SEROTONIN AND SEROTONERGIC TYPE-2 ANTAGONISTS IN HYPERTENSION AND ADULT ATOPIC ASTHMA

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Preface

The work which forms the basis of this thesis was undertaken at the Medical Research Council Blood Pressure Unit and Department of General Medicine at the Western Infirmary Glasgow. Except where indicated, I personally carried out this work. I have enjoyed collaboration with a number of colleagues and this is formally acknowledged. The writing of this thesis is entirely my own work.

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Summary

Serotonin is a naturally occuring amine with vaso-active properties; its role in the pathogenesis of hypertension is unknown. Serotonin in blood is stored in platelets, and is released during platelet aggregation. Normally, only negligable amounts of serotonin are present in the plasma. Platelets from patients with untreated essential hypertension (n=22) showed increased serotonin-induced aggregation in whole blood in-vitro compared to age and sex matched normotensive controls (n=20). Enhanced platelet aggregation could contribute to raised arterial pressure in hypertension, by increasing free plasma levels of vasoconstrictors such as serotonin. However, spontaneous platelet aggregation in whole blood was not enhanced in these hypertensive patients.

Enhanced platelet aggregation in-vivo might be expected to cause a reduction in platelet contents, such as serotonin, and an increase in free plasma levels of platelet contents, such as beta-thromboglobulin and serotonin. I developed therefore a highly reproducible (intra and interassay co-efficients of variation 2.2% and 7.1% respectively), sensitive method (limit of detection <5pmol/ml) using high pressure liquid chromatography with electrochemical detection to allow accurate measurement of platelet serotonin. Levels of platelet serotonin and plasma beta-thromboglobulin were not altered in hypertensive patients compared to age and sex matched normotensive controls. These findings do not support

increased platelet aggregation in-vivo as an invariable feature of essential hypertension.

Serotonin's vasoconstrictor effects are claimed to be mediated through the type-2 subset of serotonergic receptors. In-vitro studies suggest that serotonin acts not only in its own right as a vasoconstrictor but that it also potentiates the effects of other vasoconstrictors such as noradrenaline and angiotensin II. This effect can be shown in isolated blood vessels from normal and hypertensive animals, and appears to be mediated through the serotonergic type-2 binding site. The introduction of antagonists at this site capable of reducing blood pressure has lead to renewed interest in the possible role of serotonin in raising blood pressure.

Ketanserin was the first agent available for the investigation of the putative role of the serotonergic type-2 receptor in blood pressure control in man. It is a competitive serotonergic type-2 antagonist and an effective antihypertensive agent. I set out therefore to examine the claimed mechanism of action of this drug. Although initially disputed, ketanserin also has alpha-1 adrenoreceptor antagonist activity when given in doses used to treat hypertension in man; this may contribute to its antihypertensive effects. In a double blind randomised placebo controlled study of 8 healthy subjects ketanserin 40mg twice daily for 3 days reduced significantly mean arterial pressure but did not attenuate the pressor response to infused angiotensin II. This confirmed that the attenuation of response to alpha-1 agonists caused by

ketanserin was related to an alpha-1 adrenoreceptor effect, and indicated, in contrast to in-vitro findings, that serotonin does not potentiate the vasoconstrictor effects of angiotensin II in-vivo. It follows that the short-term reduction of blood pressure caused by ketanserin in these subjects was not likely to be related to inhibition of angiotensin II-induced vasoconstriction.

Ketanserin reduces blood pressure by vasodilatation but unexpectedly does not seem to cause an appropriate reflex tachycardia even after acute administration. The drug reduced mean arterial pressure and heart rate by 5.7 ± 1.8mmHg (mean$\pm$standard error; $p<0.05$) and 3.5 ± 1.5 beats/minute ($p<0.05$) respectively compared to placebo, at 1 hour after dosing on day 4. This reduction in heart rate was accompanied by a tendency (not statistically significant) for cardiac vagal outflow, assessed by the heart rate responses to standing, deep breathing and the Valsalva maneouvre, to be reduced rather than increased after ketanserin compared to placebo. Thus the reduction in heart rate caused by ketanserin is unlikely to be caused by increased cardiac parasympathetic outflow.

Ketanserin 40mg twice daily for 3 days caused prolongation of the electrocardiographic QT and QT_C intervals by 26-40 milliseconds compared to placebo. This finding is consistent with Class III antiarrhythmic activity. It is likely that this effect is mediated through the serotonergic type-2 rather than the alpha-1 adrenoreceptor, as the highly selective serotonergic type-2 antagonist ritanserin, given in a dose of 10mg

twice daily for 4 weeks to patients with essential hypertension, also caused prolongation of the QT_c interval, by 39±11 milliseconds ($p<0.05$ compared to placebo). Although a minor prolongation this is an important observation as in some situations, for example hypokalaemia, patients may be predisposed to ventricular arrythmias.

A considerable body of evidence has accrued, mainly from in-vitro studies, to indicate a role for serotonin in the regulation of plasma renin, aldosterone and cortisol. Reduced activity of the renin-angiotensin system or adrenal cortex could contribute to the antihypertensive effects of ketanserin. However, 3 days of ketanserin 40mg twice daily did not affect basal plasma renin, aldosterone, angiotensin II, and cortisol, or importantly the rise in plasma aldosterone and suppression of plasma renin caused by infused angiotensin II in normotensive subjects. These findings do not support a major role for the serotonergic type-2 receptor in the control of plasma renin, aldosterone or cortisol in healthy man.

To try and dissect out further the role of serotonin in blood pressure control, studies were undertaken using ritanserin, a new highly selective serotonergic type-2 antagonist. This drug differs from ketanserin in that it is virtually devoid of alpha-1 adrenoreceptor binding affinity in vitro. When given in a dose of 10mg orally, ritanserin has been shown to have central effects, significantly altering sleep patterns in man. Ten patients with untreated essential hypertension were given single

oral doses of ritanserin 10 and 20mg, ketanserin 40mg and placebo in a double blind randomised crossover study. Ketanserin caused a significant reduction in sitting mean arterial pressure ($p<0.05$ compared to placebo), while ritanserin had no significant effects on sitting or standing blood pressure in either dose, compared to placebo. This did not support the concept that serotonin antagonism plays an independent role in acute blood pressure reduction caused by ketanserin. To look for an effect of more chronic therapy, ritanserin 10mg twice daily was given for 4 weeks in a double blind randomised placebo controlled parallel group study of patients with untreated essential hypertension. Ten patients received ritanserin and 8 placebo. Chronic ritanserin treatment inhibited serotonin-induced platelet aggregation ($p<0.05$ compared to placebo), an effect mediated through the serotonergic type-2 receptor, but did not significantly lower blood pressure in patients with essential hypertension. Ritanserin had no significant effect on forearm blood flow or venous tone, measured by mercury-in-strain-gauge plethysmography. These findings do not support a major independent role for the serotonergic type-2 receptor in the maintenance of raised arterial pressure in essential hypertension or in the regulation of tone of forearm resistance vessels or veins in these patients. Furthermore, it is unlikely that enhanced serotonin-induced platelet aggregation in patients with untreated essential hypertension is a direct contributory factor to raised arterial pressure in these patients.

Serotonin has been claimed to be a possible mediator of brochoconstriction. A logical extension to the main thrust of this work was then to examine the effects of serotonergic antagonists in patients with asthma. In a double blind randomised placebo controlled crossover study of 8 adult atopic asthmatics, ketanserin 40mg orally had no significant acute effects compared to placebo on resting bronchomotor tone or in preventing exercise induced asthma. These findings do not support a major contributory role of the serotonergic type-2 receptor in the regulation of resting bronchomotor tone or in exercise induced bronchoconstriction in adult atopic asthma, but suggest that ketanserin is likely to be safe for use as an alternative antihypertensive agent in patients with this condition.

Chapter 1; Introduction

1.1 Discovery of serotonin

In 1918, Janeway et al reported that serum contained a factor which caused vasoconstriction. The vasoconstriction was dependent on blood clotting, as carefully prepared plasma did not have this effect. The substance responsible for this vasoconstrictor activity was isolated and identified by two separate groups of workers, and initially given the name of serotonin (Rapport et al, 1948) or enteramine (Erspamer and Asero, 1952).

1.2 Biosynthesis and metabolism of serotonin

Serotonin (5-hydroxytryptamine) biosynthesis (figure 1) requires the essential aromatic amino-acid tryptophan (Culley et al., 1963). The rate limiting step is the hydroxylation of tryptophan by the enzyme tryptophan hydroxylase to 5-hydroxytryptophan (Tong & Kaufman, 1975). This reaction is highly dependent on the presence of oxygen and the co-factor tetrahydrobiopterin (Baumann, 1985). Synthesis of serotonin can only take place in tissues which contain tryptophan hydroxylase. The ubiquitous enzyme aromatic L-amino-acid decarboxylase then catalyses the step from 5-hydroxytryptophan to serotonin (Bouchard & Roberge, 1979). The main mode of metabolism of serotonin (figure 1) is by monoamine oxidase A, producing the unstable intermediate compound

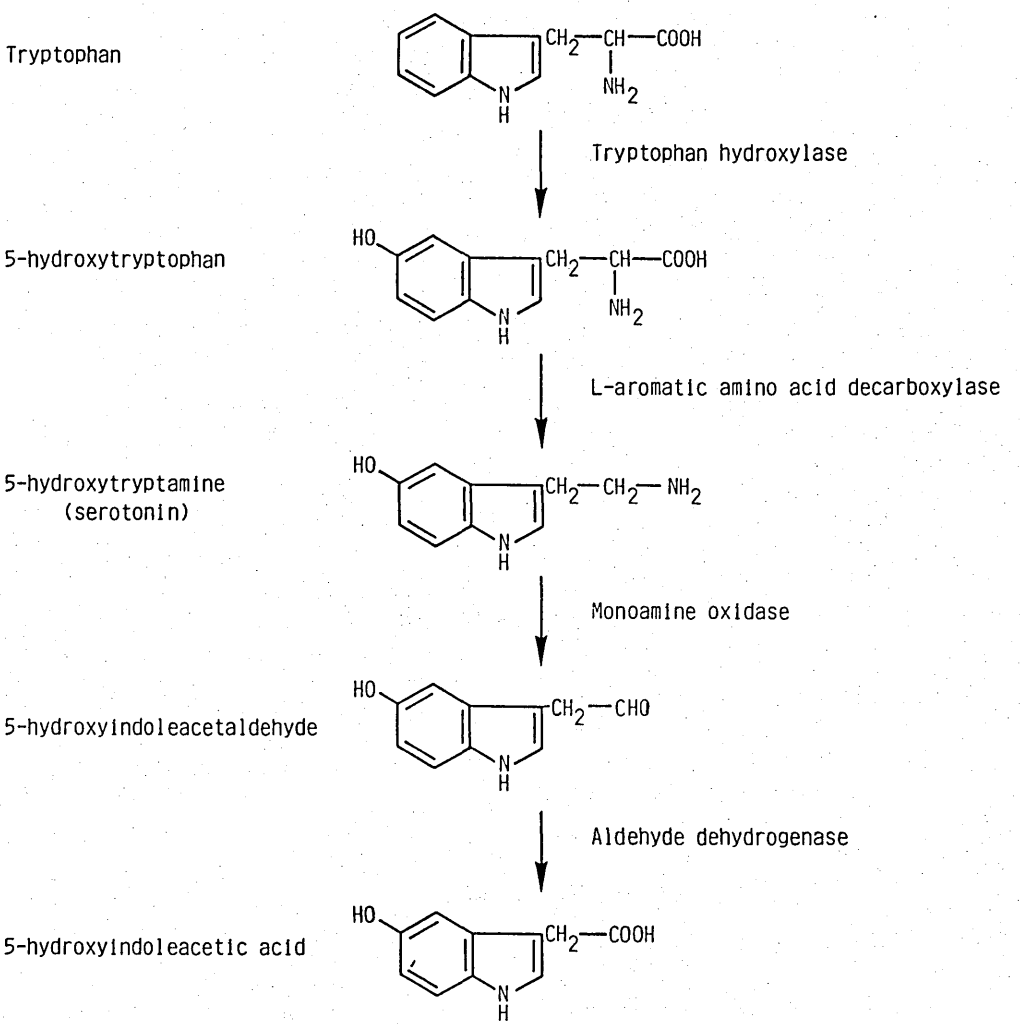


Figure 1
Serotonin biosynthesis and major pathway of metabolism.

5-hydroxyindoleacetaldehyde (Youdim & Ashkenazi, 1982). This aldehyde undergoes oxidation, catalysed by aldehyde dehydrogenase (Erwin & Deitrich, 1966), resulting in the major final metabolite 5-hydroxyindole acetic acid, which is excreted in the urine (Tyce et al., 1983). The aldehyde can also be reduced by aldehyde reductase forming 5 hydroxytryptophol (Tyce et al., 1968), although this pathway is usually of less importance than oxidation.

1.3 Serotonin receptor sites

The first classification of serotoninergic receptors was by Gaddum and Picarelli (1957). They discovered that serotonin-induced contractions in isolated ileal segments were antagonised by morphine and dibenzyline (phenoxybenzamine). These two drugs appeared to act at different sites, designated by their first letters as M and D binding sites, associated with cholinergic nerves and smooth muscle cells respectively. Although this classification remains valid it did not prove to be of major use in understanding the cardiovascular effects of serotonin (Fozard 1984).

In 1979 Peroutka and Snyder published the results of receptor binding studies in rat brain which were to provide the basis of much of our current understanding of the effects of serotonin in cardiovascular regulation. They demonstrated two separate serotonin binding sites which preferentially bound tritium labelled serotonin and spiroperidol, and designated them 5HT-1 and 5HT-2

respectively. The serotonergic type-2 binding site is thought to be equivalent to Gaddum and Picarelli's D receptor (Fozard, 1984).

Peripherally, serotonin is thought to act through 5HT-1 receptor sites to cause vasodilatation and at 5HT-2 sites to cause vasoconstriction (1.5.2). Radioligand binding studies suggest that several different subtypes of the 5HT-1 site exist (Pedigo, Yamamura & Nelson, 1981; Middlemiss & Fozard, 1983; Pazos, Hoyer & Palacios, 1985). Gaddum and Picarelli's M receptor has now been redesignated 5HT-3 (Gothert & Schliker, 1987). There is also evidence that several different subtypes of the 5HT-3 binding site exist. A subtype, 5HT-3A, has been identified on substance P containing neurones in guinea pig ileum (Buchheit et al., 1985). Pain caused by applying serotonin to blistered skin is not inhibited by methysergide (a 5HT-1 and -2 antagonist) but is prevented by the compound ICS 205-930, acting at a site named 5HT-3B (Richardson et al., 1985). The Bezold-Jarisch reflex, by which serotonin causes reflex vagal bradycardia by a direct action on the coronary chemoreceptor, appears to be mediated through yet another subtype, designated 5HT-3C (Richardson et al., 1985). These subtypes of 5HT-1 and -3 binding sites have not yet been shown to have a definite role in the regulation of cardiovascular function in man.

Many of the ligands which have been used to identify serotonin binding sites are structurally very different from serotonin itself. Furthermore, agents such as ketanserin, trazodone, and spiperone have widely differing

affinities for supposed serotonergic type-2 receptors in different tissues (Leff & Martin, 1986). In marked contrast, tryptamine analogues, compounds which are structurally very similar to serotonin, have very consistent affinities for serotonin binding sites in different tissues (Leff, Martin & Morse, 1986). Thus, it is possible that serotonin binding sites identified by serotonin antagonists which are structurally dissimilar from serotonin do not correspond with true serotonin receptor subtypes.

1.4 Serotonin in blood platelets and plasma

1.4.1 Gut enterochromaffin cells; the major source of blood serotonin.

It is likely that most of the serotonin present in peripheral blood comes from enterochromaffin cells in the gut (Pletscher, 1968). In mammals, the largest store of serotonin in the body is in gut enterochromaffin cells (Erspamer, 1954), from where it is released by parasympathetic or sympathetic nerve stimulation, and increased intraluminal intestinal pressure and acidity (Forsberg & Miller, 1983). Serotonin may play a role in the regulation of gut peristalsis (Ruckebusche, 1984) and secretory function (Cho & Ogle, 1986; Jaffe, Ferrara & Sherlock, 1986). Some of the serotonin released from enterochromaffin cells finds its way into portal blood and then passes through the liver, where it is substantially

inactivated by hepatic monoamine oxidase A (Thomas & Vane, 1967). Serotonin which escapes metabolism by the liver is transported to the lungs, where most of the free amine that remains is removed by pulmonary endothelial cells. These cells have an avid uptake mechanism for serotonin (Lee & Fanburg, 1986) which they then metabolise through the monoamine oxidase A pathway (Gillis & Pitt, 1982).

1.4.2 Blood platelets and serotonin:

Almost all the serotonin in blood is stored in platelets (Tranzer, Da Prada & Pletscher, 1966). Platelets cannot synthesise serotonin as they do not contain tryptophan hydroxylase (Morrissey, Walker & Lovenberg, 1977). Hence, platelet stores of serotonin are entirely dependent on uptake of serotonin from plasma. Platelets actively take up serotonin from plasma when the concentration of free serotonin is $< 5\mu M$ (Hardisty & Stacey, 1955). This mode of uptake is inhibited by tricyclic antidepressants such as amitryptyline and imipramine (Peters & Grahame-Smith, 1980). When the concentration of free serotonin is $> 10\mu M$, the predominant mode of uptake changes to a passive mechanism (Malmgren et al., 1981). However, as maximal free serotonin levels in-vivo are in the low nanomolar range (Molyneux & Clark, 1985) the passive mechanism is unlikely to play any physiological role. After uptake through the external platelet membrane, serotonin is stored in the dense granules as high molecular weight complexes with 5'di- and tri-phosphonucleotides and

divalent cations (Pletscher, 1968). Storage in the dense granules is inhibited by reserpine (Da Prada & Pletscher, 1969).

Serotonin and other platelet contents are released from platelets when platelet aggregation occurs. Aggregation occurs in a stepwise manner. Initially there is a reversible change in platelet shape from a disc to a spiny sphere, then platelet clumping occurs, and finally there is release of platelet contents (Holmsen, 1977). The contents of the dense granules, including serotonin, adenosine diphosphate, adenosine triphosphate, calcium and inorganic pyrophosphate, are released before the contents of the alpha granules, which include beta-thromboglobulin, platelet factor 4, acid hydrolases and fibrinogen (Holmsen, 1977; De Clerck, 1986). Adenosine diphosphate and serotonin can cause further platelet aggregation, initiating a positive feedback loop (Holmsen, 1977). Serotonin induces complete aggregation of cat platelets with platelet content release. This effect is inhibited by 5HT-2 antagonists (De Clerck, Van Nueten & Reneman, 1984). In man, serotonin is a weak stimulant for platelet aggregation in platelet rich plasma (Holmsen & Karpatkin, 1983). In healthy subjects serotonin usually causes reversible platelet shape change in platelet rich plasma without proceeding to full platelet aggregation and content release. This shape change is inhibited by serotonergic type-2 antagonists (De Clerck, David & Janssen, 1982). Serotonin also potentiates the effects of threshold concentrations of powerful stimulants to

platelet aggregation, such as collagen and adenosine diphosphate. This potentiation is again inhibited by serotonergic type-2 antagonists (De Clerck et al., 1984). Serotonin induced platelet aggregation appears therefore to be largely mediated through the serotonergic type-2 receptor.

Vasoactive substances released during platelet aggregation can cause smooth muscle contraction in isolated blood vessels. This effect is inhibited by serotonergic type-2 antagonists (Vanhoutte & Cohen, 1983). This suggests that serotonin released from aggregating platelets can affect vascular tone.

1.4.3 Free plasma serotonin:

Problems of ex-vivo platelet aggregation and serotonin release make the estimation of free plasma serotonin difficult and prone to artefact. With scrupulous collection and preparation of samples, levels are virtually undetectable, < 1ng/ml (Molyneux & Clarke, 1985). There are several mechanisms for removing serotonin from plasma, including metabolism by the liver (Thomas & Vane, 1967) and lungs (Gillis & Pitt, 1982), and uptake by platelets (Peters & Grahame-Smith, 1980). Furthermore there is a high affinity serotonin binding protein, serotonectin, a glycoprotein which circulates in plasma both in the free state and bound to leucocyte and platelet membrane surfaces (Gershon & Tamir, 1984). Albumin has low affinity for serotonin, but can bind appreciable amounts,

because of the large amount of this protein in plasma compared to serotonectin (Pignatti & Cavalli-Sforza, 1975). Protein binding effectively reduces the amount of serotonin circulating free in plasma.

1.4.4 Blood serotonin and the carcinoid syndrome.

Patients with the carcinoid syndrome, caused by a tumour of enterochromaffin cells, secrete large quantities of serotonin into their blood. However this syndrome is not a good model for studying the effects of serotonin excess on blood pressure, as other vasoactive hormones, such as Substance P, are also secreted (Emson et al., 1984).

1.5 Peripheral cardiovascular effects of serotonin

1.5.1 Effects of serotonin on different parts of the vasculature.

Serotonin causes contractions in in-vitro preparations of most large arteries, including rat caudal artery (Cohen, Fuller & Wiley, 1981) and rabbit femoral artery (Van Nueten et al., 1982), and in large veins (Victorzon, Tapparelli & Muller-Schweinitzer, 1986). Concentrations of 10^{-6} to 10^{-7} M are required to elicit these effects. There is a close correlation between the affinity of antagonists for the serotonergic type-2 binding site and their ability to antagonise serotonin induced vasoconstriction in these preparations (Van Nueten et al., 1982). This suggests that

serotonin-induced vasoconstriction is mediated through the type-2 binding site. Serotonin also amplifies the effect of other vasoconstrictors including noradrenaline, adrenaline, angiotensin II, prostaglandin F2 alpha and arginine vasopressin (Kubu & Su, 1983; Van Nueten et al., 1982). These amplifying effects are also inhibited by antagonists drugs in correlation with their serotonergic type-2 binding affinity. However, serotonin induced vasoconstriction is not always mediated through the serotonergic type-2 binding site. Serotonin induced contraction of isolated rabbit basilar artery and dog saphenous vein is not affected by the serotonergic type-2 antagonist ketanserin (Bradley, Humphrey & Williams, 1986). As the serotonergic type-1 agonist 5-carboxamidotryptamine causes vasoconstriction in these preparations, it has been concluded that the receptor mediating serotonin induced vasoconstriction in these cases is likely to be similar to the serotonergic type-1 binding site (Bradley, Humphrey & Williams, 1986). The serotonergic type-2 antagonists ketanserin, trazodone and spiperone have variable effects on serotonin induced vasoconstriction in different tissues, not corresponding to their serotonergic type-2 receptor binding affinity (Leff & Martin, 1986). There are two main possible explanations for these findings. The binding sites for serotonin may differ between animal species. However, using tryptamine analogues (compounds structurally similar to serotonin) no differences could be found in serotonin receptors mediating vasoconstriction in blood vessels from

different animal species (Leff, Martin & Morse, 1986). This suggests that serotonergic binding sites identified by ligands such as ketanserin, trazodone or spiperone (compounds which are structurally dissimilar from serotonin) do not represent true serotonin receptor sites.

In small arteries and arterioles, serotonin causes vasodilatation. This effect appears to be mediated through the serotonergic type-1 binding site as it is blocked by methysergide, a non-specific serotonergic type-1 and -2 antagonist, but not by the serotonergic type-2 antagonist ketanserin (Feniuk, Humphrey & Watts, 1983, Marwood & Stokes, 1983). The 5HT-1 agonist 5-carboxamidotryptamine also relaxes arterioles (Saxena & Verdouw, 1985). Serotonin-induced vasodilatation of isolated contracted coronary arteries appears to be mediated through the 5HT-1 receptor. Interestingly, this vasodilatation does not occur in vessels stripped of endothelium (Cohen & Vanhoutte, 1985). The vasodilatation caused by serotonin in this preparation may be due to release of a vasorelaxant factor from endothelial cells, such as endothelium derived relaxant factor.

1.5.2 Peripheral cardiovascular effects of serotonin in intact animals and man.

Serotonin does not readily cross the blood brain barrier (Lexchin, Cude-Simpson & Stancer, 1979), therefore intravenous or intraperitoneal administration of serotonin can be used to investigate its action in the periphery. It

is an unpleasant drug to administer intravenously (Page & M^{C}Cubbin, 1953), causing nausea, dizziness, tingling and transient hyperventilation. Hence very few studies have examined the effects of intravenous serotonin in man.

Serotonin has complex actions on the cardiovascular system when administered intravenously to intact animals. An intravenous bolus of serotonin initially causes a reduction in blood pressure. This is due to a marked bradycardia caused by a direct action of serotonin on vagal afferents in the coronary vasculature initiating the Bezold Jarisch reflex, thought to be mediated through the M receptor (Kalkman, Engel & Hoyer, 1984; Saxena & Lawang, 1985; Saxena, Mylecharane & Heiligers, 1985). The binding site mediating this reflex also has been designated 5HT-3c (Richardson et al., 1985). After the initial depressor response there is a rise in blood pressure, caused by serotonin-induced vasoconstriction and a positive inotropic effect on the heart. This rise in blood pressure is thought to be mediated through the serotonergic type-2 receptor as it is inhibited by serotonergic type-2 antagonists (Kalkman et al., 1984; Saxena, Mylecharane & Heiligers, 1985). Lastly there is a prolonged period of hypotension due to serotonin induced peripheral vasodilatation thought to be mediated through the serotonergic type-1 receptor, as it is inhibited by drugs with serotonergic type-1 antagonistic activity such as methysergide but not by the serotonergic type-2 antagonist ketanserin (Marwood & Stokes, 1983; Kalkman et al., 1985; Saxena & Lawang, 1985). Similar triphasic responses have

been reported in the rat, the dog and man (Page & M^{C}Cubbin, 1953; Marwood & Stokes, 1984).

1.5.3 Interactions between serotonin and the sympathetic nervous system in the periphery.

Serotonin may play a role in the regulation of noradrenaline release from sympathetic nerve endings. Low concentrations of serotonin decrease the release of noradrenaline (Langer, 1981; Shepherd & Vanhoutte 1981). Serotonin released from aggregating platelets has been shown to inhibit adrenergic neurotransmission in isolated arteries and veins (Lorenz & Vanhoutte, 1985). This effect is probably mediated through a presynaptic serotonergic type-1 like receptor (Lorenz & Vanhoutte, 1985). Although high concentrations of serotonin (10^{-5}M) cause noradrenaline release (M^{C}Grath, 1977), it is unlikely that this mechanism plays any physiological role as free plasma serotonin levels are well below this range (1.3.3).

The effects of infused serotonin in the intact animal also depend on sympathetic nervous activity. Intravenous serotonin has a strong depressor effect in dogs made hypertensive by increasing sympathetic nervous outflow by sectioning buffer nerves. However, when sympathetic nervous activity is blocked serotonin causes a marked rise in blood pressure (Page & M^{C}Cubbin, 1953). Similarly, Phillips, Mylecharane & Shaw, (1985) have shown that serotonin causes vasodilatation in the femoral bed of the dog in the presence of increased sympathetic tone. This

effect of serotonin is thought to be mediated through a presynaptic inhibitory serotonergic receptor. In contrast, serotonin potentiates the vasoconstrictor response to intra-arterial noradrenaline in the forearm in man (Scroop & Walsh, 1968). Furthermore, serotonin augments the vasoconstrictor effects of noradrenaline in isolated blood vessels (Manzini, Maggi & Meli, 1986), an effect thought to be mediated through the serotonergic type-2 receptor (1.5.1). It has even been suggested that there may be structural overlap between serotonergic type-2 and alpha-1 adrenoreceptors in the periphery (Marwood & Stokes, 1984).

In conclusion there is evidence that the action of serotonin at postsynaptic serotonergic type-2 receptors, augmenting noradrenergic vasoconstriction, may be opposed by its effects at presynaptic serotonergic type-1 receptors where serotonin seems to inhibit noradrenaline release.

1.5.4 Effects of serotonin on the heart.

Serotonin is present in myocardial cells, which can sythethise and metabolise the amine (Sole, Schum & Van Loon, 1979). Serotonin may therefore have a local effect on myocardial function. Serotonin has a positive inotropic effect on cardiac muscle due to a local increase in noradrenaline concentration (Buccino et al., 1967; Reid, 1970). This action may be important when ventricular function is impaired (Sole et al., 1979). The receptor mediating the chronotropic effects of serotonin is

controversial. In isolated kitten atrial cells, serotonin has a positive chronotropic effect which does not appear to be mediated through serotonergic type-1 or -2 receptors (Kaumann, 1983). In contrast the tachycardia response to infused serotonin in the rat appears to be mediated through a serotonergic type-2 like receptor (Saxena & Lawang, 1985), and in the cat through a serotonin type-1-like receptor (Saxena, Mylecharane & Heiligers, 1985). It is not clear whether these conflicting findings represent genuine differences between species, or are simply due to the use of different serotonergic antagonists. These drugs may have variable effects as they may not act at true serotonin receptor sites, as previously discussed (1.3).

1.6 Serotonergic neurones and the central regulation of heart rate

Serotonergic neurones may play a role in the central regulation of heart rate by modulating sympathetic and parasympathetic outflow. In the cat, the reduction in heart rate caused by the serotonin precursor 5-hydroxytryptophan appears to be due to a centrally mediated decrease in sympathetic nervous activity (Baum & Shropshire, 1975). In the dog (Bhargava & Tangri, 1959), the cat (Baum & Shropshire, 1975) and the rat (Lambert et al., 1978) intracerebroventricular serotonin causes a fall in heart rate associated with reduced sympathetic nervous activity. Therefore, increased levels of brain serotonin

appear to reduce heart rate at least partly by lowering sympathetic nervous activity.

Serotonergic neurones may also influence parasympathetic control of heart rate. In the rat, intracerebroventricular serotonin reduces heart rate by increasing parasympathetic outflow as well as reducing sympathetic outflow (Dalton, 1986). The precursor 5-hydroxytryptophan, given to increase cerebral serotonin levels, attenuates reflex vagal bradycardia in the rat (Lin & Chern, 1979). Baroreceptor mediated reflex vagal bradycardia in the cat is also inhibited by intracerebroventricular 5-hydroxytryptophan (Tadepalli, 1980). This effect appears to be dependent on central conversion of 5-hydroxytryptophan to serotonin, as it is prevented by peripheral decarboxylase inhibition (Tadepalli, 1980). Conversely, depletion of serotonin in the rat brain with intracerebroventricular para-chlorophenyalanine or 5,7-dihydroxytryptamine enhances reflex vagal bradycardia caused by peripheral infusion of adrenaline (Lin & Chern, 1979).

Thus, there is substantial evidence from animal studies that serotonergic neurones play a role in the regulation of heart rate by altering sympathetic and parasympathetic cardiac nervous outflow. It is not clear at which receptor site or sites serotonin mediates these effects.

1.7 Serotonergic neurones and the central regulation of blood pressure

1.7.1 Effects of alteration of serotonergic neuronal activity on blood pressure.

The serotonin precursors tryptophan and 5-hydroxytryptophan, administered centrally or peripherally, are used to increase cerebral serotonin levels. Tryptophan usually does not alter blood pressure, except in very high doses in rats (Sved, Fernstrom & Wurtman, 1979) or after pretreatment with a monoamine oxidase inhibiter in cats (Florez & Armijo, 1974). High doses of tryptophan are not required to achieve maximal increases in brain serotonin levels, therefore the alteration of blood pressure seen by Sved et al. (1979) is probably due to a direct effect of tryptophan itself. Monoamine oxidase inhibitors can themselves alter blood pressure, also they affect the degradation of noradrenaline, a substance unequivocally implicated in blood pressure control (Kuhn, Wolf & Lovenberg, 1980).

Administration of the serotonin precursor 5-hydroxytryptophan usually causes a reduction in blood pressure. In the decerebrate and intact cat 5-hydroxytryptophan reduces blood pressure (Baum & Shropshire, 1975; Tadepalli, Mills & Schanberg, 1977; Florez & Armijo, 1974; Freed, Echizen & Bhaskaran, 1985). Intravenous and intracerebroventricular 5-hydroxytryptophan decrease blood pressure in dogs

(M^{C}Cubbin, Kameko & Page, 1960; Rabinowitz & Lown, 1978). This effect is associated with reduced sympathetic nervous activity (M^{C}Cubbin et al., 1960). However, other investigators found that 5-hydroxytryptophan causes an increase in blood pressure in dogs (Dunkley et al., 1972). In all these studies, effects of 5-hydroxytryptophan on blood pressure are prevented by decarboxylase inhibition, suggesting that these effects are dependent upon conversion to serotonin. Thus, the data on the effects of 5-hydroxytryptophan on blood pressure are conflicting. A possible explanation for this comes from a recent study which suggests that the effects of centrally administered 5-hydroxytryptophan may be dose dependent. In the rat, large doses of 5-hydroxytryptophan cause a reduction in blood pressure, whereas small doses produce a rise in blood pressure (Dalton et al., 1986).

Conflicting data have also arisen from studies of the effects of electrical stimulation of serotonergic neurones in the medullary raphe; blood pressure has been found to increase or decrease in different animal species (Coote & M^{C}Leod, 1974; Koss & Wang, 1972; Neumayer, Hare & Franz, 1974). Furthermore, activation of serotonergic neurones in different anatomical areas can cause opposite effects on blood pressure in the one animal species (Adair et al., 1977).

Para-chlorophenylalanine (PCPA) is an irreversable inhibitor of tryptophan hydroxylase. It can therefore be used to inhibit serotonergic neuronal activity. However this substance has had variable effects on blood pressure.

In the rat, intraperitoneal and intracerebroventricular PCPA increase blood pressure (Ito & Schanberg, 1972), whereas oral PCPA decreases blood pressure (Jarrott et al., 1975). PCPA appears to have no effect on blood pressure in cats (Helke et al., 1976; Helke, Quest & Gillis, 1978) or rabbits (Wing & Chalmers, 1974).

The neurotoxins 5,6- and 5,7-dihydroxytryptamine are taken up into serotonergic and noradrenergic neurones. Pretreatment with desimipramine inhibits noradrenaline uptake and increases the specificity of these neurotoxins for serotonergic neurones. Using these methods intracerebroventricular 5,7-dyhydroxytryptamine reduces blood pressure in the spontaneously hypertensive rat (Gothert & Klupp, 1978). Injection of 5,7-dihydroxytryptamine into the dorsal raphe inhibits the pressor response to electrical stimulation of this area in the Sprague Dawley rat (Robinson, Austin & Gibbens, 1985).

The great variability of effects of activation or inhibition of the serotonergic nervous system is probably due to several factors. The various mechanisms used in these experiments lack specificity. There are definite differences between species. Effects of these studies depend also on the activity of other counter-regulatory mechanisms for blood pressure control. As in the periphery, different serotonergic receptor subtypes may have opposing effects on blood pressure (1.5.1). It is not possible to draw firm conclusions on the role of serotonergic neurones in the central regulation of blood pressure in man from these studies.

1.7.2 Effects of alterations of blood pressure on serotonergic neuronal activity.

During drug induced hypertension serotonergic neuronal acivity in the dorsal raphe and nucleus tractus solitarius is increased. This effect is blocked by sino-aortic denervation, and is therefore thought to be mediated through the baroreceptors (Freed et al., 1985). This provides indirect evidence that changes in serotonergic neuronal activity may be involved in baroreceptor mediated regulation of blood pressure.

1.7.3 Interaction between serotonergic and noradrenergic neurones in the central regulation of blood pressure.

It is likely that there is an interaction between serotonergic neurones and sympathetic control of blood pressure. Serotonergic neuronal tracts have a close anatomical association with noradrenergic neuronal pathways which are involved in blood pressure control. Several studies in cats and dogs suggest that increased levels of brain serotonin cause a reduction in sympathetic nervous outflow (Baum & Shropshire 1975; Tadepalli et al., 1977; Antonaccio & Taylor, 1977; Coote & M^{C}Leod, 1974; Koss & Wang, 1972; Neumayer et al., 1974).

1.8 Serotonergic neurones and behaviour

Serotonergic neurones may play a role in the regulation of neuropsychological function. In man, the serotonergic agonists lysergic acid diethylamide (LSD) and M-chlorophenylpiperazine cause anxiety, activation and euphoria (Mueller, Murphy & Sunderland, 1985). Serotonergic type-2 antagonists such as ritanserin inhibit LSD induced behavioural changes in animal models (Colpaert et al., 1985) and improve symptoms in patients with anxiety disorders (Ceulemans et al., 1985). In animals, serotonin, serotonin precursors and serotonergic agonists cause behavioural excitation; these effects appear to be mediated through the serotonergic type-2 receptor (Leysen, 1984). In view of all these findings, there is great interest in the potential use of serotonergic type-2 antagonists as anxiolytics.

Chronic treatment with known effective antidepressant drugs generally down-regulates serotonergic type-2 receptor sites, although the situation is complex as serotonergic neuronal transmission is usually increased due to inhibition of serotonin uptake (Willner, 1985). The precise role of serotonergic neurones in the pathogenesis of depression remains controversial.

Drugs that act at serotonergic type-1 receptor subtypes affect behaviour in mice and rats (Goodwin & Green, 1985). However serotonergic type-1-like receptors have not yet been shown to have a definite role in the control of behaviour in man.

1.9 Effects of serotonin on the renin-angiotensin system and adrenocortical function

It has been claimed that serotonin plays a role in the regulation of plasma renin, aldosterone and cortisol. Serotonin and its precursor tryptophan increase plasma renin in rats (Meyer & Hertting, 1974) and man (Modlinger, Schonmuller & Arora, 1979) respectively. Stimulation of serotonergic neurones with p-chloroamphetamine in rats causes a rise of plasma renin (Van De Kar & Richardson-Morton, 1986). This effect does not appear to be due to alterations in sympathoadrenal activity (Van De Kar & Richardson-Morton, 1986). Serotonin uptake blockers inhibit fenfluramine induced rises in plasma renin in the rat (Van De Kar, Richardson-Morton & Urban, 1985). In man, non-selective serotonergic type-1 and -2 blockade with cyproheptidine inhibits the rise in renin caused by sodium depletion (Epstein & Hamilton, 1977). It is thought that all these effects are mediated centrally through serotonergic neurones.

Serotonin causes increased production of aldosterone and corticosterone in isolated rat zona glomerulosa cells. This effect appears to be mediated through the serotonergic type-2 receptor as it is inhibited by ketanserin, a serotonergic type-2 antagonist with alpha-1 adrenoreceptor blocking activity, but not by the alpha-1 adrenoreceptor antagonist prazosin (Williams, Shaikh & Edwards, 1984). The increased production of aldosterone and cortisol from zona glomerulosa cells caused by

angiotensin II is inhibited by ketanserin, suggesting that adrenal serotonergic type-2 receptors may modify angiotensin II stimulated aldosterone production (Rocco et al., 1985). Serotonin induced aldosterone production in isolated rat zona glomerulosa cells appears to be linked through the serotonergic type-2 receptor to increased adenylate cyclase activity (Matsuoka et al., 1985). Thus there is good evidence from these in-vitro studies that serotonin acts in the periphery at the serotonergic type-2 receptor in the adrenal zona glomerula to enhance steroidogenesis.

Serotonergic neurones may be involved in the central regulation of steroidogenesis. The increase in serum corticosterone levels in rats caused by the non-specific serotonergic agonist quipazine is inhibited by ketanserin (Fuller & Snoddy, 1984). This is thought to be a central serotonergic type-2 effect, as enhanced steroidogenesis caused by quipazine is not blocked by peripheral serotonergic antagonists such as xylamidine (Fuller & Snoddy, 1983). Serotonergic agonists increase corticotrophin releasing factor release from in vitro preparations of rat hypothalamus (Holmes et al., 1982). In the intact rat, serotonergic agonists increase serum coricotrophin releasing factor (Gibbs & Vale, 1983) and adrenocorticotrophic hormone (Bruni, Hawkins & Yen, 1982). In man, the serotonergic type-2 antagonist ketanserin attenuates the rise in adrenocorticotrophic hormone caused by hypoglycaemia (Prescott et al., 1984). The serotonin precursor tryptophan increases plasma aldosterone and

cortisol in healthy man (Modlinger et al., 1979). Similarly, M-chlorophenylpiperazine, a serotonin agonist, increases plasma cortisol in healthy subjects (Mueller et al., 1985). The serotonin precursor 5-hydroxytryptophan increases plasma aldosterone in healthy man, but this effect is not inhibited by either the serotonergic type-2 antagonist ketanserin or the non-selective serotonergic type-1 and -2 antagonist methysergide (Shenker, Gross & Grekin, 1985a). Thus it is not clear which receptor type mediates the rise in aldosterone caused by 5-hydroxytryptophan. However, inhibition of peripheral decarboxylase activity with carboxydopa potentiates the effects of 5-hydroxytryptophan, suggesting that 5-hydroxytryptophan induced aldosterone production is mediated through central conversion to serotonin, as 5-hydroxytryptophan itself does not increase aldosterone production in isolated zona glomerulosa cells (Shenker, Gross & Grekin, 1985b). The serotonergic type-1 and -2 antagonist cyproheptidine reduces plasma aldosterone in patients with idiopathic hyperaldosteronism (Gross et al., 1981). This effect also is probably mediated through a central pathway, as peripheral decarboxlase inhibition potentiates the effects of cyproheptidine (Gross et al., 1981).

Thus, there is evidence to suggest that serotonin is involved in both the central and the peripheral regulation of aldosterone and cortisol production.

1.10 Effects of serotonin on bronchial smooth muscle

In the cat and dog exogenously administered serotonin has a direct action on bronchial smooth muscle, causing constriction of both central and peripheral airways (Colebatch, Olsen & Nadel, 1966). The direct effect of serotonin on respiratory smooth muscle appears to be mediated through the serotonergic type-2 receptor (Cohen et al., 1985; Saxena & Lawang, 1985). Serotonin may have also an effect on the efferent vagal pathway, potentiating vagal bronchoconstriction, as central bronchoconstriction caused by stimulation of the vagus nerve in the dog is potentiated by inhaled serotonin (Sheller et al., 1982). However not all investigators have confirmed this finding; parasympathetic mediated constriction in isolated guinea pig trachea is not affected by serotonin (M^{C}Caig, 1986). Serotonin-induced bronchoconstriction in the cat is partly mediated by inhibition of the non-adrenergic non-cholinergic neural system (Bai, Macklem & Martin, 1986). Despite the in-vitro and animal data, the role of serotonin in the regulation of airway calibre in man remains unclear.

If serotonin is an important mediator of bronchoconstriction in man, it is likely that the main source would be from enhanced platelet aggregation and content release. Although platelet aggregation has been claimed to be increased in both allergen (Knauer et al., 1981) and exercise induced asthma in man (Johnson et al., 1984), not all studies have confirmed this (Durham et al.,

1984). An association between enhanced platelet aggregation and exercise or allergen induced bronchoconstriction in man has not been firmly established.

1.11 Non-specific serotonergic type-1 and -2 antagonists

Of the non-specific serotonergic type-1 and -2 antagonists available, the effects on the cardiovascular system of cyproheptidine and methysergide have been the most thoroughly investigated. Cyproheptidine is also a potent histamine type-1 receptor antagonist (Stone et al., 1961). It has no detectable effects on blood pressure (Stone et al., 1961; Marwood & Stokes, 1983). Methysergide has partial agonist activity at serotonergic type receptor sites (Saxena, Bolt & Dhasmana, 1987). It acts centrally, reducing blood pressure by lowering sympathetic outflow (Antonaccio & Taylor, 1977). It is not clear whether this central effect is mediated through 5HT-1 or -2 receptors. Methysergide has no peripheral effects on the cardiovascular system (Antonaccio & Taylor, 1977). However these findings do not exclude a role for serotonin in the regulation of blood pressure in the periphery, as serotonergic type-1 and -2 antagonism may have opposing effects on vascular tone (1.5.1).

1.12 The serotonergic type-2 antagonist ketanserin

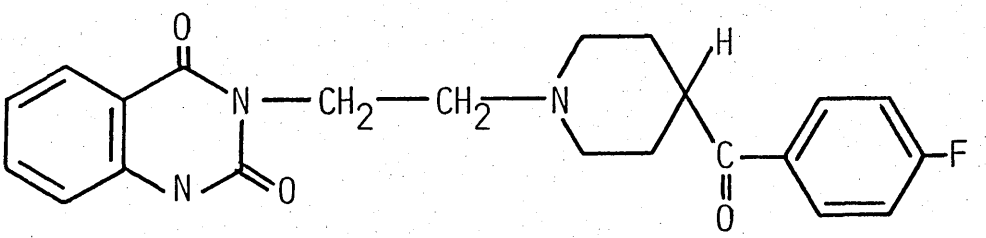
1.12.1 Receptor binding affinity of ketanserin.

Ketanserin is the first clinically available serotonergic type-2 antagonist which is virtually devoid of serotonergic type-1 binding affinity (Leysen et al, 1981). In most tissues it is a competitive serotonergic type-2 antagonist (Van Nueten et al., 1981; Marwood & Stokes, 1983). Importantly, ketanserin has no partial agonist effects (Leysen et al., 1981). However, ketanserin also has appreciable alpha-1 adrenoreceptor binding affinity, approximately one fifth of its affinity for the serotonergic type-2 receptor (Leysen et al., 1981). The major mode of metabolism of ketanserin is by reduction of the ketone to alcohol (figure 2). The resulting metabolite, ketanserinol, has one thousand times less affinity than ketanserin for the 5HT-2 receptor (Frenken & Kaumann, 1984).

