STUDIES ON THE EFFECTS OF AGEING ON THE PHYSIOLOGY AND PHARMACOLOGY OF THE AUTONOMIC NERVOUS SYSTEM

by

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DECLARATION

The studies described in this thesis were carried out by myself. The clinical monitoring of the patients involved in the studies on noradrenaline and prazosin disposition was shared with Peter Rubin D.M. The computer modelling of drug response was carried out by Peter Meredith Ph.D. The recruitment of hypertensive patients was carried out by their General Practitioners, Mairi Scott M.R.C.G.P. and James Hosie M.R.C.P. The writing of this thesis was entirely my own work.

SUMMARY

The work described in this thesis involved investigations into the effect of ageing on the physiology and pharmacology of the sympathetic nervous system.

Noradrenaline is the principal neurotransmitter of the peripheral sympathetic nervous system. It is found in increased concentration in the plasma of elderly people. The reasons for this increase could be: [1] An increase in the spillover of noradrenaline from the nerve terminal, [2] A reduction in clearance of noradrenaline from the plasma, and [3] a combination of both.

The rates of noradrenaline release into, and clearance from the circulation were measured in two groups of young and elderly volunteers. The resting noradrenaline concentrations were higher in the elderly subjects. There was no change in the rate of clearance of noradrenaline between the groups but the elderly had a higher rate of noradrenaline spillover into the circulation.

The activity of the cardiac beta-1-adrenoceptor reduces with age. There was no corresponding information concerning the activity of the alpha-1-adrenoceptor. The second study described in this thesis examined the responses of human isolated arteries to noradrenaline. This provided evidence of the sensitivity of the alpha-1-adrenoceptor free from the complicating influence of the

baroreceptors. There was no change in the sensitivity of this receptor across a wide age range.

Prazosin is an alpha-1-adrenoceptor antagonist. It has been used in the treatment of hypertension, heart failure and prostatism. The third study examined the pharmacokinetics of prazosin in two groups of young and elderly volunteers. Prazosin was administered intravenously and orally on two separate days. By this means, a measure of the drug bioavailability and clearance may be measured.

In the elderly, there was no change in the rate of clearance of prazosin but there was a reduction in bioavailability.

The fourth study reported in this thesis concerned a combined examination of the pharmacokinetics and pharmacodynamics of three antihypertensive drugs: Trimazosin, an alpha-1-adrenoceptor antagonist; acebutolol, a beta-1-adrenoceptor antagonist; and tolmesoxide, a non-specific vasodilator.

This study not only provided information about the relative effect of age on the clearance of these drugs but also on the effect of age on the sensitivity to the drugs (as measured by fall in systolic blood pressure per unit of plasma drug concentration). There was an increased effect of trimazosin with increasing age. This was due to a reduction in drug clearance. The sensitivity of the alpha-1-adrenoceptor remained unchanged. There was a

reduced effect of acebutolol with increasing age. This was due to an decrease in the sensitivity of the beta-1-adrenoceptor, the drug clearance remaining unchanged. There was no change in the sensitivity or clearance of tolmesoxide with age.

The increase in the plasma concentration of noradrenaline and the lack of change in the sensitivity of the alpha-1-adrenoceptor provides evidence for the suggestion that alpha-1-adrenoceptor antagonists might be useful in the treatment of hypertension in the elderly. Prazosin is limited by a short duration of action and by a first dose hypotensive effect. Doxazosin, which is chemically related to prazosin, has a longer half life, and the potential for once daily dosing.

The last study in this thesis describes the effect of doxazosin on elderly hypertensive patients. Doxazosin produced statistically significant, but modest, falls in diastolic pressure 24 hours post dose. There was no change in systolic pressure.

CHAPTER 1

BACKGROUND AND SCOPE OF THESIS

Old age is not synonymous with ill health. There are alterations in physiology, but many of the changes seen are of no clinical significance; for example greying hair. Other changes can be less benign. Baroreceptor function has been shown to decline with age (1). This can result in postural hypotension, which in one series, was demonstrated in 24% of subjects over the age of 64 (2). This diminished ability to respond to postural stress contributes to the increased risk of falls seen in the elderly, particularly with the onset of disease (3).

The changes that occur with ageing are not just important for physiology and pathology, but also pharmacology. Old age is associated with changes in drug distribution and metabolism as well as drug activity. The importance of these changes is illustrated by the increased risk from adverse drug reactions (ADRs) experienced by the elderly (4).

1.1 PROBLEMS WITH DRUG THERAPY IN THE ELDERLY

There is ample evidence for the increased incidence of ADRs in elderly patients (5,6,7,8). In one survey in the U.K. as many as 12% of admissions to geriatric medical units were recorded as being partly or wholly due to adverse drug reactions (9). Most of the severe ADRs are

due to commonly prescribed drugs (10).

Not all drugs have an increased risk of ADRs with age. Skin reactions to drugs do not appear to increase with age (11). Occasionally an ADR becomes less common with age, as with acute dystonic reactions due to phenothiazines and butyrophenones (12), perhaps because there is an age related decline in the dopamine levels in the basal ganglia.

The causes of the age-related increase in ADRs are complex. There is an age-related increase in the number of drug prescriptions given to inpatients (9,8,13). This reflects in part the necessities imposed by multiple pathology which is often seen in the elderly. There is a strong correlation between the number of drugs prescribed and the risk of an ADR. In one study (13), the risk increased from 5% with 1 drug, to 100% if 10 drugs are prescribed. The increased number of prescriptions will also increase the risk of adverse drug interactions. These are not particularly common, but they can be serious (14).

Not surprisingly, increasing dose has been shown to be related to the incidence of ADRs; e.g. unwanted sedation due to flurazepam in the elderly increases from 1.3% at a dose of 15 mg/day to 12.3% with a dose of 30 mg/day (15).

Poor drug compliance in the elderly is often blamed for ADRs. Compliance is difficult to measure but studies have

shown that up to 75% of drugs are not taken correctly (16). Interestingly, compliance is probably no worse in the young than the old (17,18). Poor compliance usually results in the patient failing to take a drug, which may help prevent ADRs.

The elderly frequently demonstrate changes in the disposition and clearance of drugs (Review: 4). Bioavailability (the proportion of an orally administered drug that enters the systemic circulation) is almost never measured in pharmacokinetic studies and evidence for any changes in the elderly is scarce. Pre-systemic elimination (first pass metabolism) has been studied in the elderly but with conflicting results. Propranolol was shown to have reduced pre-systemic elimination in the elderly in one study (19) but not in another (20). The main difference between these two studies was that the first involved elderly patients who were suffering from chronic disease and the second, normal volunteers. The influence of disease is also seen on the binding of drugs to protein. The plasma concentration of albumin in the elderly tends to decrease with disease (21), and as a result the binding of drugs to albumin, e.g. diazepam, decreases in the elderly sick (22). This increases the concentration of the free active drug and, as a result, the incidence of unwanted sedation.

Once a drug has been absorbed, it will be distributed throughout the body in a pattern mainly determined by its physico-chemical properties. Ageing is accompanied by

substantial changes in body composition. There is a decrease in lean body mass and body weight with an accompanying increase in body fat (23). Thus for fat-soluble drugs such as diazepam, the volume of distribution increases with age (24) and for water soluble drugs such as the model drug antipyrine, there is a decrease (25). The main importance of these changes is that there is tendency to prolong the elimination half life of fat soluble drugs (e.g. diazepam) which may prolong effect.

Drug metabolism may also be reduced with age, probably related to the reduction in blood flow and size of the liver (4). Any reduction in drug metabolism with age is unlikely to have great clinical importance because the changes are generally of small magnitude, and inter-subject variability may be far more important. Vestal et al (26) demonstrated that there was a 600% variability between subjects in hepatic antipyrine clearance, while age had only a 3% effect.

Renal excretion of drugs is frequently reduced in the elderly (4); e.g. streptomycin (27), cimetidine (28) and digoxin (29). However, atenolol also undergoes renal elimination and this appears to be unchanged with age (30).

Once a drug has been absorbed and distributed throughout the body it reaches its site of action: the drug receptor. These are macromolecular complexes found in many sites in

the cell, including mitochondriae, lysosomes and cell membranes (31). Pharmacological effect is produced by the combination of the drug and the receptor. Drugs are said to possess affinity and intrinsic activity in relation to their receptors. Affinity is defined as the efficiency with which the drug binds to its receptor, and intrinsic activity as its power to generate a stimulus. A drug which has both affinity and intrinsic activity is termed an agonist, and one which has only affinity but no intrinsic activity, an antagonist.

Drug receptor interactions may be affected by age (Review: 32). Studies on the changes in receptor activity and age are difficult to design in humans. There may be changes in the absorption, distribution and excretion of the drug which can modify response. There appears to be increased sensitivity with age, e.g. nitrazepam (33), and warfarin (34). Most of experimental work on drug sensitivity and age has concerned autonomic nervous system receptors. Exercise-induced tachycardia is inversely related to age (35). This may be due to reduced activity of the cardiac beta-1-adrenoceptor but the interpretation of the results of the study is complicated by the reduction in baroreceptor reflexes seen in the elderly (1). In contrast the beta-2-adrenoceptor does not seem to change with age (36).

The studies described in this thesis were designed to examine the effect of age on aspects of the physiology and pharmacology of the peripheral sympathetic nervous system.

The thesis is first concerned with the changes seen in the disposition of the neurotransmitter, noradrenaline, and on the sensitivity of the post-synaptic alpha-1-adrenoceptor. The therapeutic implications of these changes are then explored with particular reference to the treatment of hypertension in the elderly.

1.2 NORADRENALINE DISPOSITION IN THE ELDERLY.

The first study describes an attempt to identify the reason for the increased plasma concentration of noradrenaline seen in the elderly (37).

Noradrenaline is the principal neurotransmitter of post-ganglionic sympathetic nerves (38). It is synthesised in the neurone from tyrosine and then stored in vesicles in the terminal axon. The contents of the vesicles are discharged into the synaptic cleft by a calcium dependent exocytosis which is activated by neuronal action potentials. Noradrenaline then combines with adrenergic receptors which are present both on the pre-synaptic and post-synaptic membranes. Following release most of the noradrenaline is recovered by the neurone through a reuptake process (39). Approximately 20% of the released noradrenaline escapes from the synaptic cleft and a proportion of this fraction enters the circulating plasma (Figure 1).

The concentration of noradrenaline in the plasma may not

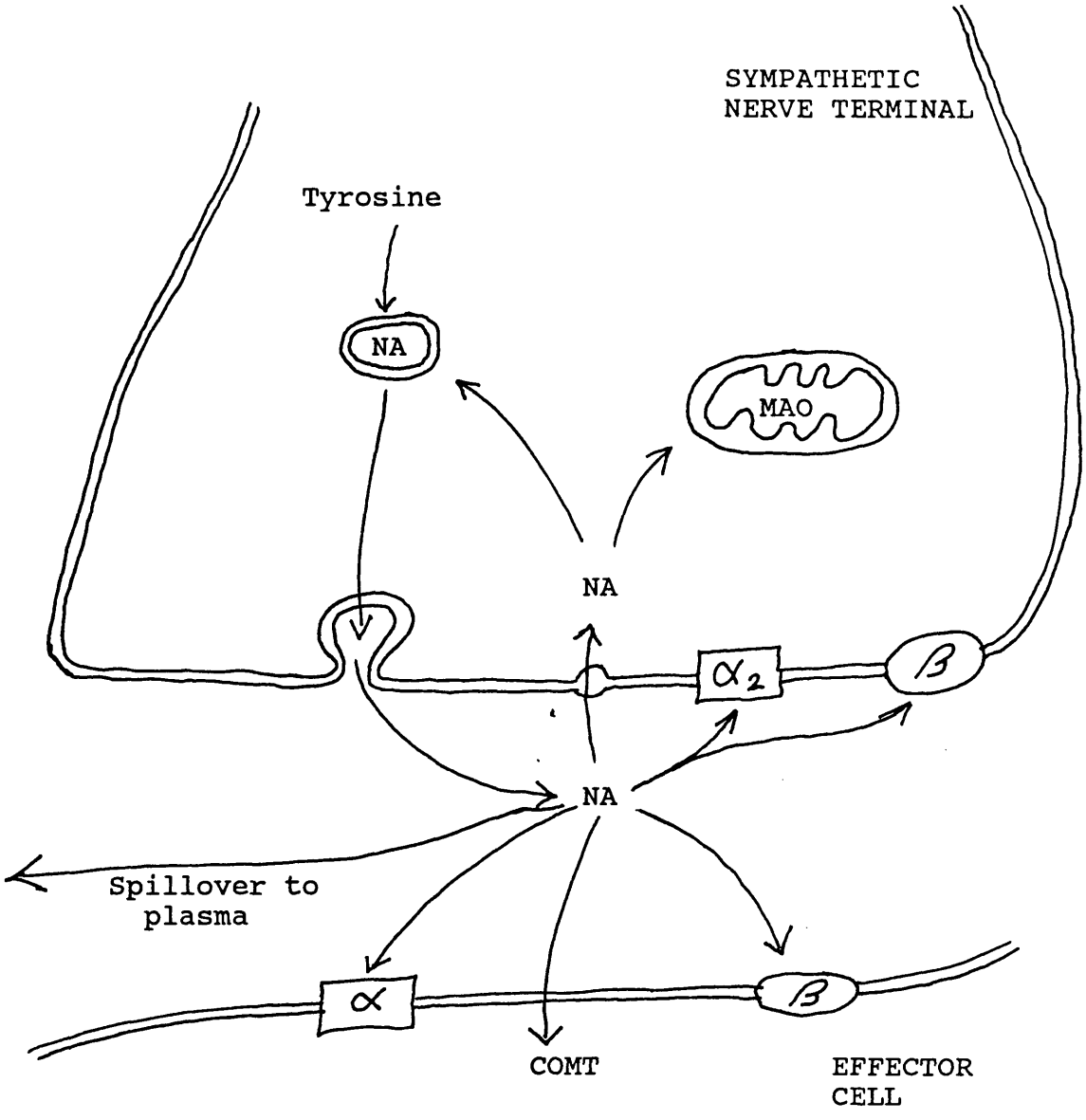

Figure 1.

Noradrenaline (NA) is produced from tyrosine, is then stored in vesicles and released into the synaptic cleft. It undergoes reuptake into the nerve terminal whete it is either oxidised by monoamine-oxidase (MAO) or is taken up once more by the vesicles. Some NA may also be taken into the effector cell where it undergoes metabolism by catechol-O-methyl transferase (COMT). The remainder of the NA escapes from the synaptic cleft and a proportion of this will reach the plasma.

reflect accurately sympathetic nervous activity. Plasma noradrenaline concentration is not a static measurement as it reflects a balance between release into, and clearance from the circulation. Although noradrenaline enters the circulation effectively from one source - the sympathetic nerve terminals - its clearance is more complex. Most of the released noradrenaline undergoes reuptake into the sympathetic neurones. Once regained by the neurone the noradrenaline is either stored once more in the vesicles or metabolised by cytoplasmic mono-amine oxidase (MAO). The noradrenaline which is not removed by the post-ganglionic sympathetic nerves may be metabolised by catechol-O-methyl transferase (COMT). Uptake may also occur into non-neuronal tissue such as muscle but this appears not to be an important pathway in humans (40,41).

It is therefore important when considering sympathetic activity to measure not only the plasma noradrenaline concentration but the rates of release and clearance of noradrenaline into and from the circulation.

Plasma noradrenaline concentration has been examined in essential hypertension (Review: 42). It became apparent that plasma noradrenaline not only increases in some hypertensive patients - usually young, newly diagnosed cases - but also increased independently with age (37). The incidence of systolic hypertension also increases with age (43) and this raises the question of a relationship between the blood pressure and the increased plasma noradrenaline concentration found in the elderly (44).

The elderly also have a greater sympathetic response to stress. With isometric exercise and cold pressor testing there is an approximate doubling of the rise in plasma noradrenaline and blood pressure between the 2nd and 6th decades (45). Under mental stress there is an increase in plasma noradrenaline in the elderly but not in the young (46).

The site of blood sampling for assessing noradrenaline kinetics has to be considered carefully. Sympathetic nerve activity and release of noradrenaline is not uniform, but may vary considerably between organs (47). There may also be changes in the secretion of noradrenaline from individual organs without any overall change in the peripheral noradrenaline concentration (48). The left atrium or pulmonary artery are probably the nearest to ideal sites to sample plasma for accurate estimation of whole body noradrenaline kinetics, however, such sites are too invasive for studies on elderly volunteers. Noradrenaline kinetics calculated from antecubital venous blood are influenced by local release and clearance in the tissues of the forearm (47) but this site is easily accessible and is highly acceptable to the volunteer subjects.

1.3 AGE AND THE RESPONSE OF HUMAN ISOLATED ARTERIES

This series of experiments involved an examination of arteries harvested from patients of a wide age range who

were undergoing abdominal surgery. I was able to quantify the contractile responses of these arteries to noradrenaline.

Catecholamines are responsible for the regulation of a large variety of endocrine and cardiovascular functions. They have influence on plasma glucose, renin and potassium concentrations as well as on heart rate and blood pressure. The principal catecholamines, adrenaline and noradrenaline initiate these actions by combining with discrete sites located on the plasma cell membrane: the adrenoceptors. These receptors also bind agonist, partial agonist and antagonist drugs. An agonist is capable of producing a maximum response, while a partial agonist causes a response that is qualitatively similar but of a lesser magnitude. An antagonist, when in combination with the receptor, has no effect other than preventing the effect of agonists.

Ahlquist (49) was the first to divide adrenergic receptors into alpha and beta. The alpha-adrenoreceptor was responsible for vasoconstriction and the beta-adrenoceptor for the production of cardiac acceleration. It is now recognised that the alpha-adrenoceptors are subdivided into alpha-1 and alpha-2 and the beta-adrenoceptors into beta-1 and beta-2 (Table 1). These receptors may be found at a sympathetic nerve terminal, either on the pre-synaptic or post-synaptic membrane or may be found at sites distant from innervation, e.g. platelets and lymphocytes.

ARTERIOLES		
coronary	alpha beta-2	constriction dilatation
skin	alpha	constriction
mucosa	alpha	constriction
skeletal muscle	alpha beta-2	constriction dilatation
cerebral	alpha	constriction
abdominal vicera	alpha beta-2	constriction dilatation
renal	alpha-1 beta-1 beta-2	constriction dilatation dilatation
VEINS		
systemic	alpha-1 beta-2	constriction dilatation
INTESTINE		
motility	alpha-1 beta-1 beta-2	decrease decrease decrease
EYE		
iris	alpha-1	contraction
ciliary muscle	beta	relaxation
PRESYNAPTIC SYMPATHETIC NERVE TERMINAL		
alpha-2	decreased release of neurotransmitter	
beta	increased release of neurotransmitter	

TABLE 1: Classification of adrenergic receptors.

At rest, the major mechanism determining peripheral vascular resistance is the tone of the smooth muscle cells, generated as a result of intravascular pressure (50). The influence of the sympathetic nervous system via the vasoconstrictor alpha-1-adrenoceptor is present, but is not dominant. However, during physical activity or stress other influences become predominant, including the alpha-1-adrenoceptor, vasopressin and angiotensin.

Increasing age and disease have been shown to affect the activity of the sympathetic nervous system. There is almost universal agreement that the effect of cardiac beta-1-adrenoceptor stimulation is diminished (Reviews: 51,52). There is diminished cardiac acceleration in elderly subjects following intravenous administration of the beta-adrenoceptor agonist, isoprenaline.

The number and activity of adrenoceptors are regulated under conditions of acute or chronic over and under-stimulation (53). Tissues which are denervated become supersensitive to adrenergic agonists due to an increased number and activity of the adrenoceptors. Similarly, tissues which are exposed to increased concentrations of adrenergic agonists may demonstrate 'down regulation' of the adrenoceptors. In the elderly there is an increase in the concentration of noradrenaline in the plasma (37). It is attractive to explain the diminished response of the cardiac beta-1-adrenoceptor in the elderly by 'down-regulation' due to increased exposure

to circulating noradrenaline, although there is no strong evidence to support this theory.

1.4 PRAZOSIN DISTRIBUTION AND AGE

Prazosin is a short acting alpha-1-adrenoceptor antagonist. It has limited use in the treatment of hypertension. This study investigated the differences in prazosin distribution between two groups of young and old volunteers.

Very few pharmacokinetic studies attempt to measure the oral bioavailability of drugs. To achieve this the drug has to be given on two separate occasions by the oral and the intravenous routes. The calculations are made on the assumption that a drug given by the intravenous route will have 100% bioavailability. The use of the intravenous method of administration has another advantage; the accurate calculation of drug clearance, which is not possible with oral dosing.

This study involved the administration of prazosin by the oral and intravenous routes and measured the bioavailability, distribution and clearance of prazosin in the two groups of volunteers. An attempt was also made to compare the hypotensive effect of prazosin in these subjects.

