CARDIOVASCULAR EFFECTS OF SEROTONERGIC AGENTS.

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THESIS SUBMITTED FOR THE DEGREE OF MD.

DEPARTMENT OF MEDICINE AND THERAPEUTICS

UNIVERSITY OF GLASGOW

JANUARY 1999

Abstract

Serotonin (5-hydroxytryptamine, 5-HT) is a neurotransmitter which exerts its cardiovascular effects predominantly by interaction with specific 5-HT_1 and 5-HT_2 receptors. The effects of serotonergic agents differ between *in vivo* and *in vitro* preparations and display wide inter-species variation. It is therefore impossible to extrapolate results from animal or *in vitro* studies to the clinical situation. The role of these two receptors has therefore been studied in patients with suspected coronary artery disease using 3 different 5-HT_1 agonists and ketanserin, a 5-HT_2 antagonist.

Sumatriptan and naratriptan, 5-$HT_{1B/D}$ receptor agonists, vasoconstrict the systemic and pulmonary circulations. Sumatriptan-induced vasoconstriction appears more pronounced in the pulmonary circulation suggesting a greater density of 5-HT_1 receptors in the pulmonary compared to the systemic circulation. Although left ventricular end diastolic pressure and pulmonary artery wedge pressure rose after sumatriptan, there was no change in peak rate of left ventricular pressure rise, indicating the absence of a negative inotropic action. Naratriptan, an analogue of sumatriptan, displayed no significant effect on coronary artery diameter, a finding previously noted with sumatriptan.

Eletriptan, a selective 5-HT_{1D} agonist with less 5-HT_{1B} activity, had little vasoconstrictor effect on the systemic, pulmonary or coronary circulation perhaps suggesting that the 5-HT_{1B} receptor subtype mediates vasoconstriction.

The effect of sumatriptan on systolic time intervals and forearm blood flow was also assessed. The results suggest that STI's are of potential use in the non-

invasive assessment of 5-HT_1 agonists. No significant effect on forearm blood flow was observed but plasma noradrenaline levels fell after subcutaneous sumatriptan.

Ketanserin, a 5-HT_2 antagonist, acted as a vasodilator in the systemic and pulmonary circulation but failed to vasodilate the coronary arteries, presumably because the patients in this study had stable angina without platelet activation and therefore had low circulating levels of serotonin.

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Declaration and acknowledgements

This thesis was conducted and written by myself. Assistance, however, was received from a number of colleagues and the following people are gratefully acknowledged:

Professor W.S. Hillis who acted as advisor and provider of endless encouragement and advice.

Drs D. Birnie, L. Swan, A. Nasser, P.D. MacIntyre and D. Muir who assisted with cardiac catheterisations, analysing pressure tracings and in measuring coronary artery diameters in the various studies.

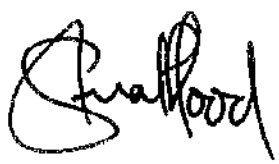
Dr J Petrie for training me in the technique of venous occlusion plethysmography and for performing the forearm blood flow study on myself.

Dr L.S. Murray, senior lecturer in statistics, Department of Medicine and Therapeutics, for her statistical expertise.

Mrs J.O. Clark for her secretarial skills

The staff of the cardiac catheterisation suite in the Western Infirmary.

Finally, my wife Lorna and our children Hannah and Ruairidh for their patience and support.



Stuart Hood

CHAPTER 1

INTRODUCTION: CARDIOVASCULAR SEROTONERGIC RECEPTORS

introduction

Over a century ago it was recognised that coagulated blood contained a substance with vasoconstrictor properties. In 1948 Rapport isolated this substance from serum and named it serotonin (5-hydroxytryptamine, 5-HT).[1] Serotonin was noted to be released from platelets as they were degraded in the clotting process. Subsequent work soon demonstrated that serotonin also existed in the enterochromaffin cells of the gastrointestinal tract where it enhanced gastrointestinal motility.[2] Within a very short period of time serotonin was isolated in the brain and thereafter its role as a neurotransmitter was acknowledged. Despite a further four decades of extensive research, many aspects of the physiological and pathophysiological role of 5-HT remain unclear. This thesis will therefore examine aspects of the role of serotonergic agents on the cardiovascular system.

Synthesis of Serotonin

The structure and pathway of serotonin synthesis are outlined in figures 1.1 and 1.2. Tryptophan, the aminoacid precursor of 5-HT is obtained in the diet. Approximately 1% of dietary tryptophan is converted to 5-hydroxytryptophan via the enzyme tryptophan-hydroxylase which is present in the enterochromaffin cells of the small intestine, but not in platelets. Serotonin in the CNS is synthesised locally as it crosses the blood brain barrier poorly.

Tissue Stores of Serotonin

Serotonin is located in the following sites:

1. Intestine

The enterochromaffin cells of the intestinal tract account for approximately 90%. 10% of the remaining 5-HT is found in the myenteric plexus of the intestine, where it is believed to act as an excitatory neurotransmitter increasing GI motility.

2. Blood

5-HT is present in high concentrations in platelets. On platelet aggregation, 5-HT is rapidly released and, together with thromboxane A_2, a potent vasoconstrictor effect can be observed. Platelets also avidly re-uptake serotonin and therefore circulating serum 5-HT levels are normally low in the absence of platelet activation.

3. Central Nervous System

5-HT can also be identified in the brain, particularly in the midbrain and spinal cord. At these sites 5-HT serves a plethora of relatively poorly understood functions.

Metabolism of 5-hydroxytryptamine

Oxidative deamination is the main mechanism by which 5-HT is metabolised. Within the brain, liver and in several other sites, the enzymes monoamineoxidase, methyl transferase and aldehyde dehydrogenase degrade serotonin to an aldehyde, and subsequently an acid (Figure1.3). The principle metabolite of 5-hydroxytryptamine is 5-hydroxyindole 3-acetic acid (5-HIAA) which is excreted in the urine. Measurement of 5-HIAA in the urine acts as an indicator of endogenous 5-HT metabolism and is of clinical use in the carcinoid syndrome, allowing the response to treatment, for example, to be assessed.

Classification of 5-HT Receptors

Gaddam and Picarelli (1957) were the first to attempt clarification of serotonergic receptors, describing D and M receptors following work on the guinea pig ileum.[3] M receptors were classified on the basis that Morphine inhibited the 5-HT mediated release of acetyl choline from myenteric cells of the guineapig ileum. Phenoxybenzamine was however observed to antagonise 5-HT-induced contractions of the same preparation and the D nomenclature was therefore established. This nomenclature persisted for nearly three decades

until further studies recognised various binding sites for 5-HT.[4] The observation that certain functions of serotonin, for example serotonin-induced vasoconstriction, were not mediated by D or M receptors altered thinking. In 1979 Peroutka and Snyder identified high and low affinity binding sites for [^{3}H] 5-HT in rat cortex.[5] The low affinity binding sites were subsequently shown to correspond with Gaddam and Picarelli's 'D' receptor and were re-classified as 5-HT_2 receptors.[6,7] The high affinity binding sites were classified as 5-HT_1 receptors. Further characterisation of 5-HT_2 receptors was advanced by the discovery of a potent, selective reversible antagonist at this receptor site, namely ketanserin.[8,9] In the last decade, the development of new specific receptor agonists and antagonists, together with genetic engineering techniques has led to the recognition of new receptors and subtypes. Current nomenclature recognises seven main types of serotonin receptor; 5-HT_1, 5-HT_2, 5-HT_3, 5-HT_4, 5-HT_5, 5-HT_6 and 5-HT_7 receptors. Each of these receptor characteristics are discussed below, paying particular attention to those which exist at vascular sites. Variation in receptor density at differing vascular sites within the same species and inter-species variations will become evident.

5-HT_1 Receptor Characteristics

Radioligand binding studies have confirmed the 5-HT_1 receptor to be heterogeneous in nature comprising of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C}, 5-HT_{1D} and 5-HT_{1E} subtypes. The functional status of these binding sites has also been established by the identification of second messenger coupling systems, but there still appears to be a lack of correlation between all receptor subtypes and functional activity.[10,11] The term 5-HT_1-like receptor is therefore sometimes used in this setting.

5-HT_{1A} Receptors

The 5-HT_{1A} receptor is a 421 aminoacid structure. The observation that serotonin agonist activity at 5-HT_{1A} receptors inhibits synaptic activity in the rat

locus coerulus provides evidence that the 5-HT_{1A} receptor exhibits autoreceptor activity.[12] This receptor therefore appears to be located primarily at pre-synaptic nerve terminals where it inhibits neuronal transmission. DNA analysis of the 5-HT_{1A} receptor reveals areas of similarity to ß adrenoreceptors and this may explain mutual receptor agonists and antagonists. Cyclic adenosine monophosphate (cAMP) acts as a second messenger system at this receptor. Stimulation of 5-HT_{1A} receptors leads to a reduction in cAMP, K^+ channel activation and hyperpolarisation.[13] The primary locations of 5-HT_{1A} receptors are cerebral cortex, raphe nucleus and the hippocampus.

5-HT_{1B} Receptor

This 386 amino acid receptor has been characterised best in the rat, but is now recognised to be the rodent homologue of the 5-HT_{1D} receptor. It was initially believed not to exist in human brain[14], but subsequently has been recognised. The 5-HT_{1B} receptor is also negatively coupled to adenylate cyclase, reducing cAMP accumulation. Its autoreceptor activity reduces acetylcholine and noradrenaline release.

5-HT_{1C} Receptor

The 5-HT_{1C} receptor has not yet been convincingly demonstrated due to the lack of selective agents. Similarities however exist between the 5-HT_{1C} and 5-HT_2 receptors. There is a high degree of amino acid sequence homology and phosphatidylinositol acts as a second messenger for both. The two receptors however do not show similar orders of potency for agonists and antagonists.[16,17] For example, 5-HT is more potent in stimulating 5-HT_{1c} mediated responses and spiperone is approximately 1000 times weaker at 5-HT_{1c} receptors.[16]

5-HT_{1D} Receptor

This receptor was first identified in bovine brain in 1987 [18] and in humans in 1991.[15] In pig and human cortex it inhibits 5-HT release from its location on

pre-synaptic nerve terminals.[19] 5-HT_{1D} receptors are found in the substantia nigra, caudate, globus pallidus and intracranial blood vessels. This receptor is also negatively coupled to cAMP.

5-HT_{1E} Receptor

A fifth 5-HT_1-like receptor subtype has been cloned from human and rat brain.[11,20] Although sharing a degree of amino acid identity with other 5-HT_1-like receptors and being negatively coupled to adenylate cyclase, this receptor displays different binding affinities, confirming its separate identity. 5-HT_{1E} receptors are found in the cortex, hippocampus and striatum.

5-HT_2 Receptors

5-HT_2 receptors have well recognised, potent selective antagonists (ketanserin, mianserin and ritanserin). αmethyl 5-HT is a selective agonist at these receptors. 5-HT_2 receptors are widely distributed and therefore activity at these sites mediate a variety of complex actions. 5-HT_2 receptors are predominantly found in the cortex, arteries, veins, platelets and GI tract. As already discussed, phosphatidylinositol is the second messenger system.

5-HT_3 Receptors

5-HT_3 receptors act via Na and K^+ channels to depolarise membranes leading to neuronal excitation. The structure of these receptors is now known.[21] 5-HT_3 receptors exist in the brain and in enteric neurones. Antagonist activity at these sites, with drugs such as ondansetron and granisetron, proves efficacious in the treatment of nausea.

