.3	0.3 ± 0.2
210	2.7 ± 1.4	3.4 ± 2.0	3.7 ± 1.5	3.5 ± 0.8
240	1.5 ± 0.6	2.9 ± 3.0	2.2 ± 1.3	2.6 ± 1.6
360	0.3 ± 0.3	0.4 ± 0.2	0.5 ± 0.3	0.5 ± 0.6
		p>0.05	p>0.05	p>0.05

propranolol or atenolol (Table 4.3.4). During the adrenaline infusion maximum heart rates were similar on all three active treatments (Table 4.3.4, Figure 4.3.2).

Systolic blood pressure was not significantly different on the four study days (Table 4.3.5). Diastolic blood pressure fell on the placebo study day during the adrenaline infusion ($p<0.001$). Diastolic pressure was unchanged during the adrenaline infusion following oxprenolol but rose slightly following propranolol. On the atenolol study day there was a significant fall in diastolic pressure compared to the other two active treatments ($p<0.05$, Table 4.3.6).

Plasma Potassium Concentrations:

Potassium concentrations fell during the adrenaline infusion from 3.8±0.2 to 3.3±0.4 mmol l^{-1} on placebo ($p<0.001$). No fall in plasma potassium was seen on any of the active treatments including the selective $beta_1$-antagonist, atenolol. There was a slight rise in potassium concentrations after propranolol, and to a lesser extent after oxprenolol. All active treatments were significantly different from placebo ($p<0.01$) but there was no significant differences between the active treatments (Figure 4.3.3, Table 4.3.7).

4.3.4 Discussion.

The persistence of the vasopressor response to adrenaline, a $beta_2$-adrenoceptor mediated action, after one weeks treatment with atenolol supports the

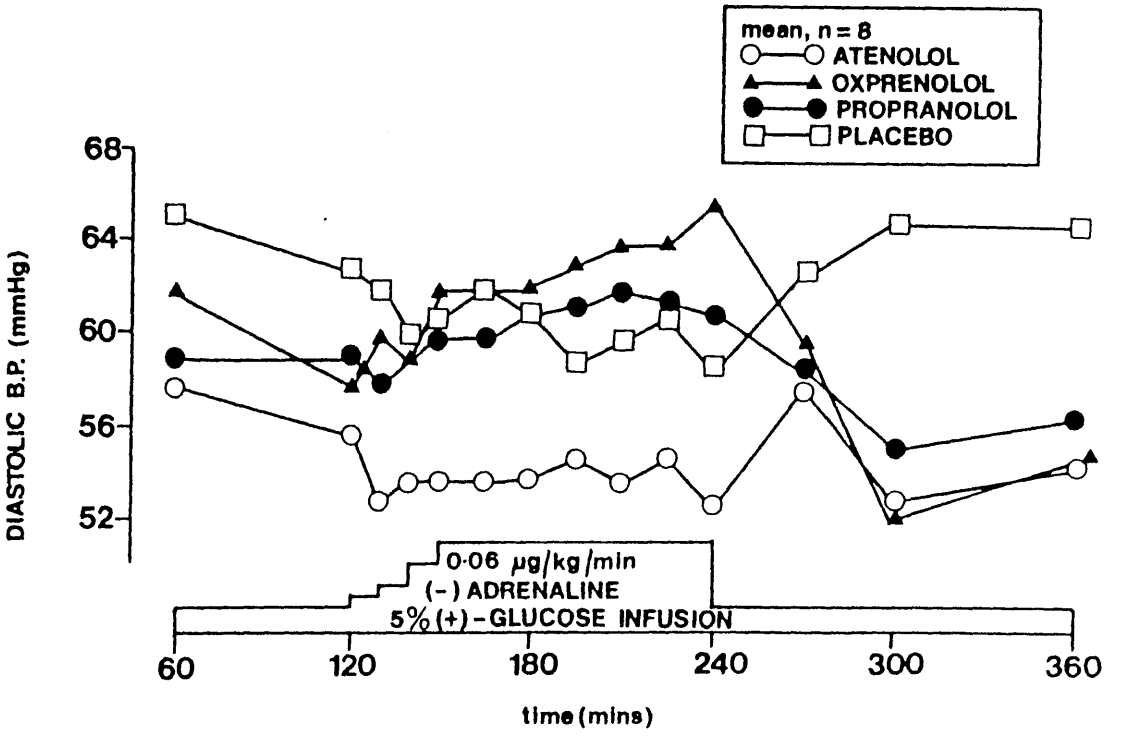


Fig. 4.3.2. CHANGES IN DIASTOLIC BLOOD PRESSURE DURING THE INFUSION

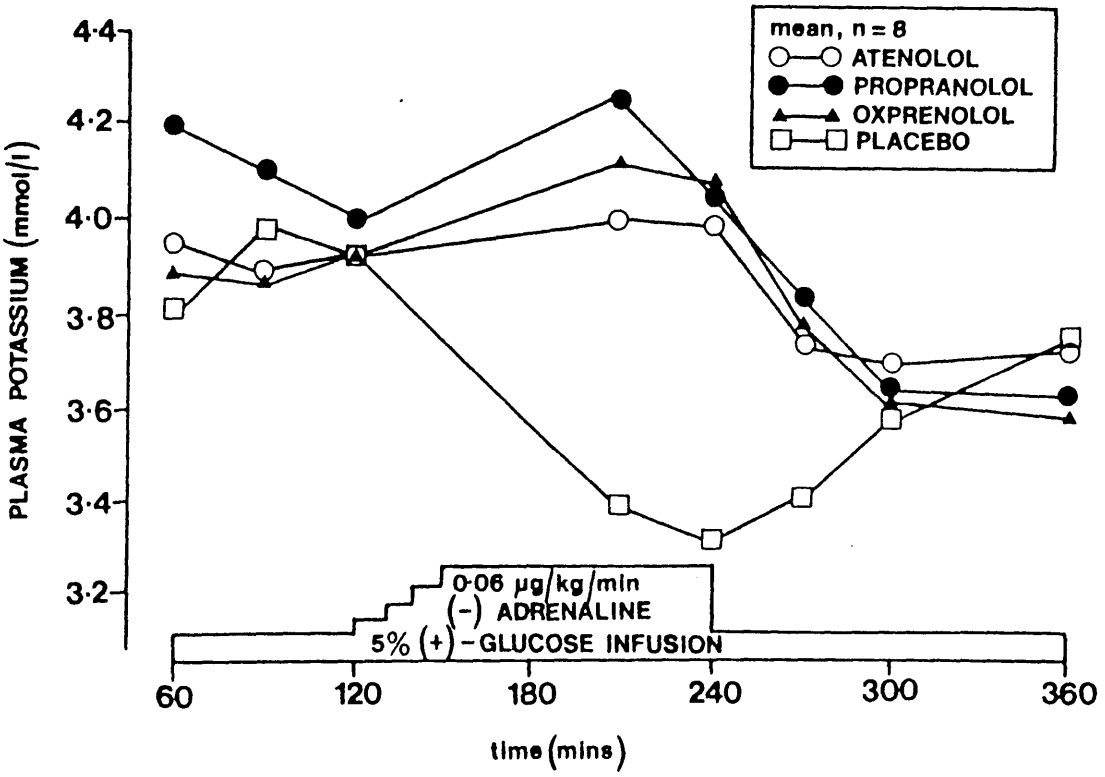


Fig. 4.3.3.: CHANGES IN PLASMA POTASSIUM DURING THE INFUSION

Table 4.3.4: - Mean heart rate at each time point on each treatment.

(beats min^{-1}, n=8; mean ± S.D.).

Time (mins.)	Placebo	Propranolol	Oxprenolol	Atenolol
60	71 ± 11	60 ± 5	64 ± 9	60 ± 5
120	66 ± 11	58 ± 8	65 ± 8	58 ± 8
130	67 ± 8	57 ± 7	64 ± 10	57 ± 7
140	73 ± 10	57 ± 7	61 ± 8	57 ± 7
150	72 ± 9	58 ± 5	60 ± 7	58 ± 5
160	74 ± 12	57 ± 5	56 ± 6	57 ± 5
175	78 ± 13	60 ± 7	62 ± 10	60 ± 7
190	77 ± 11	60 ± 6	59 ± 7	60 ± 6
210	77 ± 10	61 ± 6	62 ± 8	61 ± 6
225	78 ± 13	62 ± 8	62 ± 9	62 ± 8
240	79 ± 16	60 ± 5	59 ± 4	60 ± 5
270	75 ± 13	63 ± 7	70 ± 9	63 ± 7
300	74 ± 11	63 ± 9	69 ± 9	63 ± 9
360	75 ± 14	65 ± 12	69 ± 10	65 ± 12
		$p<0.001$	$p<0.001$	$p<0.001$

Table 4.3.5:- Mean systolic blood pressure at each time point on each treatment.

(mm Hg, n=8; mean ± S.D.).

Time (mins.)	Placebo	Propranolol	Oxprenolol	Atenolol
60	118 ± 10	110 ± 8	113 ± 10	109 ± 7
120	115 ± 11	104 ± 8	111 ± 12	106 ± 8
130	115 ± 11	105 ± 13	110 ± 13	104 ± 8
140	113 ± 10	107 ± 13	110 ± 15	105 ± 8
150	117 ± 15	104 ± 12	112 ± 13	104 ± 6
160	121 ± 15	107 ± 8	114 ± 14	107 ± 6
175	122 ± 12	109 + 12	117 ± 12	109 ± 5
190	121 ± 12	109 ± 10	113 ± 16	108 ± 8
210	121 ± 12	110 ± 10	118 ± 18	107 ± 10
225	121 ± 10	113 ± 7	117 ± 18	109 ± 10
240	121 ± 12	113 ± 9	114 ± 16	108 ± 8
270	119 ± 14	112 ± 10	114 ± 15	107 ± 11
300	121 ± 10	109 ± 18	109 ± 14	107 ± 9
360	116 ± 7	110 ± 12	109 ± 14	108 ± 8
		p>0.05	p>0.05	p>0.05

Table 4.3.6:- Mean diastolic blood pressure at each time point on each treatment.

(mm Hg, n=8; mean ± S.D.).

Time (mins)	Placebo	Propranolol	Oxprenolol	Atenolol
60	65 ± 7	59 ± 4	62 ± 8	58 ± 4
120	63 ± 5	59 ± 8	58 ± 6	56 ± 4
130	62 ± 5	58 ± 9	60 ± 8	53 ± 4
140	60 ± 6	59 ± 12	59 ± 9	54 ± 4
150	61 ± 7	62 ± 9	60 ± 9	54 ± 6
160	62 ± 10	62 ± 7	60 ± 8	54 ± 4
175	60 ± 6	62 ± 8	61 ± 8	54 ± 4
190	58 ± 7	63 ± 9	59 ± 10	55 ± 4
210	59 ± 6	64 ± 8	62 ± 9	54 ± 5
225	60 ± 8	64 ± 8	61 ± 8	55 ± 4
240	58 ± 6	66 ± 7	61 ± 10	53 ± 3
270	63 ± 4	60 ± 7	59 ± 5	58 ± 7
300	65 ± 7	52 ± 9	55 ± 6	53 ± 5
360	65 ± 6	55 ± 6	57 ± 7	54 ± 5
		p>0.05	p>0.05	p>0.05

Table 4.3.7:- Mean plasma potassium at each time point on each treatment.

(mmol l^{-1}, n=8; mean ± S.D.).

Time (mins)	Placebo	Propranolol	Oxprenolol	Atenolol
60	3.80 ± 0.21	4.20 ± 0.31	3.96 ± 0.25	3.89 ± 0.14
90	3.99 ± 0.24	4.11 ± 0.29	3.90 ± 0.25	3.88 ± 0.10
120	3.94 ± 0.16	4.01 ± 0.29	3.94 ± 0.24	3.93 ± 0.14
210	3.39 ± 0.41	4.28 ± 0.22	4.14 ± 0.16	4.01 ± 0.37
240	3.31 ± 0.41	4.05 ± 0.26	4.09 ± 0.23	3.99 ± 0.32
270	3.41 ± 0.27	3.84 ± 0.26	3.79 ± 0.31	3.75 ± 0.13
300	3.58 ± 0.23	3.64 ± 0.18	3.63 ± 0.25	3.70 ± 0.20
360	3.76 ± 0.25	3.64 ± 0.26	3.59 ± 0.20	3.74 ± 0.31
		$p<0.01$	$p<0.01$	$p<0.01$

interpretation that the dose of atenolol used in this study selectively blocked $beta_1$-adrenoceptors. The non-selective beta-adrenoceptor antagonists, propranolol and oxprenolol, abolished or reversed this response.

However the hypokalaemic response to adrenaline was abolished by all three beta-adrenergic antagonists. There are a number of possible explanations for the abolition of the hypokalaemic response to adrenaline with atenolol. Adrenaline induced hypokalaemia may not be mediated through a beta-adrenoceptor at all. Though possible it would be surprising in view of the published evidence that in other mammals adrenaline induced hypokalaemia exists and is mediated by a $beta_2$-adrenoceptor (Clausen & Flatman, 1977). An alternative explanation for the persistent vasopressor response to adrenaline following atenolol would be that adrenaline induced hypokalaemia is linked to the $beta_2$-adrenoceptor but that different $beta_2$-adrenoceptor populations in different tissues, in this study the vascular $beta_2$-receptors and the skeletal muscle membrane $beta_2$-adrenoceptors, may vary in their susceptibility to beta-adrenoceptor antagonists. There may also be differences in the efficiency of agonist coupling to functional responses at different $beta_2$-receptors.

In view of the findings of this study which contrasted with the other published evidence it was clear that further studies were required. The logical first step

being to repeat the study with a more selective $beta_1$-antagonist. Though atenolol is the most highly selective beta-antagonist currently available in the British National Formulary there is a more selective $beta_1$-antagonist currently under development, CGP 17/582B. A similar study was therefore carried out using CGP 17/582B.

4.4 Adrenaline induced hypokalaemia: the effect of a new, highly cardioselective beta-adrenoceptor antagonist, CGP 17/582B.

4.4.1 Introduction.

CGP17/582B is a new beta adrenoceptor antagonist (Fig 4.4.1) which, on experimental studies, appears to combine $beta_1$-receptor blocking selectivity with partial agonist activity. It is claimed to be a highly specific $beta_1$-adrenoceptor antagonist and in view of the results of the previous study would be ideal for studying the mechanism of adrenaline induced hypokalaemia.

The haemodynamic and hypokalaemic effects of adrenaline were compared following pretreatment for 7 days with oral CGP 17/582B; with similar pretreatment with propranolol (non-selective beta blocker with no ISA) and metoprolol (selective $beta_1$-blocker with no ISA) and an inactive placebo control.

4.4.2 Methods.

Eight healthy subjects on no regular drug therapy and

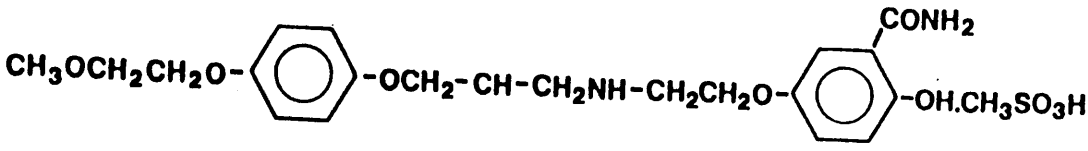


Fig.4.4.1.: STRUCTURE OF CGP 17/582B

with normal haematology, biochemistry and electrocardiograms (4 females, 4 males; age-19-37 years; weight 51-67 kgs.) were studied.

This was a placebo controlled, double blind study with supplies being dispensed by the Hospital Pharmacy. Subjects received all four treatments in a randomised Latin square design order with at least two weeks between treatments to ensure adequate washout. All drugs were matched using a disguising gelatine capsule. The four treatments were i) placebo capsule twice daily; ii) Propanolol 80 mg twice daily; iii) Metoprolol 100mg twice daily; iv) CGP 17/582B 100 mgs twice daily and each therapy was administered for one week. Prior to each treatment period, and on the second and fifth day of each treatment, subjects underwent a standard submaximal exercise test, 2 mins of exercise at 100 watts min^{-1}, using a bicycle ergometer in order to confirm and compare the degree of beta blockade achieved. Subjects attended the laboratory on the seventh day of each therapy having abstained from caffeine, food, alcohol and tobacco for 10 hours.

The drug and a standard light breakfast were given together and cannulae inserted into each forearm. Subjects were studied supine throughout. After one hour a 5% (+)-glucose (dextrose) infusion (12 mls $hour^{-1}$) containing ascorbic acid (1 mg ml^{-1}) was given, via a Braun VI perfusor pump, for one hour followed by an

infusion of (-)-adrenaline in 5% (+)-glucose which was increased stepwise in the standard protocol (Chapter 3.3) to a rate of 0.06 μg kg^{-1} min^{-1} which was continued for 90 minutes (Fig 4.4.2). Blood pressure, heart rate and plasma potassium and catecholamine levels were measured at regular intervals (Fig 4.4.2). Following the adrenaline infusion the (+)-glucose vehicle infusion was continued for a further two hours.

Blood pressure and heart rate were measured by a semi-automated sphyghmomanometer. An electrocardiogram was displayed continously throughout the study period.

Plasma potassium samples and plasma catecholamine samples were handled in the standard manner. Plasma catecholamines were measured by high performance liquid chromatography (Chapter 3.2).

Results are expressed as mean±standard deviation. Repeated measures analysis of variance was used for comparisions between treatments. Measurements before and after a manoeuvre (adrenaline infusion, exercise, etc.) were compared using a paired Student's 't' test.

4.4.3 Results

No subjects reported any side effects during the period beta-adrenoceptor antagonists were given. During the adrenaline infusion two subjects were aware of an increase in their heart rate on all treatments but no reduction in infusion rate was necessary.

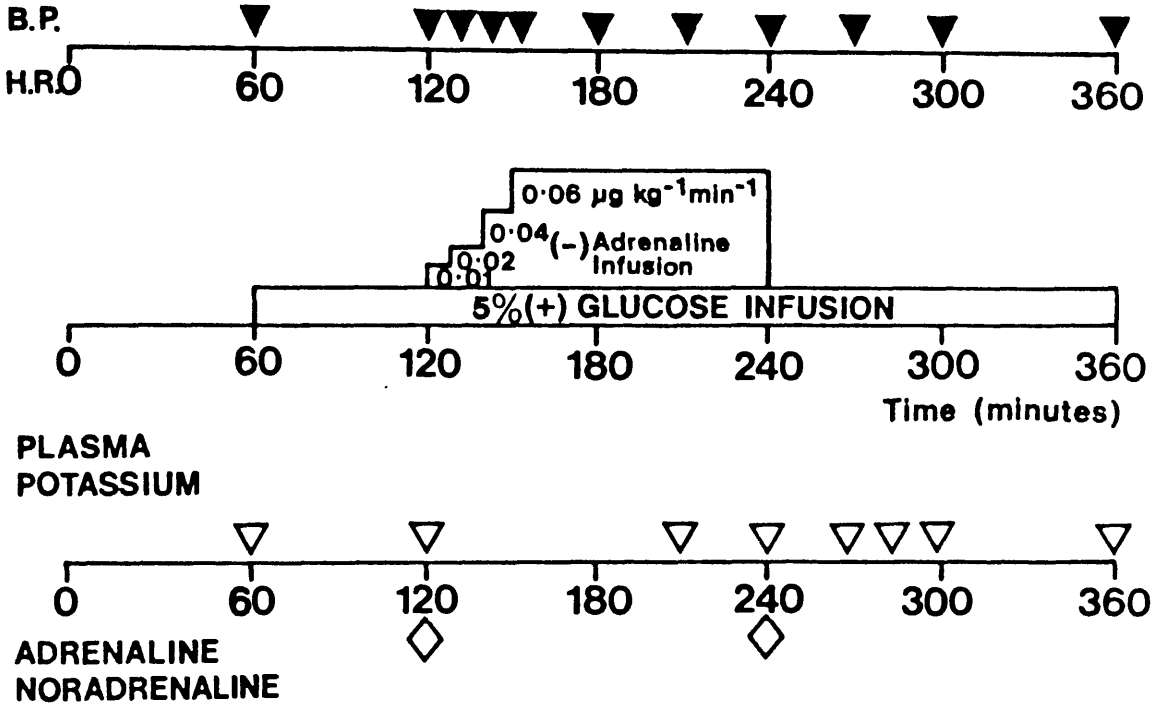


Fig. 4.4.2.: OUTLINE OF STUDY DAY

Effect of beta-adrenoceptor antagonists at rest and after exercise:-

These results are shown in Table 4.4.1 and there was no difference in pretreatment baseline heart rates between treatment periods. Following 5 days of placebo there was no change in exercise induced tachycardia after 2 mins exercise at 100 watts min^{-1}. All active treatments significantly reduced exercise induced tachycardia (metoprolol - 32% inhibition, $p<0.001$; CGP 17/582B - 24% inhibition, $p<0.01$ and propranolol - 19% inhibition, $p<0.05$) confirming beta adrenoceptor blockade after all three active treatments.

Plasma Catecholamine Concentrations:

The plasma adrenaline concentrations prior to the adrenaline infusions were not significantly different between placebo and any of the active drugs (Table 4.4.2). Following adrenaline administration the adrenaline levels were comparable to previous studies. There were no significant differences in the adrenaline concentrations throughout the infusion period between treatments compared to placebo. As the infusion rate was the same in all studies this suggests that adrenaline clearance was not altered by any of these beta antagonists. The baseline plasma noradrenaline levels were not different after beta-blockers. However noradrenaline levels decreased significantly during the

Table 4.4.1:- Mean post exercise heart rate on each treatment.

[beats min $^{-1}$; mean ± S.D.; n=8, p-values refer to comparison of heart rate at end of exercise period (2 mins at 100W^{-1}) prior to therapy and heart rate at the end of exercise on day 5 of each treatment by paired students 't'-test; N.S.D.=no significant difference.]

Treatment	Prior to Therapy		Day 5 of therapy
Placebo	129±17		125±24
		N.S.D.	
CGP 17/582B	131±9		99±16
		p<0.01	
Metoprolol	129±16		88±8
		p<0.001	
Propranolol	115±15		93±22
		p<0.05	

adrenaline infusion on the placebo, metoprolol and propranolol study days but not following CGP 17/582B, (Table 4.4.2).

Effect of adrenaline infusion on blood pressure and heart rate:

Changes in heart rate, systolic and diastolic blood pressure are shown in Figure 4.4.3. The resting heart rate was significantly reduced, compared to the placebo study day, by metoprolol ($p<0.01$) and by propranolol ($p<0.01$) but not by CGP 17/582B ($p>0.05$). No rise in heart rate was seen during the adrenaline infusion with either metoprolol or propranolol whereas heart rates rose with placebo and CGP 17/582B. Correcting for differences in baseline measurements on the different study days demonstrates significant differences in heart rate between metoprolol and propranolol when compared with CGP 17/582B or placebo following the adrenaline infusion. At the end of the study period, these changes in heart rate were still evident (Figure 4.4.3, Table 4.4.3).

Baseline systolic blood pressure was significantly reduced by metoprolol and propranolol when compared to placebo and CGP 17/582B (Table 4.4.4). However when corrected for baseline differences there were no significant differences in systolic blood pressure between treatments during the adrenaline infusion (Figure 4.4.3). The baseline diastolic blood pressure was significantly lower on propranolol compared to placebo

Table 4.4.2:- Plasma adrenaline and noradrenaline levels on each treatment.

(mean ± S.D.; n=8, p-values refer to comparison between 120' and 240' by students 't' test).

	Adrenaline Concentration ($nmol\ l^{-1}$)		Noradrenaline Concentration ($nmol\ l^{-1}$)	
Time (mins)	120'	240'	120'	240'
Treatment				
Placebo	0.2 ± 0.3	3.7 ± 1.4	2.1 ± 1.0	1.6 ± 0.6
			p<0.05	
CGP	0.1 ± 0.2	4.0 ± 2.0	1.8 ± 0.7	1.8 ± 0.8
			N.S.D.	
Metoprolol	0.1 ± 0.2	3.8 ± 0.5	2.3 ± 0.9	1.7 ± 0.7
			p<0.01	
Propranolol	0.1 ± 0.1	3.7 ± 2.2	2.5 ± 0.7	1.3 ± 0.4
			p<0.01	

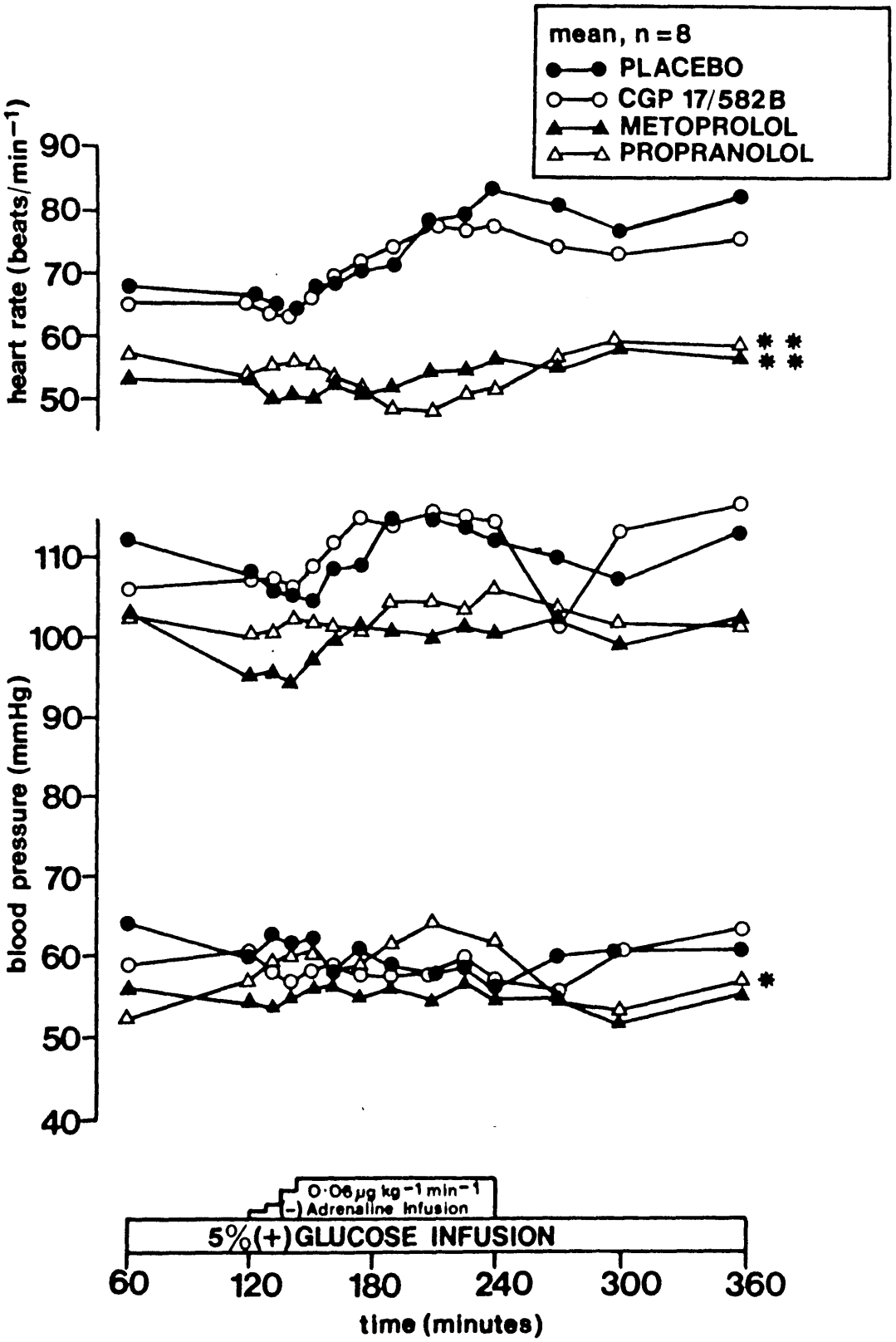


Fig. 4.4.3 : CHANGES IN HEART RATE AND BLOOD PRESSURE ON EACH STUDY DAY
(* - $p < 0.05$; * * - $p < 0.01$)

Table 4.4.3:- Mean heart rate at each time point on each treatment.

[beats min^{-1}; mean ± S.D.; n=8]

Time (mins)	Placebo	CGP 17/582B	Metoprolol	Propranolol
60	68 ± 10	66 ± 6	54 ± 4	58 ± 5
120	66 ± 11	66 ± 8	54 ± 4	54 ± 4
130	64 ± 10	64 ± 7	51 ± 4	56 ± 7
140	64 ± 9	63 ± 6	52 ± 6	56 ± 8
150	67 ± 12	66 ± 6	51 ± 4	56 ± 10
160	68 ± 8	69 ± 7	54 ± 4	54 ± 7
175	71 ± 7	71 ± 5	52 ± 4	53 ± 6
190	71 ± 8	73 ± 6	52 ± 3	50 ± 3
210	77 ± 11	77 ± 8	55 ± 5	49 ± 2
225	78 ± 10	76 ± 5	55 ± 5	52 ± 6
240	82 ± 10	77 ± 6	57 ± 7	52 ± 5
270	80 ± 9	74 ± 7	56 ± 5	57 ± 6
300	76 ± 7	72 ± 5	59 ± 6	59 ± 6
360	80 ± 8	74 ± 7	57 ± 8	58 ± 9
		p>0.05	p<0.01	p<0.01

Table 4.4.4:- Mean systolic blood pressure at each time point on each treatment.