1.5 EFFECT OF AGE ON THE METABOLISM AND EFFECT OF TRIMAZOSIN, ACEBUTOLOL AND TOLMESOXIDE.

This study represents a more complex examination of the age associated changes in pharmacokinetics and dynamics. It involved measurement of the effect and plasma concentrations of three different hypotensive drugs. Using a computer program it is possible to integrate data from plasma drug concentrations with drug effect (in this case: the drop in systolic blood pressure). It is then possible to produce a measure of concentration-effect; i.e. the fall in blood pressure produced by 1 ng/ml plasma drug concentration (54).

This study provided another refinement in technique over that used in the previous examination of prazosin kinetics; a range of volunteers was used that provided a continuous spectrum of age, rather than two discontinuous groups of young and elderly. This allowed for the use of regression analysis of age against drug effect or clearance, which proved to be a more sensitive measure of change.

It was therefore possible not only to comment on the age related changes in drug kinetics but also on the changes in receptor sensitivity to these drugs. This provided more evidence on the effect of ageing on the alpha-1 and beta-1 adrenoceptors as well as on the non-adrenergic receptor which produces vascular relaxation with tolmesoxide.

1.6 TREATMENT OF THE ELDERLY HYPERTENSIVE PATIENT WITH DOXAZOSIN

This clinical study into the treatment of hypertension in the elderly was designed to test a hypothesis which arose from the results of the experiments on noradrenaline disposition and alpha-1-adrenergic sensitivity.

Cardiovascular disease is the single largest cause of morbidity and mortality in the elderly. The Framingham study has identified hypertension as the dominant risk factor for arterial disease (43). In 'developed' societies, the incidence of hypertension increases with age. Between the ages of 85 and 94 years the incidence of hypertension in males is 38% and in females 48% (43). The European study into the treatment of hypertension (EWHE) in the elderly demonstrated a reduction in cardiovascular deaths with treatment at least up to the age of 80 (55).

The choice of antihypertensive drugs is now considerable and most have been shown to be effective in the elderly. The European study used a thiazide diuretic with or without methyldopa. Beta-adrenoceptor antagonists have been shown to be useful (56) despite the theoretical objections of reduced beta-1-adrenoceptor activity with increasing age. Experience with the more recent drugs such as angiotensin converting enzyme inhibitors is more limited but they appear to be effective and well tolerated by the elderly (57).

Increasing age is associated with an increase in the plasma concentration of noradrenaline (Chapter 2) but there appears to be no change in the sensitivity of the alpha-1-adrenoceptor (Chapters 3,5). Hypertension in the elderly is associated with high peripheral resistance and low cardiac output (58) which is quite unlike hypertension in the young in whom, especially in the early stages, there is a high resting cardiac output. These factors taken together have led to the suggestion that vasodilator drugs are the logical choice for the elderly hypertensive - particularly calcium antagonists (59). However, the combination of high circulating noradrenaline levels together with unchanged vasoconstrictor alpha-1-adrenoceptor activity suggests the use of alpha-1-adrenoceptor antagonists in the elderly hypertensive patient.

This study used a double blind cross over design with doxazosin against placebo, with three months on each treatment. At the end of the study plasma drug concentrations were measured over a 24 hour period, giving an indication of the elimination half life of this drug in the elderly.

The results of this study give an indication of the usefulness of once daily doxazosin therapy in the treatment of the elderly hypertensive patient.

CHAPTER 2

THE EFFECT OF AGE ON THE RELEASE AND CLEARANCE OF NORADRENALINE

THE EFFECT OF AGE ON THE RELEASE AND CLEARANCE OF NORADRENALINE.

2.1 INTRODUCTION

The increased plasma noradrenaline concentration found in the elderly could be due to an increase in spillover from the nerve terminals, a reduction in clearance from the plasma, or both. This study was designed to investigate the influence of age on noradrenaline release into and clearance from plasma taken from the antecubital vein.

2.2 METHODS

Measurement of noradrenaline release and clearance

The method of assessing noradrenaline release and clearance in this study involves the intravenous infusion of low concentrations of noradrenaline. The rate of noradrenaline infusion is intended to cause minimal or no change in blood pressure or heart rate. The infusion is continued until a constant plasma concentration of noradrenaline is achieved.

Noradrenaline clearance from plasma is then calculated using the relationship:-

$$Cl = \frac{\text{Infusion rate}}{Css - Cb}$$

Cl = clearance, Css = mean steady state noradrenaline

concentration and Cb = mean basal (pre-infusion) supine nordrenaline concentration (60).

The endogenous rate of noradrenaline release (Rn) into the circulation ($nmol.min^{-1}$) under steady state conditions prior to the infusion is calculated by the formula:-

$$Rn = Cl \times Cb$$

Volunteer selection

Eight young and eight elderly male volunteers were recruited. The young consisted of hospital laboratory technicians who had not taken part in similar studies before. The median age of the young group was 29 years with a range of 21-36 years. The elderly volunteers were recruited with the aid of the age-sex register of a local general practitioner. The median age was 70 years with a range of 65-78 years.

Both groups of volunteers were made familiar with the clinical laboratory, the equipment and experimental techniques before the study days. In particular the elderly subjects visited the hospital and clinical laboratory prior to the study. None of the subjects had experienced any previous serious illnesses and none were taking any drugs. All were living independently at home. All subjects had a physical examination prior to the study day and all had normal haematological and biochemical indices as well as a normal electrocardiogram.

Study protocol

At approximately 0830hrs on the study day, a venous cannula was inserted in each forearm and filled with saline containing 20 units.ml^{-1} of heparin. An arm cuff was fitted for blood pressure recording using an automated sphygmomanometer (Bosomat). The subjects then rested quietly for 20 minutes after which time they stood for 5 minutes on three separate occasions during the next 30 minutes. At the end of each 5 minute period blood was drawn via one of the intravenous cannulae for catecholamine analysis. Pulse and blood pressure recordings were made frequently. The subjects then rested for a further 20 minutes and thereafter three further samples were withdrawn for catecholamine analysis. Blood pressure and heart rate were measured simultaneously.

Noradrenaline (Levophed, Winthrop Laboratories) was then infused into the second intravenous cannula using a constant rate pump (Braun). The subjects remained supine throughout. Noradrenaline solutions were made up in normal saline and to prevent oxidation 1% ascorbic acid was added, and both the syringe and connecting tubing were protected from the light. The infusion solutions were analysed at the end of the study for catecholamine concentration.

Noradrenaline was infused at increasing rates of 0.01, 0.02 and 0.03 microgram.kg^{-1}.min^{-1} for 10 minutes each. At the eighth and tenth minute of each infusion rate, blood samples were withdrawn for noradrenaline analysis.

Noradrenaline was then infused at 0.06 microgram.kg^{-1}.min^{-1} for 180 minutes, blood samples being taken at 30 minute intervals. Blood pressure measurements were carefully recorded throughout the infusion period to detect any excessive rise which would have resulted in the study not proceeding to the administration of a higher noradrenaline concentration.

Blood for noradrenaline analysis was drawn into chilled plastic syringes, transferred to cold heparinised tubes and immediately centrifuged at 4°C. The plasma was separated and then stored at -20°C until analysed.

Analysis of noradrenaline in plasma

In the adrenal medulla, in chromaffin cells and in some neurones in the central nervous system, phenylethanolamine-N-methyl transferase (PNMT) converts noradrenaline to adrenaline using a methyl group derived from S-adenosyl-methionine (SAME). This biochemical pathway has been used to develop an assay for noradrenaline (61). The assay for plasma noradrenaline in this study uses SAME with a radiolabelled methyl group. Partially purified PNMT obtained from bovine adrenal glands is used to transfer this tritiated methyl group to the noradrenaline, forming tritiated-adrenaline. This tritiated-adrenaline is then separated from the labelled precursor by absorption onto alumina and then extraction into perchloric acid. The concentration of tritiated-adrenaline is then measured by liquid scintillation and this result is proportional to the amount of noradrenaline

originally present. The assay is highly sensitive and specific for noradrenaline. None of the naturally occuring catecholamines or their metabolites either interfere with or are detected by the assay.

Statistical analysis

Results are expressed as the median and range for the group. Values for young and old are compared by two-tailed Wilcoxon rank sum test. Noradrenaline clearance at the four infusion rates was compared by Friedman two-way analysis of variance. The possibility of a relationship between the rate of noradrenaline entry into the circulation and blood pressure for all sixteen subjects was investigated using a computer program for generalized linear interactive modelling (62).

2.3 RESULTS

The demographic data and baseline blood pressure recordings for the volunteers are shown in Table 2. The systolic and diastolic pressures for the elderly were signficantly higher than for the young in both supine and standing positions. The basal heart rate did not differ between young and old in either position. In both groups the blood pressure increased on moving from the supine to the standing position by the same proportion, (systolic pressure increased by a median of 4.6% in the elderly and 4.3% in the young; diastolic pressure increased by a median of 9.0% in the elderly and 9.2% in the young).

	SYSTOLIC BP (mmHg) (median range)	DIASTOLIC BP (mmHg) (median range)	PULSE (per min) (median range)
YOUNG			
median 29 range 21-36 years			
Supine	117 *	72 ***	60
	98-113	69-92	53-65
Erect	119 **	87 ****	74
	101-138	61-94	53-83
ELDERLY			
median 70 range 65-78 years			
Supine	146 *	102 ***	62
	127-187	78-127	60-70
Erect	157 **	107 ****	64
	105-205	83-133	60-79

* $p < 0.005$ ** $p < 0.05$ *** $p < 0.01$

**** $p < 0.01$

TABLE 2: Volunteer erect and supine blood pressure and pulse measurements.

Plasma noradrenaline concentrations were significantly higher in the elderly group in both supine and standing positions (Table 3). As with blood pressure the median incremental rise on moving from the supine to standing position was the same in each group (76% in the elderly and 77% in the young).

At the highest noradrenaline infusion rate supine systolic pressure increased by a median of 8.5% in the old and 10% in the young,while diastolic pressure increased by 3.0% in the old and 8.0% in the young. There was no significant difference in blood pressure changes between the groups during noradrenaline infusion (Table 4). In neither old nor young was there any change in pulse rate during the noradrenaline infusions.

The analysis of the infused noradrenaline solutions at the end of each experiment confirmed the expected concentrations within $\pm$7.0%.

Pre-infusion, basal plasma noradrenaline levels were stable by 20 minutes and no further rise in noradrenaline concentration occured after 5 minutes standing. Steady state plasma levels of noradrenaline were achieved by the eighth minute of the noradrenaline infusions.

Noradrenaline clearance in the elderly subjects was not significantly different from values obtained for the young. The values for clearance at each of the four infusion rates were the same in the two age groups

	SUPINE (median range)	ERECT (median range)
YOUNG (n.mol/l)	2.6 *	4.7 **
	1.4 - 3.4	1.8 - 6.0
ELDERLY (n.mol/l)	4.0 *	6.8 **
	2.5 - 6.2	4.5 - 10.1
	* $p < 0.01$	** $p < 0.01$

TABLE 3: Plasma noradrenaline concentration in young and elderly volunteers: erect and supine position

INFUSION RATE ug/kg/min	0	0.01	0.02	O.04	0.06
YOUNG					
SYST BP (mmHg)					
median	117	114	113	130	127
range	98-133	95-125	100-135	108-135	111-145
DIAS BP (mmHg)					
median	72	80	80	86	79
range	69-92	70-95	78-100	72-90	68-85
PULSE (per min)					
median	60	58	59	50	59
range	53-65	50-61	50-62	44-62	52-80
ELDERLY					
SYST BP (mmHg)					
median	154	140	135	160	165
range	126-187	130-193	128-205	140-205	130-214
DIAS BP (mmHg)					
median	102	105	100	100	100
range	78-127	80-127	80-130	85-128	73-121
PULSE (per min)					
median	63	68	60	60	67
range	50-77	50-70	55-70	60-75	57-75

TABLE 4: Effect of noradrenaline infusion rates on pulse and blood pressure

(Table 5). Since the elderly subjects were generally heavier than the young the clearance data were also calculated with regard to subject weight but again there was no significant difference between the two groups: old, 55 $ml.kg^{-1}.min^{-1}$ (range 33-111), young, 67 $ml.kg^{-1}.kg^{-1}.min^{-1}$).

The rate of spillover of noradrenaline was significantly greater in the elderly both in the supine and standing positions (Table 6). Median values were (supine position) 10.3 $nmol.min^{-1}$ in the young and 19.7 $nmol.min^{-1}$ in the old ($p < 0.05$) and (standing position) 17.2 $nmol.min^{-1}$ in the young and 29.2 $nmol.min^{-1}$ in the old ($p < 0.01$).

The possibility that the increased rate of noradrenaline release in the elderly was associated with their generally higher level of blood pressure (pre-infusion) was investigated by generalized linear interactive modelling (Table 7). When systolic and diastolic blood pressure were removed from the full model there was no significant effect on residual sums of squares in either supine or standing positions. However age was found significantly to influence the sum of squares in both positions.

2.4 DISCUSSION

This study confirmed that in the elderly there was a higher basal plasma noradrenaline concentration compared to the young. This higher concentration was related to an

Infusion rate (microgm/kg/min)	0.01	0.02	0.04	0.06
Young Noradrenaline clearance (l/min)				
(median	3.9	4.5	3.3	4.8
range)	1.5-9.8	2.9-7.1	2.5-5.2	2.7-6.1
Elderly Noradrenaline clearance (l/min)				
(median	3.6	3.6	3.9	4.1
range)	2.3-5.7	2.7-7.7	2.6-5.7	2.6-8.2

TABLE 5: Noradrenaline clearance calculated at four infusion rates.

No difference between clearance values calculated at the different infusion rates (Friedman two-way alalysis of variance)

	SUPINE (median range)	ERECT (median range)
YOUNG Noradrenaline release (n.mol/min)	10.3* 5.3-17.6	17.2** 11.0-36.4
ELDERLY Noradrenaline release (n.mol/min)	19.7* 10.1-53.8	29.2** 21.1-121.0

* $p < 0.05$ ** $p < 0.01$

TABLE 6: Plasma noradrenaline spillover; erect and supine positions

Model 1. $Rn = a_1 + a_2(SBP) + a_3(DBP) + a_4(age)$

Model 2. $Rn = b_1 + b_2(SBP) + b_3(DBP)$

Model 3. $Rn = c_1 + c_2(age)$

	Sum of squares	F	P
Supine			
Model 1	523		
Model 2	745	5.1	0.05
Model 3	545	0.25	NS
Erect			
Model 1	922		
Model 2	1625	9.2	0.01
Model 3	1194	1.8	NS

TABLE 7: Investigation of relationship between rate of noradrenaline entry to the circulation, blood pressure and age.

increase in the spillover from noradrenergic terminals with no change in the rate of clearance from the plasma.

Calculation of noradrenaline kinetics and selection of sampling site

Before giving consideration to the reasons for the increase in transmitter spillover it is important to examine the origin of plasma noradrenaline taken from the antecubital vein. Noradrenaline in the forearm venous plasma originates from two main sources: (i) release from the total peripheral noradrenergic nervous system and, (ii) local release from the sympathetic nerves in the forearm (see Figure 2). Noradrenaline is both cleared from and released into the circulation by the tissues of the forearm. Approximately half the noradrenaline in the plasma taken from the antecubital vein originates from the forearm tissues (47). The concentration of noradrenaline in plasma taken from the arterial tree or from right heart catheterisation (mixed venous blood) may be more representative of "total body" spillover, but the lungs are also responsible for extraction and release of noradrenaline. There is therefore probably no site for blood sampling that can be considered ideal for the assessment of total body noradrenaline kinetics. Local variations in sympathetic activity may occur without altering the plasma noradrenaline concentration measured in peripheral plasma, e.g. in some patients with renal artery stenosis there is an increased spillover from the ischaemic kidney without any change in the venous plasma noradrenaline concentration (48).

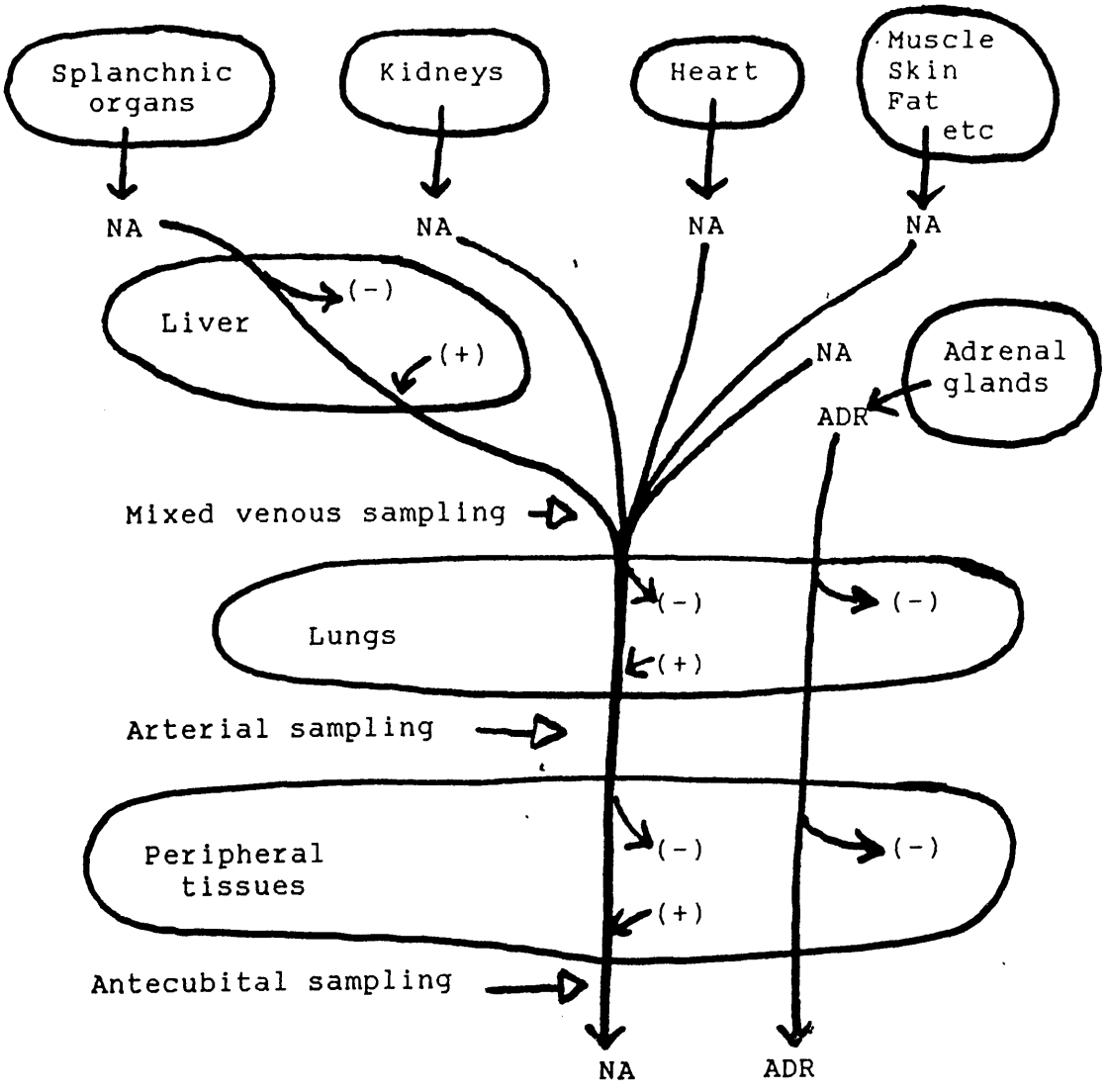

Figure 2: Diagramatic representation of the source of noradrenaline in blood taken from the antecubital vein. Approximately half of this noradrenaline originates from the tissues of the forearm.

There is a greater extraction of catecholamines by deep rather than superficial forearm tissue and therefore the antecubital vein is preferable to the brachial when investigating noradrenaline clearance with infused noradrenaline (48). The antecubital vein has the additional advantage because sampling is relatively non-invasive and is easily repeatable. Furthermore the antecubital plasma noradrenaline concentration has been shown to be similar to that obtained from simultaneous sampling from the femoral artery and vein and the pulmonary artery (63).

The method of calculating noradrenaline spillover and clearance used in this study was described by Fitzgerald et al (60). Calculation of noradrenaline clearance is made following intravenous infusion of exogenous noradrenaline to a steady state concentration. The endogenous spillover of noradrenaline is then measured simply by the product of the basal plasma noradrenaline concentration and the calculated clearance. In making these calculations three critical assumptions are made: (1) steady state concentration has been reached, (2) the infusion of noradrenaline does not affect clearance or (3) the infusion does not affect endogenous spillover of noradrenaline.

The validity of the steady state assumption is supported by the results of the study and by the observations of other workers (60). It has been demonstrated that steady state supine levels are achieved by 20 minutes. In the

standing position, no further rise in plasma noradrenaline concentration occurs after 5 minutes (37). These observations were confirmed by the results of this study. The concentration of noradrenaline at the eighth and tenth minutes of the infusion are the same.