5-HT_4 Receptors

The 5-HT_4 receptor is a more recent discovery. It was first identified in 1989 in the brain [22] but is also located in the gastrointestinal system and myocardium. It is positively coupled to adenylate cyclase increasing local accumulation of

cAMP and depolarising cell membranes. A lack of selective agonists and antagonists at the 5-HT_4 receptor has curtailed expansion in our knowledge of it. 5-HT_4 receptor activation with cisapride however, results in release of acetylcholine in the myenteric plexus, probably by an action at a pre-synaptic site.[23] 5-HT_4 receptors therefore appear to play an important role in gastrointestinal motility. In addition, 5-HT_4 receptors have also been noted in the cerebral cortex and human right atrium. 5-HT_4 agonism on isolated human atrial cells elicits a positive inotropic response.[24,25]

5-HT_5 Receptors

The 5-HT_5 receptor has recently been cloned in rodent species. Subdivided into 5-HT_{5A} and 5-HT_{5B} both receptor subtypes exhibit low affinity for sumatriptan and serotonin, distinguishing these two receptors from the human 5-HT_1-like receptor.[26,27]

5-HT_6 Receptors

These receptors have also been cloned in rodent species and are positively coupled to adenyl cyclase.[28,29] It is thought they may be involved in brain development.

5-HT_7 Receptors

5-HT_7 receptors were first identified in 1993 from molecular cloning in rat[30] and human[31] brains. In humans this receptor is predominantly detected in the brain, but also in the coronary arteries and in the gastrointestinal tract.[31] The second messenger coupling system suggests that the 5-HT_7 receptor may be implicated in the mediation of vasorelaxation.

VASOACTIVE 5-HT RECEPTORS

5-HT receptors may influence the cardiovascular system by many diverse methods. Direct actions on vascular smooth muscle, indirect actions on

vascular endothelium or sympathetic nerve terminals, or more complex interplays with cardiovascular reflexes may all be implicated in changes in vascular tone. These issues are discussed below.

Direct vasoconstriction mediated by 5-HT_1 receptor stimulation

The presence of a 5-HT_1-like mediated vascular constriction was first demonstrated in the saphenous vein of the dog.[32,33] Subsequent work has demonstrated similar responses in a wide variety of arterial and venous preparations from many species including humans (See table 1.1).

5-HT_1-like receptors often co-exist however with 5-HT_{2A} receptors in vascular beds. In the absence of highly selective 5-HT_1 agonists therefore, it can be difficult to isolate individual receptor properties. The rabbit saphenous vein preparation appears to be an exception as contractions are mediated purely by 5-HT_1-like receptors.[34] The presence of occult functional 5-HT_1 activity may however be overlooked and underestimated. Several tissues may appear devoid of 5-HT_1 mediated vasoconstriction under resting conditions, but may elicit powerful responses when pre-stimulated with other vasospastic agents. For example, 5-HT causes isolated rabbit femoral artery rings to contract.[46] Spiperone and ketanserin (5-HT_2 receptor antagonists) antagonised these responses suggesting that the 5-HT response was mediated by 5-HT_2 receptors. A small component of the contraction (10%) was however 5-HT_1 mediated as it was resistant to spiperone and ketanserin. In the presence of the thromboxane A_2 mimetic U46619, angiotensin II or histamine however, responses became largely resistant to the effects of spiperone and about 60% of the total response to 5-HT was mediated by 5-HT_1-like receptors.[46] This suggests a synergistic effect between 5-HT_1-like agonists and thromboxane A_2, histamine and angiotensin II. Similar synergy has been observed in rabbit renal artery [47] and human coronary arteries.[45,48] The biochemical basis of this synergy is uncertain but may have some connection with the respective

receptor coupling systems as it occurs with other receptors negatively coupled to adenylate cyclase. Interestingly thromboxane A_2, histamine (H_1) and angiotensin II receptors are all positively coupled to the phosphoinositol signalling system.[46] This infers that cells already exposed to phosphoinositol drive may react differently on account of competing or complimentary influences. The potential importance of this synergistic phenomenon *in vivo* is obvious and should not be ignored when investigating *in vitro* responses.

Direct Vasoconstriction Mediated by 5-HT_2 Receptor Stimulation

Powerful vasoconstrictive effects of 5-HT have been observed in a wide range of vessels in many species (See Table 1.2). The availability of ketanserin, a potent 5-HT_2 antagonist, has allowed the functional role of 5-HT_2 receptors in these responses to be assessed. Evidence also exists from in vivo studies that 5-HT_2 receptors may influence total peripheral resistance via actions on resistance arterioles. Transient, 5-HT_2 receptor-induced hypertensive responses have been noted in the rat.[62,63] Intravenous α-methyl 5-HT (5-HT_2 agonist) produced dose-related increases in blood pressure in both DOCA-salt hypertensive and normotensive rats but the magnitude of response was greater in hypertensive rats.[62] These responses were dose-dependently antagonised by ketanserin confirming 5-HT_2 mediated vasoconstriction. In anaesthetised rats 5-MeODMT and TFMMP (partially selective 5-HT_1 agonists) produced hypotensive effects which were preceded by a ketanserin-sensitive increase in blood pressure.[63] These transient hypertensive response were therefore ascribed to 5-HT_2 receptor stimulation. In pithed rat preparations, these pressor responses are associated with an increase in total peripheral resistance.[64] Cats exhibit similar physiology with 5-HT induced pressor responses being antagonised by selective 5-HT_2 antagonists.[65,66] A different effect is observed in the dog however, with indirect 5-HT induced pressor effects mediated via 5-HT_2 receptor stimulated release of catecholamines from the adrenal medulla.[67,68]

5-HT_2 receptors overall appear to play a minor role in 5-HT induced changes in vascular resistance, presumably as they exist in greater density in conductance vessels. 5-HT_2 mediated vasospasm can occur, however, as demonstrated in the rabbit renal vascular bed where 5-HT_2 receptor activation produced flow limiting spasm.[69,70] The effect of 5-HT_2 receptor activation may be influenced by the presence or absence of atherosclerosis.[71-74] For example, in normal monkeys serotonin had little effect on mesenteric blood flow. In contrast, serotonin virtually abolished blood flow to the colon in atherosclerotic counterparts.[71] Also in monkeys, there was a greater than 10-fold increase in constrictor responses to serotonin in the hindlimb of atherosclerotics compared to normals.[73] This raises the possibility that, in atherosclerotic arteries, 5-HT_2 receptor activation may exacerbate already impaired vascular flow, such that a relatively benign vascular response may have more severe implications resulting in flow-limiting constriction.[71-74] Local 5-HT administration may even result in tissue ischaemia due to collateral circulation vasospasm in hind limb vessels of the rat[75], cat[76] and dog.[77]

5-HT RECEPTORS MEDIATING VASORELAXATION DIRECTLY

As previously observed, 5-HT administered *in vitro* results in prominent contractile responses on blood vessels. If however, it is administered slowly to anaesthetised animals, the net response is vasodilatation. The mechanism behind this was initially unclear, but more recently evidence for a direct vasorelaxant action has been identified. Histamine contracted sheep and goat pulmonary vein rings will relax in response to submicromolar concentrations of 5-HT.[78,79] These responses are resistant to morphine and dibenamine and it was concluded that neither "D" or "M" receptors mediated this vasodilatory effect. It is only relatively recently that the 5-HT receptor types involved have been identified as belonging to the 5-HT_1-like and 5-HT_4 classes.[80]

Smooth Muscle 5-HT_1-like Receptors

5-HT induced relaxation of tonically constricted isolated blood vessels has been widely reported [81] (Table 1.3) but in some cases, only becomes evident when 5-HT receptors which mediate vasoconstriction are simultaneously blocked.[9,82] In addition, many of the studies have been performed in the presence of intact endothelium and therefore some of the responses may be mediated via endothelial-derived relaxing factor (EDRF) rather than direct 5-HT actions on vascular smooth muscle. A number of endothelium denuded preparations have been studied and the evidence suggests involvement of the same receptor type in all tissues.[54,83-88] Although specific agonists and antagonists do not exist for this receptor, the rank order of potency for a number of agents [carboxamidotryptamine (5-CT) > 5-HT >>α methyl-5-HT (α-Me-5-HT) > sumatriptan] concurred with a single 5-HT_1 receptor subtype mediating direct vasorelaxation. This theory is reinforced by the resistance to block by selective 5-HT_2 (ketanserin) and 5-HT_3 (ondansetron, MDL72222) antagonists. Moreover methiothepin, a non specific 5-HT_1 antagonist, could block these responses at nanomolar concentrations.

The 5-HT_1 receptor mediating direct vasorelaxation shares many properties with, but is not identical to, the 5-HT_1 receptor mediating vasoconstriction. Minor differences in pharmacological properties have allowed clear distinctions to be drawn between the two receptor subtypes, and from other 5-HT receptors. The features which differentiate the 5-HT_1 receptor mediating vasorelaxation from that responsible for vasoconstriction are (i) No effect of sumatriptan (ii) poor agonist activity of α-Me-5-HT and (iii) antagonism mediated by spiperone (5-HT_2 antagonist). Equally there is no evidence to implicate this receptor as a correlate of any of the central 5-HT receptors. Several studies with a number of agents show that this 5-HT_1 receptor does not meet the recognised criteria for classification as 5-HT_{1A}, 5-HT_{1B}, 5-HT_{1C} or 5-HT_{1D}. This vasorelaxant receptor has therefore not been defined in terms of a CNS binding site.[84,88]

In keeping with other 5-HT_1 receptors, this receptor also appears coupled to cAMP as a second messenger system. In distinction to the others however, agonism at this receptor site increases cAMP production (positive coupling).[85,86,88] Cyclic AMP increases four fold in pig venacava in response to 5-HT and carboxamidotryptamine (5-CT). The time course of this rise is compatible with a vasorelaxant action.[88] In canine femoral veins and mesenteric arteries, this action appears to be associated with an enhanced K^+ efflux which may theoretically inhibit calcium influx. The exact mechanism here remains uncertain however.[89-91]

Smooth Muscle 5-HT_4 Receptors

A novel 5-HT receptor, (5-HT_4) positively coupled to adenylate cyclase was first described in brain tissue by Dumuis et al.[92] Its ubiquitous distribution has now been acknowledged through identification on peripheral autonomic neurones [93,94] G.I. smooth muscle [95,96], cardiac muscle [97-99] and vascular smooth muscle.[80] 5-HT_4 receptors are not antagonised by selective 5-HT_1, 5-HT_2 or 5-HT_3 receptor ligands.[95] The first recognised 5-HT_4 antagonist was tropesitron, an indole 5-HT_3 receptor antagonist [100], but more recently GR113808 has been utilised to radiolabel 5-HT_4 receptors in the brain.[101] The most potent agonist at the 5-HT_4 receptor is 5-HT itself, but no DNA clone for the receptor has been identified. The receptor is therefore characterised by rank order of potency and resistance to blockade by selective 5-HT_1, 5-HT_2 and 5-HT_3 antagonists (metergoline, ketanserin and ondansetron) respectively. Despite well documented cardio-active effects there is a paucity of reports describing vascular responses mediated by 5-HT_4 receptors. In guineapig [96,99] ,piglet [90] and human atrial tissues [97-102] positive inotropic or chronotropic responses are observed. 5-HT_4 receptor mediated vascular responses have only been observed in sheep pulmonary vein [80] where the potent relaxant effect of 5-HT was resistant to ketanserin (5-HT_2 antagonist), methysergide and methiothepin,

(non-specific 5-HT_1 antagonists/5-HT_2 antagonists) and MDL72222 (5-HT_3 antagonist). The responses, however, were competitively antagonised by high concentrations of tropesitron, as were responses to αMe-5-HT and a novel agonist BIMU8. Together with a lack of efficacy of sumatriptan and 2-Me-5-HT these results were in keeping with a 5-HT_4 mediated response. 5-CT however, also behaved as a potent agonist. Unlike 5-HT it was not sensitive to tropesitron, but was sensitive to methiothepin.

When high concentrations of tropesitron were applied, initial antagonism of 5-HT mediated relaxation was lost and sensitivity to methiothepin was then observed. The conclusion was therefore that 5-HT_1 and 5-HT_4 receptors co-exist in the sheep pulmonary vein and that 5-HT_1 phenomena only become apparent amid high local 5-HT concentrations.