[mm Hg; mean ± S.D.; n=8]

Time (mins)	Placebo	CGP 17/582B	Metoprolol	Propranolol
60	112 ± 8	106 ± 11	103 ± 8	103 ± 4
120	108 ± 11	107 ± 4	95 ± 5	100 ± 4
130	105 ± 8	107 ± 8	95 ± 6	100 ± 4
140	105 ± 7	106 ± 3	94 ± 5	102 ± 3
150	104 ± 5	108 ± 4	97 ± 3	101 ± 8
160	108 ± 7	111 ± 7	100 ± 7	101 ± 5
175	108 ± 13	114 ± 5	101 ± 7	101 ± 6
190	114 ± 9	114 ± 8	101 ± 6	104 ± 5
210	114 ± 10	115 ± 7	100 ± 5	104 ± 8
225	113 ± 9	114 ± 7	101 ± 5	103 ± 9
240	112 ± 10	114 ± 6	100 ± 6	106 ± 9
270	109 ± 7	100 ± 15	102 ± 5	103 ± 7
300	106 ± 11	113 ± 4	99 ± 12	100 ± 4
360	112 ± 8	115 ± 5	101 ± 6	101 ± 5
		p>0.05	p>0.05	p>0.05

and this difference was maintained throughout the study period. After propranolol pre-treatment there was a rise in diastolic pressure during the adrenaline infusion compared to placebo ($p<0.05$). After metoprolol there was a modest difference from placebo throughout which was not significant when corrected for baseline differences ($p>0.05$). On the CGP 17/582B study day changes in diastolic blood pressure during the adrenaline infusion did not differ significantly from those seen on the placebo study day (Table 4.4.5).

Plasma Potassium:

Changes in plasma potassium are shown in Figure 4.4.4. (Table 4.4.6). There was no significant effect on baseline plasma potassium with any beta-blocker pretreatment. Following placebo pretreatment potassium fell significantly during the adrenaline infusion and a similar fall was seen with CGP 17/582B. A small but insignificant rise in plasma potassium was observed with propranolol but plasma potassium fell during the adrenaline infusion by an insignificant amount following metoprolol. The pattern of plasma potassium response to the adrenaline infusion was significantly different from placebo following both metoprolol and propranolol ($p<0.05$) but not following CGP 17/582B pretreatment (Figure 4.4.4, Table 4.4.6). One hour after the end of the adrenaline infusion plasma potassium was not significantly different between the four study periods.

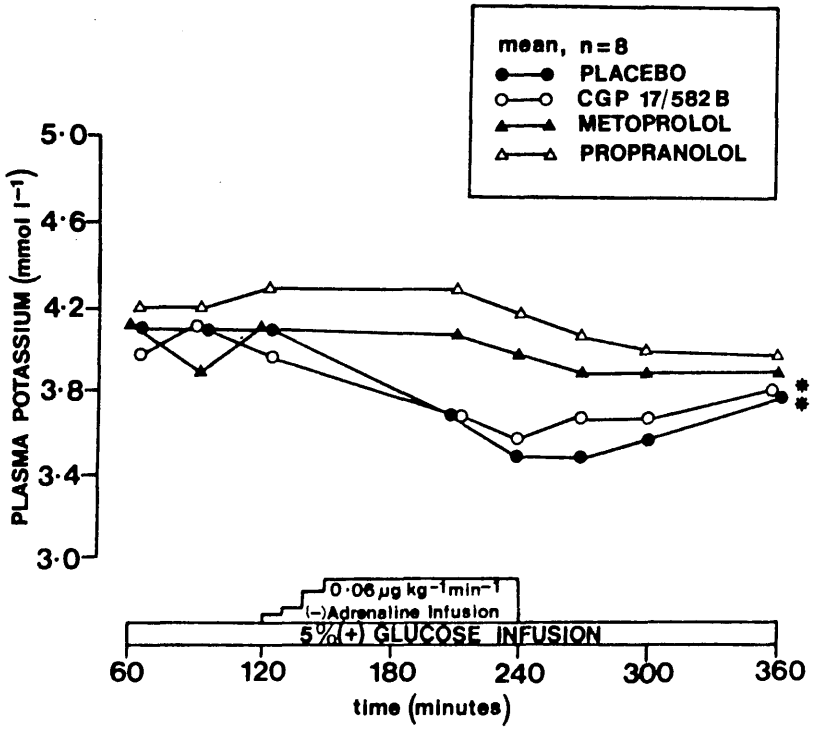


Fig. 4.4.4 : CHANGES IN POTASSIUM ON EACH STUDY DAY
(*-$p < 0.05$)

Table 4.4.5:- Mean diastolic blood pressure at each time point on each treatment.

[mm Hg; mean ± S.D.; n=8]

Time (mins)	Placebo	CGP 17/582B	Metoprolol	Propranolol
60	63 ± 6	58 ± 7	55 ± 7	52 ± 6
120	60 ± 7	60 ± 5	54 ± 6	56 ± 3
130	62 ± 5	57 ± 2	52 ± 5	57 ± 5
140	60 ± 7	55 ± 7	54 ± 4	58 ± 4
150	61 ± 8	57 ± 3	55 ± 7	59 ± 5
160	56 ± 9	58 ± 7	56 ± 5	57 ± 5
175	60 ± 7	57 ± 5	54 ± 4	58 ± 5
190	58 ± 3	56 ± 3	56 ± 8	60 ± 6
210	56 ± 5	56 ± 3	54 ± 4	62 ± 7
225	57 ± 5	59 ± 7	56 ± 5	58 ± 10
240	54 ± 7	56 ± 4	54 ± 3	61 ± 8
270	59 ± 7	54 ± 11	54 ± 5	54 ± 5
300	59 ± 6	59 ± 8	50 ± 3	52 ± 5
360	59 ± 7	62 ± 7	54 ± 8	55 ± 5
		p>0.05	p>0.05	p<0.05

Table 4.4.6:- Mean plasma potassium at each time point on each treatment.

[mmol l^{-1}; mean ± S.D., n=8].

Time (mins)	Placebo	CGP 17/582B	Metoprolol	Propranolol
60	4.1 ± 0.3	4.0 ± 0.4	4.1 ± 0.2	4.2 ± 0.3
90	4.1 ± 0.2	4.1 ± 0.4	3.9 ± 0.3	4.2 ± 0.3
120	4.1 ± 0.3	4.0 ± 0.4	4.1 ± 0.3	4.3 ± 0.4
210	3.7 ± 0.4	3.7 ± 0.3	4.1 ± 0.2	4.3 ± 0.2
240	3.5 ± 0.3	3.6 ± 0.3	4.0 ± 0.3	4.2 ± 0.2
270	3.5 ± 0.3	3.7 ± 0.4	3.9 ± 0.3	4.1 ± 0.3
300	3.6 ± 0.3	3.7 ± 0.4	3.9 ± 0.2	4.0 ± 0.3
360	3.8 ± 0.3	3.8 ± 0.3	3.9 ± 0.2	4.0 ± 0.4
		$p>0.05$	$p<0.05$	$p<0.05$

4.4.4 Discussion.

CGP 17/582B is highly selective $beta_1$-adrenoceptor antagonist with intrinsic sympathomimetic activity (Klepzig & Strauer, 1986). Though its basic structure is a phenoxypropranolamine, common to most beta-blockers, it has two unusual features. The methoxy-ethoxy group is in the para position, leading to cardioselectivity and mild intrinsic sympathomimetic activity, and the amino-group has a salicylate moiety, which may explain the very high receptor affinity of this compound (Fig 4.4.1).

The heart rate response to exercise allowed accurate tritration of the dose of each individual beta-blocker to achieve adequate $beta_1$-blockade. Using a dose of each beta-blocker sufficient to reduce exercise induced heart rate by 25% should ensure that, with selective beta-blockers such as CGP, excessive beta-blockade does not occur. This is important as with all 'selective' beta-antagonists the degree of selectivity is relative and with increasing doses a degree of $beta_2$-adrenoceptor blockade occurs (Lertora et al, 1975).

Propranolol, a non-selective beta-blocker, abolished completely adrenaline induced hypokalaemia whereas metoprolol, which has a degree of selectivity, though not as highly selective as CGP, attenuated adrenaline induced hypokalaemia. CGP, which is a highly selective $beta_1$-adrenoceptor antagonist, had no effect on adrenaline induced hypokalaemia. The results of the present study

with propranolol and metoprolol are consistent with the hypothesis that the fall in potassium which follows adrenaline infusion is mediated by a membrane bound Na^+/K^+ ATPase linked to a beta adrenoceptor of the $beta_2$ subtype. The haemodynamic and metabolic effects are in keeping with non-selective and relatively selective $beta_1$ blockade respectively.

The failure of CGP to alter the pressor effects of adrenaline provides evidence that the dose of CGP used in this study was selective to the $beta_1$-adrenoceptor and that $beta_2$-adrenoceptors on peripheral blood vessels were unaffected. Additional evidence is provided by the noradrenaline levels with each drug. Beta agonist infusion leads to increases in plasma noradrenaline due to stimulation of presynaptic facilitatory $beta_2$ receptors (Stjarne & Brundin, 1975; Yamaguchi et al , 1977; Majewski et al, 1980; Vincent et al, 1982). Adrenaline which has both $beta_1$- and $alpha_2$- agonist activity did cause plasma noradrenaline to fall in this study. This could be due to an imbalance in adrenaline's effects, at the concentrations achieved during the infusion, on the facilitatory $beta_2$- and inhibitory $alpha_2$-presynaptic mechanisms (Musgrave, Backmann & Gordon, 1984). After betablockade it might be expected that unopposed $alpha_2$-inhibitory action of adrenaline would lead to a fall in plasma noradrenaline. Noradrenaline fell during the adrenaline infusion and

this was greater with propranolol and metoprolol pretreatment than with placebo. There was no effect on noradrenaline with CGP 17/582B suggesting that, due to its high selectivity, the facilitatory presynaptic $beta_2$-adrenoceptor was not blocked.

The results of this study agree with the hypothesis that adrenaline induced hypokalaemia is mediated via a $beta_2$-adrenoceptor. The partial agonist action of CGP 17/582B had no effect on either basal plasma potassium levels or adrenaline induced hypokalaemia suggesting it is of no relevance.

4.5 Summary.

The results of the three studies described in this chapter demonstrate that adrenaline and salbutamol induced hypokalaemia are not the result of changes in circulating insulin levels. Secondly, that salbutamol, a selective $beta_2$-agonist, induces hypokalaemia to a similar extent as adrenaline suggesting that adrenaline induced hypokalaemia is the result of $beta_2$-stimulation.

The results from the first beta-blocker study were more difficult to interpret and would appear to conflict with this conclusion. The abolition of the hypokalaemic response to adrenaline by atenolol would suggest that either the hypokalaemic response is not mediated via the $beta_2$-adrenoceptor or that this dose of atenolol was too high and, though selective for some $beta_2$-adrenoceptor

populations, e.g. vascular $beta_2$-adrenoceptors, the dose was sufficient to blockade $beta_2$-adrenoceptors on skeletal muscle. This may be the result of differences in the efficiency of agonist coupling to functional responses at different $beta_2$-receptors. Other workers (Vincent et al, 1984) have also reported abolition of this response with a similar dose of atenolol whereas with a lower dose of atenolol a further group have reported attenuation of the hypokalaemic response to adrenaline (Struthers et al, 1984).

The results of the third study with the highly selective $beta_1$-antagonist, CGP 17/582B, strongly suggest that adrenaline induced hypokalaemia is mediated via the $beta_2$-adrenoceptor and not via the $beta_1$-adrenoceptor. In this study the vascular tissue response was similar to the hypokalaemic response, unlike the second study using atenolol.

In conclusion these studies support the thesis that adrenaline induced hypokalaemia is mediated via the $beta_2$-adrenoceptor. The final proof would be to study the recently developed agent ICI-118551 which is a highly selective $beta_2$-adrenoceptor antagonist. Studies with this expensive, and scarce drug, have been carried out by two groups of workers (Struthers & Reid, 1984; Brown, Brown & Murphy, 1983). Both these studies have confirmed that ICI-118551 totally abolishes adrenaline induced hypokalaemia, confirming that the hypokalaemic response to adrenaline is mediated via the $beta_2$-adrenoceptor.

CHAPTER 5

DIURETICS AND HYPOKALAEMIA

5. DIURETICS AND HYPOKALAEMIA.

5.1 Diuretic induced hypokalaemia.

As previously discussed (Chapter 2), hypokalaemia is potentially dangerous to patients with both ischaemic heart disease and hypertension. Diuretic therapy is a well recognised cause of hypokalaemia. Surprisingly the exact mechanisms of potassium losses remains uncertain, and the significance of low serum levels as an indicator of total body depletion is unclear.

As plasma potassium is not protein bound 100% is filtered at the glomerulus, 80-90% of the filtered load is reabsorbed in the ascending loop of Henle and the remainder beyond so that by the distal tubule all the filtered potassium has been reabsorbed. The source of urinary potassium is secretion along the late distal tubule and the collecting duct, resulting in a fractional secretion of 5-15% (Giebisch, Malnic & Berliner, 1981). As discussed previously (Chapter 1.4) the exact mechanisms and control of renal tubular active and passive secretion of potassium are not known but evidence suggests that they are dependent on intraluminal sodium concentration and rate of flow of tubular fluid (Figure 1.4.1).

The effect of diuretics on potassium excretion depends on the site of action of the diuretic (Figure 5.1.1). As

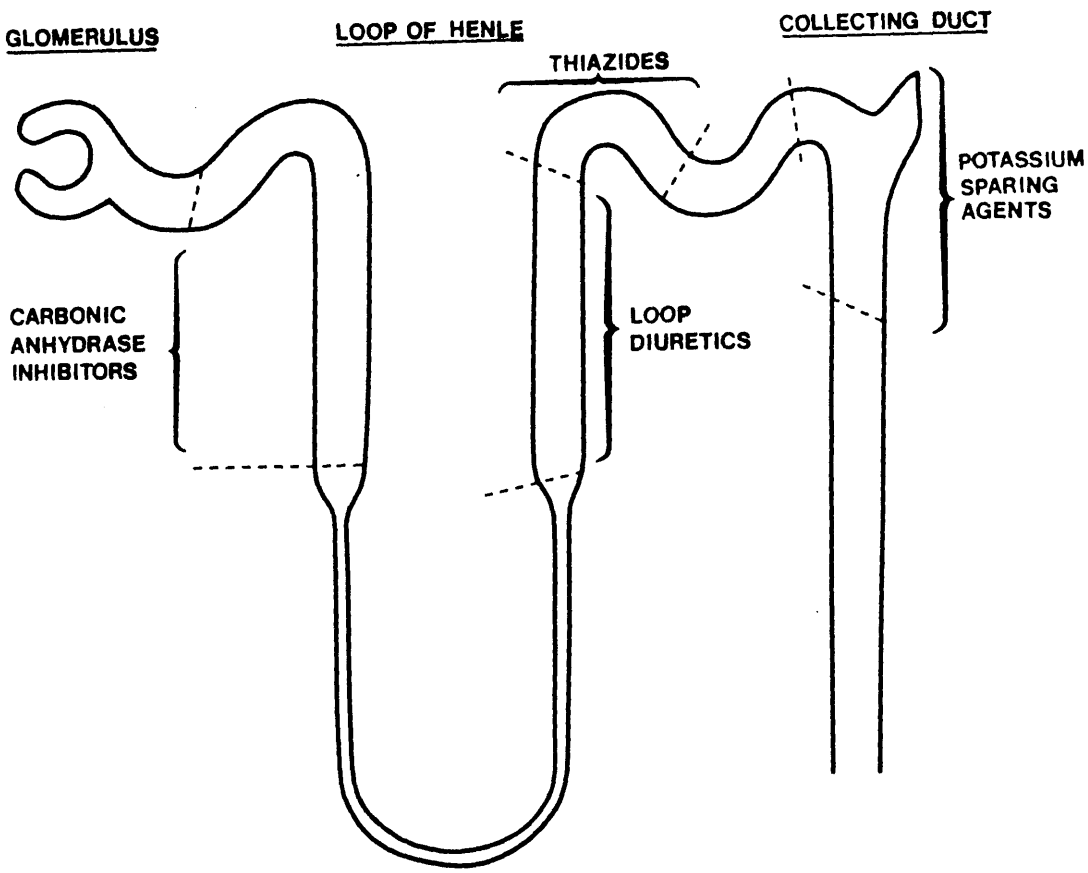


Fig. 5.1.1. SITE OF ACTION OF PRINCIPAL GROUPS OF DIURETICS

a general rule, diuretics that act at sites proximal to the potassium secretory sites, i.e. late distal tubule and collecting ducts, will increase urinary potassium secretion by increasing the delivery of sodium and fluid to the potassium secretory sites. The increased luminal sodium concentration alters potassium secretion, possibly by creating a larger transepithelial gradient. Diuretics such as frusemide and the thiazides also enhance potassium excretion by increasing aldosterone levels, thus increasing intraluminal sodium concentrations. In addition they induce a metabolic alkalosis (Knochel, 1984; Chapter 1.4) which results in an ingress of potassium into cells. Indeed it has been argued that diuretics do not lead to whole body potassium depletion but only alter internal balance and disposition of potassium (Kassirer & Schwartz, 1966). There is, however, convincing recent evidence that diuretics do increase urinary potassium loss and deplete whole body potassium stores. After several weeks of diuretic therapy potassium excretion does fall into the 'normal' range. This excretion is, however, abnormal when measured as fractional excretion of whole body potassium, which has itself fallen as a result of the diuretic therapy (Schuck, 1982).

Agents which act in the late distal tubule and the collecting duct do not cause potassium loss and are often referred to as 'potassium-saving' diuretics. Two groups

of drugs have been developed; sodium-channel blockers such as amiloride and triamterene; and the aldosterone antagonists such as spironolactone.

'Loop' Diuretics

Diuretics acting in the ascending loop of Henle, e.g. frusemide, bumetanide and ethacrynic acid, result in large increases in intraluminal sodium concentrations, increased tubular fluid flow and hence increased secretion of potassium. The incidence of hypokalaemia in patients receiving frusemide is unclear from the literature but is probably dose-dependent. In a study of adverse reactions to frusemide in hospitalized patients only a 3.5% incidence of hypokalaemia was reported (Lowe et al, 1979), confirming a previous smaller study (Greenblatt et al, 1977).

Thiazide diuretics

Thiazide diuretics act on the 'early' distal tubule (Figure 5.1.1) and lead to significant potassium losses, though there is controversy over both the incidence and clinical significance of such losses. Potassium losses are related to both the dose (Giebisch et al, 1981) and duration of treatment (Whelton & Watson, 1986). The mechanism of the potassium loss is still disputed. One suggestion is that the effect of thiazides are purely due to increased fluid flow in the distal tubule and are not influenced by intraluminal sodium changes or any change in transepithelial gradient (Constazo & Windhager, 1978).

Effect of diuretic therapy on tissue potassium levels.

A review of the literature (Dyckner & Wester, 1987a) reported that all studies had shown numerically lower potassium values after diuretics and in 11 of the 23 studies quoted this difference was statistically significant. A recent study by the same authors demonstrated that 48% of diuretic treated patients with congestive cardiac failure had decreased skeletal muscle potassium (Wester & Dyckner, 1985). These authors have also shown that the combination of a thiazide with a potassium sparing diuretic increases skeletal muscle potassium (Dyckner & Wester, 1984).

Serum and muscle potassium levels have been found to correlate, though only weakly in patients with congestive cardiac failure. However, the inter-individual variability in response makes serum levels a poor predictor of muscle potassium levels (Dyckner & Wester, 1987b).

5.2 The effect of diuretic therapy with bendrofulazide, frusemide and spironolactone on plasma potassium during adrenaline infusions.

5.2.1 Introduction.

The most popular groups of diuretics, thiazides and loop diuretics, are frequently given to patients with ischaemic heart disease and hypertension and these diuretics can cause hypokalaemia. Such patients with

hypokalaemia secondary to diuretic therapy could, therefore, be at greater risk from adrenaline induced hypokalaemia during acute stress.

Spironolactone is an aldosterone antagonist and has not been reported to reduce plasma potassium concentrations (Ochs et al, 1978).

The effect on adrenaline induced hypokalaemia of pretreatment with three diuretics: bendrofluazide, a thiazide; frusemide, a 'loop' diuretic; and spironolactone was studied in healthy normotensive subjects using a double blind, placebo controlled, crossover design. The dose of each diuretic chosen for this study was the standard and most commonly used daily dose.

In addition, the effect of increased circulating adrenaline levels in the presence and absence of diuretic pre-treatment on erythrocyte intracellular potassium concentrations was studied. The erthyrocyte was chosen because it is simple to obtain samples, though it is not known if erythrocytes have membrane bound $beta_2$-adrenoceptors.

5.2.2 Methods.

Eight healthy normotensive volunteers (4 males, 4 females; age range 21-37 years) were studied. All subjects had normal routine haematology, biochemistry and electrocardiograms and were on no regular drug therapy.

The study was placebo controlled and double blind with

supplies being dispensed by the hospital pharmacy. Subjects received four separate treatments in a randomised order using a Latin square design with at least two weeks between treatments to ensure adequate washout. All drugs were matched using a disguised gelatine capsule. The four treatments were i) bendrofluazide 5 mgs daily; ii) placebo capsule one daily; iii) frusemide 40 mg daily; and iv) spironolactone 100mgs daily. Each therapy was given for two weeks.

On the fourteenth day of each treatment limb subjects attended the laboratory having abstained from all food, caffeine, alcohol and tobacco for 10 hours.

The drug and a standard light breakfast were given and cannulae inserted. Subjects were studied supine throughout and after one hour of rest they received a period of a control 5% (+)-glucose solution followed by stepwise increases in the rate of adrenaline infusion in the standard infusion protocol (Chapter 3.3).

Baseline measurements were made during the one hour control glucose infusion. Exact details of the timing of all measurements are shown in Figure 5.2.1.

Blood pressure and heart rate were measured in duplicate by a semi-automated sphygmomanometer. An electro-cardiogram was displayed continously throughout the study period.

Plasma potassium was measured by standard methods on an autoanalyser. Venous samples for catecholamine

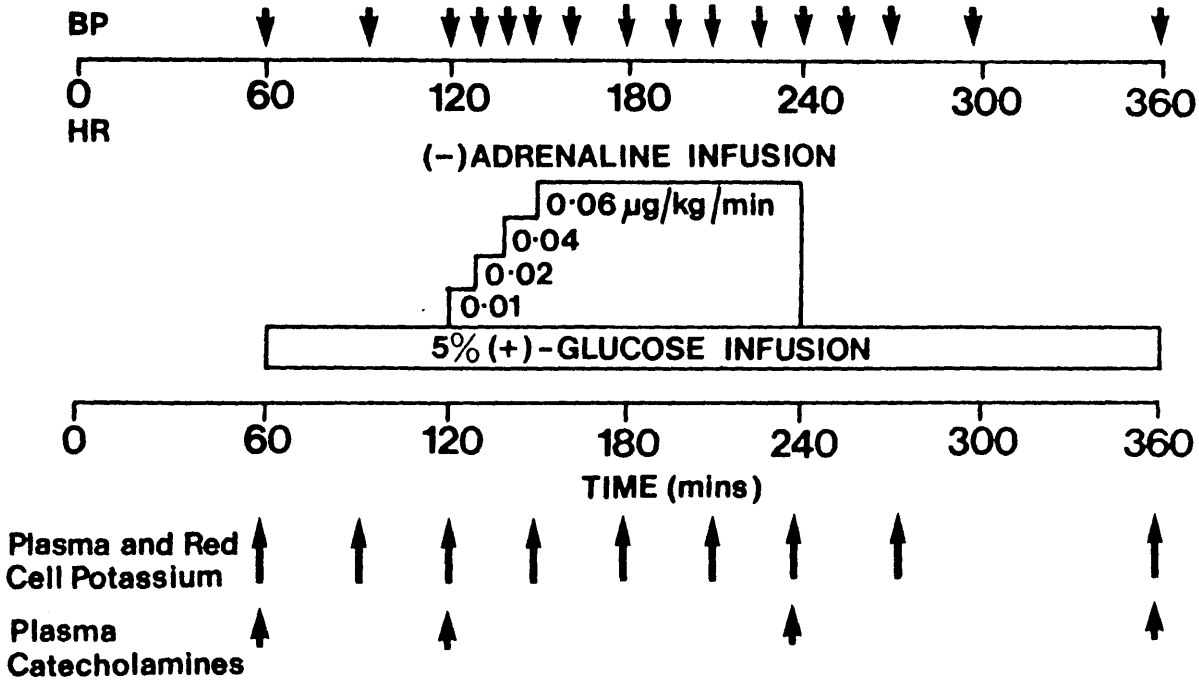


Fig. 5.2.1. : OUTLINE OF STUDY DAY

concentrations were collected in the standard fashion and were analysed by the high performance liquid chromatography assay method (Chapter 3.2).

Red cell potassium was measured by direct sampling of whole blood into lithium sulphate using a flame photometer (Corning Model 430).

Results are expressed as mean ± standard deviation. Comparisons between treatments were made by repeated measures analysis of variance with corrections made for baseline differences. Measurements before and after a manoeuvre (e.g. adrenaline infusion) were compared using a paired Students t-test.

5.2.3 Results.

All subjects completed the study and no subject reported any side-effects, although several commented on increased urine volume while taking frusemide.

Plasma catecholamines.

Baseline adrenaline and noradrenaline were not significantly different on the four treatments. The adrenaline levels at the end of the adrenaline infusion showed considerable variation between study days in the same subject and between subjects but the levels were increased at least 20 fold by the infusion. Adrenaline levels were not significantly different on the four study days (Table 5.2.1).

Haemodynamic measurements.

There were no differences in the baseline heart rates

for all four treatments. Following the adrenaline infusion at a rate of 0.06µg kg^{-1} min^{-1} for 90 minutes the heart rate was similar for all treatments (Table 5.2.2). The mean increase with the infusion of adrenaline was 10±3 beats min^{-1}. No cardiac rhythm disturbances were noted. There were no significant differences in either systolic or diastolic blood pressure between treatments (Table 5.2.3 & 5.2.4).

Plasma potassium

Baseline potassium levels were not significantly different on any of the three active therapies compared to placebo (Table 5.2.5). The rank order was that potassium was lowest on bendrofluazide and unaltered on spironolactone and frusemide (placebo - 4.1±0.1; spironolactone - 4.1±0.3; frusemide - 4.1±0.5; and bendrofluazide - 3.8 $\pm$ 0.5 mmol l^{-1}). Plasma potassium fell during the period of the adrenaline infusion on all four treatments (Figure 5.2.2). This fall was larger on the frusemide (0.5mmol l^{-1}) and bendrofluazide (0.4mmol l^{-1}) study days. As the baseline values were lower on bendrofluazide this fall resulted in hypokalaemia with a nadir of 3.3 mmol l^{-1}. The fall on the placebo and spironolactone study days was the same.

Red cell potassium

Though baseline red cell potassium was lower on spironolactone (101 mmol l^{-1})and bendrofulazide (101 mmol l^{-1}) these were not significantly less than the values on

Table 5.2.1 Mean plasma catecholamines at each time point on each study day.

(nmol l^{-1}; n=8, mean ± S.D.)

Adrenaline Concentrations

Time (mins)	PLACEBO	SPIRONOLACTONE	FRUSEMIDE	BENDROFLUAZIDE
60	0.1±0.05	0.1±0.05	0.1±0.05	0.1±0.4
120	0.1±0.05	0.1±0.1	0.1±0.05	0.1±0.1
240	2.2±1.7	4.1±5.8	4.0±5.0	2.3±1.2
360	0.1±0.1	0.4±0.5	2.4±6.0	0.3±0.7
		p>0.05	p>0.05	p>0.05

Noradrenaline Concentrations

Time (mins)	PLACEBO	SPIRONOLACTONE	FRUSEMIDE	BENDROFLUAZIDE
60	2.0±0.9	1.9±1.0	2.1±1.0	2.2±1.1
120	2.0±0.7	2.4±1.3	2.2±1.2	2.0±0.6
240	2.1±0.4	2.0±1.2	3.6±4.0	2.1±0.9
360	2.0±1.0	1.2±0.5	2.1±1.5	2.0±0.7
		p>0.05	p>0.05	p>0.05

Table 5.2.2 Mean heart rate at each time point on each study day.

(beats min^{-1}; n = 8; mean ± S.D.)

Time (mins)	Placebo	Spironolactone	Frusemide	Bendrofluaz.
60	70 ± 6	74 ± 9	73 ± 10	71 ± 9
90	68 ± 7	69 ± 8	70 ± 11	68 ± 7
120	69 ± 5	69 ± 7	72 ± 10	69 ± 7
130	71 ± 6	68 ± 7	71 ± 10	70 ± 10
140	73 ± 4	68 ± 4	73 ± 10	68 ± 8
150	73 ± 5	71 ± 6	74 ± 9	70 ± 9
160	71 ± 5	73 ± 10	72 ± 10	73 ± 10
175	77 ± 9	74 ± 9	76 ± 10	76 ± 10
190	75 ± 6	77 ± 8	76 ± 10	77 ± 10
210	79 ± 9	77 ± 10	81 ± 11	79 ± 9
225	81 ± 10	78 ± 13	80 ± 12	79 ± 9
240	85 ± 13	79 ± 12	78 ± 8	77 ± 12
270	81 ± 12	78 ± 14	83 ± 15	84 ± 8
300	77 ± 6	76 ± 10	79 ± 8	77 ± 10
360	81 ± 8	77 ± 11	81 ± 7	81 ± 6
		p>0.05	p>0.05	p>0.05

Table 5.2.3 Mean systolic blood pressure at each time point on each study day.

(mm Hg; n = 8; mean ± S.D.)

Time (mins)	Placebo	Spironolactone	Frusemide	Bendrofluaz.
60	118 ± 9	117 ± 8	121 ± 9	116 ± 16
90	113 ± 10	117 ± 8	117 ± 10	123 ± 23
120	114 ± 11	115 ± 11	115 ± 11	116 ± 6
130	112 ± 13	117 ± 11	119 ± 10	116 ± 8
140	111 ± 14	114 ± 9	117 ± 12	115 ± 7
150	115 ± 12	118 ± 11	117 ± 8	117 ± 11
160	114 ± 8	120 ± 15	118 ± 8	117 ± 8
175	118 ± 9	118 ± 10	120 ± 8	122 ± 9
190	118 ± 8	123 ± 10	126 ± 10	127 ± 9
210	120 ± 11	124 ± 14	128 ± 14	127 ± 7
225	123 ± 13	124 ± 10	126 ± 12	124 ± 8
240	122 ± 10	121 ± 8	127 ± 13	120 ± 8
270	120 ± 13	122 ± 9	122 ± 14	120 ± 10
300	117 ± 13	119 ± 10	120 ± 10	119 ± 10
360	116 ± 10	117 ± 10	116 ± 8	120 ± 10
		p>0.05	p>0.05	p>0.05

Table 5.2.4 Mean diastolic blood pressure at each time point on each study day.