1.12.2 Mechanism of the antihypertensive action of ketanserin.

Ketanserin is an effective antihypertensive agent. It reduces blood pressure in both acute (Wenting et al., 1982; Wenting et al., 1984; Zoccali et al., 1983; Zabludowski et al., 1984) and chronic dosage (De Cree et al., 1981; Fagard et al., 1984; Woittiez et al., 1986). However, the mechanism of reduction of blood pressure

Ketanserin



Ketanserinol

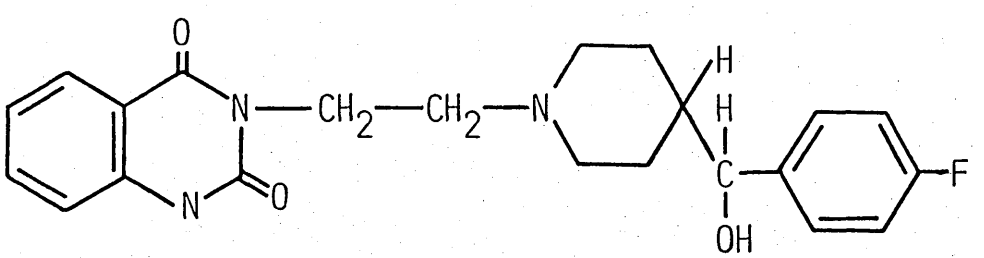


Figure 2
Structural formulae of ketanserin and its major metabolite ketanserinol.

caused by ketanserin remains controversial.

Ketanserin inhibits serotonin induced platelet aggregation in acute (De Clerck & Xhonneux, 1985) and chronic dosage in man (Vermylen et al., 1986). Serotonin induced platelet aggregation is thought to be mediated through the serotonergic type-2 binding site (1.4.2). Thus, in keeping with its pharmacological profile, ketanserin seems to be an effective serotonergic type-2 antagonist when given in doses known to lower blood pressure in essential hypertension.

It is possible that ketanserin reduces blood pressure by acting through the serotonergic type-2 receptor to inhibit serotonin induced vasoconstriction in the periphery. Ketanserin has been shown to inhibit serotonin induced vasoconstriction in isolated blood vessels (Van Nueten et al., 1982). Serotonin also amplifies the effect of other vasoconstrictor agents such as noradrenaline and angiotensin II (Van Nueten et al., 1981; Van Nueten et al., 1982). This amplification is inhibited by serotonergic antagonists in close correlation with their serotonergic type-2 binding affinity (Van Nueten et al., 1982). In is possible that ketanserin could act on these amplification mechanisms in-vivo to reduce arterial blood pressure.

Ketanserin may also have a central effect on serotonergic neurones involved in the regulation of blood pressure. Ketanserin is not lipid soluble and therefore crosses the blood-brain barrier poorly (Leysen et al., 1981), however the blood brain barrier is deficient in

certain areas, some of which lie close to parts concerned with cerebrovascular regulation. Undoubtably, radiolabelled ketanserin does cross the blood-brain barrier in rats (Laduron, Jannsen & Leysen, 1982). Furthermore, ketanserin has been shown to have central effects in-vivo in animals and man. Neuropsychological effects of serotonin agonists in rats are prevented by ketanserin (Niemegeers et al., 1983). In man, ketanserin can cause side effects of drowsiness and headache (Staessen et al., 1985). Therefore, ketanserin should not be considered to act solely in the periphery.

Another possible mechanism for the reduction in blood pressure caused by ketanserin is by decreasing activity of the renin-angiotensin-aldosterone system. Ketanserin may act directly on serotonergic type-2 receptors on zona glomerulosa cells in the adrenal cortex to reduce aldosterone and corticosterone synthesis (Williams et al., 1984; Rocco et al., 1985), or have a central effect on serotonergic type-2 receptors possibly involved in the regulation of plasma renin (Epstein & Hamilton, 1977), aldosterone (Shenker et al., 1985b) and cortisol (Modlinger et al., 1979; Mueller et al., 1985).

Although there has been considerable interest raised at the possibility that ketanserin reduces blood pressure by its effects as a serotonergic antagonist, ketanserin could be acting through the alpha-1 adrenoreceptor to reduce blood pressure. Alpha-1 adrenoreptor antagonists such as prazosin have a direct effect on vascular smooth muscle causing vasodilatation (Stanaszek et al., 1983). Central

alpha-1 adrenoreceptor antagonism may also reduce blood pressure by lowering sympathetic nervous outflow (M^{c}Call & Humphrey, 1982; M^{c}Call & Schuette, 1984).

The possibility that ketanserin reduces blood pressure by peripheral alpha-1 antagonism has been studied in-vivo in man by examining the pressor response to intravenous infusion or bolus injection of alpha-1 agonists such as phenylephrine and methoxamine. Acute intravenous administration of ketanserin can lower blood pressure in hypertensive patients without attenuating the pressor response to phenylephrine (Wenting et al., 1982; Zabludowski et al., 1984). These results suggest that acutely ketanserin reduces blood pressure by mechanisms other than peripheral alpha-1 antagonism. In contrast, Reimann & Frohlich (1983) have shown that an intravenous infusion of ketanserin attenuated the pressor response to methoxamine. Three days of ketanserin 40mg twice daily administered to healthy subjects attenuated the pressor response to infused phenylephrine (Zabludowski, Ball & Robertson, 1985). Four weeks of ketanserin 40mg twice daily given to hypertensive patients attenuated the pressor response to methoxamine (Fagard et al., 1984). These results suggest that intravenous infusion or chronic oral administration of ketanserin causes alpha-1 antagonism, and that the reduction of blood pressure caused by the drug in these circumstances may be at least partly due to this effect.

1.12.3 Effects of ketanserin on heart rate.

Ketanserin reduces blood pressure by peripheral vasodilatation (Wenting et al., 1982; Wenting et al., 1984: Fagard et al., 1984). Drugs which cause vasodilatation usually stimulate a baroreceptor mediated increase in heart rate, due to increased cardiac sympathetic outflow and reduced cardiac vagal activity (Man In'T Veld et al., 1980). However, the acute reduction in blood pressure caused by intravenous ketanserin administration is accompanied by only a small rise in heart rate (Wenting et al., 1982; De Cree et al., 1981; Zoccali et al., 1983). Similarly, chronic oral ketanserin has been reported to reduce blood pressure either without increasing heart rate (De Cree et al., 1981; Woittiez et al., 1986), or even with a small reduction in heart rate (Fagard et al., 1984). This lack of reflex tachycardia suggests that ketanserin may modify baroreceptor arc activity, although the precise mechanism of this action is unclear.

1.13 The serotonergic type-2 antagonist ritanserin

Ritanserin (figure 3) is a new, highly selective serotonergic antagonist (Leysen et al., 1985). It is a non-competitive serotonergic type-2 antagonist. Importantly, like ketanserin, it has no partial agonist activity at this site. Ritanserin is virtually devoid of receptor binding affinity at serotonergic type-1 and

Ritanserin

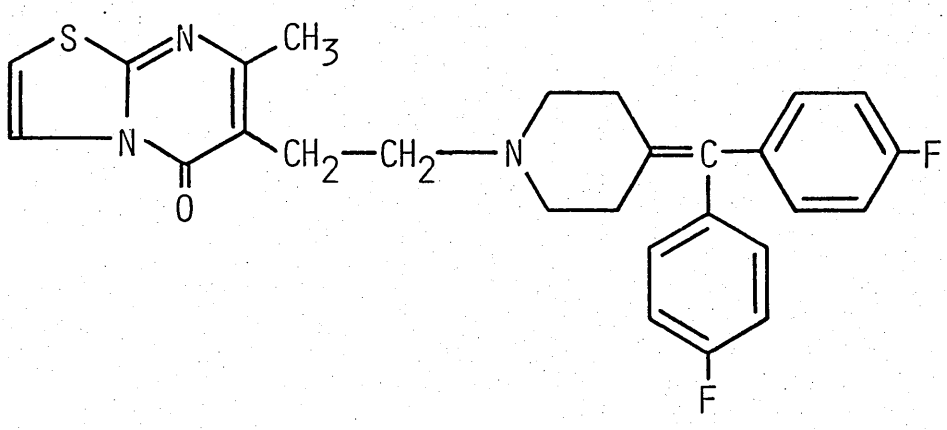


Figure 3
Structural formula of ritanserin.

alpha-1 receptor sites; it binds to histamine type-1 receptors with one twenty-fifth of its affinity at the serotonergic type-2 receptor. Ritanserin is a very long acting agent. In the rat, serotonergic type-2 receptor sites are occupied for over 24 hours after a single dose (Leysen et al., 1985). It is more lipophilic than ketanserin, and therefore should cross the blood brain barrier more readily. Ritanserin inhibits head twitch behaviour in mice (Goodwin & Green 1985) and has anxiolytic effects in rats (Colpaert et al., 1985). In man a single oral dose of ritanserin 10mg has detectable central effects 12 hours after dosing, causing alterations in sleep pattern (Idzikwski & Mills, 1986). In a placebo controlled study, ritanserin 10mg daily for 2 weeks improved symptoms in patients with anxiety disorders (Ceulemans et al., 1985).

The main advantage of ritanserin over ketanserin as a research tool is its lack of alpha-1 adrenoreceptor binding affinity (Leysen et al., 1981; Leysen et al., 1985). This makes ritanserin a more appropriate agent to investigate the role of the serotonergic type-2 receptor in the regulation of blood pressure and heart rate.

1.14 Background and objectives of the research

1.14.1 Serotonin and platelet function in essential hypertension.

Serotonin is a vasoactive amine that may play a role in the development and maintenance of raised arterial pressure. Nearly all the serotonin circulating in blood is stored in platelets. Platelet contents, including serotonin and beta-thromboglobulin, are released during platelet aggregation (Holmsen, 1977). Serotonin released from platelets may cause vasoconstriction and raised arterial pressure. Serotonin is itself a weak stimulant of platelet aggregation (De Clerck, 1986) and may cause therefore a further increase in platelet aggregation and platelet content release.

I have studied serotonin induced platelet aggregation, spontaneous platelet aggregation, platelet serotonin levels and plasma beta-thromboglobulin in patients with untreated essential hypertension compared to age and sex matched normotensive controls.

1.14.2 Assay of platelet serotonin levels by high pressure liquid chromatography with electrochemical detection.

In view of its high sensitivity, specificity, safety and relatively low cost compared to other techniques (Kissinger, Bruntlett & Shoup, 1981; Sasa et al., 1985)

high pressure liquid chromatography with electrochemical detection is potentially a useful method for serotonin assay. I have investigated the use of this method in the assay of serotonin in human platelet rich plasma and whole blood.

1.14.3 Mechanism of action of the serotonergic type-2 antagonist ketanserin.

The serotonergic type-2 antagonist ketanserin is an effective antihypertensive agent. However its effects may not be wholly due to serotonergic antagonism. Short-term oral ketanserin has been shown to reduce the pressor response to the alpha-1 adrenoreceptor agonists phenylephrine (Zabludowski et al., 1985) and methoxamine (Fagard et al., 1984), suggesting that the drug acts at least partly as an alpha-1 antagonist. However, alternative explanations for these findings have been suggested. The reduction in blood pressure caused by ketanserin is due primarily to peripheral vasodilatation, which could in itself reduce the vascular response to any pressor agent. Also there may be an important interplay between serotonergic type-2 and alpha-1 adrenoreceptor sites, in that in vitro, serotonin can be shown to amplify the vasoconstriction caused by noradrenaline. This effect is also seen with other vasoconstrictors such as angiotensin II (Van Nueten et al., 1981). This amplification is inhibited by serotonin antagonists in close correlation with their serotonergic type-2 binding

affinity, suggesting that it is mediated through the 5HT-2 receptor (Janssen, 1985). Therefore, I have studied the effects of ketanserin on the pressor response to infused angiotensin II, a vasoconstrictor largely devoid of alpha-1 adrenergic activity, in healthy subjects.

Ketanserin inhibits the increase in production of aldosterone caused by serotonin and angiotensin II in isolated rat zona glomerulosa cells (Rocco et al., 1985; Williams et al., 1984). Serotonergic neurones may also play a role in the central regulation of aldosterone (Shenker et al., 1985b), cortisol (Prescott et al., 1984) and renin (Epstein & Hamilton, 1977). I have examined the effects of ketanserin on the renin-angiotensin-aldosterone system and plasma cortisol before and after graded intravenous infusion of angiotensin II in healthy subjects.

The peripheral vasodilatation and reduction in blood pressure, caused either by acute (Zabludowski et al., 1984) or chronic (Fagard et al., 1984) ketanserin administration is not associated with the expected reflex increase in heart rate. It has been claimed that this effect may be due to changes in cardiac autonomic outflow. I have studied the effects of ketanserin on cardiac parasympathetic outflow, measured by the heart rate responses to change of posture from lying to standing, deep breathing and Valsalva manoeuvre.

In vitro, ketanserin causes prolongation of the action potential and effective refractory period (Saman, Thandroyen & Opie, 1985), effects consistent with class

III antiarhythmic activity. I have examined the effects of ketanserin on the QT interval, representing action potential duration, in healthy subjects.

1.14.4 Effects of ritanserin in essential hypertension:

Ritanserin is a new serotonergic type-2 antagonist which differs from ketanserin in that it is virtually devoid of alpha-1 adrenoreceptor binding affinity (Leysen et al., 1985). This makes it a particularly useful agent to investigate the role of the serotonergic type-2 receptor in hypertension. Ritanserin is more lipophilic than ketanserin and should penetrate the blood brain barrier more readily (Leysen et al., 1985). Central serotonergic antagonism may cause changes in psychological function. Serotonin-induced platelet aggregation is mediated through the serotonergic type-2 receptor, making this a useful marker to demonstrate drug compliance and adequate dosing.

I have studied; (a) the effects of acute ritanserin administration on blood pressure, heart rate and psychological function, and (b) the effects of chronic oral ritanserin on serotonin induced platelet aggregation, blood pressure, heart rate, forearm blood flow and venous compliance, QT interval, and psychological function, in patients with essential hypertension.

1.14.5 Effects of ketanserin on resting bronchomotor tone and exercise-induced bronchospasm in adult atopic asthma:

Serotonin, acting through the type-2 receptor, may cause contraction of respiratory smooth muscle (Colebatch et al., 1966; Cohen et al., 1985). It has been suggested that serotonin released from platelets may play a role in the pathogenesis of asthma. I have studied the effects of acute administration of the serotonergic type-2 antagonist ketanserin on resting bronchomotor tone and exercise-induced bronchoconstriction in adult patients with atopic asthma.

Chapter 2; Methods

2.1 Measurement of serotonin in platelet rich plasma and whole blood by high pressure liquid chromatography with electrochemical detection

2.1.1 Materials.

Serotonin, 5-hydroxyindoleacetic acid and prostaglandin E_1 were purchased from Sigma Laboratories. Methanol (HPLC grade) was purchased from Rathburn Chemicals. All other routine laboratory chemicals (Analar grade) were purchased from Sigma laboratories. All water used was doubly glass distilled.

2.1.2 Methods of measurement.

Serotonin levels were measured by high pressure (3000 P.S.I.) liquid chromatography (HPLC) with electrochemical detection (oxidation potential +0.7V). The following equipment was used: a glassy carbon electrochemical detector (Bioanalytical Systems), a Gilson model 302 pump with a model 802 manometric module, a Gilson 231 automated sample injector, and a Shimadzu C-R3A Chromatopac integrator. The HPLC column used was a Spherisorb 5 ODS2, length 30cm and diameter 2mm (HPLC technology). The flow rate was 0.2ml/minute. The automated injector was set to sample 50µL. The electrochemical detector was set at 1 nanoamp/volt. All chromatography was performed at room

temperature.

The mobile phase consisted of a citrate / acetate buffer, pH 5.2, containing 0.25g/l octyl sulphate and 17.5% methanol, giving retention times for serotonin and 5-hydroxyindoleacetic acid of 13.9 minutes (n=19, coefficient of variation 2.2%) and 0.4 minutes (n=19, coefficient of variation 5.9%) respectively.

Standard solutions of serotonin were prepared immediately before each assay. For assay of serotonin in platelet rich plasma and whole blood, a standard solution of 200 nanogrammes serotonin / ml (0.494nmol/ml) was prepared by dissolving 10mg of serotonin creatinine sulphate (molecular weight 405) in 100ml 0.1M hydrochloric acid, and further diluting 200µL of this solution to 100ml with 0.1M hydrochloric acid. Standard solutions remained stable at room temperature for up to 5 hours (figure 4). Standard solutions of serotonin were run in duplicate prior to and after every 6 platelet rich plasma or whole blood samples. The peak height for serotonin, as measured by electrochemical detection, was found to be directly proportional to known concentrations of serotonin in 0.1 N hydrochloric acid over the range 1-50ng/50µL (20-1000ng/ml; figure 5), which embraces the range required for assay of serotonin in platelet rich plasma. The limit of detection of serotonin by these methods was < 5 pmol/ml.

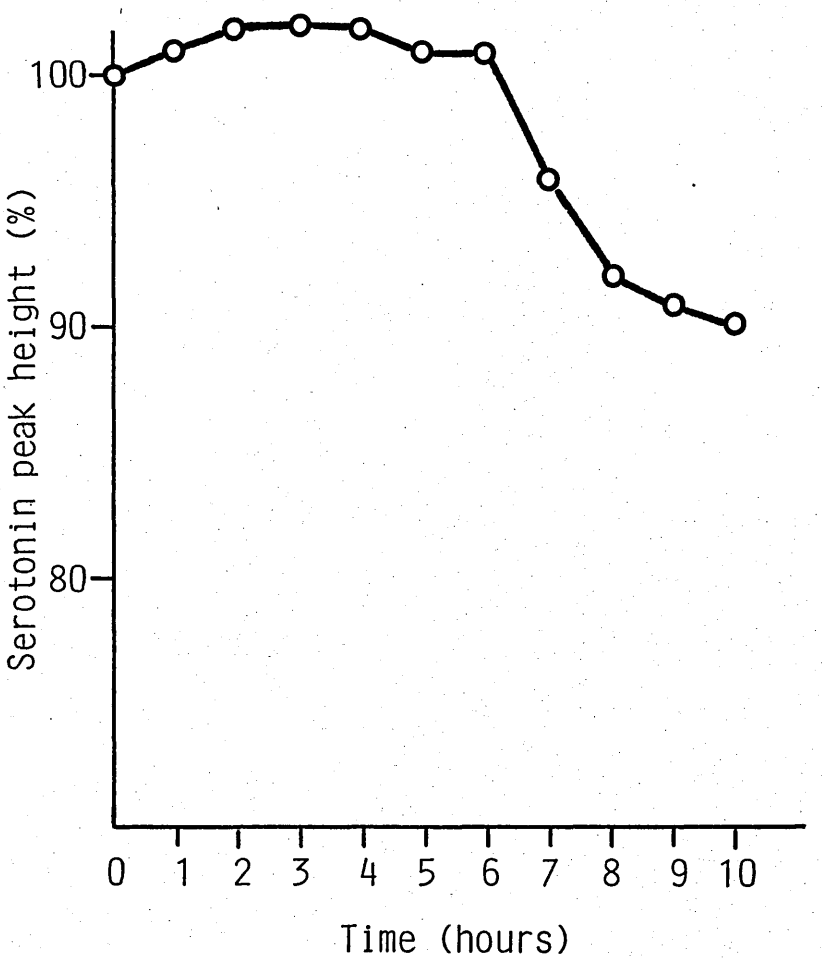


Figure 4
Serial measurements of a standard solution of serotonin (0.494nmol/ml in 0.1M hydrochloric acid) at room temperature using high pressure liquid chromatography with electrochemical detection. Initial peak height (at time 0 hours) = 100%.

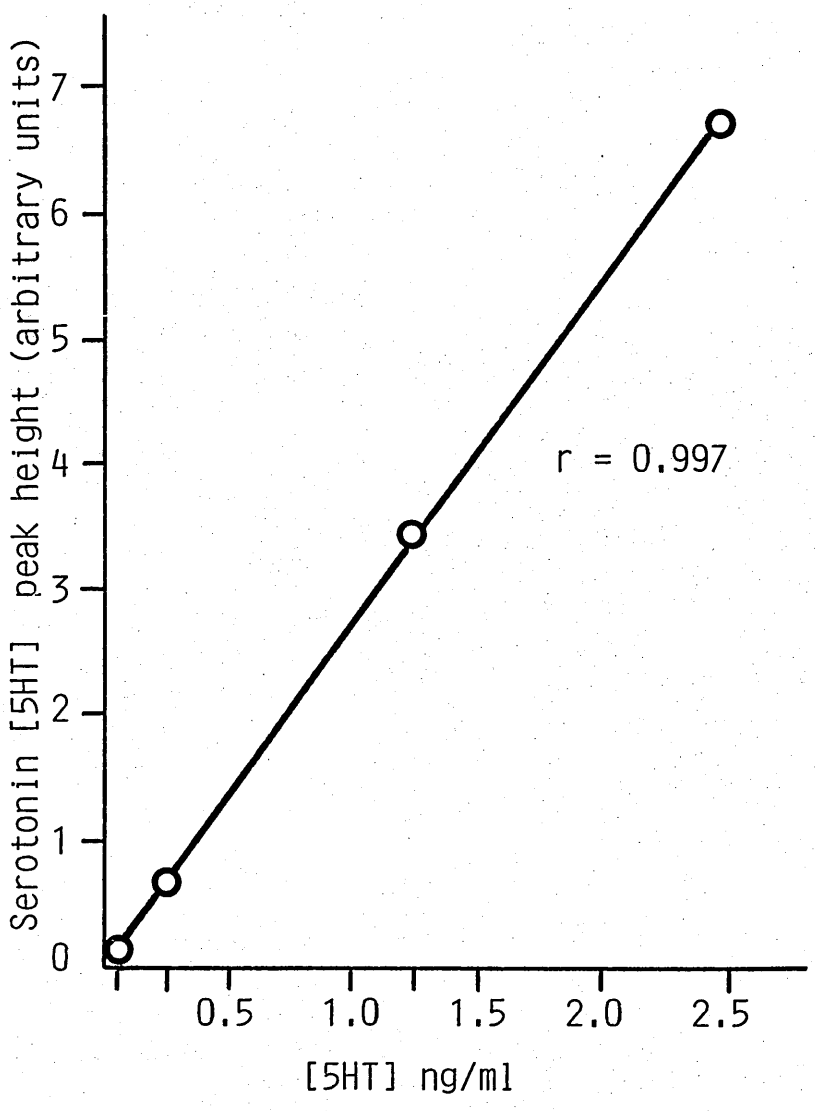


Figure 5
Serotonin peak height, measured by high pressure liquid chromatography with electrochemical detection, verses serotonin concentration in 0.1M hydrochloric acid.

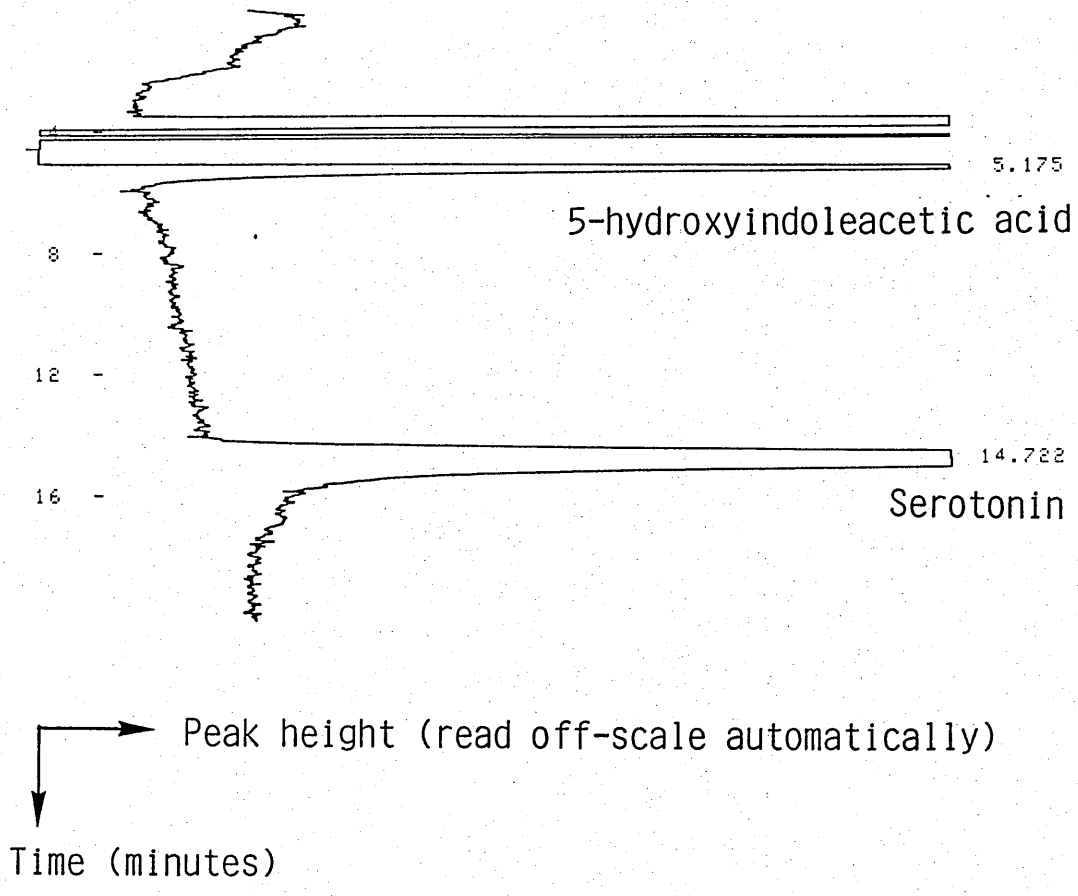


Figure 6
Chromatogram of serotonin (0.494nmol/ml) and 5-hydroxyindoleacetic acid (1.235nmol/l) in 0.1M hydrochloric acid, measured by high pressure liquid chromatography with electrochemical detection.

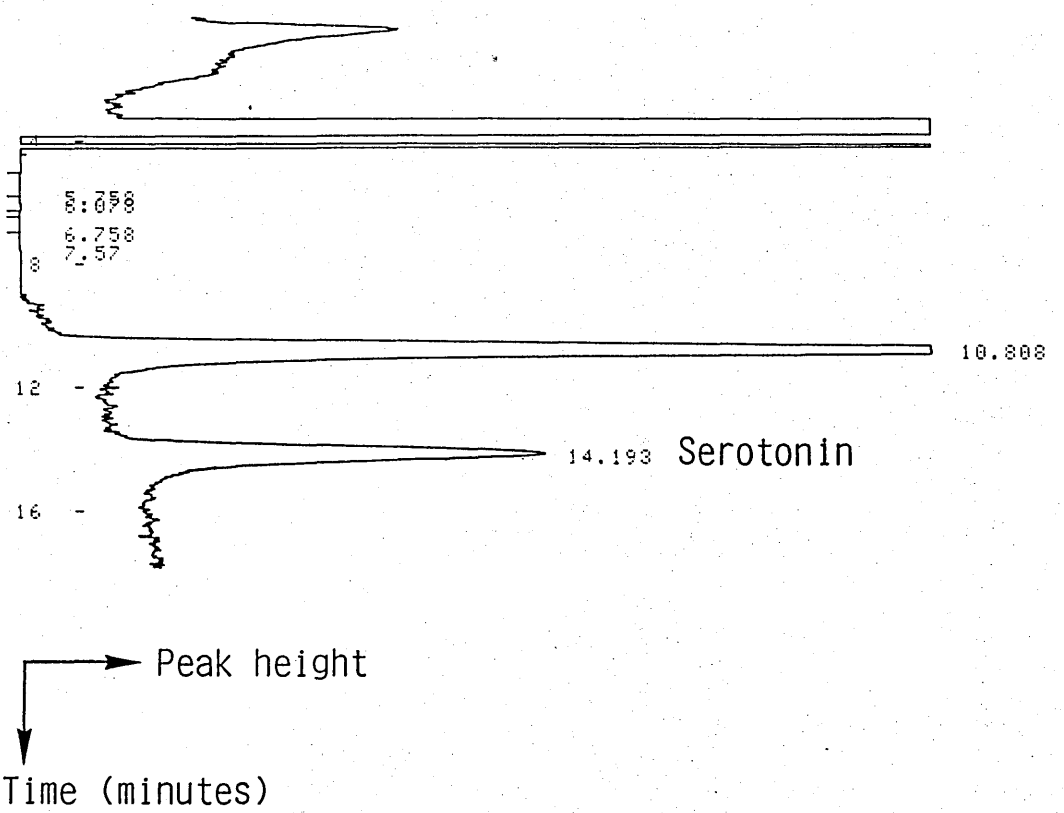


Figure 7
Chromatogram of serotonin in platelet rich plasma, measured by high pressure liquid chromatography with electrochemical detection.

2.1.3 Collection of blood.

Venous blood for assay of serotonin was collected from the ante-cubital fossa or other prominant arm veins using a 19 gauge Butterfly cannula, without venous stasis. During development of the assay blood was donated by healthy male volunteers from the medical and laboatory staff. Samples were transferred immediately to the appropriate collection media and placed on ice. All samples which were not analysed immediately were prepared and stored at -70^{o}C within 30 minutes of collection.

2.1.4 Serotonin in platelet rich plasma.

Samples for assay of serotonin in platelet rich plasma were prepared as follows; 5mls whole blood was added to 200uL containing 50ng prostaglandin E_1, sodium metabisulphite 25mM and ethylenediamine tetra-acetic acid 15%, and spun at 160G for 10 minutes to obtain platelet rich plasma (PRP). An aliquot of PRP was used for determination of platelet count using a Coulter Counter, and the remainder was stored at -70^{o}C. At the time of assay samples were thawed and 0.5ml distilled water was added to 0.5ml PRP and left on ice for 5 minutes to ensure platelet lysis. Samples were deproteinised by adding 50uL sodium hydroxide (1M) and 50µL zinc sulphate 20% with centrifugation at 10,000G in a cold-room at 4^{o}C for 5 minutes. Further sample purification was achieved by taking 0.5ml of the supernatent, adding 100µL perchloric

acid (4M) and repeat centrifugation at 10,000G at $4^{o}C$ for 5 minutes. The resulting supernatant was then assayed for serotonin. Each sample was assayed in duplicate.

These preparation steps resulted in the dilution of platelet rich plasma by a factor of 2.76. The serotonin content of PRP, expressed per 10^9 platelets can be calculated as follows, using the dilution factor, the peak heights of serotonin from the standard solution of serotonin and platelet rich plasma, and the platelet count in that platelet rich plasma:

$$\text{Platelet serotonin } (\text{nmol}/10^9 \text{platelets}) = \frac{\text{PRP peak} \cdot 2.76 \cdot [5HT]_{\text{standard}} (\text{nmol/ml})}{\text{standard peak height} \cdot \text{number platelets in PRP } (10^9/\text{ml})}$$

With the retention time for serotonin of 13-15 minutes there was good discrimination between serotonin and other peaks in all patients. However, the 5-hydroxyindoleacetic acid peak at 0.4 minutes could not be reliably separated from other interfering peaks in platelet rich plasma (figures 6 and 7).

Using these methods the inter-assay co-efficient of variation for 6 aliquots of platelet rich plasma prepared in parallel was 7.1%. The intra-assay co-efficient of variation was 2.2% (n=10). There was no detectable reduction of serotonin peak height in platelet rich plasma after 5 hours at room temperature (n=6, change = $0.0 \pm 9.2\%$, mean$\pm$standard deviation). The recovery of 1000ng serotonin

in 50μL 0.1 N hydrochloric acid spiked into 0.5ml platelet rich plasma was 102+10 % (n=6, mean±standard deviation).

2.1.5 Serotonin in whole blood.

Samples for analysis of serotonin content of whole blood were collected as follows: 2mls whole blood were added to 200μL containing 15% ethylenediamine tetra-acetic acid and 25mM sodium metabisulphide. Samples were frozen on dry ice, then thawed at room temperature. An alternative method of collection of whole blood into 200μL ethylenediamine tetra-acetic acid 15% and ascorbic acid 3%, as used by Geeraerts, Schimpfessel & Crockaert, (1974) in a fluorometric assay, was found to cause a broad interfering peak on electrochemical detection. Therefore, this method of sample collection was not pursued further.

The methods of sample preparation of Kellum & Jaffe, (1976) and Korczyn et al., (1985), used prior to radio-immunoassay and fluorometric assays respectively, were investigated for applicability to high pressure liquid chromatography with electrochemical detection.

To ensure complete platelet lysis, 2mls of ice cold distilled water were added to each 2ml sample of whole blood (collected as above), and samples left on ice for 10minutes. To this was added 200μL sodium hydroxide (1M) and 200μL zinc sulphate 20%. This step caused a thick slurry which was extremely difficult to mix. This problem could not be resolved even with increased sample dilution with distilled water compared to published methods (Kellum

& Jaffe, 1976: Korczyn et al., 1985). After prolonged sample mixing, centrifugation was performed at 1500g at 4^{o}C for 10minutes, using a Beckman TJ4 centrifuge. Each 1ml of supernatant was mixed with 200uL of perchloric acid (4M), then centrifuged at 10,000g for 5minutes. This added step removed a further substantial precipitate. The resulting supernatant was assayed for serotonin.

The recovery of 2,500 and 10,000ng serotonin in 100 microlitres 0.1N hydrochloric acid, added to 2mls whole blood and prepared as above was 9 and 27% respectively. In one subject, the serotonin content of whole blood expressed per 10^9 platelets was only 23% of that found in the same subject in platelet rich plasma per 10^9 platelets. No residual serotonin could be extracted with perchloric acid (4M) from either of the precipitates obtained after the two centrifugation steps described above.

In view of the difficulties with the serotonin assay in whole blood it was abandoned in favour of assay of serotonin in platelet rich plasma (2.1.4).

2.2 Serotonin and platelet aggregation in patients with essential hypertension compared to age and sex matched normotensive controls

2.2.1 Patients and subjects.

Twenty three patients (12 males) with untreated essential hypertension, defined as a supine blood pressure of > 160/90mmHg, were compared with 20 normotensive subjects (12 males), with supine blood pressure < 160/90mmHg. All women were post-menopausal. The study protocol was approved by the Ethical Supervisory Committee of the Western Infirmary, Glasgow. Informed consent was obtained. The mean age of patients with hypertension was 60.7 (range 47-70) years compared to 59.9 (51-70) in the normotensive group.

Hypertensive patients were recruited from a local general practice and from general medical outpatient clinics. Two of these patients had a history of stable angina pectoris, 1 intermittent claudication, and 3 chronic obstructive airways disease. Fundoscopy revealed no patients with fundal haemorrhages or exudates. Urinalysis (Multistix) was negative for blood and protein in all patients. One patient had electrocardiographic evidence of left ventricular hypertrophy (S in V_2 + R in V_6 > 35mm) and strain (downsloping ST depression > 1mm in the lateral leads). All other electrocardiographs were normal. Hypertensive patients all had a cardiothoracic ratio of < 50% on postero-anterior chest X-ray.

Normotensive subjects were recruited from the same general practice and a general surgical outpatient clinic. They were healthy or had minor surgical problems such as inguinal hernia or ingrown toe-nails. The only drug allowed in either group was paracetamol. Particular care was taken to exclude those with a recent history of ingestion of aspirin, non-steroidal anti-inflammatory drugs, antihypertensive drugs, or any other agents known to have haemodynamic or antiplatelet effects.

2.2.2 Methods.

Patients attended on two occasions one week apart. At the first visit, a full medical history (including smoking habit and usual weekly alcohol intake) and examination were performed. Supine blood pressure (mean of 2 recordings, right arm) and heart rate were measured after 15 minutes lying, and erect blood pressure and heart rate after 2 minutes standing. Venous blood was taken for urea and electrolytes, blood sugar, liver function tests, and full blood count. A standard 12-lead electrocardiograph was recorded. In hypertensive patients, a chest X-ray was performed. At the second visit, body weight was recorded, then repeat measurements of blood pressure and heart rate were made as above. A size 19 gauge Butterfly cannula was then inserted into a vein in the left ante-cubital fossa for blood (without stasis) for plasma beta-thromboglobulin, serotonin induced and spontaneous platelet aggregation, whole blood viscosity and platelet

serotonin. Samples were taken in a standard order as above.

2.2.3 Methods of measurement.

Blood pressure was measured using a Hawksley Random Zero Sphygmomanometer (diastolic phase V). Pulse rate was measured by manual palpation at the wrist over 60 seconds.

Serotonin-induced platelet aggregation and spontaneous platelet aggregation in whole blood were quantified as described by Saniabadi et al., (1983). Venous blood was anticoagulated with 3.8% trisodium citrate in a proportion of 9 to 1, giving a final citrate concentration of 12.9mM. One ml aliquots of this mixture were then transferred to plastic vials, flushed with 5% carbon dioxide in air, and sealed. Ten microlitres was withdrawn from an aliquot and added to 9.1mls diluent (Laboratory Impex Limited, London) and mixed by gentle inversion. The red cell count was determined with the Clay Adams haematology analyser (HA-5), allowing determination of whole blood single platelet count by the Ultra flow 100 whole blood platelet counter (Clay Adams). The aliquots were then incubated at 37^{o}C in a Gallenkamp shaking water bath at 130-140 shakes per minute. Spontaneous platelet aggregation was quantified by measuring the reduction in platelet count caused by incubation in the shaking water bath. Serotonin-induced platelet aggregation was quantified by measuring the reduction in single platelet count caused by adding serotonin (1μM) to whole blood and incubation at

37°C in the shaking water bath for 15 minutes. A blank test was also performed with the solvent used for serotonin. The time taken from the collection of blood to the completion of the assay was less than 3 hours in all subjects.

For measurement of whole blood viscosity, venous blood was anticoagulated with ethylenediamine tetra-acetic acid (1.5g/L). Whole blood viscosity was measured at high ($94s^{-1}$) and low ($0.94s^{-1}$) shear rates using a Contraves LS30 rotational viscometer. Haematocrit was determined using a Hawksley microcentrifuge at 13,000g for 5 minutes, allowing correction of whole blood viscosity for this variable. The shear rate is defined as the gradient in velocity between adjacent fluid layers in laminar flow (arterial blood flow in-vivo is laminar); the velocity difference ($m.s^{-1}$) divided by distance between layers (m) gives the S.I. units, s^{-1}. Shear stress is the force which causes shearing (relative movement of layers) in a flowing liquid (mPa). The viscosity of fluid in laminar flow is equal to the ratio of shear stress to shear rate (Lowe, 1986).

Plasma beta-thromboglobulin was measured by radioimmunoassay (Radiochemical Centre, Amersham). Venous blood was collected into proprietary test tubes containing ethylenediamine tetra-acetic acid, theophylline and prostaglandin E_1, and placed in ice-cold water before centrifugation at 3000g at 4°C for 30 minutes. The middle third of the platelet poor plasma was then removed and stored at -70°C until assay.

Platelet serotonin was measured as described in section 2.1.4 .

Data for platelet agregation studies, plasma beta-thromboglobulin, whole blood viscosity and platelet serotonin were incomplete due to technical problems.

2.1.4 Statistical analysis.

Statistical analysis of the differences between the hypertensive and normotensive groups was by unpaired Student (t) test (2-tailed). For each subject, mean supine and erect blood pressures and heart rates from the 2 visits were calculated for analysis.

To analyse the relationship between serotonin-induced platelet aggregation and other variables, linear regression analyses were performed, using the percentage of platelets remaining after the addition of serotonin as the dependent variable and choosing the independent variables from age, body weight, supine blood pressure, plasma beta-thromboglobulin, whole blood viscosity and red blood cell count. Multiple regression analysis of the percentage of platelets remaining after the addition of serotonin as the dependent variable against the independent variables as above was then performed; in this analysis, whole blood viscosity corrected for haematocrit at high and low shear rates were used as they correlated better than uncorrected viscosities with the percentage of platelets remaining after the addition of serotonin. Only 23 patients (12 hypertensive, 11 normotensive) had

recorded data for all variables, and consequently only these patients have been included in the multiple regression analysis. To analyse the relationship between platelet serotonin content and plasma beta-thromboglobulin, a linear regression model was fitted using the platelet serotonin content as the dependent variable and plasma beta-thromboglobulin as the independent variable. Regression analyses were performed using the BMPD statistical package.

Results are expressed as the mean$\pm$standard error of the mean. Results were considered statistically significant at $p<0.05$.

2.3 The effects of short-term ketanserin treatment on blood pressure, heart rate, the renin angiotensin system, adrenocortical function, QT interval and vagal function and the response to infused angiotensin II in healthy subjects

2.3.1 Subjects.

Eight normotensive male volunteers, mean age 25 (range 20-31) years, weight 75 (64-88) Kg, were investigated. All were healthy, as determined by history, physical examination and routine laboratory screening. The study protocol was approved by the Ethical Supervisory Committee of the Western Infirmary, Glasgow. Informed consent was obtained.

2.3.2 Methods.

In a double blind randomised crossover study design, ketanserin 40mg and matching placebo were each given orally twice daily for three days, with a minimum of three days between phases. Supine (5 minutes lying) and erect blood pressure (2 minutes standing) and pulse rate were measured before (9am) and one hour after (10am) the first doses of ketanserin 40mg and placebo. On the fourth day subjects attended the ward at 8am for supervised administration of ketanserin or placebo and insertion of an intravenous cannula. Having lain supine for 45 minutes, 3 baseline measurements of blood pressure and heart rate

were made over a 15 minute period prior to a graded intravenous infusion of angiotensin II (Hypertensin, Ciba) (2,4,8,16 and 32 ng/Kg/minute, 5 minutes at each rate); blood pressure and heart rate were recorded at the end of each 5 minute period. Venesection was performed before and at the end of each infusion. Plasma concentrations of drug, renin, angiotensin II, aldosterone and cortisol were measured. Fifteen minutes after the infusion, when blood pressure had returned to baseline, supine and erect pressure and heart rate responses to the lying to standing, Valsalva and deep breathing manoeuvres were recorded (Ewing & Clarke, 1982). The timing of these observations was chosen to correspond as closely as possible with the likely time of peak plasma ketanserin concentrations (Hedner, Pettersson & Persson, 1986; Waller, Tucker & Ramsay, 1987).

The above observations and angiotensin II infusion were repeated at 3pm on day 4, 7 hours after drug or placebo, when drug concentrations were expected to be approaching trough levels (Hedner, Pettersson & Persson, 1986; Waller, Tucker & Ramsay, 1987).

2.3.3 Method of measurement of blood pressure.

Blood pressure was measured with a standard mercury sphygmomanometer (diastolic phase V). Mean arterial pressure was calculated as diastolic blood pressure + [(systolic - diastolic pressure)/3].

2.3.4 Methods of measurement of cardiac parasympathetic outflow.

Heart rate responses to change of posture from lying to standing, deep breathing, and the Valsalva manoeuvre were recorded as described by Ewing and Clarke (1982). These tests of autonomic function were performed in standard order as above. Blood pressure and heart rate were allowed to return to basal levels, with a minimum of 5 minutes rest between tests. Changes in RR' interval were recorded using a single channel electrocardiograph with a paper speed of 25mm/sec.

Changing posture from lying to standing initially causes an increase in blood pressure due to increased peripheral resistance, caused by compression of arteries and arterioles by contraction of muscles in the legs and abdomen. This increase in blood pressure lasts only a few seconds (Borst et al., 1984). Subsequently, blood pressure decreases, probably due to reduced cardiac output caused by venous pooling and reduced venous return to the heart, and stimulation of arterial baroreceptors due to the initial increase in blood pressure (Borst et al., 1984). This reduction in blood pressure is minimised by baroreceptor mediated increased sympathetic nervous outflow, causing peripheral vasoconstriction, and by an increase in heart rate, which is maximal around the 15th beat after standing. This increase in heart rate is largely abolished by atropine, suggesting that it is caused mainly by a reflex reduction in cardiac vagal

outflow (Ewing et al., 1978; Ewing et al., 1980). These reflex responses act to increase blood pressure back above supine levels. There is then a rebound reduction in heart rate, maximal around the 30th beat after standing, which is abolished by atropine suggesting that it is mediated through increased cardiac vagal outflow (Ewing et al., 1978; Ewing et al., 1980). The ratio of the longest to the shortest RR' interval, around the 30th and 15th beats respectively after standing, gives an index of cardiac parasympathetic outflow (Ewing et al., 1978; Ewing & Clarke, 1982). Changes in this ratio reflect alterations in cardiac vagal tone.

Deep breathing causes marked changes in heart rate (sinus arrhythmia) in healthy subjects. Inspiration causes activation of the stretch receptors in the lungs, causing reduced vagal outflow and an increase in heart rate. The opposite occurs during expiration. The beat to beat variation in heart rate caused by breathing is abolished by atropine but unaffected by propranolol, suggesting that it is largely under vagal control (Wheeler et al., 1973). Deep breathing, with maximal inspiration and expiration each lasting 5 seconds, was performed for 30 seconds in the sitting position (Ewing & Clarke, 1982); the mean ratio of the longest RR' interval during expiration to the shortest RR' interval during inspiration was calculated. Changes in this ratio reflect alterations in cardiac parasympathetic outflow.