Noradrenaline has been shown to increase its own clearance by a beta-adrenergic mediated mechanism (64). In this study there was no change in clearance of noradrenaline at the four different infusion rates (Table 5).

The possibility that the infusion of noradrenaline affected the rate of entry of endogenous noradrenaline into the circulation has to be considered. A possible mechanism for this would be activation of pre-synaptic alpha-adrenoceptors on the nerve terminal which would lead to a reduction in the rate of noradrenaline release. This would give an underestimate of noradrenaline clearance which would tend to become more pronounced with the increase in infusion rate. Again it should be emphasised that in this study there was no change in clearance with increase in the the infusion rate. Also the calculation of noradrenaline clearance is more dependent on the relatively large infusion rate of exogenous noradrenaline measured in micrograms per minute, compared to the rate of endogenous spillover which is measured in nanograms per minute.

The origin of the increased plasma noradrenaline concentration in the elderly has also been assessed by

other investigators (Table 8). Esler et al (65) reached the opposite conclusion to this study. They found no change in the rate of spillover and a decrease in the rate of clearance of noradrenaline. Their technique was different from that employed here, involving the infusion of tritiated-noradrenaline at tracer concentrations. This has the potential advantage of not having a pharmacological effect. In the study by Esler et al (65), measurement of noradrenaline release is dependent on achieving a steady state concentration of tritiated-noradrenaline which was measured by the amount of radioactivity present in an extract of the plasma. However it took 60 minutes for the radioactivity to reach steady state compared to the 8 minutes to steady state noradrenaline concentration in this study. Noradrenaline is rapidly metabolised in plasma with a half life of approximately 3 minutes (66). This suggests that Esler et al (65) might have been measuring a mixture of tritiated-noradrenaline and tritiated-metabolites of noradrenaline. Veith et al (67), also using a similar tritiated-noradrenaline technique, found that there was an increase in spillover of noradrenaline with age which was accompanied by a less significant reduction in clearance. Hoeldtke and Cilmi (68) demonstrated an increase in spillover with no change in clearance with age. Once again questions arise about the presence of tritiated-metabolites of noradrenaline which would introduce errors into the calculations of noradrenaline spillover and clearance.

NORADRENALINE KINETICS AND AGE		
SPILLOVER	CLEARANCE	AUTHORS
No change	Reduced	Esler et al 1981 (65)
Increased	No change	Hoeldtke et al 1985 (68)
Increased	Reduced	Veith et al 1986 (67)
Increased	No change	MacGilchrist et al 1988 (70)
Increased	No change	Poehlman et al 1990 (71)

TABLE 8: Studies of the origin of the increased plasma noradrenaline concentration in the elderly: Tritiated-noradrenaline techniques.

Howes et al (69) attempted to improve the assay for tritiated-noradrenaline in plasma by introducing a step to separate the metabolites with high performance liquid chromatography. In this study after 30 minutes of constant rate infusion with tritiated- noradrenaline only 57% of the radioactivity measured was noradrenaline and this fell to 44% after 90 minutes. Using this assay, MacGilchrist et al (70) were able to confirm the present finding of a increase in the spillover of noradrenaline with age with no accompanying change in clearance. The present study suffers the potential disadvantage of using small but pharmacological doses of noradrenaline but has the considerable advantage of a highly specific noradrenaline assay which is unaffected by the presence of metabolites.

In a recent study (71) which examined the effect of physical activity and age on the release and clearance of noradrenaline, there was no change in the rate of clearance with age. Plasma noradrenaline levels and noradrenaline spillover were highest in elderly males who took regular exercise.

Factors affecting noradrenaline kinetics in the elderly

The factors controlling noradrenaline spillover into plasma are complex and some possibilities are listed in Table 9.

An obvious reason for the increased spillover could be an increase in sympathetic activity with age. Support for

Age related factors that could affect noradrenaline spillover.

1. Extent of tissue sympathetic innervation.
2. Amount of sympathetic nerve activity
3. Amount of noradrenaline released per nerve impulse
4. Activity of presynaptic adrenoceptors involved in the regulation of noradrenaline release
5. Degree of reuptake into nerve (and or other tissue)
6. Activity of mono-amine oxidase and catechol-O-methyl transferase
7. Size of synaptic cleft.
8. Permeability of tissue between synaptic cleft and lumen of vessel

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Table 9: Some of the factors which might change with age and cause an alteration in noradrenaline spillover into the circulation.

this suggestion comes from a study where sympathetic activity in the peroneal nerve was monitored using dermal electrodes. Both plasma noradrenaline concentration and age correlated with sympathetic nerve activity (72). The concentration of noradrenaline has been measured in human posterior tibial artery, obtained at post mortem, and in biopsy specimens of the cervix uteri. In both, the concentration of noradrenaline decreases with age (66). There is also histochemical evidence of a reduction of noradrenaline in human sympathetic ganglia (73). Taken together, these findings might suggest that there is increased sympathetic activity with age with an increase in release of noradrenaline accompanied by a reduction in the stores of noradrenaline in innervated tissue.

The sympathetic control of blood vessels is influenced by baroreceptors which when stimulated cause reduction in sympathetic activity and an increase in vagal tone. The effect of age on the responses of the baroreceptor reflex has been investigated (1). The effect of infusing the pressor agent phenylephrine was investigated in a group of volunteers aged 19-66 years. Increasing age was associated with a reduction in the sensitivity of the baroreceptor reflex. Another group of investigators (74) used a variable pressure neck chamber to study the responses of the carotid baroreceptors. They confirmed the reduction in efficiency of the receptors seen in the elderly but demonstrated that this only affected the control of heart rate, as the ability to alter blood pressure was preserved. Shimada et al (75) examined the effect of age

on baroreceptor sensitivity in relation to plasma noradrenaline concentration. Using multiple regression analysis, when the influence of reduced baroreceptor sensitivity was eliminated, age was no longer associated with an increase in plasma noradrenaline. The authors therefore suggested that the reduction in sensitivity of the baroreceptor with increasing age resulted in an increase in sympathetic activity, and therefore an increase in plasma noradrenaline concentration. This view is almost certainly an oversimplification, as the studies with the variable neck chamber (74) demonstrated that the splanchnic circulation was principally involved in blood pressure regulation. However the splanchnic circulation does not contribute signficantly to the peripheral venous noradrenaline concentration (76), as noradrenaline released from the mesenteric nerves enters the portal venous system and is extensively metabolised on the first pass through the liver. On the other hand a considerable proportion of the noradrenaline measured in the peripheral venous plasma is derived from the skeletal muscle circulation, which appears not to be substantially affected by the baroreceptor reflex (74).

An attempt has been made to examine the effect of age on the rate of noradrenaline synthesis in the sympathetic nervous system (68). The technique involves an analysis of noradrenaline metabolite excretion in the urine. The authors demonstrated that there was no change in the rate of production of noradrenaline with age; however, there are limitations with this method - e.g. the difficulty in

separating catecholamines released from the sympathetic nervous system from those released from the adrenal medulla and brain.

The rate of release of noradrenaline from the sympathetic nerve terminal is modified by several different presynaptic receptors. Stimulation of presynaptic alpha-2-adrenoceptors results in a reduction of noradrenaline release. The physiological importance of the presynaptic alpha-2-adrenoceptor is not yet established. In isolated human pulmonary arteries noradrenaline release was inhibited by alpha-2-adrenoceptor agonists (77). However with studies *in vivo*, the role of these receptors is less clear. The difference in plasma noradrenaline concentration between the brachial artery and antecubital vein is not altered by arterial infusion of the alpha-2-adrenoceptor agonist clonidine (78). However this study only measured the difference in arterio-venous concentration which does not necessarly reflect noradrenaline release from nerves, as the muscles of the arm both release and remove noradrenaline from the circulation. Clonidine did produce a dose related reduction in noradrenaline spillover when a tritiated-noradrenaline tracer technique was employed (79). It is of interest that even though the release of noradrenaline was reduced by clonidine, the overall plasma noradrenaline concentration remained unchanged.

There is little information on the effect of ageing on presynaptic alpha-2-adrenoceptor function. In an

examination of isolated saphenous veins taken from patients of different ages, there was no change in presynaptic alpha-2 activity (80). This was confirmed by an *in vivo* study of the effect of clonidine on tritiated-noradrenaline kinetics in subjects of different ages (81).

There are no studies on the effect of age on other presynaptic receptors, e.g. beta-adrenoceptors or angiotensin receptors.

Spillover of noradrenaline into the plasma is not only influenced by the rate of release from the neurone but also by the rate of removal from the synaptic cleft. The principal mechanism for removal is reuptake into the neurone (uptake-1) which accounts for approximately 80% of the noradrenaline released (39). The noradrenaline is then either taken up into the intraneuronal vesicles or is metabolised by cytoplasmic mono- amine-oxidase (MAO). Extraneuronal uptake of noradrenaline (uptake-2) occurs *in vitro* (although there is evidence that uptake-2 does not occur *in vivo*) (41). There is extraneuronal metabolism of noradrenaline by catechol-0-methyl transferase (COMT).

There is some indirect evidence that uptake-1 may be deficient with increasing age (68). This evidence is derived from a study of the excretion of different metabolites of noradrenaline. With increasing age there was a reduction in the MAO metabolites with a relative increase in the COMT metabolites. This suggests an

increase in extra-neuronal metabolism, perhaps as a result of a decrease in the efficiency of uptake-1. However the same result could arise from a diminution in the efficiency of MAO with age similar to the decline in oxidation of certain drugs e.g. diazepam (82). There is no direct evidence for any change in the activity of MAO with age. There is evidence that the activity of COMT does not change with age (83).

In summary, this study demonstrated that in plasma taken from the antecubital vein the age related increase in plasma noradrenaline concentration was related to an increase in spillover rather than a decrease in clearance. There is evidence in the literature to suggest that this increased spillover of noradrenaline may be associated with an increase in sympathetic nerve traffic, or a decrease in the efficiency of neuronal reuptake, or a combination of both. However other mechanisms not yet studied, such as the influence of age on presynaptic alpha-2-adrenoceptors or angiotensin receptors, may be important.

CHAPTER 3

THE EFFECT OF AGE ON THE RESPONSES OF HUMAN ISOLATED ARTERIES TO NORADRENALINE

THE EFFECT OF AGE ON THE RESPONSES OF HUMAN ISOLATED ARTERIES TO NORADRENALINE

3.1 INTRODUCTION

Increasing age is associated with a decrease in the activity of the cardiac beta-1-adrenoceptor (84). It is not known whether this is related to the increased spillover in noradrenaline observed in Chapter 2.

This study was designed to examine the responses of the vasoconstrictor alpha-adrenoceptors with increasing age. Although it is relatively easy to stimulate alpha-adrenoceptors *in vivo*, interpretation of the results of such a study is made difficult by the presence of cardiovascular reflex activity which also has been shown to change with age in man (1). A further complication of such a study is the possibility that the disposition of the drugs used in the study would be altered by age. It was therefore decided to study human isolated arteries obtained from patients undergoing abdominal surgery. Such an *in vitro* study avoids cardiovascular reflex activity.

3.2 METHOD

Selection of arteries

Arteries were obtained from patients undergoing intra-abdominal surgery at Stobhill General Hospital or the

Southern General Hospital, Glasgow. All surgery was elective in nature and was carried out by the departments of general surgery (gastrectomy or bowel resection) or gynaecology (hysterectomy).

In each case clinical details of each patient were gathered from the hospital case records. In particular diagnosis, blood pressure, drug history and past medical history were recorded. Details of the premedication and anaesthetic agents used were also noted.

Patients were excluded from the study if they were known to suffer from autonomic neuropathy, diabetes mellitus, hypertension, ischaemic colitis, treated or untreated thyroid disease or were receiving drugs prior to surgery known to modify sympathetic vascular activity. Arteries were excluded from the study if they were macroscopically diseased.

The arteries were collected at the time of surgery. Once the bowel resection or hysterectomy was carried out, the surgical specimen was immediatedly dissected and a length of artery removed. In colonic or small intestine surgery, a section of mesenteric artery was removed and in gastrectomy specimens, a section of gastric artery was obtained. Where hysterectomy was carried out, a section of the uterine artery was removed.

The artery specimen was then placed in ice cold physiological Krebs solution which had previously been

gassed with 95% oxygen and 5% carbon dioxide, and thereafter immediately taken to the laboratory for further analysis.

Isolated artery laboratory technique

The arteries were then cannulated with 24 gauge stainless steel wire and the surrounding connective tissue dissected free with the help of a dissecting microscope. Care was taken to minimise damage to the adventitia which contains the sympathetic plexus. The artery was then cut into a helix, at an angle of 30^{o} to the longitudinal axis (85) (Figure 3). This has the effect of producing a spiral strip of vascular smooth muscle which is then placed in an organ bath containing Krebs solution, (Figure 4). The Krebs solution had the following composition (mmol/l): NaCl 118, $NaHCO_3$ 24.97, KH_2PO_4 1.25, KCl 4.75, $CaCl_2$ 2.54, $MgSO_4$ 1.19 and glucose 1.11. The solution was gassed with 95% oxygen and 5% carbon dioxide throughout the experiment. The bath was maintained at $37^{o}C$ with a water jacket and a thermostatically controlled circulation pump (Churchill). Tension was measured under isometric conditions by a Grass FT03 force displacement transducer, and recorded by a Grass Polygraph ink pen recorder (Grass Instruments).

Initial resting tension was set at 1 gm and then the tissue was left for one hour to equilibriate.

The responses of the arterial strips to noradrenaline were then assessed. Noradrenaline (Sigma) was added to the

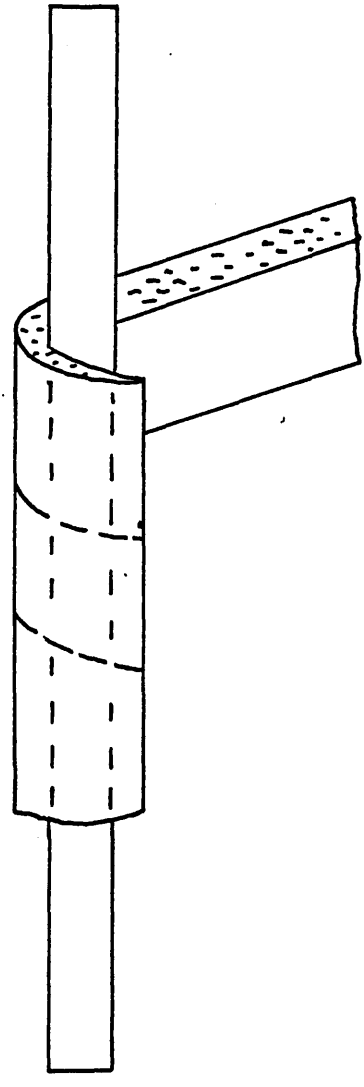


Figure 3: Preparation of the arterial helix.

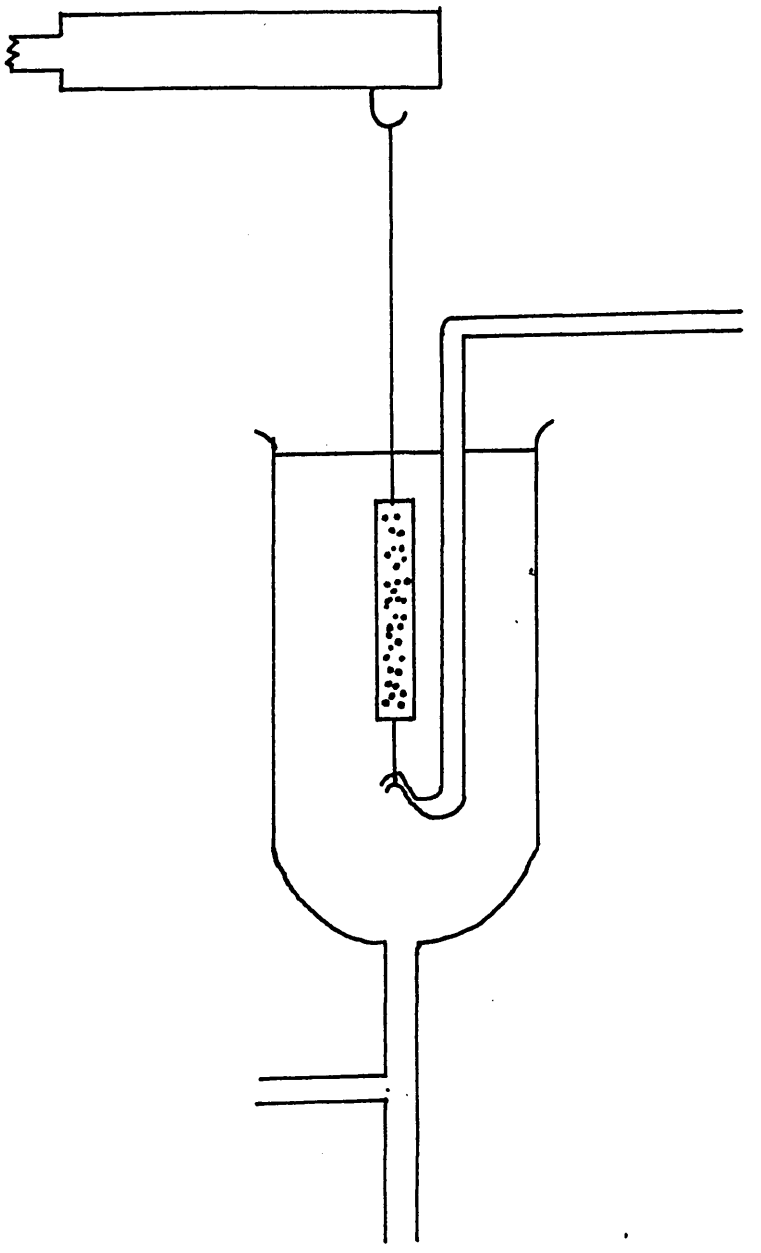


Figure 4: The arterial strip is suspended in a physiological solution maintained at 37 degrees Celcius. Tension is recorded isometrically by a Grass FT03 transducer.

organ bath in a cumulative manner. The concentration of noradrenaline in the bath ranged from 10^{-8}M to 10^{-3}M. Following the production of a maximum response the tissue was washed in fresh Krebs solution. The dose response to noradrenaline was repeated on at least two occasions for each artery. The results were taken as the mean of the responses. Dose responses to KCl were also examined in the initial studies to determine the lowest concentration at which a maximum response could be obtained ($8x10^{-2}$M). The maximum response to potassium was determined in each tissue by increasing the concentration of KCl in the bath to $8x10^{-2}$M.

Statistical analysis

The dose response curves to noradrenaline were drawn using the Hill transformation (86):

$$\log_{10}[\text{Norad}] \text{ is plotted against } \log_{10}\left(\frac{R}{Rmax - R}\right)$$

[Norad] is the concentration of noradrenaline in the bath, R is the magnitude of the corresponding contraction and Rmax is the maximum contraction produced by noradrenaline in that artery. This transformation converts the sigmoid dose-response curve into a straight line. From this line the concentrations of noradrenaline which produce a 50% maximum response, the ED_{50}, and a 5% maximum response, the ED_5, were calculated. The ED_{50} is a measure of the sensitivity of the tissue to the drug. The ED_5 may be regarded as a threshold concentration which produces a minimal response. The responses to noradrenaline are

expressed as a percentage of the maximum response to noradrenaline. This allows comparisons of the sensitivities of arteries of difference sizes.

Responses were also expressed as a percentage of the maximum response to potassium. This allows comparison of the alpha-adrenergic contraction with that produced by a non-adrenergic receptor mechanism.

Results were analysed using age as a continuous variable and also with age divided into three groups, 30-49 years, 50-69 years and above 70 years. Statistical analysis was by non-parametric methods (Spearman Rank Correlation and the Mann Whitney test).

3.3 RESULTS

Patient selection

Arteries were studied from 20 patients whose ages ranged from 35 to 83 years. The clinical details of these patients are shown in Table 10. The arteries were obtained were 7 uterine, 9 mesenteric and 4 gastric. In general pre-operative medication consisted of a narcotic analgesic with atropine and anaesthesia was maintained by nitrous oxide and oxygen with or without halothane. All patients received skeletal muscle relaxants.