(mm Hg; n = 8; mean ± S.D.)

Time (mins)	Placebo	Spironolactone	Frusemide	Bendrofluaz.
60	61 ± 7	61 ± 5	63 ± 5	62 ± 8
90	61 ± 5	64 ± 3	65 ± 5	64 ± 8
120	61 ± 8	63 ± 5	64 ± 7	67 ± 6
130	60 ± 9	63 ± 4	65 ± 6	64 ± 6
140	58 ± 8	61 ± 8	63 ± 6	63 ± 5
150	58 ± 7	62 ± 6	63 ± 5	63 ± 6
160	58 ± 4	61 ± 8	62 ± 6	62 ± 7
175	57 ± 8	61 ± 5	62 ± 7	63 ± 6
190	58 ± 7	59 ± 4	63 ± 4	60 ± 5
210	58 ± 7	62 ± 2	61 ± 7	64 ± 2
225	58 ± 7	61 ± 4	61 ± 6	61 ± 7
240	60 ± 8	61 ± 4	63 ± 8	58 ± 6
270	61 ± 7	64 ± 3	67 ± 4	65 ± 6
300	54 ± 9	65 ± 7	67 ± 3	65 ± 5
360	59 ± 6	63 ± 11	63 ± 6	66 ± 7
		p>0.05	p>0.05	p>0.05

Table 5.2.5 Mean plasma potassium at each time point on each study day.

(mmol l^{-1}; n = 8; mean ± S.D.)

Time (mins)	Placebo	Spironolactone	Frusemide	Bendrofluaz.
60	4.1 ± 0.1	4.1 ± 0.3	4.1 ± 0.5	3.9 ± 0.5
90	4.1 ± 0.2	4.2 ± 0.3	4.1 ± 0.4	3.8 ± 0.4
120	4.0 ± 0.2	4.2 ± 0.3	4.0 ± 0.4	3.8 ± 0.4
210	3.8 ± 0.4	3.9 ± 0.3	3.5 ± 0.3	3.4 ± 0.3
240	3.7 ± 0.5	3.8 ± 0.3	3.5 ± 0.2	3.4 ± 0.3
270	3.8 ± 0.3	3.8 ± 0.3	3.4 ± 0.3	3.5 ± 0.3
300	3.9 ± 0.2	3.8 ± 0.1	3.6 ± 0.2	3.5 ± 0.3
360	3.9 ± 0.2	3.8 ± 0.1	3.6 ± 0.3	3.5 ± 0.2
		p>0.05	p>0.05	p>0.05

the placebo (104 mmol l^{-1}) and frusemide study days (105mmol l^{-1}). Red cell potassium did not alter during the period of the adrenaline infusion (Table 5.2.6, Figure 5.2.3).

5.2.4 Discussion.

The fall in plasma potassium during the adrenaline infusion was similar with all three diuretics. However the nadir of the plasma potassium was dependent on the initial plasma potassium level which was, in turn, dependent on the action of each diuretic on potassium homeostasis. As baseline plasma potassium was lower on bendrofluazide and frusemide, the adrenaline infusions caused an apparently greater degree of hypokalemia. During the increased sympathoadrenal activity of severe acute illness, such as myocardial infarction, pretreatment with these diuretics may therefore place patients at increased risk of clinically significant hypokalaemia. Spironolactone, by not altering baseline potassium values, had the same effect as inactive placebo treatment.

Many cells have membrane bound Na+/K+ ATPase and in this study the effect of adrenaline on intracellular potassium concentrations of the erythrocyte was studied. No significant effect on intracellular potassium concentration was demonstrated. As the vast majority of potassium is intracellular and only a small proportion is extracellular then only a small intracellular shift is

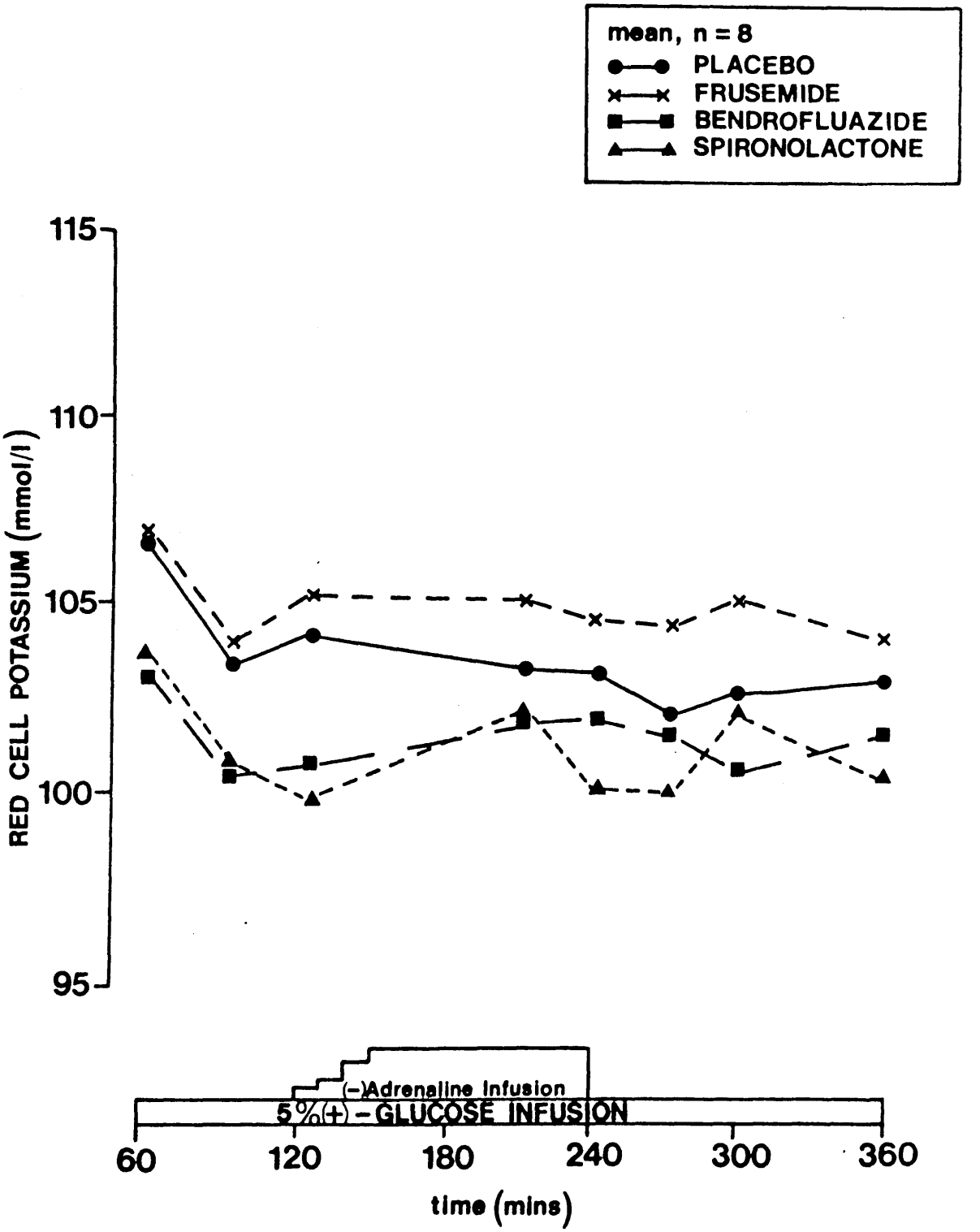


Fig. 5.2.3: CHANGES IN RED CELL POTASSIUM ON EACH STUDY DAY

Table 5.2.6 Mean red cell potassium at each time point on each study day.

(mmol l^{-1}; n = 8; mean ± S.D.)

Time (mins)	Placebo	Spironolactone	Frusemide	Bendrofluaz.
60	106 ± 5	103 ± 6	107 ± 3	103 ± 4
90	103 ± 6	101 ± 5	104 ± 4	100 ± 5
120	104 ± 4	101 ± 4	105 ± 3	101 ± 5
210	103 ± 4	102 ± 4	105 ± 3	102 ± 5
240	103 ± 5	100 ± 6	105 ± 3	102 ± 6
270	102 ± 5	100 ± 6	104 ± 3	101 ± 6
300	102 ± 5	102 ± 5	104 ± 4	101 ± 5
360	103 ± 5	99 ± 5	104 ± 3	101 ± 6
		p>0.05	p>0.05	p>0.05

necessary to cause a significant and detectable fall in plasma potassium. In contrast this intracellular shift is such a small proportion of the intracellular potassium content that it will not alter the concentration. However, it is unknown if erythrocytes have membrane bound $beta_2$-adrenoceptors and if no such receptors exist this would explain these results.

Diuretic therapy is frequently used in patients with hypertension and ischaemic heart disease. Fifty percent of hypertensive patients treated with thiazide diuretics have a resting serum potassium less than 3.5 mmol l^{-1} (Morgan & Davidson, 1980; MRC Working Party on Mild to Moderate Hypertension, 1981). The incidence of ventricular ectopic beats has been claimed to be inversely related to the serum potassium level (MRC Working Party on Mild to Moderate Hypertension,1983) but this claim is controversial (Moser, Black & Stair, 1986; Madias, Madias & Gavras, 1984; Caralis & Perez-Stable, 1986). Hypokalaemia increases the incidence of ventricular arrhythmias in patients with acute myocardial infarction (Dyckner et al, 1975)). Both hypertensive patients and those with cardiac failure are at increased risk of myocardial infarction and the accompanying rises in circulating catecholamine levels. This study suggests that patients receiving chronic thiazide therapy could be at increased risk of severe hypokalaemia and, thus, cardiac dysrhythmias following infarction. There is some

evidence from one large trial of thiazide therapy in the treatment of mild to moderate hypertension that the actively treated group did less well than the placebo group with a higher incidence of sudden death (MRFIT, 1982). It is possible that the hypokalaemic effect of thiazide diuretics increased the incidence of cardiac dysrhythmias in these patients.

The effect of pretreatment with frusemide is more difficult to interpret. Frusemide, did not lower baseline potassium levels significantly. However there was a greater fall in plasma potassium during the adrenaline infusion following frusemide. The clinical significance of this finding is unclear.

Spironolactone neither altered baseline potassium levels nor did it alter the degree of adrenaline induced hypokalaemia. This finding would suggest that spironolactone may prove a safer form of diuretic therapy in patients with an increased risk of myocardial infarction or ischaemia.

CHAPTER 6

THEOPHYLLINE AND HYPOKALAEMIA

6.1 Introduction - Interactions between methylxanthines, sympathomimetics and the sympathetic system.

Methylxanthines, principally theophylline, are probably the most commonly used bronchodilators and are frequently used in combination with sympathomimetic drugs such as salbutamol. The evidence that this combination has useful therapeutic benefit is controversial, studies investigating the potential benefit on pulmonary function or exercise tolerance in asthma (Hartnett & Marlin, 1976; Dyson & Campbell, 1977; Wolfe et al, 1979) and in chronic bronchitis (Barclay et al, 1981; Leitch et al, 1981; Alexander, Dull & Kasik 1980; Eaton et al, 1980; Evans 1984; Taylor et al, 1985) have been conflicting though the majority have suggested an additive effect. There are very few studies of the interaction between methylxanthines and sympathomimetics in other tissues. However, it is known that methylxanthines increase noradrenaline induced cardiac inotropic responses (Rall & West, 1963), aortic muscle contraction (Kalsner, 1971) and coronary artery strip contraction (Kalsner, Frew & Smith, 1975). Patients receiving both a methylxanthine and a sympathomimetic have an increased incidence of side-effects (Weinberger & Bronsky, 1975). Nevertheless,it has become common practice, with the

advent of modern slow-release theophyllines, to use combinations of theophylline and sympathomimetics, such as the $beta_2$-selective agonists, salbutamol. The safety of this combination has been questioned recently. Asthma deaths have risen in recent years, as discussed previously, and this rise has paralleled the increasing use of this combination. The Federal Food and Drug Administration sponsored a number of studies in animals of the safety of the combination and these studies reported a lower threshold for ventricular fibrillation in animals treated with the combination (F.D.A. 1981; Nicklas et al, 1982; Joseph et al,1981). The combination was associated with the development of myocardial necrotic lesions (Green, Guideri & Lehr, 1980).

There are, therefore, a number of potential interactions between theophyllines and sympathetic agonists in patients with asthma or bronchitis.

As discussed below (6.2), the mechanism of action of the methylxanthines remains unknown. One postulated mechanism is increases in circulating catecholamine concentrations. This theory has never been investigated in subjects receiving chronic therapy with these drugs. To study this question was the aim of the first study described in this chapter.

The studies outlined in this chapter were designed to answer the following questions:

1) Does chronic theophylline therapy increase circulating

adrenaline, or noradrenaline concentrations, or alter adrenaline clearance? 2) Do steady state therapeutic plasma theophylline concentrations increase the hypokalaemic or haemodynamic response to adrenaline? 3) Do steady state therapeutic plasma theophylline concentrations increase salbutamol induced hypokalaemia and, if adrenaline concentrations are also raised in severe attacks of bronchospasm, is there a further fall in plasma potassium?

6.2 The pharmacology and clinical uses of the methylxanthines.

6.2.1 Pharmacology of the methylxanthines.

The methylxanthines are a group of closely related plant alkaloids, theophylline, theobromine and caffeine, which have been used in medicinal preparations for hundreds of years (Rall, 1985). Early pharmacological studies confirmed that these compounds are central nervous system stimulants. They are derivatives of xanthine, dioxypurine, with a variety of methyl substitutions at three sites on the ring, hence methylxanthines (Figure 6.2.1). The use of the methyl-xanthines as stimulant beverages and medicinal potions led to the observation that they had a number of other actions in addition to C.N.S. stimulation, they act on the kidney to promote a diuresis, stimulate cardiac muscle and relax smooth muscle, particularly bronchial

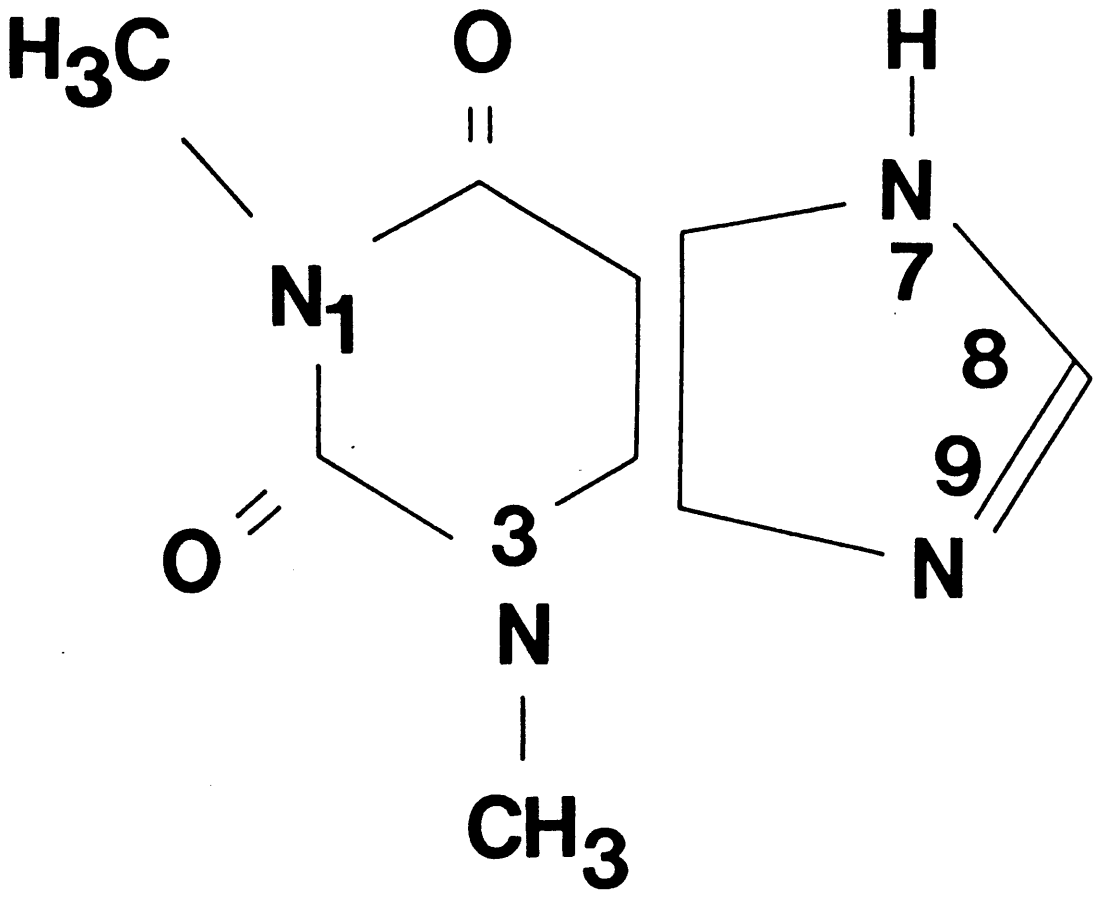


Fig. 6.2.1. : STRUCTURE OF THEOPHYLLINE

smooth muscle (Rall, 1985).

Coffee was first recommended for the treatment of asthma in 1859 (Salter, 1859). In 1888 the active agent was extracted from tea leaves, named theophylline, derived from the Greek 'divine leaf' (Kossel, 1888) and was soon identified as 1,3-dimethylxanthine (Fischer & Ach, 1895). Thereafter the other methylxanthines were identified, extracted and more recently synthetised. It is as bronchodilators that methylxanthines, and specifically theophyllines, are used today and world wide theophylline is probably the most commonly prescribed bronchodilator.

The pharmacology of the methylxanthines is complex. Theophylline has a very low solubility which has led to the production of a number of complexes, such as aminophylline (theophylline and ethylenediamine) in an attempt to improve solubility for parenteral administration.

Theophylline principally undergoes hepatic metabolism (90%) by microsomal oxidative enzymes and demethylation to 3, methylxanthine, 1,3 dimethyluric acid and 1, methyluric acid. There is a wide inter-individual variation in the clearance rate of theophylline making prediction of individual's dose requirements difficult. Many factors alter theophylline clearance, e.g. hepatic enzyme inducing drugs and hepatic congestion secondary to cor pulmonale (Ogilvie, 1978; McElnay, Smith & Helling,

1982). Thus monitoring of plasma theophylline levels is required to ensure adequate therapy.

The therapeutic plasma range of theophylline is 10-20 $\mu g\ ml^{-1}$ (Mitenko & Ogilvie, 1973), though controversy continues as to the threshold level for theophylline's actions which probably lies between 5-8 $\mu g\ ml^{-1}$ (Piafsky & Ogilvie, 1975). Theophylline has a low therapeutic index and serious toxicity can occur with plasma levels as low as 35 $\mu g\ ml^{-1}$, though rare below 50 $\mu g\ ml^{-1}$. In addition theophylline, even in therapeutic doses, may produce side-effects, with headache and upper gastrointestinal symptoms being the most common.

The plasma half life of theophylline is short, 3-4 hours, and this requires frequent drug administration, 4-6 hourly, to maintain adequate plasma levels. This problem has been surmounted by the development, in recent years, of effective slow release preparations requiring 8 or 12 hourly administration. These slow release preparations, combined with the development of relatively cheap therapeutic plasma monitoring, have led to increased use of theophyllines in the U.K., particularly in the treatment of nocturnal symptoms in asthma. Thus, though theophylline is a relatively difficult drug to use, it is widely prescribed.

6.2.2 Mechanism of action of methylxanthines.

Despite its popularity and long history theophylline's

mechanism of action remains unknown. A number of possible mechanisms of action have been proposed but evidence for the importance of each mechanism at therapeutic tissue levels is conflicting.

Theophylline is a phosphodiesterase inhibitor. Phosphodiesterase catalyses the breakdown of adenosine cyclic 3',5' monophosphate (cyclic AMP), which is the second messenger mediating many hormonal effects including the bronchodilator effect of β-agonists, to adenosine 5'-monophosphate (AMP). Thus, a phosphodiesterase inhibitor will increase cellular concentrations of cAMP and will produce bronchial smooth muscle relaxation. In vitro synergism between the two agents has been shown to affect cyclic AMP concentrations (Kaliner et al, 1971) and for many years this was thought to be the principal mechanism by which theophyllines bronchodilate (Anonymous, 1970). There are a number of pieces of evidence that suggest that phosphodiesterase inhibition is not the mechanism of action. Firstly, there are many other phosphodiesterase inhibitors described, e.g. dipyridamole, yet none of them are effective bronchodilators (Fredholm, Brodin & Strandberg, 1979). Secondly, at the tissue levels thought to occur at therapeutic plasma levels, in vivo experiments have reported that theophylline only has a minor inhibitory effect on phosphodiesterase, approximately 10% (Polson et al, 1978a; Polson et al

1978b). Finally, if theophylline's principal action is phosphodiesterase inhibition then concentrations of cAMP should increase in both cells and plasma in vivo. Two studies have reported an increase in plasma cAMP levels following an intravenous bolus of aminophylline (Campbell et al 1977; Mackay, Baldwin & Tattersfield, 1983). However, other studies have been negative (Lohmann, Miech & Butcher, 1977; Parrott et al, 1976; Trembath & Shaw 1978).

The second proposed mechanism of action is alteration of intra-cellular calcium kinetics or the sensitivity of the sarcoplasmic reticulum to calcium. Methylxanthines augment the twitch response of isolated skeletal muscle (Bianchi, 1975) and current evidence is that this is due to increased permeability of the sarcoplasmic reticulum to calcium. The sarcoplasmic reticulum is involved in the termination of the contractile process by active uptake and sequestration of calcium (Bianchi, 1975). The threshold for these effects on intracellular organelles is reported to be considerably greater than maximal therapeutic concentrations and would not, therefore, explain the observed pharmacological actions of methylxanthines (Rall, 1985).

Currently, two mechanisms are favoured as possible explanations of the observed actions of methylxanthines, a direct effect on the sympathetic system or adenosine

antagonism, and a third possiblity is emerging, an indirect effect on the sympathetic system.

Many of the observed actions of theophyllines would be consistent with increased sympathetic stimulation. A number of studies have clearly demonstrated that, following aminophylline, circulating catecholamines or urinary catecholamines increase (Atuk et al, 1967; Snider & Waldek, 1974; Robertson et al, 1978; Higbee, Kumar & Galant, 1982; Vestal et al, 1983) and that, in vitro, theophylline causes degranulation of adrenal medullary catecholamine storage granules (Aunis et al, 1975). No studies have examined the chronic effect of theophyllines on catecholamine levels. The cellular mechanism by which theophyllines could increase catecholamine release remain unknown.

Adenosine, the endogenously produced purine nucleoside which is formed from cAMP has been known for many years to have potent effects in the circulation (Rall, 1982), though the mechanism of its effects was unclear. In 1965 in a detailed report it was observed (De Gubareff & Sleator, 1965) that the circulatory effects of adenosine could be antagonized by caffeine. Yet only recently has it been appreciated that there are in fact adenosine receptors on a wide variety of cell surfaces which either inhibit (R_i or A_1) or stimulate (R_a or A_2) adenyl cyclase leading to increased cellular cyclic AMP (Wolff, Londos & Cooper, 1981). The adenosine receptors are distinct from the purinergic receptors described in the gut and the

circulation. Theophyllines are capable of adenosine antagonism in vitro and in vivo. In vitro caffeine and theophylline were found to be competitive anatagonists of adenosine in a wide variety of tissues (Rall, 1982). In vivo theophylline has been shown to antagonise the bronchoconstriction induced by adenosine (Cushley, Tattersfield & Holgate 1983, Mann & Holgate 1985). Adenosine levels rise during antigen induced bronchoconstriction. If theophylline were to inhibit A_1-receptors and thus decrease the inhibition of adenyl cyclase then intracellular cAMP levels would rise. At therapeutic levels theophylline is a competitive adenosine antagonist. However, enprofylline (3-propyl-xanthine) is a potent bronchodilator (Lunell, Anderson & Persson, 1984) yet has been shown to have no action at the A_1-receptor (Persson, 1983), though there is controversy over whether it may have effects on A_2-receptors in some tissues (Fredholm & Persson, 1982). Clearly if enprofylline has no A_1-activity then the implication is that theophyllines do not bronchodilate by blocking adenosine receptors.

Finally, yet another mechanism has been suggested as a result of recent work. Originally it was thought that methylxanthines had no effect on purinergic receptors (Rall, 1982). Recently it has been shown that purines such as adenosine and ATP may exert a co-transmitter role

at sympathetic effector sites via purinergic receptors on cell membranes. It has been demonstrated that in sympathetic nerves supplying blood vessels ATP and adenosine inhibit pre-junctional P_1-purinoceptors decreasing noradrenaline release and this effect is blocked by methylxanthines (Burnstock, 1985). Thus suggesting a possible path by which theophyllines could stimulate the sympathetic system.

In summary the mechanism of action of the theophyllines remains uncertain, though currently adenosine antagonism is the favoured mechanism, despite some opposing evidence.

6.3 The effect of chronic theophylline therapy on circulating catecholamines and adrenaline clearance.

6.3.1 Introduction:

Theophyllines may act by increasing catecholamine release, principally adrenaline, from the adrenal medulla (6.2.2). Increased catecholamine release could, in turn, to maintain homeostasis, result in an increase in adrenaline clearance.

This placebo controlled, crossover study examined the effects of chronic therapeutic theophylline therapy on circulating adrenaline and noradrenaline levels and, by infusing adrenaline, on adrenaline clearance. This design also allowed examination of the effects of chronic therapeutic theophylline therapy on adrenaline

induced hypokalaemia. The results of this subsidiary study are presented in the succeeding section (6.4).

6.3.2 Methods

Eight healthy subjects, 5 females and 3 males, aged 18 to 33 years were studied. They had normal electrocardiograms, serum biochemistry and haematology. They were on no drug therapy.

Each subject was studied at the same time of day on two occasions on the fifth day of treatment with either a slow release theophylline preparation (Neulin SA, 10-15 mg kg^{-1} in divided doses, Riker Laboratories, Loughborough, U.K.) or an identical placebo in a single blind randomised fashion. Study days were at least 10 days apart (Figure 6.3.1).

On the study day subjects abstained from caffeine containing beverages for eight hours and following a light standard breakfast which contained no caffeine, intravenous cannulae were inserted into the antecubital veins of both arms. Blood was then taken for a trough theophylline level and that morning's dose was administered by mouth. The subjects were supine throughout the study.

After one hour's rest an infusion of 5% (+)-glucose (12ml hr^{-1}) was given for one hour. Subjects then received increasing stepwise infusions for 10 minutes each, of 0.01, 0.02, 0.04 and 0.06 μg $kg^{-1}min^{-1}$ (-)-adrenaline (Antigen Ltd.) infused by Braun Perfusor

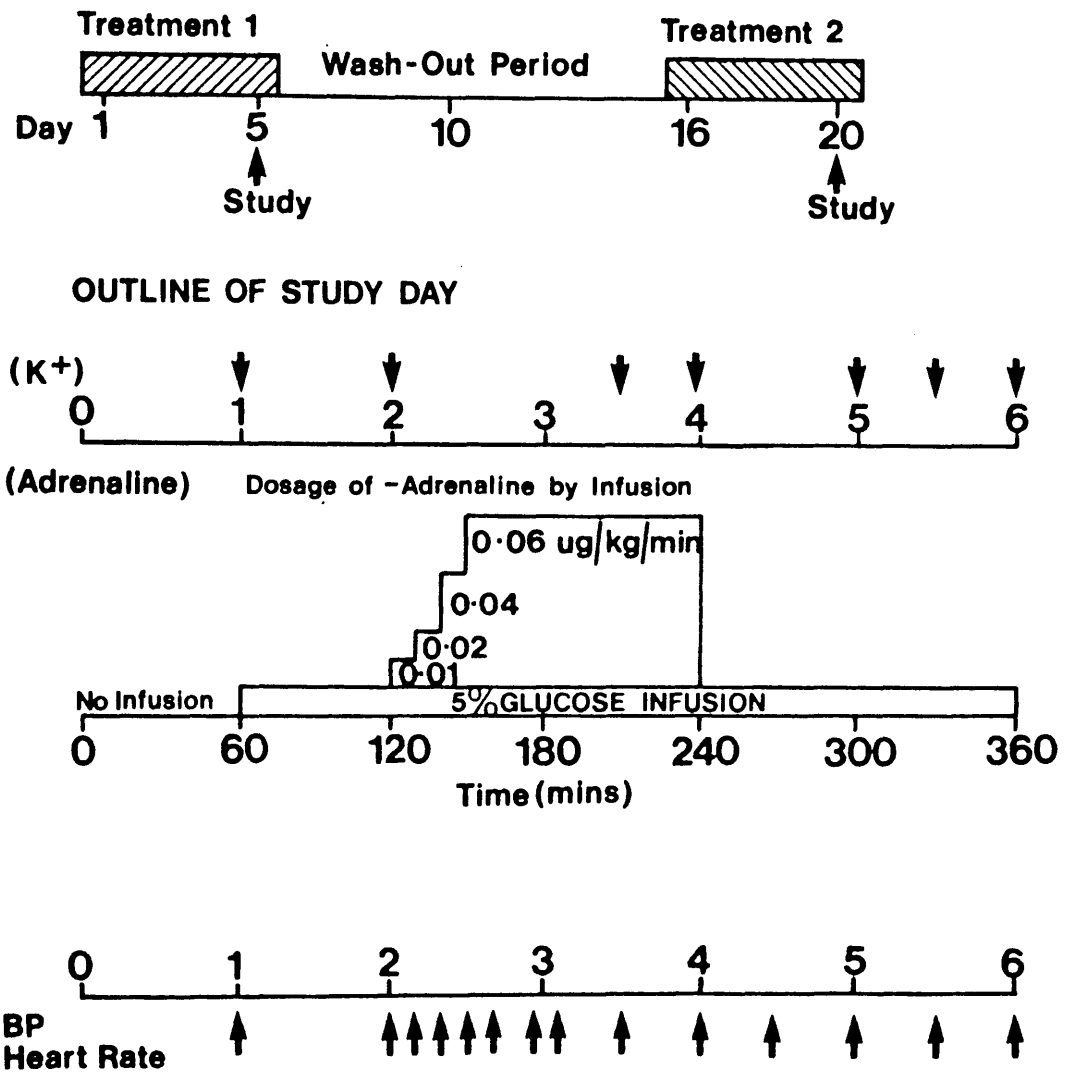


Fig. 6.3.1. : STUDY DESIGN

VI pump by the standard protocol (3.3). If no symptoms or adverse haemodynamic effects occurred then 0.06 μg $kg^{-1}min^{-1}$ (-)-adrenaline was continued for a further 90 minutes. On discontinuation of the adrenaline infusion, (+)-glucose was continued for a further two hours.