The Valsalva manoeuvre was performed at an intra-oral pressure of 40mmHg (measured by blowing into a standard

mercury sphygmomanometer) for 15 seconds while seated (Ewing & Clarke, 1982). This test was performed 3 times, each from tidal inspiration. Subjects were instructed to avoid gasping after releasing intra-oral pressure. The Valsalva manoeuvre causes a transient increase in intrathoracic pressure. The sudden increase in intrathoracic and intra-abdominal pressure caused by straining compresses blood out into peripheral arteries, causing an initial increase in arterial pressure (Eckberg, 1980). In response, there is a baroreceptor mediated reduction in heart rate due to increased cardiac parasympathetic outflow (phase I), and reduced vasoconstrictor sympathetic nervous outflow to resistance vessels. In phase II there is a reduction in venous return, with pooling of blood in the limbs, causing reduced left ventricular stroke volume and cardiac output, leading to reduced arterial pressure. In response to this there is a baroreceptor mediated increase in heart rate caused by reduced parasympathetic nervous outflow, and an increase in peripheral resistance due to augmented sympathetic vasoconstrictor tone. In phase III, immediately after the release of straining there is a further reduction in blood pressure due to the sudden reduction in intrathoracic pressure and consequent dilatation of intrathoracic vessels. This causes a further increase in heart rate mediated by reduced vagal outflow, and increased sympathetic nervous outflow to resistance vessels. In phase IV, left ventricular stroke volume and cardiac output rapidly return to normal, but arterial

resistance vessels remain constricted, causing a rise in arterial pressure above baseline levels. This in turn causes a baroreceptor mediated reduction of sympathetic vasoconstrictor outflow to resistance vessels, and a fall in heart rate due to increased parasympathetic tone (Eckberg, 1980). Thus the heart rate changes during Valsalva are mediated by alterations in cardiac vagal outflow. The mean ratio of the longest RR' interval during phase II (while straining) to the shortest RR' interval during phase IV (after straining) gives an index of cardiac parasympathetic tone (Ewing & Clarke, 1982).

2.3.5 Methods of measurement of the QT interval.

QT and QT_C intervals were calculated as the mean from four consecutive cardiac cycles, taken from a single channel electrocardiograph with a paper speed of 25mm/sec. The QT_C was calculated by Bazett's formula (QT divided by the square root of the preceeding RR' interval; Bazett, 1920). For each subject, the most appropriate standard limb lead, with a clearly defined Q and upright T wave, was selected and maintained for all further recordings. All QT intervals were measured from the beginning of the Q wave to the end of the T wave.

2.3.6 Methods of measurement of plasma renin, angiotensin II, aldosterone, cortisol, ketanserin and ketanserinol.

Plasma active renin (Millar et al., 1980), angiotensin II (Dusterdieck & M^{C}Elwee, 1971), aldosterone and cortisol (Nicholls et al., 1980) were measured by radio-immumoassay; plasma ketanserin and ketanserinol by high pressure liquid chromatography with fluorescence detection (Davies, 1983). Interassay coefficients of variation were less than 15% for all methods.

2.3.7 Statistical analysis.

Data were analysed by Student's paired (t) test (2-tailed). All comparisons are of ketanserin verses placebo. For analysis of the blood pressure responses to infused angiotensin II regression lines of blood pressure change against infusion rate were calculated by the method of least squares after drug and placebo for each subject. All results are expressed as the mean$\pm$standard error of the mean. Results were considered statistically significant at $p<0.05$.

2.4 Effects of single oral doses of ritanserin and ketanserin on blood pressure, heart rate and psychological function in patients with untreated essential hypertension

2.4.1 Patients.

Ten patients (8 males), mean age 62 (range 40-79) years, weight 73 (40-103) kg, with untreated essential hypertension, defined as a blood pressure of greater than 160/95mmHg measured in the sitting position on at least 2 occasions, were studied. The study protocol was approved by the Ethical Supervisory Committee of the Western Infirmary, Glasgow. Informed consent was obtained from all patients.

2.4.2 Methods.

In a double blind randomised four-way crossover study, ritanserin 10mg, ritanserin 20mg, ketanserin 40mg and placebo were each given as a single dose orally, with a minimum of 1 week between phases. Patients attended at 9am. Two baseline measurements of sitting and standing blood pressure and heart rate were made, after 30mins sitting in quiet surroundings. The drug or placebo was then administered, and repeat measurements of blood pressure and heart rate made at 30, 60 and 90 mins, and 2, 4, 6 and 8hrs later. Psychological function testing (6 patients only) was performed prior to, and at 1, 2, 4, 6 and 8 hrs after, drug and placebo administration.

2.4.3 Methods of measurement of blood pressure, heart rate and psychological function.

Blood pressure (phase V diastolic) was measured using a Hawksley Random Zero Sphygmomanometer. Mean arterial pressure was calculated as diastolic blood pressure + [(systolic - diastolic pressure)/3]. At each time point 3 measurements of blood pressure and pulse rate after 5 mins sitting, and 2 measurements immediately on standing, were taken. Pulse rate was measured by manual palpation at the wrist over 60 seconds.

Psychological function testing was performed using a standard visual anologue scale of 18 subjective feelings (Herbert, Johns & Dore, 1976; appendix 1) which allows calculation of scores for tranquillity and alertness, and the Multiple Affect Adjective Check List (Zuckerman & Lubin, 1965; appendix 2), which gives scores for anxiety, depression and hostility.

2.4.4 Statistical analysis.

Analyses of blood pressure and pulse rate were done by calculating the area under the curve after each treatment for each patient, and tests of psychological function by calculating mean changes from baseline after drug and placebo. Blood pressure and pulse rate changes after drug administration were then compared with changes after placebo using the Student's paired (t) test (2-tailed); psychological function test changes were compared

similarly but using the Wilcoxon signed rank test. The tranquillity and alertness scores (Herbert, Johns & Dore, 1976) are expressed as percentages of the maximal possible scores. All results are expressed as mean$\pm$standard error of the mean. Differences were considered statistically significant at $p<0.05$.

2.5 Effects of chronic oral ritanserin on blood pressure, heart rate, forearm blood flow and venous compliance, QRS and QT intervals, serotonin-induced platelet aggregation and psychological function in patients with untreated essential hypertension

2.5.1 Patients.

Nineteen patients (11 males) with untreated essential hypertension were studied. All women were post-menopausal. Age and weight ranged from 42-71 (mean 60) years and 55.4-100.9 (74.2) Kg respectively. The study protocol was approved by the Ethical Supervisory Committee of the Western Infirmary, Glasgow. Informed consent was obtained from all patients. The study was discontinued prematurely (the study protocol was for 30 patients) when reports of possible adverse effects on the electrocardiograph of healthy volunteers were received from another centre. These changes proved subsequently to be machine artifact.

2.5.2 Methods.

After 4 weeks single-blind placebo run-in (weeks -4 to 0), patients received either ritanserin 10mg twice daily (10 patients) or matching placebo (9 patients) for a further 4 weeks (weeks 0 to 4) in a randomised double-blind parallel group study. One patient was removed from the study after developing a supra-ventricular tachycardia following randomisation to placebo. Analysis

has been restricted to the 18 patients who completed the study.

Investigations were performed in a standard order, with measurements repeated at the same time of day within individuals. At weeks -4, -2, 0, 2 and 4 body weight was recorded prior to measurement of supine blood pressure (twice) and pulse rate after 15 minutes lying, and erect blood pressure (once) and pulse rate 2 minutes after standing. Blood pressure was measured in the right arm by Hawksley Random Zero Sphygmomanometer (diastolic phase V). Pulse rate was measured by manual palpation at the wrist over 60 seconds.

At weeks 0 and 4 a Butterfly cannula (19 gauge) was then inserted into the left antecubital fossa and blood taken for platelet aggregation studies, platelet serotonin levels, urea and electrolytes and full blood count. An electrocardiograph was recorded and tests of psychological function then administered. Finally, forearm blood flow and venous compliance were measured in the right arm.

Measurements of blood pressure, pulse rate, body weight, urea and electrolytes and full blood count were made on, and tests of psychological function administered to, all 18 patients. The electrocardiograph was recorded, and platelet aggregation studies measured in 14 patients (7 on ritanserin). Spontaneous and serotonin-induced platelet aggregation were measured as previously described (2.2.3).

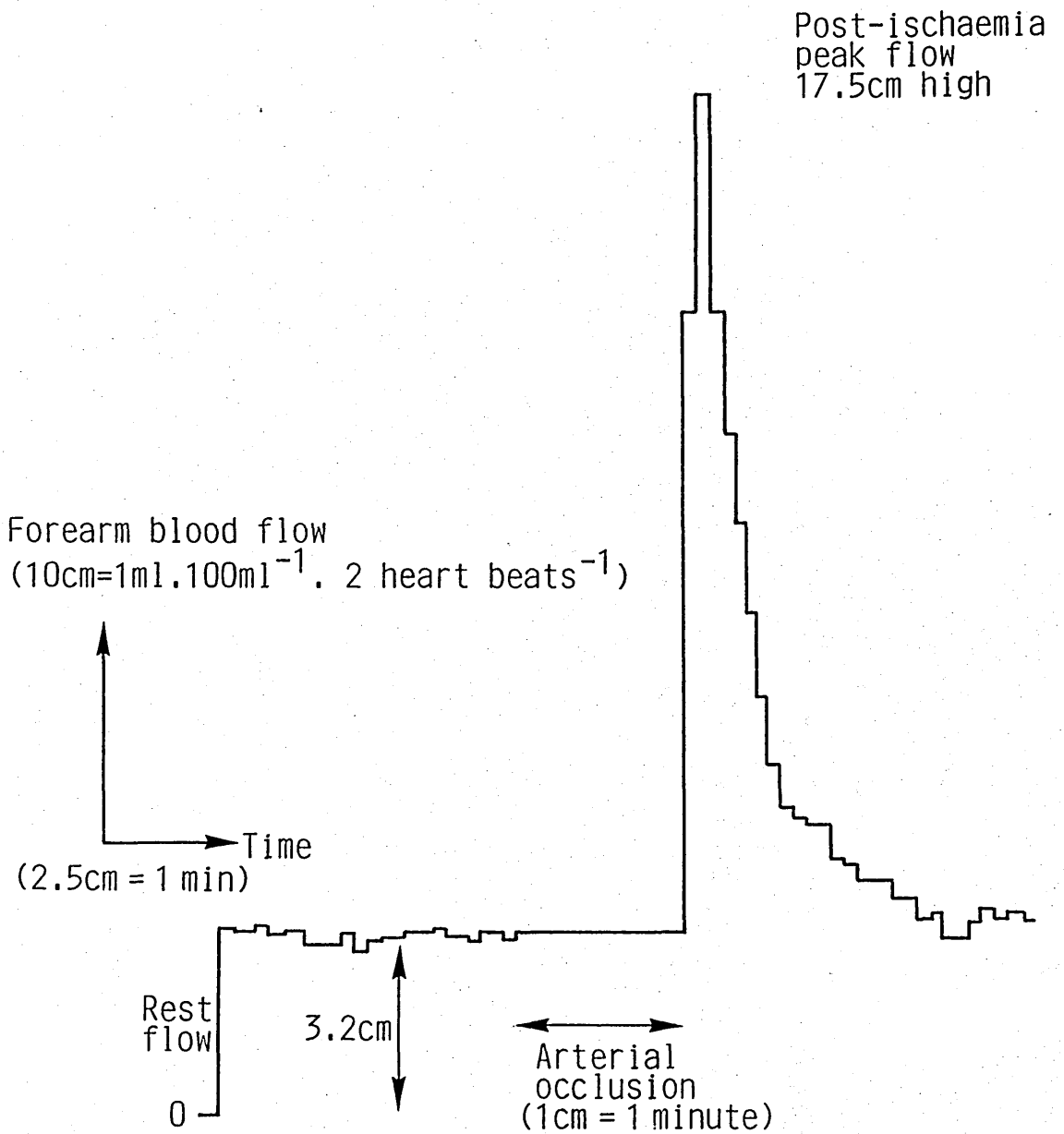


Figure 8
Forearm blood flow at rest and after 3 minutes of arterial occlusion, measured by mercury-in-strain guage venous occlusion plethysmography.

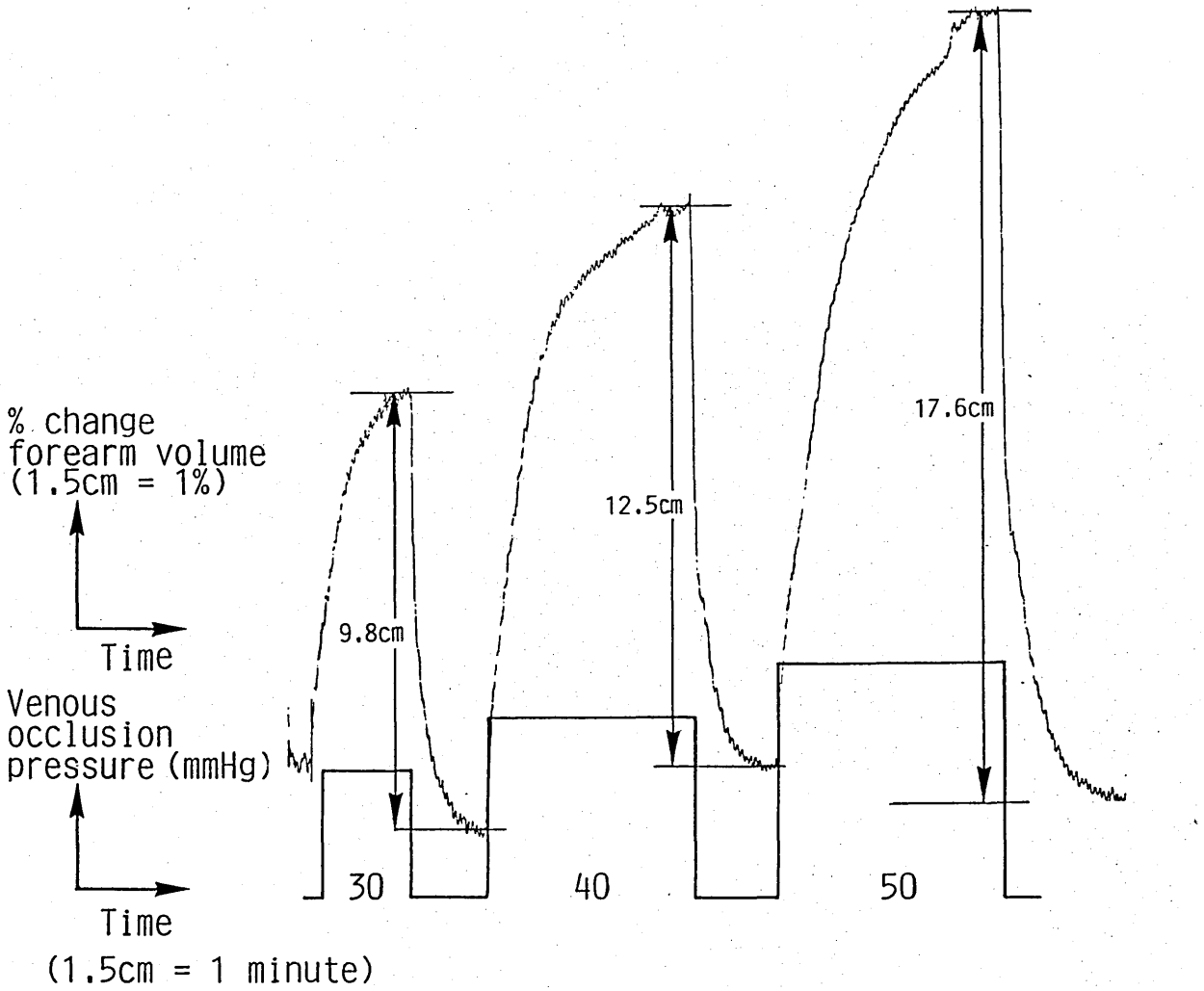


Figure 9
Forearm venous compliance, quantified by measuring the increase in forearm volume at venous occlusion, pressures of 30, 40, 50 and 60mmHg, by mercury-in-strain guage venous occlusion plethysmography.

2.5.3 Measurement of forearm blood flow and venous compliance by mercury-in-strain gauge venous occlusion plethysmography.

Forearm blood flow and venous compliance were measured by mercury-in-strain gauge venous occlusion plethysmography using the Janssen Periflow. This is a simple, safe non-invasive technique. If we assume that the forearm is approximately cylindrical in shape:

$c = 2\pi r$ or $r = c/2\pi$

$V = L\pi r^2 = L\pi (c/2\pi)^2 = Lc^2/4\pi$

(Where c is the circumferance, r the radius, V the volume and L the length of a cylinder).

Therefore the volume of the forearm is proportional to the square of the circumference.

As the amount of mercury in the strain guage is constant, any increase (n) in length of the mercury column causes a proportional decrease (n) in cross-sectional area.

$R = SL/a$

$v = la = nla/n$

(where R is the resistance of the column, S the specific resistance of mercury, l the length and a the cross sectional area of the mercury column)

Therefore, $R = Sn^2l/a$, and any change in resistance of the mercury column is proportional to the square of the change in length of the column. The length of the mercury column is equal to the circumferance of the forearm. Thus, changes in both the resistance of the mercury column and

the volume of the forearm are proportional to the square of the change in forearm circumferance. It follows that changes in volume of the forearm are directly proportional to changes in resistance of the mercury strain gauge. These assumptions have been shown to be valid by studies of blood flow measured by mercury-in-strain gauge venous occlusion plethysmography during isolated limb perfusion (Englund, Hallbrook & Ling, 1972).

Co-ordination of measurements of change in forearm volume with the R wave on an electrocardiograph (representing the begining of ventricular systole) allows accurate semi-continuous measurement of limb arterial flow (Barendsen, Venema & Van Den Berg, 1971). An appropriate delay for the passage of the pulse wave from the heart to the measured area can be set on the Periflow: for the forearm 60 milliseconds was chosen. Forearm blood flow was measured as follows: forearm venous return was occluded with a cuff pressure of 40mmHg for 3 heart beats (RR' intervals). The increase in forearm volume was measured over the second and third heartbeats. The initial heartbeat was ignored to minimise artifact caused by the rapid inflation of the cuff. The cuff pressure was then released for 2 heart beats to allow forearm volume to return towards normal. After determination of resting blood flow (RF, figure 8), post-ischaemic flow (PF) was measured after 3 minutes of arterial occlusion (d'Inverno, 1980, figure 8) at a cuff pressure of 40mmHg greater than systolic blood pressure. Blood flow was expressed as ml/100ml/min. Basal vascular resistance and minimal

vascular resistance were calculated as mean arterial pressure divided by rest flow and post-ischaemic flow respectively (Strano et al., 1985).

Venous compliance was quantified by measuring the % increase in forearm diameter at continuous venous occlusion pressures of 30, 40, 50 and 60mmHg. The slope of change in limb volume against change in pressure gives an index of venous compliance (d'Inverno, personal communication, figure 9).

All measurements were made in a draught-free room, the daily temperature of which was 20-22^{o}C and relative humidity 40-60%. Patients lay supine for 15 minutes rest before recordings began. The right arm was supported along its length with the elbow held in extension and the hand raised 20cm above the bed. An occluding cuff was placed just proximal to the elbow, and a mercury strain gauge around the widest part of the forearm. A light blanket covered the arm to avoid fluctuations in temperature of the mercury strain gauge which would alter its electrical resistance.

2.5.4 Methods of measurement of QRS and QT intervals.

Electrocardiographic QT, QT_C (Bazett, 1920) and QRS interval durations were calculated as the mean from 5 consecutive cardiac cycles, taken from a 6-channel (standard limb leads I-aVL) electrocardiograph with a paper speed of 100mm/sec. The most appropriate limb lead, with a clearly defined Q wave and upright T wave, was

chosen for all measurements for each subject. QRS and QT intervals were measured from the begining of the Q to the end of the S and T waves respectively. QT_c was calculated as the QT interval divided by the square root of the preceeding RR' interval.

2.5.5 Psychological function.

Subjective feelings were measured by 6 standard 10cm visual analogue scales (Herbert, Johns & Dore, 1976). Co-ordination and concentration were assessed using the Digit Substitution Test (Oswald et al., 1978), in which standard symbols are substituted for digits (0-9); the score was counted as the number of correct substitutions in 3 minutes.

2.5.6 Statistical analysis.

Statistical analysis of platelet aggregation, QT, QT_c and psychological function was by Wilcoxon rank sum test for non-parametric data. All other data were analysed by Student's unpaired (t) test (2-tailed). For analysis of venous compliance, regression lines of change in forearm volume against venous occlusion pressure were calculated by the method of least squares for each subject. All results are expressed as the mean$\pm$standard error of the mean. The 95% confidence limits (2-tailed) were calculated as described by Gardner & Altman (1986). Results were considered statistically significant at $p<0.05$.

2.6 Effects of the serotonergic type-2 antagonist ketanserin on resting bronchomotor tone and exercise induced bronchoconstriction in adult atopic asthma

2.6.1 Patients.

Eight atopic asthmatic patients aged 17-40yrs (table 1) were studied. Six had positive prick skin tests to one or more common allergens and all had eosinophilia in blood and/or sputum. The study protocol was approved by the Ethical Supervisory Committee of the Western Infirmary, Glasgow, and each subject gave fully informed consent.

Patients taking inhaled bronchodilators discontinued them for at least 8h before each test; subjects on inhaled corticosteroids continued these during the study. No patients were taking sodium cromoglycate or oral steroids.

2.6.2 Methods.

In a double blind randomised balanced crossover trial the effects of a single oral dose of ketanserin (40mg) were compared with that of a matching placebo, with a minimum of 4 days between phases.

Measurements of FEV_1, FVC, $\dot{V}_{50(c)}$ and $\dot{V}_{25(p)}$ were made at the same time of day in each patient in the sitting position, in a draught-free room, the daily temperature of which was 20-22^{0}C and relative humidity 40-60%. Baseline recordings of pulmonary function and of sitting blood pressure and heart rate were made before and 45mins after

Patient	Age (Years)	Sex	Height (cm)	Baseline* FEV_1 (L)	FEV_1 % predicted	Current treatment
1	22	F	168	1.78	52	F,Bt
2	40	F	155	2.73	108	F/I,B
3	25	F	165	2.43	74	S,B
4	30	M	179	2.65	63	S
5	17	M	172	4.50	119	S
6	28	M	175	2.88	72	S,B
7	24	M	178	4.15	96	S
8	25	M	170	4.63	117	none

* = Mean of study days
M = male, F = female
B = beclomethosone dipropionate inhaler
Bt = betamethasone valerate inhaler
F = fenoterol inhaler
F/I = fenoterol and ipratropium bromide inhaler
S = salbutamol inhaler

Table 1:

Characteristics of adult atopic asthmatics studied.

both drug and placebo. Subjects then exercised on the treadmill for 8mins at 6kph at 10^0. Blood pressure and heart rate were recorded at the end of exercise. Pulmonary function was assessed at the end of exercise and every 5mins for 20min after exercise.

Venous blood was taken at 45 and 75mins (20mins after exercise) after drug and placebo for measurements of plasma ketanserin and its metabolite ketanserinol.

2.6.3 Methods of measurement.

Pulmonary function tests were performed using a Morgan 12L Spiroflow with a flow volume differentiator and modified Rikadenki X-Y plotter (RW 201-T); measurements of forced expiratory volume in 1 second (FEV_1), forced vital capacity (FVC) and maximal flow rates at 50% ($\dot{V}_{50(c)}$) and 25% ($\dot{V}_{25(p)}$) of forced vital capacity (figure 10) were made in triplicate (90secs apart), with the maximal values of FEV_1 and FVC, and the mean values of $\dot{V}_{50(c)}$ and $\dot{V}_{25(p)}$ being used for analysis. Initially a forced expiration was made from tidal breathing for measurement of $\dot{V}_{25(p)}$. This expiration was held for 1-2 seconds before a maximal inspiration, which was also held for 1-2 seconds before a full forced expiration, allowing measurement of FEV_1 and $\dot{V}_{50(c)}$. Maximal inspiratory volume was assumed to be equal in each series of 3 measurements for the purpose of calculating maximal expiratory flow rates (Boushey & Dawson, 1982).

Arterial blood pressure (diastolic phase V) was measured

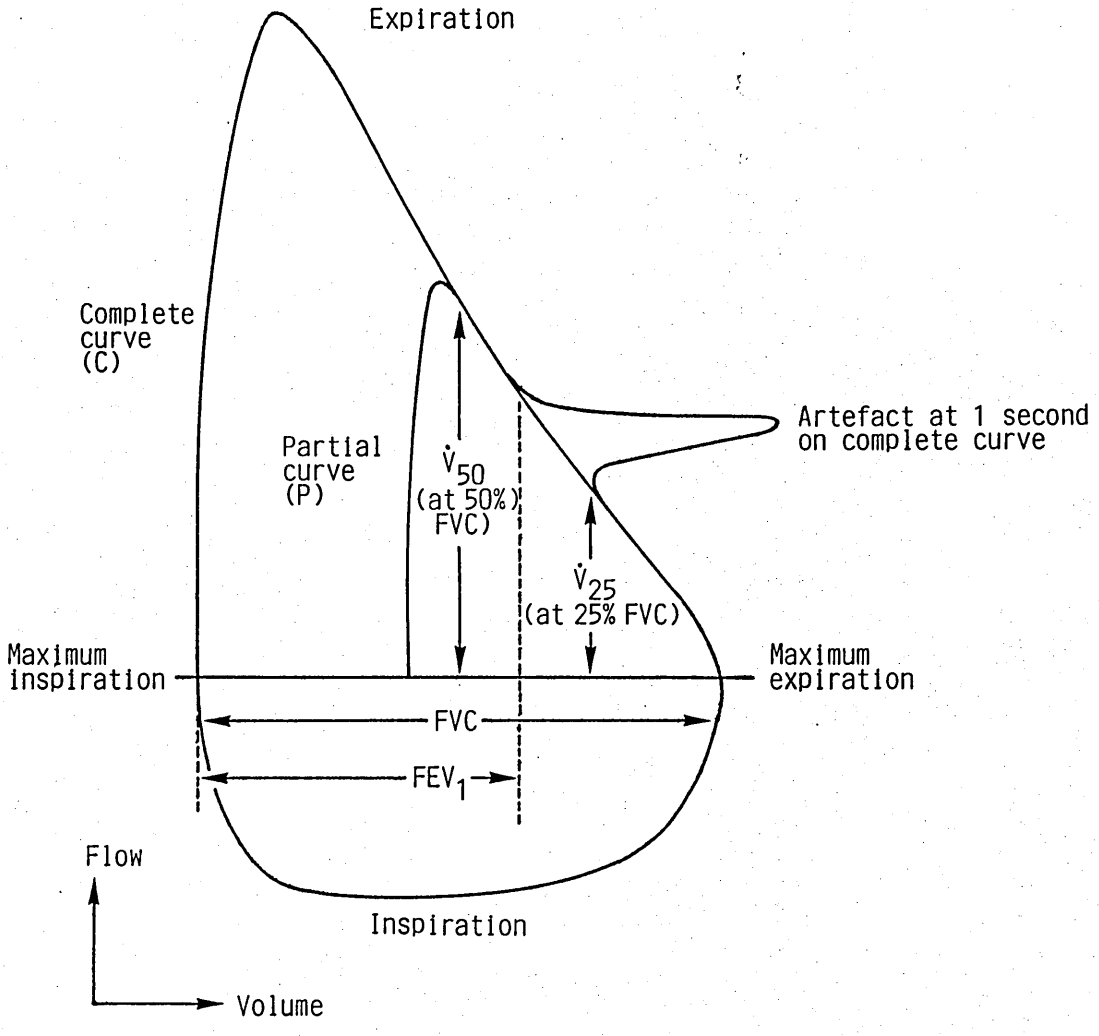


Figure 10
Respiratory flow volume loop, showing forced vital capacity, forced expiratory volume in 1 second, expiratory flow rate at 50% of forced vital capacity and expiratory flow rate at 25% of forced vital capacity.

in the right arm with the patient seated, using a standard mercury sphygmomanometer. Mean arterial pressure was calculated as the diastolic + [(systolic - diastolic)/3]. Baseline measurements of blood pressure and heart rate were performed after 5 minutes sitting.

Plasma ketanserin and ketanserinol were measured by high pressure liquid chromatography with fluorescence detection (Davies, 1983).

2.6.4 Statistical analysis.

Analysis of changes in resting bronchomotor tone, blood pressure and heart rate after ketanserin compared to placebo were by Student's paired (t) test (2-tailed). Post-exercise pulmonary function values were expresssed as a percentage of the immediate pre-exercise values. Statistical analysis of pulmonary function tests after exercise was by repeated measures of analysis of variance, using the GENSTAT statistical package. The 95% confidence limits (2-tailed; Gardner & Altman, 1986) of the effect of ketanserin compared to placebo on resting bronchomotor tone and maximal percentage reduction in pulmonary function after exercise were also calculated. All results are expressed as the mean$\pm$standard error of the mean. Differences were considered statistically significant at $P<0.05$.

3.1 Serotonin and platelet aggregation in patients with essential hypertension compared to age and sex matched normotensive controls

3.1.1 Blood pressure and heart rate.

Mean supine and erect blood pressures in the hypertensive group were 176.7±3.6 / 98.6±1.1 and 162.6±4.1 / 99.6±1.7 mmHg respectively, compared to 135.5±2.2 / 77.0±1.3 and 122.1±2.5 / 78.3±1.5 in the normotensive group. Mean supine heart rate was higher in the hypertensive group at 78.2±1.7 beats / minute compared to 70.0±2.1 in the control group ($p<0.005$). There was a similar tendency for erect heart rate to be increased in the hypertensive group, at 85.9±1.9 compared to 81.6±2.0, although this difference did not achieve statistical significance ($p=0.17$). Four (17%) of the hypertensives and 3 (15%) of the normotensives had a known family history of a first degree relative with hypertension.

3.1.2 Body weight, smoking habit, and alcohol.

Mean body weight was significantly higher ($p<0.01$) in the hypertensive patients (73.6±3.7Kg) than the normotensives (66.5±3.3). Seventeen (74%) of the hypertensive patients were non or ex-smokers, compared to 15 (75%) in the normotensive group. Six (26%) of the

hypertensives were non-drinkers, 6 (26%) drank less than 1 unit (10g) of alcohol per week, and the 11 (48%) regular drinkers consumed 5.5±1.7 units per week. Of the normotensives, 8 (40%) were non-drinkers, 4 (20%) drank less than 1 unit per week and 8 (40%) drank 8.1±2.5 units per week.

3.1.3 Platelet aggregation.

There was no significant difference between the hypertensive and normotensive groups in initial platelet count measured prior to the aggregation studies (table 2). The percentage of platelets remaining after serotonin-induced platelet aggregation in whole blood was less in the hypertensives, 50.0±3.8%, than the control group, 56.9±2.4% ($p<0.05$, figure 11). Thus serotonin-induced platelet aggregation was enhanced in the hypertensives compared to the normotensive group. Serotonin-induced platelet aggregation did not appear to be selectively increased in those patients with known ischaemic heart disease, peripheral vascular disease or chronic obstructive airways disease (figure 11). When the data from the hypertensive and normotensive groups were combined (table 3) there was a statistically significant linear correlation ($p<0.001$) between the percentage of platelets remaining after the addition of serotonin to whole blood and systolic (table 4, figure 12) but not diastolic blood pressure (table 4, figure 13). The percentage of platelets remaining after the addition of

serotonin to whole blood was also significantly correlated with patient age (table 4, figure 14) and sex (table 4, figure 15). There was no significant difference in spontaneous platelet aggregation between the hypertensive and normotensive groups (table 2, figure 16). The percentage of platelets remaining after the addition of serotonin to whole blood could not be adequately described by the nine independent variables considered (table 5; age, sex, weight, supine systolic and diastolic blood pressure, red blood cell count, plasma beta-thromboglobulin and whole blood viscosity at low and high shear rates corrected for haematocrit); even with all nine variables in the model this only accounted for 43.2% of the variability.

3.1.4 Platelet serotonin and plasma beta-thromboglobulin.

Platelet serotonin and plasma beta-thromboglobulin levels did not differ significantly between the hypertensive and normotensive groups, although there was a tendency for beta-thromboglobulin to be reduced in the hypertensive group (table 2, figures 17 and 18). There was no significant linear correlation between platelet serotonin and plasma beta-thromboglobulin levels (figure 19).

3.1.5 Full blood count, urea and electrolytes, blood sugar and liver function tests.

Red blood cell count, haemoglobin and haematocrit were all slightly higher in the hypertensive than the control group, although these differences did not reach statistical significance (table 3). Mean cell volume did not differ between the two groups (table 3). There were no significant differences in serum sodium, potassium, urea, creatinine, gamma glutamyl transferase or blood sugar between the hypertensive and normotensive groups (table 6).

	Hypertensive		Normotensive	
Platelet count (10^9/L)	205±10	(n=23)	206±15	(n=20)
Platelets remaining after serotonin-induced aggregation (%)	*50.0±3.8	(n=22)	56.9±2.4	(n=20)
Platelets remaining after spontaneous aggregation (%)	49.5±4.6	(n=23)	47.2±4.8	(n=20)
Platelet serotonin (nmol/10^9 platelets)	3.7±0.4	(n=20)	3.7±0.4	(n=19)
Beta-thromboglobulin (ng/mL)	41.5±4.4	(n=20)	54.3±12.4	(n=17)

(* = $p<0.05$, unpaired Student (t) test, 2-tailed)

Table 2:

Platelet count, percentage of platelets remaining after serotonin-induced and spontaneous aggregation, platelet serotonin content, and plasma beta-thromboglobulin, in patients with untreated essential hypertension compared to an age and sex matched normotensive control group.

	Hypertensive	Normotensive
Red blood cell count (10^{12}/L)	4.76±0.09 (n=23)	4.58±0.09 (n=20)
Haemoglobin (g/dL)	14.8±0.3 (n=23)	14.1±0.2 (n=20)
Haematocrit (%)	43.6±0.9 (n=23)	41.9±0.7 (n=20)
Mean cell volume (fL)	91.7±1.0 (n=23)	91.6±0.7 (n=20)
$94s^{-1}$	5.01±0.17 (n=18)	5.10±0.12 (n=10)
$94s^{-1}$(c)	4.96±0.13 (n=18)	5.37±0.19 (n=10)
$0.94s^{-1}$	20.2±1.1 (n=18)	18.3±0.7 (n=10)
$0.94s^{-1}$(c)	19.7±0.6 (n=18)	19.8±0.6 (n=10)

$94s^{-1}$ = whole blood viscosity at high shear corrected (c) and uncorrected for haematocrit

$0.94s^{-1}$ = whole blood viscosity at low shear corrected (c) and uncorrected for haematocrit

Table 3:

Red blood cell count, haemoglobin, mean cell volume, haematocrit and whole blood viscosity at high and low shear rates in patients with untreated essential hypertension compared to an age and sex matched normotensive control group.

Linear regression analysis of the percentage of platelets remaining after serotonin-induced aggregation verses each independent variable.

Independent variable	Number patients	Regression coefficient	t-statistic	p	% fit
Sex	42	-12.35	-3.32	0.002	21.6
Age (years)	42	-0.90	-2.82	0.007	16.6
Body weight (Kg)	42	0.12	0.91	0.37	2.1
Supine SBP (mmHg)	42	-0.26	-3.60	<0.001	24.5
Supine DBP (mmHg)	42	-0.26	-1.49	0.15	5.2
βtG_{-1} (ng/mL)	37	0.08	1.43	0.16	5.5
$94s^{-1}$	27	2.13	0.54	0.60	1.1
$94s^{-1}$ (c)	27	-2.38	-0.58	0.34	1.3
$0.94s^{-1}$	27	-0.09	-0.14	0.89	<0.1
$0.94s^{-1}$ (c)	27	-1.43	-1.48	0.15	8.1
RBC(10^{12}/L)	42	4.69	0.92	0.37	2.1

Sex (male=1, female=2)
SBP = systolic blood pressure
DBP = diastolic blood pressure
βtG_{-1} = beta-thromboglobulin
$94s^{-1}$ = whole blood viscosity at high shear corrected (c) and uncorrected for haematocrit
$0.94s^{-1}$ = whole blood viscosity at low shear corrected (c) and uncorrected for haematocrit
RBC = red blood cell count

Table 4:

Linear regression analysis of serotonin-induced platelet aggregation in whole blood (dependent variable) against sex, age, body weight, supine systolic and diastolic blood pressure, plasma beta-thromboglobulin, whole blood viscosity at high and low shear rates and red blood cell count (independent variables).

Multiple regression analysis of the percentage of platelets remaining after serotonin-induced aggregation verses all 9 independent variables (23 patients).

Independent variable	Regression coefficient	t-statistic	p
Sex	-11.31	-1.53	0.15
Age (years)	0.22	0.34	0.74
Body weight (Kg)	-0.14	-0.68	0.51
Supine SBP (mmHg)	-0.06	-0.33	0.75
Supine DBP (mmHg)	-0.10	-0.25	0.81
btG_{-1} (ng/ml)	0.06	0.68	0.51
$94s^{-1}$ (c)	-1.00	-0.16	0.87
0.94^{-1} (c)	-1.80	-1.28	0.22
RBC (10^{12}/l)	-2.39	-0.27	0.79

Sex (male=1, female=2)
SBP = systolic blood pressure
DBP = diastolic blood pressure
btG_{-1} = beta-thromboglobulin
$94s^{-1}$(c) = whole blood viscosity high shear corrected for haematocrit
$0.94s^{-1}$(c) = whole blood viscosity low shear corrected for haematocrit
RBC = red blood cell count

Table 5:

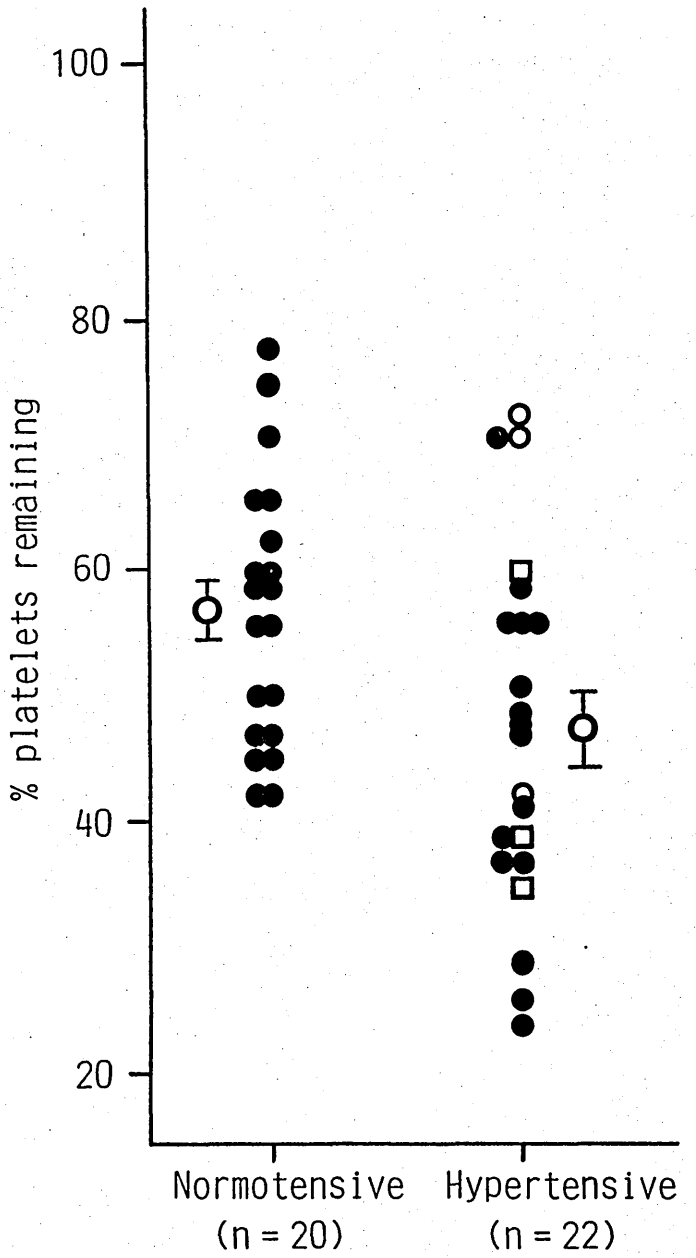
Multiple regression analysis of serotonin-induced platelet aggregation in whole blood (dependent variable) against sex, age, body weight, supine systolic and diastolic blood pressure, plasma beta-thromboglobulin, whole blood viscosity at high and low shear rates (corrected for haematocrit) and red blood cell count (independent variables).

		Hypertensive (n=23)	Normotensive (n=20)
Sodium	(mmol/L)	141.7±0.5	142.3±0.4
Potassium	(mmol/L)	4.16±0.07	4.05±0.05
Urea	(mmol/L)	4.7±0.2	5.1±0.3
Creatinine	(μmol/L)	85.8±4.5	81.9±3.3
γGT	(U/L)	29.5±5.1	27.1±6.0
Blood sugar	(mmol/L)	5.3±0.2	5.1±0.2

(γGT = gamma glutamyl transferase)

Table 6:

Serum urea and electrolytes, gamma glutamyl transferase and blood sugar in patients with untreated essential hypertension compared to age and sex matched normotensive subjects.



○ known ischaemic heart disease/peripheral vascular disease

□ chronic obstructive airways disease

Figure 11

Percentage of single platelets remaining after the addition of serotonin (1μM) to whole blood, in patients with untreated essential hypertension compared to age and sex matched normotensive subjects.

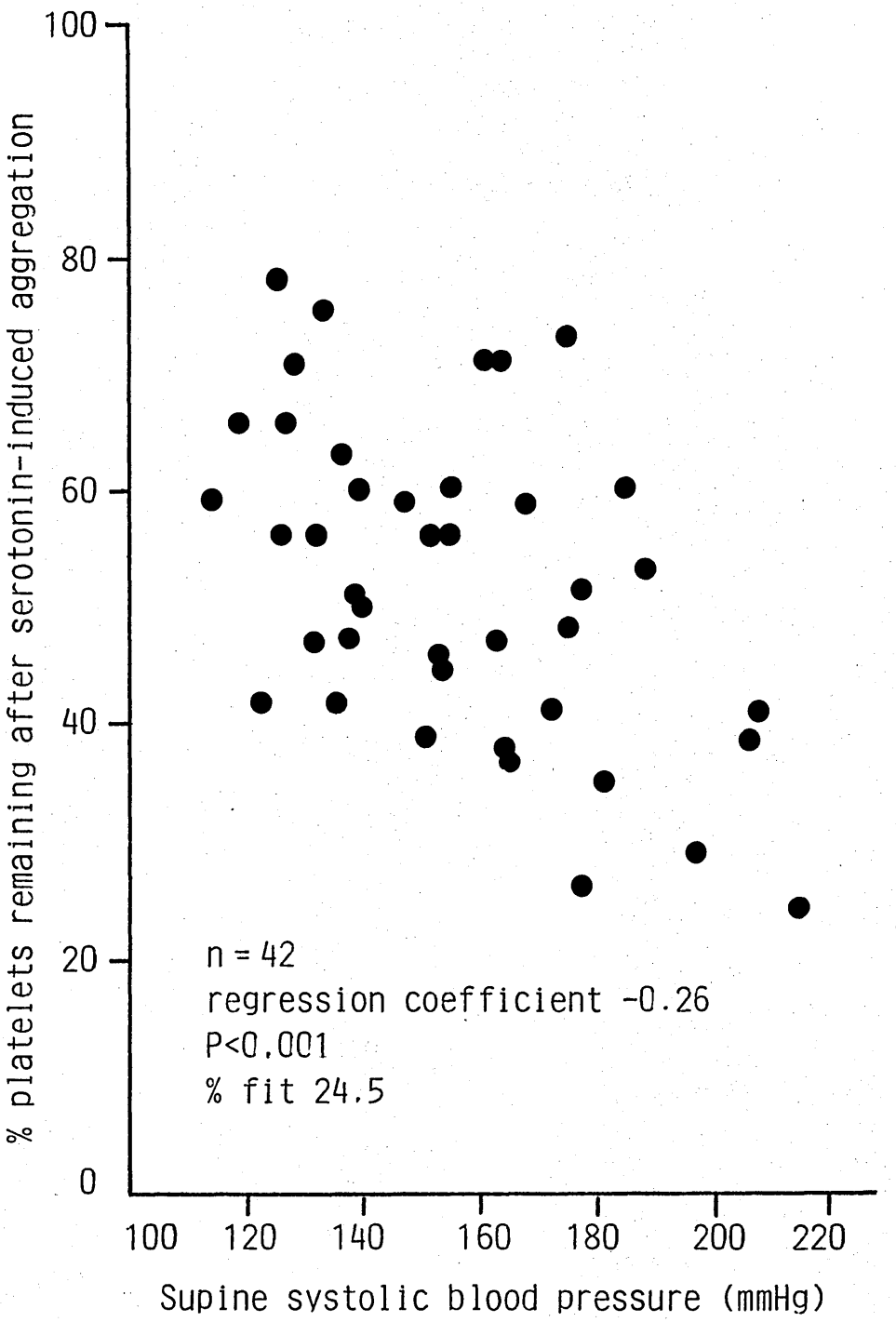


Figure 12
Percentage of single platelets remaining after the addition of serotonin (1μM) to whole blood. verses supine systolic blood pressure.