Responses to noradrenaline

Figure 5 shows the responses to noradrenaline for three

AGE	SEX	ARTERY	DIAGNOSIS	DRUG HISTORY
36	f	uterine	menorrhagia	none
39	f	uterine	menorrhagia	none
40	f	uterine	pelvic inflam	none
42	f	mesenteric	Crohns disease	salazopyrin
45	f	uterine	menorrhagia	diazepam
46	f	uterine	menorrhagia	phenobarbitone
49	f	uterine	menorrhagia	none
52	f	mesenteric	carcinoma	paracetamol
53	f	uterine	fibroids	none
59	m	mesenteric	carcinoma	none
62	m	mesenteric	carcinoma	none
64	f	mesenteric	carcinoma	none
71	f	mesenteric	carcinoma	none
71	m	mesenteric	diverticular disease	thiazide lactulose
71	f	mesenteric	carcinoma	none
72	f	gastric	carcinoma	none
73	m	gastric	carcinoma	none
77	m	gastric	peptic ulcer	cimetidine
81	f	gastric	carcinoma	none
83	f	mesenteric	carcinoma	none

TABLE 10: Climical details (Isolated artery study)

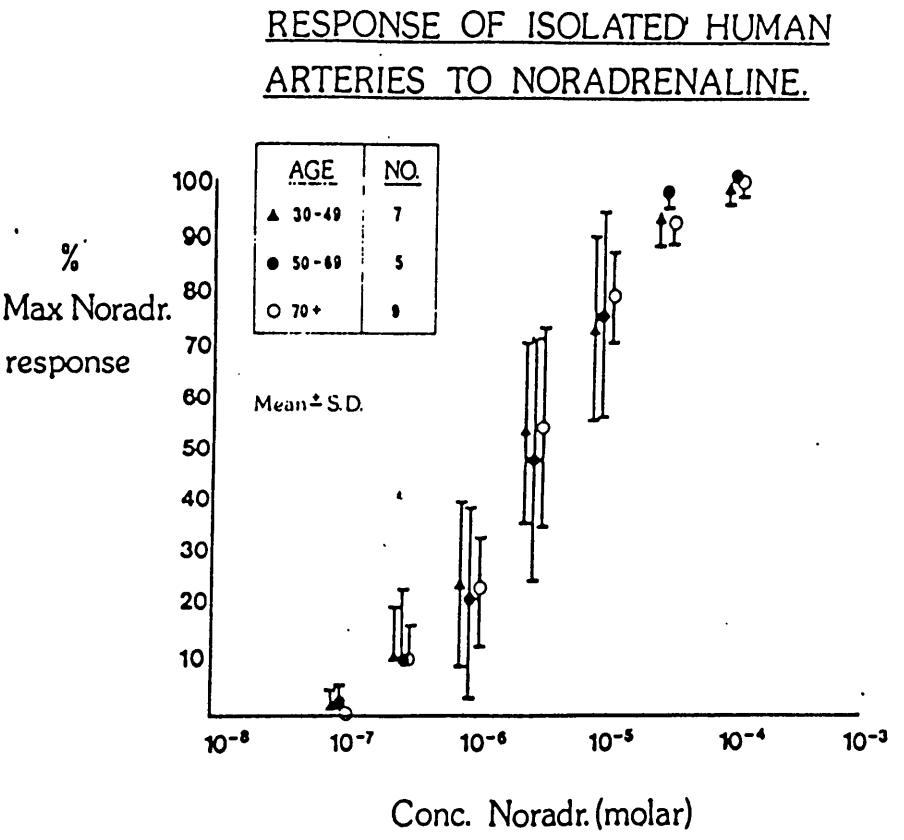


Figure 5: Responses of arterial strips to noradrenaline in the three age groups:
Young (30 - 49 years, n = 7)
Middle aged (50 - 69 years, n = 5)
Elderly (70 + years, n = 8)

age groups; young (median 42, range 36-49 years, n = 7), middle-aged (median 59, range 52-64 years, n = 5) and elderly (median 75, range 71-83 years, n = 8). Noradrenaline response is expressed as a percentage of the maximum noradrenaline response. There was no signficant difference in the dose response curves of the three groups.

The values for ED_{50} and ED_5 for the arteries of the different age groups are shown in Table 11. Once again there is no significant difference between the groups. The responses to noradrenaline, expressed as a percentage of the maximum potassium chloride response were similar at all ages: median 103% (range 75-229%).

Analysis of the subgroups demonstrated that there was no difference between the ages of the males (median 71, range 59-77 years) and females (mean 52, range 36-83 years). There was no differences in the ED_5 or ED_{50} between the sexes. However there was a significant difference between the ages of patients with benign conditions (mean 50, range 36-77 years) and those suffering from malignant diseases (mean 69, range 52-83 years) ($p = 0.007$) but there was no difference in the ED_5 or ED_{50} (Table 12).

The mean ages of the patients from whom uterine arteries were obtained (44 years) was significantly lower than that for the mesenteric arteries (64 years) ($p = 0.006$) and the gastric arteries (76 years) ($p = 0.01$) (Table 13). There was a significant difference in the threshold response to

	AGE median (range)	$ED_5 x10^{-8} M$ median (range)	$ED_{50} x10^{-6} M$ median (range)
YOUNG (n=7)	42 36-49	21.8 5.3-48.2	2.9 1.1-7.8
MIDDLE AGED (n=5)	59 52-64	31.2 7.2-91	2.7 0.9-6.1
ELDERLY (n=8)	72 71-83	40.8 4.5-282	2.8 1.9-22.4

TABLE 11: Noradrenaline response in young, middle aged and elderly: Threshold (ED_5) and sensitivity (ED_{50})

	AGE median (range)	$ED_5 x10^{-8} M$ median (range)	$ED_{50} x10^{-6} M$ median (range)
BENIGN (n=10)	50 * 36-77	27 4.5-57.3	3 1.1-7.8
MALIGNANT (n=10)	69 * 52-83	71 7.2-282	5 0.9-22.4

* $p = 0.007$

TABLE 12: Noradrenaline response in arteries from patients suffering from malignant and benign pathology: Threshold (ED_5) and sensitivity (ED_{50}).

	AGE median (range)	$ED_5 x10^{-8} M$ median (range)	$ED_{50} x10^{-6} M$ median (range)
UTERINE (n=7)	44 * 36-53	22 ** 5.2-42.6	3 1.1-6.4
MESENTERIC (n=9)	64 * 43-83	51 ** 7.2-94.6	4 0.9-7.8
GASTRIC (n=4)	76 * 72-81	91 4.5-282	8 2.0-22.4

* Uterine vs mesenteric age p = 0.006
Uterine vs gastric age p = 0.01

** Uterine vs mesenteric threshold p = 0.03

TABLE 13: Noradrenaline response in uterine, mesenteric and gastric arteries: Threshold (ED_5) and sensitivity (ED_{50}).

noradrenaline (ED_5) between the uterine arteries (mean 2.3 x 10^{-7}mol) and the mesenteric arteries (mean 5.1 x 10^{-7}mol) (p = 0.03). This difference, although statistically significant, is small in comparison with the range of noradrenaline concentrations used in this study. The difference is also small in comparision with the range of threshold concentrations, uterine, 5.3 x 10^{-8} to 4.3 x 10^{-7}M and mesenteric, 7.2 x 10^{-8} to 9.5 x 10^{-7}M.

Systolic blood pressure rose with the age of the patient (p = 0.003). There was no significant difference with diastolic pressure with age. However although the median diastolic pressure was the same in both sexes (90 mmHg) the males had marginally higher diastolic pressures (range 90 - 130mmHg) than the females (range 60 - 95mmHg)(p = 0.04). There was no relationship between the systolic or diastolic pressure and the threshold response (ED_{50}) or the sensitivity of the arteries to noradrenaline (ED_{50}).

3.4 DISCUSSION

Responses of arteries from different sites

In these arteries there was no change in response to noradrenaline over the age range 35 - 83 years. However, these arteries came from different regional circulations. The responses to noradrenaline of human isolated human arteries taken from different sites have been studied and found to be similar (87). Another study demonstrated that human pulmonary and crural vessels had a greater maximum

response with noradrenaline when compared to potassium but in mesenteric arteries, the noradrenaline response was the greater (88). In the uterine, colonic and gastric arteries studied in this series there was no significant difference in the sensitivity to noradrenaline (ED_{50}). There was, however a small difference between the threshold response (ED_5) in the uterine arteries compared with mesenteric vessels. The uterine arteries came from a younger population of patients than the mesenteric or gastric arteries and it is possible that the change in threshold response or other unrecognised differences between the reponses of the different arteries have obscured an age related change.

Influence of sex

A recent study has shown differences in responses to adrenergic drugs between the sexes (89). In men, but not women, brachial artery infusion of clonidine and phenylephrine produced a dose related vaso- constriction and isoprenaline a dose related vasodilatation. In the present study there were no differences in the responses between the sexes. Nevertheless the youngest patients were all female (Table 10) and this disparity also could obscure age related changes.

Influence of disease

Malignancy and diabetes are recognised causes of neurological disease including autonomic neuropathy. There are no reports of the effect of autonomic neuropathy on the responses of human isolated arteries. In these

arteries malignancy was only found in middle aged and oldest patients. Malignancy-induced autonomic changes may have gone unrecognised but there was no clinical evidence of such disorder in the case records of these patients.

Hypertension has been shown to reduce the sensitivity of post-mortem human digital arteries to noradrenaline (90). Other studies using temporal (91) and intra-abdominal (92) arteries taken from living patients have not confirmed this finding. The current study found no relationship between systolic or diastolic blood pressure and the sensitivity to noradrenaline. The diastolic blood pressure recordings were higher in the males, one patient had a pre-operative level of 130mmHg. He had not previously been recognised as hypertensive. The incidence of atherosclerosis increases with age. Moderate or severe atherosclerosis has been shown to inhibit the responses of human isolated coronary arteries to the vasodilatation produced by isoprenaline but not to the vasoconstrictor effect of nordrenaline (93). In this study no artery had macroscopic evidence of atherosclerosis.

Influence of tissue storage

Storage of innervated human arteries (3 - 14 days) has been shown to increase the sensitivity and maximum response to nordrenaline (94). This can probably be explained by denervation supersensitivity due to receptor up-regulation. Stored arteries also tended to produce spontaneous rhythmic contractions which may be due to

unstable membrane potentials or even prostaglandin synthesis. In this study the arteries were examined within 2 hours of surgery.

Site of vascular alpha-adrenoceptors

Noradrenaline produces contraction of vascular smooth muscle by activation of alpha-adrenoceptors. It is now recognised that both alpha-1 and alpha-2-adrenoceptors are present on the smooth muscle of human mesenteric veins (95,96). They may also be present in human mesenteric arteries but appear to be functionally unimportant. It is thought that the alpha-1-adrenoceptor is situated at the neuroeffector junction and is predominantly affected by neuronally-released noradrenaline. On the other hand the alpha-2-adrenoceptors occur near the intima of blood vessels and are stimulated by circulating catecholamines (97). There was no attempt in this study to identify or differentiate between the responses of the different alpha-adrenoceptors.

Tissue preparation

The techniques used to study human isolated arteries have been reviewed by Moulds (98). The spiral strip preparation is easy to prepare and gives excellent and reproducable concentration-effect curves. They have the disadvantage of being less physiological than studies which involve the measurement of tension in ring segments of arteries. Both techniques are less physiological than intraluminal perfusion experiments. However with perfusion studies responses are not as easy to compare

beteeen vessels of different size. There is also a longer delay between administration of drug and response than is seen in the spiral strip method.

Isometric tension was measured in this study even though it is less physiological than isotonic tension (98). Isotonic monitoring is more sensitive but there tends to be an "all or none" response which makes the construction of dose response curves difficult.

Another problem in the interpretation of studies of isolated arteries is the influence of endothelium-derived relaxing factor (EDRF). This is a powerful vasodilator substance (possibly nitrous oxide) that is released by the endothelial cells of almost all blood vessels in both reptiles and mammals (Reviewed: 99). There is some experimental evidence to show that ageing impairs endothelium-dependent vasodilatation (100). The removal of endothelium from rat arteries during *in vitro* studies enhanced the effect of alpha-2-adrenoceptor mediated contraction (101). The effect was less on alpha-1-adrenoceptor mediated contraction. There have been very few studies on the role of endothelium in human isolated arteries. The contractions of human isolated coronary arteries to noradrenaline (102) and isoprenaline (103) are endothelium-independent while contraction to substance P is endothelium-dependent (102). In the present study it is not known whether the endothelium was intact. The use of the 24 gauge stainless steel wire in the production of the spiral strip is more than likely to

cause endothelial damage. This damage would have occurred with all the arteries and would not have been expected to affect any age group more than any other.

Results of other studies on age and alpha-adrenoceptors

Since publication of the results of this study (104) there have been two further studies involving age and the sensitivity of human isolated arteries to nordrenaline. The first involved the responses of isolated digital arteries and metacarpal veins obtained at at post mortem (105). There was a significant positive correlation between patient age and maximum response to noradrenaline when expressed as a percentage of the maximum potassium response in males but not females. There was no change in the sensitivity of the arteries as measured by the ED_{50}. The second study was on the effects of drugs on human isolated coronary arteries (93). These arteries were obtained from kidney donor patients whose hearts were not used for transplantation or from hearts intended for transplantation but not used because of complications in the recipient. A third group of coronary arteries were obtained from patients dying of cardiac disease. The responses to noradrenaline were unaffected by age or disease. Obviously arteries can undergo autolysis post-mortem or alternatively could demonstrate the denervation supersensitivity that occurs with storage (94). However it has been shown that there is no significant change in the sensitivity of postmortem digital arteries for up to 60 hours after death, when the

body is kept in refrigeration (87).

There is therefore general agreement that in the human isolated arteries studied there is no change in the sensitivity to noradrenaline with increasing age. It is clearly possible that the relative contribution of alpha-1 and alpha-2-adrenoceptors to the overall contraction could change with age. This would not be demonstrated with the use of noradrenaline which is a non- selective alpha-agonist. However, due to the insignificant contribution of alpha-2-adrenoceptors to the contraction of human mesenteric arteries (95,96) this seems unlikely. It is therefore reasonable to conclude that there was no change in the sensitivity of the alpha-1-adrenoceptor with increasing patient age.

Unlike the human mesenteric artery, contraction of the the saphenous vein is mediated principally by alpha-2-adrenoceptors. In an elegant study, the effects of the alpha-1- adrenoceptor antagonist prazosin and alpha-2-adrenoceptor antagonist yohimbine were compared in veins precontracted with noradrenaline (80). Yohimbine was less potent with increasing age while there was no change with prazosin. This provides evidence that while the alpha-1-adrenoceptor remains unchanged with increasing age, the postsynaptic alpha-2- adrenoceptor becomes less sensitive. These results are even more interesting because it was shown that the sensitivity of the presynaptic alpha-2-adrenoceptor remained unchanged with age (80). There was no age-related difference in the

potency of alpha-2-adrenergic blockade at inhibiting the overflow of tritium evoked by electrical stimulation from veins pre-incubated with [^{3}H]-noradrenaline. This lack of change in the presynaptic alpha-2-adrenoceptor has been confirmed by an *in vivo* study of noradrenaline kinetics where the the effect of the alpha-2-adrenoceptor agonist clonidine was unchanged with age (81).

There have been few investigations in to the activity of the alpha-1-adrenoceptor with age *in vivo*. There is indirect evidence of a reduction in alpha-1-adrenoceptor activity from a study of groups of young and elderly volunteers (106). Comparisons were made of systemic blood pressure responses to intravenous infusions of phenylephrene before and after 1 mg oral prazosin. Responsiveness was expressed as the dose of phenylephrine required to raise mean arterial pressure by 20 mm Hg (PD_{20}). This dose was significantly higher in the elderly group before prazosin. Prazosin produced comparable falls in blood pressure in both groups. Following prazosin administration the PD_{20} was the same for both groups. The authors cautiously suggest that these results are not inconsistent with an age-related reduction in alpha-1-adrenoceptor responsiveness. A virtually identical study was carried out by Klein et al (107). They came to the conclusion that although prazosin appeared to have a greater effect in the elderly, there was no change in the sensitivity of the alpha-1-adrenoceptor.

Other investigators have tried to eliminate the influence of baroreceptor reflexes which may have complicated these *in vivo* studies (106,107). Vasoconstrictor tone was quantified by measuring the vasodilatation induced in the forearm during an intra-brachial arterial infusion of phentolamine (108). There was no difference in the increase in blood flow observed with age of the volunteer. The use of the non-selective alpha-antagonist phentolamine may have complicated the results by inhibiting, not only post-synaptic alpha-1 and alpha-2- adrenoceptors, but also presynaptic alpha receptors as well, causing an increased release of noradrenaline. Two studies have examined age related responses of the dorsal hand vein to noradrenaline (109) and phenylephrine (110). This technique involves measurement of movement in the wall of a dorsal hand vein with a freely moveable electrical coil which rests over the centre of the vein. The rate of infusion of drugs required was too small to cause measurable systemic effect (e.g. noradrenaline 50 ng/min). This method also allows for measurement of the dose required to produce a maximum response which is very unusual in *in vivo* research. As a result a reasonably accurate measure of drug sensitivity is possible - in this case the dose required to produce a 50% maximum constriction (ED_{50}) was similar to *in vitro* studies. There was no difference in the ED_{50} in young or old volunteers, although the overall dose range was large (1.5 - 360 ng/min). Similarly there was no change in the dose of phenylephrine required to produce 50% or maximum venoconstriction.

The dilator pupillae muscle is the only other alpha-adrenergic innervated tissue, known to me, to be studied for the effect of age. The alpha receptors are presumed to be of the alpha-1 type. The diameter of the dark-adapted pupil is reduced in the elderly perhaps due to decreased sympathetic dilator tone (111). The dilatation produced by a single dose of phenylephrine is greater in the elderly, although the maximum dilatation reached is similar in young and old. This has been interpreted by the authors as evidence for diminished sympathetic nerve activity combined with an increase in postsynaptic alpha-1- adrenoceptor sensitivity.

Noradrenaline causes aggregation in human platelets due to activation of alpha-2-adrenoceptors. Some workers have reported that platelet aggregation occurs more readily in the elderly however others find no such relationship (Review: 112). In the platelet, cyclic adenosine mono-phosphate (cyclic AMP) production is stimulated by prostaglandin E_1 ($PGE_{1)}$ and inhibited by alpha-2-adrenoceptor stimulation. Baseline cyclic AMP levels and production in response to PGE_1 are unrelated to age but the ability of noradrenaline to inhibit cyclic AMP production was reduced in a fit group of healthy elderly (112). This reduction in inhibition with noradrenaline in the elderly was small and was not seen with the more potent agonist adrenaline. It is unlikely to be of clinical importance.

Platelets offer a readily accessible tissue and several

groups have tried to study the effects of increasing age on adrenoceptor number. This is accomplished by measuring the binding of radio-labelled agonists or antagonists to intact platelets or to a preparation of platelet membranes (113). Rather surprisingly, for what should be a relatively standard experiment, the results of these studies have been conflicting (Review: 114). There are reports of a reduction (115) or an increase (116) in the maximum number of binding sites with increasing age, while other studies have not demonstrated any change (112,117,118,119). The use of different ligands in these studies may help to explain the variation in results. However, using [^{3}H]-yohimbine as the ligand, an increase (116), a reduction (115) and no change (112,118) in platelet receptor number with age have all been demonstrated. Overall, there is no strong evidence to suggest a consistent change in receptor number with age.

Considerable caution should be exercised in the interpretation and extrapolation of results from platelet alpha receptor to alpha-1-adrenoceptors at other sites (120). The platelet alpha-2-adrenoceptor may be similar to other non-innervated peripheral receptors, for example the post junctional alpha-2-adrenoceptor. Both appear to respond to circulating catecholamines and will be regulated by the level of peripheral sympathoadrenal activity. In contrast peripheral presynaptic receptors will be under intrasynaptic regulation.

To date our knowledge of the influence of ageing on the

sensitivity of human alpha-adrenoceptors is summarized in Table 14. There appears to be no change in the sensitivity of the adrenoceptors principally associated with the neuroeffector junction: the postsynaptic alpha-1-adrenoceptor and presynaptic alpha-2-adrenoceptor. There is evidence for a reduction in the sensitivity of the post synaptic alpha-2-adrenoceptor and for a slight reduction of the effect of the platelet alpha-2-adrenoceptor. There appears to be no change in the number of alpha-2-adrenoceptors on platelets. Both the post-synaptic alpha-2-adrenoceptor and platelet adrenoceptor are under the control of circulating catecholamines. Why then does age have such a differential effect on the ageing of human adrenoceptors ?