Blood pressure, heart rate, E.C.G., catecholamines and serum potassium were measured at intervals throughout the study (Fig 6.3.1). Blood for plasma theophylline level was taken at the start of the study and during the period of the adrenaline infusion (Fig 6.3.1).

Plasma adrenaline and noradrenaline samples were collected in the standard manner (Chap 3.4) and assayed by radioimmunoassay (Chap. 3.3). Theophylline plasma concentration was measured by high pressure liquid chromatography (Gere & Benje, 1977).

The plasma clearance of adrenaline was calculated from the difference between steady state adrenaline plasma levels during infusions of 0.06μg kg^{-1} min^{-1} adrenaline and basal adrenaline during the 5% (+)-glucose infusion using the formula (Clutter et al, 1980):-

$$\text{Clearance} = \frac{\text{Infusion Rate}}{\text{Steady state adrenaline} - \text{basal adrenaline}}$$

Results are expressed as mean±standard deviation. Placebo treatment was compared with theophylline by Student's t-test.

6.3.3 Results

Theophylline concentrations.

The mean plasma concentration of theophylline measured

before dosing on the fifth day (trough concentration) was 14.3±6.3 μg ml^{-1}. Individual values are given in Table 6.3.1. The therapeutic range is 10-20 μg ml^{-1}. Very small amounts of theophylline, <1.0 μg ml^{-1}, were detected after placebo in 3 subjects despite the 10 day washout period. It is probable that the assay was detecting dietary xanthines such as caffeine or theobromine.

Adrenaline and noradrenaline concentrations and adrenaline clearance.

Basal plasma adrenaline concentration, prior to the adrenaline infusion, was 0.37±0.43 nmol l^{-1} on theophylline and 0.49±0.96 nmol l^{-1} on placebo (p>0.05). Plasma adrenaline concentration at steady-state during the (-)-adrenaline infusions was 4.14±0.16 nmol l^{-1} on placebo, compared to 3.11±2.86 nmol l^{-1} on theophylline (p>0.05, Table 6.3.2). Individual's adrenaline clearance varied widely but there was no significant difference between study days (10.0±8.5 l kg^{-1} hr^{-1} on theophylline; 5.5±4.4 l kg^{-1} hr^{-1} on placebo. Table 6.3.2). Basal plasma noradrenaline concentrations were not significantly different between the two study days (Table 6.3.2).

6.3.4 Discussion.

Therapeutic plasma concentrations of theophylline were achieved after 5 days of oral dosing. There was no

Table 6.3.1:- Plasma theophylline concentrations on the placebo and theophylline study days.

(n=8)

Volunteer No.	Theophylline concentration (μg ml^{-1}).	
	Placebo Day	Theophylline Day
1	0	10.3
2	0.1	9.8
3	0	8.0
4	0	5.8
5	0	18.3
6	0.7	25.0
7	0.2	16.2
8	0.6	17.5
Mean	0.2	14.3
S.D.	0.1	6.3

Table 6.3.2:- Plasma adrenaline, noradrenaline and adrenaline clearance on each study day.

(n=8; volunteer (8) plasma adrenaline undetectable (u.d.)).

Subject No.	Adrenaline Concentration (nmol l^{-1})		Noradrenaline Concentration (nmol l^{-1})		Adrenaline Clearance (l kg-1 hr-1)	
	Placebo	Theoph.	Placebo	Theoph.	Placebo	Theoph.
1	0.13	0.10	2.5	1.9	6.3	12.3
2	0.96	0.30	3.5	2.0	5.2	27.8
3	0.17	0.15	1.0	2.1	4.7	7.0
4	0.22	0.20	1.0	2.1	14.9	9.2
5	0.19	0.16	1.0	2.3	2.3	7.3
6	2.8	0.30	2.1	6.5	2.8	2.2
7	0.4	0.30	4.2	1.4	2.8	4.4
8	u.d.	u.d.	2.1	3.2	-	-
Mean	0.63	0.17	2.2	2.7	5.5	10.0
S.D.	0.97	0.09	1.2	1.6	4.4	8.5

significant difference in circulating adrenaline levels during supine rest between theophylline and placebo. Similarly, noradrenaline levels were unaltered by theophylline. These findings are in conflict with previous studies of the effects of acute doses of methylxanthines on catecholamine levels.

In 1967 Atuk and colleagues infused 485mg of theophylline into volunteers and reported significant rises in urinary catecholamines concentrations (Atuk et al, 1967). Robertson and co-workers administered oral caffeine, 250mg, in normal subjects and demonstrated a rise in circulating adrenaline (207%) and noradrenaline (75%) (Robertson et al, 1978). Similarly, a small single oral dose of theophylline (5mg kg^{-1}) was shown to increase circulating adrenaline levels 3 hours after ingestion, and this was accompanied by an insignificant rise in noradrenaline (Higbee et al, 1982). More recently Vestal found that a short infusion of adrenaline resulted in a rise in both plasma adrenaline and noradrenaline (Vestal et al, 1983).

The effect of methylxanthines on isolated bovine adrenal glands is to stimulate release of catecholamines, principally adrenaline, from the adrenal medulla, independently of the nervous system (Poisner, 1973). This study also suggested that there may be two independent mechanisms involved, one calcium independent and one calcium dependent. Relatively high concentrations of

xanthines have been shown to be required to trigger such release (Snider & Waldeck, 1974). Finally, intravenous theophylline increases plasma dopamine beta-hydroxylase activity suggesting that theophylline triggers degranulation of catecholamine storage granules (Aunis et al, 1975).

All these studies consistently showed increases in catecholamines but all used single doses or very short infusions of theophylline and are, therefore, not directly comparable to this study of the effects of chronic theophylline therapy. An alternative explanation for the conflicting results between chronic and acute therapy could be that adrenaline clearance increases in response to increased catecholamine release to maintain homeostasis. No significant increase in adrenaline clearance was demonstrated in this study. These results suggest that catecholamine release is not stimulated by chronic theophylline therapy and, thus, increased circulating catecholamine concentrations cannot explain the chronic actions of theophylline.

6.4 The effect of theophylline and circulating adrenaline on plasma potassium

6.4.1 Introduction

Hypokalaemia has not been reported with therapeutic plasma theophylline levels, though plasma potassium levels do fall on acute dosing (Zangvoort et al., 1986). Hypokalaemia is common following self-poisoning with

large doses of theophylline (Buckley et al, 1983). In a placebo controlled trial of normal subjects the effect of steady-state therapeutic theophylline concentrations on adrenaline induced hypokalaemia was examined.

6.4.2 Methods

The subjects studied and the protocol followed was as outlined in the previous study (6.3.2).

Each subject was studied at the same time of day on two occasions on the fifth day of treatment with either a slow release theophylline preparation (Neulin SA, 10-15 mg kg^{-1} in divided doses, Riker Laboratories, Loughborough, U.K.) or identical placebo in a single blind randomised fashion. Study days were at least 10 days apart (Fig 6.3.1).

Blood pressure, heart rate, E.C.G., catecholamines and serum potassium were measured at intervals throughout the study (Fig 6.3.1) and by the methods outlined in the preceding sections (3.4 and 6.3.2).

Results are expressed as mean$\pm$standard deviation. Placebo treatment day is compared with theophylline treatment by analysis of variance, as applied to repeated measures.

6.4.3 Results

Side-effects

Seven of the eight volunteers complained of side-effects on active therapy, theophylline, compared to

only one on placebo. Six had nausea, the seventh had insomnia and 2 volunteers complained of headache and tremor, in addition, to nausea and insomnia.

Theophylline concentrations.

The plasma concentration of theophylline measured before dosing on the fifth day (trough) was 14.3±6.3 μg ml^{-1} within the therapeutic range. Very small amounts of theophylline (<1.0 μg ml^{-1}) were detected after placebo in 3 subjects (Table 6.3.1).

Adrenaline and noradrenaline concentrations.

As discussed above there was no significant differences in baseline adrenaline and noradrenaline concentrations or in adrenaline concentrations at steady-state during the adrenaline infusions (see Table 6.3.2).

Plasma potassium concentrations.

Mean plasma potassium concentrations are given in Table 6.4.1. Though baseline plasma potassium was 3.88±0.32 mmol l^{-1} on theophylline compared to 4.08±0.16 mmol l^{-1} on placebo prior to the (-)-adrenaline infusion, this was not significantly different. Plasma potassium levels at the end of the (-)-adrenaline infusion, were significantly lower on theophylline (3.03±0.48 mmol l^{-1}) compared to the placebo (3.41±0.20 mmol l^{-1}; $p<0.02$, [Fig 6.4.1], with plasma potassium consistently lower on theophylline. There was wide individual variation in the fall in plasma potassium with adrenaline, 0.25-1.4 mmol l^{-1} (Fig 6.4.2). During the theophylline study day plasma potassium levels as low as 2.35 mmol l^{-1} were noted

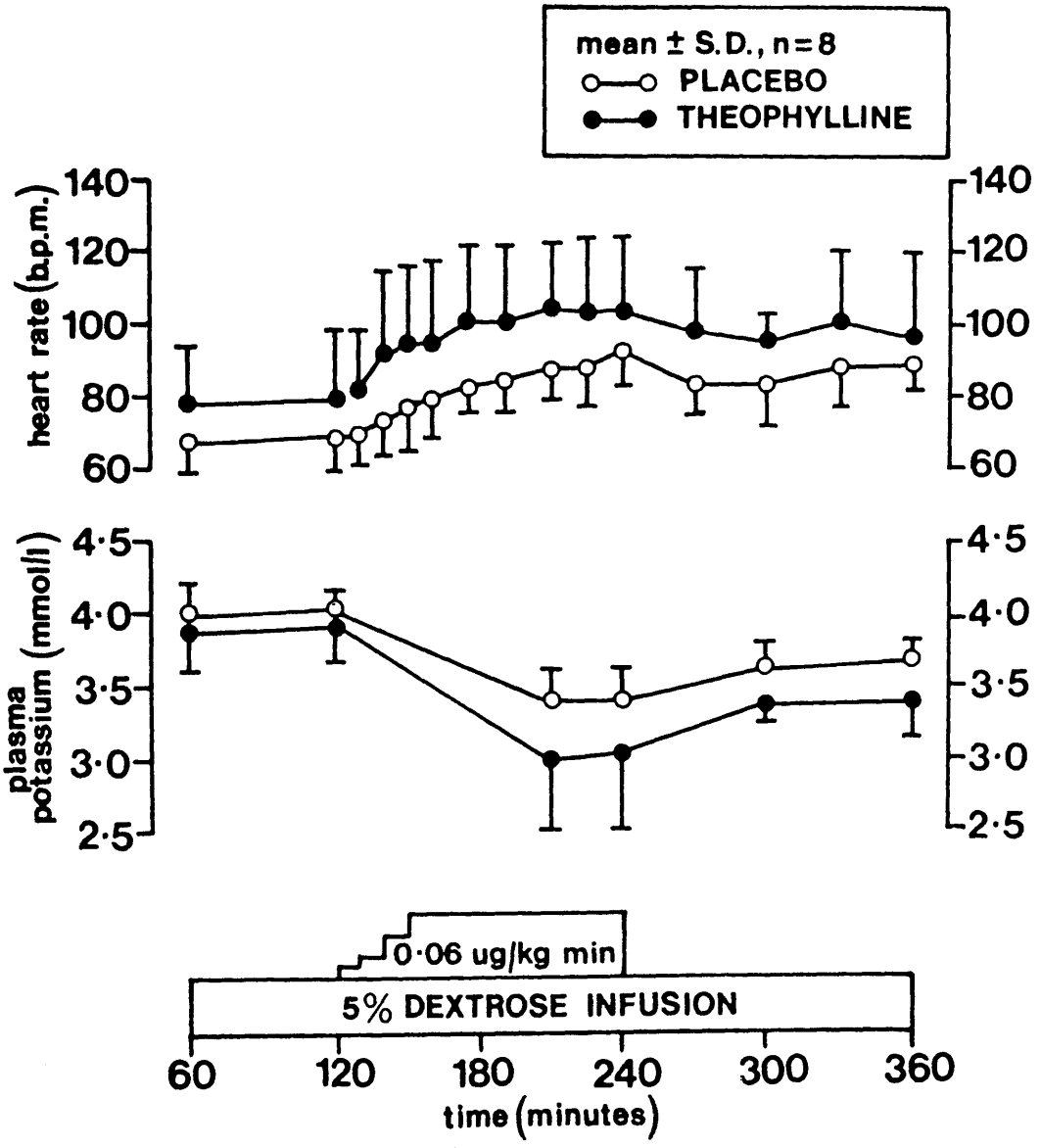


Fig. 6.4.1 : HEART RATE AND PLASMA POTASSIUM ON EACH STUDY DAY

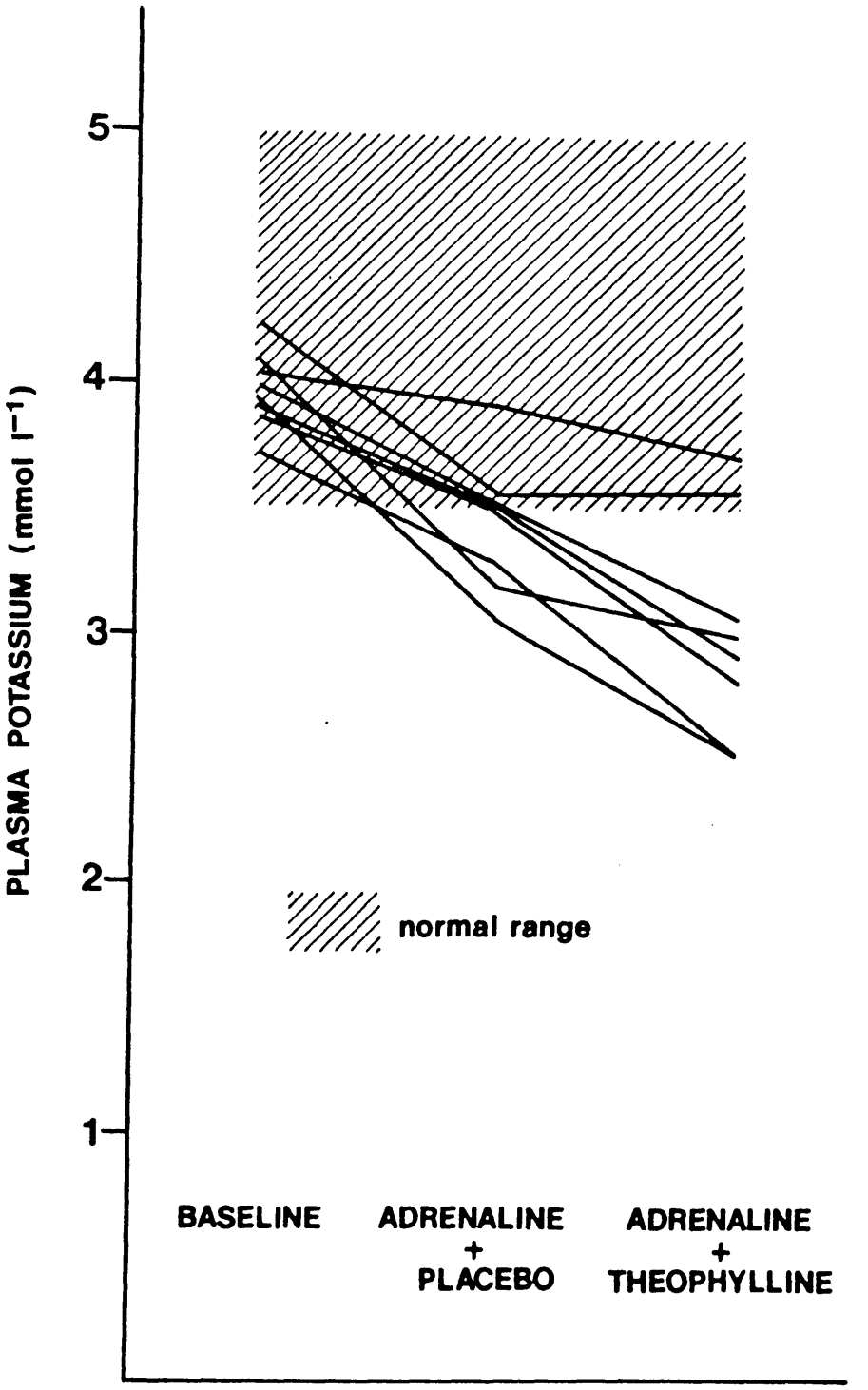


Fig. 6.4.2 : INDIVIDUAL SUBJECTS FALL IN PLASMA POTASSIUM FOLLOWING ADRENALINE INFUSION

during the adrenaline infusion.

Haemodynamic measurements.

Mean heart rates at each time are given in Table 6.3.2. Baseline heart rate, prior to the (-)-adrenaline infusion, on theophylline (78.5±14.9 beats min-1) was higher compared to placebo (68.9±9.0) but this difference was not statistically significant. Heart rate, throughout the study period, was significantly higher ($p<0.05$) on theophylline compared to placebo when corrected for baseline differences (Fig 6.4.3).

Mean systolic and diastolic blood pressure results are given in Table 6.4.3. Baseline systolic blood pressure was unchanged on theophylline (117±13 mmHg) compared to placebo (113±7) ($p>0.05$). Systolic blood pressure was, however, significantly higher during the whole study period on the theophylline compared to placebo ($p<0.05$). Diastolic blood pressure was not significantly different between study days.

All subjects remained in stable sinus rhythm throughout the infusions.

6.4.4 Discussion

Plasma concentrations of theophylline within the therapeutic range were achieved following 5 days of oral dosing. There was significant potentiation of the haemodynamic and hypokalaemic effects of adrenaline by theophylline. There has been one previous report of

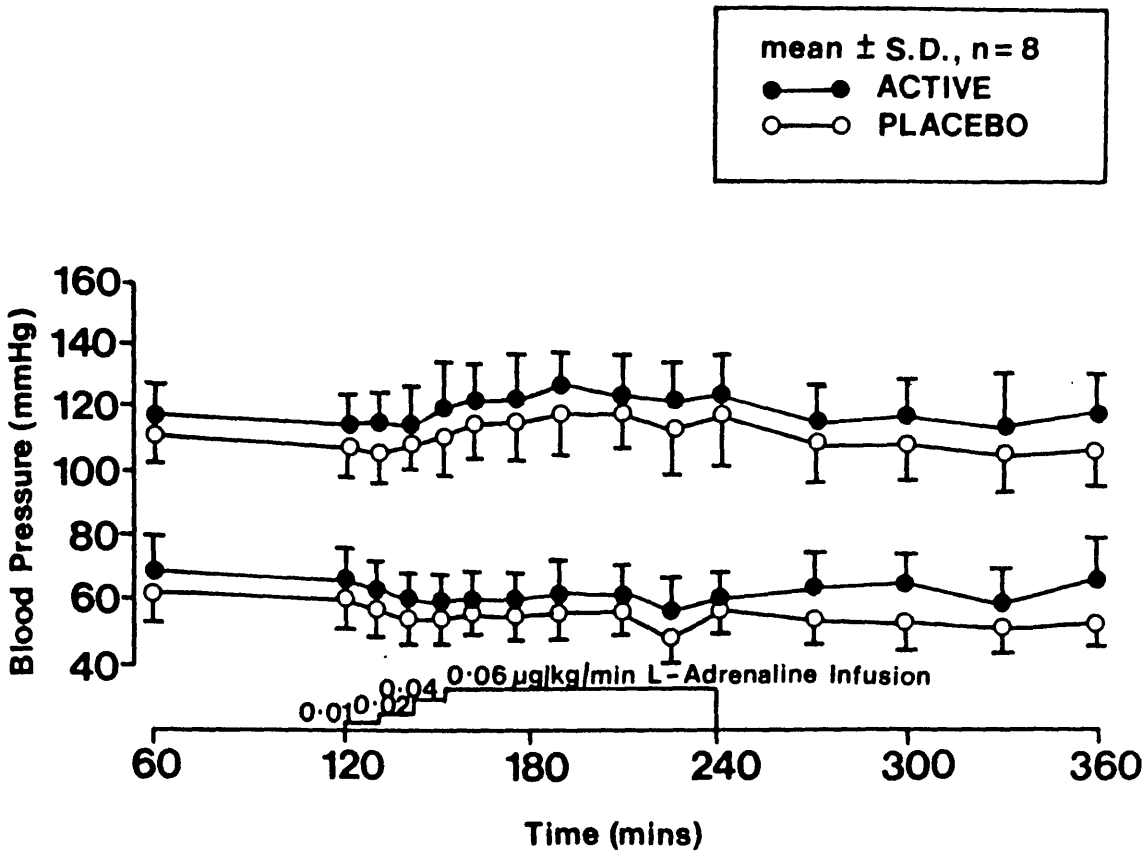


Fig. 6.4.3. : CHANGES IN SYSTOLIC AND DIASTOLIC BLOOD PRESSURE DURING EACH STUDY DAY

Table 6.4.1:- Mean plasma potassium concentrations at each time point on each study day.
(n=8; mean ± S.D.)

Time (mins)	Plasma Potassium (mmol l^{-1}) Placebo	Theophylline
60	4.08 ± 0.16	3.88 ± 0.32
120	4.06 ± 0.10	3.90 ± 0.28
210	3.42 ± 0.22	3.00 ± 0.50
240	3.41 ± 0.20	3.03 ± 0.48
300	3.62 ± 0.18	3.33 ± 0.32
360	3.78 ± 0.22	3.40 ± 0.38

$p<0.02$

Table 6.4.2:- Mean heart rate at each time point on each study day.

(n=8; mean±S.D.).

Time (mins)	Heart Rate (beats min^{-1}). Placebo Study Day	Theophylline Study Day
60	69 ± 9	79 ± 15
120	68 ± 9	79 ± 18
130	70 ± 9	81 ± 18
140	74 ± 11	93 ± 22
150	78 ± 13	95 ± 23
160	81 ± 12	96 ± 23
175	84 ± 9	102 ± 21
190	85 ± 9	101 ± 24
210	89 ± 9	105 ± 20
225	89 ± 10	104 ± 20
240	92 ± 9	104 ± 21
270	84 ± 10	98 ± 17
300	84 ± 12	95 ± 15
330	88 ± 11	106 ± 13
360	88 ± 7	96 ± 22

$p<0.05$

Table 6.4.3:- Mean systolic and diastolic blood pressure at each time point on each study day.
(n=8; mean ± S.D.)

	Systolic B.P. (mmHg)		Diastolic B.P. (mmHg)	
Time (mins)	Placebo	Theophylline	Placebo	Theophylline
60	113±13	116±13	70±10	64±8
120	112±10	116±12	69±9	65±12
130	111±13	118±10	66±10	61±11
140	114±11	116±16	61±11	60±11
150	115±14	123±16	62±11	61±11
160	121±14	125±14	63±12	61±9
175	121±15	127±16	62±11	60±10
190	122±13	131±12	64±11	60±10
210	124±13	128±12	62±12	61±9
225	119±15	129±12	61±12	53±9
240	122±18	128±13	61±10	61±11
270	114±14	120±10	67±11	58±9
300	114±13	123±12	68±10	58±10
330	112±13	120±19	65±10	58±10
360	114±11	125±12	71±15	57±8
	$p<0.05$		$p>0.05$	

theophylline potentiating the actions of another beta-adrenoceptor agonist, terbutaline, resulting in increased hypokalaemia (Smith & Kendall, 1986). It was well recognised when combinations of sympathomimetics, such as ephedrine and theophylline, were widely used that there was an increased incidence of side-effects with such combination therapy though there is a paucity of published data in vivo (Weinberger & Bronsky, 1975). This study shows that therapeutic doses of theophylline and pathophysiological levels of adrenaline interact resulting in increased tachycardia and hypokalaemia.

There are still a number of oral combination tablets containing theophylline and ephedrine available in the U.K. (British National Formulary, 1989) and some authorities, particularly in North America, still recommend the parenteral administration of adrenaline and aminophylline in the therapy of severe asthma (Zagelbaum & Pare, 1982).

The results of this study suggest that there is the potential for interaction between theophylline and either endogenous adrenaline or exogenously administered sympathomimetics resulting in increased hypokalaemia, tachycardia and possibly cardiac dysrhythmias in severe attacks of asthma.

6.5 The effect of theophylline and therapeutic doses of salbutamol on plasma potassium

6.5.1 Introduction

The previous study, 6.4, demonstrated that theophylline increases adrenaline induced hypokalaemia. A previous study (Chap 4.1) suggested that the mechanism of the hypokalaemic effect of the $beta_2$-agonist, salbutamol, was by stimulation of a membrane bound $beta_2$-adrenoceptor possibly linked to Na^+/K^+ATPase.

Combination therapy with salbutamol, or similar selective $beta_2$-agonists, and theophylline is common in acute attacks of bronchospasm. This study aimed to examine the changes in plasma potassium and haemodynamics when therapeutic doses of theophylline were added to conventional intravenous doses of salbutamol.

As previously discussed (Chap 2.3) circulating adrenaline concentrations may be raised in lifelong attacks of bronchospasm and a secondary aim was to study the effect on the hypokalaemia and haemodynamic changes induced by salbutamol in combination with theophylline of raising circulating adrenaline concentrations into the pathophysiological range.

6.5.2 Methods

Fourteen healthy volunteers (7 males, 7 females; age range 19-29 years) who had normal haematology, biochemistry and electro-cardiographs were studied in a single-blind, randomised, placebo controlled, balanced

Latin square crossover design arranged so that the active agents adrenaline, salbutamol and theophylline with appropriate placebos, could be compared in the combinations of interest in the course of three study days. The combinations studied were:-

(1) Intravenous salbutamol + placebo theophylline + vehicle control adrenaline;

(2) Intravenous salbutamol + theophylline + vehicle control adrenaline;

(3) Intravenous salbutamol + theophylline + (-)-adrenaline;

A diagram of the study design is given in Figure 6.5.1.

In the run-in phase each individual subject's theophylline dose requirement of a slow release theophylline preparation (Uniphyllin Tablets, Napp Laboratories) taken twice daily (5-7.5 mg kg^{-1} day^{-1} for 2 days then 10-15 mg kg^{-1} day^{-1} for 5 days) was determined. If tolerated then a trough theophylline level was measured by H.P.L.C. (Gere & Benje, 1977) and the required daily dose to achieve a therapeutic plasma level throughout was calculated using a microcomputer based optimisation programme (OPT, Nodecrest Ltd.). This dose was used for the remainder of the study.

Subjects attended the laboratory on the seventh and ninth day of active theophylline treatment period and on only the seventh day of their placebo phase, having fasted for 10 hours and abstained from caffeine, alcohol and smoking for 12 hours. By careful conduct of the

procedure it was possible to do this without breaking the blind. A light standard breakfast was given along with the morning dose of the drug. Intravenous cannulae were inserted into each forearm. Subjects were studied supine throughout the study period. The procedure on each separate study day was identical except for the infusions administered. Following a control 5% (+)-glucose infusion, an (-)-adrenaline infusion or an identical control adrenaline vehicle solution was commenced and after one hour salbutamol was added.

Salbutamol 4 μg kg^{-1} (16 nmol kg^{-1}) was administered as an intravenous loading dose over 5 minutes followed by a maintenance infusion 8μg kg^{-1} $hour^{-1}$ (32 nmol kg^{-1} $hour^{-1}$) continued for 55 minutes.

The (-)-adrenaline infusion was modified from previous studies and adrenaline was commenced at a rate of 0.015 μg kg^{-1} min^{-1} for 10 minutes and, if tolerated, the infusion rate was doubled at 10 minute intervals to 0.06 μg kg^{-1} min^{-1}, if there were no side effects, and this dose continued for a further 100 minutes. Both the (-)-adrenaline infusion and the control adrenaline vehicle infusion contained ascorbic acid (1 mg ml^{-1}) to prevent oxidation of the adrenaline.

At the end of the second hour of active infusions the salbutamol and (-)-adrenaline infusions were both stopped and a 5% (+)-glucose infusion was continued for a further two hours.

Throughout the study period heart rate and blood pressure were measured frequently and blood taken for plasma potassium and catecholamine assay (Chap 3.3 & 3.4; Fig 6.5.1). Catecholamine concentrations were measured by high performance liquid chromatography (Chapter 3.2).

Before the start of the study and at 180 minutes trough and peak theophylline concentrations were measured by a competitive binding assay based on fluorescence polarization (T.D.X. system, Abbot Laboratories Ltd.). Blood was taken at 150 and 180 minutes to measure plasma salbutamol concentrations by high performance liquid chromatography in the Clinical Research Unit of Glaxo Laboratories.

Results are presented as mean $\pm$ standard deviation. Repeated measures analysis of variance was used to compare individual variables on separate study days.