5-HT Receptors Mediating Vasorelaxation Indirectly

In addition to directly mediated vasorelaxation, 5-HT can also induce vasorelaxation indirectly by stimulating release of EDRF. This process was first identified in canine and porcine coronary arteries [55] but has since been observed in pig venacava [103] and pulmonary arteries.[56] The jugular vein in a number of species also exhibits a similar phenomenon.[84,104-107] In all of these studies, the observed relaxation occurred despite tonic contraction of vessel preparations. The implication therefore was that EDRF release had been stimulated by 5-HT. Preparations with denuded endothelium are known to show augmented contractile responses to serotonin, but this may simply be explained by removal of inhibition of release of EDRF.[108,109] A clear distinction should therefore be drawn between studies demonstrating direct EDRF stimulation and those studies employing denuded endothelium preparations. Current evidence supports the premise that 5-HT-induced endothelial dependent relaxations are mediated by 5-HT_1-like receptors. Spiperone (5-HT_2), ondansetron (5-HT_3) and tropesitron (5-HT_4) failed to antagonise

endothelium-dependent responses in rings of coronary artery [110-112] and guinea pig jugular vein [107]. Methiothepin, however, exhibits potent antagonist activity and the order of agonist potency (5-CT > 5-HT > αMe-HT≥ sumatriptan >8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) > 2-Me-HT) suggests a 5-HT_1-like receptor profile.[107] Although this receptor is not identical to the 5-HT_{1D} receptor mediating vasoconstriction, major similarities cannot be overlooked. For example the potency of agonist drugs at these receptors follow the same order as the 5-HT_1-like receptor mediating vascular contraction. Furthermore, the second messenger characteristics appear to overlap. 5-HT-induced relaxations of the pig coronary artery are inhibited by pertussis toxin, consistent with coupling to a G protein and adenylate cyclase.[113] Negative receptor coupling to adenylate cyclase has already been described for the vasoconstrictive 5-HT_1 counterpart.[114,115]

The distribution and function of these 5-HT_1 receptors within the vascular system are poorly understood. Invasive haemodynamic studies with sumatriptan confirm vasoconstrictor effects, but these responses may be dampened by a simultaneous 5-HT_1 induced endothelium dependent relaxation. In anaesthetised beagles, the vasoconstrictor effects of sumatriptan are heightened when nitric oxide (NO) release is blocked.[116] The overall role of 5-HT_1 receptors in controlling the vasculature therefore remains unknown. A similar common identity for these two 5-HT_1 receptors is suggested, but the exact relationship requires further clarification.

Atypical 5-HT_1 Receptors on Vascular Endothelium

Before identification of the above described 5-HT_1-like receptor on vascular endothelium, the ability of 5-HT to effect endothelium dependent relaxations via an atypical 5-HT receptor had been observed in a number of species and tissues.[56,84,103-106] The ability of L-arginine analogues to inhibit such relaxation suggested that the receptor stimulated NO release. As is the case for the 5-

HT_1-like receptor on vascular endothelium, 5-HT_2 (ketanserin) and 5-HT_3 antagonists (MDL72222, ondansetron) are unable to block these responses, whereas methysergide, methiothepin and cyproheptadine are potent antagonists.[84,105] The pharmacological profile of agonists differs however, excluding a 5-HT_1-like characterisation.[117] A selective agonist (723C86) has been identified for this receptor, but no specific antagonist exists.[118] At present this atypical receptor does not have a formal allocation in the Bradley specification scheme.[4]

Isolated tissue studies have demonstrated the presence of this receptor in arteries and veins, but confirmation of an important vascular role is still awaited. Intravenous infusions of 723C86 in conscious rabbits produces no consistent change in systemic or regional haemodynamics.[119] Further studies are required to clarify the role of this atypical 5-HT receptor.

5-HT_3 Receptor Mediated Reflex Vasodilatation

Evidence exists for a serotonergic mechanism causing reflex vasodilatation. Human forearm [120,121] and cat intestinal [122] blood flow studies have recognised the involvement of 5-HT receptors in a vasodilator reflex. It has been proposed that mechanical stimulation of the intestinal mucosa releases 5-HT from enterochromaffin cells, which, via a neural reflex results in the secretion of vasoactive intestinal peptide, a known vasodilator hormone.[123] A role for an unknown 5-HT receptor has been suggested since dihydroergotamine, a substance with serotonergic properties, inhibits this response.[122] Low doses of 5-HT cause a rapid transient increase in blood flow which is abolished by the selective 5-HT_3 receptor antagonist, tropisetron, suggesting that 5-HT_3 receptors may be implicated.[121]

5-HT Receptors and Prostaglandin Secretion

5-HT has been observed to stimulate vascular smooth muscle synthesis and release of PGI_2 (prostacyclin) in cell cultures of bovine aorta.[124] Studies of antagonist potency demonstrate this phenomenon to be 5-HT_2 receptor mediated.[125] A similar 5-HT-induced prostacyclin release has been noted in intact blood vessels. In the dog saphenous vein, 5-HT responses were mimicked by α-Me-5-HT and blocked by ritanserin, again implicating a 5-HT_2 receptor.[126] In the rat aorta, however, methysergide, cyproheptadine and mianserin all were effective antagonists, but ketanserin was an exception.[127] The receptor subtype involved in the rat aorta is therefore a subject of conjecture, as is the premise that 5-HT receptor mediated release of prostacyclin plays a role in influencing vascular smooth muscle tone.

HEART RATE RESPONSES TO 5-HT AND RELATED DRUGS

Serotonin can elicit both bradycardic and tachycardic responses.

Bradycardia

In the presence of an intact CNS and vagus nerves, 5-HT will produce profound transient bradycardia in a variety of species. These responses can be eliminated by vagotomy, spinal section or ganglion blockade.[128,129] This suggests it originates from activation of afferent cardiac neurons (i.e. von Bezold-Jarisch-like reflex). Intravenous bolus injections of 5-HT, phenylbiguanide and 2-Me-5-HT (5-HT_3 agonist) in anaesthetised rats result in transient bradycardia sensitive to a number of 5-HT_3 receptor antagonists including MDL72222 and ICS205-390.[130-136] 5-HT also elicits bradycardia in the rabbit.[70,136,137] Phenylbiguanide (5-HT_3 agonist) and 5-carboximadotryptamine (probably by release of platelet 5-HT) can mimic this response [70,137] which is antagonised by MDL72222 [70], 2 naphthylguanidine and bufotenine [138], but not by ketanserin (5-HT_2 antagonist).[70]

Rapid bolus injections of 5-HT in the cat cause bradycardic responses sensitive to bilateral vagotomy, atropine and MDL72222.[139,140] When injected into the left ventricle[141], left coronary artery[142] or pericardial sac[143], a reflex bradycardia is also induced which is abolished by MDL72222 (5-HT_3 antagonist). Injections of 5-HT into the ascending aorta produce no bradycardic response.[141] The above evidence therefore supports a role for 5-HT_3 receptors in this cardiac sensory receptor reflex (von Bezold-Jarisch-like reflex).

Cardiogenic Hypertensive Chemoreflex

A cardiogenic hypertensive chemoreflex that increases efferent vagal and sympathetic nerve activity is induced by 5-HT in the dog. This leads to an initial brief slowing of the heart followed by tachycardia.[144,145]. A role for 5-HT_3 receptors is again supported since ICS205-930 could antagonise this cardiogenic hypertensive chemoreflex.[146]

Central 5-HT_{1A}Mechanism

8-OH-DPAT, a 5-HT_{1A} receptor ligand reduces heart rate in normotensive, spontaneously hypertensive and induced hypertensive rats. This response is antagonised partly by pimozide and fully by atropine plus propranolol, but methysergide, methiothepin and cinanserin among others have no effect.[147-149] Extensive studies by Fozard et al have demonstrated the bradycardic and hypotensive responses in spontaneously hypertensive rats to be centrally mediated, and that the central actions of 8-OH-DPAT are mediated by 5-HT_{1A} receptors.[150] In the cat, agents with 5-HT_{1A} agonist activity lower heart rate in contrast to 5-HT_{1B} agonists which have no effect.[151-157] These studies imply that bradycardia and hypotension result from increased vagal and decreased sympathetic tone secondary to central 5-HT_{1A} receptor activation, as discussed below.

Pre-Synaptic Inhibition of Sympathetic Activity

Preganglionic stimulation of sympathetic nerves in rats results in a positive chronotropic response which is inhibited by 5-methoxytryptamine via an unidentified receptor.[158] In the dog 5-HT can abolish electrically stimulated tachycardia.[159] Cyproheptadine is ineffective in blocking this response excluding a role for 5-HT_2 receptors.

Stimulation of Cholinergic Neurons

Isolated perfused hearts of reserpine treated rabbits become bradycardic when exposed to 5-HT. MDL 72222 abolishes this response, suggesting an influential role of 5-HT_3 receptors on post ganglionic cholinergic nerve endings.[132] It may be however that 5-HT_3 receptors are located on cardiac vagal ganglia as 5-HT_3 receptors have been identified on parasympathetic ganglia.[4,160]

TACHYCARDIC ACTION

A variety of mechanisms are responsible for 5-HT induced tachycardia which is a species dependent phenomenon. A discussion of these mechanisms follows:

Tyramine-like action:

Intravenous administration of 5-HT in the spinal guinea-pig results in tachycardia which is insensitive to ketanserin, MDL72222 or methiothepin, but is antagonised by ß adrenoreceptor antagonists and by the 5-HT uptake inhibitor indalpine. Pretreatment with reserpine failed to modify the first response to 5-HT but tachyphylaxis quickly developed to subsequent challenges.[161] Unlike the spinal guinea-pig, isolated guinea-pig atria displayed a reserpine insensitive tachycardic response to 5-HT.[162] The evidence therefore suggests that the chronotropic response to 5-HT in the guinea-pig heart arises from stimulation of catecholamine release in a manner akin, but not identical, to tyramine. Another possibility is that 5-HT stimulates the release of calcitonin gene-related peptide from the guinea-pig sinus node to accelerate the

heart rate.[73,163] In isolated dog atria the chronotropic response to 5-HT is attenuated by both desipramine and ß adrenoreceptor blockers, suggesting an indirect sympathomimetic action.[74,164,165] Similar conclusions have been drawn using high dose 5-HT *in vivo*.[166]

5-HT_1-like receptor stimulation

Trendelenberg demonstrated that lysergide can abolish 5-HT mediated tachycardia in cat atria, but that reserpine, dichlorisoprenaline and cocaine were all ineffective.[167] He concluded that this cardioactive action was mediated by the "D" 5-HT receptor which may belong to either 5-HT_1-like or 5-HT_2 type.[128] In kitten atria pre-treated by reserpine, the responses could be blocked by methysergide or phenoxybenzamine, but not by ketanserin, MDL72222 or yohimbine.[168] In cats with sectioned spinal chords, 5-HT induced tachycardia is only partially susceptible to blockade by guanethidine, propranolol, burimamide, 5-HT_2 receptor antagonists [132] or bilateral adrenalectomy despite the possibility that 5-HT may stimulate catecholamine secretion from the adrenal medulla.[162] This action of 5-HT is interrupted by mixed 5-HT_1 and 5-HT_2 receptor antagonists (methiothepin, methysergide) and mimicked by 5-CT to confirm that 5-HT_1-like receptors mediate tachycardia in the cat.[66,139,170]

5-HT_2 receptor stimulation

In pithed rats 5-HT (100 µg/kg) increases heart rate and blood pressure and these responses are effectively antagonised by cyproheptadine. In contrast, another 5-HT_2 receptor antagonist, R50656, suppressed the pressor response, but not the tachycardia.[171] In contrast to 5-HT, 5-CT fails to increase heart rate in ganglion blocked rats and the tachycardia induced by 5-HT is ketanserin and cyproheptadine sensitive, suggesting a 5-HT_2 receptor function.[172] 5-methoxytryptamine also elicits tachycardia, but 8-OH-DPAT and DOI have no effect on pithed, normotensive or spontaneously hypertensive rats. DOI exhibited partial agonist activity at 5-HT_2 receptors mediating pressor

responses but completely failed to influence heart rate or to antagonise 5-HT induced tachycardia.[173,174] The unexpected findings with DOI and R50656 point toward an "atypical" 5-HT_2 receptor modulating chronotropic responses in the rat. It cannot be ignored however, that 5-HT at high doses (100 µg/kg) may increase heart rate via a tyramine-like action sensitive to desipramine or propranolol.[175]

Serotonin induced tachycardia in anaesthetised dogs is widely reported.[176,177] A concomitant elevation of noradrenaline concentration in coronary sinus and venacaval blood is also observed, but this can be abolished by autonomic blockade.[166] Later work has shown that 5-HT induced tachycardia in vagotomised, cardiac decentralised and ganglion blocked dogs is accompanied by raised catecholamine blood levels which can be suppressed by cyproheptadine, methysergide, syrosingopine (a catecholamine depleting agent) and bilateral adrenalectomy (43% reduction).[158,159] 5-HT may therefore induce tachycardia by 5-HT_2 receptor-mediated catecholamine release from the adrenal medulla in addition to a tyramine-like effect.