Continued exposure to a drug or hormonal agonist often leads to a blunted response to that agonist (113). It would be attractive to relate the increased circulating levels of noradrenaline seen in the elderly to down-regulation of the postsynaptic alpha-2-adrenoceptor and to the lesser inhibition of cyclic AMP production seen in elderly platelets exposed to noradrenaline. Attempts have been made to demonstrate down-regulation of platelet adrenoceptors *in vitro*. Incubation of platelets with high concentrations of adrenaline resulted in decreased radio-ligand binding of [^{3}H]-dihydroergotryptine, suggesting loss of active receptor sites (121). However this could not be repeated using the ligand [^{3}H]-yohimbine (122). This may be due to the non-specific nature of dihydroergotryptine which is known to bind to other

IN VIVO (PRESYNAPTIC)			
Alpha-2	Saphenous vein	No change	(80)
IN VIVO (POSTSYNAPTIC)			
Alpha-1	Arteries and veins	No change	(93,104,105)
Alpha-2	Saphenous vein	Reduction	(80)
PLATELET LIGAND BINDING STUDIES			
Alpha-2		Increase	(116)
		No change	(112,117,118, 119)
		Reduction	(115)
IN VIVO (PRESYNAPTIC)			
Alpha-2		No change	(81)
IN VIVO (POSTSYNAPTIC)			
Alpha-1		Decrease	(106, 111)
		No change	(107,108,109, 110)
Beta-2		No change	(36)

TABLE 14: Summary of studies of ageing and human adrenoceptors.

receptors, e.g. dopamine receptors. Platelets also accumulate adrenaline during *in vitro* incubation. This retained adrenaline can later block the binding of radio-ligands leading to an incorrect conclusion that down regulation has occurred (113). There is now evidence from animal studies that the ageing may be associated with a loss of the ability to down-regulate autonomic receptors. Beta-adrenoceptors on young rat lymphocytes down-regulate with the acute stress of immobilisation (123). Receptors accessable to surface ligand binding were reduced by 45% (total receptor numbers remained unaltered). This was interpreted as an internal redistribution of receptors in response to stress. With middle aged and older rats (12 and 26 months) there was no redistribution of beta-receptors with age.

The regulatory influences on the alpha-adrenoceptors at the neuroeffector junction are difficult to define. Nothing is known about the influence of age on the synaptic concentrations of nordrenaline. The evidence of increased spillover of noradrenaline in the forearm circulation has been presented (Chapter 2). This does not necessarily mean that there is an increased concentration of neuro-transmitter at the synapse as clearly it can escape from the synapse into the surrounding cells, extracellular space and eventually into the circulation.

In summary, the evidence from human experiments virtually all agree with the findings of this study, that there is no change in the sensitivity of the vascular post-synaptic

alpha-1-receptor with age.

I have not included in this discussion references to many animal studies. These frequently compare immature with mature animals rather than mature with senescent. Futhermore the relevance of studies on aged rats and other animals to human physiology remains unclear.

CHAPTER 4

THE INFLUENCE OF AGE ON THE DISPOSITION AND EFFECT OF PRAZOSIN

THE INFLUENCE OF AGE ON THE DISPOSITION AND EFFECT OF PRAZOSIN

4.1 INTRODUCTION

Increasing age is associated with a rise in the plasma concentration of noradrenaline (Chapter 2) but there is no change in the sensitivity of the vasoconstrictor alpha-1-adrenoceptor (Chapter 3). These observations not only relate to the physiology of ageing but also may be important to the actions of adrenergic drugs. Prazosin is a selective alpha-1-adrenergic antagonist, having high affinity but no intrinsic activity. The higher plasma concentration of noradrenaline in the old could in theory lead to a relative resistance to the hypotensive action of prazosin.

In man, prazosin also undergoes first pass metabolism with only 40-60% of an orally administered dose reaching the systemic circulation unchanged (124). This study was designed to examine the effect of increasing age on the disposition of prazosin and the associated changes in blood pressure and pulse in groups of young and elderly volunteers who were living independently in the community.

4.2 METHODS

Volunteer selection

Fourteen volunteers took part in this study. Seven

subjects were young and their ages ranged from 22 to 32 and the other seven were elderly with an age range of 66 to 78 years. All were living at home independently. The young were recruited from hospital staff and the elderly were recruited by the help of their General Practitioners. All subjects underwent a physical examination prior to the study and none had significant pathology. No volunteer had a past history of serious illness or was receiving any medication. All had normal haematological, biochemical indices and had normal electrocardiograms.

Experimental technique

Each subject was studied on two occasions at least 1 week apart. On one study day subjects received 1 mg of prazosin orally 3 hours after a light breakfast and 5ml blood samples were drawn through an indwelling venous cannula at 0, 15, 30, 60, 90, 120, 150, 180, 240, 300, 360, 420 and 480 minutes with the subject supine. On the other study day the young subjects received 1 mg and the elderly subjects 0.5 mg of prazosin gluconate (Pfizer) by rapid intravenous injection and samples were drawn from the other arm at the times given above and in addition at 1, 2, 5, 10, 20 and 40 minutes.

Erect and supine blood pressure was recorded frequently during the injection of prazosin and at regular intervals during both study days using an automated sphygmomanometer (Bosomat).

Plasma prazosin analysis

Whole blood was stored at -20°C until analysed by high pressure liquid chromatography using fluorescence detection (125). This method has a coefficient of variation of 5 - 10 % over the concentration range 2 nmol/l to 100 nmol/l. All samples were analysed within 4 weeks of being collected. Prazosin is stable in whole blood stored at -20°C for at least 6 months (125).

Pharmacokinetic analysis

Blood concentration *v* time data following intravenous administration were fitted to a biexponential function of the form:

$$Cp(t) = Ae^{-alpha(t)} + Be^{-beta(t)}$$

using a nonlinear least squares regression programme based on the Marquardt algorithm (126) with reciprocal weighting on a Varian V70 series computer. The slope of the disappearance phase after oral prazosin was estimated by fitting the terminal log-linear phase of the prazosin concentration-time curve to a monoexponential decay function using the nonlinear least squares computer programme. The area under the concentration versus time curve during the blood sampling period was calculated using the trapezoids rule. The extrapolated area beyond the last data point was calculated by dividing the concentration at the time of the last sample by the terminal slope obtained from the computer fit of the data following the intravenous injection. The total area under the curve (AUC) for oral or intravenous dosing was the sum of these two areas.

The absolute bioavailability (F), defined as the fraction of the oral dose which reaches the systemic circulation in unchanged form, and clearance (Cl) were determined by the following expression:

$$F = \frac{AUC_{oral}/dose_{oral}}{AUC_{i.v.}/dose_{i.v.}}$$

$$Cl = \frac{Dose_{i.v.}}{AUC_{i.v.}}$$

The first pass metabolism of prazosin is not saturable over a wide dose range: the AUC/mg following 5 mg to young subjects (127) is not greater than the AUC seen in the present study. The coefficients of the biexponential equation were used to calculate the volume of distribution at steady state according to standard techniques assuming a two compartment open model with elimination from the central compartment.

Statistical analysis

Comparisons between young and old were performed using non-parametric analysis: the Wilcoxon rank sum test and the Mann Whitney test.

4.3 RESULTS

The young subjects ranged in weight from 61 to 87.2 kg (mean 77.5) and the elderly from 59.5 to 98 kg (mean 77.8).

Blood pressure changes following oral prazosin are shown in Figures 6-9 (changes expressed as percentage falls from baseline). In general, there were no differences between the falls in pressure observed between the young and elderly. Supine blood pressure fell less than that measured in the erect position.

Table 15 shows the coefficients of the biexponential equation to which the intravenous data was fitted. The elimination half-life of prazosin was the same following intravenous and oral administration both for the young group who received the same dose by each route (123 min i.v.; 126 min oral) and for the elderly subjects who received a lower intravenous compared to oral dose (194 min i.v.; 188 min oral).

The half-life, clearance, volume of distribution and bioavailability of prazosin in young and elderly subjects are shown in Table 16. Half life was significantly longer in the elderly subjects but clearance did not differ between the two groups whether when expressed in terms of unit weight (Table 16) or in absolute terms (308 $\pm$ 80 $ml.min^{-1}$ in the young, 274 $\pm$ 84 $ml.min^{-1}$ in the elderly; $P > 0.1$). Volume of distribution at steady state was

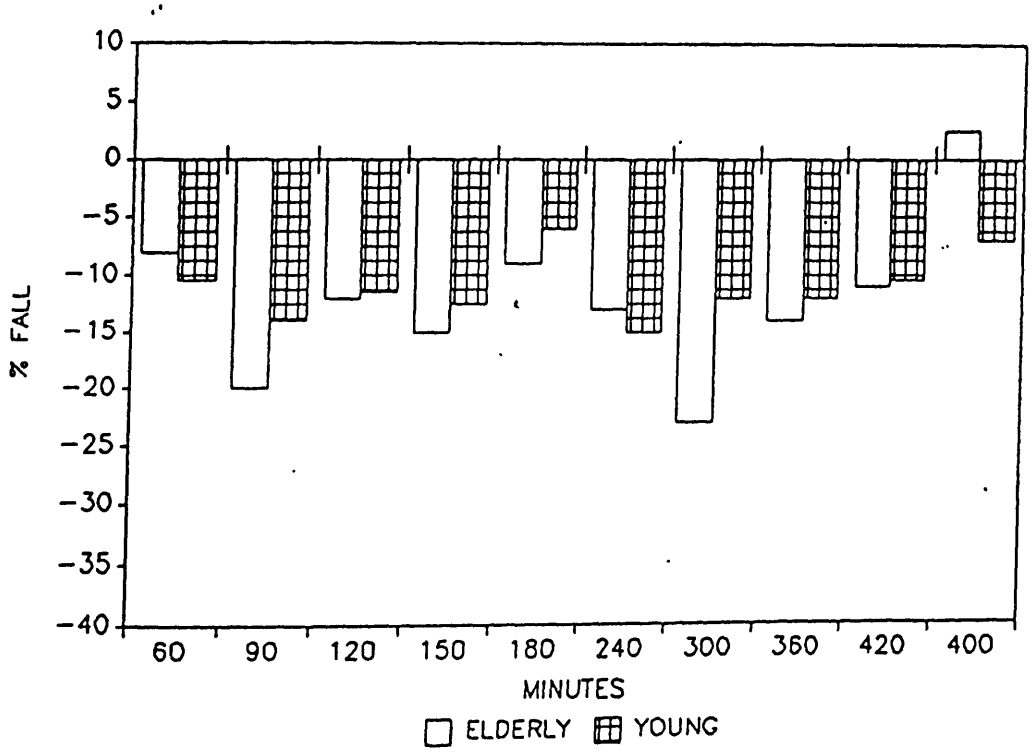


Figure 6: Percentage fall in systolic blood pressure (erect position) after 1mg oral prazosin.

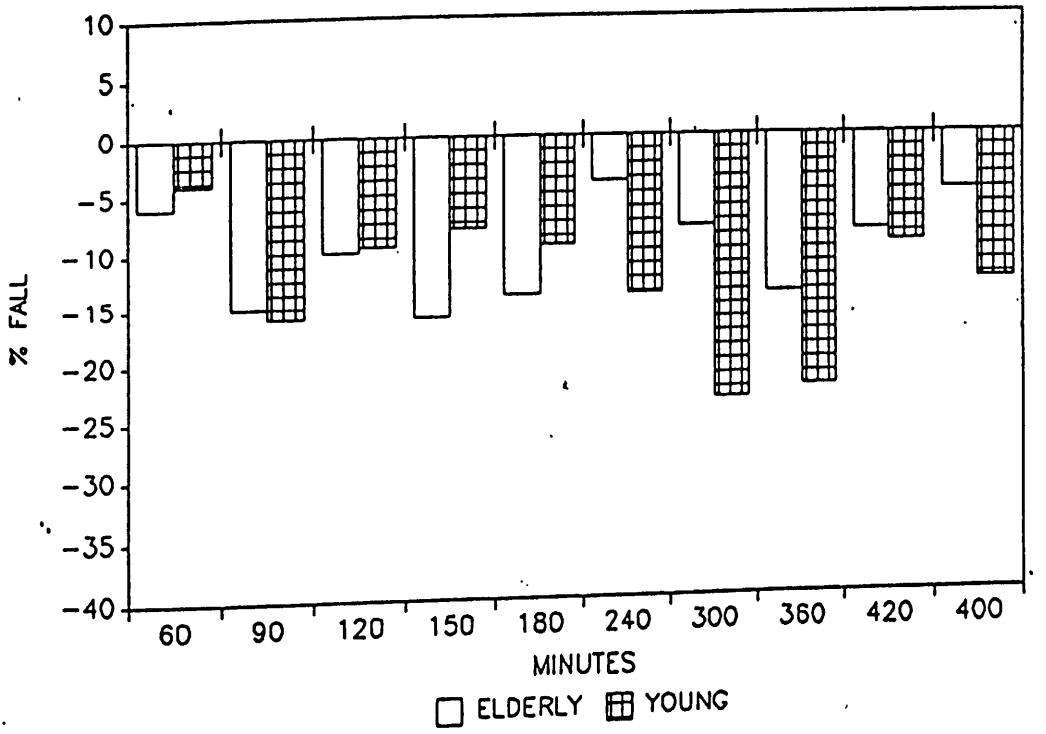


Figure 7: Percentage fall in diastolic blood pressure (erect position) after 1mg oral prazosin.

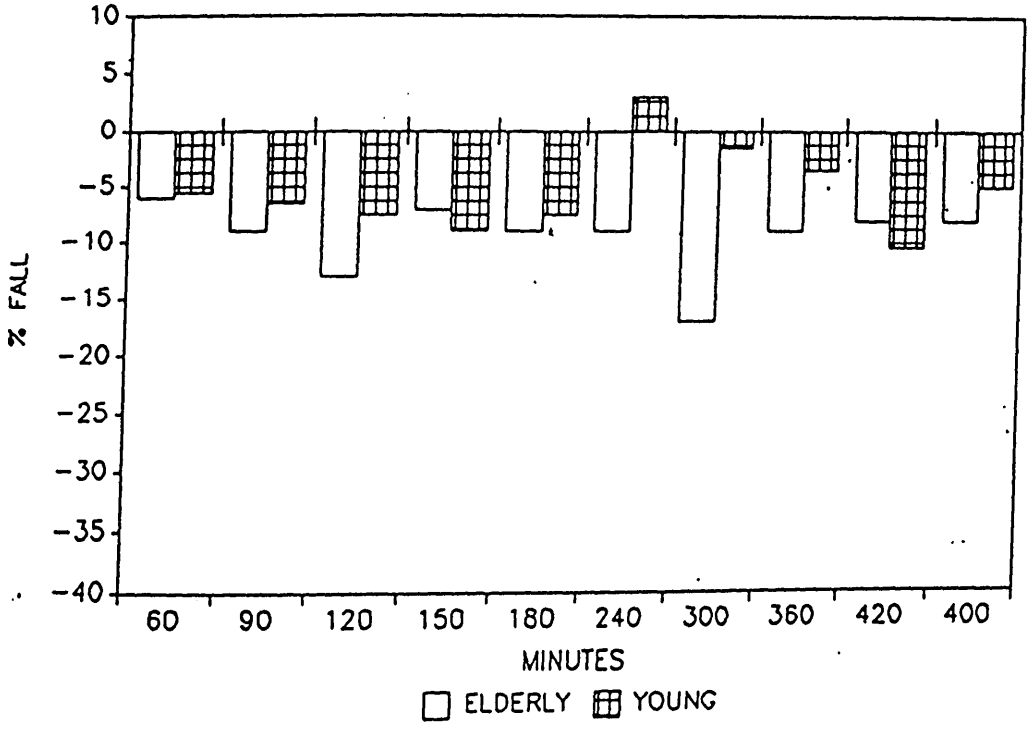


Figure 8: Percentage fall in systolic blood pressure (supine position) after 1mg oral prazosin.

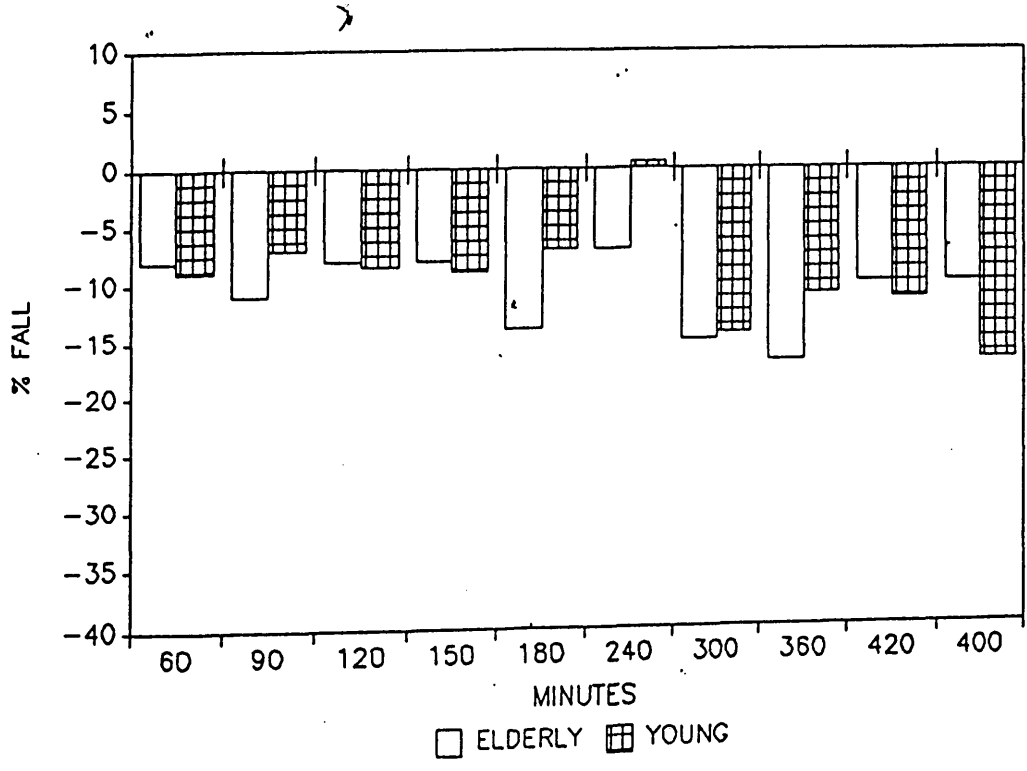


Figure 9: Percentage fall in diastolic blood pressure (supine position) after 1mg oral prazosin.

	A	alpha	B	beta
Young	202 ±187	0.232 ±0.187	45.8 ±13.7	0.00573 ±0.0009
Old	84.5 ±42.4	0.172 ±0.13	18.0 ±3.1	0.00368 ±0.0006

TABLE 15: Coefficients of the equation $Cp(t) = Ae^{-alpha\ t} + Be^{-beta\ t}$. Young subjects received 1 mg and elderly subjects 0.5 mg of prazosin intravenously. Data are expressed as means ± s.d. The mean coefficients of determination for the fitted curves were 0.97 (young) and 0.933 (old). A and B have the units of $nmol^{-1}$; alpha and beta have the units of min^{-1}

	$T_{1/2}$ (min ± sd)	Cl ($ml.min^{-1}.kg^{-1}$ ± sd)	V_Dss (l^{-1} ± sd)	F (± sd)
Young	123 ** ± 19.4	3.94 ± 0.73	0.63 * ± 0.14	0.68 * ± 0.17
Elderly	194 ** ± 36.0	3.53 ± 1.0	0.89 * ± 0.26	0.48 * ± 0.16

** $P < 0.01$ * $P < 0.05$

$T_{1/2}$ = elimination half life

Cl = clearance

V_dss = volume of distribution at steady state

F = bioavailability

TABLE 16: Pharmacokinetic indices of prazosin in young and elderly subjects (mean ± sd)

significantly greater in the elderly both when in expressed in absolute terms (49.4 ± 14.8 l in the young and 69.2 ± 20.0 l in the elderly; $P < 0.05$) and when analysed in terms of unit weight (Table 16).

Bioavailability was significantly lower in the elderly than the young, less than half the orally administered drug reaching the systemic circulation in unchanged form (Table 16). The total area under the curve per mg ($umol.min^{-1}$) were as follows: Intravenous: 9.0 ± 2.5 young; 10.2 ± 2.9 old; oral: 6.1 ± 1.7 young; 4.6 ± 0.4 old.

4.4 DISCUSSION

The elimination half life of many drugs that undergo hepatic degradation has been shown to be prolonged in old age (4,82). However a knowledge of half-life alone provides only limited information about the changes in disposition that might occur in the elderly. This is because half-life ($T_{1/2}$) is itself determined by both clearance (Cl) and volume of distribution (V_d):

$$T_{1/2} = \frac{V_d}{Cl} \times 0.693$$

Volume of distribution and clearance can be determined properly only from data produced by intravenous

administration. Few pharmacokinetic studies in the elderly have been performed using intravenous drug administration, e.g: antipyrine (128), diazepam (22,24), propranolol (19), chlormethiazole and lignocaine (129), nifedipine (130), aminophylline (131), nitrazepam (132) labetolol (133), levodopa (134). Increasing age had no consistent effect on the disposition of these drugs. Antipyrine, nifedipine, levodopa and chlormethiazole showed a decreased clearance in the elderly. There is no change in the clearance of labetolol or nitrazepam. Aminophylline clearance is unchanged with age in healthy non-smoking volunteers. Nitrazepam, diazepam and lignocaine showed a significant prolongation of elimination half-life in the elderly but this was due to an increase in the volume of distribution rather than a fall in the clearance. There is no change in the elimination half life of levodopa with age - but this is because there is a parallel fall in both the rate of clearance and the volume of distribution with age.