6.5.3 Results.

Tolerance

One volunteer withdrew during the initial treatment period because of nausea, her plasma theophyline level was 14.6 $\mu g\ ml^{-1}$. Two volunteers withdrew during the main study. One subject was unable to tolerate the salbutamol infusions on two occasions due to salbutamol induced vomiting. One subject was withdrawn as a result of developing premature supraventricular beats at a rate of 6-8 beats min-1 during the combination of (-)-adrenaline and salbutamol infusions during active theophylline therapy. These extrasystoles persisted for 4 hours after

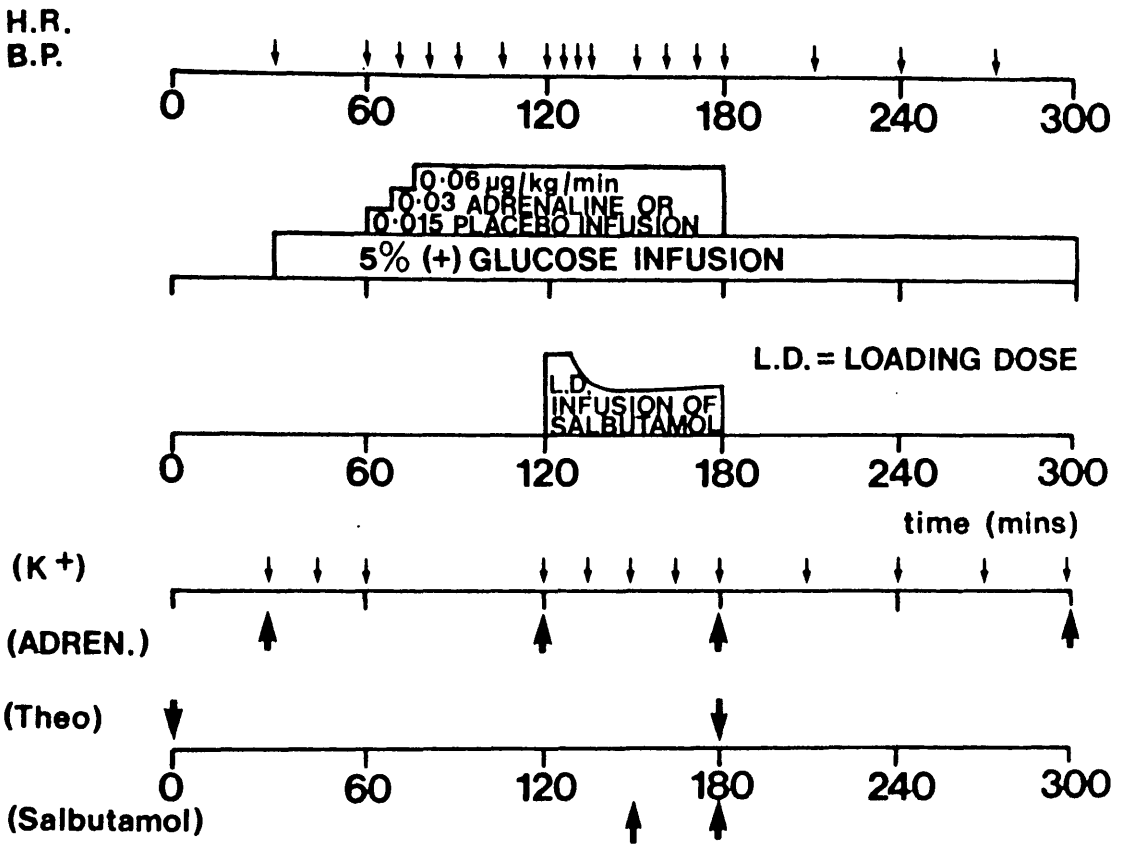


Fig. 6.5.1.: OUTLINE OF INFUSION REGIMEN AND TIMES OF OBSERVATIONS DURING STUDY DAY

stopping the infusions. Data from the remaining 11 subjects is presented.

All subjects noted palpitations during the salbutamol loading dose. No dysrhythmias were seen but heart rate increased. The salbutamol infusion caused tremor of the hands in two subjects. No other adverse effects occurred.

Serum Theophylline Levels

Theophylline levels are shown in Table 6.5.1 The mean theophylline concentration on active therapy at time 0' (trough) was 11.9µg ml^{-1} (range- 6.3-17.9µg ml^{-1}) and 13.5µg ml^{-1} (range- 8.3-23.5 µg ml^{-1}) at time 180' (peak). On placebo therapy theophylline was not detected.

Plasma Salbutamol Concentrations

Plasma salbutamol levels were between 5.0-12.0ng ml^{-1} (Table 6.5.1). With this infusion regimen some subjects demonstrated a small fall in salbutamol concentrations between the first and second measurement and others modest rises. Neither theophylline, nor the adrenaline infusion significantly altered salbutamol levels (Table 6.5.1).

Plasma Adrenaline Concentrations

Baseline plasma adrenaline levels were similar on all study days (Table 6.5.1) and did not alter with the control infusions of the adrenaline vehicle. On the active adrenaline infusion plasma adrenaline concentration increased from 1.55±3.34 to 4.28±4.23 nmol

Table 6.5.1: Plasma catecholamine and salbutamol concentrations.

(n=11; mean ± S.D.; T = theophylline; A = Adrenaline infusion period)

[catecholamine levels before (30') and during the adrenaline infusion period (180') and salbutamol concentrations during (150')and at the end of the salbutamol infusion (180') on each study day.]

	Adrenaline Conc. ($nmol\ l^{-1}$)		**Noradrenaline Conc.** ($nmol\ l^{-1}$)		**Salbutamol Conc.** ($ng\ ml^{-1}$)	
Time	30'	180'	30'	180'	150'	180'
Plac. T + Plac. A	1.44±2.96	1.48±3.37	1.97±0.91	3.15±0.97	6.74±0.90	7.74±1.14
Active T + Plac. A	0.77±1.05	0.78±1.28	3.75±1.97	4.70±2.25	7.34±0.64	7.71±1.70
Active T + Active A	1.55±3.34	4.28±4.23	3.47± 1.84	4.45±1.85	7.12±1.01	7.58±1.10

l^{-1} at the end of the (-)-adrenaline infusion, falling to 0.88±1.01 nmol l^{-1} by the end of the study period.

Plasma Potassium Concentrations

Baseline plasma potassium concentrations were similar on each study day. Salbutamol infusion (8 μg kg^{-1} hr^{-1}), without the active (-)-adrenaline infusion or theophylline, caused a fall in plasma potassium from 4.0± 0.3mmol l^{-1} to 3.0±0.3 mmol l^{-1} (Fig 6.5.2, Table 6.5.2). Salbutamol induced hypokalaemia was significantly greater following chronic pretreatment with theophylline, plasma potassium falling from 3.9±0.3 mmol l^{-1} to 2.6±0.3 mmol l^{-1} ($p<0.05$). The combination of (-)-adrenaline and theophylline, in addition to salbutamol, did not cause any further fall of potassium, the nadir being similar, 2.6±0.3 mmol l^{-1}. By the end of the study plasma potassium levels were similar on each of the study days. Plasma potassium concentrations had still not, however, returned to baseline levels two hours after the active infusions (Fig 6.5.2, Table 6.5.2).

Haemodynamic Measurements

There was no difference in baseline heart rate at 60' on any study day (Table 6.5.3). Heart rate increased during the salbutamol infusion on all three study days and this rise was significantly greater on the two study days when active theophylline was given compared to the placebo theophylline study day ($p<0.05$). Adding the (-)-

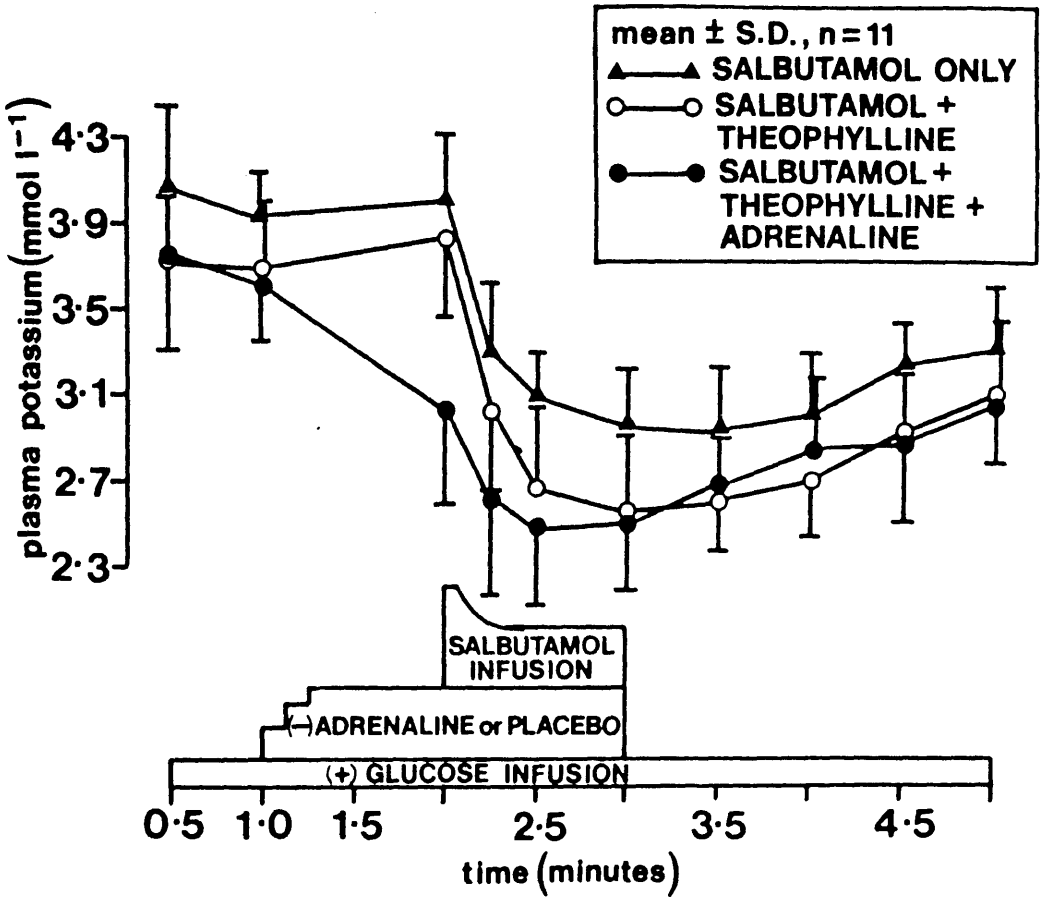


Fig. 6.5.2 : CHANGES IN PLASMA POTASSIUM CONCENTRATIONS ON EACH STUDY DAY

Table 6.5.2: Mean plasma potassium concentrations at each time point on each study day.

(n=11; mean ± S.D.).

Time (mins)	PLASMA POTASSIUM (mmol l^{-1})		
	Placebo + Placebo	Theophylline + Placebo	Theophylline + Adrenaline
30	4.1 ± 0.4	3.7 ± 0.3	3.8 ± 0.4
60	3.9 ± 0.2	3.7 ± 0.3	3.6 ± 0.3
120	4.0 ± 0.3	3.9 ± 0.3	3.1 ± 0.4
135	3.3 ± 0.3	3.1 ± 0.4	2.7 ± 0.4
150	3.1 ± 0.2	2.7 ± 0.4	2.6 ± 0.3
180	3.0 ± 0.3	2.6 ± 0.3	2.6 ± 0.3
210	3.0 ± 0.3	2.7 ± 0.3	2.7 ± 0.3
240	3.1 ± 0.3	2.8 ± 0.3	2.9 ± 0.3
270	3.3 ± 0.2	3.0 ± 0.3	3.0 ± 0.4
300	3.4 ± 0.3	3.2 ± 0.3	3.1 ± 0.3
		$p<0.05$	$p<0.05$

adrenaline infusion to the combination of theophylline and salbutamol caused only a further small rise in heart rate (Fig 6.5.3). Heart rate fell after the end of the active infusions but remained elevated at the end of the study period on all treatments, compared to baseline values (Fig 6.5.3).

Salbutamol consistently lowered diastolic blood pressure and increased systolic blood pressure on all three study days but there was no significant difference between the three study days (Table 6.5.4, 6.5.5 & Fig 6.5.3).

6.5.4 Discussion

The results demonstrate that theophylline increases the potassium lowering effect of salbutamol but (-)-adrenaline makes no further difference. The theophylline concentrations were well within the established therapeutic range and achieved by an oral slow release formulation employed in conventional doses. The dose of salbutamol used was in the lower half of the recommended intravenous dosage schedule for the treatment of asthma (A.B.P.I., 1988).

The addition of the adrenaline infusion, leading to high concentrations of adrenaline, did not alter the absolute extent of hypokalaemia seen with the salbutamol and theophylline combination, suggesting the $beta_2$-receptor linked Na^+/K^+ ATPase pump was already maximally stimulated. Alternatively, rapid changes in $beta_2$-receptor affinity or number may have occurred, even in

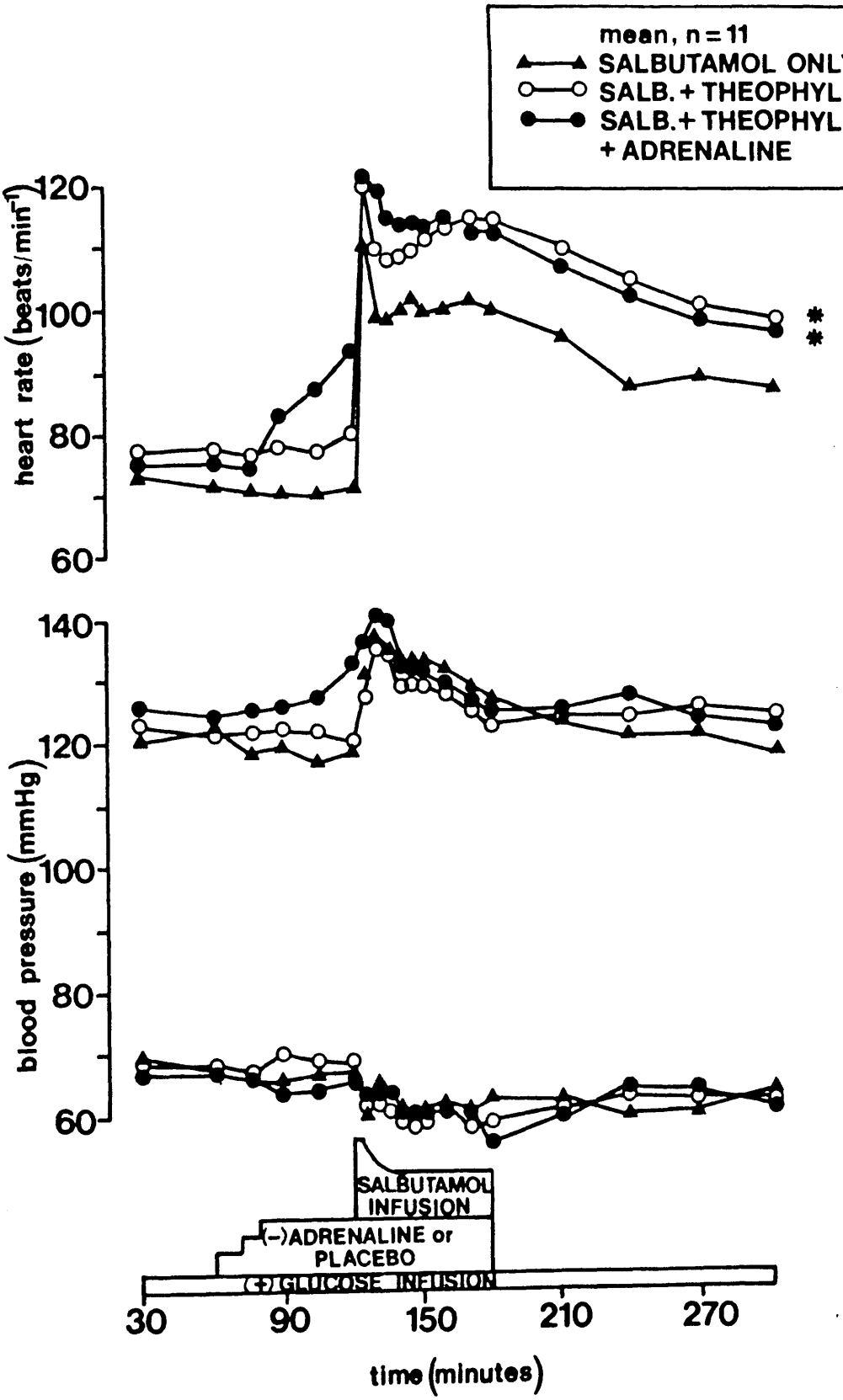


Fig. 6.5.3 : CHANGES IN HEART RATE AND BLOOD PRESSURE ON EACH STUDY DAY
(* - $p < 0.05$)

Table 6.5.3: Mean heart rate at each time point on each study day.

(n=11; mean ± S.D.).

MEAN HEART RATE (beats min^{-1}).

Time (mins)	**Placebo + Placebo**	**Theophylline + Placebo**	**Theophylline + Adrenaline**
30	74 ± 8	77 ± 14	75 ± 5
60	72 ± 7	78 ± 12	76 ± 6
75	70 ± 7	77 ± 13	75 ± 8
90	70 ± 8	79 ± 13	83 ± 10
105	71 ± 9	78 ± 11	86 ± 11
120	72 ± 8	80 ± 14	94 ± 13
125	111 ± 18	121 ± 22	122 ± 19
130	99 ± 15	110 ± 19	119 ± 20
135	99 ± 15	108 ± 19	114 ± 14
140	101 ± 13	109 ± 19	113 ± 12
145	103 ± 14	110 ± 20	113 ± 11
150	101 ± 15	112 ± 17	113 ± 13
160	101 ± 12	115 ± 17	114 ± 12
170	103 ± 15	115 ± 18	113 ± 13
180	101 ± 15	115 ± 16	113 ± 13
210	97 ± 14	110 ± 17	106 ± 12
240	88 ± 13	104 ± 16	102 ± 10
270	90 ± 8	101 ± 15	99 ± 11
300	87 ± 9	99 ± 15	97 ± 8
		$p<0.05$	$p<0.05$

Table 6.5.4: Mean systolic blood pressure at each time point on each study day.

(mean±S.D., n=11).

	MEAN SYSTOLIC BLOOD PRESSURE (mmHg)		
Time (mins)	**Placebo + Placebo**	**Theophylline + Placebo**	**Theophylline + Adrenaline**
30	120 ± 11	123 ± 8	126 ± 8
60	122 ± 13	121 ± 8	125 ± 7
75	119 ± 10	121 ± 6	125 ± 9
90	120 ± 11	122 ± 9	125 ± 6
105	118 ± 10	122 ± 7	127 ± 8
120	119 ± 11	120 ± 7	132 ± 13
125	132 ± 14	128 ± 18	136 ± 15
130	138 ± 13	136 ± 8	141 ± 13
135	135 ± 11	135 ± 13	141 ± 11
140	134 ± 12	130 ± 12	133 ± 10
145	134 ± 9	130 ± 13	132 ± 12
150	134 ± 8	130 ± 10	133 ± 9
160	133 ± 10	129 ± 10	130 ± 11
170	130 ± 7	127 ± 11	127 ± 8
180	128 ± 7	123 ± 9	126 ± 7
210	125 ± 10	126 ± 12	126 ± 8
240	121 ± 8	125 ± 11	128 ± 6
270	121 ± 8	126 ± 9	125 ± 8
300	119 ± 9	125 ± 6	124 ± 7
		p>0.05	p>0.05

Table 6.5.5: Diastolic blood pressure at each time point on each study day.

(mean±S.D., n=11).

	DIASTOLIC BLOOD PRESSURE (mm Hg)		
Time (mins)	**Placebo + Placebo**	**Theophylline + Placebo**	**Theophylline + Adrenaline**
30	69 ± 7	69 ± 4	67 ± 5
60	68 ± 5	68 ± 5	67 ± 5
75	65 ± 5	66 ± 6	66 ± 5
90	65 ± 6	70 ± 7	63 ± 5
105	67 ± 5	69 ± 6	64 ± 5
120	67 ± 6	68 ± 9	66 ± 6
125	60 ± 8	61 ± 13	63 ± 8
130	66 ± 7	62 ± 7	64 ± 9
135	61 ± 5	61 ± 8	64 ± 10
140	61 ± 6	59 ± 7	61 ± 10
145	61 ± 6	58 ± 9	61 ± 11
150	60 ± 5	59 ± 8	60 ± 9
160	61 ± 9	61 ± 9	60 ± 7
170	60 ± 8	58 ± 6	60 ± 10
180	62 ± 9	59 ± 6	55 ± 9
210	62 ± 6	61 ± 11	60 ± 9
240	60 ± 5	63 ± 9	63 ± 8
270	61 ± 4	62 ± 5	63 ± 6
300	64 ± 4	62 ± 8	61 ± 5
		p>0.05	p>0.05

the short time of these experiments, with agonist induced receptor down regulation limiting the extent of the response. These explanations are clearly not mutually exclusive. Recent studies with radioligand binding to guinea pig skeletal muscle membranes suggest that agonists do indeed cause down regulation of beta-receptors (Elfellah & Reid, 1987) and that such changes may occur relatively rapidly (Snaveley, Zeiler & Insel, 1985).

Theophylline potentiated adrenaline-induced hypokalaemia by an unknown mechanism but failed to increase adrenaline induced vasodilation in a previous study (Chap 6.4). Similarly, in this study, during the salbutamol infusion, no enhanced vasodilator effect, the result of stimulation of $beta_2$-adrenoceptors on peripheral vascular smooth muscle, of either theophylline or adrenaline was seen. The fall in diastolic blood pressure during the salbutamol infusion was similar on the placebo and active theophylline study days. Other cardiovascular homeostatic responses may have been recruited to maintain blood pressure.

A previous unconfirmed report has suggested theophylline has a beta-agonist action (Mackay et al, 1983). The enhancement of the chronotropic effects of salbutamol, a $beta_1$-adrenoceptor action, by theophylline seen in this study suggests that the action of theophylline is not specific to $beta_2$-adrenoceptors. As theophylline and not

adrenaline increased salbutamol effects it appears that the former is either exerting its action independently of the beta-adrenoceptors or by activating second or subsequent messengers in the cell membrane or intracellularly.

Acknowledgement I would like to acknowledge the able assistance of Dr. C. Reid in the supervision of some of these studies in my absence.

6.6 Summary

In summary, the results of the studies presented in this chapter demonstrate a metabolic and haemodynamic interaction between theophylline and adrenaline, by an unknown mechanism which does not involve increased adrenomedullary release of catecholamines.

Steady state therapeutic theophylline levels increase adrenaline induced hypokalaemia and tachycardia, at pathophysiological concentrations of adrenaline. The clinical importance of this finding requires further study, especially as most asthmatics probably do not have high circulating levels of adrenaline during attacks. However, in very severe attacks of asthma adrenaline levels may be raised and such patients could be at increased risk of cardiac dysrhythmias.

Of greater clinical importance is the demonstration that theophylline and salbutamol, a widely used combination, can interact both metabolically and haemodynamically.

However, it is important to emphasise that these interactions were seen with intravenous infusions of salbutamol which achieve much higher plasma and tissue levels than are seen after inhalation therapy, the commonest route of administration. Oral preparations of salbutamol are available which achieve plasma levels intermediate between inhaled and intravenous. New slow release preparations are now available which are already resulting in a rapid increase in the use of oral salbutamol therapy and thus higher plasma salbutamol levels.

The clinical importance of this interaction is likely to be limited with inhaled therapy but may be more relevant if oral β_2-agonist therapy is used. It is in life-threatening attacks of bronchospasm, accompanied by hypoxia and acidosis, which lead to an increased risk of cardiac dysrhythmias, when large doses of theophyllines and beta$_2$-agonists would be given parenterally then the interaction demonstrated in these studies could precipitate dysrhythmias.

It is noteworthy that theophylline and salbutamol maximally stimulated the Na^+/K^+ ATPase, or led to down regulation, such that further stimulation by pathophysiological levels of adrenaline had no effect. If the mechanism was down regulation in such a short period, one hour, then it suggests powerful homeostatic mechanisms exist to attenuate acute adrenergic induced

falls in plasma potassium. Such tachyphylaxis may further limit the risks that the interactions demonstrated in these studies pose to patients.

CHAPTER 7

CATECHOLAMINES AND MAGNESIUM.

7: CATECHOLAMINES AND MAGNESIUM.

7.1 Background

Magnesium, the second most abundant intracellular cation, is present in the plasma, but in relatively small amounts. As discussed in Chapter 1 the control of magnesium ion movements across cell membranes is poorly understood.

Reviewing the literature, it is clear that hypomagnesaemia has been reported to occur in a number of situations in which circulating adrenaline is known to be raised. These situations include myocardial infarction (Dyckner, 1980), cardiac surgery (Holden et al, 1972; Schiemann et al, 1969) and insulin induced hypoglycaemia (Lindsay, 1976). Hypomagnesaemia has also been reported following the selective $beta_2$-agonist, salbutamol (Philips et al, 1980). Hypomagnesaemia increases the risks of cardiac dysrhythmias, both in isolation and when combined with hypokalaemia (Chapter 2).

The studies described in this chapter had the aim of examining the following questions:

1) Does increasing circulating adrenaline cause hypomagnesaemia?

2) Is a $beta_2$-adrenoceptor involved in adrenaline induced hypomagnesaemia?

3) Finally, as diuretics may causehypomagnesaemia,

is there any interaction between diuretic induced hypomagnesaemia and adrenaline induced hypomagnesaemia?

7.2 Adrenergic control of plasma magnesium.

7.2.1 Introduction

As discussed above, the published literature suggests that catecholamines could have a role in the movement of magnesium across cell membranes. In this study the effects of increased adrenaline levels on plasma magnesium was examined in volunteer subjects.

It has been suggested that insulin may be involved in the internal regulation of magnesium (Lindsay, 1976) and, as adrenaline alters insulin release, changes in plasma insulin and glucose levels were also studied.

7.2.2 Methods

Eight healthy subjects, four males and four females, receiving no drugs, aged 22-37 years participated.

The protocol followed was identical to that described in Chapter 4.2, except that subjects only received three treatments and were studied on three separate occasions one week apart. In a single-blind study subjects received each of three treatments, placebo salbutamol and placebo adrenaline; placebo salbutamol and active adrenaline; or active salbutamol and placebo adrenaline, given in a random order by a balanced Latin square design. Subjects fasted overnight then received a light standard breakfast

30 minutes before commencing the study period and, thereafter, fasted throughout the study period. After 30 minutes supine rest an infusion of 5% (+)-glucose (12mls h^{-1}) was administered for one hour. Subjects then received either an active solution of (-)-adrenaline or an identical vehicle control solution. If after 10 minutes of infusion of (-) adrenaline at a rate of 0.03µg kg^{-1} min^{-1} there were no symptoms or adverse effects then the rate was increased to 0.06µg kg^{-1} min^{-1} and this rate was continued for 110 minutes, a total of 120 minutes of (-)-adrenaline.

During the last 60 minutes of the (-)-adrenaline infusion period either an active salbutamol infusion or an identical vehicle control infusion was commenced at a dose of 40ng kg^{-1} min^{-1} and increased by increments at 15 minute intervals to a dose of 120ng kg^{-1} min^{-1} which was then continued for 30 minutes, a total of 60 minutes of salbutamol infusion. Thereafter a 5% (+)-glucose control solution was continued for a further 1 hour (Figure 7.2.1).

Plasma potassium was measured by standard flame photometric methods. Magnesium concentrations were measured by atomic absorption spectro-photometry using a Perkin Elmer AA Spectrophotometer. Glucose levels were measured using Trinders Glucose oxidase method on a Technicon RA 1000 analyser. Insulin samples were measured by an identical method to that described in section 4.2.

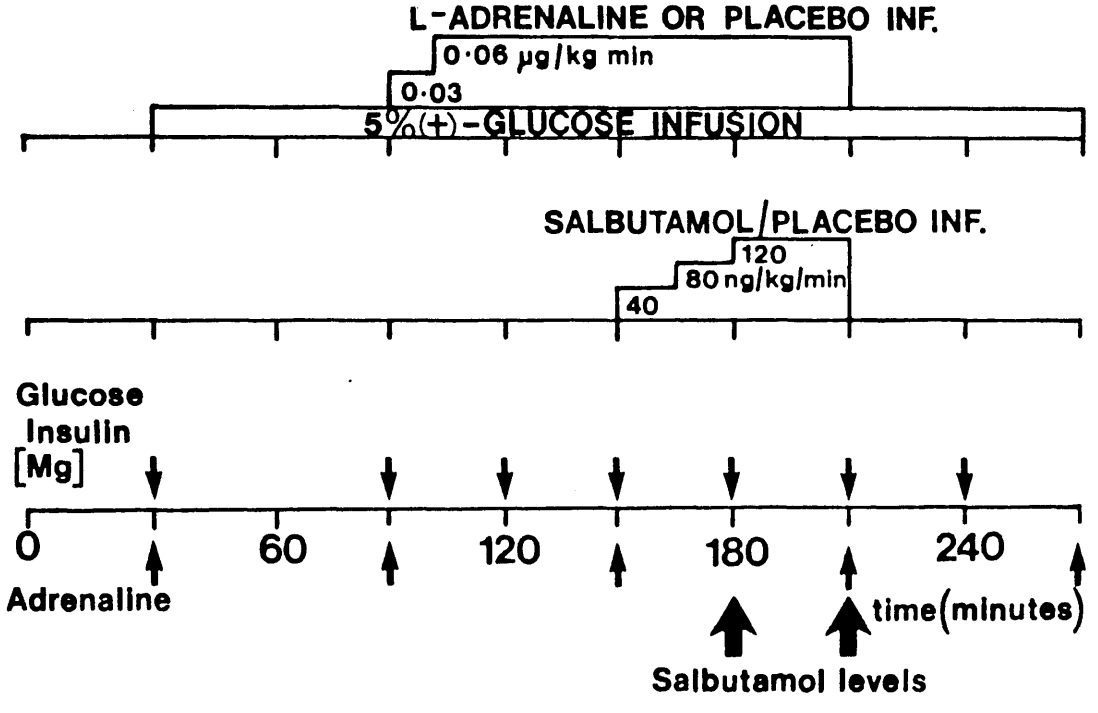


Fig. 7.2.1. OUTLINE OF STUDY PERIOD

Plasma salbutamol levels were measured by high performance liquid chromatography system with electrochemical detection, courtesy of Glaxo Laboratories.