5-HT_3 Receptor Stimulation

In isolated rabbit atria, 5-HT stimulates noradrenaline release, resulting in tachycardia. Extensive studies in perfused heart preparations have demonstrated that while reserpine, propranolol, cocaine, MDL72222 and ICS 205-930 (5-HT_3 antagonist) inhibit this tachycardia; desipramine, methiothepin and methysergide are without effect.[130,133,178,179] The indirect cardio-active effect of 5-HT in the rabbit is therefore mediated via 5-HT_3 receptors stimulating release of noradrenaline from post-ganglionic sympathetic nerve terminals. 5-HT_3 receptors also mediate tachycardia in conscious instrumented dogs.[180]

5-HT_4 Receptor Stimulation

In anaesthetised pigs, 5-HT elicits a tachycardia which is insensitive to drugs affecting autonomic receptors, histamine H_1 and H_2 receptors, dopamine receptors, calcium channels, 5-HT_1-like and/or 5-HT_2 and 5-HT_3 receptors.[181] Furthermore, selective agonists at 5-HT_1-like and 5-HT_3 receptors do not increase heart rate. Therefore, 5-HT-induced tachycardia in the pig is not mediated via endogenous catecholamines, histamine or calcium transport into the SA node, nor does it result from an action on 5-HT_1, 5-HT_2 or 5-HT_3 receptors.[181] 5-methoxytyptamine, renzapride and alpha-methyl 5-HT mimicked the effects of 5-HT on the pig heart, the responses being antagonised by ICS205-930. This evidence therefore implies that 5-HT-induced tachycardia in the pig is controlled by 5-HT_4 receptors. 5-HT_4 receptors also appear to play a role in human atria where a positive inotropic effect is observed.[24,25,97,182]

Unidentified Mechanisms

In hamster isolated atria, 5-HT increases heart rate probably by combination of a direct effect on the myocardium and an indirect effect via the liberation of noradrenaline from cardiac sympathetic nerves, since the responses are essentially unaffected by phentolamine plus propranolol or phenoxybenzamine.[183,184]

BLOOD PRESSURE RESPONSES TO 5-HT AND RELATED DRUGS

5-HT exerts a complex triphasic effect on arterial blood pressure consisting of rapid, intense but brief hypotension followed by a moderate hypertension and, finally, a more sustained hypotension.[185,186] The late sustained hypotensive effect is more potent than either the initial hypotensive effect or the pressor response and therefore the term serotonin is actually inconsistent with its action.[172] As holds for many 5-HT induced responses, the hypertensive phase varies largely depending on the species and experimental conditions. In the dog, particularly after ganglion blockade, the hypertensive phase is prominent

[67,187], whereas this phase is marginal in the rabbit [70,136,187], cat[139,187] or pigs.[188-190] These differences may be accounted for by distribution and population variations in 5-HT receptor subtypes in different species.

Initial Transient Depressor Response

The initial hypotensive response to 5-HT arises from an initial bradycardia with consequent reduction in cardiac output. This occurs secondary to stimulation of 5-HT_3 receptors on afferent cardiac vagal nerves. Some evidence however suggests a transient 5-HT_3 mediated vasodilatation which may arise from an axon-like reflex.[120]

Pressor Response

The pressor phase has been attributed to 5-HT_2 receptor effects as this phase is sensitive to ketanserin and related drugs. The activation of 5-HT_2 receptors may occur on the blood vessels as in the rat [131,172-174,186,191], and cat [66,139,170] or on the adrenal medulla to stimulate catecholamine release as occurs in the dog.[67,159] The contractile effects on arteries and veins are generally mediated by 5-HT_2 receptors, although in cranial blood vessels of different species (including humans) and in dog saphenous vein, 5-HT_1-like receptors also mediate vasoconstrictor responses.[33,39,40,57,192-198] In some vessels and species 5-HT_1-like and 5-HT_2 receptors appear to co-exist where they both mediate contractile responses. This appears true in the dog[33,39,52,195-197,199] and monkey.[39,40,196]

5-HT may also influence sympathetic nerve activity. Pontomedullary serotonergic neural connections and 5-HT_2 receptors exert an excitatory effect on pre-ganglionic sympathetic activity.[200] Whether these nerves have a tonic influence, however, is doubtful. The 5-HT-induced increase in sympathetic activity may also involve a 5-HT_1-like receptor. Intrathecal administration of 5-

CT (but not 8-OH-DPAT) in the rat increases sympathetic activity, an action which is not antagonised by ketanserin, MDL72222 or ICS 205930.[201]

Late Depressor Response

Selective 5-HT_2 or 5-HT_3 receptors fail to inhibit the late depressor effect of 5-HT. Methysergide and methiothepin can however abolish this response.[131,170,172,186] 5-CT can evoke a similar depressor response which is also blocked by methiothepin and methysergide.[170,172,190] Furthermore, there is good correlation between the hypotensive activity of tryptamine analogues and their affinity for the 5-HT_1-like receptor binding site.[191] The late hypotensive response therefore appears to be mediated via the 5-HT_1-like receptor, but a number of different mechanisms are involved depending on experimental preparation and species. These mechanisms include central vasomotor inhibition, vascular smooth muscle responses, inhibition of noradrenergic neurons and release of endothelium-derived relaxant factor (EDRF).

Central Vasomotor Inhibition

CNS injections of 5-HT are known to cause pressor, depressor or biphasic responses depending on the site of application, dose used, whether the animals were conscious or anaesthetised, normotensive or hypertensive.[200-207] 5-HT neuronal function appears to vary depending on the site within the CNS. Dorsal and median raphe, anterior hypothalamus and ventrolateral medullary raphe areas appear associated with pressor responses, whereas midline medullary raphe nuclei may elicit pressor or depressor actions.[207,208] Different 5-HT receptors seem to mediate the central pressor and depressor responses.[200]

A number of 5-HT_{1A} agonists have been observed to decrease arterial blood pressure and heart rate.[200] Urapadil is one of these agents, which although it also possesses α-adrenergic antagonist activity still relies on 5-HT_{1A} agonism for some of its effects.[209,210] The role of the CNS in 5-HT_{1A} mediated

cardiovascular responses has been confirmed in several studies. Urapidil, flesinoxan and 8-OH-DPAT when administered into the cat CNS decrease heart rate and blood pressure more potently than when administered intravenously.[153-155,200,210,211] 5-HT_{1A} receptor antagonism abolishes these responses.[63,150,153,200,209] Additionally, 8-OH-DPAT, flesinoxan and/or urapidil reduce sympathetic nerve activity [151,152,200,212,213] especially of the renal nerves [214], leading to renal vasodilatation.[215,216] Hypotension, bradycardia and a reduction in renal nerve activity has also been demonstrated to occur following 5-HT administration into the fourth ventricle of the cat [207] as has the centrally-induced increase in vagal tone in the rat.[205,207] 5-HT may also act at a spinal level, with 5-HT_2 receptors implicated in the inhibitory effect on sympathetic activity. Intrathecal α-Me-5-HT mimics this effect which is antagonised by ketanserin, but unaffected by prazosin, MDL72222 or ICS 205930.[201]

Inhibition of Noradrenergic Neurons

In the isolated dog saphenous vein 5-HT has a dual action on post-ganglionic sympathetic neurons. Low concentrations of 5-HT inhibit noradrenaline release following transmural stimulation, while higher concentrations initiate catecholamine release by a tyramine-like reaction.[217] The inhibitory effect of 5-HT and related drugs on transmitter release from post-ganglionic sympathetic nerves has been confirmed in various organs of different species including canine and human saphenous vein,[217,218] canine coronary [219] and carotid arteries[220,221] and rat kidney[222] vena cava[223]and heart.[158] The involvement of 5-HT_1-like receptors is suggested by the high agonist potency of 5-CT and 5-HT [222,223] and antagonism by methiothepin metergoline and methysergide [158,219,222,224] but not ketanserin, LY53857 or metoclopramide.[158,219.222] Obviously interference with sympathetic neurotransmission by 5-HT will modify direct cardiovascular effects. An example of this occurs in the canine external carotid vascular bed when 5-HT causes vasodilatation when sympathetic vascular tone is high and vasoconstriction when it is low.[220.225]

Vascular Smooth Muscle Responses and Release of EDRF

These two mechanisms by which vascular relaxation may be mediated by 5-HT have already been discussed earlier in this chapter.

SEROTONIN RECEPTORS ON CORONARY ARTERIES

Both 5-HT_1-like and 5-HT_2 receptors exist in human coronary arteries. As might be expected from the foregoing discussion, the role of these two receptors in the coronary circulation is equally complex. The actions of serotonin and related compounds on the coronary arteries may also vary depending on the presence or extent of atherosclerosis. In acute ischaemic syndromes (i.e. unstable angina and myocardial infarction) it is known that fissuring of an atherosclerotic plaque occurs with subsequent platelet aggregation and serotonin release. Serotonin may therefore play an important pathophysiological role in such situations. In patients with complex coronary artery lesions, elevated serotonin levels have been observed in the coronary sinus.[226] Plasma coronary samples are also capable of inducing canine coronary artery vasoconstriction *in vitro*, an action which is inhibited by serotonin receptor antagonists.[227]

In anaesthetised dogs, serotonin causes dose-dependent constriction of coronary arteries when the endothelium has been injured.[228,229] In conscious dogs with an intact endothelium, serotonin displays a biphasic response with an initial vasodilatation, followed by constriction.[230] Isolated coronary artery segments with an intact endothelium relax in response to serotonin but will contract following endothelial removal.[55,231]The presence of an intact endothelium (e.g. no atherosclerosis) therefore appears an important factor influencing the response to serotonin.

Human coronary artery responses

In vitro responses using human epicardial coronary artery rings from explanted hearts have shown that serotonin causes contraction.[43,44] The smooth muscle contraction was mediated by both 5-HT_1-like and 5-HT_2 receptor subtypes but the 5-HT_2 receptors were thought to be functionally more important as ketanserin partly antagonised these responses. A residual contraction (30% of the maximum effect) was 5-HT_1-like mediated.[44] It was also postulated that the relative effects of 5-HT_1-like and 5-HT_2 receptors may vary depending on the presence of coronary artery atheroma.