This study demonstrates that prazosin also has a prolonged elimination half-life in the elderly which is due to an increase in the volume of distribution. The volume of distribution is largely determined by the physico-chemical characteristics of the drug. Lipophilic drugs will be able to distribute widely across membranes and also accumulate in adipose tissue. Ageing is associated with a reduction in lean body mass and increase in fat (23). It might therefore be expected that lipophilic drugs would have an increased volume of distribution in the elderly.

Prazosin is only modestly lipophilic (135) and the 40% increase in the volume of distribution in the elderly is small in comparison with the 200-300% increase in the volume of distribution of the much more lipophilic drug diazepam (24).

Prazosin, diazepam and lignocaine have in common not only the increase in the volume of distribution with no change in clearance in the elderly, but also their route of elimination: demethylation. However it is not possible to assume that demethylation is unaffected by age as chlordiazepoxide which is also metabolised by this pathway, has reduced clearance in the elderly (136).

The clearance of propranolol in the elderly has been studied by two groups of investigators. Castleden and George (19) demonstrated a reduction in clearance with age while Shneider et al (20) demonstrated that there was no change. The most likely explanation for this discrepancy is that the first study compared young fit volunteers with institutionalised, chronically disabled elderly. The second study examined young and elderly volunteers who were living at home. It is therefore than likely that the original conclusion that clearance is reduced by age alone is wrong - and that clearance is reduced by age in combination with disease. In the present study, all volunteers were living at home independently and none was receiving any medication.

The bioavailability of a drug is related to the degree of

gastrointestinal absorption as well as to the degree of first pass metabolism. This study showed that in the elderly, there was 40% less unchanged drug reaching the systemic circulation compared with the young. It is probable that the enzymic pathways responsible for first pass metabolism are the same as that for systemic clearance. As there was no change in the systemic clearance of prazosin it is unlikely that the first pass metabolism is significantly altered with age. It is therefore possible to conclude that there is a reduction in the gastrointestinal absorption of prazosin with increasing age. The physiological changes that occur with age may help to explain this observation since there is a decrease in the liver mass (137), a fall in intestinal blood flow (138) and the number of gastro-intestinal absorbing cells probably also decrease (139). There does not appear to be any change in the hepatic concentration of drug metabolising enzymes (140).

In this study there were only minor differences in the fall in blood pressure between the young and elderly groups. Basal blood pressure was higher in the elderly than the young and falls were expressed as a percentage of basal. The falls in blood pressure with intravenous administration of prazosin were similar, but the dose given to the elderly was half that given to the young. There were no consistent differences in the responses to 1mg oral prazosin given to young and old. This is in contrast to a more recent study where elderly volunteers had a significantly greater fall in supine blood pressure

when given 1 mg prazosin, erect blood pressure was not assessed (107).

Since the publication of the results of this study (141) there has been one other study into the effect of age on the disposition of prazosin (142). This study involved two groups of normal volunteers: 10 young (19-30 years) and 5 older (54-62 years). No change was seen in the area under the concentration-time curves or in the elimination half-life, between these two groups. The use of oral only dosing ignores any changes in bioavailability that may have occurred. The lack of change in the elimination half-life may reflect the relative youth of their 'older' volunteers when compared to the elderly in the present study.

The pharmacokinetic changes for prazosin described here are unlikely to be of major clinical importance. A prolonged elimination half-life caused by an increased volume of distribution will result in a longer time to reach steady state plasma concentration with regular dosing - but the final concentration will not differ from that seen in the young. However even the time to steady state is of little clinical importance for a drug with such a short half life as prazosin. The reduction in absorption is also unlikely be of significance for a drug where dose is titrated against effect.

A more important conclusion of this study is the importance of using of intravenous drug administration in studies which attempt to measure drug clearance.

CHAPTER 5

THE INFLUENCE OF AGE ON THE DISPOSITION AND HYPOTENSIVE EFFECT OF TRIMAZOSIN, ACEBUTOLOL AND TRIMAZOSIN

THE INFLUENCE OF AGE ON THE DISPOSITION AND EFFECT OF TRIMAZOSIN, ACEBUTOLOL AND TRIMAZOSIN

5.1 INTRODUCTION

The study described in this chapter adopts an experimental method which combines the measurement of drug effect and relates this to plasma drug concentration (54). Using this approach it is possible to describe age related changes not only in the pharmacokinetics (drug distribution and metabolism) but also in the pharmacodynamics (effect) of drugs.

Three antihypertensive drugs were studied: trimazosin, an alpha-1-adrenergic antagonist; acebutolol, a beta-1-adrenoceptor antagonist; and tolmesoxide, a peripheral vasodilator which acts directly on vascular smooth muscle.

Trimazosin is a quinazoline derivative, related to prazosin. It has a longer elimination half-life in the young than prazosin (143) and is extensively metabolised to 1-hydroxytrimazosin. This metabolite also has antihypertensive properties (144).

Acebutolol first undergoes hydrolysis and then N-acetylation to form diacetolol which is inactive (145).

Tolmesoxide undergoes hepatic metabolism to its sulphone metabolite which is then O-demethylated and excreted in

the urine as a glucuronide conjugate (146).

This single blind, placebo controlled study was designed to examine the effect of age on the pharmacokinetics and pharmacodynamics of trimazosin, acebutolol and tolmesoxide. Drug effect was measured by the fall produced in systolic blood pressure.

5.2 METHODS

Volunteer selection

The study was undertaken in 30 subjects aged 21 to 77 years (15 male and 15 female) none of whom had any serious illness and who all had normal haematological and biochemical parameters. No volunteer smoked or was receiving drug therapy (including oral contraceptives). The subjects were recruited so as to obtain as far as possible a continuous spectrum of age; they were however subsequently arbitrarily divided into equal three groups of ten (5 male, 5 female) the three groups being classified as young, middle aged and elderly (Table 17).

Experimental technique

The subjects attended fasting at 8 a.m. on 4 separate occasions with intervals of at least a week. An indwelling catheter was inserted into the antecubital vein and the subjects were then rested for at least half an hour at which time baseline measurements of erect and supine blood pressure and heart rate were made.
The drugs were administered in random order by intravenous

		Age years	Weight kg	Male:Female
Young	mean	24	61.0	5 : 5
	s.d.	2	10.8	
	range	21-28	48-77	
Middle aged	mean	43	68.9	5 : 5
	s.d.	5	12.8	
	range	39-52	52-85	
Elderly	mean	69	64.7	5 : 5
	s.d.	5	9.5	
	range	62-77	55-79	

TABLE 17: Volunteer Demographic Data for study of pharmacokinetics and dynamics of trimazosin acebutolol and tolmesoxide

injection into the other arm of either: placebo; trimazosin 100 mg; acebutolol 20 mg; and tolmesoxide 100 mg.

Blood samples for drug analysis and blood pressure measurements were made at regular intervals over the following 10 hours.

Pharmacokinetic and pharmacodynamic analysis

The drug and metabolite concentration data were fitted to a series of one and two compartment models and the parameters estimated by extended least squares fitting (147). The goodness of fit of each model was judged using both the coefficient of determination and the z value for runs in the residuals. The models considered include those which incorporate an effect associated with both parent drug and metabolite (144). Model comparison was based on the Akaike Information Criterion values (148). From these fits the following parameters were derived; drug clearance, volume of distribution, drug elimination half-life and the ratio of the area under the metabolite and drug concentration time curves which represents a measure of the relative clearance of the metabolite.

Conventional analysis of blood pressure and heart rate was made on the basis of repeated measures analysis of variance and all results are expressed as mean $\pm$ 1 standard deviation. Pharmacokinetic and pharmacodynamic data was examined by two way analysis of variance and linear regression.

Trimazosin

Trimazosin and its major metabolite 1-hydroxytrimazosin were measured simultaneously by high pressure liquid chromatography (HPLC) (149). The limit of detection of the assay for the parent drug and metabolite is 1.0ng.ml^{-1} and the inter-assay coefficients of variation across the assay range was 5.2% and 4.6% respectively.

The drug and metabolite concentrations were more appropriately fitted to an integrated 3-compartment model with two compartments describing drug disposition and one compartment for the metabolite concentrations. The most appropriate effect model was one in which effect was attributed to both parent drug and metabolite. This confirmed previous findings with intravenously administered trimazosin (144).

Acebutolol

The plasma concentrations of acebutolol and its major metabolite diacetolol were determined by a sensitive, specific HPLC assay (150). The limit of detection for both drug and metabolite was 20 ng.ml^{-1} while the respective inter-assay coefficients of variation across the assay range were 4.2% and 5.6%.

The most appropriate kinetic model combined two compartments for drug disposition and one for metabolite arising from the central drug compartment.

Tolmesoxide

Tolmesoxide and its major sulphone metabolite were measured by an HPLC assay (151). The assay limit for parent drug and metabolite was 5 $ng.ml^{-1}$ and the inter-assay coefficients of variation across the assay range were 6.8% and 7.5% respectively. The most appropriate model was a two compartment (drug) and one compartment (metabolite).

5.3 RESULTS

Trimazosin

The pharmacokinetic parameters derived from simultaneously fitting the plasma drug and metabolite concentration data are given in Table 18. Although the drug clearance in the elderly (0.6 ± 0.1 $ml.min^{-1}.kg^{-1}$) appeared to be less than that in the young (0.9 ± 0.2 $ml.min^{-1}.kg^{-1}$) the difference did not reach statistical significance. There was a related apparent difference in the ratio of the areas under the drug and metabolite concentration curves. The ratio for the young was 1.1 ± 0.2 in the young and 0.8 ± 0.1 in the old, again this did not reach statistical significance. However when age was treated as a continuous spectrum with the application of linear regression analysis there was a significant negative correlation with age ($P < 0.005$, $r = -0.59$) as shown in Figure 10. There was also a corresponding negative correlation between the ratios of the areas under the drug and metabolite concentration curves and age ($p < 0.005$, $r = -0.54$), shown in Figure 11.

		Clearance	AUC_M/ AUC_D	$Beta_{T1/2}$ (min)	Vd_{SS} ($l.kg^{-1}$)
Young	mean	0.9	1.1	160	0.2
	s.d.	0.2	0.2	40	0.04
	range	0.5-1.1	0.9-1.3	110-221	0.1-0.3
Middle aged	mean	0.8	0.8	188	0.2
	s.d.	0.3	0.2	57	0.06
	range	0.3-1.3	0.6-1.2	147-337	0.1-0.3
Elderly	mean	0.6	0.8	177	0.1
	s.d.	0.1	0.1	34	0.03
	range	0.3-0.9	0.6-0.9	144-225	0.1-0.2

AUC_M Area under metabolite time/concentration curve.

AUC_D Area under drug time/concentration curve.

$Beta_{T1/2}$ Elimination half life.

Vd_{SS} Volume of distribution at steady state.

TABLE 18: Trimazosin pharmacokinetic parameters

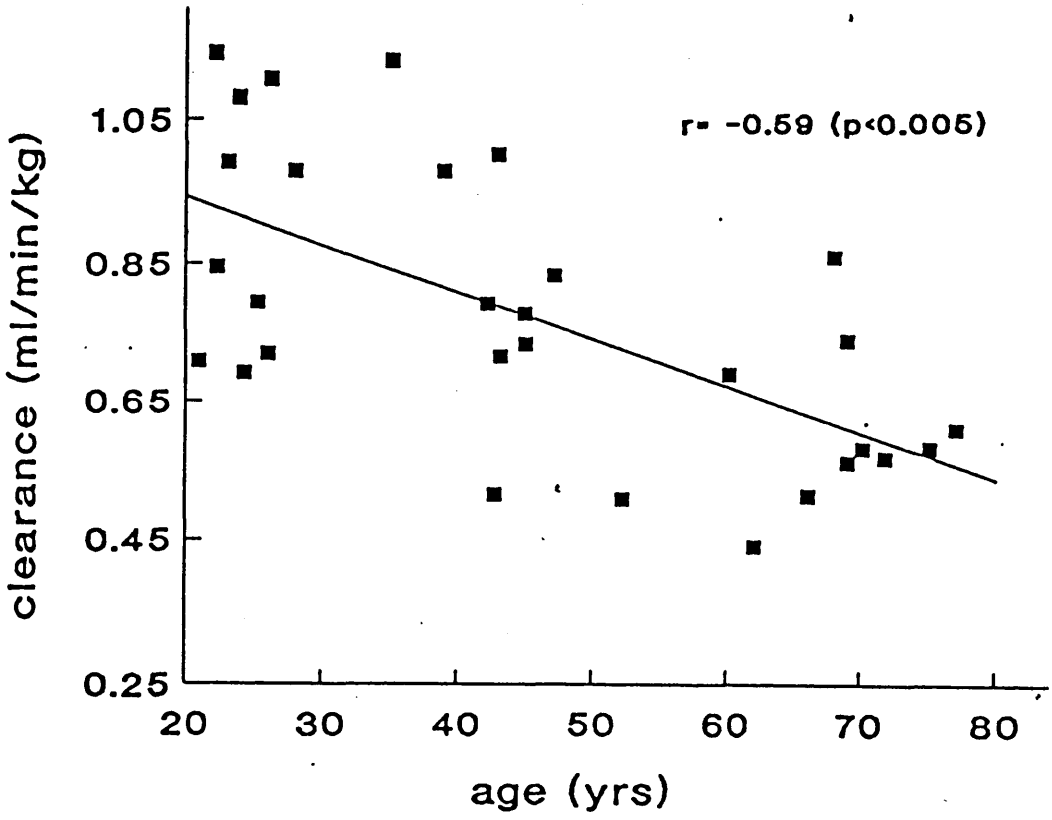


Figure 10: Age and the clearance of trimazosin. Linear regression analysis.

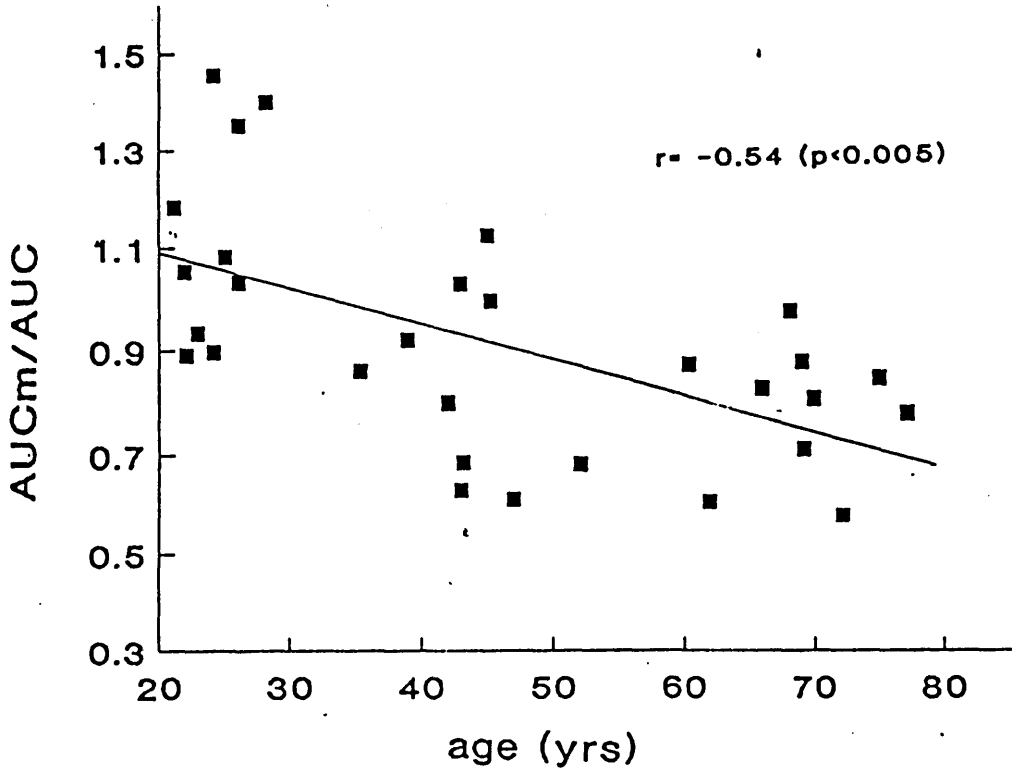


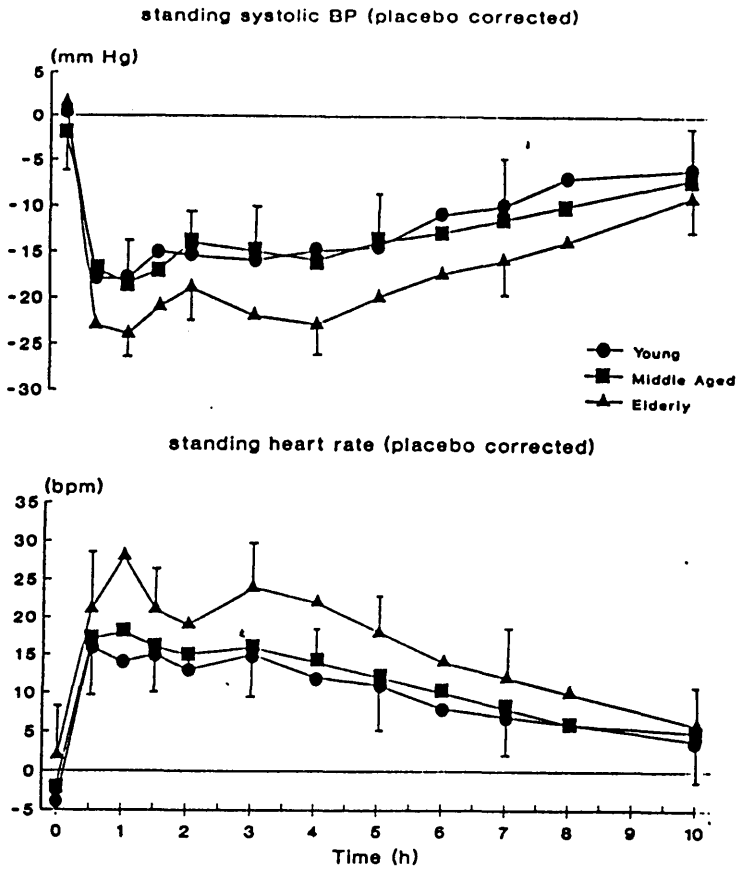
Figure 11: Relationship between age and the ratios of the areas under the trimazosin and hydroxytrimazosin time-concentration curves. Linear regression analysis.

Examination of the mean blood pressure profiles demonstrated a significantly greater effect in the elderly subjects than in the middle aged and young ($P < 0.05$), Figure 12. A biphasic response was shown in all subjects by the concentration effect model. The first response being due to the drug and the second to the active metabolite. The mean effect model parameters are given in Table 19 and Table 20. There were no significant effects of age upon the sensitivity to trimazosin expressed in terms of placebo corrected fall in erect systolic blood pressure per unit of drug concentration. There were no significant effects of age upon Keq, the first order rate constant describing the disequilibrium between concentration and effect. (Effectively this reflects the time lag between the plasma concentration and the corresponding lowering of blood pressure.) Similarly there was no relationship between the sensitivity to the drug and age when linear regression analysis was applied.

The greater response to trimazosin observed in the elderly can be attributed to the decline in drug clearance with age. The sensitivity to this alpha-1-adrenoceptor antagonist did not change with age.

Acebutolol

The pharmacokinetic parameters for acebutolol are shown in Table 21. There were no significant differences between; clearance, elimination half-life, volume of distribution or the ratio of the area under the drug and metabolite concentration curves (relative metabolite clearance).



(mean + standard deviation)

Figure 12: Mean change in systolic blood pressure and pulse rate in response to intravenous trimazosin: young, middle aged and elderly volunteers.

		Responsiveness ($mmHg.ng^{-1}.ml^{-1}$)	
		Drug	Metabolite
Young	mean	1.5	3.1
	s.d.	0.6	1.0
	range	0.9-2.3	2.0-5.1
Middle aged	mean	1.7	3.3
	s.d.	0.6	0.7
	range	0.7-2.3	2.5-4.6
Elderly	mean	1.7	3.6
	s.d.	0.6	0.8
	range	0.9-2.8	2.5-3.6

TABLE 19: Trimazosin responsiveness (placebo corrected fall in erect systolic blood pressure per unit plasma drug/metabolite concentration.

		keq ($l.min^{-1}$)	
		Drug	Metabolite
Young	mean	0.10	0.01
	s.d.	0.01	0.004
	range	2.00-0.11	0.008-0.015
Middle aged	mean	0.10	0.01
	s.d.	0.01	0.004
	range	0.08-0.11	0.008-0.014
Elderly	mean	0.09	0.01
	s.d.	0.01	0.004
	range	0.08-0.11	0.008-0.013

TABLE 20: Trimazosin "keq" - A measurement of the discrepancy between the plasma concentration and the hypotensive effect of the drug.