Samples for plasma adrenaline levels were measured by radio-enzymatic assay (Chap 3.2). Results are expressed as mean and standard deviation. Placebo treatment is compared to active treatments, salbutamol and adrenaline, by analysis of variance, as applied to repeated measures, and corrected for variations in baseline measurements prior to analysis.

7.2.3 Results

Plasma adrenaline concentrations were <0.1 nmol l^{-1} prior to and at the end of the placebo infusions on the placebo adrenaline + placebo salbutamol study day and 0.12 and <0.1 nmol l^{-1} before and at the end of the infusion period on the placebo adrenaline + salbutamol study day. On the active adrenaline + placebo salbutamol study day adrenaline concentrations rose from <0.1 to 2.33 nmol l^{-1} by the end of the adrenaline infusion (Table 7.2.1).

Baseline magnesium levels were not significantly different on the three study days (Table 7.2.2). There was a significant fall in magnesium at the end of the active adrenaline + placebo salbutamol infusion (0.67±0.07 mmol l^{-1}; $p<0.02$) compared to the placebo salbutamol + placebo adrenaline study day. The fall in

Table 7.2.1: Mean plasma adrenaline and salbutamol concentrations on each study day.

(n=8; mean ± S.D.)

[A=adrenaline; S=salbutamol; U.D.=undetectable]

	Salbutamol Concentration ($ng\ ml^{-1}$)		**Adrenaline Concentration** ($nmol\ l^{-1}$)	
Time	180'	210'	30'	210'
Treatment				
Vehicle A + **Vehicle S**	U.D.	U.D.	<0.1	<0.1
Adrenaline + **Vehicle S**	U.D.	U.D.	0.16±0.16	2.33±1.62
Vehicle A + **Salbutamol**	2.6±0.8	4.6±0.8	0.12±0.03	<0.1

Table 7.2.2: Mean plasma magnesium at each time point on each study day.

(mmol l^{-1}; n=8; mean ± S.D.).

Time (mins)	Placebo	Adrenaline	Salbutamol
30	0.73 ± 0.04	0.69 ± 0.06	0.74 ± 0.05
90	0.74 ± 0.04	0.70 ± 0.06	0.76 ± 0.05
120	0.75 ± 0.04	0.71 ± 0.06	0.76 ± 0.04
150	0.75 ± 0.04	0.71 ± 0.04	0.75 ± 0.04
165	0.75 ± 0.04	0.68 ± 0.05	0.73 ± 0.05
180	0.75 ± 0.04	0.67 ± 0.04	0.73 ± 0.04
195	0.75 ± 0.04	0.67 ± 0.06	0.72 ± 0.05
210	0.75 ± 0.03	0.67 ± 0.07	0.70 ± 0.06
240	0.75 ± 0.03	0.66 ± 0.06	0.69 ± 0.07
270	0.74 ± 0.04	0.66 ± 0.07	0.68 ± 0.06
		$p<0.02$	$p<0.05$

magnesium during the active salbutamol + placebo adrenaline study day was slower and continued after the end of the infusion to 0.68±0.06 mmol l^{-1} ($p<0.05$, Figure 7.2.2).

Plasma potassium concentrations fell on the active adrenaline + placebo salbutamol ($p<0.005$) and on the active salbutamol + placebo adrenaline ($p<0.01$). On the placebo adrenaline + placebo salbutamol study day potassium levels rose (Figure 7.2.2, Table 7.2.3).

Insulin concentrations fell during the run-in period on all study days. During the placebo salbutamol + placebo adrenaline infusion and during the active adrenaline + placebo salbutamol infusion insulin levels fell but they then rose sharply after the adrenaline infusion. During the period of the active salbutamol + placebo adrenaline infusion insulin levels did not alter. There were no significant differences between the three study days (Figure 7.2.3, Table 7.2.4). Glucose levels were unchanged during the placebo salbutamol + placebo adrenaline infusion and the active salbutamol + placebo adrenaline infusion but rose significantly during the active adrenaline + placebo salbutamol infusion ($p<0.005$, Figure 7.2.3, Table 7.2.5).

7.2.4 Discussion

As discussed previously (Chapter 3.2) the infusion of adrenaline at this dose (0.06µg kg^{-1} min^{-1}) leads to

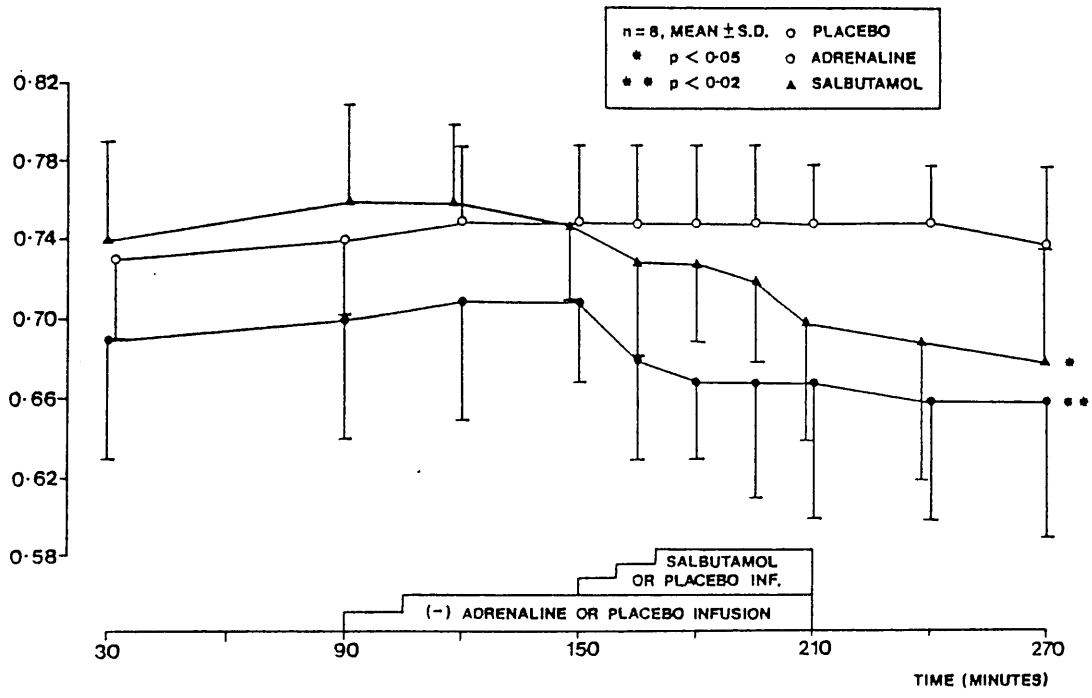


Fig. 7.2.2.: CHANGES IN PLASMA MAGNESIUM ON EACH STUDY DAY

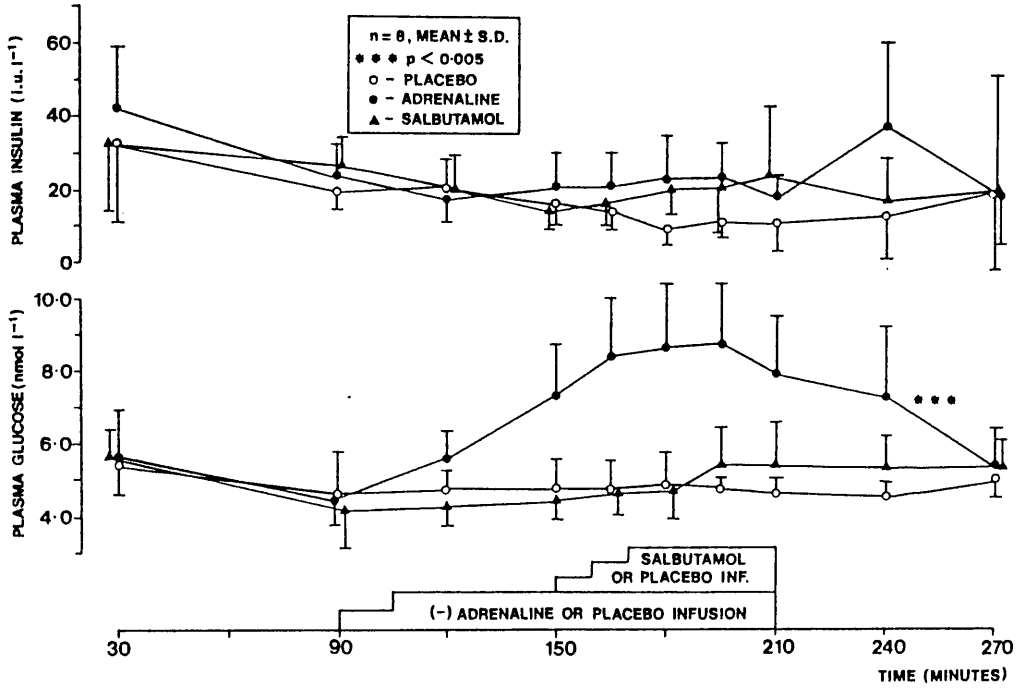


Fig. 7.2.3: CHANGES IN PLASMA INSULIN AND GLUCOSE ON EACH STUDY DAY

Table 7.2.3 Mean plasma potassium at each time point on each study day.

(mmol l^{-1}; n=8; mean ± S.D.).

Time (mins)	Placebo	Adrenaline	Salbutamol
30	3.8 ± 0.3	3.8 ± 0.5	3.9 ± 0.3
90	3.9 ± 0.2	4.0 ± 0.3	3.8 ± 0.2
120	4.1 ± 0.3	3.6 ± 0.6	4.0 ± 0.2
150	4.1 ± 0.2	3.5 ± 0.6	4.0 ± 0.2
165	4.1 ± 0.2	3.4 ± 0.5	4.0 ± 0.2
180	4.1 ± 0.2	3.4 ± 0.4	3.9 ± 0.4
195	4.1 ± 0.2	3.4 ± 0.4	3.6 ± 0.3
210	4.0 ± 0.3	3.4 ± 0.5	3.4 ± 0.2
240	4.0 ± 0.2	3.5 ± 0.4	3.5 ± 0.3
270	4.0 ± 0.3	4.0 ± 0.4	3.7 ± 0.4
		$p<0.005$	$p<0.01$

Table 7.2.4: Mean plasma insulin at each time point on each study day.

(i.u. l^{-1}; n=8; mean ± S.D.)

Time (mins)	Placebo	Adrenaline	Salbutamol
30	33 ± 22	42 ± 27	33 ± 19
90	20 ± 5	24 ± 9	27 ± 8
120	21 ± 8	18 ± 6	20 ± 10
150	17 ± 6	22 ± 9	16 ± 6
165	15 ± 5	23 ± 8	17 ± 6
180	10 ± 4	24 ± 12	21 ± 7
195	13 ± 5	24 ± 10	22 ± 13
210	12 ± 8	19 ± 6	26 ± 18
240	14 ± 12	39 ± 23	18 ± 12
270	20 ± 21	19 ± 13	21 ± 32
		p>0.05	p>0.05

Table 7.2.5: Mean plasma glucose at each time point on each study day.

(mmol l^{-1}; n=8; mean ± S.D.).

Time (mins)	Placebo	Adrenaline	Salbutamol
30	5.4 ± 0.8	5.6 ± 1.3	5.6 ± 0.8
90	4.7 ± 1.1	4.6 ± 0.8	4.2 ± 1.0
120	4.8 ± 0.5	5.6 ± 0.8	4.3 ± 0.5
150	4.8 ± 0.8	7.4 ± 1.4	4.5 ± 0.5
165	4.8 ± 0.8	8.5 ± 1.6	4.7 ± 0.6
180	4.9 ± 0.9	8.7 ± 1.9	4.9 ± 0.9
195	4.8 ± 0.3	8.8 ± 1.7	5.5 ± 1.0
210	4.7 ± 0.4	8.0 ± 1.6	5.5 ± 1.2
240	4.6 ± 0.4	7.4 ± 1.9	5.4 ± 0.9
270	5.1 ± 0.5	5.5 ± 1.0	5.5 ± 0.7
		$p<0.005$	$p>0.05$

levels similar to those observed in acute myocardial infarction. This study shows that such adrenaline levels result in a small, though significant, fall in plasma magnesium levels. Falls in magnesium did not parallel the falls in potassium; they were slower in onset and did not recover during the hour long observation period following adrenaline. Magnesium ions are small, highly polarised, have a large hydrated size in solution and will therefore pass with difficulty through water filled channels. These physical characteristics will make a highly specific, high affinity magnesium binding site unlikely. However there is evidence that there are moderate affinity binding sites which do select magnesium ions (Flatman, 1984). This difference in the likely affinity of binding sites for magnesium compared to the highly specific binding sites for potassium may explain the different time courses in the change in each ion's concentration in response to the infusions.

Plasma potassium levels rose after the end of the active infusions and magnesium levels did not rise. Adrenaline inhibits insulin release and glucose levels rose during the adrenaline infusion resulting in rebound hyperinsulinaemia after the adrenaline infusion (Figure 7.2.3). Lindsay has reported that insulin-induced hypoglycaemia is associated with falls in magnesium levels and postulated an insulin stimulated magnesium transport system (Lindsay, 1976). As insulin also

stimulates potassium transport into cells and potassium levels rose during this period of relative hyperinsulinaemia, but when insulin levels still did not exceed baseline levels, it is unlikely that this mechanism would explain these findings. An alternative explanation for these findings would be increased renal excretion, and this cannot be excluded as urinary magnesium concentrations were not examined. In support of this hypothesis is a recent report demonstrating that urinary magnesium levels increase during terbutaline infusion, a $beta_2$-agonist (Bos, Postma & Doormaal, 1988).

A therapeutic dose of salbutamol caused a significant fall in magnesium levels, though the time course of this fall was slower and more prolonged than with adrenaline. The salbutamol infusion regimen used in this study would have failed to achieve steady-state salbutamol levels. Salbutamol clearance is low and this may explain the continued fall in plasma magnesium levels following the salbutamol infusion.

These results suggest that magnesium may flux between extracellular and intracellular compartments under the influence of beta-adrenergic receptors, possibly of the $beta_2$ subtype. Two studies in animals indirectly support this hypothesis. Devane and his colleagues demonstrated that major surgery in dogs resulted in hypokalaemia and hypomagnesaemia with an increase in erythrocyte and

lymphocyte concentrations of both cations during the period of adrenomedullary stress (Devane, Donnelly & Ryan, 1980). Rayssiguer reported adrenaline-induced hypomagnesaemia in sheep which was abolished by beta-adrenoreceptor blockade with propranolol (Rayssiguer,1979). This worker also suggested another possible mechanism by which adrenaline could lower plasma magnesium levels. Adrenaline stimulates lipolysis and, hence, increases free fatty acids which, in turn, bind magnesium thus lowering plasma magnesium. One group of workers have shown in acute alcohol withdrawal, another situation with increased circulating adrenaline levels, that plasma magnesium falls as free fatty acid levels rise. (Flink et al., 1979; Flink, Brick & Shane, 1981).

Hypomagnesaemia has commanded less attention than hypokalaemia, possibly because deficiency of both cations often co-exists and hypokalaemia is a much more common cause of cardiac dysrhythmias. However, hypomagnesaemia is associated with an increased incidence of cardiac dysrhythmias even in the absence of a low potassium (Chadda et al, 1973; Flink, 1980). The clinical significance of such a small fall in plasma magnesium, as seen in this study, is uncertain and further studies in man are required to determine the mechanism of adrenaline-induced hypomagnesaemia and its clinical relevance.

7.3 The effect of diuretic pre-treatment on adrenaline induced hypomagnesaemia.

7.3.1 Introduction

As previously discussed diuretic therapy is widely used in patients with ischaemic heart disease. Therapy with loop diuretics and thiazides may result in hypomagnesaemia and, potentially, cause cardiac dysrhythmias.

Diuretic associated hypomagnesaemia

Renal handling of magnesium is very different from potassium (Fig 1.4.1). As some plasma magnesium is protein bound only approximately 80% is filtered at the glomerulus and only 25% reabsorbed in the proximal tubule. The principal site of reabsorption is the loop of Henle (50-60%), probably controlled by two factors. A sodium chloride/potassium co-transporting system and, secondly, backflux of sodium and potassium creating a trans-epithelial gradient favouring magnesium reabsorption. At the distal tubule a small amount (5%) of further reabsorption occurs. Thus, urinary magnesium is between 3-5% of the filtered load. Controversy exists as to the possible existence of mechanisms for active secretion of magnesium. Such a system is almost certainly not operative under normal circumstances, but may operate in certain pathologies (Sachtjan, Meyer & Massry 1979; Brunette, Vigneault & Carriere, 1974).

The effect of diuretics on magnesium differ from there

effects on potassium (Fig 5.1.1). Diuretics, such as carbonic anhydrase inhibitors, which act on the proximal tubule, may cause significant potassium losses but do not affect magnesium excretion. Presumably any blockade of magnesium reabsorption in the proximal tubule is compensated for by increased reabsorption in the loop of Henle. Diuretics which act on the loop of Henle, e.g. frusemide, have profound effects on magnesium reabsorption and result in major increases in urinary magnesium. Thiazides, which act in the early distal tubule, a site of only minor magnesium reabsorption, would not be expected to have significant effects on magnesium excretion and, indeed, short term animal studies have shown this to be the case (Ryan, 1986). However, a number of clinical studies of chronic thiazide therapy have demonstrated significant magnesium losses and either hypomagnesaemia (Dyckner & Wester, 1979) or muscle magnesium deficiency (Wester & Dyckner 1985). Further studies found that hypomagnesaemia following thiazides was dependent on dose (Hollifield & Slaton, 1981, Hollifield 1986) and duration (Dyckner & Wester 1979). In contrast, studies over 3 to 10 years in hypertensives have failed to demonstrate significant intracellular magnesium depletion (Ryan 1986). It has been suggested that the effects of thiazides are indirect, secondary to delayed thiazide induced alterations in the renin-angiotensin-aldosterone system

and/or due to alterations in calcium metabolism which may interfere with magnesium reabsorption (Ryan 1986).

The two groups of agents which act in the late distal tubule and collecting ducts act differently. Current evidence suggests that triamterene and amiloride conserve magnesium. The situation is less clear with spironolactone, acute studies in animals show little effect (Devane & Ryan, 1983) whilst long term studies in man have demonstrated increased skeletal muscle magnesium when spironolactone is added to chronic conventional diuretic therapy (Dyckner & Wester, 1986).

The effect of pretreatment with each of these diuretics, frusemide, bendrofluazide and spironolactone on plasma potassium and magnesium changes during an adrenaline infusion was studied in healthy subjects.

7.3.2 Methods

Subjects

Eight healthy normotensive volunteers (4 males, 4 females; age range 21-37 years) were studied. All subjects had normal routine haematology, biochemistry and electrocardiograms and were on no regular drug therapy.

Treatment Protocol

This was a placebo controlled, double blind study with supplies being dispensed by the hospital pharmacy. Subjects received four separate treatments in a randomised order using a Latin square design with at

least two weeks between treatments to ensure adequate washout. All drugs were matched using a disguised gelatine capsule. The four treatments were:

i) bendrofluazide 5 mgs daily;

ii) placebo capsule one daily;

iii) frusemide 40 mg daily;

iv) spironolactone 100mgs daily;

and each therapy was administered for two weeks.

On the fourteenth day of each treatment limb subjects attended the laboratory having abstained from all food, caffeine, alcohol and tobacco for 10 hours. The drug and a standard light breakfast were given and cannulae inserted into each forearm. Subjects were studied supine throughout and, after one hour of supine rest a 5% (+)-glucose solution, containing ascorbic acid (1 mg ml^{-1}) to prevent oxidisation of the adrenaline, was commenced, via a Braun VI perfusor pump, at a rate of 12 ml hr^{-1}. Baseline measurements were made during the one hour control (+)-glucose infusion. (-)-adrenaline infusion (Antigen Ltd.) was commenced at a rate of 0.01µg kg^{-1} min^{-1} and, if after ten minutes there were no adverse effects, then the infusion rate was increased stepwise at 10 minute intervals to 0.06µg kg^{-1} min^{-1} in the standard protocol, as described in Chapter 3. This dose was continued for 90 minutes. Following the adrenaline infusion the control (+)-glucose infusion was continued for a further two hours (Fig 7.3.1).

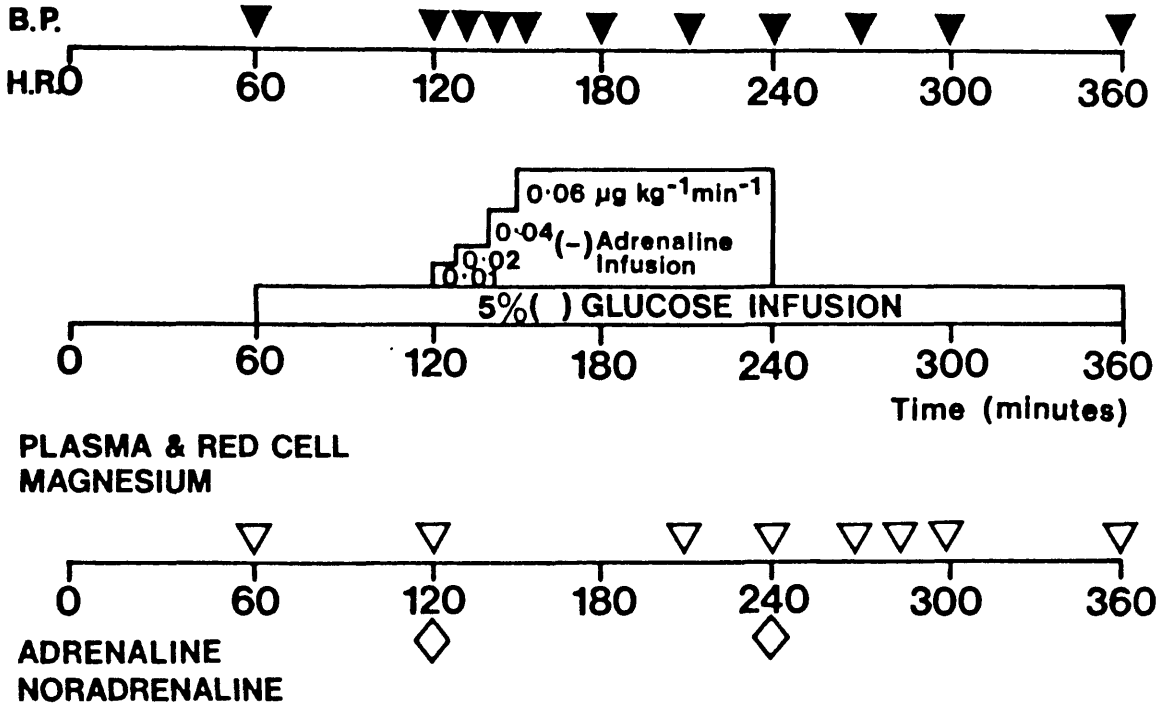


Fig. 7.3.1. OUTLINE OF STUDY PERIOD

Plasma magnesium was measured by spectrophotometry using a Perkins-Elmer atomic absorption spectrophotometer (Perkins-Elmer Ltd.).

Red cell magnesium was measured by dilution of the red cells into lanthanum nitrate and then measuring the magnesium level by spectrophotometry.

Samples for plasma catecholamines were collected in the standard fashion and analysed by high performance liquid chromatography (Chap 3.3).

Results are expressed as mean and standard deviation. Repeated measures analysis of variance was used for comparisions between treatments. Measurements before and after a manoeuvre (e.g. adrenaline infusion) were compared using a paired Students t test.

7.3.3 Results

All subjects completed the study and none reported any adverse effects.

Plasma catecholamines.

Baseline adrenaline, steady-state adrenaline during the infusion periods and noradrenaline were not significantly different on the four treatments. Adrenaline levels at the end of the adrenaline infusion showed considerable variation between study days in the same subject, and between subjects, but the levels were increased at least 20 fold by the infusion (Table 7.3.1).

Table 7.3.1: Mean plasma adrenaline and noradrenaline at each time on each study day.

(n=8; mean S.D.; ADR=adrenaline; NADR=noradrenaline).

Adrenaline Concentration (nmol l^{-1})

Time (mins)	PLACEBO	SPIRONOLACTONE	FRUSEMIDE	BENDROFLUAZ.
60	0.1±0.05	0.1±0.05	0.1±0.05	0.1±0.4
120	0.1±0.05	0.1±0.1	0.1±0.05	0.1±0.05
240	2.2±1.7	4.1±5.8	4.0±5.0	2.3±1.2
360	0.1±0.1	0.4±0.5	2.4±6.0	0.3±0.7
		p>0.05	p>0.05	p>0.05

Noradrenaline Concentration (nmol l^{-1}).

Time (mins)	PLACEBO	SPIRONOLACTONE	FRUSEMIDE	BENDROFLUAZ.
60	2.0±0.9	1.9±1.0	2.1±1.0	2.2±1.1
120	2.0±0.7	2.4±1.3	2.2±1.2	2.0±0.6
240	2.1±0.4	2.0±1.2	3.6±4.0	2.1±0.9
360	2.0±1.0	1.2±0.5	2.1±1.5	2.0±0.7
		p>0.05	p>0.05	p>0.05

Plasma and red cell magnesium

Baseline plasma magnesium levels were not altered by any of the three active therapies compared to the placebo study day (placebo - 0.76 mmol l^{-1}; spironolactone - 0.75 mmol l^{-1}; frusemide - 0.78 mmol l^{-1} and bendrofulazide - 0.79 mmol l^{-1}). During the adrenaline infusion plasma magnesium fell by 0.3-0.6 mmol l^{-1} (Figure 7.3.2, Table 7.3.2). The effect of adrenaline on plasma magnesium was prolonged beyond the period of the infusion and the lowest magnesium values were seen between 30 and 60 minutes after the adrenaline infusion. The plasma magnesium changes induced by the adrenaline were identical with the placebo study day on all the active treatments (Fig 7.3.2).

Baseline red cell magnesium levels were higher on spironolactone and frusemide (2.1 mmol l^{-1}) compared to placebo and bendrofluazide (1.9 and 2.0 mmol l^{-1} respectively). Red cell magnesium did not alter during the adrenaline infusion on any study day (Fig 7.3.3, Table 7.3.3).

7.3.4 Discussion.

Pathophysiological adrenaline levels again caused plasma magnesium to fall as in the previous study (7.2).

In this study no pretreatment, with any of the diuretics, altered baseline plasma magnesium. This contrasts with previous published work demonstrating

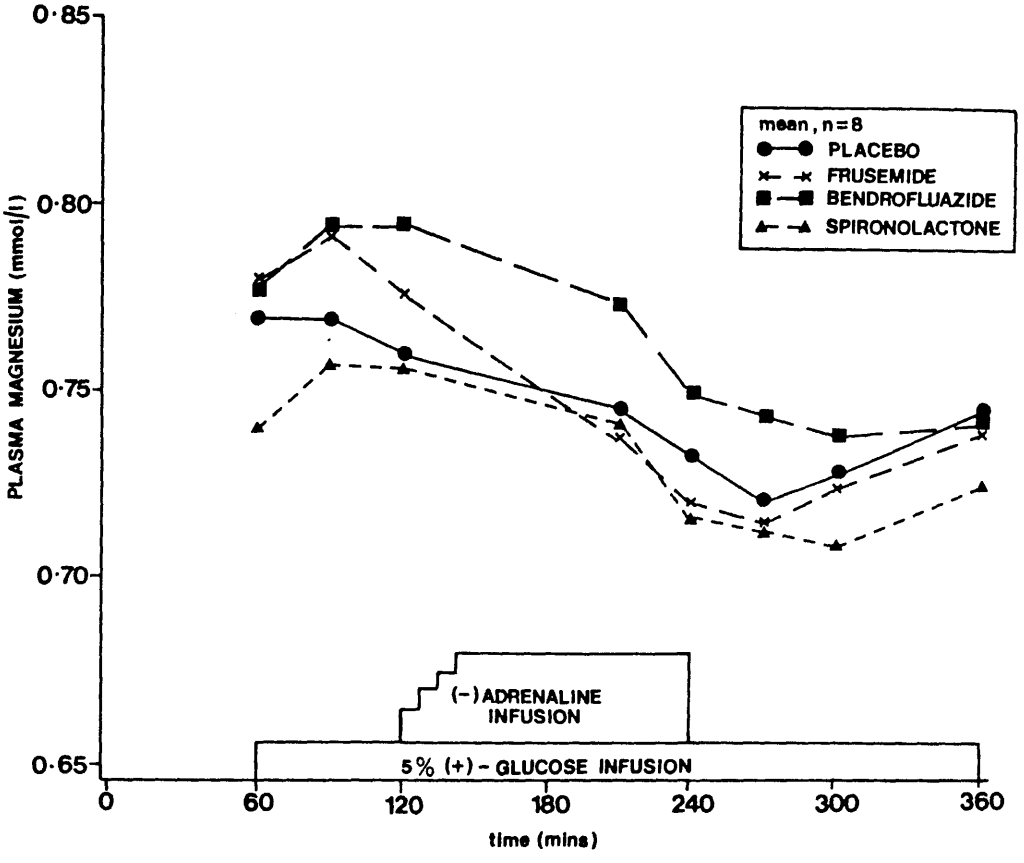


Fig. 7.3.2: CHANGES IN PLASMA MAGNESIUM ON EACH STUDY DAY

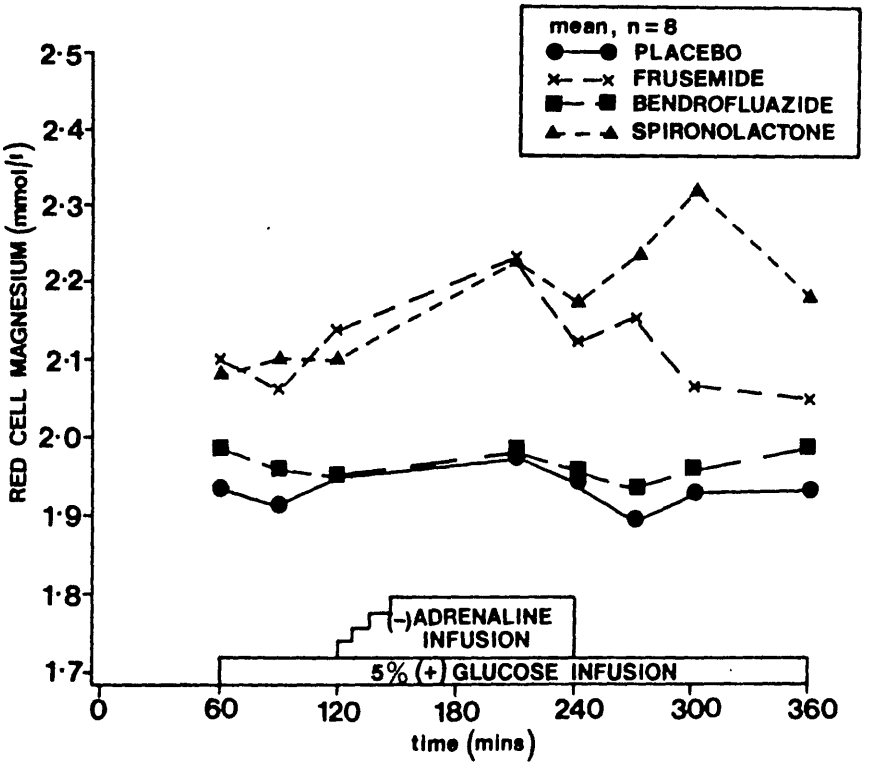


Fig. 7.3.3.: CHANGES IN RED CELL MAGNESIUM ON EACH STUDY DAY

Table 7.3.2: Mean plasma magnesium at each time point on each study day.