More recently Kaumann and colleagues reassessed the relative participation of 5-HT_1-like and 5-HT_2 receptors in human epicardial coronary arteries.[232] They found that both constrictor 5-HT_1-like and 5-HT_2 receptors existed in coronary arteries and that their relative ratio was not modified by atheroma. In addition, they reported the predominance of 5-HT_1-like over 5-HT_2 receptors in mediating serotonin-evoked contraction. In the same study they also confirmed selective 5-HT_1-like mediated contractions with sumatriptan.

Serotonin administration *in vivo* displays divergent effects on the coronary arteries.[233] In patients with normal coronary arteries serotonin acted as a vasodilator and increased coronary artery blood flow. This effect was potentiated by ketanserin, indicating that the vasodilator action of serotonin in normal human coronary arteries is non-5-HT_2 mediated (i.e. possibly 5-HT_1-like). In patients with coronary atherosclerosis, serotonin-evoked (ketanserin sensitive) vasoconstriction and reduced blood flow, suggesting that vasoconstriction arises secondary to direct 5-HT_2 activation.

In another study, intracoronary infusion of 5-HT in patients with angiographically normal coronary arteries caused a biphasic response in proximal segments, with dilatation at low doses and constriction at high doses.[234] In the same

study, intracoronary infusion of 5-HT in patients with coronary artery disease caused only vasoconstriction, leading to myocardial ischaemia. In patients with stable angina, ischaemia arose from distal (small) vessel constriction, whereas patients with variant angina displayed coronary artery spasm at doses of serotonin which dilate normal vessels. Ketanserin was unable to attenuate neither coronary artery vasospasm nor distal vessel constriction.[235] This observation suggests that activation of 5-HT_1-like receptors, either at hyper-reactive sites in patients with variant angina, or in the distal epicardial vessel of patients with chronic stable angina, may contribute to or cause myocardial ischaemia when 5-HT is released from aggregating platelets.

Two recent studies have confirmed an important role for 5-HT_2 receptors when vasoconstriction is observed during percutaneous transluminal coronary angioplasty (PTCA).[236,237] Following balloon dilatation, coronary artery vasoconstriction has been noted distal to the angioplasty site. The degree of vasoconstriction was related to the amount of serotonin released into the circulation[236] and was attenuated, at least partly, by ketanersin in both studies. It therefore must be assumed that both 5-HT_1-like and 5-HT_2 receptors are implicated in serotonin-induced human coronary artery vasoconstriction.

The clinical importance of 5-HT_1-like receptors in mediating coronary artery vasoconstriction has been highlighted with the use of sumatriptan, a selective 5-HT_1-like agonist, in the treatment of acute migraine. Isolated case reports of coronary spasm with angina [238], myocardial infarction [239] and ventricular fibrillation[240] following sumatriptan have raised concerns over its use in patients with ischaemic heart disease. Coronary artery vasoconstriction following intravenous[241] and subcutaneous[242] sumatriptan has been observed, but detailed ECG monitoring following sumatriptan administration revealed that 99% had no ECG changes.[243] 5-HT_1-like agonism with sumatriptan may therefore unmask underlying coronary artery disease.

Despite initial claims of selective intracranial vasoconstriction, sumatriptan has also been shown to exert vasopressor responses on the systemic and pulmonary circulation.[241,242] A relative percentage increase in pulmonary artery pressure greater than the change in systemic arterial pressure by a factor of 2-3 may suggest greater density of 5-HT_1-like receptors in the pulmonary compared to the systemic circulation.[242] The number of patients, however, was small and may therefore not be truly representative. The effects of 5-HT_1 agonism on left ventricular function *in vivo* have not been studied.

SUMMARY

The effects of serotonin on the cardiovascular system are therefore complex, comprising tachycardia or bradycardia, hypotension or hypertension and vasoconstriction or vasodilatation. The eventual response depends on a number of factors including species, vascular bed under study, and dose employed. As the effects of serotonin are receptor mediated, the net effect will rely on the relative densities of 5-HT receptor subtype population within the vascular bed under study. The lack of suitably selective antagonists and agonists has limited progress in the investigation of serotonin-mediated responses. Present knowledge implies that 5-HT acts on seven main classes of receptors, but these subtypes are defined in terms of functional pharmacology rather than agonist/antagonist potency orders. Increased understanding of the roles of each receptor can only be expected when more selective agents become available.

The physiological role of 5-HT remains controversial, with a plethora of *in vitro* findings which may bear no clinical or *in vivo* relevance. The pathophysiological role for 5-HT has however been identified in a number of clinical settings namely, migraine, acute myocardial ischaemia and nausea/vomiting. Heightened awareness of 5-HT pharmacology may promote further therapeutic interventions in these areas.

This thesis will investigate some aspects of the cardiovascular actions of serotonergic agents, looking particularly at the role of 5-HT_1-like and 5-HT_2 receptors *in vivo*. The specific aims are listed below.

AIMS

1. To investigate the mechanism of the rise in pulmonary artery wedge pressure following sumatriptan injection and determine whether this is secondary to an acute increase in afterload or due to negative inotropic effects.

2. To examine the possibility that sumatriptan has a greater 5-HT_1 induced vasopressor response in the pulmonary compared to the systemic circulation.

3. To assess whether systolic time intervals may be a potential non-invasive tool to investigate the haemodynamic effects of 5-HT_1 agonists.

4. To assess whether the 5-$HT_{1B/D}$ agonist, naratriptan has a similar in vivo pharmacological profile to sumatriptan.

5. To investigate the haemodynamic effects of eletriptan, a 5-HT_{1D} agonist with limited 5-HT_{1B} activity, and comment on whether the absence of 5-HT_{1B} activity potentially alters drug pharmacodynamics.

6. To examine whether sumatriptan induces 5-$HT_{1B/D}$ vasoactive effects on the peripheral circulation using forearm blood flow plethysmography.

7. To investigate the effect of sumatriptan on plasma noradrenaline levels.

8. To assess whether ketanserin, a 5-HT_2 antagonist, has vasodilator properties on the coronary arteries and if so, to infer on the possibility of basal serotonergic tone in patients with stable angina symptoms.

ARTERY/VEIN	SPECIES	REFERENCE NO.
Saphenous Vein	Rabbit	34
	Human	35,36
Basilar Artery	Sheep	37
	Dog	38,39
	Primate	38,39
	Human	40
Umbilical Artery	Human	41
Pulmonary Artery	Human	42
	Bovine	42
Coronary Artery	Human	43-45

Table 1.1 Tissues in which 5-HT_1-like receptors mediate vascular contraction

ARTERY/VEIN	SPECIES	REFERENCE NO.
Rabbit	Aorta	49
	Carotid Artery	50
Dog	Carotid Artery	51
	Femoral Artery	51
	Gastrosplenic Vein	52
Cat	Middle Cerebral Artery	53
	Saphenous Vein	54
Pig	Carotid Artery	50
	Coronary Artery	55
	Pulmonary Artery	56
Rat	Basilar Artery	57
	Jugular Vein	58
	Mesenteric Vein	59
Human	Femoral Artery	60
	Temporal Artery	61
	Coronary Artery*	43

* Part contribution from 5-HT_1-like receptors

Table 1.2 Tissues in which 5-HT_2 receptors mediate vascular contraction.

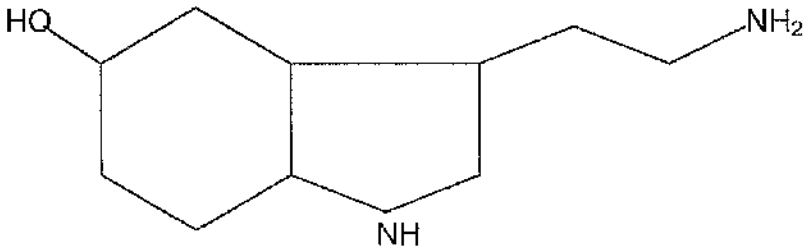
SPECIES	VEIN	RECEPTOR TYPE	REFERENCE
Cat	Saphenous vein	5-HT_1-like	54
Rabbit	Jugular vein	5-HT_1-like	84,105
Neonatal Pig	Vena Cava	5-HT_1-like	85,86
Sheep	Pulmonary Vein	5-HT_1-like + 5-HT_4	78,80

Table 1.3 Tissues in which 5-HT mediates vasorelaxation directly.

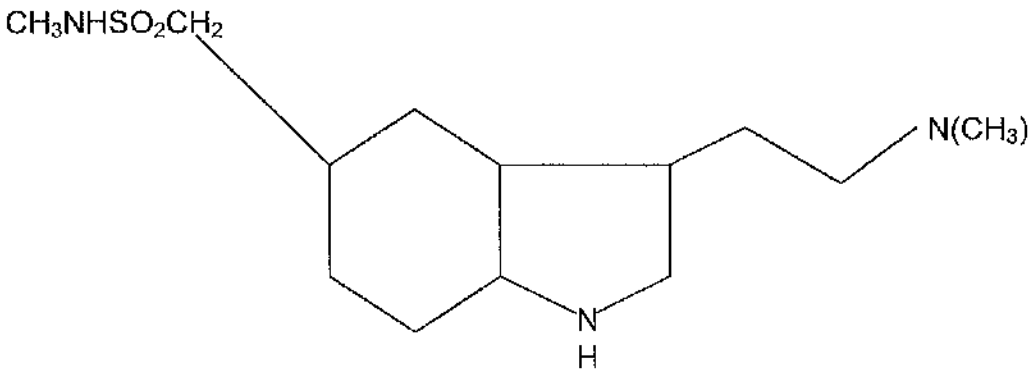
RECEPTOR	AGONIST	ANTAGONIST
5-HT$_{1A}$	5-HT 8-OH-DPAT Urapidil	Methiothepin Methysergide Metergoline WAY 100135
5-HT$_{1B}$	5-HT 5-CT Sumatriptan Naratriptan	Methiothepin Metergoline
5-HT$_{1C}$	5-HT α-Methyl-5-HT	Methysergide Methiothepin Metergoline Mesulergine
5-HT$_{1D}$	5-HT 5-CT Sumatriptan Naratriptan Eletriptan	Methiothepin Methysergide Metergoline
5-HT$_2$	5-HT α-Methyl-5-HT Mianserin	Ketanserin Ritanserin Spiperone Methiothepin Metergoline Mesulergine Methysergide Cyproheptadine
5-HT$_3$	5-HT 2-Me-5-HT	Ondansetron Granisetron Tropisetron MDL 72222 ICS 205-930
5-HT$_4$	5-HT Cisapride	GR113808 Tropesitron ICS 205-930

5-CT= Carboxamidotryptamine

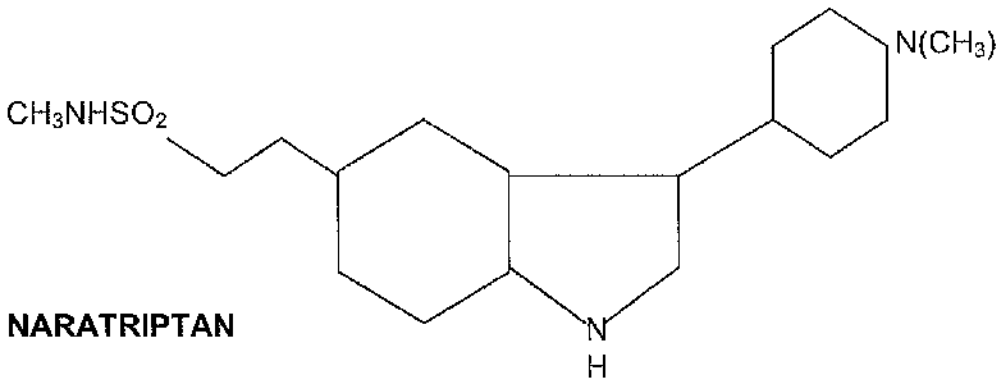
Table 1.4 Serotonin receptor agonists and antagonists.



5- HYDROXYTRYPTAMINE



SUMATRIPTAN



NARATRIPTAN

Figure 1.1 Structural formulae of 5-hydroxytryptamine, sumatriptan and naratriptan.