		Clearance	AUC_M/AUC_D	$Beta_{T1/2}$ (min)	Vd_{SS} (l/kg)
Young	mean	387	0.5	184	1.3
	s.d.	168	0.2	63	0.3
	range	192-653	0.3-0.6	115-307	0.9-1.6
Middle aged	mean	393	0.6	175	1.2
	s.d.	122	0.4	52	0.56
	range	158-592	0.3-1.5	119-293	0.7-2.2
Elderly	mean	402	0.5	175	1.2
	s.d.	132	0.2	74	0.3
	range	265-638	0.04-0.9	93-351	0.8-1.7

AUC_M Area under metabolite time/concentration curve.

AUC_D Area under drug time/concentration curve.

$Beta_{T1/2}$ Elimination half life.

Vd_{SS} Volume of distribution at steady state.

TABLE 21: Acebutolol pharmacokinetic parameters

Linear regression analysis also failed to demonstrate any effect of age on these parameters.

Conventional analysis of blood pressure and heart rate profiles revealed no significant effects of age when comparisons are made between the groups. Significant falls in blood pressure were seen in all three groups.

The mean effect model parameters are given in Table 22. there were no significant differences between the groups as assessed by analysis of variance. However, linear regression analysis demonstrated that there was a significant negative correlation between sensitivity (unit fall in placebo corrected erect systolic blood pressure per unit of drug concentration) and age ($P < 0.005$; $r = -0.51$) Figure 13. Regression analysis failed to demonstrate any other significant effects of age on Keq which describes the disequilibrium between concentration and effect.

Tolmesoxide

The pharmacokinetic parameters are shown in Table 23. There were no significant differences between the groups in any of the parameters, nor were there any with linear regression analysis.

Tolmesoxide produced significant changes in blood pressure and heart rate in all three groups. The mean effect model parameters are shown in Table 24. There was no significant effect of age upon the responsiveness of

		Responsiveness ($mmHg.ng^{-1}.ml^{-1}$)	keq ($l.min^{-1}$)
Young	mean	0.2	0.009
	s.d.	0.1	0.001
	range	0.1-0.3	0.008-0.01
Middle aged	mean	0.1	0.009
	s.d.	0.04	0.001
	range	0.1-0.2	0.007-0.01
Elderly	mean	0.1	0.009
	s.d.	0.03	0.002
	range	0.1-0.2	0.007-0.01

TABLE 22: Acebutolol - Concentration effect modelling parameters: Responsiveness and keq (a measure of the discrepancy between the plasma concentration and hypotensive effect

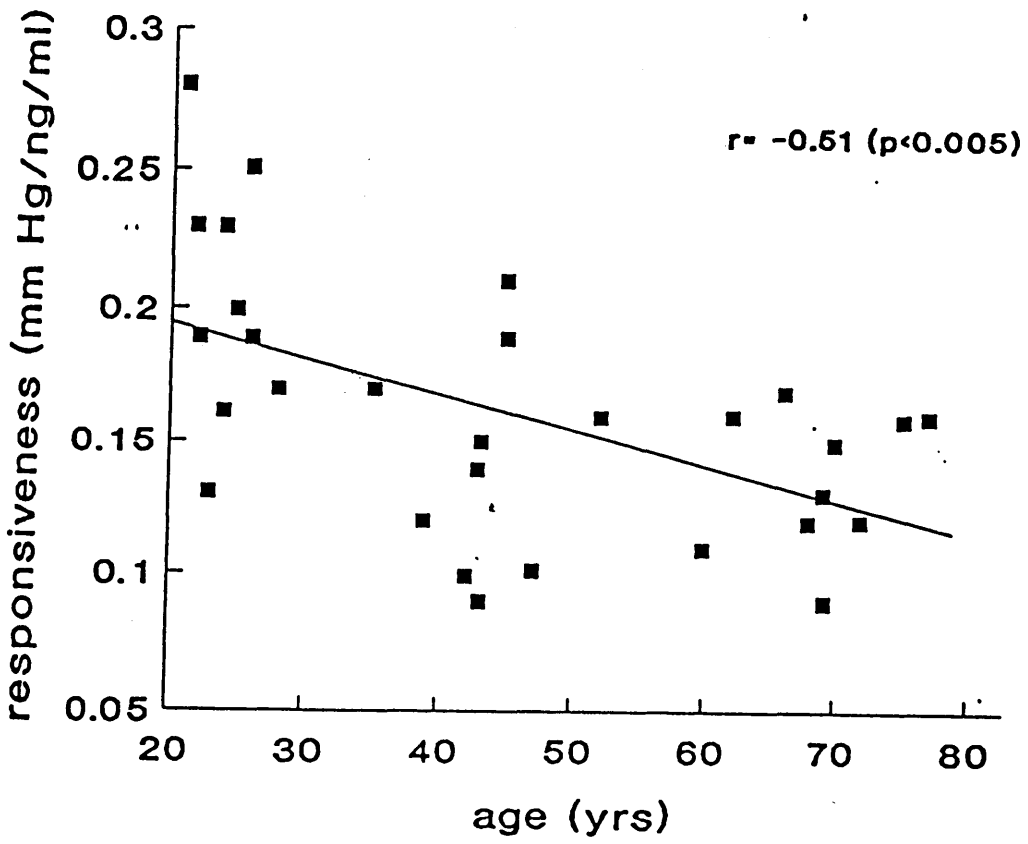


Figure 13: Relationship between the sensitivity to acebutolol (unit fall in placebo corrected systolic blood pressure per unit of drug concentration) and age.

		Clearance	AUC_M/AUC_D	$Beta_{T1/2}$ (min)	Vd_{SS} ($l.kg^{-1}$)
Young	mean	6.0	0.7	290	2.3
	s.d.	3.7	0.2	67	0.5
	range	2.3-11.0	0.5-1.5	97-307	1.0-2.7
Middle aged	mean	4.2	0.5	175	1.0
	s.d.	1.2	0.1	45	0.36
	range	2.0-5.1	0.3-0.8	104-236	0.7-1.4
Elderly	mean	4.5	0.6	182	1.1
	s.d.	1.4	0.1	46	0.3
	range	2.9-6.5	0.4-1.0	124-172	0.8-1.7

AUC_M Area under metabolite time/concentration curve.

AUC_D Area under drug time/concentration curve.

$Beta_{T1/2}$ Elimination half life.

Vd_{SS} Volume of distribution at steady state.

TABLE 23: Tolmesoxide pharmacokinetic parameters

		Responsiveness ($mmHg.ng^{-1}.ml^{-1}$)	keq ($l.min^{-1}$)
Young	mean	12.6	0.02
	s.d.	2.1	0.01
	range	9.8-15.6	0.01-0.02
Middle aged	mean	10.8	0.02
	s.d.	1.7	0.021
	range	8.6-13.0	0.01-0.02
Elderly	mean	11.3	0.02
	s.d.	2.1	0.01
	range	8.7-13.4	0.01-0.03

TABLE 24: Tolmesoxide - Concentration effect modelling parameters: Responsiveness and keq (a measure of the discrepancy between the plasma concentration and hypotensive effect

tolmesoxide in terms of placebo corrected fall in erect systolic blood pressure per unit of drug concentration. There was also no difference in the Keq values. Once again linear regression analysis failed to show any differences when age was treated as a continuous spectrum.

5.4 DISCUSSION

This study demonstrated that, with increasing age, in normal volunteers, the hypotensive effect of trimazosin was increased, that of acebutolol was decreased, while there was no change in the response to tolmesoxide. The increased hypotensive action of trimazosin in the elderly was due to reduced clearance; the blood pressure fall per unit drug concentration remained unchanged. The reduced effect of acebutolol in the elderly was due to decreased drug sensitivity; drug clearance remaining unchanged. Tolmesoxide clearance and effect were both unaltered by age.

Drug sensitivity

The unchanged sensitivity to trimazosin adds to the evidence that the activity of the alpha-1-adrenoceptor remains unaffected by age (Chapter 2). However, the influence of age on baroreceptor reflexes could not be controlled in this study. It is therefore not possible to make firm conclusions about adrenoceptor sensitivity. The same difficulty exists in the interpretation of other similar *in vivo* studies. Two studies have been carried

out examined the effect of age on the responses to the alpha-1-adrenoceptor agonist phenylephrine and antagonist prazosin in groups of normal volunteers (106,107). Elliott et al (106) produced evidence for a slight fall in receptor sensitivity with age, but Klein et al (107) demonstrated no change in receptor sensitivity with age. In a recent study of the concentration-effect of the alpha1-adrenoceptor antagonist, doxazosin, there was no change in the sensitivity of the receptor with age (152). Overall this study supports the larger body of evidence that there is no change in the sensitivity of the alpha-1-adrenoceptor with age (Chapter 2).

The relative resistance of the older volunteers to the beta-1-adrenoceptor antagonism of acebutolol gives support to the hypothesis that the effect of the cardiac beta-1-adrenoceptor is reduced in the elderly (Review: 51,52,153). This is in agreement with Vestal et al. (84) who demonstrated that the elderly are less sensitive to the cardiac effects of the non selective beta-adrenoceptor agonist (isoprenaline) and antagonist (propranolol). However, a more recent study confirmed that while the elderly had less cardiac acceleration with isoprenaline, the sensitivity to the non-selective beta-adrenoceptor antagonist timolol was unchanged (154). The apparent conflict in the results of these two studies may be the result of differences in the proportion of free active drug available as compared to the inactive protein-bound drug. Propranolol, as a basic drug, is highly bound to alpha-1-acid glycoprotein, an acute phase protein whose

concentration is known to increase with disease and is therefore commonly present at higher plasma concentrations in the elderly (56). In contrast timolol is minimally bound to plasma proteins.

There appears to be no substantial change in the number of lymphocyte beta-adrenoceptors with increasing age (155,156) but there is evidence for a reduction in receptor affinity (157). The effect of age on the stimulation of lymphocyte adenylate cyclase by beta-adrenoceptor agonists has been shown to be unchanged (158) or reduced (159) in the elderly. There is also an age related reduction in the stimulation of adenylate cyclase activity by prostaglandin E_1 (160) and by sodium fluoride (161). In summary, the evidence from studies of lymphocyte beta-adrenoceptors points to reduced receptor affinity and to reduced activity of adenylate cyclase, but the relevance of these results to innervated cardiovascular tissue remains questionable.

The evidence concerning the activity of the beta-2-adrenoceptor and ageing is conflicting. Beta-2-adrenoceptor mediated vasodilatation to intra-arterially injected isoprenaline decreases with age (162). Similarly there is a decrease in the dilatation of the dorsal hand vein to isoprenaline with age (110). However, using plethysmography, there was no age related change in vasodilatation in the calf in response to an intravenous infusion of adrenaline (163). Furthermore, the respiratory and metabolic effects of

beta-2-adrenoceptor stimulation does not appear to change with age (36,164). The number of beta-2-adrenoceptors present on human rectus abdominis muscle biopsies has been estimated by ligand binding and does not change with age (165).

Overall the evidence points to a reduction in the activity of the elderly beta-1-adrenoceptor which may be due to a small reduction in affinity but more significantly to changes in the post-receptor chain of events. Further study is still required on the effect of age on both the beta-1 and beta-2-adrenoceptors.

The sensitivity of the elderly to tolmesoxide is unchanged with increasing age. Tolmesoxide acts directly on vascular smooth muscle to produce vasodilation. There is very little information available concerning the effect of age on the activity of such vasodilators. In the dorsal hand vein, where dilatation to isoprenaline was diminished, there was no evidence for change in the venodilatation produced by nitroglycerin (110). There is some evidence that the hypotensive response to the calcium channel blocker, nifedipine, is greater in the elderly (130), although overall there is probably little effect of age (166). In general, although there is very little clinical experimental data, this study is in agreement with there being no age related change in the sensitivity of vascular smooth muscle to dilator drugs which act independently of adrenoceptors.

Pharmacokinetic changes with age

There are 3 other studies on the effect of age on the pharmacokinetics of quinazoline alpha-adrenoceptor antagonists. Two of these studies included intravenous drug administration, of prazosin (Chapter 3) and doxazosin (167). With increasing age, prazosin had an increase in volume of distribution but no change in clearance (Chapter 3). With doxazosin, there was greater variability in the drug kinetic measurements in the old, but there was a significant increase in the volume of distribution and this was associated with an increase in clearance. In a study of orally administered prazosin and terazosin involving normal volunteers (young 19-30 years and 'old' 54-62 years) (142) there was no change in the area under the time-concentration curve or in the elimination half-life of prazosin. With terazosin, there was an increase in both the area under the time-concentration curve and elimination half-life in the older subjects.

The present study does not show any effect of age on acebutolol metabolism and elimination. This confirms a previous study (168), however an earlier report suggested that the elimination half-life of acebutolol was prolonged in the elderly when given by the oral but not intravenous route (169). The major metabolite, diacetolol, has been reported to have beta-adrenoceptor antagonist properties (170). Using the concentration- effect model, the present study provides no evidence that diacetolol contributed to the hypotensive effect.

There are no other studies on the effect of age on the pharmacokinetics of tolmesoxide.

Study design

This study illustrates the benefit of using a continuous age spectrum in an examination of the effect of age in clinical pharmacology. Analysis of data arbitrarily divided into age groups did not demonstrate significant differences which only became apparent when all subjects were analysed together by linear regression. Once again this study demonstrates the importance of intravenous drug administration for the calculation of volume of distribution and drug clearance.

In 30 normotensive volunteers, there was a reduction in the clearance of trimazosin and no change in the clearance of acebutolol or tolmesoxide with age. Using concentration-effect analysis, it has been shown that the sensitivity to acebutolol decreases with age while that to trimazosin and tolmesoxide remain unchanged.

CHAPTER 6

DOUBLE BLIND CROSS-OVER COMPARISON OF DOXAZOSIN AND PLACEBO IN ELDERLY HYPERTENSIVE PATIENTS

DOUBLE BLIND CROSS-OVER COMPARISON OF DOXAZOSIN AND PLACEBO IN ELDERLY HYPERTENSIVE PATIENTS

6.1 INTRODUCTION

Doxazosin is a quinazoline derivative with selective alpha-1-adrenoceptor blocking activity comparable to the related drug, prazosin (171), but with a more gradual onset of hypotensive activity (172). Combined pharmacokinetic and dynamic studies in young and elderly volunteers have indicated that doxazosin has a duration of action suggesting the potential for once daily dosing (167,152). The efficacy of once daily doxazosin in the treatment of essential hypertension has previously been demonstrated in younger patients (173,174).

This study was designed to investigate the efficacy and safety of once daily doxazosin in elderly hypertensive patients living in the community. The pharmacokinetic profile was also investigated after steady-state oral dosing.

6.2 METHOD

Patient selection

Patients aged 65 and over who had mild to moderate hypertension, with diastolic blood pressures of 95 to 120 mmHg took part. All lived at home. Patients with angina

pectoris requiring treatment, cardiac failure not controlled by diuretics or other major concurrent diseases were excluded. Concomitant treatment with diuretics was allowed, provided the dose remained unchanged throughout the study, but treatment with other antihypertensive drugs was not allowed during the study or for 4 weeks before entering the placebo run-in period.

Study design

The study was a two centre, double-blind, randomized, cross-over comparison of once daily doxazosin and placebo, preceded by a 4 week single-blind placebo run-in period. At the end of the run-in period patients with a diastolic blood pressure greater or equal to 95 mmHg both supine and standing were randomized to treatment with doxazosin or placebo for 10 weeks. At the end of this period the patients were given the alternative treatment for a further 10 weeks.

Treatment was given once daily in the morning. The dose was titrated at two-weekly intervals by using double-blind identically matching tablets of doxazosin and placebo. During the doxazosin period the initial dose was 1 mg and subsequently daily doses were 2 mg, 4 mg, 8 mg and 16 mg.

The patients were reviewed at two-weekly intervals throughout the study. They were told to take their tablets 24 hours before the review visits and to record the time on a diary card. On the day of the review they did not take their medication until after the blood

pressure measurements had been made. Compliance with drug therapy was checked at every visit with a tablet count.

The target antihypertensive effect was defined as a reduction in both supine and standing blood pressures to below 95 mmHg and by at least 10 mmHg measured 24 hours after the dose.

Clinical measurements

At each review visit resting blood pressure and radial pulse were measured in duplicate after 5 minutes in the supine position and after 2 minutes standing. Blood pressure was measured using a random zero sphygmomanometer (Hawksley) and Phase V diastolic blood pressure was recorded.

A 12-lead ECG was recorded on entry to the study, at the end of the placebo run-in period and at the end of each treatment period.

All volunteered or observed adverse events were recorded at each review visit. Haematological and biochemical screens and routine urinalysis were performed on entry into the study, at the end of the run-in period and during and at the end of each treatment period (weeks 8, 14, 18 and 24). Body weight was recorded with electronic digital scales (Siemans) at each visit.

Steady State Pharmacokinetics

All patients who completed both phases of double-blind

treatment were eligible for entry into the pharmacokinetic study. Those who completed the double blind study on doxazosin were continued on the same dose in an open manner.

The placebo treated patients were retitrated to the doxazosin dose reached during the first period of the cross-over or to that dose which controlled blood pressure (as defined above) if this was lower. All patients were maintained at constant dose of doxazosin for at least 14 days before pharmacokinetic assessment. During the pharmacokinetic study blood samples were taken immediately before administration of a supervised dose of doxazosin and at 1 ,2, 3, 4, 6, 8, 12, 24 and 48 hours after the dose.

Plasma doxazosin analysis

The plasma was separated by centrifugation and stored at -20^{o}C until assayed for doxazosin. Samples were analysed by high performance liquid chromatography using fluorescence detection with prazosin as the internal standard (175). All samples were analysed in duplicate and calibration standards and quality controls were processed in the same way as unknowns. The lower limit of detection was 1 ng.ml^{-1}. The precision of the method of analysis (n = 4-10) expressed as the coefficient of variation was $\pm$9.6, $\pm$2.9, $\pm$3.5 and $\pm$6.8 at 1, 10, 40 and 100 ng.ml^{-1} respectively. Areas under the doxazosin plasma concentration-time curves were calculated by the linear trapezoid rule and regression lines were fitted by

least squares regression analysis. The plasma elimination half-lives within the dosage interval were calculated by applying a one compartment model with duration times of the observed terminal linear phase from 4 or 6 hours to 24 hours.

Statistical Analysis

For blood pressure and heart rate the results are expressed as mean values with 95% confidence intervals of the mean changes. Analysis of variance (with treatment, period of study and patient as factors) was carried out in order to assess treatment differences in (final-baseline) changes. P values for comparisons of blood pressure and heart rate changes are based on two-sided tests.

6.3 RESULTS

Patients

Forty Caucasian patients (16 men, 24 women) aged 65 to 82 years (mean 71.4 years) entered the double blind period, 21 being randomized to treatment with doxazosin during the first phase and 19 to placebo, The mean duration of diagnosed hypertension was 5.3 years (range 1 month to 20 years). Twenty one patients received other medication during the double-blind doxazosin phase of the study and 20 patients during the double-blind placebo phase (Table 25). Twenty-eight patients completed both phases of double-blind therapy.

Drug	Number of patients
Analgesics	7
Antacids	1
Antibacterials	11
Cyanocobalamin	1
Decongestants	2
Diuretics	7
Fenfluramine	1
Glibenclamide	1
Inositol nicotinate	1
Mianserin	1
Multivitamins	1
Nonsteroidal anti-inflammatory	9
Quinine sulphate	2
Salbutamol	1
Temazepam	2
Thyroxine	1

TABLE 25: Other drugs taken during the study.

Six patients were withdrawn from the study during doxazosin treatment and 6 during double-blind placebo treatment. The reasons for withdrawal during doxazosin treatment were: two patients developed intercurrent illnessess (multiple metastases and uncontrolled non-insulin dependent diabetes), one patient died, one patient experienced minor adverse effects and two patients moved away from the area of the study. The reasons for withdrawal during placebo treatment were: two patients suffered intercurrent illnesses (congestive cardiac failure, and hospital admission for prostatectomy), one patient moved from the area, two patients showed poor compliance (repeatedly forgetting to take the tablets) with therapy and one patient was withdrawn due to lack of efficacy.

Another two patients were excluded from the efficacy analysis, as both had a diuretic added to treatment during the double-blind phase of the study. Twenty six patients were therefore included in the efficacy analysis. The mean final daily dose of doxazosin in these patients was 8.6 mg (range 1-16 mg) and the mean durations of therapy for doxazosin and placebo treatment were 71.5 days (range 63-92) and 72.6 days (range 64-95) respectively. The distribution of doxazosin doses before final assessment for this group of patients is shown in Table 26.

Blood Pressure and Heart Rate

Blood pressure and heart rate measurements 24 hours after the dose at the end of each period of double blind

Dose (mg/day)	Number of patients
1	2
2	3
4	6
8	6
16	9

TABLE 26: Doxazosin dose before the final blood measurement in those patients analysed for efficacy.

treatment were compared with baseline values, which were taken as the mean of the measurements made at the end of Weeks 2 and 4 in the placebo run-in period. The mean baseline, mean final and mean changes (final-baseline) in blood pressures and heart rates are shown in Table 27 and Table 28.