(n=8; mean ± S.D.; mmol l^{-1})

Time (mins)	Placebo	Spironolactone	Frusemide	Bendrofluaz.
60	0.77±0.03	0.72±0.05	0.78±0.04	0.78±0.05
90	0.77±0.03	0.74±0.05	0.79±0.05	0.80±0.05
120	0.76±0.03	0.75±0.04	0.78±0.04	0.79±0.06
210	0.74±0.02	0.73±0.03	0.74±0.03	0.77±0.05
240	0.73±0.02	0.71±0.04	0.72±0.03	0.75±0.05
270	0.72±0.03	0.70±0.05	0.71±0.02	0.74±0.05
300	0.73±0.01	0.70±0.04	0.72±0.03	0.74±0.05
360	0.74±0.01	0.72±0.05	0.74±0.02	0.74±0.05
		p>0.05	p>0.05	p>0.05

Table 7.3.3: Mean red cell magnesium concentrations at each time point on each study day.

(n=8; mean ± S.D.; mmol l^{-1})

Time (mins)	Placebo	Spironolactone	Frusemide	Bendrofluaz.
60	1.9±0.3	2.1±0.2	2.1±0.2	2.0±0.3
90	1.9±0.3	2.1±0.2	2.1±0.3	2.0±0.2
120	1.9±0.2	2.1±0.2	2.1±0.3	2.0±0.3
210	2.0±0.2	2.2±0.4	2.2±0.3	2.0±0.2
240	1.9±0.2	2.2±0.3	2.1±0.3	2.0±0.2
270	1.9±0.2	2.2±0.5	2.2±0.3	1.9±0.3
300	1.9±0.3	2.3±0.6	2.1±0.4	2.0±0.2
360	1.9±0.2	2.2±0.2	2.1±0.4	2.0±0.2
		p>0.05	p>0.05	p>0.05

that both chronic thiazide and frusemide therapy may cause hypomagnesaemia (Dyckner & Wester, 1981). Possible explanations for this finding include the use of an inadequate dose, though this would seem unlikely from the published evidence (Wester & Dyckner, 1981), or too short a period of therapy, two weeks, to significantly alter plasma magnesium concentrations. Finally, not everyone develops diuretic associated hypomagnesaemia and, possibly, too few subjects were studied to detect a significant magnesium losing effect.

Adrenaline induced hypomagnesaemia did not increase following pretreatment with any of the diuretics suggesting that these diuretics would not increase the risk of severe hypomagnesaemia during periods of adrenomedullary stress, such as myocardial infarction. However, in view of the failure of this study to demonstrate diuretic induced hypomagnesaemia, a commonly reported phenomenon, such an interpretation cannot be regarded as conclusive.

In an attempt to confirm that adrenaline induced hypomagnesaemia was the result of intracellular shift of magnesium, rather than increased renal excretion, red cell magnesium was measured in this study. However no significant change in red cell magnesium concentrations were observed. This could be because a much larger proportion of magnesium in humans is intracellular and only a small proportion is extracellular. Only a small

intracellular shift of magnesium would be necessary to cause significant, and detectable, falls in extracellular concentrations. In contrast, this intracellular shift is such a small proportion of the intracellular magnesium concentration that it will be difficult to detect. It cannot, therefore, be concluded that the failure to detect any change in intracellular cation concentrations excludes intracellular shift as the mechanism of adrenaline induced hypomagesaemia.

This study suggests that patients receiving chronic thiazide or frusemide therapy would not be at increased risk of severe hypomagnesaemia and, thus, cardiac dysrhythmias during acute stress, such as myocardial infarction.

CHAPTER 8: GENERAL DISCUSSION AND CONCLUSIONS

8: GENERAL DISCUSSION AND CONCLUSIONS.

8.1 The adrenegic system and potassium homeostasis.

As demonstrated in Chapter 3, it is possible to design an effective infusion regimen to achieve circulating pathophysiological concentrations of adrenaline which can be safely administered to volunteer subjects.

Using this infusion regimen the studies presented in this thesis show that circulating adrenaline concentrations, 10-25 times basal level, similar to those seen in the stress of acute severe illness, such as myocardial infarction, have both metabolic and cardiovascular effects.

On average heart rate increased by 10-15 beats per minute and the changes in blood pressure were modest, a fall of approximately 5 mm Hg in diastolic and a rise of less than 10 mmHg in systolic blood pressure. This contrasts with the cardiovascular changes reported in situations of acute psychological stress (Anfilogoff et al, 1987) where, despite more modest rises in adrenaline, 4-5 times basal level, there are dramatic increases in heart rate (60-80 beats per minute). In situations of psychological stress noradrenaline increases 4 fold suggesting large increases in adrenergic tone from the central nervous system and, in addition, vagal tone will also alter. These factors may explain the disrepancy between the findings in these two situations. During

severe physical exercise the increases in circulating adrenaline are even more modest (approximately 2-3 fold, Struthers et al, 1988).

In all studies presented in this thesis the rise in in circulating adrenaline results in a rapid and significant fall in plasma potassium. No such fall was seen during the placebo infusions.

8.2 Mechanism of adrenaline induced hypokalaemia.

In Chapter 4 adrenaline induced hypokalaemia was shown to be independent of changes in plasma insulin. The mechanism of adrenaline induced hypokalaemia is likely to be a direct effect of adrenaline on potassium transport mechanisms across cell membranes, principally active transport of potassium by the Na^+/K^+ pump linked to its enzyme, Na^+/K^+ ATPase.

However, there is some conflicting evidence which has led to alternate hypotheses. Activity of Na^+/K^+ ATPase can be measured directly and such studies have suggested that the enzyme, as opposed to the pump, only responds to high concentrations of β-agonist (Coffey, Hadden & Hadden, 1975; Wu & Phillis, 1980). Also adrenaline stimulates Na^+/K^+ pump activity in intact leucocytes but Na^+/K^+ ATPase activity does not increase (Baron, Green & Kahn, 1985). This study may be at fault as pump activity is measured on intact cells, whilst Na^+/K^+ ATPase activity requires homogenization of the cell which may alter the link between $beta_2$-receptors and Na^+/K^+

ATPase. Similarly, another group has also reported that stimulation of Na^+/K^+ ATPase isolated from sacrolemmal membranes was not linked to receptors (Cheng, Rogus & Zierler, 1977). Yet, paradoxically, the same group demonstrated that in intact skeletal muscle the stimulation of the pump by catecholamines is specific and mediated by beta-adrenoceptors (Rogus, Cheng & Zierler, 1977). Pump activity is not stimulated by beta-agonists in all tissues, rat salivary gland (Batri et al, 1973) fails to respond. It is possible that in other tissues the Na^+/K^+ ATPase is not linked to β-receptors.

An alternative hypothesis is that the catechol moiety, rather than the intact hormone, increases Na^+/K^+ ATPase activity, possibly by chelating an inhibitory metal ion (Lee & Phillis, 1977; Schaefer, Komlos & Serge, 1979; Krogt & Belfroid 1980). However, these studies were all carried out on cortical tissue and Na^+/K^+ ATPase is known to differ in different tissues. Clearly for this theory to be valid α-agonists would also have to stimulate the Na^+/K^+ pump. This has been studied, but the evidence is conflicting, with some groups showing stimulation of the pump (Brown & Caulfield 1979; Smith 1984; Coffey et al, 1975) and others inhibition (Martinez, Quissell & Giles, 1976; Smith & Jones, 1985; Riozzi et al, 1984). Further evidence against this theory is the absence of any fall in plasma potassium during infusion of α-adrenergic agonists in intact

animals (D'Silva 1934) or in humans.

The studies in Chapter 4 suggest that adrenaline induced hypokalaemia is mediated through the sympathetic nervous system, most likely via a $beta_2$-adrenoceptor. The highly selective $beta_2$-agonist, salbutamol, results in a similar fall in plasma potassium, both in degree and in time course, as adrenaline. Adrenaline induced hypokalaemia is blocked by beta-adrenoceptor antagonists. This blockade of adrenaline induced hypokalaemia is progressively less as increasingly selective $beta_1$-antagonists are used. The effect of ICI118551, a highly selective $beta_2$-adrenoceptor antagonist, has also been studied, though not in the studies described in this thesis, and shown to completely abolish adrenaline induced hypokalaemia (Brown et al, 1983).

The mechanism by which stimulation of the $beta_2$-adrenoceptor increases Na^+/K^+ ATPase activity is uncertain. The most likely route is via the 'second messenger', cAMP, phosphorylating protein kinase which increases Na^+/K^+ ATPase activity. The combination of dibutyrl cyclic AMP plus theophylline mimics all the effects of adrenaline on ion transport (Clausen & Flatman, 1977, Rogus et al, 1977).

Do all tissues respond similarly to adrenaline? As such a large percentage of the body's potassium pool is in the skeletal muscle cells (Ginsberg & Wilde, 1954), this is clearly the predominant tissue and its response will

determine the change in plasma potassium. Other tissues which have been reported to respond similary include frog stomach smooth muscle cells (Scheid et al, 1979) and human leucocytes (Baron et al, 1985). As stated previously, rat salivary gland cells fail to respond (Batri et al, 1973). Possibly of crucial importance, in determining the risks of dysrhythmias during adrenaline induced hypokalaemia, is a report that adrenaline causes an efflux of potassium from cardiac muscle cells (Struthers et al, 1987), in contrast to its action on skeletal muscle. Rapid potassium intracellular shifts result in hyperpolarization of the cell membrane in skeletal muscle but adrenaline could, in cardiac muscle, result in hypopolarization or maintenance of a stable membrane potential, if the fall in intracellular and extracellular potassium concentrations are in a balanced ratio.

8.3 Is there an adrenergic potassium sensor?

Thus, there is considerable evidence that the adrenergic system has a role in the control of potassium homeostasis. What is less clear is, under which circumstances adrenergic control of potassium is physiologically important? From the data presented in these studies, and other studies referred to in the course of discussion, the adrenergic system will influence plasma potassium during the stress of heavy

muscular exercise and during severe adrenomedullary stress, such as myocardial infarction. In these situations the primary stimulus to catecholamine release is, clearly, not plasma potassium levels but is from the central nervous system.

Does the adrenergic system have any role in control of plasma potassium levels in healthy non-exercising humans? The rise in plasma potassium after chronic beta-blockade with propranolol (Pedersen, Pedersen & Pedersen, 1979) suggests that physiological levels of adrenaline and noradrenaline do have a role in potassium homeostasis. In support of this hypothesis, the disposal of exogenous potassium loads is impaired following beta-blockade and this impairment is unrelated to changes in circulating aldosterone or insulin levels (DeFronzo, 1987). Further the hyperkalaemia of chronically adrenalectomized animals is reversed by the physiological replacement of fasting plasma adrenaline levels (Bia et al, 1982). Finally, insulinopenia superimposed on adrenalectomy causes a marked deterioration in potassium tolerance (DeFronzo et al, 1980), suggesting that in circumstances, such as diabetes, adrenergic control plays a more central role due to the absence of another potassium controlling hormone.

If an adrenergic control system does exist for potassium homeostasis, then what is the sensor by which adrenal release of catecholamines is influenced by

plasma potassium to form a feedback loop? It has been reported that potassium can stimulate tyrosine hydroxylase, the first enzyme in catecholamine synthesis, in adrenal culture cells (Silberstein et al, 1972). However, when KCl was infused in humans to raise circulating plasma potassium levels by 0.5-1.0 mmol l^{-1} no rise in circulating catecholamine levels could be demonstrated (DeFronzo, Bia & Birkhead, 1981). Further studies of this interesting question are required.

8.4 Implications for beta-adrenoceptor blocker therapy.

Beta-adrenoceptor antagonists are widely used in the treatment of hypertension and angina pectoris. The great majority of patients are taking beta-adrenoceptor antagonists which are either unselective or, at best, only partially selective for the $beta_1$-adrenoceptor. These drugs will, therefore, interfere with adrenaline induced hypokalaemia. Is this likely to have any potential benefits or risks for these patients?

Possible benefits of beta-adrenoceptor antagonists would include protection of the patient from hypokalaemia during the acute stress of myocardial infarction, when their threshold for dysrhythmias is low. In one placebo controlled study all patients admitted with an acute myocardial infarction were given either intravenous timolol or placebo and their plasma potassium monitored over the subsequent 24 hours (Nordrehaug et al, 1985). The patients who received the beta-adrenoceptor

antagonist did have significantly higher plasma potassium levels. There have been numerous studies of the use of chronic beta-adrenoceptor blockade after myocardial infarction which have demonstrated improved survival (Practolol Multicenter International Study, 1975; Timolol Norwegian Multicenter Study group, 1981). The protective mechanism of beta-blockade remains unknown. It may be hypothesised that, by protecting patients from hypokalaemia, induced by rises in circulating adrenaline during further ischaemic events these patients are at less risk of cardiac dysrhythmias.

Postulated risks of beta-adrenoceptor blockade include the risk of hyperkalaemia during exercise. Small, but significant, rises in plasma potassium occur during chronic beta-adrenoceptor blockade (Pedersen et al, 1979) and are due to an extracellular shift, and not net retention of potassium (Pedersen et al, 1979). One probable homeostatic advantage of adrenaline induced intracellular flux of potassium would be to restore plasma potassium towards normal during strenous muscular exercise, as potassium leakage increases from exercising muscle, thus replenishing muscle potassium stores. During constant muscular exercise, such as cycling, plasma potassium levels increase by approximately 0.5mmol l^{-1} (DeFronzo, 1987). However, during beta-adrenoceptor blockade much greater rises (2-2.5 fold) in

plasma potassium occur (Carlsson et al, 1978; Castellino, Simonson & DeFronzo, 1986). There is, however, no proof that this increase in plasma potassium associated with exercise, when taking beta-adrenoceptor antagonists, is harmful. A recent report (Struthers et al, 1988) suggests that potassium fluxes during exercise are also under other influences. During the intense short bursts of muscular activity of a game of squash these authors showed very rapid transient falls in plasma potassium, unrelated to changes in circulating adrenaline and unaffected by $beta_2$-adrenoceptor blockade by ICI118551. Insulin levels fall during such exercise and, thus, the mechanism of the hypokalaemia is unclear. Possibly, vasodilatation and increased muscle blood flow, whether directly or indirectly, stimulate uptake of potassium by exercising muscle.

Currently, there is overwhelming clinical evidence that beta-adrenoceptor blockade offers patients with ischaemic heart disease significant benefits, whilst the risks remain speculative and unproven. The theoretical advantage of selective $beta_1$-blockade, as it would not interfering with adrenaline induced hypokalaemia, is not supported by current evidence, as both selective and non-selective beta-adrenoceptor antagonists have been shown to benefit patients.

8.5 Implications for diuretic therapy.

The results of the study of the effect of diuretics

(Chapter 5) demonstrated no interaction between any of the diuretics studied and adrenaline. Nonetheless, it did show that there was a potential increase in the risks associated with adrenaline induced hypokalaemia with potassium losing diuretics, simply because baseline plasma potassium levels are lower. Thus, the lower the baseline plasma potassium, then the more profound the hypokalaemia induced by adrenaline during stress and the greater the risk of dysrhythmias. Assessment of the risk of therapy with frusemide or thiazide diuretics is impossible because of the uncertainty that exists in guaging the severity of the chronic hypokalaemia and total body potassium induced by these diuretics and, in turn, the effects of such changes in potassium homeostasis on membrane polarization in cardiac muscle. If chronic diuretic therapy, despite resulting hypokalaemia, causes no change in cardiac membrane polarization due to balanced intracellular potassium losses then adrenaline induced hypokalaemia will not lead to increased risk in patients receiving such therapy. As a result, the risks of diuretic induced hypokalaemia remains a matter of continuing controversy (Moser et al, 1986; Madias et al, 1984; Caralis & Perez-Stable, 1986) and further studies are required.

8.6 Implications for bronchodilator therapy.

Theophylline has been reported 'in vitro' to increase

adrenaline induced Na^+ efflux, K^+ influx and membrane hyperpolarization suggesting an effect on Na^+/K^+ATPase activity (Clausen & Flatman 1977; Rogus et al 1977). In the first study described in Chapter 6 a similar effect was shown 'in vivo' in normal humans. This was not the result of theophylline increasing circulating levels of adrenaline. The mechanism of this interaction is probably at a cellular level and remains unclear.

The clinical implications are, that therapeutic levels of theophylline could increase adrenaline induced hypokalaemia in acute severe stress. It is impossible to quantify the size of such a risk to patients with chronic respiratory disease but it is likely it would be relatively small.

Theophylline, the most widely used bronchodilator, and the $beta_2$-adrenoceptor agonist bronchodilator, salbutamol (Chapter 6.5) do interact leading to a potential increase in the fall in plasma potassium. In Chapter 6.4 chronic theophylline therapy was shown to increase adrenaline induced hypokalaemia. In Chapter 6.5 the effect of acute intravenous dosing with salbutamol was studied. This contrasts with many clinical situations, where patients are likely to have been receiving chronic therapy with $beta_2$-agonists. Thus, it is possible that down-regulation of the receptors may have occurred in some patients with chronic therapy. The extent of down-regulation is probably dependent on both the route and the doses that the patients were

receiving. "In vivo" desensitization has been demonstrated in animals (Avner & Noland, 1978), though controversy persists in this area as there is no convincing evidence that down-regulation occurs with standard doses of $beta_2$-agonists inhaled from metered dose inhalers (Jenne et al, 1982). However, increasingly widespread use of nebulisers is resulting in patients being exposed to higher doses and increased systemic absorption of drug which has been deposited in the oropharynx and then swallowed. Whether clinically significant down-regulation of the bronchodilator action or of the hypokalaemic action of $beta_2$-agonists occurs with these higher doses is not known, though there is one preliminary report suggesting significant down regulation occurs in asthmatics (Lipworth et al, 1989). The very recent introduction of effective slow release preparations of salbutamol will also result in more patients achieving chronic steady state salbutamol tissue concentrations, unlike the transient blood levels that occur with metered dose inhalers. Tachyphlaxis may become more relevant in clinical situations more frequently.

The clinical implications of the interaction between theophylline and salbutamol are, therefore, difficult to assess. It may well be that patients with severe asthma or bronchitis, who have been exposed to large doses of

beta$_2$-agonists, but who are also likely to be the patients at highest risk of significant hypoxaemia and acidosis, will be at lower risk of theophylline potentiation of salbutamol induced hypokalaemia because of a degree of down-regulation. Patients with less severe disease, and hence not regularly take high doses of these bronchodilators, who suffer an acute severe attack of bronchoconstriction and who are then given large doses of beta$_2$-agonists, along with therapeutic doses of theophylline may be at greater risk of significant hypokalaemia resulting from the interaction between these bronchodilators. Nevertheless, it must be stated that the combination of oral slow release theophyllines and inhaled or oral beta$_2$-agonists is widely used, both acutely and chronically, often in patients with co-existent heart disease, and there is little evidence to suggest these patients are being placed at risk by this therapy. If there is a risk it is probably greatly outweighed by the benefits such therapy offers patients.

8.7 Adrenergic control of plasma magnesium.

As discussed previously little is known of the internal regulation of magnesium disposal. The results in Chapter 7 show that adrenaline, in pathophysiological doses, leads to small falls in plasma magnesium levels. The mechanism is unclear but the time course is different from the time

course of adrenaline induced hypokalaemia. It may well be an indirect effect of adrenaline rather than a primary control mechanism, possibly as suggested by Rayssiguer due to increased magnesium binding by free fatty acids secondary to adrenergic stimulation of adipocytes (Rayssigeur, 1979). Further study will be required to attempt to define the mechanisms and clinical importance of adrenaline induced hypomagnesaemia.

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APPENDIX ONE

PUBLICATIONS ARISING OUT OF THIS WORK.

PUBLICATIONS.

(1) **Desensitization of platelet B_2-adrenoreceptors after short term infusions of adrenoreceptor agonist in man.** Jones, C.R., Giembcyz, M., Hamilton, C.A., Rodger, I.W., Whyte, K.F., Deighton, N., Elliott, H.L. & Reid, J.L. Clinical Science (1986), 70, 147-153.

(2) **Adrenergic control of plasma magnesium in man.** Whyte, K.F., Addis, G.J., Whitesmith, R. & Reid, J.L. Clinical Science (1987), 72, 135-138.

(3) **The mechanism of salbutamol-induced hypokalaemia.** Whyte, K.F., Addis, G.J., Whitesmith, R. & Reid, J.L. British Journal of Clinical Pharmacology (1987), 23, 65-71.

(4) **Failure of chronic theophylline therapy to alter circulating catecholamines.** Whyte, K.F., Addis, G.J., Whitesmith, R. & Reid, J.L. European Journal of Respiratory Diseases (1987), 70, 221-228.

(5) **Haemodynamic, metabolic and lymphocyte beta2-receptor changes following chronic beta-adrenoceptor antagonism.** Whyte, K.F., Jones, C.R., Howie, C.A., Deighton, N. & Reid, J.L. European Journal of Clinical Pharmacology, (1987), 32, 237-243.

(6) **Salbutamol induced hypokalaemia: The effect of theophylline alone and in combination with adrenaline.** Whyte, K.F., Reid, C., Whitesmith, R. & Reid, J.L. British Journal of Clinical Pharmacology 1988, 25, 571-578.

(7) **Adrenaline induced hypokalaemia and hypomagnesaemia: the effect of diuretic therapy.** Whyte, K.F., Whitesmith, R. & Reid, J.L. European Journal of Clinical Pharmacology (1988), 34, 333-337.

(8) **Effect of CGP17/582, a new selective beta-adrenoceptor antagonist, on the haemodynamic and hypokalaemic response to adrenaline.** Whyte, K.F., De Vane, P.J., Whitesmith, R., Kelman, A. & Reid, J.L. British Journal of Clinical Pharmacology (1989), 27: 553-561.

(9) **Epinephrine induced hypokalemia: the role of beta-adrenoceptors.** Reid, J.L., Whyte, K.F. & Struthers, A.D. American Journal of Cardiology (1986), 57, 23-27F.

(10) **Acute and chronic regulation of alpha$_2$-adrenoreceptor number and function in man.** Jones, C.R., Hamilton, C.A., Whyte, K.F., Elliott, H.L. & Reid, J.L. Clinical Science (1985), 68(Suppl.), 129-132.

APPENDIX TWO

PRESENTATIONS TO LEARNED SOCIETIES.

PRESENTATIONS.

(1) Methyl-xanthines and adrenaline-induced hypokalaemia - possible contributions to tachyarrhythmias in asthmatic patients?.
Medical Research Society, London 1984.

(2) Chronic oral theophylline therapy and catecholamine secretion.
9th. International Congress of Pharmacology, London 1984.

(3) Increased adrenaline levels cause hypomagnesaemia.
Scottish Society of Experimental Medicine, Glasgow 1984.

(4) Effects of chronic oral theophylline therapy on catecholamine secretion and metabolism.
European Society of Pneumonology, Basle 1984.

(5) Haemodynamic, metabolic and lymphocyte $beta_2$-receptor changes following chronic B-adrenoreceptor antagonism.
British Pharmacological Society, London 1984.

(6) Chronic oral theophylline therapy: Effects on catecholamine secretion and metabolism.
Medical Research Society, Oxford 1984.

(7) Raised adrenaline levels cause hypomagnesaemia.
Medical Research Society, London 1985.

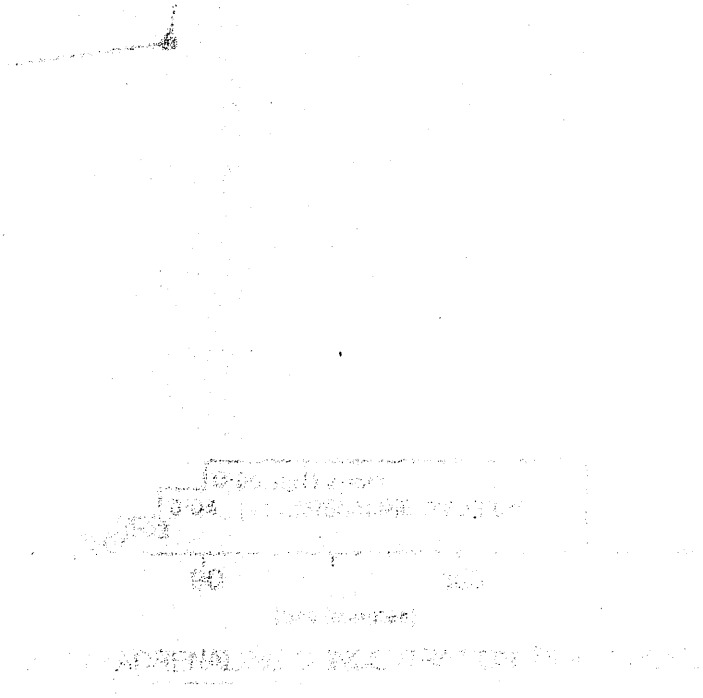
(8) Mechanism of salbutamol induced hypokalaemia.
European Society of Pneumonology, Stresa 1985.

(9) Desensitisation of platelet $alpha_2$-adrenoreceptors following short term adrenoreceptor agonist infusions in man.
Medical Research Society, Oxford 1984.

(10) Theophylline increases salbutamol-induced hypokalaemia and tachycardia.
British Thoracic Society, Edinburgh 1987.

APPENDIX THREE

ADRENALINE CONCENTRATION/TIME PROFILE FOR INDIVIDUAL SUBJECTS.