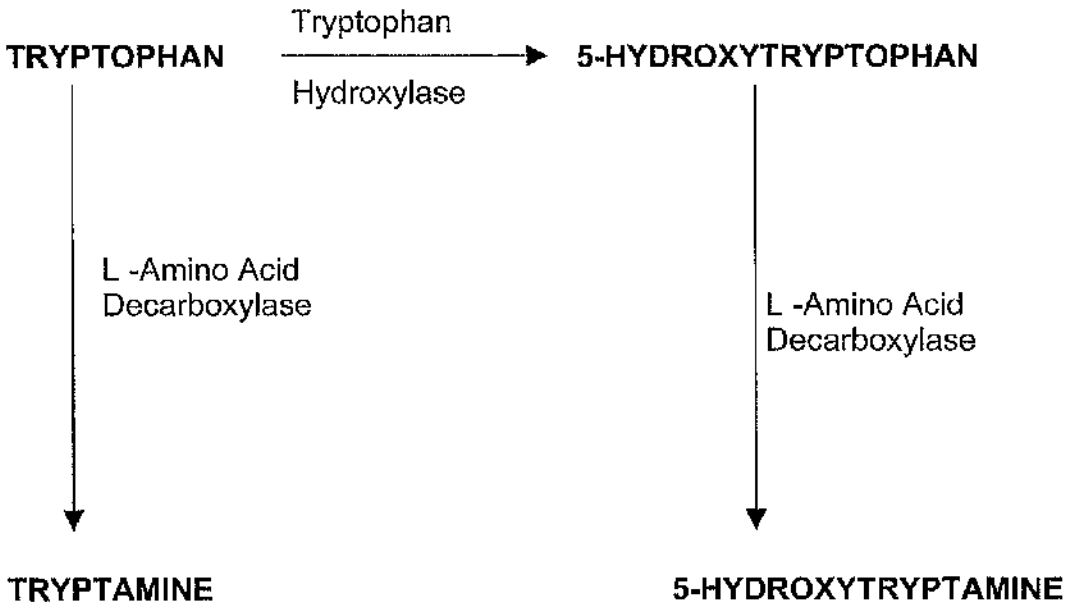


Figure 1.2. Synthesis Of 5-Hydroxytryptamine (5-HT)

CHAPTER 2

METHODS.

METHODS

Patient Groups

All patients in this thesis were studied during coronary arteriography performed for diagnostic purposes with a view to selection of patients for coronary intervention by angioplasty or coronary artery bypass surgery. All vasoactive therapy other than sublingual glyceryl trinitrate was discontinued 24 hours before study.

Standard exclusion criteria were used to exclude women of child bearing potential and patients with an unstable clinical substrate including myocardial infarction within 3 months, unstable angina, cardiac arrythmias and hypertension (diastolic BP >95 mmHg). Patients with a coronary artery stenosis >50% were excluded from the studies involving sumatriptan, eletriptan and naratriptan on account of potential coronary vasoconstrictor properties of 5-HT_1 agonists. Patients in the ketanserin study were not subject to exclusion on the basis of coronary artery anatomy. Ketanserin can however prolong the QT interval particularly in the presence of hypokaleamia. Patients were therefore not enrolled if they had a prolonged QT interval or were concurrently taking potassium-losing diuretics.

The Ethics Committee of The West Glasgow Hospitals University NHS Trust approved studies. Each patient was issued an appropriate information sheet and written informed consent was obtained.

Central Haemodynamic Monitoring

Routine left ventricular angiography and selective coronary arteriography were performed by the conventional Judkin technique. After the diagnostic procedure, a pigtail catheter was retained in the aorta to measure systolic and diastolic blood pressures, and in turn could be introduced into the left ventricle (LV) to measure LV systolic and end diastolic pressures (LVEDP). To allow the calculation of peak rate of left ventricular pressure rise ($\delta p/\delta t$), the left ventricular pressure tracing was expanded to 100mm.s^{-1} and digitised.[244] To measure pulmonary artery systolic (PASP) and diastolic (PADP) pressures, right atrial pressure (RAP) and occluded wedge pressures (PAWP), a 7f balloon flotation catheter (Swan Ganz) was positioned in the pulmonary artery. Hard copies of systemic, pulmonary and left ventricular pressure tracings were obtained at baseline and at 10 minute intervals until stable. Following administration of placebo, pressures were recorded. Thereafter active drug was administered and pressure recordings were obtained at regular intervals for 15-40 minutes depending on the individual protocol for each drug.

Cardiac outputs were measured by the thermodilution technique, a mean value of at least 3 measurements at each timepoint. The total systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) were calculated from the undernoted formulae.

$$SVR = \frac{80(A-RA)}{C.O.} \text{ dyne.s.cm}^{-5}$$

A = mean arterial pressure (mmHg)
RA = mean right atrial pressure (mmHg)

$$PVR = \frac{80(PA-PAW)}{C.O.} \text{ dyne.s.cm}^{-5}$$

PA = mean pulmonary artery pressure
PAW = mean pulmonary artery wedge pressure (mmHg)

As drug administration was performed on an open basis in all studies, pressure tracings were analysed by a blinded observer.

Systolic Time Interval Estimation

The systolic time intervals (STI's) were measured from a simultaneous recorded electrocardiogram and aortic pulse wave tracings (Figure 2.1). This allowed the calculation of total electromechanical systole (EMS) from the onset of the QRS to the incisural notch on the arterial tracing. The left ventricular ejection time (LVET) was measured from the upstroke of the arterial tracing until the trough of the incisural notch. The pre-ejection period (PEP) was derived by subtracting the LVET from the total electromechanical systole. This represents the interval from the onset of electrical depolarisation until the onset of mechanical ejection. The ratio of the PEP over LVET was calculated (PEP/LVET). All STI values were then corrected for heart rate according to the regression formula in table 2.1.

Quantitative Coronary Angiography

After base-line measurements, placebo and active drug were administered via an intravenous cannula in the right antecubital fossa (ketanserin, eletriptan) or subcutaneously into the deltoid region of the right arm (naratriptan). Quantitative angiography was performed on right and left coronary arteries. The chosen artery was determined after visualisation of the coronary artery system in the standard views. A projection was chosen that allowed simultaneous visualisation of the coronary artery system and the diagnostic 6 French catheter which was used for reference calibration. Serial angiograms were obtained with a Siemens angioscope C arm. X-ray tube to patient distance was fixed to maintain identical magnification. Non-ionic contrast medium (Ultravist) 4-7 ml was injected by hand into the coronary artery under study. Recordings of the

angiograms were stored on digital tape and analysed on a Siemens Angiographic Workstation (AWOS v4.0) or on a Cardiotrace software package with automatic edge detection. End diastolic frames from the third or fourth cardiac cycle were stored for analysis and several corresponding segments were identified along the lengths of the artery. Two blinded observers measured coronary artery diameter at these sites using the automated edge detection programme. Absolute values were obtained by using the 6 French catheter diameter (1.98mm) as a calibration factor. Stenotic areas in the arteries were also identified and the stenotic index (percentage) automatically calculated by the computer.

Reproducibility Study

Reproducibility of the methodology to measure coronary artery dimensions was assessed in 15 additional patients (8M, 7F) undergoing diagnostic coronary angiography. Immediately after the diagnostic angiogram, a second angiographic run was performed in a manner identical to that described above. Angiograms were stored on digital tape and coronary artery dimensions were measured by 2 observers on the software analytical system. A total of 53 coronary artery segments were measured (see table 2.1), with a mean (sd) diameter of 3.2 $\pm$ 0.6 mm. Mean (sd) intraobserver variability was 0.13 $\pm$ 0.13mm (subject 1) and 0.13 $\pm$ 0.12mm (subject 2). Mean interobserver variability was 0.14 $\pm$ 0.15 mm. The coefficient of variation was calculated for each observer using the technique described by Bland.[245] The intra-observer coefficients of variation were 5.3% (observer 1) and 4.8% (observer 2). The inter-observer coefficient of variation was 5.2%.

STUDY LIMITATIONS

Patient numbers

The individual studies in this thesis involved small patient numbers and this may have resulted in type 1 or type 2 errors. In those studies, which failed to show a significant change in coronary artery diameter, the possibility of type 2 errors should not be overlooked. Due to catheter laboratory constraints, coronary artery protocols were restricted to 10 patients. Assuming a 20% change in coronary artery diameter to be clinically relevant, this would require approximately 18 patients to detect such a change with 90% probability however.

Patient selection

It is important to recognise that the responses to each of the serotonergic agents in these studies are likely to have been influenced by by the functional state of the endothelium. Patients with modest coronary artery atherosclerosis are likely to have dysfunctional endothelium and could therefore respond differently to subjects with completely normal coronary angiograms. Other factors also associated with endothelial dysfunction include cigarette smoking, hypercholesterolaemia, diabetes and the post-menopausal state. The prevalence of factors predisposing to endothelial dysfunction in each of the chapters is discussed below but it is impossible to say with certainty that the endothelium was dysfunctional in all of these patients as assessment of endothelial dependent relaxation was not made for each individual. This is an important limitation as many of the subjects in this thesis may fall into the category of Syndrome X which is associated with angiographically normal coronary arteries but endothelial abnormalites. In addition, smoking status was

unknown in a number of patients and some of the earlier studies were performed prior to widespread assessment of serum cholesterol in patients with suspected coronary artery disease and therefore cholesterol levels were not recorded for all individuals.

Chapter 3:- A total of 5 patients (50%) had known factors predisposing to endothelial dysfunction. Two patients had minor coronary artery disease and a further 3 were post-menopausal women not on hormone replacement. Cholesterol levels and smoking status were not known for these patients and there were no diabetics.

Chapters 4 & 5:- Nineteen of 28 patients (68%) had known potential causes of endothelial dysfunction. This included 14 patients with mild coronary artery disease. Smoking status was not documented in all patients. Ten patients were post-menopausal of which 5 also had mild coronary artery disease. Cholesterol levels were unknown and there were no diabetics.

Chapter 6:- Eight of 10 patients had factors predisposing to endothelial dysfunction. One patient had minor coronary artery disease, 2 patients were current smokers and 4 patients were post-menopausal. One further patient was diabetic, post-menopausal and hypercholesterolaemic.

Chapter 7:- Six of ten patients (60%) had known potential causes of endothelial dysfunction. Two patients had mild coronary artery disease. Four patients were post-menopausal of which 3 and 2 respectively were also hypertensive and

hypercholesterolaemic. Two further patients were post-menopausal but were on hormone replacement. Smoking status was not recorded.

Chapter 8:- Nine of ten patients (90%) had factors predisposing to endothelial dysfunction. Eight had coronary artery disease (4 of which were current smokers, a further 3 were post-menopausal and 3 were hypercholesterolaemic). One patient with normal coronary arteries was a diet controlled diabetic and hypercholesterolaemic.

Chapter 9:- All subjects in this chapter were normal healthy volunteers with no known factors predisposing to endothelial dysfunction.

METHODOLOGICAL LIMITATIONS

Limitations of $\delta P/\delta t$ measurement

The use of fluid filled catheters/ transducer systems to measure haemodynamic pressures has inherent sources of error.[246] Accurate measurement of pressure waves requires a frequency response range of 20Hz but commonly used pressure measurement systems with 6F catheters attached to standard strain gauge manometers have frequency responses less than 10Hz. In addition, motion of the catheter within the left ventricle or against the wall of the ventricle can cause artifact. Micromanometer catheters, which have a pressure transducer mounted at the catheter tip, have superior harmonic properties and are therefore less susceptible to measurement error. Ideally therefore, a micromanometer system for $\delta P/\delta t$ measurement should have been employed but this would have had both cost and time implications (micromanometers require meticulous calibration).