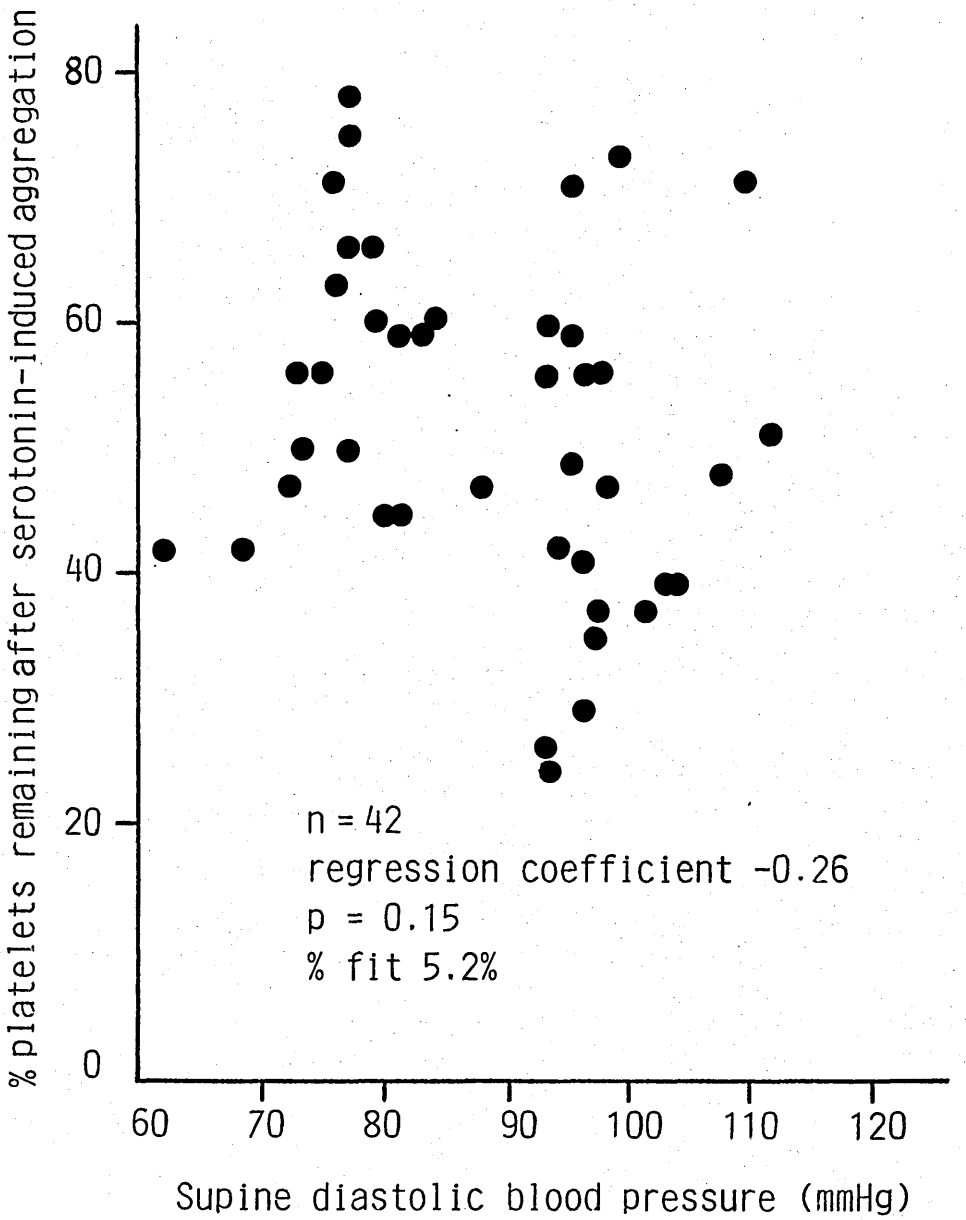


Figure 13
Percentage of single platelets remaining after the addition of serotonin (1μM) to whole blood verses supine diastolic blood pressure.

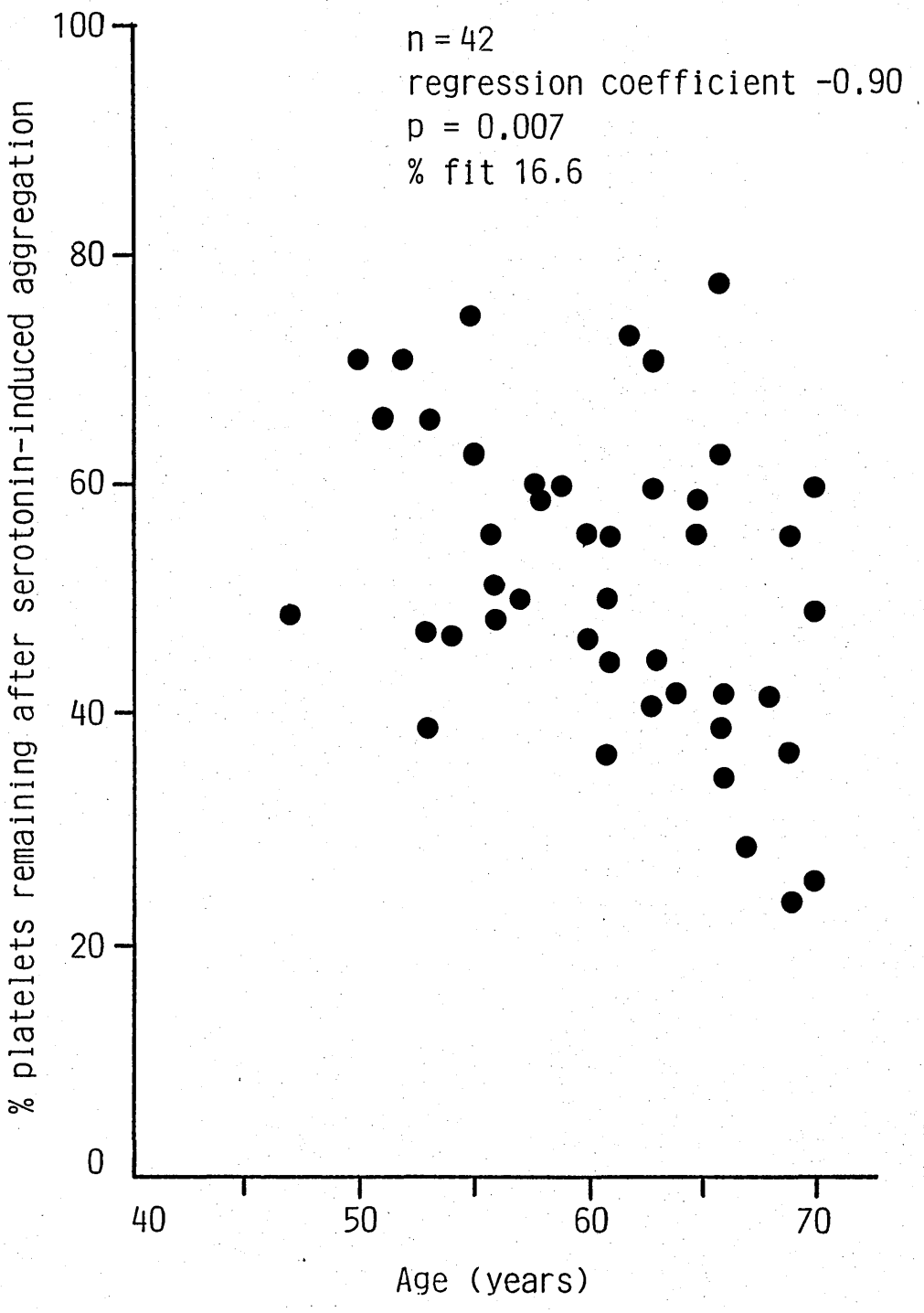


Figure 14
Percentage of single platelets remaining after the addition of serotonin (1μM) to whole blood verses age in years.

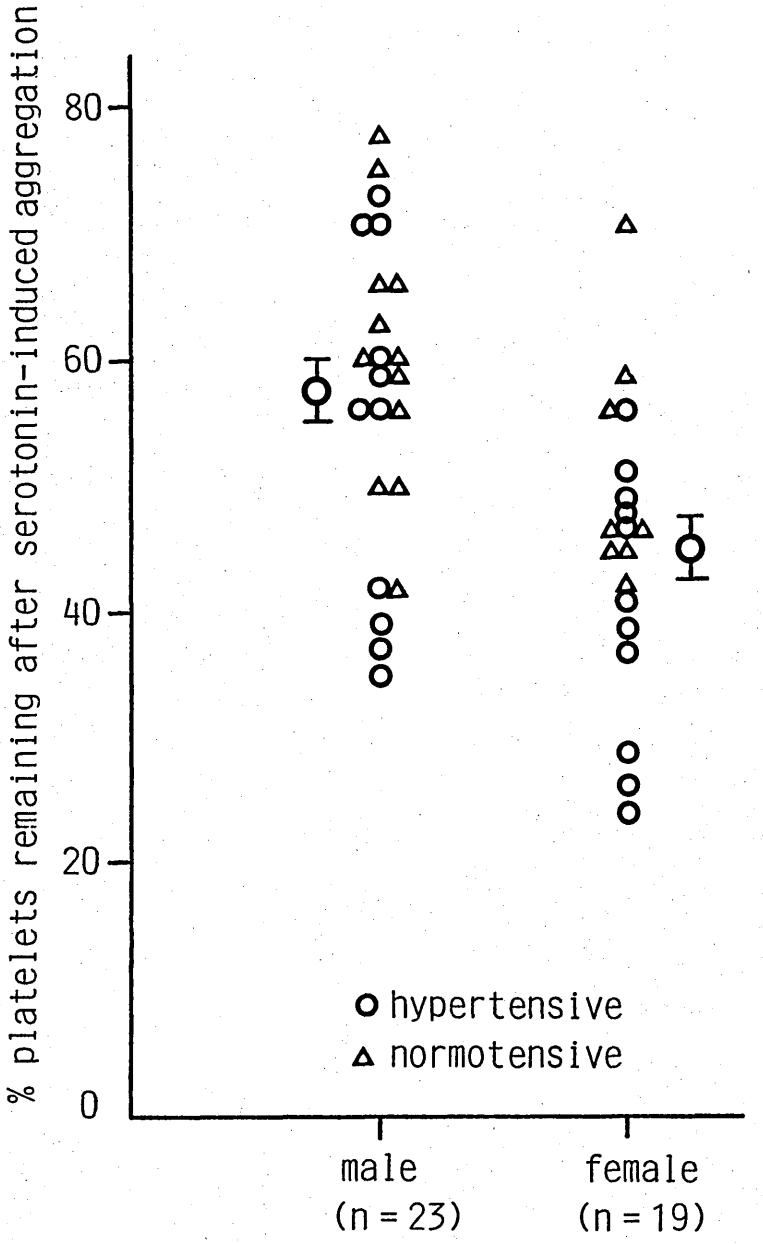


Figure 15
Percentage of single platelets remaining after the addition of serotonin (1μM) to whole blood, verses patient sex.

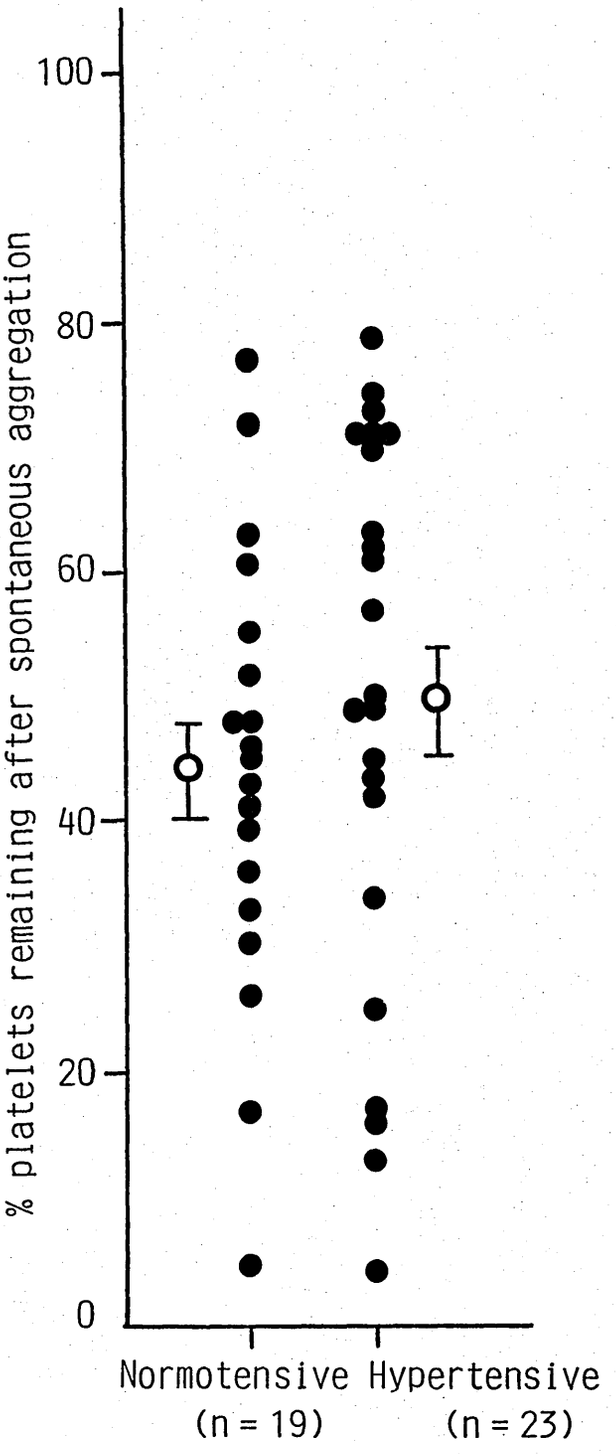


Figure 16
Percentage of single platelets remaining after spontaneous platelet aggregation in whole blood (15 minutes incubation at 130-140 shakes/minutes at 37^{o}C), in patients with untreated essential hypertension compared to age and sex matched normotensive subjects.

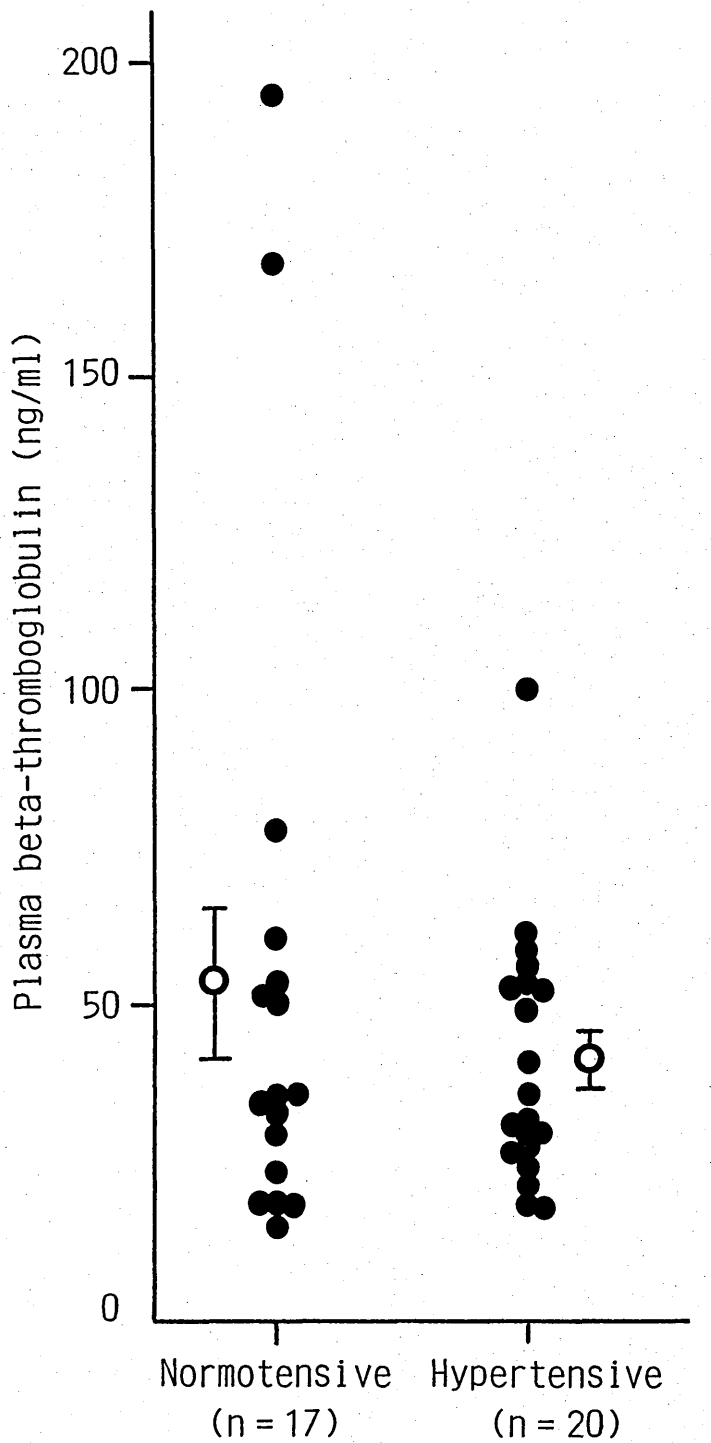


Figure 17
Plasma beta-thromboglobulin in patients with untreated essential hypertension compared to age and sex matched normotensive subjects.

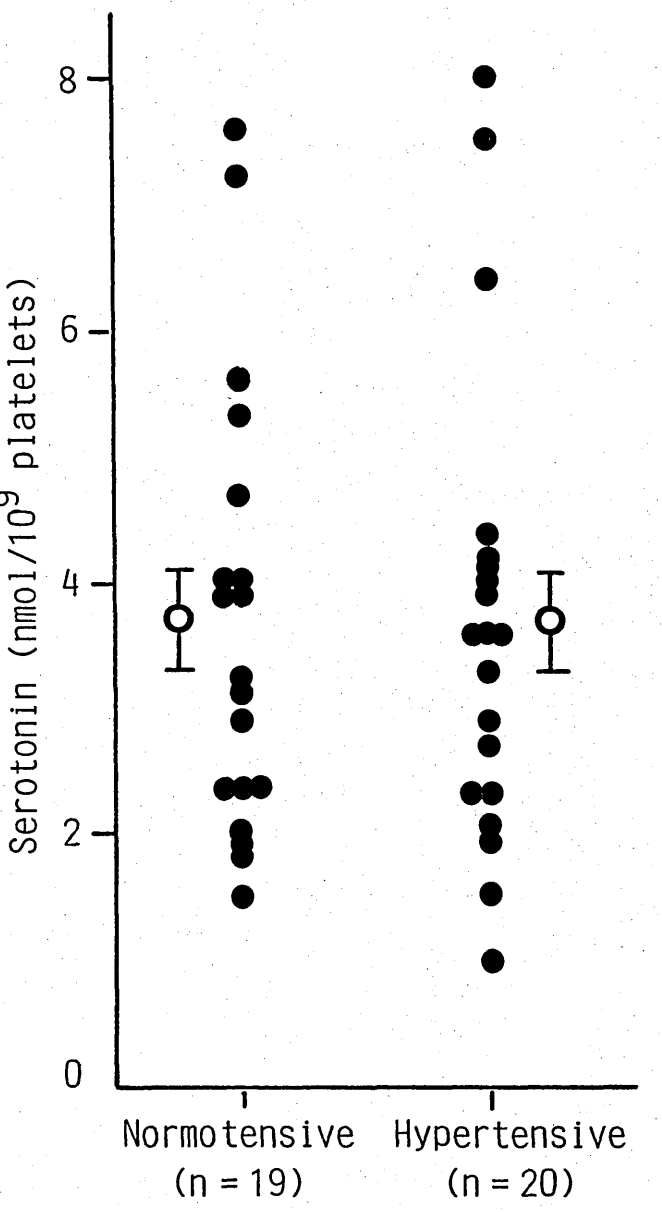


Figure 18
Platelet serotonin content in patients with untreated essential hypertension compared to age and sex matched normotensive subjects.

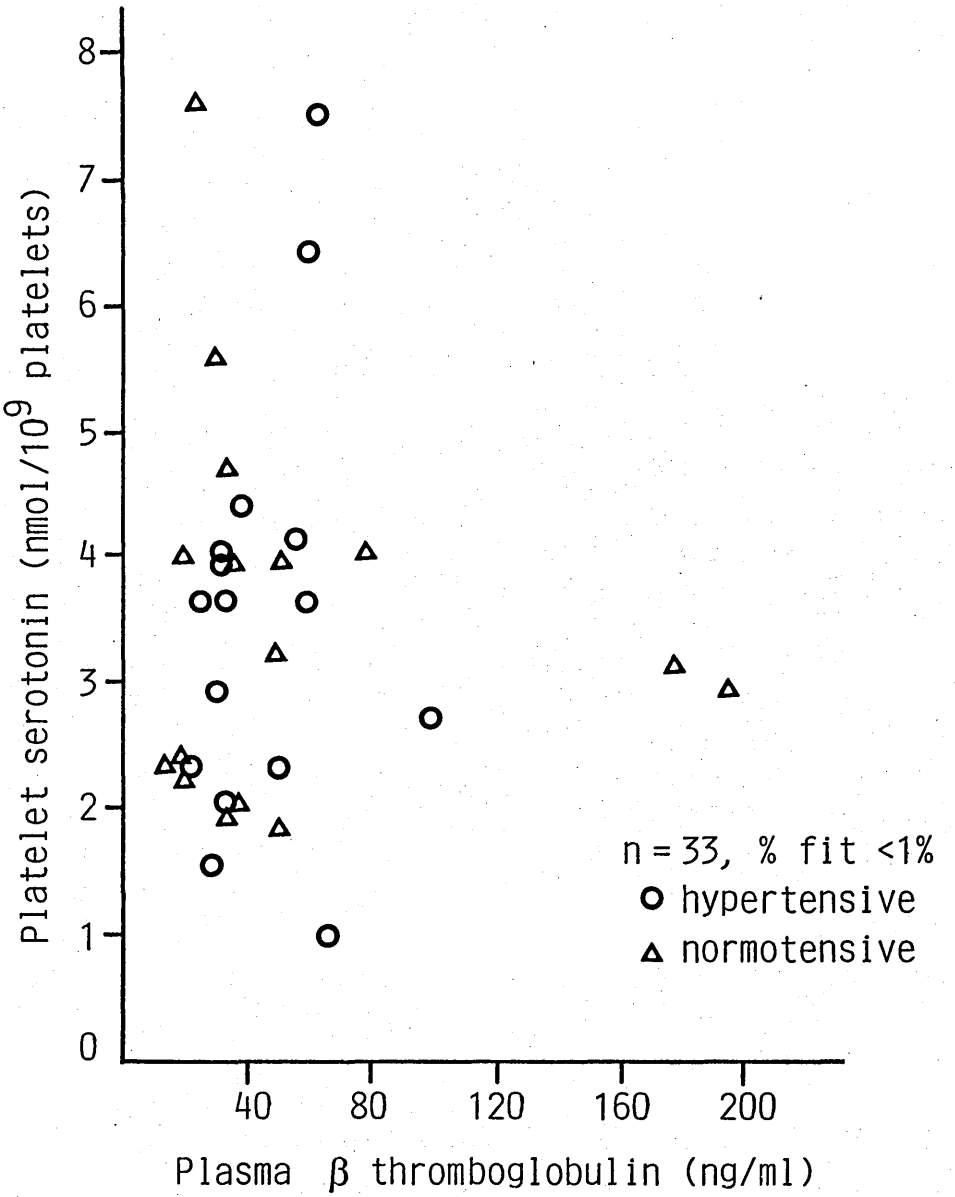


Figure 19
Linear regression analysis of platelet serotonin content (dependent variable) against plasma beta-thromboglobulin (independent variable).

3.2 The effects of short-term ketanserin treatment on blood pressure, heart rate, the renin-angiotensin system, adrenocortical function, QT interval and vagal function and the response to infusion of angiotensin II in healthy subjects

3.2.1 Blood pressure and heart rate.

a) First dose:

One subject experienced symptomatic postural hypotension after the first dose of ketanserin 40mg. His standing blood pressure and heart rate fell from 124/72 mmHg and 100 beats/min, before the drug, to 82/48 mmHg and 72 beats/min respectively 1 hour after drug administration; supine blood pressure and heart rate were 132/60 mmHg and 88 beats/min prior to ketanserin compared to 123/50 mmHg and 88 beats/min 1 hour after drug administration. However, mean changes in blood pressure 1 hour after the first dose of ketanserin were small; although standing systolic blood pressure was reduced by ketanserin compared to placebo ($p<0.05$), there were no significant changes in systolic, diastolic, mean arterial pressure or heart rate either supine or erect after ketanserin compared to placebo (table 7).

b) Chronic oral dosing.

Three days of ketanserin caused only minor but significant effects on blood pressure; mean supine arterial blood pressure was significantly lower ($p<0.02$) at 45-60 minutes after ketanserin on the morning of day 4

(80.2±2.2 mmHg) compared to placebo (85.9±1.5); there were no detectable differences around 7 hours after dosing. There were no significant changes in erect blood pressure after 3 days of ketanserin, either around 1 or 7 hours after dosing, compared to placebo (table 8).

On the morning of day 4 (1 hour after dosing) supine heart rate was reduced after ketanserin by 3.5±1.5 beats/min when compared to placebo ($p<0.05$); there were no significant differences 7 hours after dosing (table 8).

c) Angiotensin II infusion.

Ketanserin did not attenuate the rise in blood pressure in response to the graded intravenous infusions of angiotensin II at 1 and 7 hours after dosing on day 4 (figures 20 and 21). The maximal reductions in heart rate caused by the infusion of angiotensin II were 6.9±3.6 and 9.8±3.3 beats/minute at 1 and 7 hours respectively after ketanserin compared to 6.5±4.0 and 11.9±3.2 after placebo; there were no significant differences between drug and placebo.

3.2.2 Autonomic function tests.

The deep breathing, Valsalva and 30 : 15 lying to standing ratios were all reduced 90 mins after ketanserin on day 4, consistent with a decrease in cardiac parasympathetic outflow; however, individually these changes did not achieve statistical significance ($p=0.16$, $p=0.09$ and $p=0.06$ respectively). At 7 hours post drug on day 4 there were no detectable differences in the above

ratios between the ketanserin and placebo phases (table 9).

3.2.3 The QT interval.

Both the uncorrected QT and QT_C intervals were prolonged after ketanserin therapy, by 26-40 milliseconds. This effect was apparent at both 1 and 7 hours after drug administration on day 4 (table 10, figures 22 and 23).

3.2.4 Plasma renin, aldosterone, angiotensin II and cortisol.

No significant differences were observed in supine resting plasma renin, aldosterone, angiotensin II or cortisol after 3 days of ketanserin, compared to placebo (table 11). The morning angiotensin II infusion caused similar rises in plasma aldosterone after both ketanserin (4.1 ± 1.4 ng/100ml) and placebo (4.8 ± 1.0 ng/100ml). The suppression of plasma active renin by angiotensin II infusion was also not significantly affected by ketanserin, with a reduction of 5.1 ± 1.3 μU/ml, compared to 4.1 ± 1.0 after placebo.

3.2.5 Plasma ketanserin and ketanserinol levels.

Plasma ketanserin levels were 118 ± 38, 106 ± 32 and 54 ± 5 nmol/l, at 45 mins, 90mins (before and after the morning angiotensin II infusion) and 7 hours respectively after

drug administration on day 4; plasma ketanserinol levels were 219±41, 222±35 and 205±19 nmol/l.

3.2.6 Adverse drug reactions:

One subject developed syptomatic first dose hypotension as detailed above (3.2.1). Two subjects complained of nasal stuffiness while taking ketanserin.

	Placebo		Ketanserin	
Time	0	1 hour	0	1 hour
Supine SBP	121.1±3.3	118.9±3.3	125.4±2.5	121.0±2.7
Supine DBP	68.3±2.9	70.1±3.8	63.7±3.6	62.3±3.0
Supine map	85.9±2.0	86.3±3.0	84.1±4.7	81.7±1.9
Supine HR	70.9±3.6	68.6±3.2	69.7±4.0	68.6±4.7
Erect SBP	110.4±1.6	114.3±1.7	115.9±2.1	104.7±4.1*
Erect DBP	77.4±1.8	76.0±3.3	72.4±3.4	69.9±4.1
Erect map	87.9±1.5	88.7±2.0	86.9±2.4	81.0±4.0
Erect HR	85.6±3.7	85.7±3.5	83.1±5.9	85.7±6.1

(* $p<0.05$, paired Student (t) test, 2-tailed)

SBP = systolic blood pressure (mmHg)
DBP = diastolic blood pressure (mmHg)
map = mean arterial pressure (mmHg)
HR = heart rate (beats/minute)

Table 7:

Effects of ketanserin 40mg orally, compared to placebo, on supine and erect blood pressure and heart rate, before and 1 hour after dosing, in 8 healthy subjects.

	Day 4			
	1 hour after dosing		7 hours after dosing	
	Ketanserin	Placebo	Ketanserin	Placebo
Supine SBP	118.1±3.5	118.6±3.5	120.3±3.1	120.3±3.1
Supine DBP	61.3±2.9	67.7±3.0	60.0±3.6	61.3±2.7
Supine map	80.2±2.2*	85.9±1.5	80.0±2.0	80.4±1.6
Supine HR	59.5±2.8*	63.0±3.2	63.0±2.9	66.6±3.0
Erect SBP	111.4±2.1	110.4±3.1	113.3±3.4	111.5±2.2
Erect DBP	70.9±2.6	75.6±2.1	70.9±3.1	72.1±2.1
Erect map	84.4±1.8	87.3±2.3	84.9±2.7	85.4±1.9
Erect HR	83.8±4.0	80.6±4.2	84.6±2.7	87.5±3.7

(* $p<0.05$, paired Student (t) test, 2-tailed)

SBP = systolic blood pressure (mmHg)
DBP = diastolic blood pressure (mmHg)
map = mean arterial pressure (mmHg)
HR = heart rate (beats/minute)

Table 8:

Supine and erect blood pressure and heart rate, at 1 and 7 hours after dosing after 3 days ketanserin 40mg twice daily, compared to matching placebo, in 8 healthy subjects.

	Day 4			
	1 hour after dosing		7 hours after dosing	
	Ketanserin	Placebo	Ketanserin	Placebo
Lying to standing ratio	1.25±0.09	1.44±0.10	1.31±0.08	1.30±0.06
Valsalva ratio	1.52±0.04	1.63±0.06	1.55±0.08	1.62±0.08
Deep breathing ratio	1.50±0.09	1.64±0.08	1.55±0.07	1.56±0.06

Table 9:

Ratios of maximal to minimal RR' intervals during lying to standing, Valsalva and deep breathing manoevres after 3 days ketanserin 40mg twice daily compared to matching placebo, 1 and 7 hours after dosing, in 8 healthy subjects.

	Day 4			
	1 hour after dosing		7 hours after dosing	
	Ketanserin	Placebo	Ketanserin	Placebo
QT (msec)	434±17*	408±8	432±9**	392±11
QT_C (msec)	444±12*	416±10	446±9**	416±7

(* $p<0.05$, ** $p<0.01$, paired Student (t) test, 2-tailed)

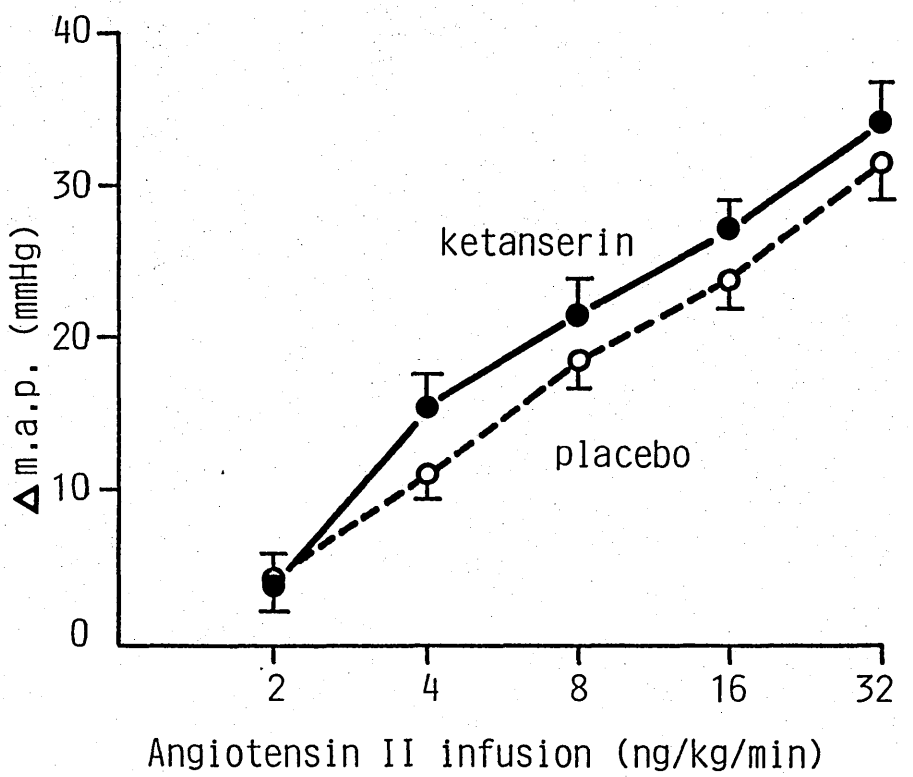
Table 10:

Electrocardiographic QT and QT_C intervals, at 1 and 7 hours after dosing after 3 days ketanserin 40mg twice daily, compared to matching placebo, in 8 healthy subjects.

		Day 4 (1 hour after dosing) Ketanserin	Placebo
Renin	(μU/mL)	18.4±3.3	18.0±2.8
Aldosterone	(ng/100mL)	4.5±0.7	3.0±0.5
Angiotensin II	(pg/mL)	5.5±1.4	4.9±1.2
Cortisol	(pg/100mL)	13.5±1.3	15.3±1.3
Potassium	(mmol/L)	4.0±0.1	4.0±0.1

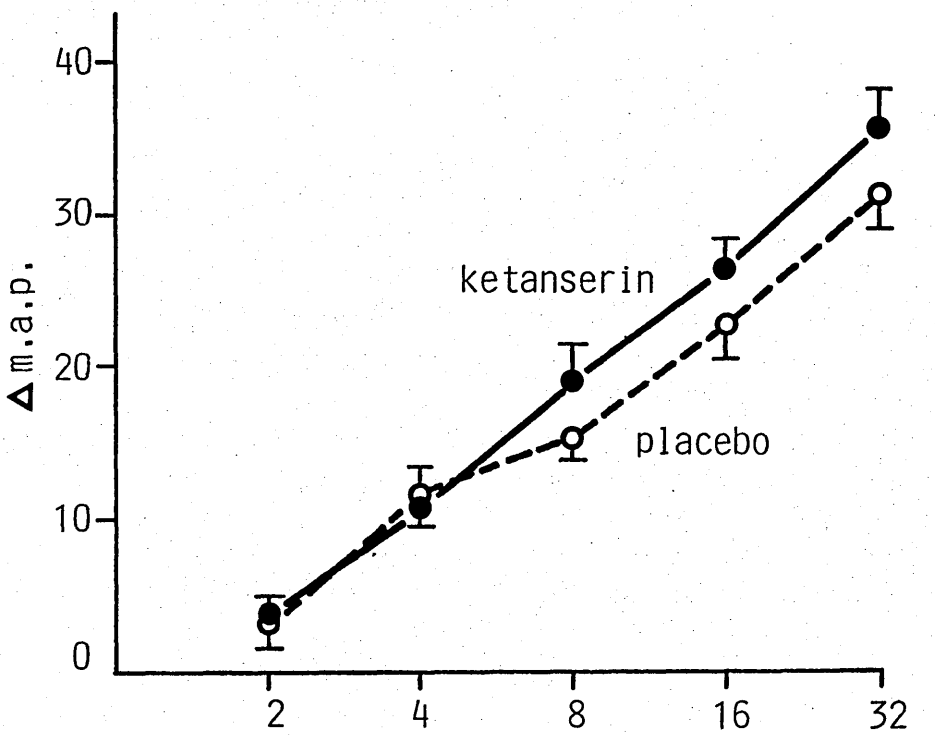
Table 11:

Plasma renin, aldosterone, angiotensin II and cortisol and serum potassium after 3 days ketanserin 40mg twice daily (1 hour after dosing) compared to matching placebo, in 8 healthy subjects.



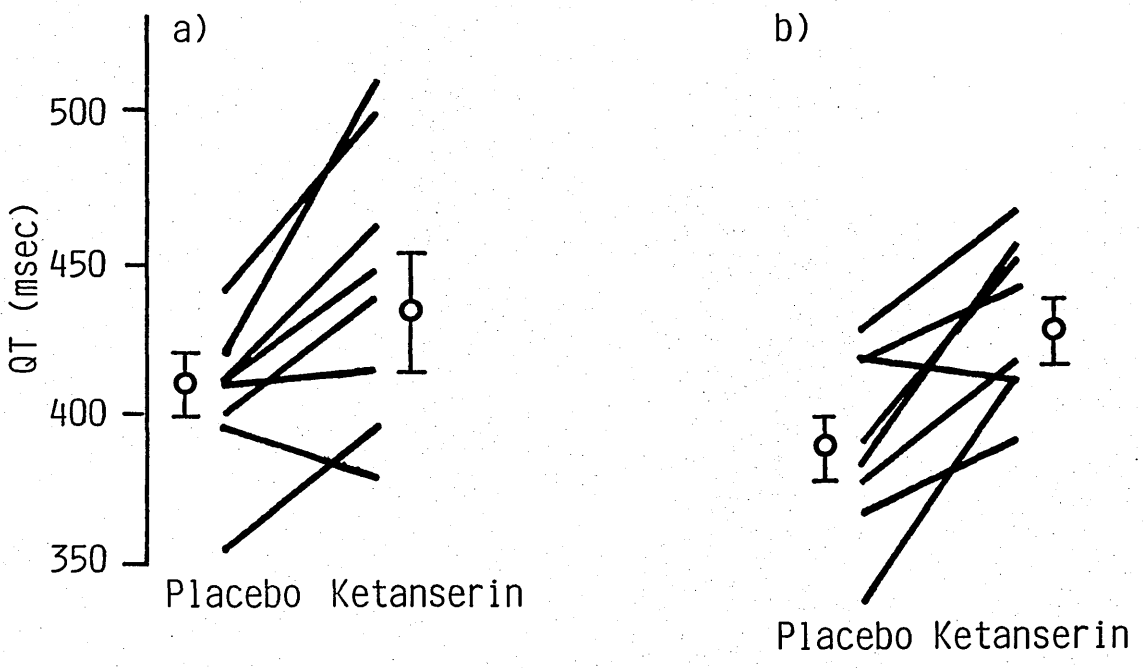
Δm.a.p. = change in mean arterial pressure

Figure 20
Change in mean arterial pressure due to incremental infusion of angiotensin II after 3 days ketanserin 40mg twice daily compared to matching placebo, 1 hour after dosing, in 8 healthy subjects.



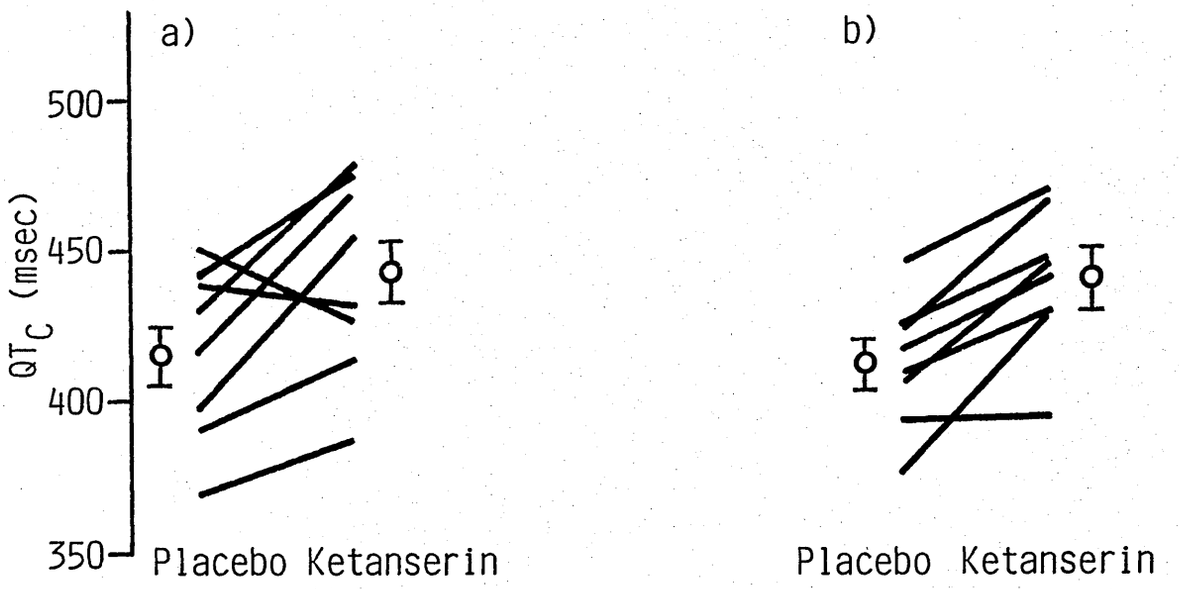
Δm.a.p. = change in mean arterial pressure

Figure 21
Change in mean arterial pressure due to incremental infusion of angiotensin II after 3 days ketanserin 40mg twice daily compared to matching placebo, 7 hours after dosing, in 8 healthy subjects.



a) 1 hour after dosing
b) 7 hours after dosing

Figure 22
Electrocardiographic QT interval after 3 days ketanserin 40mg twice daily compared to matching placebo, 1 and 7 hours after dosing, in 8 healthy subjects.



a) 1 hour after dosing
b) 7 hours after dosing

Figure 23
Electrocardiographic QT_C interval after 3 days ketanserin 40mg twice daily compared to matching placebo, 1 and 7 hours after dosing, in 8 healthy subjects.

3.3 Effects of single oral doses of ritanserin and ketanserin in patients with untreated essential hypertension

3.3.1 Blood pressure and heart rate.

Ketanserin 40mg caused a significant ($P<0.03$) reduction of the area under the curve for sitting mean arterial pressure over the 8 hours after drug administration compared to placebo (figure 24). There was a mean reduction of sitting mean arterial pressure of 15.4±3.2 (baseline = 119.4±2.4mmHg) after ketansrin, compared to 8.5±2.2 (baseline = 118.2±2.1) after placebo. Ritanserin had no significant effects on sitting mean arterial pressure when compared to placebo; there were average reductions of 9.0±2.6 (baseline = 117.0±3.3) and 10.8±1.8 (baseline = 119.8±0.9) after 10 and 20mg ritanserin respectively (figure 24). There was a trend for ketanserin to reduce erect mean arterial pressure, by 10.8±3.1 (baseline = 120.0±2.8) compared to a reduction of 6.6±3.1 (baseline = 119.8±2.1) after placebo, but there was no statistically significant difference in area under the curve (figure 25). Ritanserin 10 and 20mg had no significant effects on erect mean arterial pressure compared to placebo (figure 25).

There were no significant changes in area under the curve for sitting (figure 26) or standing heart rate (figure 27) after ritanserin or ketanserin compared to placebo. Average pulse rate over the 8 hours after placebo

was reduced by 1.6±0.9 beats / min (baseline 78.8±1.6) sitting and 2.4±1.0 (baseline 82.4±1.1) standing, compared to reductions of 3.6±2.6 (baseline 81.6±2.2) and 2.8±2.2 (baseline 82.2±1.8) after ritanserin 10mg, 2.2±0.8 (baseline 77.5±2.5) and 2.1±1.8 (baseline 80.0±2.9) after ritanserin 20mg, and 3.1±1.4 (baseline 80.3±1.2) and 0.3±1.0 (baseline 79.1±1.2) after ketanserin 40mg.

3.3.2 Psychological function tests.

There were no significant changes in tranquillity or alertness scores (Herbert, Johns & Dore, 1976) or hostility, anxiety or depression scores (Zuckerman & Lubin, 1965) after ritanserin 10 or 20mg or ketanserin 40mg compared to placebo (tables 12 and 13).

3.3.3 Adverse drug reactions.

No adverse drug reactions or side effects were noted after ritanserin or ketanserin in this study.

	Placebo	Ritanserin 10mg	Ritanserin 20mg	Ketanserin 40mg
Baseline Hostility	4.2±1.0	5.2±1.3	5.0±1.2	4.3±1.0
△ Hostility	0.7±0.2	-0.7±0.5	0.1±0.5	0.6±0.3
Baseline Anxiety	4.3±1.5	5.3±2.4	5.0±1.5	4.2±1.9
△ Anxiety	0.3±0.8	-0.6±0.8	0.1±0.9	0.3±0.7
Baseline Depression	8.2±2.1	10.3±2.8	10.0±2.3	10.2±2.5
△ Depression	1.8±0.9	-0.3±0.4	0.3±0.6	0.3±1.0

△ = mean change from baseline

Table 12:

Baseline and mean change from baseline of hostility, anxiety and depression scores during 8 hours after single oral doses of placebo, ritanserin 10mg, ritanserin 20mg and ketanserin 40mg in 6 patients with untreated essential hypertension.