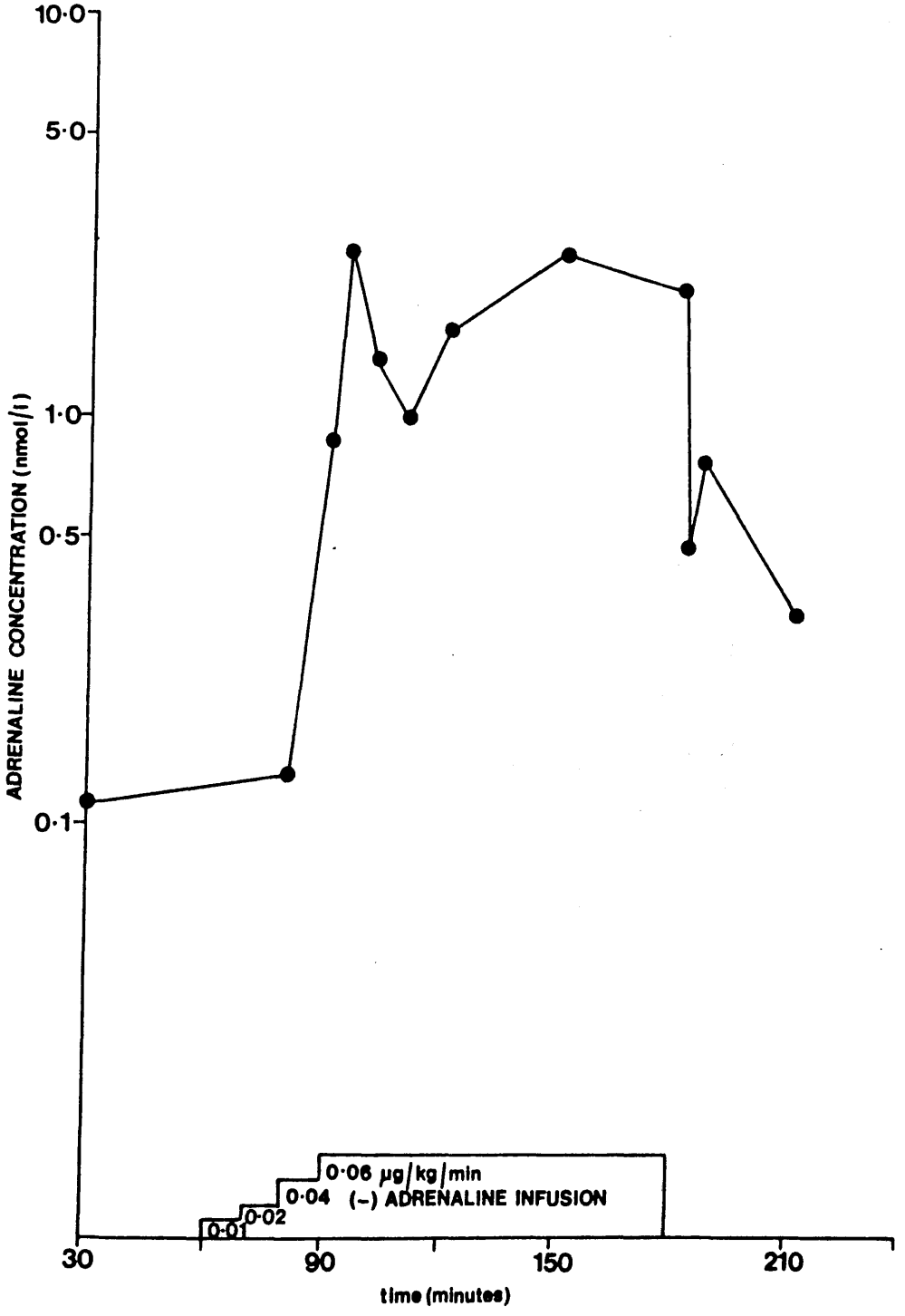


Fig: Appendix 3.1 -ADRENALINE CONCENTRATION TIME PROFILE FOR SUBJECT ONE

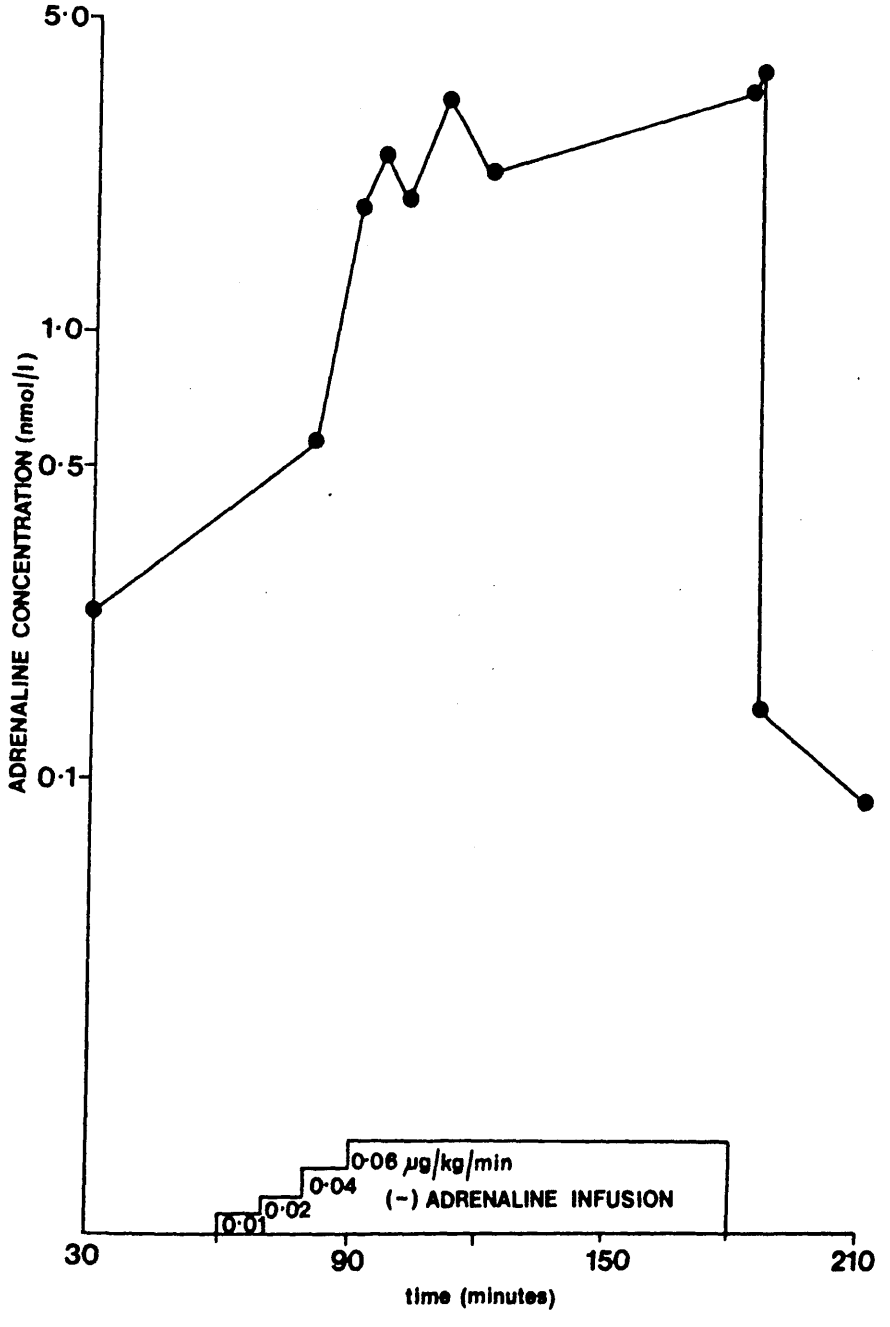


Fig.: Appendix 3.2.- ADRENALINE CONCENTRATION/TIME PROFILE FOR SUBJECT TWO

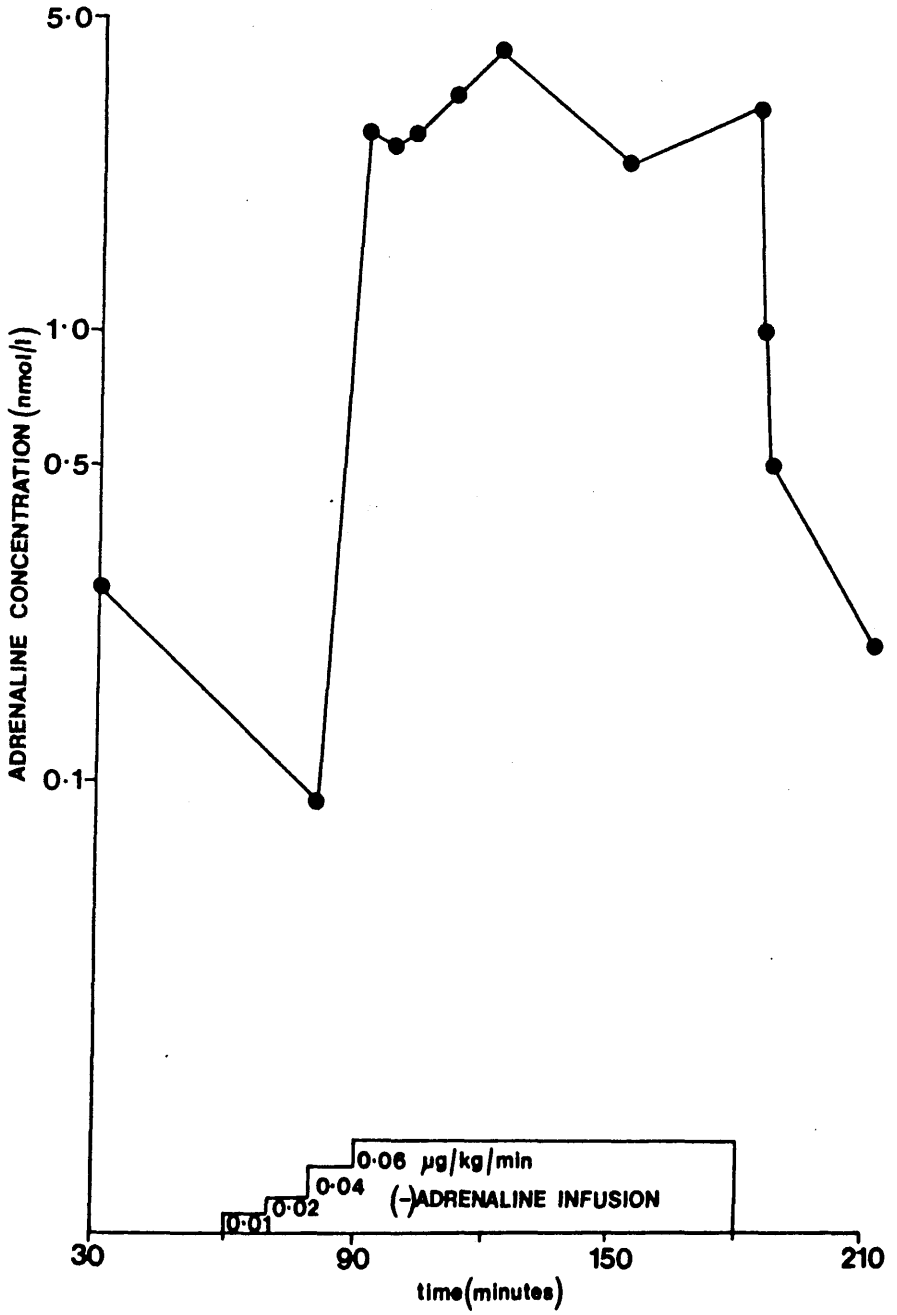


Fig.: Appendix 3.3 - ADRENALINE CONCENTRATION/TIME PROFILE FOR SUBJECT THREE

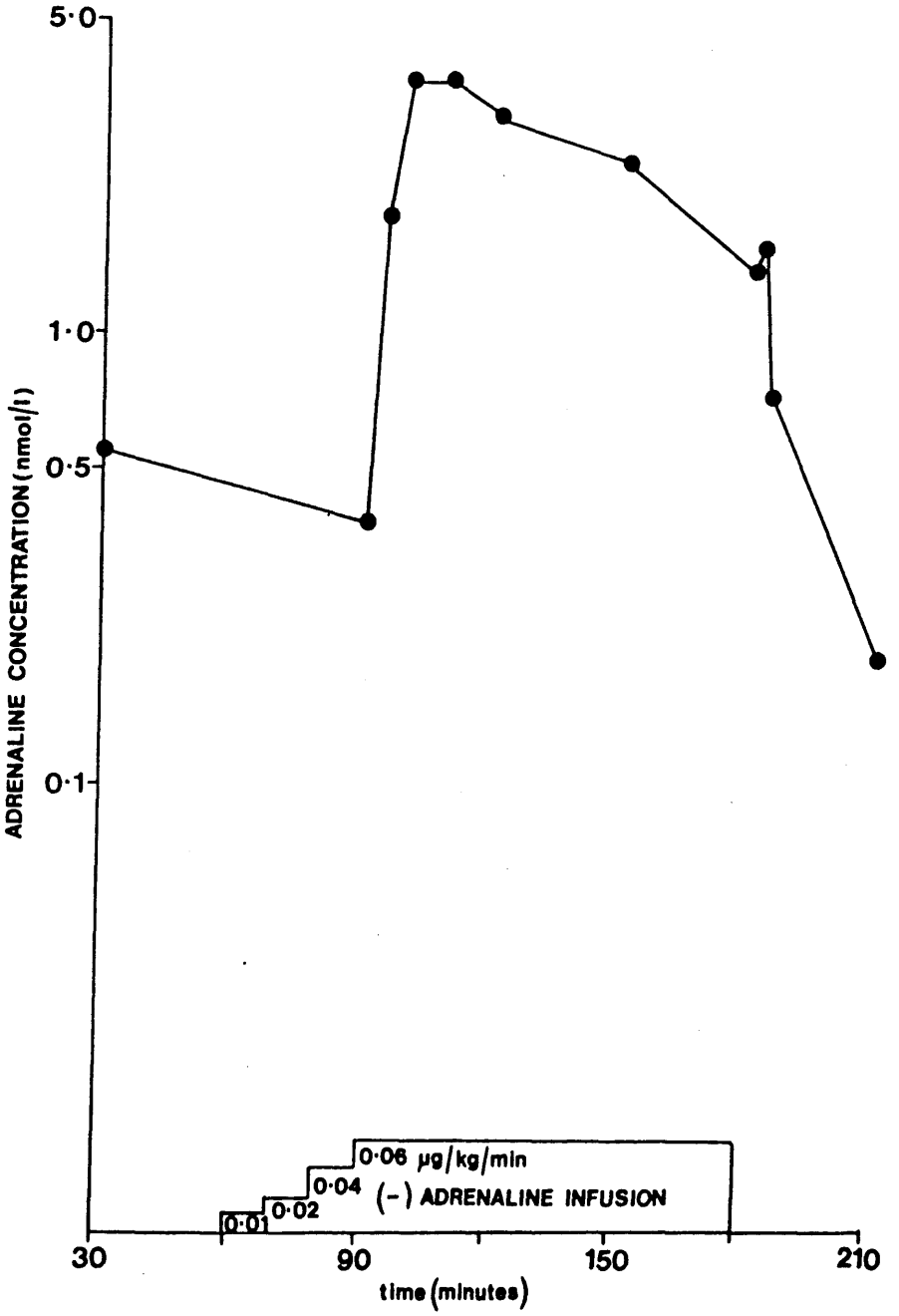
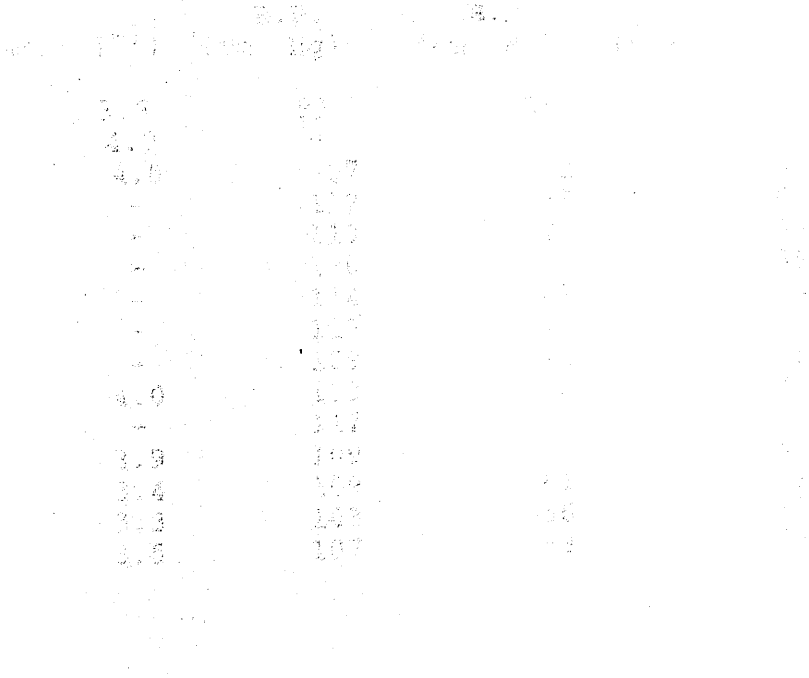


Fig: Appendix 3.4 - ADRENALINE CONCENTRATION/TIME PROFILE FOR SUBJECT FOUR

APPENDIX FOUR

INDIVIDUAL SUBJECT DATA FROM STUDY PRESENTED IN SECTION 4.2



SUBJECT NO. 1

TREATMENT: PLACEBO.

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.8	116	62	74
90	4.0	-	-	-
120	3.9	113	58	80
130	-	109	64	79
140	-	113	61	80
150	-	112	61	80
160	-	119	69	86
175	-	121	60	89
190	-	125	65	90
210	3.5	119	58	84
225	-	121	59	81
240	3.5	117	59	82
270	3.4	112	63	87
300	3.8	117	71	86
360	4.0	112	72	83

SUBJECT NO. 1

TREATMENT: PROPRANOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.8	99	55	63
90	4.0	-	-	-
120	4.0	97	62	67
130	-	112	65	60
140	-	117	73	58
150	-	120	73	55
160	-	114	68	53
175	-	113	73	56
190	-	109	73	58
210	4.0	113	60	74
225	-	117	66	56
240	3.9	109	70	65
270	3.4	109	61	67
300	3.3	103	56	85
360	3.5	107	53	72

SUBJECT NO. 1

TREATMENT: OXPRENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.2	111	62	68
90	3.9	-	-	-
120	3.9	96	56	67
130	-	103	60	65
140	-	102	58	68
150	-	103	62	63
160	-	103	66	57
175	-	113	65	68
190	-	110	67	63
210	4.2	112	68	62
225	-	102	59	65
240	4.1	111	65	60
270	3.9	104	59	71
300	3.6	98	52	64
360	3.7	101	55	69

SUBJECT NO. 1

TREATMENT: ATENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.0	101	57	60
90	4.0	-	-	-
120	4.0	98	56	56
130	-	96	53	58
140	-	100	56	57
150	-	102	56	57
160	-	105	57	54
175	-	107	56	55
190	-	106	60	63
210	4.4	106	58	63
225	-	96	52	62
240	4.1	103	56	60
270	3.7	110	59	66
300	3.5	102	53	64
360	4.0	106	58	69

SUBJECT NO.2

TREATMENT: PLACEBO

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.5	108	62	82
90	3.7	-	-	-
120	3.9	103	59	69
130	-	109	59	71
140	-	111	52	90
150	-	109	54	77
160	-	107	51	91
175	-	116	52	104
190	-	107	52	92
210	2.7	107	52	94
225	-	108	51	102
240	2.8	110	54	114
270	3.2	107	63	99
300	3.3	117	71	92
360	3.4	110	72	89

SUBJECT NO. 2

TREATMENT: PROPRANOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.1	100	52	59
90	3.9	-	-	-
120	3.9	106	53	62
130	-	104	55	54
140	-	105	56	53
150	-	105	61	51
160	-	105	65	48
175	-	112	65	48
190	-	112	64	48
210	4.1	109	64	53
225	-	110	67	52
240	4.1	116	62	56
270	3.8	105	49	73
300	3.6	100	53	73
360	3.4	99	54	69

SUBJECT NO. 2

TREATMENT: OXPRENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.8	106	55	73
90	3.7	-	-	-
120	3.8	101	55	65
130	-	107	61	66
140	-	104	63	64
150	-	112	61	56
160	-	117	63	53
175	-	116	68	65
190	-	113	58	64
210	3.9	119	59	60
225	-	121	62	69
240	3.8	109	61	64
270	3.3	106	58	71
300	3.4	107	55	81
360	3.6	111	55	77

SUBJECT NO. 2

TREATMENT: ATENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.9	110	54	66
90	3.8	-	-	-
120	3.8	102	54	65
130	-	109	44	66
140	-	98	52	63
150	-	106	48	66
160	-	103	48	65
175	-	106	48	71
190	-	107	51	72
210	3.7	101	49	75
225	-	107	53	75
240	3.7	102	50	69
270	3.5	109	56	73
300	3.6	105	54	78
360	3.6	102	57	71

SUBJECT NO. 3

TREATMENT: PLACEBO

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.1	116	69	78
90	4.0	-	-	-
120	4.1	113	64	72
130	-	111	63	67
140	-	111	61	74
150	-	111	59	79
160	-	121	59	83
175	-	119	59	79
190	-	117	59	79
210	3.0	124	61	81
225	-	131	69	93
240	3.0	127	67	81
270	3.2	115	80	75
300	3.5	110	55	76
360	4.1	111	61	79

SUBJECT NO. 3

TREATMENT: PROPRANOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.0	111	59	67
90	3.9	-	-	-
120	3.9	99	5	80
130	-	81	43	62
140	-	90	37	58
150	-	92	56	60
160	-	104	60	54
175	-	105	58	60
190	-	102	59	54
210	4.3	106	65	59
225	-	101	56	58
240	3.9	108	62	66
270	3.8	104	58	73
300	3.8	82	53	63
360	3.7	98	56	71

SUBJECT NO. 3

TREATMENT: OXPRENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.1	106	65	69
90	4.1	-	-	-
120	4.0	81	55	78
130	-	89	45	72
140	-	91	41	65
150	-	93	44	66
160	-	95	44	58
175	-	100	46	57
190	-	102	50	58
210	4.2	117	63	69
225	-	111	61	69
240	4.2	105	60	59
270	4.3	100	51	83
300	4.0	92	45	81
360	3.8	96	46	82

SUBJECT NO. 3

TREATMENT: ATENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.6	107	57	66
90	3.8	-	-	-
120	3.9	99	52	64
130	-	96	54	63
140	-	94	47	68
150	-	99	49	63
160	-	104	69	63
175	-	108	57	64
190	-	102	56	63
210	3.9	99	57	62
225	-	100	61	70
240	3.8	103	55	63
270	3.7	84	42	62
300	3.9	95	46	64
360	3.6	99	46	72

SUBJECT NO. 4

TREATMENT: PLACEBO

Time (mins)	Serum K+ (mmoll-1)	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.0	109	61	60
90	4.2	-	-	-
120	3.9	105	61	50
130	-	107	59	57
140	-	103	55	60
150	-	102	54	55
160	-	116	52	55
175	-	111	57	65
190	-	110	53	66
210	3.4	112	54	65
225	-	109	54	67
240	3.3	108	52	68
270	3.5	104	59	63
300	3.8	111	58	68
360	3.9	105	56	54

SUBJECT NO. 4

TREATMENT: PROPRANOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.1	105	60	47
90	4.0	-	-	-
120	4.1	99	56	45
130	-	99	57	45
140	-	103	58	47
150	-	93	61	48
160	-	103	58	47
175	-	107	60	45
190	-	108	65	44
210	4.3	108	69	50
225	-	117	79	50
240	4.3	114	79	48
270	4.2	114	70	51
300	4.2	115	58	57
360	3.5	100	51	52

SUBJECT NO. 4

TREATMENT: OXPRENOLOL

Time (mins)	Serum K+ ($mmol^{-1}$)	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.1	109	66	50
90	3.9	-	-	-
120	4.1	109	54	55
130	-	105	58	51
140	-	98	59	48
150	-	104	61	50
160	-	108	63	45
175	-	113	63	46
190	-	112	65	44
210	4.2	111	63	46
225	-	108	56	46
240	4.2	99	55	51
270	3.9	108	55	57
300	3.6	102	56	61
360	3.5	102	63	60

SUBJECT NO. 4

TREATMENT: ATENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.9	99	55	52
90	3.8	-	-	-
120	3.9	101	57	43
130	-	91	53	43
140	-	103	53	46
150	-	97	52	52
160	-	102	53	50
175	-	107	53	51
190	-	104	51	50
210	4.4	105	50	59
225	-	108	56	54
240	4.5	99	50	53
270	3.8	99	59	51
300	3.4	97	51	49
360	3.4	98	50	49

SUBJECT NO. 5

TREATMENT: PLACEBO

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.9	138	80	78
90	4.0	-	-	-
120	3.8	126	71	67
130	-	129	67	69
140	-	120	70	69
150	-	139	75	80
160	-	127	65	70
175	-	135	72	70
190	-	133	71	73
210	3.3	128	71	76
225	-	133	75	74
240	2.8	141	68	77
270	3.2	129	71	75
300	3.3	132	73	77
360	3.5	123	69	77

SUBJECT NO. 5

TREATMENT: PROPRANLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.0	121	65	72
90	3.8	-	-	-
120	3.8	109	63	62
130	-	112	59	58
140	-	115	69	57
150	-	112	69	54
160	-	122	71	61
175	-	128	71	64
190	-	125	76	65
210	4.3	134	77	74
225	-	124	64	59
240	4.2	124	66	61
270	3.7	121	61	71
300	3.8	119	58	69
360	4.1	123	62	74

SUBJECT NO. 5

TREATMENT: OXPRENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.7	120	77	74
90	3.8	-	-	-
120	3.8	129	67	76
130	-	124	70	82
140	-	128	63	72
150	-	128	66	69
160	-	137	67	66
175	-	136	72	70
190	-	139	71	65
210	4.0	143	71	70
225	-	145	69	72
240	4.0	142	80	60
270	3.9	130	65	80
300	3.9	127	64	75
360	3.9	127	64	81

SUBJECT NO. 5

TREATMENT: ATENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.8	138	80	78
90	3.8	-	-	-
120	3.8	126	71	67
130	-	129	67	69
140	-	120	70	80
150	-	139	75	70
160	-	127	65	70
175	-	135	72	73
190	-	133	71	76
210	3.4	128	71	74
225	-	133	75	74
240	3.5	141	68	77
270	3.7	129	71	75
300	3.7	132	73	77
360	3.7	123	69	77

SUBJECT NO. 6

TREATMENT: PLACEBO

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.6	125	63	56
90	4.3	-	-	-
120	4.2	135	71	60
130	-	135	70	59
140	-	133	67	64
150	-	143	66	68
160	-	155	81	68
175	-	145	65	70
190	-	143	60	66
210	3.8	146	59	71
225	-	132	60	69
240	3.7	134	55	66
270	3.5	146	65	67
300	3.7	138	61	65
360	3.9	125	59	65

SUBJECT NO. 6

TREATMENT: PROPRANOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.3	118	59	53
90	4.5	-	-	-
120	4.5	121	76	55
130	-	128	73	51
140	-	125	70	55
150	-	118	71	50
160	-	112	66	53
175	-	116	64	55
190	-	116	66	50
210	4.6	103	68	54
225	-	118	62	54
240	-	125	71	51
270	4.1	132	67	55
300	3.8	143	42	57
360	4.0	131	63	62

SUBJECT NO. 6

TREATMENT: OXPRENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.3	135	65	55
90	4.4	-	-	-
120	4.4	130	69	55
130	-	131	73	53
140	-	134	74	51
150	-	133	73	52
160	-	133	68	52
175	-	133	64	55
190	-	132	69	52
210	4.2	141	68	57
225	-	139	76	57
240	3.8	136	66	61
270	3.4	144	66	64
300	3.3	132	63	61
360	3.4	131	65	59

SUBJECT NO. 6

TREATMENT: ATENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.0	121	57	54
90	4.0	-	-	-
120	4.2	117	61	55
130	-	109	57	58
140	-	121	55	56
150	-	103	49	58
160	-	116	56	56
175	-	116	53	55
190	-	119	54	57
210	3.8	121	54	56
225	-	121	52	56
240	3.8	118	52	56
270	3.8	112	63	57
300	3.8	121	52	56
360	3.9	117	54	57

SUBJECT NO. 7

TREATMENT: PLACEBO

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.6	110	60	57
90	3.6	-	-	-
120	3.7	106	61	53
130	-	104	56	58
140	-	104	55	64
150	-	106	56	63
160	-	110	57	63
175	-	114	55	68
190	-	114	54	68
210	3.4	110	53	63
225	-	113	55	65
240	3.5	111	51	66
270	3.3	113	60	61
300	3.4	116	66	62
360	3.6	121	66	62

SUBJECT NO. 7

TREATMENT: PROPRANOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.6	106	58	57
90	4.2	-	-	-
120	4.3	104	56	54
130	-	104	56	52
140	-	88	46	51
150	-	88	45	48
160	-	95	51	47
175	-	87	49	51
190	-	91	46	47
210	H	101	52	47
225	-	109	59	46
240	H	108	56	51
270	4.0	104	65	57
300	3.6	104	58	55
360	3.3	113	58	53

SUBJECT NO. 7

TREATMENT: OXPRENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.6	103	54	57
90	3.6	-	-	-
120	3.6	105	55	62
130	-	105	55	61
140	-	106	55	59
150	-	113	56	62
160	-	107	57	61
175	-	108	57	76
190	-	89	42	64
210	4.0	89	43	62
225	-	93	48	61
240	4.1	99	47	62
270	3.9	107	56	64
300	3.8	112	56	62
360	3.4	106	57	62

SUBJECT NO. 7

TREATMENT: ATENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.0	113	64	56
90	4.0	-	-	-
120	4.0	107	55	53
130	-	106	55	51
140	-	107	54	49
150	-	110	56	54
160	-	108	50	53
175	-	105	52	56
190	-	96	55	56
210	4.3	109	48	57
225	-	109	51	52
240	4.2	110	52	55
270	3.9	115	63	57
300	4.0	114	56	60
360	4.3	109	57	53

SUBJECT NO. 8

TREATMENT: PLACEBO

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.9	119	66	82
90	4.1	-	-	-
120	4.0	116	61	77
130	-	115	61	75
140	-	111	59	81
150	-	114	60	75
160	-	116	61	79
175	-	118	59	82
190	-	116	50	84
210	4.0	122	61	78
225	-	118	56	75
240	3.9	117	57	74
270	4.0	122	63	74
300	3.8	123	63	63
360	-	120	61	93

SUBJECT NO. 8

TREATMENT: PROPRANOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	4.7	117	61	59
90	4.6	-	-	-
120	3.6	100	58	58
130	-	102	55	53
140	-	110	60	52
150	-	106	59	52
160	-	103	58	53
175	-	106	59	57
190	-	108	58	53
210	4.6	105	53	54
225	-	109	61	54
240	3.8	98	63	58
270	3.7	109	52	58
300	3.7	104	57	59
360	3.5	110	43	63

SUBJECT NO. 8

TREATMENT: OXPRENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.9	113	54	68
90	3.8	-	-	-
120	3.9	111	53	65
130	-	115	57	65
140	-	113	60	63
150	-	113	53	59
160	-	114	52	58
175	-	118	54	57
190	-	109	53	63
210	4.4	109	57	70
225	-	114	60	57
240	4.5	113	53	54
270	3.8	112	58	67
300	3.4	102	49	70
360	3.4	95	47	62

SUBJECT NO. 8

TREATMENT: ATENOLOL

Time (mins)	Serum K+ (mmol l^{-1})	Systolic B.P. (mm Hg)	Diastolic B.P. (mm Hg)	Heart Rate (beats min^{-1})
60	3.9	111	57	63
90	3.8	-	-	-
120	3.8	108	54	62
130	-	108	51	58
140	-	108	50	58
150	-	99	54	59
160	-	100	50	60
175	-	103	52	65
190	-	108	48	57
210	4.4	92	52	57
225	-	108	53	63
240	4.5	107	51	60
270	3.8	104	56	63
300	3.4	103	50	66
360	3.4	115	47	69