Other possible invasive methods of measuring LV function during this study would include measuring LV volumes, stroke volume and ejection fraction in response to placebo and sumatriptan injection. This would however involve repeated left ventricular angiography which is not without risk (eg. air embolus, excessive x-ray contrast and ventricular dysrhythmias). Ejection fraction could be measured non-invasively by radionuclide ventriculography but this would obviously entail radiation exposure. Trans-thoracic echocardiography can quantify LV ejection fraction but this requires high quality recordings with precise visualisation of the endocardial border which is not possible in all subjects.

Limitations of STI methodology

The technique for measuring systolic time intervals in this study was unconventional and could be criticised. The intention was to assess whether STI may represent a potential non-invasive method of assessing the cardiovascular effects of serotonergic drugs and this would obviously require confirmation using conventional non-invasive approaches. The use of fluid filled catheters/ transducers to measure aortic pressures is open to the same criticism applied to that for $\delta p/\delta T$ assessment (see above). In addition, the inherent transmission delays through the catheters results in the absolute values of the STI's being slightly longer than those obtained externally. Nonetheless a directional change may be evident.

Using the convential technique of simultaneous electrocardiography, phonocardiography and carotid pulse wave tracings would provide more accurate absolute values for the STI. This however, relies upon precise

identification of a) the onset of ventricular depolarisation on the ECG b) the early high frequency components of A2 and c) the incisural notch on the carotid tracing, which are all common sources of error.

Limitations of venous occlusion plethysmography methodology

In conventional venous occlusion plethysmography studies, active drug is given intra-arterially and the contralateral arm is used as a simultaneous control for forearm blood flow. In chapter 9 however, sumatriptan was administered subcutaneously and therefore two study days were required. Although strenuous efforts were made to standardise experimental conditions, it is possible that biological variation may have occurred between both study days and this may have influenced results. Problems with reproducibility of measurement of forearm blood flow have been described but coefficients of variation of 10-15% for this technique have been reported.[247,248]

Segment	Observer 1	Observer 1	Observer 2	Observer 2
	Run 1	Run 2	Run 1	Run 2
1	3.7	3.3	3.3	3.0
2	3.1	3.0	2.8	2.6
3	3.1	2.9	3.1	3.0
4	3.1	3.0	2.9	3.3
5	3.0	3.2	3.0	3.0
6	5.2	5.9	5.1	5.6
7	3.2	4.0	3.0	3.4
8	2.1	2.0	2.0	2.2
9	3.7	3.9	3.7	3.9
10	4.8	4.6	4.6	4.9
11	3.2	3.1	3.2	3.2
12	2.5	2.4	2.5	2.7
13	2.6	2.5	2.7	2.6
14	3.7	4.0	3.9	4.1
15	3.0	3.1	2.6	2.4
16	2.9	2.7	2.9	2.9
17	2.3	2.4	2.4	2.3
18	1.7	1.9	2.0	1.9
19	3.8	3.9	3.9	4.0
20	4.3	4.5	4.4	4.4
21	3.4	3.4	3.4	3.5
22	2.2	2.4	2.1	2.5
23	2.5	2.5	2.4	2.8
24	2.1	2.1	2.1	2.4
25	3.1	3.0	3.1	3.0
26	3.5	3.6	3.5	3.8
27	1.9	1.8	1.9	1.7
28	3.4	4.0	3.4	4.0
29	3.0	2.9	3.2	3.1
30	2.4	2.2	2.4	2.6
31	4.1	4.0	3.9	3.9
32	2.1	2.0	2.1	2.0
33	3.6	3.6	3.4	3.5
34	3.3	3.3	3.3	3.3
35	3.3	3.3	3.3	3.3
36	2.4	2.5	2.6	2.5
37	1.8	1.9	1.7	1.9
38	2.3	2.4	2.2	2.1
39	4.3	4.4	4.3	4.4
40	4.0	4.1	4.1	4.0
41	5.1	4.9	5.0	5.0
42	3.4	3.8	3.7	3.5
43	3.4	3.5	3.7	3.5
44	3.0	2.9	2.9	2.7
45	3.5	3.2	3.5	3.0
46	3.8	4.2	3.8	4.4
47	3.8	3.7	3.6	3.7
48	3.5	4.0	3.7	4.0
49	2.9	2.8	3.0	2.8
50	2.0	1.8	1.9	1.9
51	3.3	3.5	3.2	3.2
52	3.3	3.4	3.2	3.2
53	3.6	3.6	3.8	3.6

Table 2.1 Coronary artery diameters (mm) for the reproducibility study.

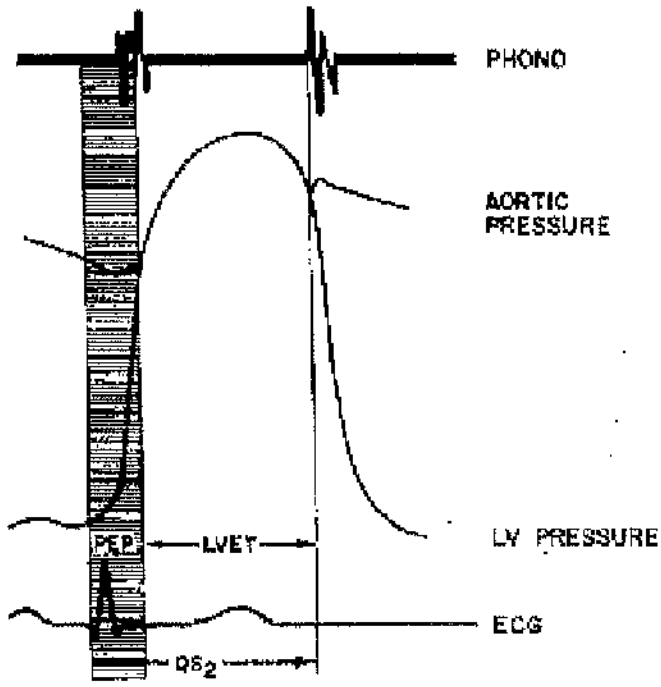


Figure 2.1: Simultaneous tracing of electrocardiogram, phonocardiogram, left ventricular and aortic pressure tracings. LVET= left ventricular ejection time, PEP= pre-ejection period, QS_2= total electromechanical systole.

CHAPTER 3

THE HAEMODYNAMIC EFFECTS OF SUMATRIPTAN, A SELECTIVE 5-$HT_{1B/D}$ AGONIST.

Serotonin, sumatriptan and their role in migraine.

Sumatriptan, a selective 5-$HT_{1B/D}$ receptor agonist, is the prototype and first clinically available 5-HT_1 agonist, which is effective in the treatment of acute migraine. Fifty years ago, serotonin (5-HT) was isolated and identified as a vasoconstrictor substance.[1] Since then the effects of serotonergic compounds on the cardiovascular system have been extensively studied. During the 1960's evidence accumulated that 5-HT played a role in migraine. Sicuteri showed that methysergide, a non specific 5-HT antagonist could reduce the frequency and severity of migraine headache.[249] Kimball et al subsequently relieved acute migraine pain by intravenous injection of 5-HT.[250] Intravenous serotonin however, was associated with side effects of nausea, dyspnoea, faintness and paraesthesia. Urinary 5-hydroxy indole acetic acid (5-HIAA), the main metabolite of 5-HT was later found to be elevated during acute migraine.[251]

In 1983 it was reported that dilatation of the frontal branches of the superficial temporal artery contributed to the pain of acute migraine in one third of patients and, in a further third of patients, vasodilatation of the carotid circulation was implicated.[252] The search for a 5-HT agent which could abolish these vasodilator responses and thereby relieve migraine pain without unacceptable side effects was therefore stepped up. Sumatriptan (GR43175) is an analogue of 5-HT with selectivity for the 5-HT_1 receptor subtype.[36,39,40,111,253] This receptor occurs in a number of vascular beds in several species and agonist activity is known to cause vasoconstriction *in vitro* [34-45] (see table 1.1).

Of clinical relevance for the acute treatment of migraine, sumatriptan constricts human isolated basilar artery.[40] More interestingly, a selective vasoconstriction

has been observed in the carotid circulation of the anaesthetised cat[193] and dog.[254] In the beagle dog, intravenous sumatriptan produced a dose-dependent rise in carotid vascular resistance with negligible effect on heart rate or blood pressure suggesting a selective action on the carotid circulation.[254] Similarly, sumatriptan and ergotamine caused dose-dependent reduction in cardiac output through arteriovenous anastamoses.[193] Whereas ergotamine caused increases in diastolic BP and total peripheral resistance, sumatriptan did not, again implying that sumatriptan had a selective action on the carotid vasculature. There is therefore sound rationale for the use of sumatriptan in acute migraine.

The efficacy of sumatriptan in acute migraine and cluster headache has been evaluated in a number of large double blind controlled trials.[255-258] Response rates in the order of 60-80% can be expected after subcutaneous sumatriptan but the mechanism of action is uncertain. Friberg et al have reported migraine pain associated with middle cerebral artery dilatation.[259] This could be reversed by sumatriptan suggesting that vasodilatation is responsible for the headache and that sumatriptan induced vasoconstriction results in relief of symptoms. There is evidence however that the anti-migraine activity of sumatriptan relates not only to vascular receptor properties but also on an ability to modulate release of certain neuropeptides responsible for neurogenic inflammation (plasma protein extravasation and vasodilatation).[260.261]

Cardiovascular Side Effects of Sumatriptan

The side effect profile of sumatriptan is favourable. Given subcutaneously, adverse events such as tingling (9%), pressure sensation (6%), flushing (6%)

and tightness (3%) occur more commonly than with placebo.[262] The mechanism of these side effects is uncertain. Life-threatening side effects have however occurred following administration of sumatriptan. Isolated case reports of coronary spasm with angina,[238] myocardial infarction [239] and ventricular fibrillation[240] together with the 3-6% incidence of chest pressure and tightness have raised cardiovascular safety concerns.

These adverse events suggest that the effects of sumatriptan are not confined to the intracranial circulation in humans. There is evidence that this is indeed the case. Systolic and diastolic blood pressure rose by a mean of 12 and 10mmHg respectively after subcutaneous administration of sumatriptan.[263] The effect was evident within 10 minutes of administration and persisted for 30-60 minutes. Vasoconstrictor responses have been noted from invasive recordings of systemic and pulmonary arterial pressure following intravenous [241] and subcutaneous [243] sumatriptan in patients undergoing diagnostic cardiac catheterisation.

In these two studies, systemic arterial pressure rose by 16-20% with 40-58% increases in mean pulmonary arterial pressure (MPAP). Furthermore there was a 14-16% reduction in coronary artery diameter implying an additional vasoconstrictor response in the coronary circulation. An additional rise in the pulmonary arterial wedge pressure (PAWP) was also observed after sumatriptan. The increase in PAWP was postulated to be secondary to pulmonary venoconstriction but direct measurement of the left atrial or left ventricular end diastolic pressure (LVEDP) was not made and therefore a

reduction in left ventricular contractility could not be excluded as the cause of the rise in PAWP.

The effect of sumatriptan on standard haemodynamic parameters was therefore investigated further in 10 patients undergoing diagnostic cardiac catheterisation, paying particular attention to the possibility of a negative inotropic action. Simultaneous measurement of the PAWP and LVEDP was performed and the peak rate of left ventricular pressure rise ($\delta P/\delta t$) was also established allowing the mechanism of the rise in PAWP to be more fully evaluated.

METHODS

Patient Group

Ten patients (3 male, 7 female, mean age 49+/- 9 years) were studied. Exclusion criteria and description of the methodology were as reported in chapter 2 (pages 49-50). Table 3.1 summarises the study protocol.

TIME (mins)	-10	0	10	20	30	40	50	60
Coronary angiogram	*							
Placebo injection		*						
Sumatriptan 6mg s.c.				*				
6 lead ECG	*	*	*	*	*	*	*	*
Cardiac Haemodynamics	*	*	*	*	*	*	*	*

Table 3.1. Study protocol: The haemodynamic effects of sumatriptan.