	Placebo	Ritanserin 10mg	Ritanserin 20mg	Ketanserin 40mg
Baseline Alertness	81.0±5.4	79.4±9.2	84.6±6.8	87.2±6.3
△ Alertness	2.7±2.9	1.2±5.7	2.3±5.1	1.9±2.4
Baseline Tranquillity	77.9±5.5	76.8±9.6	83.2±6.2	84.1±6.8
△ Tranquillity	0.0±0.9	-3.4±4.9	-0.4±0.5	1.6±2.2

Baseline is % of maximal possible score
△ = mean % change from baseline

Table 13:

Baseline and mean change from baseline of alertness and tranquillity scores during 8 hours after single oral doses of placebo, ritanserin 10mg, ritanserin 20mg and ketanserin 40mg in 6 patients with untreated essential hypertension.

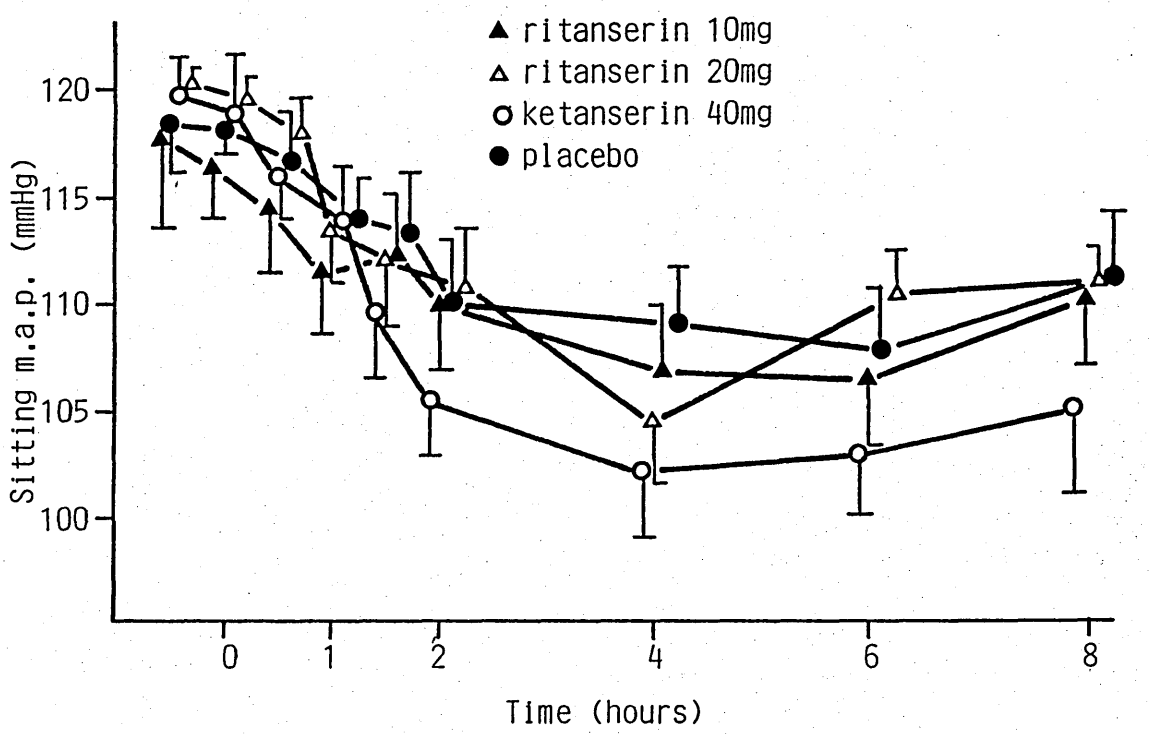


Figure 24
Sitting mean arterial pressure before and after single oral doses of ritanserin 10mg, ritanserin 20mg, ketanserin 40mg and placebo in 10 patients with untreated essential hypertension.

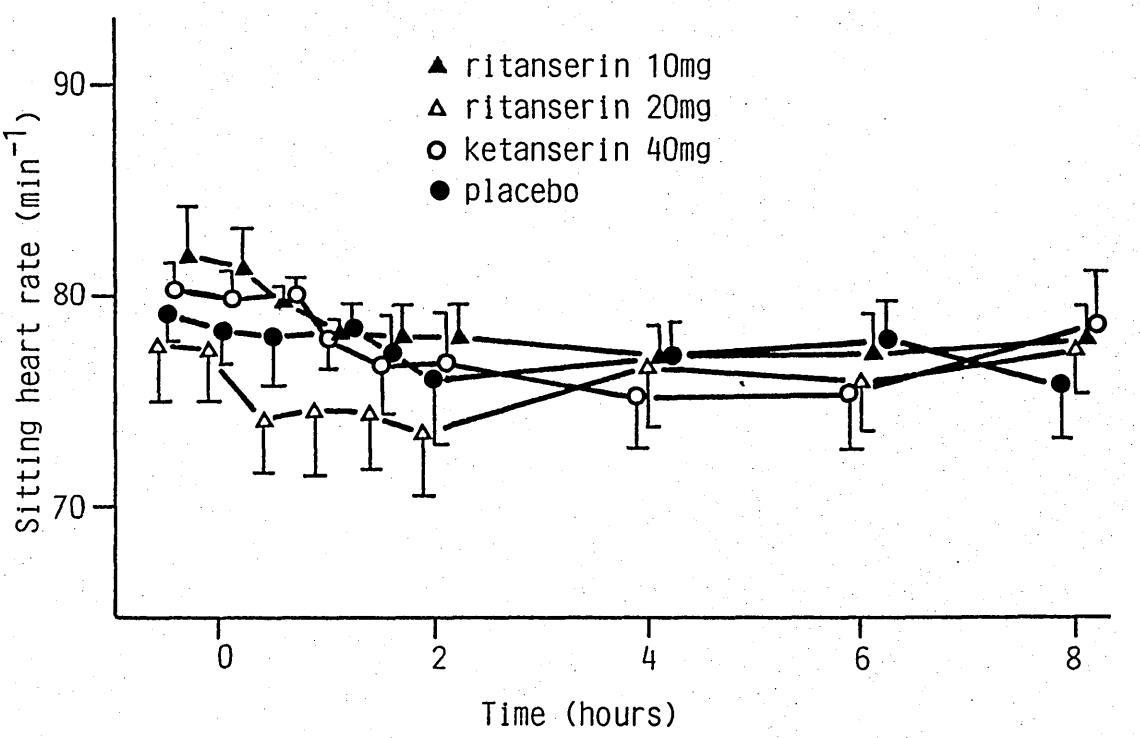


Figure 25
Sitting heart rate before and after single oral doses of ritanserin 10mg, ritanserin 20mg, ketanserin 40mg and placebo in 10 patients with untreated essential hypertension.

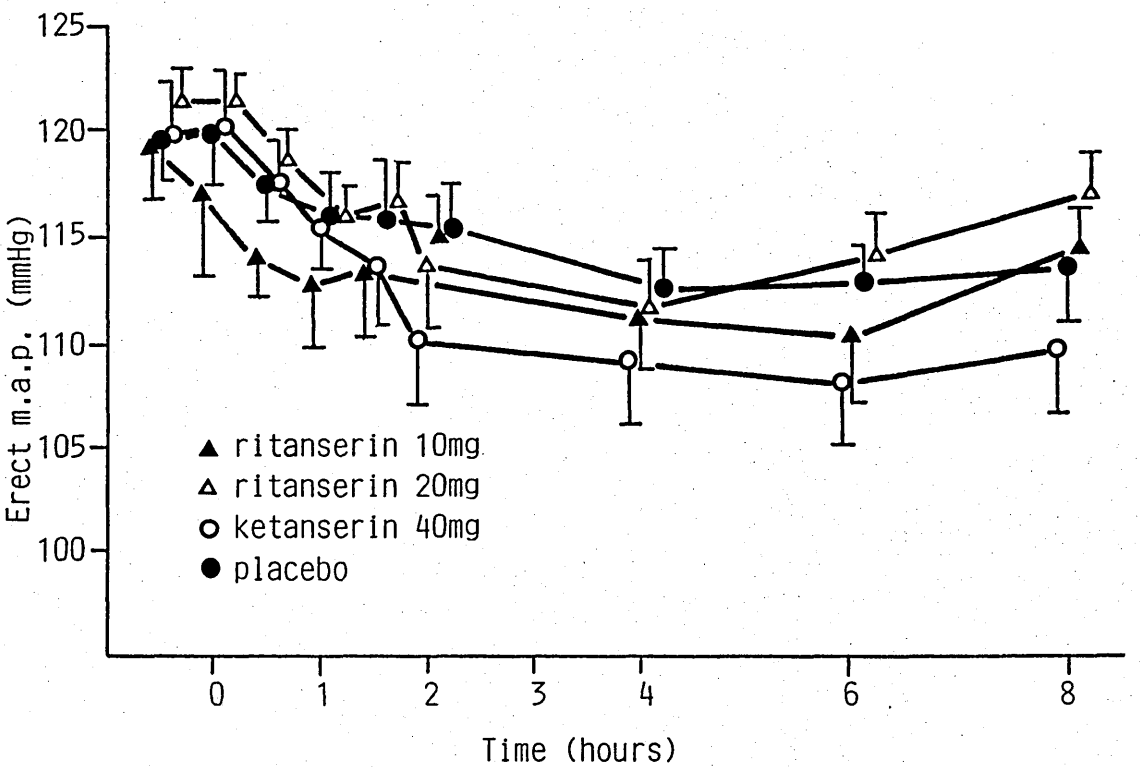


Figure 26
Standing mean arterial pressure before and after single oral doses of ritanserin 10mg, ritanserin 20mg, ketanserin 40mg and placebo in 10 patients with untreated essential hypertension.

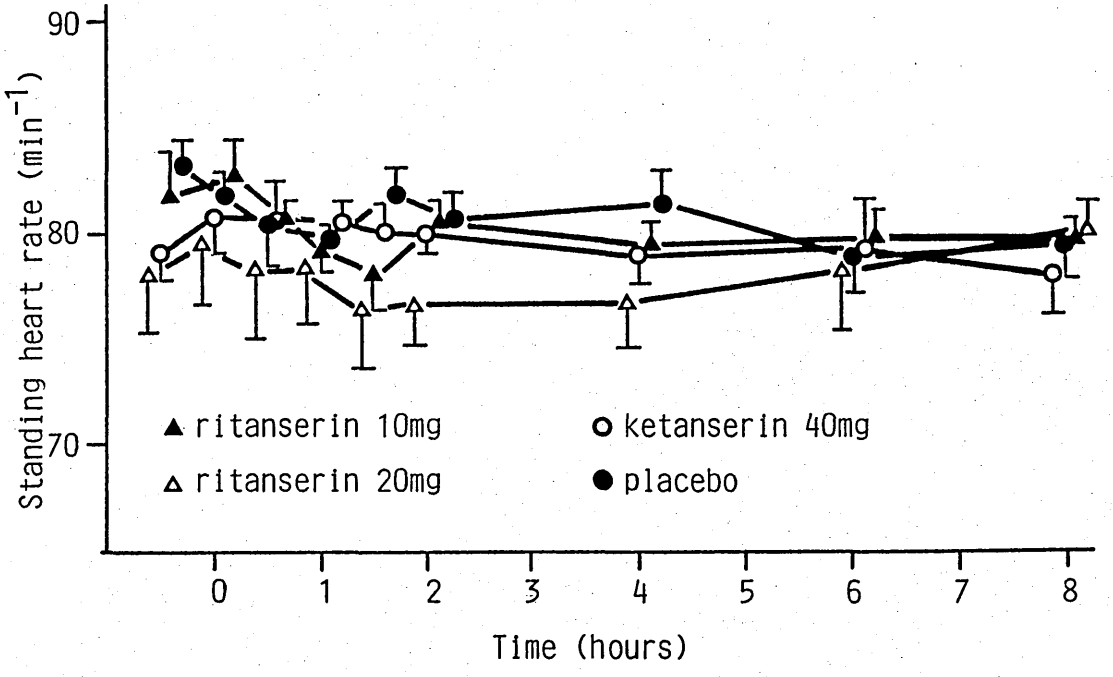


Figure 27
Standing heart rate before and after single oral doses of ritanserin 10mg, ritanserin 20mg, ketanserin 40mg and placebo in 10 patients with untreated essential hypertension.

3.4 Effects of chronic oral ritanserin in patients with untreated essential hypertension

3.4.1 Serotonin-induced and spontaneous platelet aggregation.

Ritanserin significantly inhibited serotonin-induced platelet aggregation compared to placebo ($p<0.05$, figure 28); the % reduction in platelet numbers caused by serotonin decreased from 50.4±5.8 to 21.7±7.0% over 4 weeks (week 0-4) active treatment, compared to 49.0±7.0% at week 0 and 58.7±3.9% at week 4 in the placebo control group. Ritanserin had no significant effects on total platelet numbers, spontaneous platelet aggregation or platelet serotonin content (table 14).

3.4.2 Blood pressure and heart rate.

Ritanserin had no significant effects on supine or erect blood pressure or heart rate compared to placebo (table 15, figures 29 and 30). Supine systolic and diastolic blood pressures rose by 1.2±4.1 and decreased by 0.6±0.9 mmHg respectively over 4 weeks treatment with ritanserin (weeks 0-4), compared to reductions of 7.3±7.8 and 3.1±4.0 mmHg over the corresponding period in the placebo control group; erect systolic and diastolic pressures decreased by 0.3±3.7 and 3.3±3.0 mmHg in the ritanserin group compared to 7.9±8.3 and 6.8±4.6 mmHg in the control group. Supine and erect heart rate decreased by 2.0±2.5 and 1.0±3.8

beats/min respectively over 4 weeks of ritanserin treatment, compared to reductions of 5.0±2.2 and 5.0±3.0 beats/min over the corresponding period in the control group. The 95% confidence limits (2-tailed) for the effects of ritanserin compared to placebo at 2 and 4 weeks are shown in table 16.

3.4.3 Forearm blood flow and venous compliance.

There were no significant changes in forearm blood flow at rest, of peak flow after 3 minutes of arterial occlusion or of basal or minimal forearm vascular resistance in the ritanserin treated patients compared to the placebo control group (table 17, figures 31 and 32). Forearm venous compliance also did not change significantly after drug compared to placebo (table 17, figure 33).

3.4.4 Electrocardiographic QRS and QT intervals.

Ritanserin caused prolongation of the QT_C interval by 41±11 milliseconds compared to 4.4±4.0 after placebo ($p<0.05$, table 18, figure 34). There was a similar tendency for the drug to prolong the uncorrected QT interval (figure 35), although this did not acheive statistical significance. Ritanserin had no detectable effects on QRS duration (table 18).

3.4.5 Urea and electrolytes, haematocrit and body weight.

There were no significant changes in plasma sodium, potassium, urea or creatinine during treatment with ritanserin (table 19). Weight remained steady during drug treatment, with no detectable alterations of haematocrit (table 19).

3.4.6 Tests of psychological function.

There were no significant changes caused by ritanserin (compared to placebo) in the digit subtraction substitution test score (table 20) or in subjective feelings measured by visual analogue scales (table 21).

3.4.7 Adverse drug reactions.

One patient complained of drowsiness and lethargy while taking ritanserin. No other adverse drug reactions or side effects were noted.

	Ritanserin (n=7)		Placebo (n=7)	
	Week 0	4	0	4
Platelet count (10^9/L)	202±17	217±27	203±21	214±16
Platelets remaining after blank test (%)	94.4±4.5	79.0±5.3	86.2±3.2	88.2±12.0
Platelets remaining after serotonin-induced aggregation (%)	49.6±5.8	78.3±7.0*	51.0±7.0	41.3±3.9
Platelets remaining after spontaneous aggregation (%)	47.4±8.9	56.1±6.9	55.3±9.6	41.3±8.9

(* $p<0.05$, Wilcoxon rank sum test, change after ritanserin compared to change after placebo)

Table 14:

Platelet count and platelet aggregation (quantified by the reduction in single platelet count in whole blood) in patients with essential hypertension, before and after 4 weeks of ritanserin 10mg twice daily compared to a parallel placebo control group.

	Ritanserin 10mg twice daily (n=10)			Matching placebo (n=8)		
	Week 0	2	4	0	2	4
sSBP	166.7±3.5	165.5±7.9	167.9±9.7	167.3±5.9	160.4±4.2	160.0±5.2
sDBP	97.1±3.8	96.2±2.9	96.5±3.7	90.0±2.5	88.3±2.9	86.9±3.9
sHR	80.6±4.4	78.6±3.9	78.6±3.9	72.5±2.0	68.1±2.6	67.5±2.8
eSBP	158.8±8.5	161.4±8.3	158.5±9.3	153.5±7.4	148.3±4.6	145.6±5.3
eDBP	103.6±3.4	102.6±3.3	100.3±3.6	93.5±4.7	91.9±3.2	86.8±5.0
eHR	90.2±5.0	88.8±4.4	89.2±4.3	81.5±2.4	78.0±3.5	76.5±3.1

s = supine, e = erect
SBP = systolic blood pressure (mmHg)
DBP = diastolic blood pressure (mmHg)
HR = heart rate (beats/minute)

Table 15:

Supine and erect blood pressure and heart rate in patients with essential hypertension before and after 2 and 4 weeks of ritanserin 10mg twice daily compared to a parallel placebo control group.

Mean changes in blood pressure and heart rate (95% confidence limits) after ritanserin 10mg (n=10) twice daily compared to placebo (n=8).

	Week 2	Week4
Supine SBP	5.6 (-7.8, 19.0)	8.5 (-9.3, 26.3)
Supine DBP	3.6 (-6.3, 13.5)	2.5 (-6.3, 11.3)
Supine HR	-2.4 (-7.7, 2.9)	3.0 (-4.0, 10.0)
Erect SBP	7.9 (-6.3, 22.1)	7.6 (-10.5, 25.7)
Erect DBP	0.6 (-9.7, 8.5)	3.5 (-7.6, 14.6)
Erect HR	2.9 (-3.7, 9.5)	4.0 (-6.3, 14.3)

SBP = systolic blood pressure (mmHg)
DBP = diastolic blood pressure (mmHg)
HR = heart rate (beats/min)

Table 16:

Mean changes and 95% confidence limits (2-tailed) of supine and erect blood pressure and heart rate in patients with essential hypertension, after 2 and 4 weeks of ritanserin 10mg twice daily compared to a parallel placebo control group.

	Ritanserin (n=7) Week 0	4	Placebo (n=7) 0	4
RF	11.5±2.7	12.2±1.4	8.3±1.2	9.4±1.7
PF	59.1±10.2	62.1±5.5	48.6±6.5	41.6±3.4
BVR	13.2±2.5	10.4±1.0	16.7±3.4	14.1±2.1
MVR	2.5±0.5	2.1±0.3	2.6±0.3	2.8±0.2
Intercept	-0.16±0.43	-0.13±0.51	-0.26±0.37	-0.35±0.51
Slope	0.107±0.016	0.119±0.012	0.107±0.009	0.110±0.005

RF = forearm resting blood flow (ml/100ml/min)
PF = maximal forearm post-ischaemic flow (ml/100ml/min)
BVR = basal vascular resistance = mean arterial pressure / RF
MVR = minimal vascular resistance = mean arterial pressure / PF

The intercept (a) and slope (b) describe the regression line of venous occlusion pressure in mmHg (x) against % increase in forearm volume (y), where y=a+bx

Table 17:

Forearm blood flow at rest, maximal post-ischaemic flow, basal and minimal vascular resistance and venous compliance in patients with essential hypertension, before and after 4 weeks of ritanserin 10mg twice daily compared to a parallel placebo control group.

	Ritanserin (n=7)		Placebo (n=7)	
	Week 0	4	0	4
QT (msec)	385±10	422±15	388±10	392±10
QT_C (msec)	439±12	478±12*	402±5	410±5
QRS (msec)	93±3	92±2	89±2	89±3

(* $p<0.05$, Wilcoxon rank sum test; change after ritanserin compared to change after placebo)

Table 18:

Electrocardiographic QT, QT_C and QRS interval durations in patients with essential hypertension, before and after 4 weeks of ritanserin 10mg twice daily compared to a parallel placebo control group.

	Ritanserin (n=10)		Placebo (n=8)	
	Week 0	4	0	4
Na^+ (mmol/L)	141.6±0.5	141.1±0.6	141.0±0.8	142.4±0.9
K^+ (mmol/L)	3.97±0.10	4.06±0.09	4.10±0.13	4.05±0.10
U (mmol/L)	4.8±0.3	4.9±0.2	4.9±0.4	5.2±0.4
Cr (µmol/L)	86.5±5.6	82.9±6.8	75.1±5.5	78.6±6.4
Hct (%)	43.8±1.5	43.1±1.5	41.5±0.8	40.8±1.2
Weight (Kg)	74.8±5.5	75.2±5.4	73.6±5.8	73.4±5.9

Na^+ = sodium
K^+ = potassium
U = urea
Cr = creatinine
Hct = haematocrit

Table 19:

Serum sodium, potassium, urea, creatinine, and haematocrit and body weight in patients with essential hypertension, before and after 4 weeks of ritanserin 10mg twice daily compared to a parallel placebo control group.

	Ritanserin (n=10)		Placebo (n=8)	
	Week 0	4	0	4
DST score	48.4±4.4	51.2±5.5	48.4±6.1	54.3±5.2

DST = digit substitution test
score = number of correct digit substitutions in 3 minutes

Table 20:

Digit substitution test score in patients with essential hypertension before and after 4 weeks of ritanserin 10mg twice daily compared to a parallel placebo control group.

	Ritanserin (n=10)		Placebo (n=8)	
	Week 0	4	0	4
Alert-Drowsy	34.2±5.5	28.6±4.1	34.8±10.7	34.4±11.3
Well co-ordinated-Clumsy	22.6±4.1	21.8±5.0	27.6±11.6	35.9±10.9
Tense-Relaxed	52.8±11.6	59.4±7.7	60.8±12.0	49.1±12.1
Incompetent-Proficient	69.7±6.6	73.7±6.7	64.1±8.0	82.8±4.9
Happy-Sad	28.9±7.5	31.0±6.7	20.5±8.5	17.8±6.6
Withdrawn-Sociable	80.8±8.2	76.0±6.6	76.9±6.0	79.1±4.5

Distance (mm) along line (100mm) between 2 words as shown, on visual analogue scales.

Table 21:

Subjective feelings, measured by self-administered visual analogue scales, in patients with essential hypertension before and after 4 weeks of ritanserin 10mg twice daily compared to a parallel placebo control group.

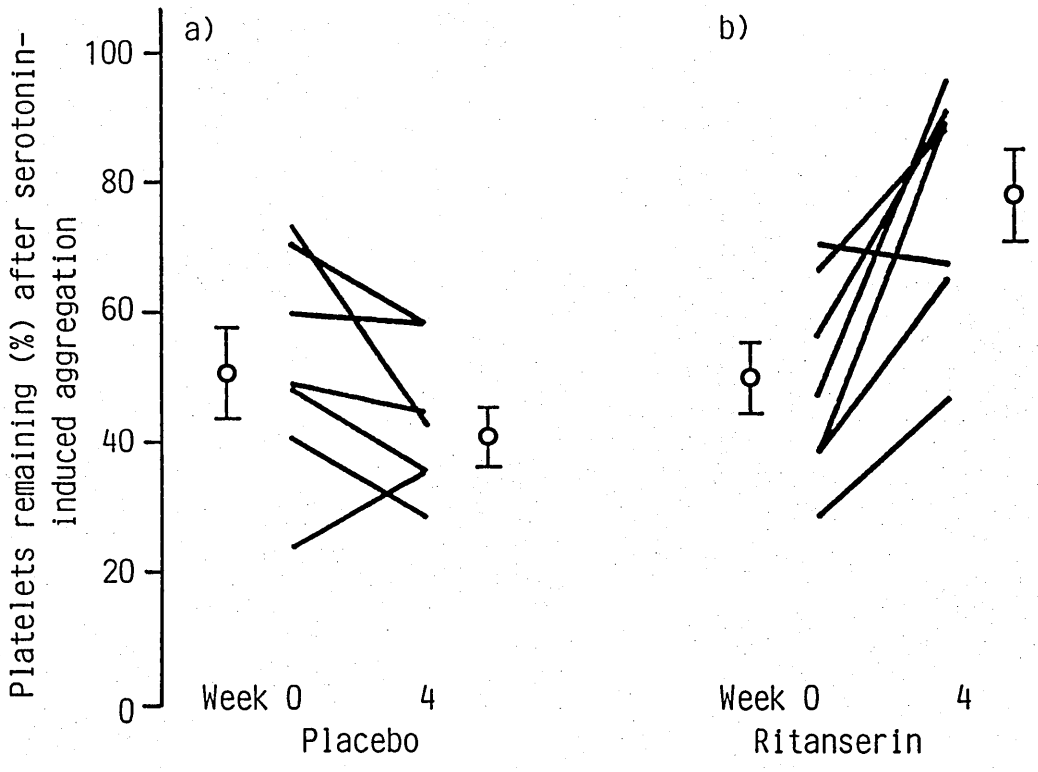


Figure 28
Serotonin - induced platelet aggregation, quantified as the reduction in single platelet count in whole blood caused by the addition of serotonin (1μM), in patients with essential hypertension before and after 4 weeks of ritanserin 10mg twice daily (n = 7) compared to a parallel placebo control group (n = 7).

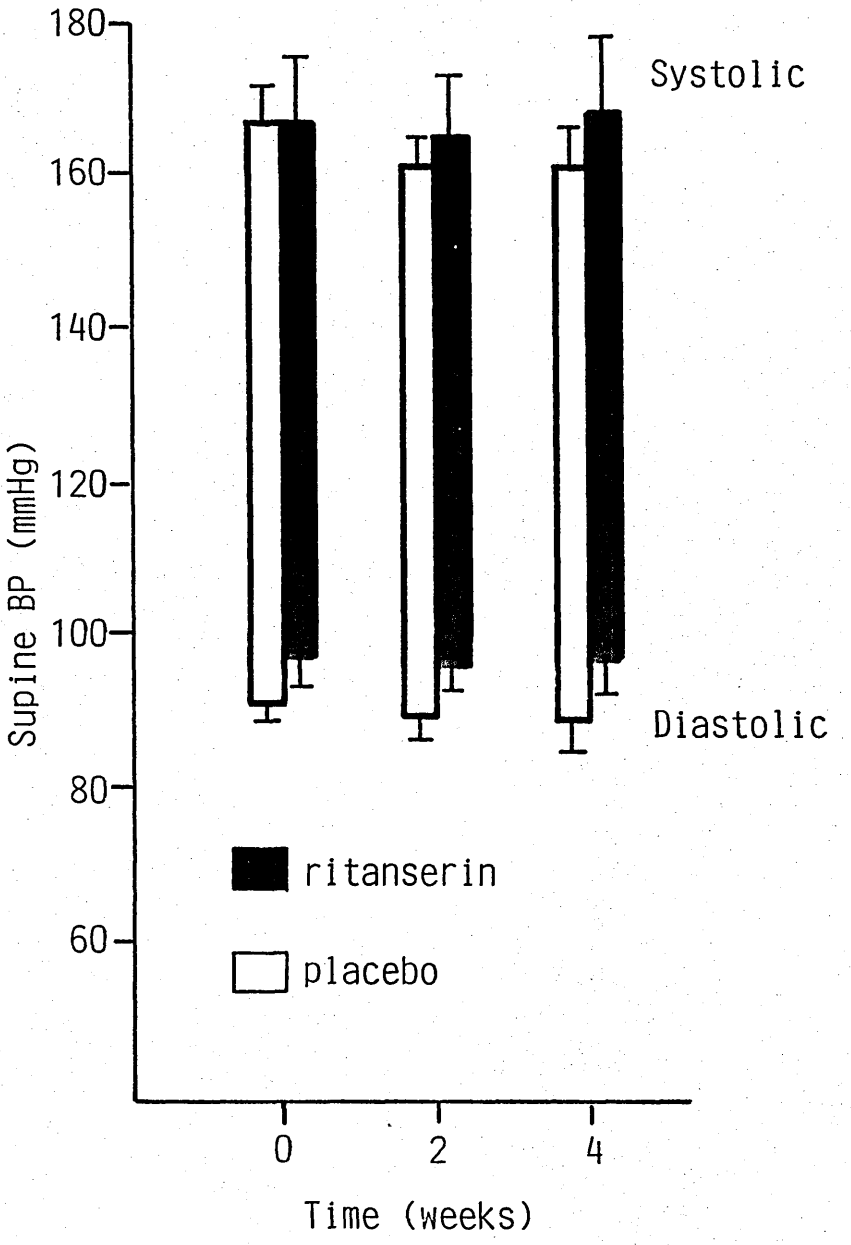


Figure 29
Supine systolic and diastolic blood pressure in patients with essential hypertension treated with ritanserin 10mg twice daily for 4 weeks (n = 10), compared to a parallel placebo control group (n = 8).

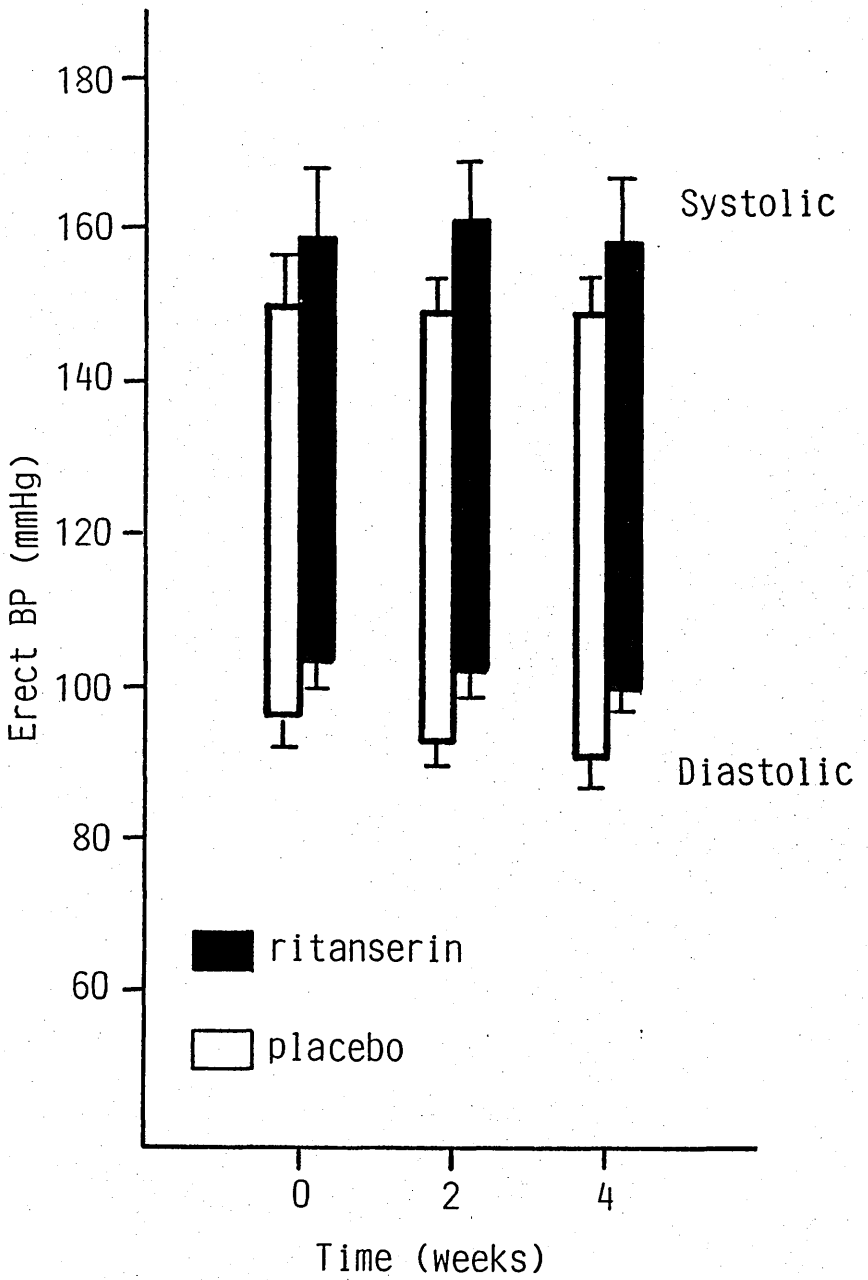


Figure 30
Standing systolic and diastolic blood pressure in patients with essential hypertension treated with ritanserin 10mg twice daily for 4 weeks, compared to a parallel placebo control group (n = 8).

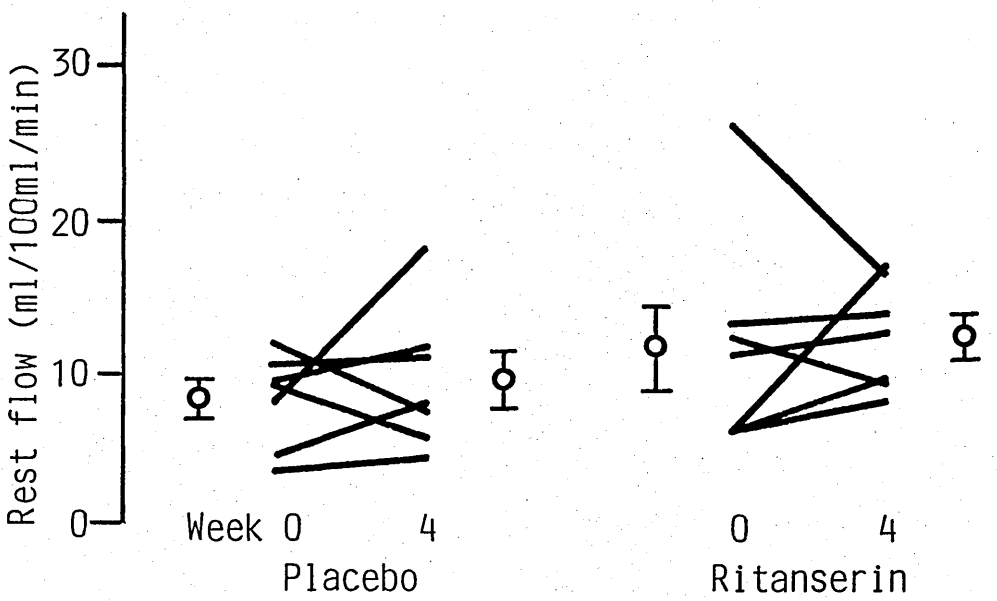


Figure 31
Forearm blood flow at rest in patients with essential hypertension before and after 4 weeks of ritanserin 10mg twice daily (n = 7) compared to a parallel placebo control group (n = 7).

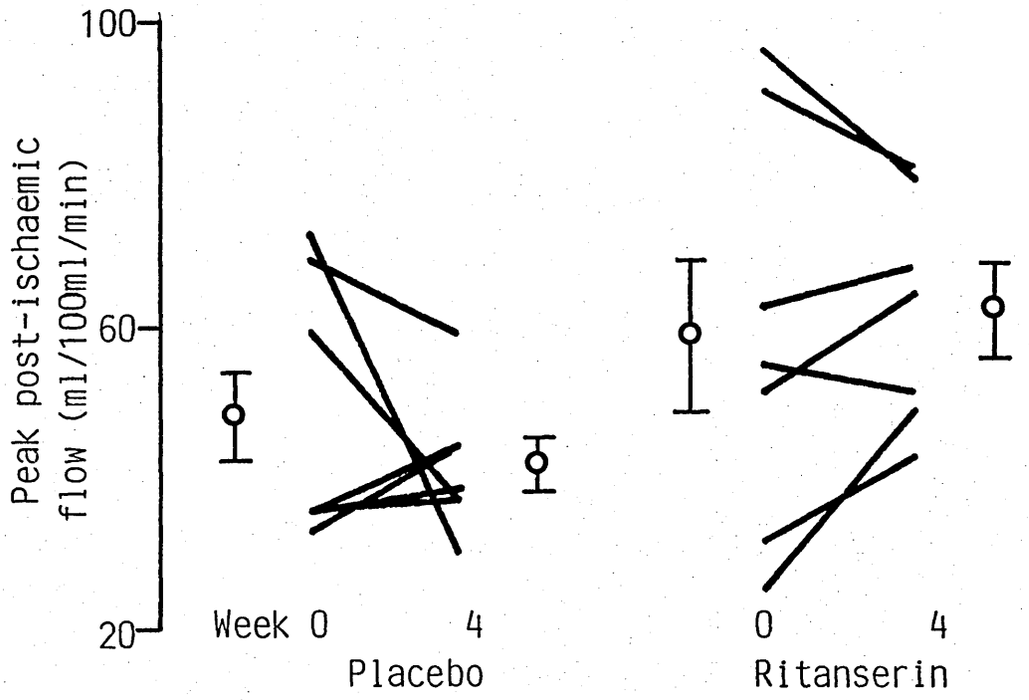


Figure 32
Post-ischaemic forearm blood flow in patients with essential hypertension before and after 4 weeks of ritanserin 10mg twice daily (n = 7) compared to a parallel placebo control group (n = 7).

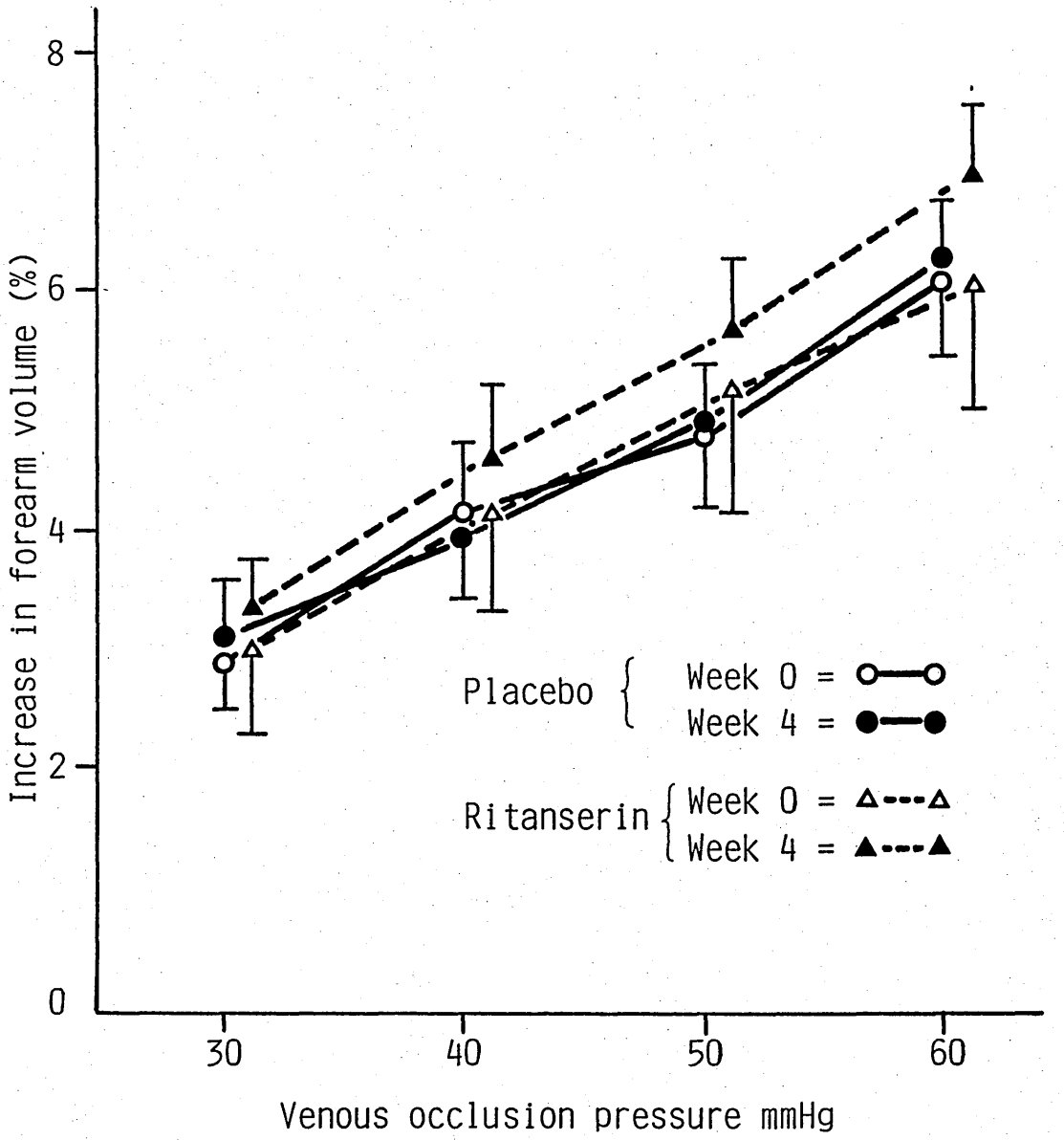


Figure 33
Forearm venous compliance, measured by percentage increase in forearm volume at increasing venous occlusion pressures, in patients with essential hypertension before and after 4 weeks of ritanserin 10mg twice daily (n = 7) compared to a parallel placebo control group (n = 7).

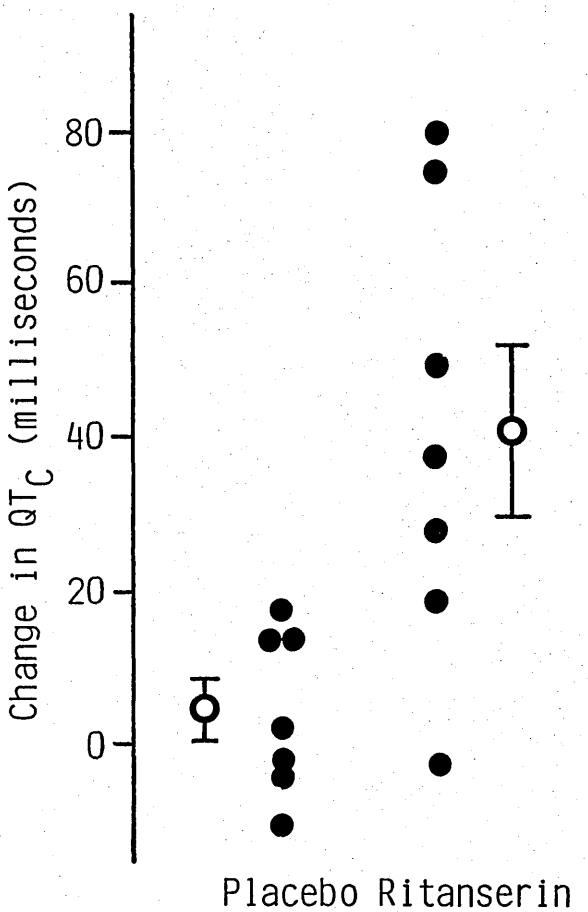


Figure 34
Change in electrocardiographic QT_C interval in patients with essential hypertension after 4 weeks of ritanserin 10mg twice daily (n = 7) compared to a parallel placebo control group (n = 7).

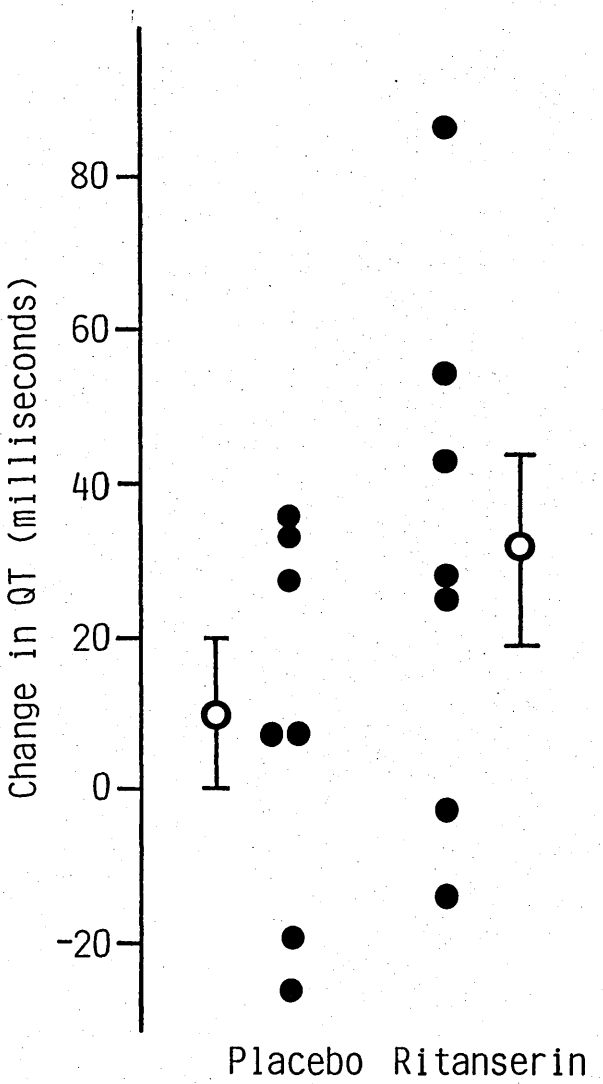


Figure 35
Change in electrocardiographic QT interval in patients with essential hypertension after 4 weeks of ritanserin 10mg twice daily (n = 7) compared to a parallel placebo control group (n = 7).