The mean changes from baseline in standing blood pressures (mmHg) were -12.7/-12.5 (systolic/diastolic) for doxazosin and -5.7/-6.9 for placebo. The corresponding mean changes in supine blood pressure were -6.1/-10.3 for doxazosin and +0.2/-4.8 for placebo (Figure 14). The mean reductions from baseline both in standing and supine diastolic blood pressures were significantly greater at the end of doxazosin treatment ($p < 0.05$). The reductions in standing and supine systolic blood pressures were not statistically significant when compared with placebo. There were rises in both standing and supine heart rates with doxazosin (3.2 and 1.7 beats.min^{-1}) compared with falls with placebo (-0.1 and -2.1 beats.min^{-1}), but these changes were not statistically significant.

Pharmacokinetics

Eighteen patients (6 men, 12 women) aged 65 to 82 years (mean 71.4 years) entered the pharmacokinetic study. The blood pressure profile over the pharmacokinetic study period (24 hours) is shown in Figure 15. The mean duration of continuous doxazosin therapy before pharmacokinetic analysis was 80.7 days (range 22 to 146 days). The distribution of doxazosin dose and mean

	Baseline mean	Placebo	
		Final mean	Mean change (95% conf)
ERECT POSITION			
Systolic (mmHg)	178.5	172.8	-5.7 (-12.4,1.6)
Diastolic (mmHg)	108.4	101.5	-6.9 (-10.1,-3.5)
Pulse (beats/min)	81.5	81.5	-0.1 (-3.0,+2.5)
SUPINE POSITION			
Systolic (mmHg)	181.4	181.6	+0.2 (-5.9,+6.1)
Diastolic (mmHg)	105.4	100.6	-4.8 (-7.5,-2.2)
Pulse (beats/min)	78.6	76.4	-2.1 (-6.3,+1.1)

TABLE 27: Changes in pulse and blood pressure following placebo therapy.

	Baseline mean	Doxazosin		Doxazosin Placebo Difference (95% conf)
		Final mean	Mean change (95% conf)	
ERECT POSITION				
Systolic (mmHg)	178.5	165.8	-12.7 (-20.0,-6.0)	-6.9 (-17.4,+2.2)
Diastolic (mmHg)	108.4	95.9	-12.5 (-15.9,-9.4)	-5.6 * (-10.4,-1.3)
Pulse (beats/min)	81.5	84.7	+3.2 (+0.7,+6.1)	+3.2 (-0.8,+7.4)
SUPINE POSITION				
Systolic (mmHg)	181.4	175.3	-6.1 (-12.0,0.0)	-6.2 (-14.5,+2.3)
Diastolic (mmHg)	105.4	95.1	-10.3 (-12.9,-7.6)	-5.5 * (-9.1,-1.8)
Pulse (beats/min)	78.6	80.2	+1.7 (-1.6,+5.9)	+3.7 (-0.4,+10.0)

* $p < 0.05$ for doxazosin-placebo difference in final baseline changes.

TABLE 28: Changes in pulse and blood pressure following doxazosin.

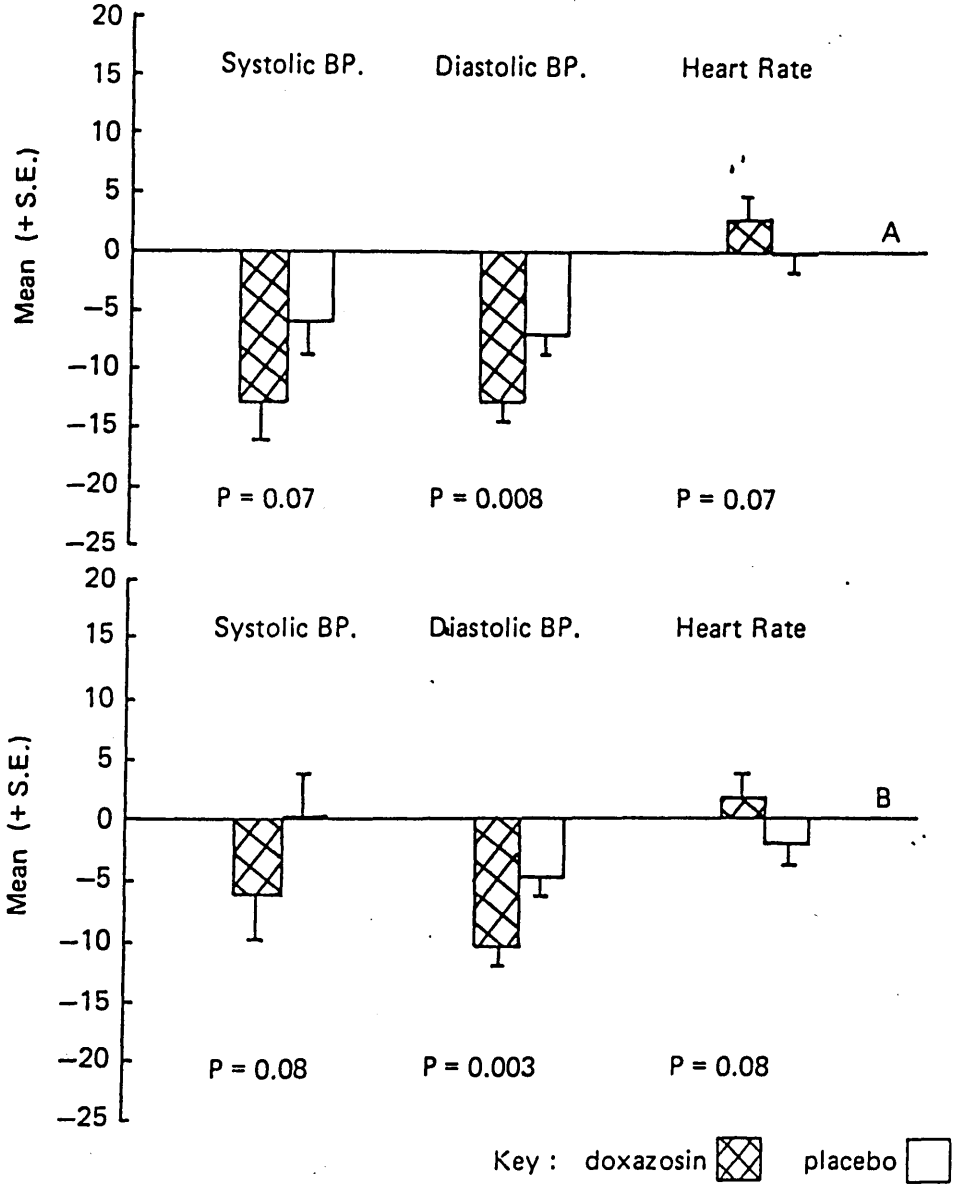


Figure 14: Mean changes in blood pressure and pulse with doxazosin and placebo.

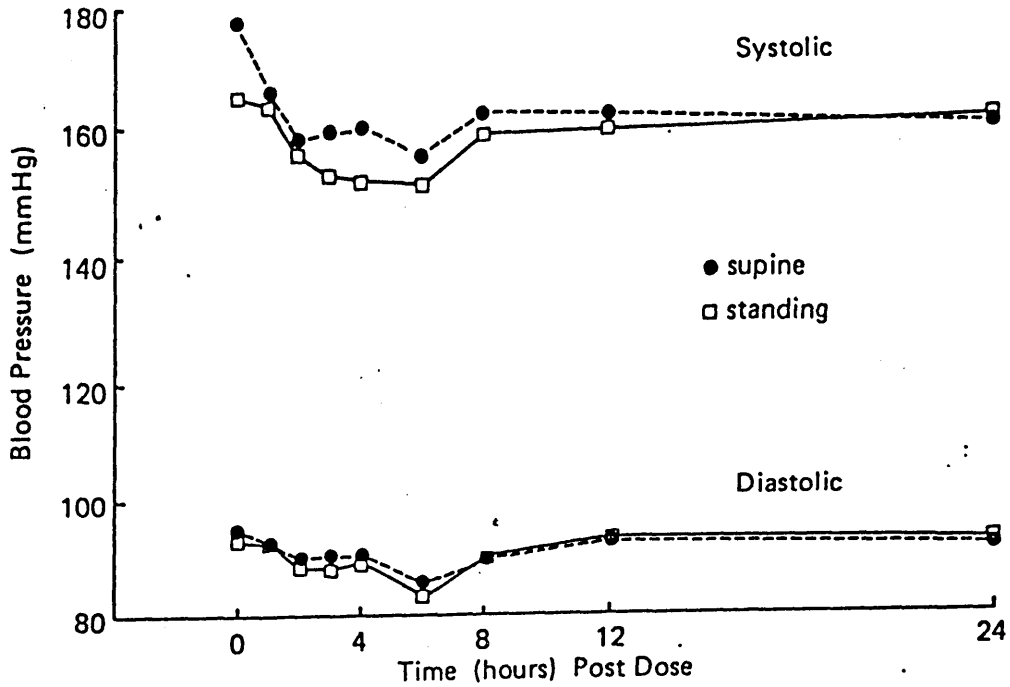


Figure 15: Blood pressure profile over 24 hours from one patient, following administration of oral doxazosin

pharmacokinetic variables are shown in Table 29. Both the time to peak plasma concentration and the plasma elimination half-life were independent of dose, with a median time to peak plasma concentration for all patients of 3 hours (range 1-4 hours) and a mean plasma elimination half-life (n = 17) within the dose interval of 16.1 hours (range 10.1 - 27.1 hours). One patient was excluded from the analysis of terminal elimination half-life because there was a poor fit to the calibration curve. Within the dosage range 1 to 16 mg, the peak plasma concentration during the dosage interval and the average concentration during the dosage interval (AUC(0-24)/24) were linearly related to dose (Figure 16).

Adverse Events

All the patients who entered double-blind treatment were evaluated for adverse events. Nine patients reported unwanted effects during doxazosin treatment and five during double-blind placebo treatment. One patient taking doxazosin (1 mg) reported headache, increased sweating and weakness and was withdrawn from the study. Dizzyness was the most frequently reported unwanted effect; it was reported in 5 patients during doxazosin treatment and four patients during placebo treatment. Dizziness in these patients disappeared with continued treatment, one doxazosin-treated (4 mg) patient receiving a reduced dose. The remaining drug-related (or possibly drug-related) adverse effects headache, dyspnoea, lethargy, nausea and ankle oedema during doxazosin treatment and nausea during placebo treatment, were minor and were generally tolerated

	Doxazosin dose (mg/day)					All[A] Patients
	1	2	4	8	16	
Number	3	4	2	5	4	18
Terminal elimination half-life (h)	14.4 ±1.7	15.7 ±3.0	18.1	16.2[C] ±7.5	16.9 ±6.7	16.1[C] ±5.0
Time to peak[B] (h)	2.0 (2-4)	2.5 (1-3)	1.5	3.0 (2-3)	3.5 (2-4	3.0 (1-4)
Peak concentration (ng/ml)	9.4 ±1.5	18.0 ±3.2	28.7	84.7 ±10.6	168 ±45.2	9.6 ±2.0
Minimum concentration (ng/ml) (24hr post dose)	3.2 ±1.0	6.7 ±1.3	11.0	26.9 ±3.5	57.8 ±26.9	3.3 ±1.0
AUC(0-24)/24 (ng/ml)	5.6 ±1.4	11.2 ±1.4	17.2	46.5 ±4.2	97 ±29.1	5.6 ±1.1

A The values for peak concentration, minimum conc-concentration, and AUC(0-24)/24 are normalized to a 1mg dose.

B The values for time to peak concentration are given as medians and ranges.

C One patient was excluded from the analysis of terminal half-life because there was a poor fit to the calibration curve.

TABLE 29: Summary of pharmacokinetic data of doxazosin (mean with s.d.)

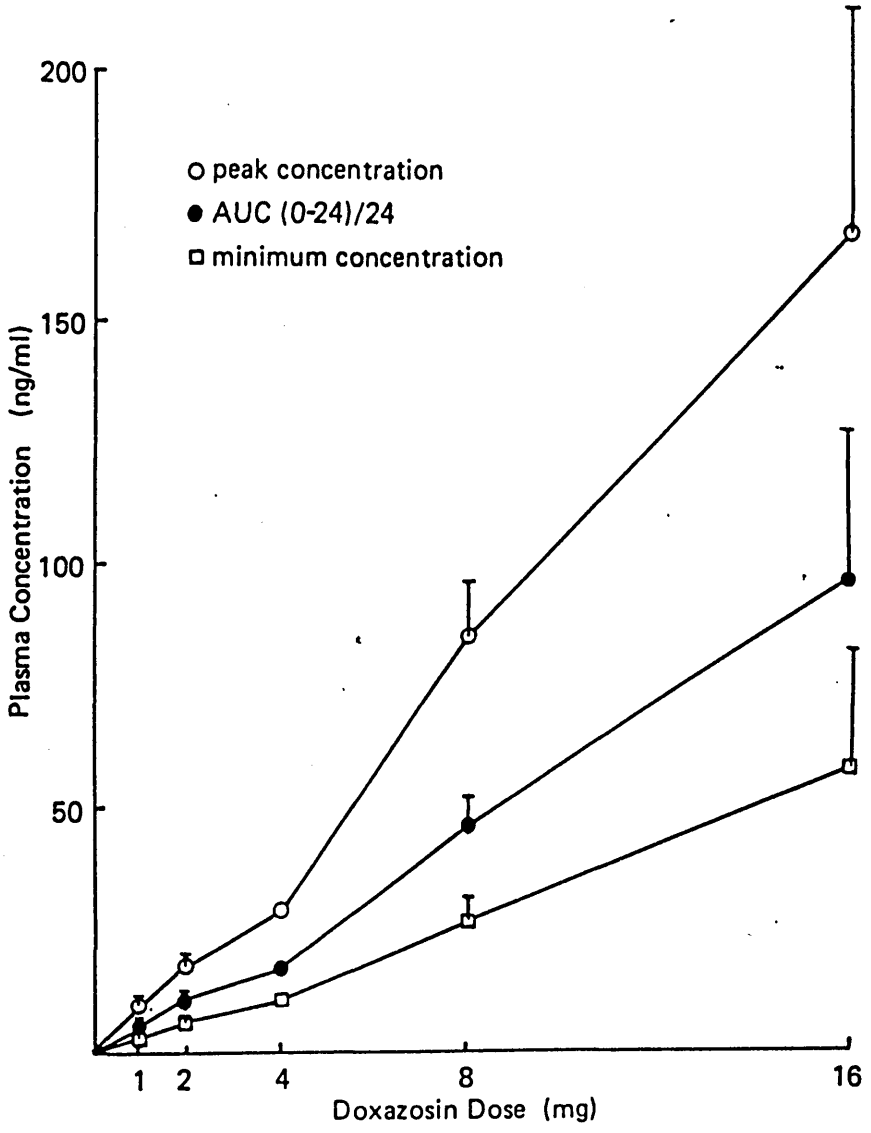


Figure 16: Relationship between dose of doxazosin with peak and minimum plasma concentration and AUC(0-24)/24. Mean with standard deviation.

or disappeared with continued therapy.

One previously untreated hypertensive man died from a suspected stroke while taking doxazosin (2 mg). Satisfactory blood pressure control in this patient had not been achieved.

There were no statistically significant difference in mean changes in body weight at the end of 10 weeks treatment with doxazosin or placebo.

No patterns of changes were observed in either treatment group with respect to haematological or biochemical measurements. The incidence of abnormalities was similar in both treatment groups (13 for doxazosin and 14 for placebo) and the range of abnormalities were also similar. There was no evidence of drug- related changes in ECG in either treatment group.

6.4 DISCUSSION

Choice of antihypertensive therapy for the elderly

The prevalence of hypertension in the elderly is very high and rises with age (43); in the Framingham study high blood pressure has been identified in approximately 40% of males and 50% of females between the ages of 65 and 94 years. Hypertension in elderly patients is associated with blunted beta-adrenoceptor mediated effects; exercise induced tachycardia and heart rate responses to

isoprenaline are reduced (108), plasma renin activity is normal or low (176,177), there is a hyper- reponsiveness to sympathetic stimuli (45) and peripheral vasodilator responses to beta-adrenoceptor stimulation are diminished (162). In contrast to the beta-adrenoceptor the activity of the vasoconstrictor alpha-1-adrenoceptor, which appears to be unchanged in the normotensive elderly (Chapter 3), may even be enhanced in the older hypertensive patient (178). These observations give rise to the suggestion that peripheral vasodilator drugs, for example, alpha-1-adrenoceptor antagonists, may have a primary role to play in the treatment of hypertension in the elderly where peripheral resistance is high (58).

The reduction in baroreceptor activity seen with age (1) contributes to the increased risk of drug induced postural hypotension seen in the elderly (4). Prazosin may cause severe postural hypotension with the first dose (179). It also has a short elimination half-life in young and elderly (Chapter 4) and has to be administered twice or three times a day. Prazosin is therefore not an ideal drug for the treatment of the elderly hypertensive.

Doxazosin and the elderly hypertensive patient

This study examined the effect of doxazosin in elderly hypertensive patients. Doxazosin and prazosin are both quinazoline derivatives, but doxazosin had been shown to have a longer elimination half-life in both young (167) and elderly (167) normal volunteers.

Forty patients entered this double-blind placebo controlled cross-over study. Twenty eight completed both phases of double blind treatment but due to the addition of diuretic therapy, two further patients were excluded from the examination of drug efficacy. Doxazosin produced a significant, but small, fall in placebo corrected diastolic blood pressure 24 hours post dosing, (-6.9 mmHg erect, -6.2 mmHg supine). There was no significant fall in systolic pressure when compared to placebo. The hypotensive efficacy of doxazosin has also been described in a multicentre study involving 903 patients (181). Doxazosin was compared with placebo in a subgroup of 172 young and old patients. Recalculation of the published data from this trial confirms the modest effect of doxazosin on placebo corrected diastolic pressure (-7.7 mmHg erect, -4.7 mmHg supine).

One patient was withdrawn from the present study due to adverse effects during doxazosin treatment. The other adverse effects were minor and were well tolerated, or dissapeared with continued therapy. The most frequently reported side effect was dizziness, which occurred in a similar proportion of doxazosin and placebo treated patients.

One patient died from a suspected stroke during doxazosin therapy. His hypertension had not been previously treated and control had not been achieved with doxazosin therapy.

Assessment of steady-state pharmacokinetics was carried

out in 18 of these patients. Over the dosage range 1-16 mg/day plasma concentrations were linearly related to dose, suggesting that absorption of doxazosin in the elderly is not dose-dependent. Peak plasma concentrations occured 1 to 4 hours after dosing. The mean plasma elimination half-life was 16.1 hours (range 10.1 - 27.1 hours), which is longer than the 8.8 $\pm$ 4.2 hours reported in an acute dosing study (180). This supports the observation that the elimination half life of doxazosin is prolonged with chronic dosing (152).

The modest fall in diastolic blood pressure, 24 hours post dose, and the lack of signficant effect on systolic pressure shown in this study suggests that doxazosin is unlikely to play a major role in the treatment of hypertension in the elderly.

The studies described in this thesis examine aspects of the effects of ageing on the physiology and pharmacology of the sympathetic nervous system.

The examination of noradrenaline disposition in age was the first to report the increased spillover of the neuro-transmitter in the elderly. Subsequent studies have all confirmed this finding.

The study on human isolated arteries was the first to report the lack of change in the sensitivity of the alpha-1-adrenoceptor with age. All subsequent studies have confirmed this finding.

The study on prazosin disposition and ageing demonstrated that there was a reduction in the bioavailability and an increase in the volume of distribution. These changes are unlikely to be of clinical significance.

The effect of age was described on the hypotensive action and metabolic fate of trimazosin, acebutolol and tolmesoxide. The clearance of trimazosin was reduced but that of acebutolol and tolmesoxide remained unchanged. The hypotensive action of acebutolol was reduced with age while that of trimazosin and tolmesoxide was unchanged.

Doxazosin, given once and day, produced a modest fall in

diastolic pressure in a group of elderly hypertensive patients. There was no significant fall in systolic blood pressure.

Research into the physiology and pharmacology of ageing is becoming increasingly important. Within a very few decades, there will be considerable increases in the numbers of elderly in almost all developed countries. The work described in this thesis was carried out over a ten year period and the studies reflect changes in experimental design over that period.

It is now necessary to study each new drug in elderly volunteers and patients. It is better to examine age as a continuous variable rather than studying two groups of young and elderly. In Clinical Pharmacology, it is not acceptable to calculate clearance of a drug from oral dosing studies.

I would hope that an increased understanding of aging and its effect of therapy will help to reduce the current epidemic of adverse drug reactions seen in our elderly patients.

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