3.5 Effects of ketanserin in adult atopic asthma

3.5.1 Resting bronchomotor tone and exercise-induced bronchoconstriction.

FEV_1, $\dot{V}_{50(c)}$ or $\dot{V}_{25(p)}$, pre- and 45 min post-treatment, were not significantly different after administration of either ketanserin or placebo (tables 22 and 23).

Ketanserin had no significant effects on the fall in FVC, FEV_1, $\dot{V}_{50(c)}$ or $\dot{V}_{25(p)}$ after exercise (figures 36, 37, 38 and 39), although there was a tendency for the fall in FEV_1 and FVC after exercise to be reduced by drug treatment. The mean maximal absolute and percentage (%) fall in FEV_1 caused by exercise was 0.48±0.09L (16.8±3.7%) after ketanserin compared to 0.55±0.08L (19.2±4.2%) after placebo; the mean maximal fall in $\dot{V}_{25(p)}$ was 0.58±0.14L/sec (32.5±5.7%) after drug compared to 0.49±0.11 (28.2±1.3%) after placebo. The 95% confidence limits of the effects of ketanserin on maximal percentage reduction in pulmonary function after exercise compared to placebo are shown in table 23.

3.5.2 Blood pressure and heart rate.

There were no significant changes in resting mean arterial blood pressure (m.a.p.) after either ketanserin or placebo; exercise caused a rise in m.a.p. of 8.3±1.1 mmHg after drug compared to 10.5±1.4 after placebo (not significant). Exercise caused a similar rise in heart

rate, 46.0±4.7 beats/min after ketanserin compared to 46.3±4.7 after placebo.

3.5.3 Plasma ketanserin and ketanserinol levels.

Plasma ketanserin and ketanserinol levels were 25.4±11.4 and 16.1±5.2 ng/ml respectively at 45 mins, and 36.5±9.6 and 23.2±6.1 ng/ml respectively 75 mins after drug.

3.5.4 Adverse drug reactions.

One patient complained of drowsiness after ketanserin. No other adverse drug effects were seen.

	Ketanserin		Placebo	
Time	0	45mins	0	45mins
FEV_1 (L)	3.27±0.37	3.24±0.33	3.16±0.39	3.23±0.40
FVC (L)	4.53±0.50	4.44±0.40	4.59±0.48	4.63±0.45
$\dot{V}_{50(c)}$ (L/sec)	3.33±0.58	3.41±0.56	3.28±0.60	3.45±0.70
$\dot{V}_{25(p)}$ (L/sec)	1.59±0.23	1.95±0.35	1.63±0.29	1.74±0.31

Table 22:

Effect of a single oral dose of ketanserin (40mg) compared to placebo on resting bronchomotor tone in 8 adult atopic asthmatic patients.

Mean % change in pulmonary function (95% confidence limits) after ketanserin 40mg compared to placebo

	At rest (0-45mins after dosing)	After exercise (maximal change)
FEV_1	-2.8 (-11.8, 6.2) %	2.4 (-6.4, 11.2) %
FVC	-2.1 (-9.7, 5.5) %	3.7 (-5.5, 12.9) %
$\dot{V}_{50(c)}$	7.6 (-8.0, 23.2) %	-4.2 (-22.5, 13.9) %
$\dot{V}_{25(p)}$	14.9 (-0.9, 30.7) %	-4.3 (-19.2, 10.6) %

Table 23:

The 95% confidence limits (2-tailed) of the effects of ketanserin 40mg compared to placebo on resting bronchomotor tone and maximal bronchoconstriction after exercise in 8 adult atopic asthmatic patients.

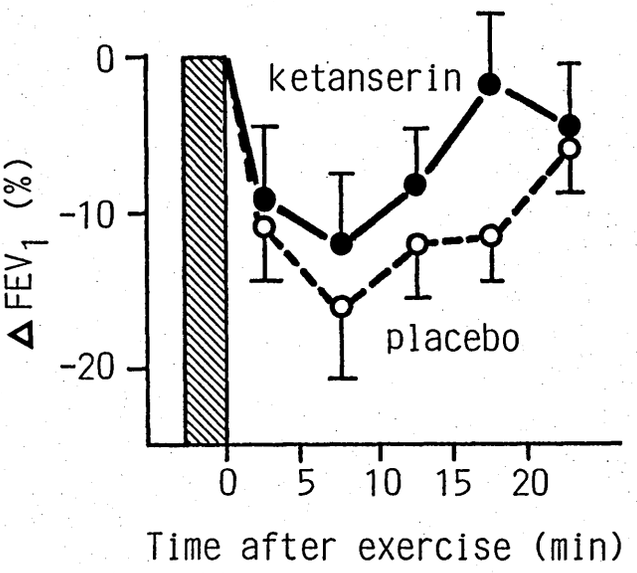


Figure 36
Percentage change in forced expiratory volume in 1 second, following exercise, 45 minutes after a single oral dose of ketanserin 40mg compared to matching placebo in 8 adult atopic asthmatic subjects.

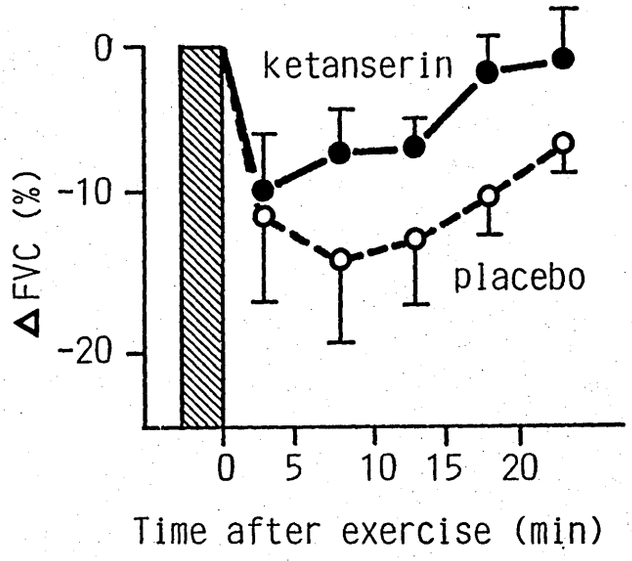


Figure 37
Percentage change in forced vital capacity after exercise, 45 minutes after a single oral dose of ketanserin 40mg compared to matching placebo in 8 adult atopic asthmatic subjects.

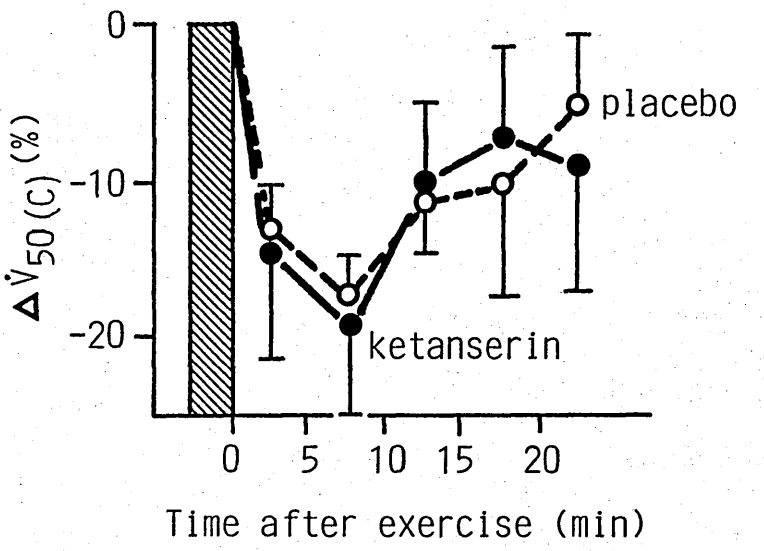


Figure 38
Percentage change in $\dot{V}_{50(C)}$ following exercise, 45 minutes after a single oral dose of ketanserin 40mg compared to matching placebo, in 8 adult atopic asthmatic subjects.

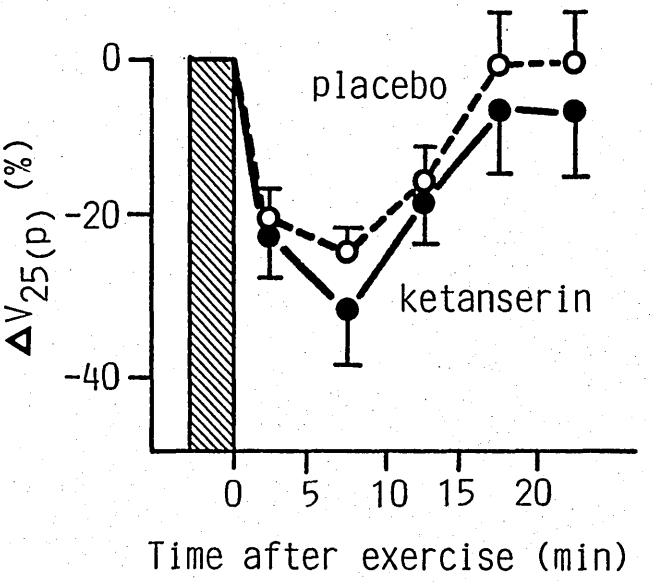


Figure 39
Percentage change in $V_{25(p)}$ following exercise, 45 minutes after a single oral dose of ketanserin 40mg compared to matching placebo, in 8 adult atopic asthmatic subjects.

Chapter 4; Discussion

4.1 Measurement of platelet serotonin by high pressure liquid chromatography with electrochemical detection

4.1.1 High pressure liquid chromatography with electrochemical detection.

Measurement of hydroxyindoles such as serotonin by high pressure liquid chromatography with electrochemical detection is highly sensitive and specific. Furthermore it is a safe method, particularly in that it avoids the use of radio-isotopes. The equipment and reagents required are relatively inexpensive compared to other methods of serotonin assay (Sasa et al., 1978). When a potential of +0.5 to +1.0 volts is applied to a solution containing serotonin, the hydroxyl group of serotonin (figure 1) is oxidised (Tagari, Boullin & Davies, 1984), giving off a small current (measured in uamps). This current is directly proportional to the concentration of serotonin in the solute (Kissinger, Bruntlett & Schoup, 1981). However, in plasma and other body fluids, there are numerous other interfering substances which are also electroactive at these potentials. Therefore a separation step, such as high pressure liquid chromatography, is required to remove these substances prior to electrochemical detection. Under the assay conditions that I have described, the concentration of serotonin in known standard solutions was directly proportional to the serotonin peak height, across

the likely range of serotonin levels in platelet rich plasma.

The retention time for serotonin on the column can be varied by adjusting the concentration of methanol in the mobile phase. Increasing the concentration of methanol allows serotonin to pass more quickly through the column, bringing the peak forwards. I have shown that, with a retention time of approximately 14 minutes, the serotonin peak in platelet rich plasma can be clearly distinguished with no interfering peaks. With this retention time up to 6 samples can be assayed in duplicate before any significant reduction of serotonin peak height occurs, at around 5 hours. Using the automated sample injector, 12 samples can be handled readily in an 8 hour working day.

With the above retention time for serotonin, the 5-hydroxyindole acetic acid peak in platelet rich plasma arrives very early and cannot be clearly separated from other interfering peaks. Although the 5-hydroxyindole acetic acid peak could be delayed, by decreasing the amount of methanol in the mobile phase, allowing satisfactory separation from interfering peaks, prolongation of the retention time for serotonin would have lead to this being measured less accurately. Furthermore, very few samples could be assayed for both serotonin and 5-hydroxytryptamine in a single day, due to the lack of stability of serotonin. Therefore, attempts have not been made to analyse plasma 5-hydroxyindole acetic acid levels at the same time as serotonin.

The major drawback of serotonin assay in platelet rich

plasma by high pressure liquid chromatography with electrochemical detection is the limited number of samples that can be processed in a single day. There are several other different basic methods by which serotonin can be measured. In early days bioassays were used (Erspamer, 1954). Although these were sensitive, they were clumsy and lacked specificity and therefore fell into disfavour. There exist several different techniques for measurement of total hydroxyindoles by fluorescence, including native fluorescence in strong hydrochloric acid (Geeraerts, Schimpfessel & Crockaert, 1974), and derivatisation with ninhydrin (Nathenas, Dexter & Katzman, 1973) or o-phthalaldehyde (Shuttleworth & O'Brien, 1981). However, to measure serotonin rather than total 5-hydroxyindoles, a separation step such as high pressure liquid chromatography is required prior to fluorescence detection (Anderson, Young & Batter, 1981). Although the limit of detection for serotonin of fluorescence assays is at best around 2.5pmol/ml (Sasa et al., 1978), compared to detection limits of as low as 0.3pmol/ml using electrochemical detection (Artigas et al., 1985), high pressure liquid chromatography with fluorescence detection is a useful alternative method for measurement of platelet serotonin content.

Radioimmunoassay techniques have also been used to measure serotonin (Kellum & Jaffe, 1979; Engbaek & Voldby, 1982). This requires the raising of antisera to serotonin, and the use of radio-isotopes. Radio-immunoassay is certainly an acceptable technique for measuring serotonin,

with claimed limits of detection of as low as 2pmol/ml (Engbaek & Voldby, 1982). Samples can be processed more rapidly by this technique than by high pressure liquid chromatography, however because of the materials used radio-immunoassay is significantly more expensive (Sasa et al., 1978) and is unlikely to have the precision of a chemical method involving high pressure liquid chromatography and oxidation at a given potential.

The limit of detection of 5pmol/ml that I found with high pressure liquid chromatography with electrochemical detection is perfectly satisfactory for measuring platelet serotonin, and is similar to that of Tagari, Boullin & Davies, (1984). Using more complicated sample preparation techniques, including the use of an internal standard and extraction into an organic solvent, Artigas et al., (1985) found a detection limit of 0.3pmol/ml for serotonin by high pressure liquid chromatography with electrochemical detection. Using a simple assay method without internal standards or an extraction procedure, Molyneux & Clarke (1985) reported a limit of detection of 1pmol/ml. For measurement of tissue or free plasma serotonin clearly the most sensitive methods should be chosen, however such low detection limits hold no advantage for measurement of the large amounts of serotonin in platelet rich plasma. Consequently, simple and quick sample preparation techniques should be used.

4.1.2 Preparation of platelet rich plasma.

I have shown that serotonin levels in platelet rich plasma can be measured sensitively and accurately using a quick and simple sample preparation technique which avoids the need for time consuming extraction into organic solvents as previously described by other investigators (Sasa et al., 1978; Artigas et al., 1985). The two deproteinisation steps used in the preparation of platelet rich plasma for serotonin analysis result in very clean samples which do not affect chromatographic efficiency. The excellent recovery of serotonin spiked into platelet rich plasma shows that this technique of sample preparation does not cause any significant loss of serotonin during sample preparation.

Sodium metabisulphite was used as an antioxidant (Molyneux & Clarke, 1985), as ascorbic acid, used in various fluorescence methods for assay of total hydroxyindoles (Geeraerts et al., 1974), caused a broad interfering peak on electrochemical detection. Other authors have found low concentrations of ascorbic acid (10uM) to be satisfactory as an antioxidant in assay of serotonin by high pressure liquid chromatogrphy (Baudouin-Legros et al., 1985). As well as ethylene diamine tetra-acetic acid, prostaglandin E_1 was used for collection of blood for platelet serotonin. It has been claimed that further inhibition of platelet aggregation and content release can be acheived by using prostaglandin E_1 (Kellum & Jaffe, 1979). It seems sensible to prevent

platelet content release until the final stages of sample preparation, as intra-platelet serotonin may be less susceptable to degradation than when in the free state.

Samples of platelet rich plasma can also be prepared for serotonin assay by adding an extra centrifugation step to form a platelet pellet. This then requires re-suspension in a solvent before sample purification can proceed (Artigas et al., 1985). These added steps do not seem to have any major advantage over the simpler and quicker technique of platelet lysis and content release by freezing and thawing and the addition of de-ionised water, except to decrease the dilution of serotonin, which may be more important with less sensitive techniques than electrochemical detection.

In the assay of serotonin by high pressure liquid chromatography with electrochemical detection, some investigators have used an internal standard (Artigas et al., 1985). As the time of the serotonin peak on electrochemical detection is very reproducible, and the peak is well separated from any other interfering compounds, it seems that the use of an internal standard is not essential in serotonin assay in platelet rich plasma (Molyneux & Clarke, 1985). Avoiding the use of an internal standard keeps sample preparation as simple as possible.

4.1.3 Assay of serotonin in whole blood.

Published methods of analysis of whole blood serotonin levels were examined for possible applicablity to high pressure liquid chromatography with electrochemical detection. It was found that deproteinisation steps using sodium hydroxide and zinc sulphate (Geeraerts et al., 1974; Kellum & Jaffe, 1979) caused a thick unmanageable slurry which was very difficult either to mix thoroughly or spin down. This problem was only partly ameliorated by increasing the dilution of whole blood with distilled water. More importantly, the recovery of spiked serotonin into frozen then thawed whole blood was very poor. Furthermore, disappointingly low levels of serotonin were detected in whole blood samples compared to levels in platelet rich plasma from the same subject. These findings are in direct contrast to those of Geerhardts et al., (1974) and Kellum & Jaffe, (1979), who found similar levels of serotonin in platelet rich plasma compared to whole blood from the same patients.

Attempts made to extract serotonin from the residual protein pellets after each of the two preparation steps yielded no detectable serotonin, therefore it is unlikely that the problem is failure of serotonin release during sample preparation. These findings suggest that serotonin is destroyed by a factor in frozen-thawed whole blood which is not present in platelet rich plasma. This factor may be haemoglobin, released from disrupted red cells after freezing and thawing. Oxidation of serotonin caused

by haemoglobin is only partly inhibited by the addition of an anti-oxidant such as ascorbic acid or sodium metabisulphide (Blum & Ling, 1959).

Data from previous publications claiming good recovery of spiked serotonin from whole blood samples (Geeraerts et al., 1974; Kellum & Jaffe, 1979) may be explained by avoiding freezing and thawing with the inevitable haemolysis that this causes. However freezing and thawing is essential if samples are to be kept for any significant period of time and assayed in batches.

In view of these difficulties in sample preparation of whole blood for serotonin assay, and the poor recovery of spiked serotonin from frozen-thawed whole blood, assay of whole blood serotonin levels was abandoned in favour of platelet rich plasma. As nearly all the serotonin present in blood is stored in platelets, whole blood serotonin assay has no theoretical advantage over platelet rich plasma.

4.2 Serotonin and platelet aggregation in patients with untreated essential hypertension compared to age and sex matched normotensive subjects

4.2.1 Characteristics of the hypertensive and normotensive control groups.

Supine heart rate was significantly higher in the hypertensive compared to the normotensive group. It is well recognised that heart rate is elevated in a proportion of patients with arterial hypertension (Julius, Esler & Randall, 1975).

The hypertensive patient group had a significantly greater mean body weight than the normotensive group. Otherwise the two groups were well matched for age, sex distribution, and had similar smoking and drinking habits. There were no significant differences between hypertensives and normotensives in renal function, based on serum urea and creatinine. Mean cell volume and serum gamma glutamyl transferase were similar in the hypertensive and normotensive groups, with no elevated values suggestive of concealed alcohol abuse.

4.1.2 Serotonin-induced platelet aggregation.

a) Is enhanced serotonin-induced platelet aggregation associated with raised arterial blood pressure?

There is good evidence that serotonin-induced platelet aggregation is mediated through the serotonergic type-2 receptor (1.4.2) and the phosphatidyl inositol messenger system (de Courcelles et al., 1985; de Courcelles et al., 1987). I have shown that serotonin-induced platelet aggregation is significantly enhanced in patients with untreated essential hypertension compared to age and sex matched normotensive subjects. Although mean body weight was higher in the hypertensive than the normotensive group, there was no significant correlation between body weight and the percentage of platelets remaining after serotonin-induced platelet aggregation, suggesting that body weight did not contribute to the difference in serotonin-induced platelet aggregation between the two groups. There was a significant negative correlation between the percentage of single platelets remaining after serotonin-induced platelet aggregation and systolic blood pressure, suggesting that a direct linear relationship exists between systolic blood pressure and serotonin-induced platelet aggregation. However, multiple regression analysis revealed no significant correlation between the percentage of single platelets remaining after serotonin-induced platelet aggregation and systolic blood pressure; thus, any independent relationship between

serotonin-induced platelet aggregation and systolic blood pressure remains unproven. No significant relationship between serotonin-induced platelet aggregation and diastolic pressure was demonstrated. It is not clear whether the enhanced serotonin-induced platelet aggregation seen in patients with untreated essential hypertension is due directly to raised arterial pressure, or conversely whether enhanced serotonin-induced platelet aggregation contributes to raised blood pressure; during platelet aggregation vasoconstrictor substances including serotonin are released (Holmsen, 1977). Thus, the antihypertensive effects of the serotonergic type-2 antagonist ketanserin could be due to inhibition of serotonin-induced platelet aggregation, reducing release of intra-platelet vasoconstrictor substances. Alternatively, increased serotonin-induced platelet aggregation in hypertension could be primarily due to atheromatous complications rather than directly related to raised arterial pressure, although this hypothesis is not supported by the data from the small number of hypertensive patients with known vascular disease in this study. Firm conclusions cannot be drawn for the cause or effect of enhanced serotonin-induced platelet aggregation in patients with essential hypertension. Nevertheless, this association between enhanced serotonin-induced platelet aggregation and hypertension has not been reported previously.

b) Is serotonin-induced platelet aggregation related to patient age or sex?

Increasing age and female sex were both associated with enhanced serotonin-induced platelet aggregation on linear regression analysis; however there were no significant relationships between these variables on multiple regression analysis. Thus, no clear independent relationships between serotonin-induced platelet aggregation and patient age or sex were demonstrated.

Other measures of in-vitro platelet aggregation may increase also with ageing. Enhanced adenosine diphosphate-induced platelet aggregation has been shown to be associated with increasing age on simple linear regression analysis (Vlachakis & Aledort, 1980; Yamanishi et al., 1985). In caucasians, plasma beta-thromboglobulin rises with increasing age (Dewar et al., 1979; Ludlum, 1979), suggesting that platelet aggregation in-vivo is enhanced in older subjects. However, in a large study of Japanese men, there was no correlation of beta-thromboglobulin or platelet factor 4 with age (Yamanishi et al., 1985). Nevertheless, most of the evidence suggests that platelet aggregation both in-vitro and in-vivo is enhanced with increasing age. Thus, the association between serotonin-induced platelet aggregation and age is only a part of the non-specific increase in platelet aggregability with ageing.

When differences in platelet aggregation between the sexes have been studied, it has been apparent that men

have enhanced in-vivo platelet aggregation compared to women (Dewar et al., 1979). Furthermore, atheromatous vascular disease, to which men are at increased risk, enhances serotonin-induced platelet aggregation in platelet rich plasma (De Clerck, 1986). Thus, the finding that serotonin-induced platelet aggregation was increased in women was surprising and is difficult to explain. However, my study was not designed specifically to address this issue, therefore interpretation must be with caution; further confirmation of these findings is required before we can be certain that serotonin-induced platelet aggregation is enhanced in women.

c) Is serotonin-induced platelet aggregation related to blood viscosity?

Raised blood viscosity could contribute to raised arterial pressure in patients with arterial hypertension. It has been claimed that serotonin may modulate red cell filterability and blood viscosity. The serotonergic type-2 antagonist ketanserin improves red cell filterability in patients with hypertension (Zannad et al., 1985), acute myocardial infarction and peripheral vascualr disease (De Crée et al., 1985a; De Crée et al., 1985b), inhibits serotonin-induced platelet aggregation (De Clerck & Xhonneux, 1985; Vermylen et al., 1986), and reduces arterial blood pressure. Improved red cell filterability after ketanserin is largely dependent on the presence of platelets (De Cree et al., 1985a). Thus, interest has been

raised in the possible relationship between serotonin-induced platelet aggregation and blood viscosity. I have found that whole blood viscosity at low and high shear rates was not significantly correlated with the reduction in single platelet count caused by serotonin-induced platelet aggregation. However, only a very small number of the patients studied had overt vascular disease. It is possible that serotonin and serotonin-induced platelet aggregation could play a role in increasing blood viscosity and raising arterial pressure in patients with atheromatous vascular disease, or after acute ischaemic events such as myocardial infarction (De Crée et al., 1985a; Dormandy, 1987). This hypothesis remains however largely unproven.

d) Platelet aggregation in-vitro in whole blood compared to platelet rich plasma.

In platelet rich plasma from healthy subjects, serotonin is a weak aggregating agent, causing platelet shape change and reversable aggregation; however, serotonin also potentiates the aggregating effects of threshold concentrations of potent platelet aggregating agents such as thrombin and adenosine diphosphate (De Clerck et al., 1984). In the presence of sub-threshold concentrations of these aggregating agents, serotonin can cause irreversable platelet aggregation and content release (De Clerck et al., 1984). Platelets from patients with atheromatous vascular disease frequently show irreversable platelet

aggregation and release of platelet contents in platelet rich plasma in response to serotonin (De Crée et al., 1985b). Thus, atheroma may cause enhanced serotonin-induced platelet aggregation in platelet rich plasma. However, measurement of platelet aggregation in whole blood may have advantages over platelet rich plasma. Platelets vary in size and density, therefore centrifugation for platelet rich plasma may select out platelets which do not fully represent platelets and platelet aggregability in-vivo (Haver & Gear, 1982). Furthermore, other cells in whole blood may influence platelet function. White blood cells can release prostacyclin, which inhibits platelet aggregation (Blackwood et al., 1978). Red blood cells are thought to augment platelet aggregation by releasing adenosine diphosphate (Gaarder et al., 1982). Mechanical factors, with jostling of platelets by other blood cells, may also influence platelet activity. Therefore, platelet aggregation studies in whole blood may reflect in-vivo conditions more closely than studies of platelet rich plasma.

4.2.3 Spontaneous platelet aggregation.

There was no significant difference in spontaneous platelet aggregation in whole blood between the hypertensive and normotensive patient groups. Adenosine diphosphate from red blood cells is thought to play an important role in spontaneous platelet aggregation in

whole blood (Saniabadi et al., 1984). Indeed, spontaneous platelet aggregation is markedly attenuated in platelet rich plasma compared to whole blood (Saniabadi et al., 1984).

Other methods have been used to investigate adenosine diphosphate mediated platelet aggregation in hypertension. Although increased platelet aggregation in platelet rich plasma in response to adenosine diphosphate has been reported in small studies of hypertensive patients (Ikeda et al., 1985; Bodzenta-Lukasyk, Krupinski & Bielawiec, 1987) other authors have found no difference compared to a normotensive control group (Mehta & Mehta, 1981; Yamanishi et al., 1982). By far the largest of these studies was by Yamanishi et al., 1982).

The threshold concentration of adenosine diphosphate required to cause platelet aggregation has been claimed to be reduced in hypertensives compared to normotensive controls (Coccheri & Fiorentini, 1971; Vlachakis & Aledort, 1980); however, in one of these studies the control group is not age matched and a number of the hypertensive patients are on treatment (Vlachakis & Aledort, 1980), and in the other no information is presented on age and sex matching of the control group (Coccheri & Fiorentini, 1971).

In an uncontrolled observational study, prazosin has been shown to reduce adenosine diphosphate-induced platelet aggregation in platelet rich plasma in hypertensive patients (Ikeda et al, 1985). However, adequately controlled studies using antihypertensive

agents with differing modes of action are required before we can be sure that reduction of blood pressure in patients with hypertension is associated with diminished adenosine diphosphate-induced platelet aggregation.

Thus, there is little evidence to suggest that raised arterial pressure is associated with enhanced adenosine diphosphate-induced platelet aggregation in hypertensive patients. Many studies reporting positive findings have been small (Ikeda et al., 1985; Bodzenta-Lukasyk et al., 1985) or inadequately controlled (Coccheri & Fiorentini, 1971; Vlachakis & Aledort, 1980; Ikeda et al., 1985). Furthermore, studies have failed to demonstrate a correlation between adenosine diphosphate-induced platelet aggregation and systolic or diastolic blood pressure.

Measurement of platelet aggregation in platelet rich plasma may be less physiological than spontaneous platelet aggregation in whole blood, as discussed above (4.2.2). The finding that spontaneous platelet aggregation in whole blood was not enhanced in patients with untreated essential hypertension compared to age and sex matched normotensive controls does not support an association between enhanced adenosine diphosphate-mediated platelet aggregation and raised arterial blood pressure.

4.2.4 Platelet serotonin.

Mean platelet serotonin content was not significantly different in hypertensives compared to age and sex matched normotensive controls. Levels were similar to those found

by other groups (Baudouin Legros et al., 1985; Kamal, Le Quan Bui & Meyer, 1984; Puri et al., 1986). Platelet serotonin levels have been claimed to be reduced in most studies of essential hypertension (Bhargava et al., 1979; Baudouin Legros et al., 1985; Kamal et al., 1984) and in some normotensive relatives of hypertensive patients (Kamal et al., 1984), although other investigators have found that platelet serotonin content was not different in hypertensives compared to a normotensive control group (Ahtee et al., 1974). It has been also claimed that platelets from hypertensives show reduced uptake of serotonin (Ahtee et al., 1974; Kamal, Le Quan Bui & Meyer, 1984) although Feltkamp, Meurer & Godehardt, (1984) found no difference in platelet serotonin uptake in hypertensives compared to normotensives. Bhargava et al., (1979) found reduced uptake of serotonin in platelets from hypertensive patients only when very high non-physiological concentrations of serotonin were used; at lower concentrations no differences could be demonstrated in platelets from hypertensive and normotensive subjects. Most of these studies are open to criticism. Many investigators either do not give important descriptive details of their control and hypertensive groups or use control groups which are unmatched for age and sex (Ahtee et al., 1974; Bhargava et al., 1979; Kamal et al., 1984; Baudouin-Legros et al., 1985).

Hypertensives may have enhanced platelet release reaction in response to various stimuli in vivo. It has been claimed that stimulation of the sympathetic nervous

system by isometric handgrip causes an increased release of serotonin from platelets in hypertensives compared to normotensive controls (Palermo et al., 1986). Enhanced platelet release reaction of serotonin might be expected to cause a detectable reduction in platelet serotonin content.

Plasma levels of free serotonin have been reported to be elevated in hypertensives (Biondi, Agostini & Marasini, 1986), in keeping with increased platelet turnover and serotonin release. However free plasma serotonin may not reflect in-vivo platelet aggregation. Even with scrupulous sample preparation, a small amount of serotonin will be released from platelets ex-vivo, resulting in artefactually high "free" plasma serotonin levels (Molyneux & Clarke, 1985). Thus it is unlikely that the so-called "free" plasma serotonin levels that Biondi et al., (1986) measure reflect the in-vivo situation.

In summary, although most studies have claimed reduced platelet serotonin levels, decreased platelet serotonin uptake, and possible increased platelet serotonin release in hypertension, these findings must be seriously questioned in view of the use of inadequate control groups and inherent difficulties with the methodology. It is not clear whether the vascular complications of hypertension, particularly atheroma, affect platelet release or uptake of serotonin. In my study, with carefully chosen controls and accurate measurement of platelet serotonin, there was no evidence to suggest that platelet serotonin is reduced in patients with essential hypertension.

4.2.5 Plasma beta-thromboglobulin.

When care is taken in the collection and preparation of blood samples, plasma beta-thromboglobulin levels are thought to give an index of in-vivo platelet aggregation and platelet content release (Yamanishi et al., 1985). I found no difference in plasma beta-thromboglobulin between patients with untreated essential hypertension and a normotensive control group. Plasma beta thromboglobulin levels in venous blood have been reported to be increased in hypertensives compared to age and sex matched normotensives in some (Bodzenta-Lukasyk et al., 1987; Ikeda et al 1985; Mehta & Mehta, 1981; Kjeldsen et al., 1983; Yamanishi et al., 1985) but not all studies (Petralito et al., 1982; Catalano et al., 1985). Even in those studies which have shown elevated venous beta-thromboglobulin levels in patients with essential hypertension, no significant correlation of beta-thromboglobulin with systolic or diastolic blood pressure has been demonstrated.

Raised venous beta-thromboglobulin levels have been found in patients with peripheral vascular disease (Cella et al., 1979), and hypertensive retinopathy (Petralito et al., 1982), suggesting that underlying vascular disease, rather than the level of arterial blood pressure, may be the cause of raised venous plasma beta-thromboglobulin seen in some of the studies of essential hypertension.

In uncontrolled studies of antihypertensive agents, the alpha-1 adrenoreceptor antagonist prazosin (Ikeda et al.,

1985) and the central sympathomimetic lofexidine (Mehta & Mehta, 1981) appeared to cause a fall in venous beta-thromboglobulin levels in hypertensive patients. However, properly controlled studies of antihypertensive agents with varying modes of action are required before we can be sure that reducing blood pressure in patients with essential hypertension reduces plasma beta-thromboglobulin.

Platelet life span, determined by chromium^{51} radiolabelled autologous platelets, is reduced in patients with severe hypertension uncontrolled by thiazide diuretics (Abrahamsen et al., 1968). Other authors have found reduced platelet life span, assessed by the return of platelet lipid peroxidation capacity after aspirin, in less severe untreated hypertension (Vlachakis & Aledort, 1979). However, in neither of these studies was the control group age and sex matched with the hypertensives.

There was no significant correlation between venous beta-thromboglobulin and platelet serotonin content in normotensive and hypertensive patients. As beta-thromboglobulin and serotonin are released during platelet aggregation (Holmsen, 1977), it might be expected that plasma beta-thromboglobulin should be inversely related to platelet serotonin content. However, serotonin may be released by platelets without full platelet aggregation and release reaction (Ossim & Wylie, 1982; Ossim & Wylie, 1983). Serotonin, contained in the dense granules, may be released from platelets without release of beta-thromboglobulin from platelet alpha granules

(Holmsen, 1977). Thus, it is perhaps not surprising that platelet serotonin levels were not significantly inversely correlated with plasma beta-thromboglobulin.

A direct association between enhanced platelet aggregation in-vivo, as measured by venous plasma beta-thromboglobulin levels, and raised arterial blood pressure remains as yet unproven. The result of my study, showing no significant difference in plasma beta-thromboglobulin between hypertensive patients and a carefully chosen age and sex matched control group, does not support enhanced platelet aggregation in-vivo as a feature of essential hypertension.

4.3 The effects of short-term ketanserin on blood pressure, heart rate, the renin-angiotensin system, adrenocortical function, QT interval and vagal function and the response to infusion of angiotensin II in healthy subjects

4.3.1 Blood pressure.

a) Is the reduction in blood pressure caused by ketanserin due to attenuation of angiotensin II-mediated vasoconstriction?

In isolated blood vessel preparations, serotonin amplifies the vasoconstriction produced by a number of agents, including noradrenaline and angiotensin II. This amplification is inhibited by ketanserin, and other serotonergic antagonists, in correlation with serotonergic type-2 antagonistic activity (Van Nueten et al., 1981; Van Nueten et al., 1982). It has therefore been deduced that serotonergic type-2 antagonism may reduce blood pressure by attenuating the effect of these non-serotonergic vasoconstrictors. However, I have shown that short term administration of the serotonergic type-2 antagonist ketanserin does not attenuate the pressor response to infused angiotensin II in healthy subjects. The timing of observations after short-term ketanserin administration, at around 1 and 7 hours after dosing, was chosen to represent the times of expected approximate peak and trough plasma ketanserin levels (Hedner et al., 1986;

Waller, Tucker & Ramsay, 1987). Subsequently, other investigators have shown that chronic oral ketanserin does not attenuate the pressor response to angiotensin II in patients with essential hypertension (Donnelly et al., 1987). Therefore, it is unlikely that ketanserin reduces blood pressure by inhibiting angiotensin II-mediated vasoconstriction, or that the serotonergic type-2 receptor amplifies angiotensin II mediated vasoconstriction in-vivo.

b) Is the reduction in blood pressure caused by ketanserin due to alpha-1 adrenoreceptor antagonism?

It has been shown that ketanserin, given in the same dose and for the same duration of time as my study, attenuates the pressor response to the alpha-1 agonist phenylephrine in healthy subjects (Zabludowski et al., 1985). Other investigators have shown a similar attenuation of pressor response to alpha-1 agonists with chronic oral ketanserin administration (Fagard et al., 1984; Donnelly et al., 1987) or intravenous infusion of ketanserin (Reimann & Frohlich, 1983). These findings suggest that short term or chronic oral ketanserin causes alpha-1 antagonism, and that this effect may be at least partly responsible for the reduction in blood pressure caused by the drug. However, other explanations for these findings have been suggested. The reduction in blood pressure caused by ketanserin is due to peripheral vasodilatation (Fagard et al., 1984). This vasodilatation

could non-specifically reduce the response to any vasoconstrictor agent. Also, there may be an important interplay or even structural overlap (Marwood & Stokes, 1984) between serotonergic type-2 and alpha-1 receptor sites. In vitro, serotonin amplifies vasoconstriction caused by noradrenaline (Van Nueten et al., 1981). This effect is inhibited by serotonin antagonists in close correlation with their serotonergic type-2 binding affinity, suggesting that it is mediated through the serotonergic type-2 receptor (Janssen, 1985).

In a study directly comparable with that of Zabludowski et al., (1985), I have now demonstrated that ketanserin does not attenuate the blood pressure response to infused angiotensin II, a vasoconstrictor agent largely devoid of alpha adrenergic activity. Although angiotensin II has a peripheral action augmenting vasoconstriction caused by the sympathetic nervous system (Zimmerman, 1980), the main mechanism by which it increases blood pressure is by a direct effect on specific angiotensin II receptors on vascular smooth muscle. Therefore, it is likely that the effect of ketanserin, attenuating the pressor response to alpha-1 agonists represents true alpha-1 adrenoreceptor blockade.

There is other supporting evidence for ketanserin causing alpha-1 blockade in vivo in man; pretreatment with the known competitive alpha-1 antagonist prazosin (compared with a dose of frusemide giving an equivalent blood pressure reduction) diminishes the antihypertensive effect of ketanserin (Wenting et al., 1984). In the

spontaneously hypertensive rat serotonergic type-2 antagonists reduce blood pressure in close correlation with their alpha-1 receptor binding affinity (Cohen et al., 1983).

However it is not possible to explain all of ketanserin's effects by alpha-1 blockade. Prazosin is a good example of a drug that reduces blood pressure by alpha-1 antagonism (Stanaszek et al., 1983). It causes marked attenuation of the pressor response to infused alpha-1 agonists. This effect can be detected even at low doses of infused alpha-1 agonist (Zabludowski et al., 1984). In comparison ketanserin has only a small effect, attenuating the pressor response only at relatively high doses of infused alpha-1 agonist (Donnelly et al., 1987). Furthermore, acute intravenous administration of ketanserin causes a reduction in blood pressure without attenuating the blood pressure response to phenylephrine (Wenting et al 1982 and 1984, Zabludowski et al 1984). It has been shown also that ketanserin will reduce blood pressure in patients with autonomic insufficiency who are unresponsive to the alpha-1 antagonist phentolamine (Wenting et al, 1984). Although pretreatment with prazosin diminishes, it does not completely block the reduction in blood pressure caused by ketanserin (Wenting et al., 1984). Therefore the antihypertensive effects of ketanserin cannot be explained entirely by alpha-1 antagonism. However, it is likely that alpha-1 antagonism contributes to the reduction in blood pressure caused by ketanserin.

c) Is the reduction in blood pressure caused by ketanserin due to serotnergic type-2 antagonism?

Both acute and chronic oral ketanserin attenuate serotonin-induced platelet aggregation in platelet rich plasma (De Clerck & Xhonneux, 1985; Vermylen et al., 1986). As serotonin induced platelet aggregation is thought to be mediated through the type-2 receptor, ketanserin is appropriately designated a serotonergic type-2 antagonist, confirming receptor binding studies (Leysen et al., 1981). However it is not clear what role this serotonergic type-2 antagonism caused by ketanserin plays in mediating the antihypertensive effects of the drug.

d) Ketanserin and first dose hypotension.

One subject experienced marked postural hypotension after the first dose of ketanserin 40mg, associated with a reduction in heart rate. Similar first dose hypotension has been reported with the alpha-1 antagonist prazosin (Stanaszek et al., 1983). Reduced venous return caused by antagonism of alpha-1 adrenoreceptor mediated venoconstriction may contribute to the first dose effects of prazosin (Schapel & Betts, 1981). Venodilatation could stimulate cardiopulmonary receptors, triggering increased vagal outflow, causing bradycardia and hypotension (Parati et al., 1987). In patients with essential hypertension a marked reduction in heart rate may accompany the fall in

blood pressure caused by the first dose of prazosin (Kobrin et al., 1983). The acute reduction in blood pressure caused by prazosin correlates well with the activity of the sympathetic nervous system, measured by plasma noradrenaline (Guthrie & Kotchen, 1983). It is notable that the subject who experienced first dose postural hypotension after ketanserin had a high basal heart rate, and that a marked reduction in standing heart rate accompanied the fall in erect blood pressure. The similarities between this "first dose" effect of ketanserin and those reported after prazosin suggest that this adverse effect of ketanserin was due to alpha-1 adrenoreceptor antagonism. However any drug causing vasodilatation may be capable of occasionally inducing severe hypotension and bradycardia in some individuals.

4.3.2 Heart rate and autonomic function.

a) Effect of ketanserin on heart rate.

The vasodilatation produced by ketanserin (Wenting et al., 1982) should cause an increase in heart rate, due to a combination of reduced parasympathetic tone and increased cardiac sympathetic outflow (Man In'T Veld et al., 1980). However, in our study ketanserin caused a slight decrease in supine heart rate at a time when mean blood pressure was significantly reduced. Other investigators have reported similar findings (Fagard et al., 1984; Zabludowski et al., 1984).

b) Is the reduction in heart rate caused by ketanserin due to alterations of autonomic nervous outflow?

There are several possible explanations for the reduction in heart rate caused by ketanserin. Animal studies suggest that serotonin and serotonergic neurones may be involved in the central regulation of heart rate. Increased brain serotonin attenuates reflex vagal bradycardia caused by intravenous pressor agents (Lin & Chern, 1979; Tadepalli, 1980); diminished brain serotonin has the opposite effect (Lin & Chern, 1979). It is conceivable therefore that central serotonergic type-2 blockade caused by ketanserin could reduce heart rate by augmenting parasympathetic outflow; although ketanserin is not lipid soluble it has been shown to cross the blood-brain barrier (1.13.2). However, I found that there was a tendency for cardiac vagal activity, measured by the heart rate responses to standing, deep breathing and the Valsalva maneouvre (Ewing and Clarke, 1982), to be diminished at around the time of expected peak plasma drug levels (Hedner et al., 1986; Waller, Tucker & Ramsay, 1987), when heart rate and mean arterial pressure were significantly reduced. Although the changes in heart rate responses to lying to standing, deep breathing and the Valsalva maneouvre did not acheive statistical significance, it is possible that the tendency for parasympathetic tone to be reduced represents a normal reflex response of the autonomic nervous system to vasodilatation and reduced arterial pressure (Man In'T

Veld et al., 1980). The results from autonomic function testing certainly do not support increased parasympathetic activity as the mechanism for the reduction in heart rate caused by ketanserin.

Serotonergic antagonists, including methysergide, cyproheptadine (non-selective serotonergic type-1 and -2 blockers) and ketanserin appear to have a central sympatholytic action in baroreceptor denervated cats; central alpha-1 adrenoreceptor blockade with prazosin has a similar effect (M^{C}Call & Schuette, 1984). It is possible that ketanserin could cause a fall in heart rate by reducing sympathetic outflow through central serotonergic type-2 or alpha-1 adrenoreceptor antagonism.

c) Is the reduction in heart rate caused by ketanserin due to a reduction in plasma angiotensin II?

Fagard et al., (1984) found reduced plasma angiotensin II levels after chronic oral ketanserin therapy. This also could cause a reduction in heart rate by influencing autonomic nervous system activity; there is evidence for angiotensin II causing an increase in heart rate by both reducing vagal activity (Lee, Ismay & Lumbers, 1980) and by activation of the sympathoadrenal system (Zimmerman, 1981). However, at a time when heart rate was significantly reduced, I did not observe any reduction in plasma angiotensin II after ketanserin.

d) Effects of ketanserin on the heart rate response to infused angiotensin II and the baroreceptor reflex.

Short term oral ketanserin did not affect the reduction in heart rate caused by the rise in blood pressure during the infusion of angiotensin II. The reduction in heart rate during angiotensin II infusion is less than that caused by equi-pressor doses of other vasoconstrictors such as alpha-1 antagonists. The reasons for this are several. Angiotensin II may itself modify baroreceptor function (Marker, Miles & Scroop, 1980), reduce vagal tone (Lee, Ismay & Lumbers, 1980) and augment sympathetic outflow (Zimmerman, 1980). These effects tend to minimise the reflex reduction in heart rate caused by the rise in blood pressure due to angiotensin II. The possibility that ketanserin causes resetting of baroreceptors has been investigated by studying its effects on changes in heart rate caused by pressor agents and hypotensive drugs. In the spontaneously hypertensive rat, ketanserin potentiates hypotension caused by sodium nitroprusside (Smits, Van Essen & Strukker-Boudier, 1987). This suggests that the antihypertensive effects of ketanserin may be partly due to resetting of the baroreflex. However, in man acute ketanserin administration does not affect the heart rate responses to drug induced hypo- or hypertension (Berdeaux et al., 1987). Chronic oral ketanserin does not alter the heart rate responses to infused alpha-1 agonist or angiotensin II in hypertensive patients (Donnelly et al., 1987). Thus it is unlikely that either the reduction in

heart rate or antihypertensive effects of ketanserin are due to resetting of the baroreflex.

e) Is the reduction in heart rate caused by ketanserin due to a direct effect on the sino-atrial node?

Another possible mechanism for the reduction in heart rate caused by ketanserin is by delaying repolarisation of the sino-atrial node, as seen with some class III antiarrhthmic drugs such as melperone (Millar & Vaughan Williams, 1983). The evidence for ketanserin having class III antiarrhythmic activity is presented in section 4.3.3.

4.3.3 Electrocardiographic QT and QT_C intervals.

Ketanserin prolongs the action potential and effective refractory period in dog purkinje cells and guinea pig papillary muscle (Saman, Thandroyen & Opie, 1985), features typical of a class III antiarrhythmic agent. These effects appear to be mediated through the serotonergic type-2 rather than the alpha-1 adrenoreceptor, as the alpha-1 antagonist prazosin has virtually no effect in the above in-vitro preparations (Saman et al., 1985). I have shown that ketanserin causes significant prolongation of the QT and QT_C intervals in normal man in keeping with the above in vitro data. Similar findings have subsequently been reported in normotensive subjects after acute intravenous ketanserin (Nadamanee et al., 1987) and in hypertensive patients

following chronic oral dosing (Waller, Solomon & Ramsay, 1987; Donnelly et al., 1987). Prolongation of the QT interval is due usually to increased action potential duration in ventricular myocardial cells. This can be caused by class Ia and III antiarrythmics. Class Ia antiarryhthmics, such as quinidine, procainamide and disopyramide, also inhibit the fast sodium channel, decreasing the slope of depolarisation in phase 0 (seen as prolongation of QRS duration on the surface electrocardiogram) and reducing the amplitude of the action potential (Vaughan Williams, 1982). With an electrocardiograph paper speed of 25mm/sec it was not possible to measure accurately QRS duration in my study. However, in-vitro, ketanserin does not affect the slope of depolarisation in ventricular myocardial cells (Saman, Thandroyen & Opie, 1985), or cause prolongation of QRS duration after acute intravenous administration (Nademanee et al., 1987). Thus, it is likely that ketanserin has class III but not Ia antiarrhythmic activity.

There are many potential pitfalls in measuring QT interval. Although the QT interval is thought to represent action potential duration, different directions of impulses during excitation and recovery may cancel each other out on the surface electrocardiogram (Abildskov, 1976; Vaughan Williams, 1982). Furthermore, drugs which do not affect ventricular action potential duration but which cause changes in heart rate will tend to cause alterations of QT interval (inversely proportional to the change in heart rate). To correct for this, it has been advocated

that changes in QT interval should be assessed by calculating regression lines of QT against RR' interval for each subject (Kelman, Whiting and Sumner, 1984), as global corrections of QT for heart rate such as Bazett's formula (QT/square root of the preceeding RR' interval) are inaccurate at low and high heart rates. However, Bazett's formula is reasonably accurate when heart rate is maintained between 60 and 80 beats/minute (Staniforth, 1983; Staniforth, 1984). Therefore, in studies in which changes in heart rate are small, remaining within the above range, calculation of the QT_c interval provides useful information. However, it is important to consider absolute QT interval values, as well as making a correction of QT for heart rate.

The change in QT and QT_c caused by ketanserin could be due to alterations in autonomic nervous outflow. When heart rate is fixed by cardiac pacing, atropine causes a reduction in QT interval in man (Yanowitz, Preston & Abildskov, 1966). However the prolongation of QT interval caused by ketanserin was not accompanied by an increase in cardiac parasympathetic tone. Reduction of sympathetic nervous activity through the right stellate ganglion causes prolongation of the QT interval. This is seen acutely during right radical neck dissection when the sympathetic outflow is disrupted (Otteni et al., 1983). The left side of the sympathetic nervous system has the opposite effect (Yanowitz et al., 1966). Left stellate ganglionectomy has been used as a treatment for prolonged QT interval (Otteni et al., 1983). Changes in

sympathetic nervous outflow cannot be excluded as a possible cause of prolonged QT after ketanserin.

The prologation in QT interval caused by ketanserin was not accompanied by any reduction in serum potassium, a stimulus which is known to prolong the QT interval (Surawicz and Knoebel 1984).

Prolongation of the QT interval may be associated with life threatening cardiac arrhythmias, ventricular tachycardia or polymorphous ventricular tachycardia (torsades de pointes). It is not known if there is a critical threshold for QT interval for ventricular arrhythmias (Surawicz & Knoebel, 1984). Prolongation of the QT interval by 20ms caused by the cholesterol lowering agent probucol did not increase premature ventricular complexes (Browne et al., 1984). However, the combination of the class III antiarrhythmic agent sotalol with hydrochlorthiazide, disopyramide or tricyclic antidepressants has been reported to cause syncope and polymorphous ventricular tachycardia (M^{C}Kibben et al., 1984). The QT_C in these cases was markedly prolonged (>580ms). In view of these findings it seems sensible to advise that ketanserin should not be used in combination with potassium loosing diuretics, or other drugs known to prolong action potential duration.

4.3.4 Effects of ketanserin on the renin-angiotensin-aldosterone system and plasma cortisol.

There is in-vitro evidence that serotonergic type-2 receptors localised on adrenal zona glomerulosa cells may play a role in the regulation of adrenal steroidogenesis. Ketanserin inhibits the increased production of aldosterone and corticosterone caused by serotonin (Williams et al., 1984) and angiotensin II (Rocco et al., 1985) in isolated rat zona glomerulosa cells; this inhibition appears to be mediated through serotonergic type-2 antagonism (1.9). and not by alpha-1 adrenoreceptor antagonism, as the alpha-1 antagonist prazosin does not inhibit serotonin-induced steroidogenesis (Williams et al., 1984). Serotonergic neurones in the central nervous system also may play a role in the regulation of steroidogenesis. The serotonin precursors tryptophan 5-hydroxytryptophan increases plasma aldosterone in healthy subjects (Modlinger et al., 1979; Shenker et al., 1985b); there is good evidence that the rise in plasma aldosterone is mediated centrally as it is potentiated by peripheral decarboxylase inhibition with carbidopa (Shenker et al., 1985b). However, the rise in plasma aldosterone caused by 5-hydroxytryptophan is not affected by pretreatment with either ketanserin or methysergide (Shenker et al., 1985a), suggesting that this effect of 5-hydroxytryptophan is not mediated through serotonergic type-1 or -2 receptor sites. Central serotonergic neurones may also play a role in the

regulation of plasma cortisol (1.9). The centrally acting serotonin agonist M-chlorophenylpiperazine and the serotonin precursor tryptophan increase plasma cortisol in healthy subjects (Modlinger et al., 1979; Mueller et al., 1985). In some circumstances, serotonergic neurones may have a central action in the regulation of adrenocorticotrophic hormone release; ketanserin diminishes the adrenocorticotrophic hormone response to hypoglycaemia in man (Prescott et al., 1984). However the drug does not affect basal plasma levels of adrenocorticotrophic hormone (Gordin, Mustajoki & Pelkonen, 1985). I found that, in-vivo, ketanserin caused no changes to either basal plasma aldosterone, or to the increase of plasma aldosterone in response to infused angiotensin II. Basal plasma cortisol was also unaffected by chronic oral ketanserin. Acute intravenous ketanserin also does not alter plasma cortisol (Gordin et al., 1985). These findings do not support a major role for the serotonergic type-2 receptor in the regulation of plasma aldosterone or cortisol in healthy man. Furthermore, it is unlikely that the reduction in blood pressure caused by ketanserin is due to inhibition of adrenal steroidogenesis.

Serotonin and its precurser tryptophan increase plasma renin in rats (Meyer and Hertting, 1974) and man (Modlinger et al., 1979) respectively. Also, in man, non-selective serotonergic blockade with cyproheptidine inhibits the rise in renin caused by sodium depletion (Epstein and Hamilton, 1977). These effects are thought to

be mediated through central serotonergic neurones. Acute intravenous administration of ketanserin tends to increase plasma noradrenaline, renin and angiotensin II (Reimann et al., 1985; Wenting et al., 1982; Zoccali et al, 1983; Zabludowski et al., 1984). However, these changes are probably due to a reflex increase in sympathetic nervous activity in response to the acute reduction in blood pressure, rather than a specific pharmacological effect of ketanserin. Chronic oral ketanserin has variable effects on basal plasma catecholamines, renin and aldosterone. Fagard et al., (1984) found an increase in plasma noradrenaline and adrenaline, a reduction in plasma renin and angiotensin II, and no change in plasma aldosterone during the chronic treatment of hypertensives with ketanserin. However in a similar study (Woittiez et al., 1986) no changes in basal catecholamines, renin or aldosterone were found. I found that short-term oral ketanserin did not affect basal plasma renin or angiotensin II in normotensive subjects; normal feedback inhibition of renin secretion by infusion of angiotensin II was also maintained. Therefore, it is unlikely that the serotonergic type-2 antagonist ketanserin reduces blood pressure by inhibiting the renin-angiotensin-aldosterone system.

4.4 Effects of the serotonergic type-2 antagonist ritanserin in patients with untreated essential hypertension

4.4.1 Serotonin induced and spontaneous platelet aggregation.

Ritanserin, given in a dose of 10mg twice daily for 4 weeks, caused inhibition of serotonin-induced platelet aggregation in whole blood in patients with untreated essential hypertension. Serotonin-induced platelet aggregation is thought to be mediated through the serotonergic type-2 receptor (1.4.2). Therefore, in keeping with its receptor binding profile (Leysen et al., 1985), ritanserin is an effective serotonergic type-2 antagonist.

Ritanserin had no significant effects on spontaneous platelet aggregation in whole blood. This form of aggregation is thought to be mediated largely through release of adenosine diphosphate from red blood cells (4.1.2). The serotonergic type-2 antagonist ketanserin also does not affect adenosine diphosphate-induced platelet aggregation, in platelet rich plasma (De Clerck et al., 1982; Zannad et al., 1985). Thus it is unlikely that the serotonergic type-2 receptor site plays a role in modulating adenosine diphosphate-mediated platelet aggregation.

4.4.2 Blood pressure.

The highly selective serotonergic type-2 antagonist ritanserin caused no significant change in blood pressure when in single oral doses of 10 and 20mg to patients with essential hypertension. In contrast, ketanserin (40mg), a drug with serotonergic type-2 and alpha-1 antagonistic properties (Leysen et al., 1981), caused a significant reduction in supine mean arterial pressure in the same group of patients. It has been shown that 10mg of ritanserin causes significant alteration of sleep patterns in man (Idzikwski & Mills, 1986). Ritanserin 20mg has been found to increase somnolence in young healthy subjects (Barone et al., 1986). Therefore, the doses of ritanserin which I chose to study have been previously shown to have central effects in man.

There were no significant changes in blood pressure during 4 weeks of ritanserin 10mg twice daily in patients with untreated essential hypertension. Ritanserin had no detectable effects on forearm blood flow or vascular resistance either at rest or after ischaemia. Furthermore, ritanserin did not cause any change in body weight or venous haematocrit suggestive of counter-regulatory responses to vasodilatation. Measurement of serotonin-induced platelet aggregation showed that adequate doses of the drug had been given, causing serotonergic type-2 antagonism (4.4.1). These results, of both acute and chronic dosing with ritanserin, do not support a major independent role for the serotonergic

type-2 receptor in the regulation of peripheral vascular tone and arterial blood pressure in patients with essential hypertension. The conclusions from animal studies are similar. Acute ritanserin administration inhibits the rise in blood pressure caused by serotonin in the pithed rat, but does not attenuate the pressor response to noradrenaline (Conolan, Quinn & Taylor, 1986). In the same animal preparation, ketanserin is less potent in inhibiting serotonin induced vasoconstriction than ritanserin, but does inhibit noradrenaline induced vasoconstriction. In anaesthetised rats, acute ritanserin administration causes only a very small reduction in blood pressure compared to ketanserin (Conolan et al., 1986). In the spontaneously hypertensive rat chronic oral ritanserin, given in a dose that antagonises the pressor response to serotonin, has no effect on basal or stressed blood pressure (Gradin et al., 1985).

Serotonergic antagonists which lower blood pressure include methysergide and ketanserin. Detailed discussion of the mechanism of ketanserin's antihypertensive effects is given in section 4.3.1; it is far from clear that ketanserin reduces blood pressure by its action at the serotonergic type-2 receptor. Methysergide is a non-selective serotonergic type-1 and -2 antagonist with partial agonist activity. It acts centrally, reducing blood pressure by decreasing sympathetic nervous outflow; it has no peripheral effects on the cardiovascular system (Antonaccio & Taylor, 1977).

Ritanserin is the first serotonergic type-2 antagonist

to be studied which is virtually devoid of activity at other receptor sites which play a role in cardiovascular homeostasis (Leysen et al., 1985). Ritanserin differs from ketanserin in that it has greater serotonergic type-2 receptor binding affinity, it is a non-competitive antagonist, and it has a longer duration of action; ritanserin is also more lipophilic than ketanserin, and therefore should penetrate the blood brain barrier more readily (Leysen et al., 1985). It is unlikely that any of these differences explain the contrasting effects of ritanserin and ketanserin on blood pressure. However, ritanserin is virtually devoid of alpha-1 receptor binding affinity compared to ketanserin (Leysen et al., 1985). This difference probably explains the lack of effect of ritanserin on blood pressure compared to ketanserin, both in patients with essential hypertension and in the rat.

4.4.3 Heart rate.

Serotonergic neurones may be involved in the regulation of heart rate (1.6). In animal studies, serotonin has a central action, increasing heart rate by inhibiting reflex bradycardia (Lin & Chern, 1979; Tadepalli, 1980). Therefore, serotonergic type-2 antagonists might cause a reduction in heart rate. Although ketanserin causes a reduction in heart rate, it is not clear that this effect is due to serotonergic type-2 antagonism; it is possible that ketanserin reduces heart rate by central alpha-1 adrenoreceptor antagonism (4.3.2). In contrast to

ketanserin, the serotonergic type-2 antagonist ritanserin is virtually devoid of alpha-1 adrenoreceptor binding affinity (Leysen et al., 1985). There were no significant alterations in heart rate in patients with essential hypertension after either acute or chronic oral dosing with ritanserin. These findings do not support a major independent role for the serotonergic type-2 receptor in the regulation of heart rate in man.

4.4.4 Venous compliance.

Serotonin causes constriction of large veins in-vitro (Fenuik et al., 1985; Victorzon, Tapparelli & Muller-Schweinitzer, 1986). Serotonin-induced venoconstriction appears to be mediated through both serotonergic type-1 and -2-like receptors. The serotonergic type-1 receptor agonist 5-carboxamidotryptamine causes contraction of isolated human and dog saphenous veins (Fenuik et al., 1985; Victorzon et al., 1986). The serotonergic type-2 antagonists ketanserin and spiperone cause only partial antagonism of serotonin induced venoconstriction in the same preparations. However the situation may be more complex than this. Serotonin and serotonin type-1 agonists both inhibit noradrenaline release from sympathetic nerves in human saphenous vein (Gothert et al., 1986). This suggests that serotonin may act through a serotonergic type-1-like receptor to inhibit the venoconstrictor effects of the sympathetic nervous system.

It has not been clearly established that serotonin plays a role in the regulation of venous tone in-vivo. I have shown that forearm venous compliance in patients with essential hypertension was not significantly altered by chronic administration of the serotonergic type-2 antagonist ritanserin. This does not support a major independent role for the serotonergic type-2 receptor in the regulation of venous tone in man.

4.4.5 Electrocardiographic QRS and QT intervals.

Chronic oral ritanserin caused prolongation of the QT_c interval without affecting the QRS duration, features consistent with class III antiarrhythmic activity (Millar & Vaughan-Williams, 1983). Hypokalaemia can cause prolongation of the action potential duration, measured as an increase in QT interval on the electrocardiograph (Surawicz & Knoebel, 1984). However, the prolongation of QT_c interval caused by ritanserin was not accompanied by any reduction in serum potassium. The prolongation of QT_c interval was very similar to that caused by ketanserin (4.3.3). Both of these drugs are serotonergic type-2 antagonists, suggesting that serotonergic type-2 antagonism is the mechanism by which ketanserin and ritanserin prolong QT_c. Further studies are required to determine whether the electrophysiological effects of ritanserin are due to a direct effect on the heart as with ketanserin (4.3.3), and whether the degree of QT_c prolongation is dose dependent. The significance of

increased QT_C interval duration, and pitfalls in interpretation are discussed in section 4.3.3 .

4.4.6 Psychological function.

Serotonin precursors and serotonin agonists that cross the blood brain barrier can cause anxiety and depression, effects mediated through the serotonergic type-2 receptor (Leysen, 1984). Therefore, there is interest in the possible effects of serotonergic type-2 antagonists as anxiolytics. The serotonergic type-2 antagonist ritanserin is lipid soluble and readily crosses the blood brain barrier. I found no significant changes in hostility, anxiety or depression scores, measured on the Multiple Affect Adjective Check List, or alertness and tranquillity, measured by visual analogue scales, after single oral doses of ritanserin were given to patients with essential hypertension. Ritanserin in an acute oral dose of up to 20mg has been shown to be well tolerated in young healthy subjects, with no significant changes in psychological function found on a self-reporting symptom questionnaire (Barone et al., 1986). Ritanserin 10mg daily causes significant increase in slow wave sleep, at 1 and 14 days in healthy subjects (Idzikwski & Mills, 1986). I found no evidence of day-time sedation, measured by visual analogue scale and digit substitution test, or change in mood, measured by visual analogue scales, after chronic oral ritanserin administration to patients with essential hypertension. Therefore, serotonergic type-2 antagonism

does not seem to cause significant sedation or clinically important changes in mood in patients with normal psychological function. However, it is possible that quite different psychological effects might be seen after ritanserin in patients who present with mood disorder such as anxiety or depression. It has been shown that ritanserin 10mg daily for 2 weeks reduces symptoms in patients with anxiety disorders (Ceulemans et al., 1985).

4.5 Effects of the serotonergic type-2 antagonist ketanserin in adult atopic asthma

A single oral dose of ketanserin 40mg did not affect significantly resting bronchomotor tone in adult atopic asthmatic patients. Although there was a tendency for ketanserin to attenuate the reduction in forced expiratory volume and forced vital capacity caused by exercise, 95% confidence limits show that it is unlikely that the drug has any major acute effect in preventing exercise induced bronchoconstriction in adult atopic asthmatic patients. The drug was given in a dose which has been shown to inhibit serotonin induced platelet aggregation (De Clerck & Xhonneux, 1985). In an open study, other investigators found no significant change in resting bronchomotor tone or attenuation of exercise induced asthma in 7 patients given 10mg ketanserin intravenously (So, Lam & Kwan, 1985). In contrast, patients with chronic obstructive airways disease have been shown to increase their FEV_1 after intravenous ketanserin; patients with the most severe disease appeared to recieve the most benefit (Cazzola et al., 1987).

In exercise induced asthma the maximal reduction in airways function occurs within 15 minutes of exercise (Rubinstein et al., 1987). Bronchoconstriction after exercise is due to decreased mucosal temperature, caused by heat and water loss to air which has not been fully warmed and humidified during its passage into the airways (Strauss et al., 1978; Deal et al., 1979). The site of

airways obstruction in exercise induced asthma is heterogeneous in terms of small or large airways (M^{C}Fadden et al., 1977).

Ketanserin has both serotonergic type-2 and alpha-1 adrenoreceptor antagonist activity (4.3.1). Inhalation of the alpha-1 antagonist prazosin has no effect on airways resistance in asthma (Barnes, Ind & Dollery, 1981). Similarly, inhalation of the alpha-1 agonist phenylephrine has no effect on airways function in asthmatic subjects pretreated with an inhaled beta-blocker and atropine (Thomson, Daniel & Hargreave, 1982). Thus the alpha-1 adrenoreceptor probably does not play a major role in the regulation of airway function in asthma in man.

Serotonin is present in large quantities in the dense granules of platelets (Da Prada & Pletscher, 1969) and during platelet aggregation this potential bronchoconstrictor is released. Platelet aggregation has been claimed to be directly involved in mechanisms of both allergen (Knauer et al., 1981) and exercise induced asthma in man (Johnson et al., 1984), using elevated levels of platelet factor 4 as a marker of aggregation. However other workers (Durham et al., 1984) have measured circulating concentrations of both beta-thromboglobulin and platelet factor 4 after exercise, allergen and methacholine induced bronchoconstriction, and could not find evidence of enhanced platelet aggregation. Although platelet activating factor causes bronchoconstriction in the guinea pig, associated with accumulation of platelets in the pulmonary vasculature, other platelet aggregating

agents such as adenosine diphosphate and thrombin do not cause bronchoconstriction despite similar platelet accumulation in the lungs (Robertson & Page, 1987). It is not firmly established that enhanced platelet aggregation causes bronchoconstriction, or that exercise or allergen induced bronchoconstriction are associated with enhanced platelet aggregation.

Animal studies suggest that serotonin can cause bronchoconstriction by three separate mechanisms (1.10); by a direct action on serotonergic type-2 receptors in respiratory smooth muscle (Cohen et al., 1985; Colebatch, Olsen & Nadel, 1986), by potentiating the action of the vagus (Sheller et al., 1982), and by inhibition of the nonadrenergic-noncholinergic neural system (Bai, Macklem & Martin, 1986). How ever, the bronchoconstrictor effects of serotonin demonstrated in these animal models may result from the use of supraphysiological concentrations of the monoamine; although large amounts of serotonin circulate stored in platelets, very small quantities are likely to be present in the free state in plasma (Molyneux & Clarke, 1985). Furthermore, there may be differences in response between species. Indeed, inhaled serotonin has no effect on pulmonary function in healthy subjects or adult asthmatics, even when administered in supraphysiological concentrations (Tonnesen, 1985; Cushley, Lee & Holgate, 1986).

The asthmatic patients showed no significant reduction in sitting blood pressure after ketanserin 40mg, but this is not an unexpected response to any antihypertensive

agent given to normotensive subjects. Ketanserin did not affect the pressor response or rise in heart rate caused by exercise. In contrast, chronic oral ketanserin attenuates the rise in systolic blood pressure and heart rate in patients with essential hypertension (Hedner et al., 1987).

The lack of any major effect of ketanserin on airways function suggests that serotonin is unlikely to be an important mediator affecting resting bronchomotor tone or causing exercise induced bronchoconstriction in adult atopic asthma, although a small beneficial effect of ketanserin on exercise-induced bronchoconstriction could not be excluded. Further studies of chronic dosage and inhaled serotonergic antagonists are required to clarify this issue. While oral ketanserin does not appear to have immediate clinically relevant beneficial effects in adult atopic asthma, it may prove useful as a safe alternative antihypertensive agent in patients with this condition.

References

1) Abildskov, J.A. (1976) Adrenergic effects on the QT interval of the electrocardiogram. American Heart Journal, **92**, 210-216.

2) Abrahamsen, A.F. (1968) Platelet survival studies in man with special reference to thrombosis and atherosclerosis. Scandinavian Journal of Haematology, **Suppl 3**, 7-53.

3) Adair, J.R., Hamilton, B.L., Scappaticci, K.A., Helke, C.J. & Gillis, R.A. (1977) Cardiovascular responses to electrical stimulation of the medullary raphe area of the cat. Brain Research, **128**, 141-145.

4) Ahtee, L., Pentikainen, L., Pentikainen, P.J. & Paasonen, M.K. (1974) 5-hydroxytryptamine in the blood platelets of cirrhotic and hypertensive patients. Experentia, **30**, 1328-1329.

5) Anderson, G.M., Young, J.G. & Batter, D.K. (1981) Determination of indoles and catechols in rat brain ans pineal using liquid chromatography with fluorometric and amperometric detection. Journal of Chromatography, **223**, 315-320.

6) Antonaccio, M.J. & Taylor, D.J. (1977) Reduction in blood pressure, sympathetic nerve discharge and centrally evoked pressor responses by methysergide in anesthetised cats. European Journal of Pharmacology, **42**, 331-338.

7) Artigas, F., Sarrias, M.J., Martinez, E. & Gelpi, E. (1985) Serotonin in body fluids: Characterization of human plasmatic and cerebrospinal fluid pools by means of a new

HPLC method. Life Sciences, **37**, 441-447.

8) Bai, T.R., Macklem, P.T., Martin, J.G. (1986) The effects of parasympathectomy on serotonin-induced bronchconstriction in the cat. American Review of Respiratory Diseases, **133**, 110-115.

9) Barendsen, G.J., Venema, H. & Van Den Berg, Jw. (1971) Semicontinuous blood flow measurement by triggered venous occlusion plethysmography. Journal of Applied Physiology, **31**, 288-291.

10) Barnes, P.J., Ind, P. & Dollery, C.T. (1981) Inhaled prazosin in asthma. Thorax, **36**, 378-381.

11) Barone, J.A., Bierman, R.H., Cornish, J.W., Hsuan, A., Drake, N.D. & Collaizi, J.L. (1986) Safety evaluation of ritanserin - an investigational serotonin antagonist. Drug Intelligence and Clinical Pharmacy, **20**, 770-775.

12) Baudouin-Legros, M., Le Quan-Bui, K.H., Guicheney, P., Kamal, L.A. & Meyer, P. (1985) Platelet serotonin in essential hypertension and in menta depression. Journal of Cardiovascular Pharmacology, **7(suppl 7)**, S12-S14.

13) Baum, T. & Shropshire, A.T. (1975) Inhibition of efferent sympathetic nerve activity by 5-hydroxytryptophan and centrally administered 5-hydroxytryptamine. Neuropharmacology, **14**, 227-223.

14) Baumann, P. (1985) Transport systems and enzymes involveed in the metamorphosis of tryptophan into serotonin. Pharmacopsychiatry, **18**, 188-192.

15) Bazett, H.C. (1920) An analysis of the time relations of electrocardiograms. Heart, **7**, 353-370.

16) Berdeaux, A., Edouard, A., Samii, K. & Giudicelli,

J.-F. (1987) Ketanserin and the arterial baroreceptor reflex in normotensive subjects. European Journal of Clinical Pharmacology, **32**, 27-33.

17) Bhargava, K.P. & Tangri, K.K. (1959) The central vasomotor effects of 5-hydroxytryptamine. British Journal of Pharmacology and Chemotherapy, **14**, 411-414.

18) Bhargava, K.P., Raina, N., Misra, N., Shanker, K. & Vrat, S. (1979) Uptake of serotonin by human platelets and its relevance to CNS involvement in hypertension. Life Sciences, **25**, 195-200.

19) Biondi, M.L., Agostini, A. & Marasini, B. (1986) Serotonin levels in hypertension. Journal of Hypertension, **4(Suppl 1)**, S39-S41.

20) Blackwood, G.J., Flower, R.J., Russell-Smith, N., Salmon, J.A., Thorogood, P.B. & Vane, J.R. (1978) Prostacyclin is produced in whole blood. British Journal of Pharmacology, **64**, 436p.

21) Blum, J.J. & Ling, N.S. (1959) Oxidation of serotonin and 5-hydroxyindoles during the denaturation of oxyhaemoglobin. Biochemical Journal, **73**, 530-535

22) Bodzenta-Lukasyk, A., Krupinski, K. & Bielawiec, M. (1987) Platelet function and kallikrein system in patients with essential hypertension. Thrombosis and Haemostasis (Stuttgart), **1**, 398.

23) Borst, C., Van Brederode, F.M., Wieling, W., Van Montfrans, G.A. & Dunning, A.J. (1984) Mechanisms of initial blood pressure response to postural change. Clinical Science, **67**, 321-327.

24) Bouchard, S. & Roberge, A.G. (1979) Biochemical

properties and kinetic parameters of dihydroxyphenylalanine-5- hydroxytryptophan decarboxylase in brain, liver and adrenals of cat. Canadian Journal of Biochemistry, **57**, 1014-1018.

25) Boushey, H.A. Jr. & Dawson, A. (1982) Spirometry and flow-volume curves. In Pulmonary Function Testing, Guidelines and Controversies, Equipment Guidelines and Normal Values, ed. Clausen, J.L. & Zarins, L.P. **Ch. 7**, pp 61-82, New York: Academic Press.

26) Bradley, P.B., Humphrey, P.P.A. & Williams, R.H. (1986) Evidence for the existence of 5-hydroxytryptamine receptors, wich are not of the 5-HT_2 type, mediating contraction of rabbit isolated basilar artery. British Journal of Pharmacology, **87**, 3-4.

27) Brown, K.F., Prystowski, E.N., Heger, J.J. Cerimele, B.J., Fineberg, N. & Zipes, D.P. (1984) Prolongation of the QT interval induced by probucol: Demonstration of a method for determining QT interval change induced by a drug. American Heart Journal, **107**, 680-684.

28) Bruni, J.F., Hawkins, R.L. & Yen, S.S.C. (1982) Serotonergic mechanism in the control of beta-endorphin and ACTH release in male rats. Life Sciences, **30**, 1247-1254.

29) Buccino, R.A., Covell, J.W., Sonnenblick, E.H. & Braunwald, E. (1967) Effects of serotonin on the contractile state of the myocardium. American Journal of Physiology, **213**, 483-486.

30) Buchheit, K.H., Engel, G., Mutscher, E. & Richardson, B. (1985) Study of the contractile effect of

5-hydroxytryptamine (5-HT) in the isolated longitudinal muscle strip from guinea-pig ileum. Evidence for two distinct release mechanisms. Naunyn-Schmiedeberg's Archives Pharmacology, **329**, 36-41.

31) Catalano, M., Russo, V., Belletti, S., & Libretti, A. (1985) BTG and PF_4 values before and after effort in patients treated with ketanserin. Thrombosis and Haemostasis (Stuttgart), **54**, 300.

32) Cazzola, M., D'Amato, G., Lobefalo, G., Guillaro, B., Sepe, J., Assogna, G., Pietroletti, R. & Lauria, D. (1987) Ketanserin, a new blocking agent of serotonin S_2-receptors. Respiratory functional effects in chronic obstruction of the airways. Chest, **92**, 863-866.

33) Cella, G., Zahavi, J., de Haas, H.A. & Kakkar, V.V. (1979) b-thromboglobulin, platelet production time and platelet function in vascular disease. British Journal of Haematology, **43**, 127-136.

34) Ceulemans, D.L.S., Hoppenbrouwers, M.-L.J.A., Gelders, Y.G. & Reyntjens, A.J.M. (1985) The influence of ritanserin, a serotonin antagonist, in anxiety disorders: a double-blind placebo-controlled study verses lorazepam. Pharmacopsychiatry, **18**, 303-305.

35) Cho, C.H. & Ogle, C.W. (1986) The inhibitory action of 5-hydroxytryptamine on gastric secretory function in rats. British Journal of Pharmacology, **87**, 371-377.

36) Coccheri, S. & Fiorentini, P. (1971) Platelet adhesiveness and aggregation in hypertensive patients. Acta Medica Scandinavica, **Suppl 525**, 273-275.

37) Cohen, M.L., Fuller, R.W. & Wiley, K.S. (1981)

Evidence for 5-HT_2 receptors mediating contraction invascular smooth muscle. Journal of Pharmacology and Experimental Therapeutics, **218**, 421-425.

38) Cohen, M.L., Fuller, R.W. & Kurz, K.D. (1983) Evidence that blood pressure reduction by serotonin antagonists is related to alpha receptor blockade in spontaneously hypertensive rats. Hypertension, 5, 676-681.

39) Cohen, M.L., Schenck, K.W., Colbert, W. & Wittenauer, L. (1985) Role of 5-HT_2 receptors in serotonin induced contractions of non-vascular smooth muscle. Journal of Pharmacology and Experimental Therapeutics, **232**, 770-774.

40) Cohen, R.A. & Vanhoutte, P.M. (1985) Platelets, serotonin and endothelial cells. In Serotonin and the cardiovascular system, ed. Vanhoutte, P.M., pp 105-112, New York: Raven Press.

41) Colebatch, H.J.H., Olsen, C.R. & Nadel, J.A. (1966) Effect of histamine, serotonin and acetylcholine on the peripheral airways. Journal of Applied Physiology, **21**, 217-226.

42) Colpaert, F.C., Meert, T.F., Niemegeers, C.J.E. & Janssen, P.A.J. (1985) Behavioural and 5-HT antagonist effects of ritanserin: a pure and selective antagonist of LSD discrimination in the rat. Psychopharmacology, **86**, 45-54.

43) Conolan, S., Quinn, M.J. & Taylor, D.A. (1986) In vivo and in vitro activity of selective 5-hydroxytryptamine$_2$ receptor antagonists. British Journal of Pharmacology, **89**, 129-135.

44) Coote, J.H. & Macleod, V.H. (1974) The influence of bulbospinal monoaminergic pathways on sympathetic nerve activity. Journal of Physiology (London), **241**, 453-475.

45) Culley, W.J., Saunders, R.N., Mertz, E.T. & Jolly, D.H. (1963) Effect of a tryptophan deficient dieton brain serotonin and plasma tryptophan level. Proceedings of the Society of Experimental Biology and Medicine, **113**, 645-648.

46) Cushley, M.J., Lee, W.H. & Holgate, S.T. (1986) The effect of inhaled 5-hydroxytryptamine (5-HT, serotonin) on airway calibre in man. British Journal of clinical Pharmacology, **22**, 487-490.

47) Dalton, D.W. (1986) The cardiovascular effects of centrally administered 5-hydroxytryptamine in the concious normotensive and hypetensive rat. Journal of Autonomic Pharmacology, **6**, 67-75.

48) Da Prada, M. & Pletscher, A. (1969) Different localisation of reserpine and tyramine within the 5-hydroxytryptamine organelles of blood platelets. Experentia, **25**, 923-924.

49) Davies, C.L. (1983) Determination of ketanserin in plasma by reversed phase high performance chromatography. Journal of Chromatography, **275**, 232-233.

50) Deal, E.C. Jr., M^{C}Fadden, E.R. Jr., Ingram, R.H. Jr. & Jaeger, J.J. (1979) Hypernea and heat flux. The initial reaction sequence in exercise-induced asthma. Journal of Applied Physiology, **46**, 476-483.

51) De Clerck, F., David, J-L. & Janssen, P.A.J. (1982) Inhibition of 5-hydroxytryptamine-induced and amplified

human platelet aggregation by ketanserin (R 41 468), a selective 5-HT_2-receptor antagonist. Agents and Actions, **12**, 388-397.

52) De Clerck, F., Van Nueten, J.M. & Reneman, R.S. (1984) Platelet-vessel wall interactions: implications of 5-hydroxytryptamine. A review. Agents and Actions, **15**, 616-626.

53) De Clerck, F. & Xhonneux, B. (1985) Continuous inhibition of platelet S_2-serotonergic receptors during chronic administration of ketanserin in man. Journal of Cardiovascular Pharmacology, **7(suppl 7)**, S23-S25.

54) De Clerck, F. (1986) Review. Blood platelets in human essential hypertension. Agents and Actions, **18**, 563-580.

55) de Courcelles, D.de C., Leysen, J.E., De Clerck, F., Van Belle, H. & Janssen, P.A.J. (1985) Evidence that phospholipid turnover is the signal transducing system coupled to serotonin-S_2 receptor sites. Journal of Biological Chemistry, **260**, 7603-7608.

56) de Courcelles, D.de C., Roevens, P., Wynants, J. & Van Belle, H. (1987) Serotonin-induced alterations in inositol phospholipid metabolism. Biochimica et Biophysica Acta, **927**, 291-303.

57) De Crée, J., Geukens, H., Leempoels, J. & Verhaegen, H. (1985a) The effects of ketanserin, a 5-HT_2 receptor antagonist on the impaired blood cell filtration in patients with acute myocardial infarction. Clinical Hemorheology, **5**, 949-957.

58) De Crée, J., Leempoels, J., Demoen, B., Roels, V. &

Verhaegen, H. (1985b) Effect of ketanserin on the hyperreactivity of platelets to 5-hydroxytryptamine in patients with cardiovascular diseases. Journal of Cardiovascular Pharmacology, **7(suppl 7)**, S26-S28.

59) Dewar, H.A., Marshall, T., Weightman, D., Prakash, V. & Boon, P.J. (1979) Beta-thromboglobulin in ante-cubital vein blood - the influence of age, sex and blood group. Thrombosis and Haemostasis (Stuttgart), **42**, 1159-1163.

60) d'Inverno, E. (1980) Clinical use of plethysmography in the diagnosis of deep venous thrombosis and arterial occlusive disease. Angiology, **31**, 437-447.

61) Donnelly, R., Elliot, H.L., Meridith, P.A., Reid, J.L. (1987) Acute and chronic ketanserin in essential hypertension: antihypertensive mechanisms and pharmokinetics. British Journal of clinical Pharmacology, **24**, 599-606.

62) Dormandy, J.A. (1987) Serotonin and haemorrheology. International Journal of Cardiology, **14**, 213-219.

63) Dunkley, B., Sanghri, I., Friedman, E. & Gershon, S. (1972) Comparison of behavioural and cardiovascular effects of L-DOPA and 5-HTP in concious dogs. Psychopharmalogia (Berlin), **26**, 161-172.

64) Durham, S.R., Dawes, J., Pepper, D.S., Lee, T.H. & Kay, A.B. (1984) Platelet function in exerise and allergen induced asthma. Thorax, **39**, 707.

65) Dusterdieck, G. & McElwee, G. (1971) Estimation of angiotensin II concentration in human plasma by radio-immunoassay. An improved method and its application

to some physiological and clinical states. European Journal of Clinical Investigation, 2, 32-38.

66) Eckberg, D.L. (1980) Parasympathetic cardiovascular control in human disease: a critical review of methods and results. American Journal of Physiology, 239, H581-H593.

67) Emson, P.C., Gilbert, R.T.F., Martensson, H. & Nobin, A. (1984) Elevated concentrations of substance P and 5HT in plasma in patients with carcinoid tumours. Cancer, 54, 715-718.

68) Engbaek, F. & Voldby, B. (1982) Radio-immunoassay of serotonin (5-hydroxytryptamine) in cerebrospinal fluid, plasma, and serum. Clinical Chemistry, 28, 624-628.

69) Englund, N., Hallbrook, T. & Ling, L.G. (1972) The value of strain guage plethysmography. Journal of Clinical and Laboratory Investigation, 29, 155-158.

70) Epstein, S. & Hamilton, S. (1977) Cyproheptidine inhibition of stimulated plasma renin activity. Journal of Clinical Endocrinology and Metabolism, 45, 1235-1237.

71) Erspamer, V. & Asero, B. (1952) Identification of enteramine, the specific hormone of the enterochromaffin cell system, as 5-hydroxytryptamine. Nature (London), 169, 800-801.

72) Erspamer, V. (1954) Quantitative estimation of 5-HT in gastrointestinal tract, spleen and blood of vertebrates. In Ciba Symposium on Hypertension, ed. Wolstenholme, G. & Cameron, P., pp 78-84, London: Churchill.

73) Erwin V.G. & Deitrich, R.A. (1966) Brain aldehyde dehydrogenase. Localisation, purification and properties.

Journal of Biological Chemistry, **241**, 3533-3539.

74) Ewing, D.J., Campbell, I.W., Murray, A., Neilson, J.M.M. & Clarke, B.F. (1978) Immediate heart rate response to standing. British Medical Journal, **1**, 145-147.

75) Ewing, D.J., Hume, L., Campbell, I.W., Murray, A., Neilson, J.M.M. & Clarke, B.F. (1980) Autonomic mechanisms in the initial heart rate response to standing. Journal of Applied Physiology, **49**, 809-814.

76) Ewing, D.J. & Clarke, B.F. (1982) Diagnosis and management of diabetic autonomic neuropathy. British Medical Journal, **285**, 916-917.

77) Fagard, R., Fiocchi, R., Lijnen, P., Staessen, J., Moerman, E., De Schaepdriver, A. & Amery, A. (1984) Haemodynamic and humeral responses to chronic ketanserin treatment in essential hypertension. British Heart Journal, **51**, 149-156.

78) Feltkamp, H., Meurer, K.A. & Godehardt, E. (1984) Tryptophan-induced lowering of blood pressure and changes of serotonin uptake by platelets in patients with essential hypertension. Klinische Wochenschrift, **62**, 1115-1119.

79) Fenuik, W., Humphrey, P.P.A. & Watts, A.D. (1983) 5-hydroxytryptamine-induced relaxation of isolated mammalian smooth muscle. European Journal of Pharmacology, **96**, 71-78.

80) Fenuik, W., Humphrey, P.P.A., Perren, M.J. & Watts, A.D. (1985) A comparison of 5-hydroxytryptamine receptors mediating contraction in rabbit aorta and dog saphenous vein: evidence for different receptor types obtained by

use of selective agonists and antagonists. British Journal of Pharmacology, **86**, 697-704.

81) Florez, J. & Armijo, J.A. (1974) Effect of central inhibition of the 1-aminoacid decarboxylase on the hypotensive action of 5-HT precursors in cats. European Journal of Pharmacology, **26**, 108-110.

82) Forsberg, E.J. & Miller, R.J. (1983) Regulation of serotonin release from rabbit intestinal enterochromaffin cells. Journal of Pharmacology and Experimental Therapeutics, **227**, 755-766.

83) Fozard, J.R. (1984) Neuronal 5-HT receptors in the periphery. Neuropharmacology, **23**, 1473-1486.

84) Freed, C.R., Echizen, H. & Bhaskaran, D. (1985) V. Brain serotonin and blood pressure regulation: studies using in vivo electrochemistry and direct tissue assay. Life Sciences, **37**, 1783-1793.

85) Frenken, M. & Kaumann, A.J. (1984) Interaction of ketanserin and its metabolite ketanserinol with $5HT_2$ receptors in pulmonary and coronary arteries of calf. Naunyn Schmiedeberg's Archives of Pharmacology, **326**, 334-339.

86) Fuller, R.W. & Snoddy, H.D. (1983) Antagonism of the quipazine-induced elevation of serum corticosterone in rats by ketanserin, pirenperone and other antagonists of $5HT_2$ receptors. Federal Proceedings, **42**, 459.

87) Fuller, R.W. & Snoddy, H.D. (1984) Central serotonin antagonist activity of ketanserin. Research Communications in Chemical Pathology and Physiology, **46**, 151-154.

88) Gaarder, A., Jonssen, J., Lalands, J., Hellem, A. &

Owren P.A. (1961) Adenosine diphosphate in red cells as a factor in adhesiveness of human blood platelets. Nature, **192**, 531-532.

89) Gaddum, J.H. & Picarelli, Z.P. (1957) Two kinds of tryptamine receptors. British Journal of Pharmacology, **12**, 323-328.

90) Gardner, M.J. & Altman, D.J. (1986) Statistics in medicine: confidence intervals rather than P values: estimation rather than hypothesis testing. British Medical Journal, **292**, 746-750.

91) Geeraerts, F., Schimpfessel, L. & Crockaert, R. (1974) A simple routine method to preserve and determine blood serotonin. Specialia, **30**, 837.

92) Gershon, M.D. & Tamir, H. (1984) Serotonectin and the family of proteins that bind to serotonin. Biochemical Pharmacology, **33**, 3115-3118.

93) Gibbs, D.M. & Vale, W. (1983). Serotonergic modulation of corticosterone releasing factor and vasopressin secretion into hypophysial portal blood. Society of Neurosciences Abstracts, **9**, 703.

94) Gillis, C.N. & Pitt, B.R. (1982) The fate of circulating amines within the pulmonary circulation. Annual Reviews of Physiology, **44**, 269-281.

95) Goodwin, G.M. & Green, A.R. (1985) 5HT agonists and behaviour. British Journal of Pharmacology, 743-753.

96) Gordin, A., Mustajoki, P. & Pelkonen, R. (1985) Ketanserin without effects on basal anterior pituitary hormone secretion in healthy subjects. Journal of Endocrinological Investigation, **8**, 73-75.

97) Gothert, M. & Klupp, N. (1978) Cardiovascular effects of neurotoxic indolethylamines. Annals of the New York Academy of Sciences, **305**, 457.

98) Gothert, M., Kollecker, P., Rohm, N. & Zerkowski, H.-R. (1986) Inhibitory presynaptic 5-hydroxytryptamine (5-HT) receptors on the sympathetic nerves of the human saphenous vein. Naunyn Schmiedeberg's Archives of Pharmacology, **332**, 317-323.

99) Gothert, M. & Schlicker, E. (1987) Classification of serotonin receptors. Journal of Cardiovascular Pharmacology, **10(suppl 3)**, S3-S7.

100) Gradin, K., Pettersson, A., Hedner, T. & Persson, B. (1985) Chronic 5-HT_2 receptor blockade with ritanserin does not reduce blood pressure in the spontaneously hypertensive rat. Journal of Neural Transmission, **64**, 145-149.

101) Gross, M.D., Grekin, R.J., Gniadek, T.C., Villareal, J.Z. (1981) Suppression of aldosterone by cyproheptidine in idiopathic aldosteronism. New England Journal of Medicine, **305**, 181-185.

102) Guthrie, C.P. & Kotchen, T.A. (1983) Effects of prazosin and clonidine on sympathetic and baroreflex function in patients with essential hypertension. Journal of Clinical Pharmacology, **23**, 348-354.

103) Hardisty, R.M. & Stacey, R.S. (1955) 5-Hydroxytryptamine in normal human platelet. Journal of Physiology (London), **130**, 711-720.

104) Haver, V.M. & Gear, A.R.L. (1982) Functional fractionation of platelets: Aggregation kinetics and

glycoprotein labelling of differing platelet populations. Thrombosis and Haemostasis (Stuttgart), **48**, 211-216.

105) Hedner T., Pettersson, A. & Persson, B. (1986) Blood pressure reduction and pharmokinetics of ketanserin in hypertensive patients. Journal of Hypertension, **4(Suppl 1)**, S91-S93.

106) Hedner, T., Andersson, O.K., Pettersson, A., Persson, B. (1987) Cardiovascular effects of ketanserin during cold pressure and during isometric and dynamic exercise in hypertensive patients. Journal of Cardiovascular Pharmacology, **10(Suppl 3)**, S73-S77.

107) Helke, C.J., Souza, J.D., Hamilton, B.L., Morgenroth, V.H. & Gillis, R.A. (1976) Evidence for a role of central serotonergic neurones in digitalis-induced cardiac arrhthmias. Nature, **263**, 246-248.

108) Helke, C.J., Quest, J.A. & Gillis, R.A. (1978) Effects of serotonin antagonists on digitalis induced arrhthmias. European Journal of Pharmacology, **47**, 443-449.

109) Herbert, M., Johns, M.W. & Dore, C. (1976) Factor analysis of anologue scales measuring subjective feelings before and after sleep. British Journal of Medical Psychology, **49**, 373-379.

110) Holmes, M.C., Di Renzo, G., Beckford, U., Gillham, B. & Jones, M.T. (1982) Role of serotonin in the control of secretion of corticotrophin releasing factor. Journal of Endocrinology, **93**, 151-160.

111) Holmsen, H. (1977) Prostaglandin endoperoxide-thromboxane synthesis and dense granule secretion as positive feedback loops in the propagation of platelet

response during the basic platelet reaction. Thrombosis and Haemostasis (Stuttgart), **38**, 1030-1041.

112) Holmsen, H. & Karpatkin, S. (1983) Metabolism of platelets. In Haematology, ed. Williams, W.J., Beutler, E., Erslev, A.J. & Lichtman, M.A., pp 1149-1176, New York: M^{C}Graw-Hill.

113) Idzikwski, C. & Mills, F.J. (1986) 5-HT$_2$ antagonist causes sustained increase in human slow wave sleep. Clinical Science, **70(suppl 13)**, 89P.

114) Ikeda, T., Nonaka, Y., Goto, A. & Ishii, M. (1985) Effects of prazosin on platelet aggregation and plasma beta-thromboglobulin in essential hypertension. Clinical Pharmacology and Therapeutics, **37**, 601-605.

115) Ito, A. & Schanberg, S.M. (1972) Central nervous system mechanisms responsible for blood pressure elevation caused by p-chlorophenyalanine. Journal of Pharmacology and Experimental Therapeutics, **181**, 65-74.

116) Jaffe, B.M., Ferrara, A. & Sherlock, D.J. (1986) Comparative effects of ketanserin, atropine and methysergide on the gastrointestinal effects of hyperserotoninaemia in the awake dog. Journal of Pharmacology and Experimental Therapeutics, **238**, 536-541.

117) Janeway, T.C., Richardson, H.B. & Park, E.A. (1918) Experiments on the vasoconstrictor action of blood serum. Archives of Internal Medicine, **21**, 565-603.

118) Janssen, P.A.J. (1985) Pharmacology of potent and selective S$_2$-serotonergic antagonists. Journal of Cardiovascular Pharmacology, **7(Suppl 7)**, S2-S11.

119) Jarrot, B., McQueen, A., Graf, L. & Louis, W.J.

(1975) Serotonin levels in vascular tissue and the effects of a serotonin synthesis inhibitor on blood pressure in hypertensive rats. Clinical and Experimental Pharmacology and Physiology, **2(Suppl)**, 201-205.

120) Johnson, C.E., Davis, S., Belfield, P.W., Cooke, N.J. & Davies, J.A. (1984) Platelet activation during exercise induced asthma. Thorax, **39**, 706.

121) Julius, S., Esler, M.D., Randall, O.S. (1975) Role of the autonomic nervous system in mild human hypertension. Clinical Science and Molecular Medicine, **48(Suppl)**, 243S-252S.

122) Kalkman, H.O., Engel, G. & Hoyer, D. (1984) Three distinct subtypes of serotonergic receptors mediate the triphasic blood pressure response to serotonin in rats. Journal of Hypertension, **2(suppl 3)**, 143-145.

123) Kamal, L.A., Le Quan-Bui, K.H. & Meyer, P. (1984) Decreased uptake of ^{3}H-serotonin and endogenous content of serotonin in blood platelets in hypertensive patients. Hypertension, **6**, 568-573.

124) Kaumann, A.J. (1983) A classification of heart serotonin receptors. Naunyn Schmiedeberg's Archives of Pharmacology, **322(suppl)**, R42.

125) Kellum, J.M. & Jaffe , B.M. (1976) Validation and application of a radioimmunoassay for serotonin. Gastroenterology, **70**, 516-522.

126) Kelman, A.W., Whiting, B. & Sumner, D.J. (1984) Correction equation for ECG time intervals. British Journal of clinical Pharmacology, **18**, 113-114.

127) Kissinger, P.T., Bruntlett, C.S. & Shoup, R.E.

(1981) I. Minireview: Neurochemical applications of liquid chromatography with electrochemical detection. Life Sciences, **28**, 455-465.

128) Kjeldsen, S.E., Gjelsdal, K. Eide, I., Aakessan, I., Amunden, R., Per Foss, A. & Leren, P. (1983) Increased beta-thromboglobulin in essential hypertension: interactions between arterial plasma adrenaline, platelet function and blood lipids. Acta Medica Scandinavica, **213**, 369-373.

129) Knauer, K.A., Lichtenstein, L.M., Adkinson, N.F. Jr. & Fish, J.E. (1981) Platelet activation during antigen induced airway reactions in asthmatic subjects. New England Journal of Medicine, **304**, 1404-1407.

130) Kobrin, I., Stessman, J., Yagie, J. & Ben-Ishay, D. (1983) Prazosin induced bradycardia in acute treatment of hypertension. Archives of Internal Medicine, **143**, 2019-2023.

131) Korczyn, A.D., Lozaravits, J., Dvilanski, A., Eshel, Y. & Nathan, I. (1985) 5-hydroxytryptamine in platelets of torsion dystonia patients. Acta Neurologica Scandinavica, **71**, 171-173.

132) Koss, M.C. & Wang, S.C. (1972) Brainstem loci for sympathetic activation of the nictifating membrane and pupil in the cat. American Journal of Physiology, **222**, 900-905.

133) Kubo, T. & Su, C. (1983) Effects of serotonin and some other neurohumeral agents on adrenergic neurotransmission in spontaneously hypertensive rat vasculature. Clinical and Experimental Hypertension (A),

5, 1501-1510.

134) Kuhn, D.M., Wolf, W.A. & Lovenberg, W.A. (1980) Review of the role of the central serotonergic neuronal system in blood pressure regulation. Hypertension, **2**, 243-255.

135) Laduron, P.M., Janssen, P.F.M. & Leysen, J.E. (1982) In vivo binding of [^{3}H]ketanserin on serotonin S_2-receptors in rat brain. European Journal of Pharmacology, **81**, 43-48.

136) Lambert, G.A., Friedmen, E., Buchweitz, E. & Gershon, S. (1978) Involvement of 5-hydroxytryptamine in the central control of respiration, blood pressure and heart rate in the anesthetised rat. Neuropharmacology, **17**, 807-813.

137) Langer, S.Z. (1981) Presynaptic modulation of the release of catecholamines. Pharmacological Reviews, **28**, 255-272.

138) Lee, S.-L. & Fanburg, B.L.. (1986) Serotonin uptake by bovine pulmonary artery endothelial cells in culture. 1. Characterisation. American Journal of Physiology, **250**, C761-C765.

139) Lee, W.B., Imsay, M.J. & Lumbers, E.R. (1980) Mechanisms by which angiotensin II affects the heart rate of the concious sheep. Circulation Research, **47**, 286-292.

140) Leff, P. & Martin, G.R. (1986) Peripheral 5-HT_2-like receptors. Can they be classified with the available antagonists? British Journal of Pharmacology, **88**, 585-593.

141) Leff, P., Martin, G.R., Morse, J.M. (1986) The

classification of peripheral 5-HT_2-like receptors using tryptamine agonist and antagonist analogues. British Journal of Pharmacology, **89**, 493-499.

142) Lexchin, J.L., Cude-Simpson, K.D. & Stancer, H.C. (1979) Brain and blood indole metabolites after peripheral administration of ^{14}C-5-HT in rat. Neurochemical Research, **2**, 39-45.

143) Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vandenberk, J. & Janssen, P.A.J. (1981) Receptor binding profile of R41 468, a novel antagonist at $5HT_2$ receptors. Life Sciences, **28**, 1015-1022.

144) Leysen, J.E. (1984) Problems in in-vitro receptor binding studies and identification and role of serotonin receptor sites. Neuropharmacology, **23**, 247-254.

145) Leysen, J.E., Gommeron, W., Van Gompel, P., Wynants, J., Janssen, P.F. & Laduron , P.M. (1985) Receptor bimding properties invitro and in vivo of ritanserin, a very potent and long acting S_2 antagonist. Molecular Pharmacology, **27**, 600-611.

146) Lin, M.T. & Chern, S.I. (1979) Effect of brain 5-hydroxytryptamine alterations on reflex bradycardia in rats. American Journal of Physiology, **236**, R302-R306.

147) Lorenz, R.R. & Vanhoutte, P.M. (1985) Prejunctional adrenergic inhibition by aggregation platelets in canine blood vessels. American Journal of Physiology, **249**, H685-H689.

148) Lowe, G.D.O. (1986) Blood rheology in arterial disease. Clinical Science, **71**, 137-146.

149) Ludlum, C.A. (1979) Evidence for the platelet

specificity of beta-thromboglobulin and studies on its plasma concentration in healthy individuals. British Journal of Haematology, **41**, 271-278.

150) M^{C}Caig, D.J. (1986) Autonomic responses of the isolated, innervated trachea of the guinea pig: interaction with autonomic drugs, histamine and 5-hydroxytryptamine. British Journal of Pharmacology, **88**, 239-248.

151) M^{C}Call, R.B. & Humphrey, S.J. (1982) Involvement of serotonin in the central regulation of blood pressure: Evidence for a facilitating effect on sympathetic nerve activity. Journal of Pharmacology and Experimental Therapeutics, **222**, 94-101.

152) M^{C}Call, R.B. & Schuette, M.R. (1984) Evidence for an alpha-1 receptor mediated central sympatho-inhibitory action of ketanserin. Journal of Pharmacology and Experimental Therapeutics, **228**, 704-710.

153) M^{C}Cubbin, J.W., Kameko, Y. & Page, I.H. (1960) Ability of serotonin and norepinephrine to mimic the central effects of reserpine on vasomotor activity. Circulation Research, **8**, 849-858.

154) M^{C}Fadden, E.R. Jr., Ingram, R.H. Jr., Haynes, R.L. & Wellman, J.J. (1977) Predominant site of flow limitation and mechanism of post-exertional asthma. Journal of Applied Physiology, **42**, 746-752.

155) M^{C}Grath, M.A. (1977) 5-hydroxytryptamine and neurotransmitter release in canine blood vessels. Circulation Research, **41**, 428-435.

156) M^{C}Kibben, J.K., Pocock, W.A., Barlow, J.B., Scott

Millar, R.N. & Obel I.W.P. (1984) Sotalol, hypokalaemia, syncope and torsades de pointes. British Heart Journal, **51**, 157-162.

157) Malmgren, R., Olsson, P. & Unge, G. (1981) The effect of various inhibitors on the 5HT binding and uptake by human platelets. Thrombosis Research, **22**, 469-480.

158) Man In'T Veld, A.J., Wenting, G.J., Boomsma, F., Verhoeven, R.P. & Schalekamp, M.A.D.H. (1980) Sympathetic and parasympathetic components of reflex cardiostimulation during vasodilator treatment of hypertension. British Journal of clinical Pharmacology, **9**, 547-551.

159) Manzini, S., Maggi, C.A. & Meli, A. (1986) 5-hydroxytryptamine delays relaxation time of nor-epinephrine-induced vasoconstrction. American Journal of Physiology, **250**, H121-H130.

160) Marker, J.D., Miles, T.S. & Scroop, G.C. (1980) Modulation of the baroreceptor reflex by angiotensin II and other vasoactive drugs in anaesthetised greyhounds. Clinical Science, **58**, 7-13.

161) Marwood, J.F. & Stokes, G.S. (1983) Vascular smooth muscle and serotonin and alpha-adrenergic receptors. Clinical and Experimental Pharmacology and Physiology, **10**, 265-267.

162) Marwood, J.F. & Stokes, G.S. (1984) Review article. Serotonin (5HT) and its antagonists: involvement in the cardiovascular system. Clinical and Experimental Pharmacology and Physiology, **11**, 439-456.

163) Matsuoka, H., Ishii, M., Goto, A. & Sugimoto, T. (1985) Role of serotonin type 2 receptors in regulation of

aldosterone production. American Journal of Physiology, **249**, E234-E238.

164) Mehta, J. & Mehta, P. (1981) Platelet function in hypertension and effect of therapy. American Journal of Cardiology, **47**, 331-334.

165) Meyer, D.K. & Hertting, G. (1974) Influence of serotonin on water intake and the renin-angiotensin system in the rat. Archives of Internal Pharmacodynamics and Therapeutics, **212**, 130-140.

166) Middlemiss, D.N. & Fozard, J.R. (1983) Hydroxy-2-(di-n-propyl-amino)-tetralin discriminates between subtypes of the $5HT_1$ recognition site. European Journal of Pharmacology, **90**, 151-153.

167) Millar, J.A., Leckie, B.J., Morton, J.J., Jordan, J. & Tree, M. (1980) A microassay for total and active renin concentration in human plasma based on antibody trapping. Clinica Chimica Acta, **101**, 5-15.

168) Millar, J.S. & Vaughan Williams, E.M. (1983) Pharmacological mapping of regional effects in the rabbit heart of some new anti-arrhythmic drugs. British Journal of Pharmacology, **79**, 701-709.

169) Modlinger, R.S., Schonmuller, J.M. & Arora, S.P. (1979) Stimulation of aldosterone, renin and cortisol by tryptophan. Journal of Clinical Endocrinology and Metabolism, **48**, 599-603.

170) Molyneux, S.G. & Clarke, E.E. (1985) Precise determination of 5-hydroxytryptamine in platelets and platelet poor plasma. Clinical Chemistry, **31**, 1573-1574.

171) Morrissey, J.J., Walker, M.N. & Lovenberg, W.

(1977) The absence of tryptophan hydroxylase activity in blood platelets. Proceedings of the Society of Experimental Biology and Medicine, **154**, 496-499.

172) Mueller, E.A., Murphy, D.L. & Sunderland, T. (1985) Neuroendocrine effects of M-chorophenylpiperazine, a serotonin agonist, in humans. Journal of Clinical Endocrinology and Metabolism, **61**, 1179-1184.

173) Nademanee, K., Lockhart, E., Pruitt, C. & Singh, B. (1987) Cardiac electrophysiologic effects of intravenous ketanserin in humans. Journal of Cardiovascular Pharmacology, **10(Suppl 3)**, S81-S85.

174) Nathenas, J., Dexter, J. & Katzman, R. (1973) The application of ninhydrin (5HT) reaction to the spectrophotofluorometric assay of plasma serotonin. Biochemical Medicine, **8**, 259-267.

175) Neumayer, R.J., Hare, B.D. & Franz, D.N. (1974) Evidence for bulbospinal control of sympathetic preganglionic neurones by monoaminergic pathways. Life Sciences, **14**, 793-806.

176) Nicholls, M.G., Tree, M., Brown, J.J. Douglas, B.H., Fraser, R., Hay, G.D., Lever, A.F., Morton, J.J., Robertson, J.I.S. (1980) Angiotensin II/aldosterone dose response curves in the dog; effects of changes in sodium balance. Endocrinology, **102**, 485-493.

177) Niemegeers, C.J.E., Colpaert, F.C., Leysen, J.E., Awouters, F. & Janssen, P.A.J. (1983) Mescaline-induced head twitches in the rat: an in-vivo method to evaluate serotonin S2 antagonists. Drug Development and Research, **3**, 123-135.

178) Ossim, E.E. Wyllie, J.H. (1982) Evidence for loss of 5-hydroxytryptamine from circulating platelets. Journal of Physiology, **326**, 25P-26P.

179) Ossim, E.E. Wyllie, J.H. (1983) Sites at which circulating platelets deposit 5-hydroxytryptamine. Journal of Physiology, **33?**, 141P-142P.

180) Oswald, I.,, Adam, K., Borrow, S. & Idzikowski, C. (1979) The effects of two hypnotics on sleep, subjective feelings and skilled performance. Pharmacology of the states of alertness, ed. Passouant, P. & Oswald, I., pp 51-63, Oxford: Pergamon Press.

181) Otteni, J.C., Pottecher, T., Bronner, G., Flesch, H. & Diebolt, J.R. (1983) Prolongation of the Q-T interval and sudden cardiac arrest following right radical neck dissection. Anesthesiology, **59**, 358-361.

182) Page, I.H. & M^{C}Cubbin, J.W. (1953) The variable arterial response to serotonin in laboratory animals and man. Circulation Research, **1**, 354-362.

183) Palermo, A., del Rosso, G., Constantini, C., Bertalero, P., Rizzi, S. & Libretti, A. (1986) Platelet content of serotonin and response to stress. Journal of Hypertension, **4(Suppl 1)**, S43-S45.

184) Parati, G., Grassi, G., Coruzzi, P., Musiari, L., Ravogli, A., Novarini, A. & Mancia, G. (1987) Influence of cardiopulmonary receptors on the bradycardic responses to carotid baroreceptor stimulation in man. Clinical Science, **72**, 639-645.

185) Pazos, A., Hoyer, D. & Palacios, J.M. (1985) The binding of serotonergic ligands to the porcine choroid

plexus: characterization of a new type of serotonin recognition site. European Journal of Pharmacology, **106**, 539-546.

186) Pedigo, N.W., Yamamura, H.I. & Nelson D.L. (1981) Discrimination of multiple [^{3}H]5-hydroxytryptamine binding sites by the neuroleptic spiperone in rat brain. Journal of Neurochemistry, **36**, 220-226.

187) Peroutka, S.J. & Snyder, S.H. (1979) Multiple serotonin receptors: Differential binding of ^{3}H-5-hydroxytryptamine, ^{3}H-lysergic acid diethylamide and ^{3}H-spiroperidol. Molecular Pharmacology, **16**, 687-699.

188) Peters, R. & Grahame-Smith, D.G. (1980) Human platelet 5-HT receptors: characterisation and functional association. European Journal of Pharmacology, **68**, 243-256.

189) Petralito, A., Fiore, C.E., Mangiafico, R.A. & Malatino, L.S. (1982) Beta-thromboglobulin plasma levels in different stages of arterial hypertension. Thrombosis and Haemostasis (Stuttgart), **48**, 241.

190) Phillips, C.A., Mylecharane, E.W. & Shaw, J. (1985) Mechanisms involved in the vasodilator action of 5-hydroxytryptamine in the dog femoral artery circulation in vivo. European Journal of clinical Pharmacology, **113**, 325-334.

191) Pignatti, P.F. & Cavalli-Sforza, L.L. (1975) Serotonin binding proteins from human blood platelets. Neurobiology, **5**, 65-74.

192) Pletscher, A. (1968) Metabolism, transfer and storage of 5-hydroxytryptamine in blood platelets. British

Journal of Phasmacology and Chemotherapy, **32**, 1-16.

193) Prescott, R.W.G., Kendall Taylor, P., Weightman, D,R., Watson, J.M., Ratcliffe, W.A. (1984) The effect of ketanserin, a specific serotonin antagonist, on the PRL, GH, ACTH and cortisol responses to hypoglycaemia in normal subjects. Clinical Endocrinology, **20**, 137-142.

194) Rabinowitz, S.H. & Lown, B. (1978) Central neurochemical factors related to serotnin metabolism and cardiac ventricular vulnerability for repetitive electrical activity. American Journal of Cardiology, **41**, 516-522.

195) Rapport, M.M., Green, A.A. & Page I.H. (1948) Serum vasoconstrictor (serotonin). IV Isolation and characterisation. Journal of Biological Chemistry, **176**, 1243-1251.

196) Reid, J.V.O. (1970) Trial of tryptophan prophylaxis inpatients liable to "African" cardiomyopathy. South African Medical Journal, **44**, 732-735.

197) Reimann, I.W. & Frohlich, J.C. (1983) Mechanism of antihypertensive action of ketanserin in man. British Medical Journal, **287**, 381-383.

198) Reimann, I.W., Ratge, D., Wisser, H. & Klotz, U. (1985) Effect of intravenous ketanserin on plasma catecholamines and renin activity in normal volunteers. European Journal of clinical Pharmacology, **28**, 273-277.

199) Richardson, B.P., Engel, G., Donatsch, P. & Stadler, P.A. (1985) Identification of serotonin M-receptor subtypes and their specific blockade by a new class of drug. Nature, **316**, 126-131.

200) Robertson, D.N. & Page, C.P. (1987) Effect of platelet agonists on airway reactivity and intrathoracic platelet accumulation. British Journal of Pharmacology, **92**, 105-111.

201) Robinson, S.E., Austin, M.J.F. & Gibbens, D.M. (1985) The role of serotonergic neurons in dorsal raphe, median raphe and anterior hypothalamic pressor mechanisms. Neuropharmacology, **24**, 51-58.

202) Rocco, S., Boscaro, M., D'Agostino, D., Armanini, D., & Mantero, F. (1985) Effect of ketanserin on the in vitro reglation of aldosterone. Journal of Hypetension, **4(Suppl 1)**, S51-S54.

203) Ruckebusch, Y. (1984) Enhancement of the cyclic motor activity of the ovine small intestine by lysergic acid derivatives. Gastroenterology, **87**, 1049-1055.

204) Rubinstein, I., Levinson, H., Slutsky, A.S., Hak, H., Wells, J., Zamel, N. & Rebuck, A.S. (1987) Immediate and delayed bronchoconstriction after exercise in patients with asthma. New England Journal of Medicine, **317**, 482-485.

205) Saman, S., Thandroyen, F. & Opie L.H. (1985) Serotonin and the heart: effects of ketanserin on myocardial function, heart rate, and arrhythmias. Journal of Cardiovascular Pharmacology, **7(Suppl 7)**, S70-S75.

206) Saniabadi, A.R., Lowe, G.D.O., Belch, J.J.F., Forbes, C.D., Prentice, C.R.M. & Barbenel, J.C. (1983) The novel effect of a new prostacyclin anologue ZK36374 on the aggregation of human platelets in whole blood. Thrombosis and Haemostasis (Stuttgart), **50**, 718-721.

207) Saniabadi, A.R., Lowe, G.D.O., Barbenel, J.C. & Forbes , C.D. (1984) A comparison of spontaneous platelet aggregation in whole blood with platelet rich plasma: additional evidence for a role of ADP. Thrombosis and Haemostasis (Stuttgart), **51**, 115-118.

208) Sasa, S., Blank, C.L.-R., Wenke, D.C. & Sczupak, C.A. (1978) Liquid-chromatographic determination of serotonin in serum and plasma. Clinical Chemistry, **24**, 1509-1514.

209) Saxena, P.R. & Lawang, A. (1985) A comparison of cardiovascular and smooth muscle effects of 5-hydroxytryptamine and 5-carboxamidotryptamine, a selective agonist of 5-HT_1 receptors. Archives Internationales de Pharmacodynamie et de Therapie, **277**, 235-252.

210) Saxena, P.R., Mylecharone, E.J. & Heiligers, J. (1985) Analysis of the heart rate effects of 5-hydroxytryptamine in the cat; mediation of tachycardia by 5-HT_1-like receptors. Naunyn Schmiedeberg's Archives of Pharmacology, **330**, 121-129.

211) Saxena, P.R. & Verdouw, P.D. (1985) 5-carboxamide tryptamine, a compound with high affinity for 5-HT_1 binding sites, dilates arterioles and constricts arteriovenous anastamoses. British Journal of Pharmacology, **84**, 533-544.

212) Saxena, P.R., Bolt, G.R., Dhasmana, K.M. (1987) Serotonin agonists and antagonists in experimental hypertension. Journal of Cardiovascular Pharmacology, **10(Suppl 3)**, S12-S18.

213) Schapel, G.J. & Betts, W.H. (1981) The effect of a single oral dose of prazosin on venous reflex response, blood pressure and pulse rate in normal volunteers. British Journal of clinical Pharmacology, **12**, 875-881.

214) Scroop, G.C. & Walsh, J.A. (1968) Interactions between angiotensin, noradrenaline and serotonin on the peripheral blood vessels in man. Australian Journal of Experimental Biology and Medical Science, **46**, 573-580.

215) Sheller, J.R., Holtzman, M.J., Skoogh, B. & Nadel, J.A. (1982) Interaction of serotonin with vagal- and ACh-induced bronchoconstriction in canine lungs. Journal of Applied Physiology, **52**, 964-966.

216) Shenker, Y., Gross, M.D. & Grekin, R.J. (1985a) Peripheral serotonin$_2$ receptor blockade does not inhibit 5-hydroxytryptophan-induced aldosterone stimulation. Journal of Clinical Endocrinology and Metabolism, **61**, 1201-1204.

217) Shenker, Y., Gross, M.D. & Grekin, R.J. (1985b) Central serotonergic stimulation of aldosterone release. Journal of Clinical Investigation, **76**, 1485-1490.

218) Shepherd, S.T. & Vanhoutte, P.M. (1981) Local modulation of adrenergic neurotransmission. Circulation, **64**, 655-666.

219) Shuttleworth, R.D. & O'Brien, J.R. (1981) Intraplatelet serotonin and plasma 5-hydroxyindoles in health and disease. Blood, **57**, 505-509.

220) Smits, J.F.M., Van Essen, C.M. & Strukker-Boudier, H.A.J. (1987) Effects of ketanserin on haemodynamics and baroreflex effects in concious spontaneously hypertensive

rats. Journal of Cardiovascular Pharmacology, **10**, 1-8.

221) So, S.Y., Lam, W.K. & Kwan, S. (1985) Selective 5-HT_2 receptor blockade in exercise-induced asthma. Clinical Allergy, **15**, 371-376.

222) Sole, M.J., Schum, A. & Van Loon, G.R. (1979) Serotonin: Metabolism in the normal and failing heart. Circulation Research, **45**, 629-634.

223) Staessen, J., Fagard, R., Fiocchi, R., Lijnen, P., Rorive, G. & Amery, A. (1985) Double-blind comparison of ketanserin with propranolol in hypertensive patients: interim report. Journal of Cardiovascular Pharmacology, **7(Suppl 7)**, S140-S147.

224) Stanaszek, W.F., Kellerman, D., Brogden, R.N. & Romankiewicz, J.A. (1983) Prazosin update. A review of its pharmacological properties and therapeutic use in hypertension and congestive heart failure. Drugs, **25**, 339-384.

225) Staniforth, D.H. (1983) The QT interval and cycle length: the influence of atropine, hyoscine and exercise. British Journal of clinical Pharmacology, **16**, 615-621.

226) Staniforth, D.H. (1984) Correction equation for ECG time intervals. British Journal of clinical Pharmacology, **18**, 115.

227) Stone, C.A., Wenger, H.C., Ludden, C.T., Stavorski, J.M. & Ross, C.A. (1961) Antiserotonin-antihistamine properties of cyproheptadine. Journal of Pharmacology and Experimental Therapeutics, **131**, 111-120.

228) Strano, A., Novo, S., Pinto, A., Fasulo, S. & Alamo, G. (1985) Calf blood flow and vascular resistance

in middle-aged and elderly hypertensives. Journal of Hypertension, **3(suppl 3)**, S351-S353.

229) Strauss, R.H., M^{C}Fadden, E.R., Ingram, R.H. Jr. et al (1978) Influence of heat and humidity on the airways obstruction induced by exercise in asthma. Journal of clinical Investigation, **61**, 433-440.

230) Surawicz, B & Knoebel, S.B. (1984) Long QT: good, bad or indifferent? Journal of the American College of Cardiology, **4**, 398-413.

231) Sved, A.F., Fernstrom, J.D. & Wurtman, R.J. (1979) Tyrosine administration reduces blood pressure and enhances norepinephrine release in spontaneously hypertensive rats. Proceedings of the National Academy of Sciences of the USA, **76**, 3511-3514.

232) Tadepalli, A.S., Mills, E. & Schanberg, S.M. (1977) Central deppression of carotid baroreceptor pressor response, arterial pressure and heart rate by 5-hydroxytryptophan: Influence of supracollicular areas of the brain. Journal of Pharmacology and Experimental Therapeutics, **202**, 310-319.

233) Tadepalli, A.S. (1980) Inhibition of reflex bradycardia by a central action of 5-hydroxytryptophan. British Journal of clinical Pharmacology, **69**, 647-650.

234) Tagari, P.C. Boullin, D.J. & Davies, C.L. (1984) Simplified determination of serotonin in plasma by liquid chromatography with electrochemical detection. Clinical Chemistry, **30**, 131-135.

235) Thomas, D.P. & Vane, J.R. (1967) 5-Hydroxytryptamine in the circulation of the dog. Nature

(London), **216**, 335-338.

236) Thomson, N.C., Daniel, E.E. & Hargreave, F.E. (1982) Role of smooth muscle alpha receptors in non-specific bronchial responsiveness in asthma. American Review of Respiratory Diseases, **126**, 521-525.

237) Tong, J.H. & Kaufman, S. (1975) Tryptophan hydroxylase. Purification and some properties of the enzyme from rabbit hindbrain. Journal of Biological Chemistry, **250**, 4152-4158.

238) Tonnesen, P. (1985) Bronchial challenge with serotonin in asthmatics. Allergy, **40**, 136-140.

239) Tranzer, J.P., Da Prada, M. & Pletscher, A. (1966) Ultra-structural localisation of 5-hydroxytryptamine in blood platelets. Nature (London), **212**, 1574-1575.

240) Tyce, G.M., Flock, E.V. & Owen, C.A. Jr. (1968) Uptake and metabolism of 5-hydroxytryptamine by the isolated perfused rat liver. American Journal of Physiology, **215**, 611-619.

241) Tyce, G.M., Stockhard, J., Sharples, N.S. & Muenter, M.D. (1983) Excretion of amines and their metabolites by two patients in hepatic coma treated with L-dopa. Clinical Pharmacology and Therapeutics, **34**, 390-398.

242) Urban, J.H., Van de Kar, L.D., Schmitt, S.L. & Brownfield, M.S. (1985) In vitro evidence for a blood-borne renin-releasing factor. Life Sciences, **37**, 1335-1342.

243) Van De Kar, L.D., Richardson-Morton, K.D. & Urban, J.H. (1985) Serotonin and norepinephrine-dependent effects

of fenfluramine on plasma renin activity in concious male rats. Neuropharmacology, **24**, 487-494.

244) Van De Kar, L.D. & Richardson-Morton, K.D. (1986) Serotonergic regulation of the release of renin is not mediated by the autonomic nervous system but involves beta adrenoreceptors. Neuropharmacology, **25**, 487-492.

245) Vanhoutte, P.M. & Cohen, R.A. (1983) The elusory role of serotonin in vascular function and disease. Biochemical Pharmacology, **32**, 3671-3674.

246) Van Nueten, J.M., Janssen, P.A.J., Van Beek, J., Xhonneux, R., Verbeuren, T.J. & Vanhoutte, P.M. (1981) Vascular effects of ketanserin (R 41468), a novel antagonist of 5HT-2 serotonergic receptors. Journal of Pharmacology and Experimental Therapeutics, **218**, 217-230.

247) Van Nueten, J.M., Janssen, P.A.J., De Ridder, W., & Vanhoutte, P.M. (1982) Interaction between 5-hydroxytryptamine and other vasoconstrictor substances in the isolated femoral artery of the rabbit: Effect of ketanserin (R 41 468). European Journal of Pharmacology, **77**, 281-287.

248) Vaughan Williams, E.M. (1982) QT and action potential duration. British Heart Journal, **47**, 513-514.

249) Vermylen, J., Arnout, J., Deckmyn, H., Xhonneux, B. & De Clerck, F. (1986) Continuous inhibition of the platelet S_2-serotonergic receptors during the long term administration of ketanserin. Thrombosis Research (Stuttgart, **42**, 721-723.

250) Victorzon, M., Tapparelli, C. & Muller-Schweinitzer, E. (1986) Comparison of the actions

of serotoninergic agents on human saphenous veins and platelets. European Journal of Pharmacology, **124**, 107-111.

251) Vlachakis, N.D. & Aledort, N. (1980) Hypertension and propranolol therapy: effect on blood pressure, plasma catecholamines and platelet aggregation. American Journal of Cardiology, **45**, 321-325.

252) Waller, P.C., Solomon, S.A. & Ramsey, L.E. (1987) Comparison of ketanserin and slow-release nifedipine added to the treatment of hypertensive patients uncontrolled by a thiazide diuretic plus beta-adrenoreceptor blocker. British Journal of clinical Pharmacology, **24**, 591-597.

253) Waller, P.C., Tucker, G.T. & Ramsey, L.E. (1987) The pharmokinetics of ketanserin after a singlr dose and at steady-state in hypertensive subjects. European Journal of Clinical Pharmacology, **33**, 423-426.

254) Wenting, G.J., Man In'T Veld, A.J., Woittiez, A.J., Boomsma, F. & Schalekamp, M.A.D.H. (1982) Haemodynamic effects of ketanserin, a selective 5-hydroxytryptamine (serotonin) receptor antagonist, in essential hypertension. Clinical Science, **63**, 435S-438S.

255) Wenting, G.J., Woittiez, A.J., Man In'T Veld, A.J. & Schalekamp, M.A.D.H. (1984) 5-HT. alpha-adrenoceptors, and blood pressure. Effects of ketanserin in essential hypertension and autonomic insufficiency. Hypertension, **6**, 100-109.

256) Wheeler, T. & Watkins, P.J. (1973) Cardiac denervation in diabetics. British Medical Journal, **4**, 584-586.

257) Williams, B.C., Shaikh, S. & Edwards, C.R. (1984)

The specificity of ketanserin in the inhibition of serotonin -induced steroidogenesis in the rat adrenal zona glomerulosa. Journal of Hypertension, **4(Suppl 1)**, S51-S54.

258) Willner, P. (1985) Antidepressants and serotonergic neurotransmission: an integrative review. Psychopharmacology, **85**, 387-404.

259) Wing, L.M.H. & Chalmers, J.P. (1974) Participation of central serotonergic neurons in the control of the circulation of the unanesthetized rabbit. Circulation Research, **35**, 504-512.

260) Woittiez, A.J.J., Wenting, G.J., Van den Meiracker, A.H., Van Eck, H.J.R., Man In'T Veld, A.J., Zantvoort, F.A., Schalekamp, M.A.D.H. (1986) Chronic effect of ketanserin in mild to moderate hypertension. Hypertension, **8**, 167-173.

261) Yamanishi, J., Sano, H., Saito, K., Furuta, Y. & Fukazaki, H. (1985) PLasma concentrations of platelet-specific proteins in different stages of arterial hypertension: interactions between platelet aggregation, blood lipids and age. Thrombosis and Haemostasis (Stuttgart), **54**, 539-543.

262) Yanowitz, F., Preston, J.B. & Abildskow, J.A. (1966) Functional distribution of right and left stellate innervation to the ventricles: Production of neurogenic electrocardiographic changes by unilateral alteration of sympathetic tone. Circulation Research, **18**, 416-428.

263) Youdim, M.B.H. & Ashkenazi, R. (1982) Regulation of 5HT catabolism. Advances in Biochemical Psychopharmacology, **34**, 35-60.

264) Zabludowski, J.R., Zoccali, C., Isles, C.G., Murray, G.D., Robertson, J.I.S., Inglis, G.C., Fraser, R. & Ball, S.G. (1984) Effect of the 5-hydroxytryptamine type-2 receptor antagonist, ketanserin, on blood pressure, the renin-angiotensin system and sympatho-adrenal function in patients with essential hypertension. British Journal of clinical Pharmacology, **17**, 309-316.

265) Zabludowski, J.R., Ball, S.G. & Robertson, J.I.S. (1985) Ketanserin and alpha-1 adrenergic antagonism in man. Journal of Cardiovascular Pharmacology, **7(Suppl 7)**, S123-S125.

266) Zannad, F., Voisin, Ph., Pointel, J.P., Schmitt, C., Frietag, B. & Stoltz, J.F. (1985) Effects of ketanserin on platelet function and reed cell filterability in hypertension and peripheral vascular disease. Journal of Cardiovascular Pharmacology, **7(Suppl 7)**, S32-S34.

267) Zimmerman, B.G. (1981) Adrenergic facilitation by angiotensin: does it serve a physiological function. Clinical Science, **60**, 343-348.

268) Zoccali, C., Zabludowski, J.R., Isles, C.G., Murray, G.D., Inglis, G.C., Robertson, J.I.S., Fraser, R. & Ball, S.G. (1983) The effect of a new 5HT antagonist, ketansrin, on blood pressure, the renin angiotensin system and sympatho-adrenal function in normal man. British Journal of clinical Pharmacology, **16**, 305-311.

269) Zuckerman, M. & Lubin, B. (1965) Manual for the multiple affect adjective check list, San Diego: Edits.

Communications and Publications

At the time of submission of this thesis, the following communications had been given and publications made relating to this work.

Communications to learned societies.

1) Stott, D.J., Ball, S.G. & Robertson J.I.S. (June 1985) Specificity of the serotonergic antagonist ketanserin. Second European Meeting on Hypertension, Milan, Italy.

2) Stott, D.J., M^{C}Lenachan, J.M. & Ball, S.G. (September 1985) Ketanserin, the QT interval and autonomic function testing in normal subjects. British Pharmacological Society, University of Edinburgh.

3) Stott, D.J., Roberts, J.A., Thomson, N.C. & Ball S.G. (December 1985) Effects of serotonergic type-2 blockade in exercise induced asthma. Medical Research Society, The London Hospital.

4) Hosie, J., Stott, D.J., Robertson, J.I.S. & Ball, S.G. (September 1985) Does serotonergic type-2 antagonism reduce blood pressure? 11th Scientific Meeting of The International Society of Hypertension, Heidelberg, West Germany.

5) Stott, D.J., Saniabadi, A.R., Hosie, J., Lowe, G.D.O. & Ball, S.G. (January 1987) Effects of ritanserin, a new selective serotonergic type-2 antagonist, on blood pressure and serotonin induced platelet aggregation in

patients with essential hypertension. Medical Research Society, Charing Cross Medical School, London.

6) Stott, D.J., Saniabadi, A.R., Hosie, J., Lowe, G.D.O. & Ball, S.G. (May 1988) Serotonin and platelet aggregation in patients with essential hypertension compared to a normotensive control group. 12th Scientific Meeting of the International Society of Hypertension, Kyoto, Japan.

Publications:

1) Stott, D.J., Ball, S.G. & Robertson, J.I.S. (1985) Specificity of the serotonergic antagonist ketanserin. Journal of Hypertension, **3(Suppl 3)**, S191-S193.

2) Stott, D.J., Roberts, J.A., Thomson, N.C. & Ball S.G. (1988) Effects of the serotonergic type-2 antagonist ketanserin in adult atopic asthma. European Journal of Clinical Pharmacology, in press.

3) Stott, D.J., Robertson, J.I.S., M^{C}Lenachan, J.M. & Ball S.G. (1988) Effects of short-term ketanserin treatment on the QT interval and vagal function in healthy subjects. Journal of Autonomic Pharmacology, in press.

4) Stott, D.J., Saniabadi, A., Hosie, J., Inglis, G.C., Lowe, G.D.O. & Ball S.G. (1988) Effects of the serotonergic type-2 antagonist ritanserin on blood pressure and serotonin induced platelet aggregation in patients with untreated essential hypertension. European Journal of Clinical Pharmacology, in press.

5) Stott, D.J., Saniabadi, A.R., Inglis, G.C., Hosie, J., Lowe, G.D.O. & Ball, S.G. (1988) Serotonin and

platelet aggregation in patients with essential hypertension compared to a normaotensive control group. Drugs, in press.

6) Hosie, J., Stott, D.J., Robertson, J.I.S. & Ball, S.G. (1987) Does acute serotonergic type-2 antagonism reduce blood pressure? Comparative effects of single doses of ritanserin and ketanserin in essential hypertension. Journal of Cardiovascular Pharmacology, **10(Suppl 3)**, S86-S88.

7) Stott, D.J., M^{C}Lenachan, J.M. & Ball, S.G. (1986) Ketanserin, the QT interval and autonomic function testing in normal subjects. British Journal of clinical Pharmacology, **21**, 84P.

8) Stott, D.J., Roberts, J.A., Thomson, N.C. & Ball, S.G. (1986) Effects of serotonergic type-2 blockade in exercise induced asthma. Clinical Science, **70(Suppl 13)**, 64P.

9) Stott, D.J., Saniabadi, A.R., Hosie, J., Inglis, G.C., Lowe, G.D.O. & Ball, S.G. (1987) Effects of the serotonergic type-2 antagonist ritanserin on blood pressure and serotonin-induced platelet aggregation in patients with untreated essential hypertension. Clinical Science, **72(Suppl 16)**, 19P.

Appendix 1: Visual analogue scale questionnaire (18 factor)

Instructions: Please rate the way you feel in terms of the descriptions below. Regard the lines as representing the full range of each symptom. Rate your feelings as they are at the moment. Mark clearly and perpendicularly across each line.

Alert	_____	Drowsy
Calm	_____	Excited
Strong	_____	Feeble
Muzzy	_____	Clear headed
Well co-ordinated	_____	Clumsy
Lethargic	_____	Energetic
Contented	_____	Discontented
Troubled	_____	Tranquill
Mentally slow	_____	Quick witted
Tense	_____	Relaxed
Attentive	_____	Dreamy
Incompetent	_____	Proficient
Happy	_____	Sad
Antagonistic	_____	Friendly
Interested	_____	Bored
Withdrawn	_____	Sociable
Depressed	_____	Elated
Self centred	_____	Outward going

(10cm lines)

Calculation of alertness and tranquillity scores from the 18 factor visual analogue scale (Herbert, Johns & Dore, 1976) are made by calculating the sum of [distance from the first to the second word (mm) for each visual analogue scale multiplied by the individual factor loading].

Alertness: Scales	Factor loading
Quick-witted/Mentally slow	0.878
Alert/Drowsy	0.865
Attentive/Dreamy	0.864
Energetic/Lethargic	0.856
Proficient/Incompetent	0.826
Strong/Feeble	0.793
Clear headed/Muzzy	0.775
Well co-ordinated/Clumsy	0.759
Elated/Depressed	0.592
Outward-going/Self-centred	0.591
Interested/Bored	0.539

Tranquillity: Scales	Factor loading
Tranquill/Troubled	0.823
Calm/Excited	0.798
Contented/Discontented	0.785
Relaxed/Tense	0.782
Happy/Sad	0.744
Friendly/Antagonistic	0.701
Sociable/Withdrawn	0.648

Appendix 2: Multiple Affect Adjective Check List

Anxiety, depression and hostility scores are calculated as the number of "plus words" minus the number of "minus words" selected by the patient from the Multiple Affect Adjective Check List (Zuckerman & Lubin, 1965).

Anxiety

Plus words (n=11)

Afraid, Desperate, Fearful, Frightened, Nervous, Panicky, Shaky, Tense, Terrified, Upset, Worrying

Minus words (n=10)

Calm, Cheerful, Contented, Happy, Joyful, Loving, Pleasant, Secure, Steady, Thoughtful

Depression

Plus words (n=20)

Alone, Awful, Blue, Destroyed, Discouraged, Forlorn, Gloomy, Hopeless, Lonely, Lost, Low, Miserable, Rejected, Sad, Suffering, Sunk, Terrible, Tormented, Unhappy, Wilted

Minus words (n=20)

Active, Alive, Clean, Enthusiastic, Fine, Fit, Free, Gay, Glad, Good, Healthy, Inspired, Interested, Lucky, Merry, Peaceful, Safe, Strong, Whole, Young

Hostility

Plus words (n=16)

Angry, Bitter, Cruel, Disaggreeable, Discontented, Disgusted, Enraged, Furious, Irritated, Mad, Mean, Offended, Outraged, Stormy, Unsociable, Vexed

Minus words (n=12)

Aggreeable, Amiable, Cooperative, Friendly, Good-natured, Kindly, Polite, Sympathetic, Tame, Tender, Understanding, Willful

Appendix 3: Visual analogue scale questionnaire (6 factor)

Instructions: Please rate the way you feel in terms of the descriptions below. Regard the lines as representing the full range of each symptom. Rate your feelings as they are at the moment. Mark clearly and perpendicularly across each line.

Alert	_____	Drowsy
Well co-ordinated	_____	Clumsy
Tense	_____	Relaxed
Incompetent	_____	Proficient
Happy	_____	Sad
Withdrawn	_____	Sociable

(10cm lines)