Data analysis

A baseline value was obtained for each haemodynamic parameter by averaging two observations. As any response occurred within 10 minutes and persisted for the 40 minute study period, the average of the haemodynamic recordings at 10, 20, 30 and 40 minutes after sumatriptan injection were used to summarise the response to sumatriptan.

Statistics

Post-placebo and post-sumatriptan results were compared with baseline measurements using a paired t-test/confidence interval as the data appeared normally distributed.

RESULTS

Results are summarised in table 3.2 and in figures 3.1 to 3.6.

Electrocardiography

There were no changes in the duration of standard ECG intervals (PR,QRS, QT and QTc) and no changes in ECG morphology as assessed from the hard copies of six lead ECG's taken at 10 minute intervals throughout the study.

Heart Rate

Mean (sd) baseline heart rate was 66 (8) beats per minute. After placebo, mean change in heart rate was 0.7bpm (95%CI -1.6 to 3.0, p=0.6). Mean change in heart rate after sumatriptan was 3.8bpm (95%CI –0.2 to 7.8, p=0.15).

Cardiac Output

Mean baseline cardiac output (C.O.) was 5.1(0.9) $l.min^{-1}$. No significant change occurred after either placebo or sumatriptan. After sumatriptan, mean change in cardiac output was –0.1 $l.min^{-1}$. (95% C.I. –0.3 to 0.1, p=0.09).

Systemic Arterial Pressure

Baseline systolic (SAP), diastolic (DAP) and mean (MAP) aortic pressure were 118 (19)mmHg, 63.3 (8.7)mmHg and 87 (11)mmHg respectively. After placebo injection, no significant changes occurred. Following sumatriptan, SAP rose significantly by a mean of 23mmHg (95% C.I. 18 to 28, p=0.00001). DAP rose on average by 10mmHg (95% C.I. 5.4 to 14.6, p=0.03). MAP rose on average by 14mmHg (95% C.I. 11.2 to 16.8, p=0.00002).

Pulmonary Arterial Pressure

Baseline mean pulmonary artery systolic (PASP), diastolic (PADP) and mean (MPAP) pulmonary artery pressures were 24.3 (5.6)mmHg, 8.7 (2.9)mmHg and 14.6 (4.0)mmHg respectively. After placebo injection, no significant change in PASP or PADP occurred but MPAP fell by 0.8mmHg (95% C.I. 0.3 to 1.3, p=0.02). Following sumatriptan injection, PASP rose on average by 3.0mmHg (95% C.I. 1.1 to 4.9, p=0.024), PADP by 2.0mmHg (95% C.I. 0.6 to 3.4, p=0.05) and MPAP by 2.0mmHg (95% C.I. 0.6 to 3.4, p=0.039).

Pulmonary Artery Wedge Pressure (PAWP)

Mean (sd) baseline PAWP was 6.5 (2.5)mmHg. No significant change occurred after placebo injection but PAWP rose on average by 2.2mmHg after sumatriptan (95% C.I. 0.5 to 3.9, p=0.045).

Right Atrial Pressure (RAP)

Mean (sd) baseline RAP was 4.8 (2.4)mmHg. No significant change occurred after placebo injection but RAP rose after sumatriptan by an average of 1.3mmHg (95% C.I. 0.5 to 2.1, p=0.02).

Left Ventricular End Diastolic Pressure (LVEDP)

Mean (sd) baseline LVEDP was 7.2 (2.6)mmHg. No significant change occurred after placebo injection. After sumatriptan injection, LVEDP rose by an average of 4.4mmHg (95% C.I. 3.0 to 5.8, p=0.0005).

Peak Rate of Left Ventricular Pressure Rise ($\delta P/\delta t$)

Mean (sd) baseline $\delta P/\delta t$ was 2304 (823)mmHg.s^{-1}. No significant change occurred after placebo. Sumatriptan injection resulted in a non significant mean change in $\delta P/\delta t$ of -187mmHg.s^{-1} (95%C.I. -335 to -39, p=0.24).

Systemic Vascular Resistance (SVR)

Mean (sd) baseline SVR was 1326 (70) dyne.s.cm^{-5}. After placebo, SVR rose on average by 129 dyne.s.cm^{-5} (95% C.I. 82 to 176, p=0001). Mean increase from placebo after sumatriptan injection was 237 dyne.s.cm^{-5} (95% C.I. 165 to 309, p=0.0003).

Pulmonary Vascular Resistance (PVR)

Mean (sd) baseline PVR was 119 (21) dyne.s.cm^{-5}. No significant change occurred after placebo injection but sumatriptan injection was associated with a mean increase in PVR of 23.3 dyne.s.cm^{-5} (95% C.I. 8.1 to 38.5, p=0.02).

DISCUSSION

These results show that subcutaneous injection of sumatriptan causes arterial vasoconstriction in extracranial vascular beds, in keeping with previous *in vivo* human studies.[241,242] In the absence of changes in cardiac output and heart rate, it is assumed that the pressor responses are due to a direct sumatriptan induced vasoconstrictor effect. Procedure related effects cannot be fully excluded but this appears unlikely.

The observed 20% increase in systemic arterial pressure and 18% rise in SVR in this study is entirely consistent with previous invasive haemodynamic findings following intravenous or subcutaneous sumatriptan.[241,242] In the treatment of acute migraine with sumatriptan, significant changes in blood pressure, as measured non invasively, have not been consistently reported.[255,259,264] Some observers have however noted sumatriptan induced pressor responses in migraineurs.[265] The present study confirms the systemic vasoconstrictive effects of sumatriptan.

In agreement with previous findings, a sumatriptan-induced pulmonary vasopressor effect was also observed. Intravenous sumatriptan to a total dose of 48$\mu g.kg^{-1}$ caused a 53% rise in PASP, 77% rise in PADP and 58% rise in MPAP.[241] Subcutaneous injection of 6mg sumatriptan increased PASP and PADP by 40% and 33% respectively. Pulmonary vascular resistance rose by 40%.[242] In the present study PASP,PADP and total PVR rose by 12%, 23% and 20% respectively. The rise in pulmonary arterial pressures and PVR is of a smaller magnitude than previously recorded and may simply reflect biological variation. Previous studies showing an increase in PAP greater than the change

in systemic arterial pressure by a factor of 2-3 may suggest a greater density of 5-HT_1 receptors in the pulmonary compared to the systemic circulation. The current findings cannot refute this idea but pooling of the current results with those in the 2 previous studies[241,242] has been performed in chapter 4.

Equipotent systemic and pulmonary vasopressor effects were observed in this study. The reason for this disparity is not immediately evident as the individuals in all 3 studies were selected from the same clinical substrate. This study group included a higher percentage of females (70% compared to 40% in both previous studies). In addition, baseline values of aortic and pulmonary artery pressures were slightly lower in the present compared to the previous studies. Pulmonary artery wedge pressure increased from a mean placebo value of 6mmHg to a maximum of 9.3mmHg (55%) compared with the 80% increase (8.7mmHg to 15.6mmHg) in the previous subcutaneous study.[242] Simultaneous measurement of the LVEDP showed a rise of a similar degree to the PAWP and it may be that the change in PAWP is a reflection of the rise in LVEDP rather than a pulmonary venoconstrictive effect.

The rise in LVEDP could be secondary to increased afterload or reflect a negative inotropic effect. A negative inotropic effect has not previously been noted with serotonergic agonists and studies with human ventricular muscle preparations show a lack of functional 5-HT receptors in terms of contractile effects.[24,25] Human atrial preparations, however, elicit a 5-HT_4 mediated positive inotropic effect. A negative inotropic action of sumatriptan seems unlikely in view of the above and the lack of a significant change in the peak rate of left ventricular pressure rise ($\delta P/\delta t$). It is therefore assumed that the rise in LVEDP

and PAWP are a consequence of the acute increase in afterload. The minor non-significant reduction in $\delta P/\delta t$ could therefore reflect the ventricular response to raised aortic pressure and is unlikely to be of clinical significance.

Right atrial pressure also rose following sumatriptan. The acute increase in pulmonary artery pressure seen in this study would be expected to raise right ventricular end diastolic pressure (cf LVEDP) hence accounting for the increase in RAP. The rise in RAP exceeded the magnitude of the rise in MPAP however, (28% versus 17%) and it may be that part of the rise in RAP reflects systemic venoconstriction in addition to pulmonary artery vasoconstriction. *In vitro,* sumatriptan contracts human saphenous veins.[35,36] In high doses, applied locally, sumatriptan also induced venoconstriction in the hand veins of migraineurs.[264] This effect was blocked by ketanserin and was not evident following a standard clinical dose of 6mg subcutaneously. This ketanserin-sensitive response was therefore postulated to be 5-HT_2 mediated as a consequence of high local concentrations following intravenous injection.

In summary, a standard clinical dose of 6mg sumatriptan (s.c.) caused equipotent vasopressor responses in the systemic and pulmonary circulation. Left ventricular end diastolic pressure and pulmonary artery wedge pressure both increased after sumatriptan with no effect on the peak rate of left ventricular pressure rise ($\delta P/\delta t$) excluding a negative inotropic action of sumatriptan. The rise in right atrial pressure raises the possibility of an additional venoconstrictive action. There is no evidence that any of these effects are of clinical significance.

	Mean baseline value (S.D.)	Mean change after placebo (95% C.I.)	P Value	Mean change after sumatriptan (95% C.I.)	P value
HR (bpm)	66 (8)	0.7 (-1.6 to 3.0)	0.6	3.8 (-0.2 to 7.8)	0.15
CO ($l.min^{-1}$)	5.10 (0.9)	0.2 (-0.5 to 0.1)	0.12	-0.1 (-0.3 to 0.1)	0.09
SAP (mmHg)	118 (19)	3.0 (-0.3 to 6.3)	0.12	23 (18 to 28)*	0.00001
DAP (mmHg)	63.3 (8.7)	2.4 (0.3 to 4.5)	0.07	10.0 (5.4 to 14.6)*	0.03
MAP (mmHg)	87(11)	1.6 (-0.4 to 3.6)	0.18	14 (11 to 17)*	0.00002
SVR ($dyne.s.cm^{-5}$)	1326 (70)	129 82 to 176)	0.001	336 (282 to 420)*	0.00004
PASP (mmHg)	24.3 (5.6)	-0.4 (-1.2 to 0.4)	0.35	3.0 (1.1 to 4.9)*	0.024
PADP (mmHg)	8.7 (2.9)	-0.4 (-1.1 to 0.3)	0.27	2.0 (0.6 to 3.4)*	0.05
MPAP (mmHg)	14.6 (4.0)	-0.8 (-0.3 to – 1.3)	0.02	2.0 (0.6 to 3.4)*	0.039
PVR ($dyne.s.cm^{-5}$)	119 (21)	2.3 (-13.3 to 17.9)	0.78	23.3 (8.1 to 38.5)*	0.02
PAWP (mmHg)	6.5 (2.5)	-0.5 (-1.4 to 0.4)	0.48	2.2 (0.5 to 3.9)*	0.045
LVEDP (mmHg)	7.2 (2.6)	0.5 (-0.2 to 1.2)	0.25	4.4 (3.0 to 5.8)	0.0005
$\delta P/\delta t$ ($mmHg.s^{-1}$)	2304 (823)	-123 (-336 to 90)	0.32	-187 (-335 to –39)	0.24
RAP (mmHg)	4.8 (2.4)	-0.4 (-1.1 to 0.3)	0.26	1.3 (0.5 to 2.1)*	0.02

Table 3.2: Cardiac haemodynamics at baseline and changes after placebo and sumatriptan injection. Figures given in parentheses represent 95% confidence intervals for the mean change from baseline. In each case statistical comparisons are made with baseline values.

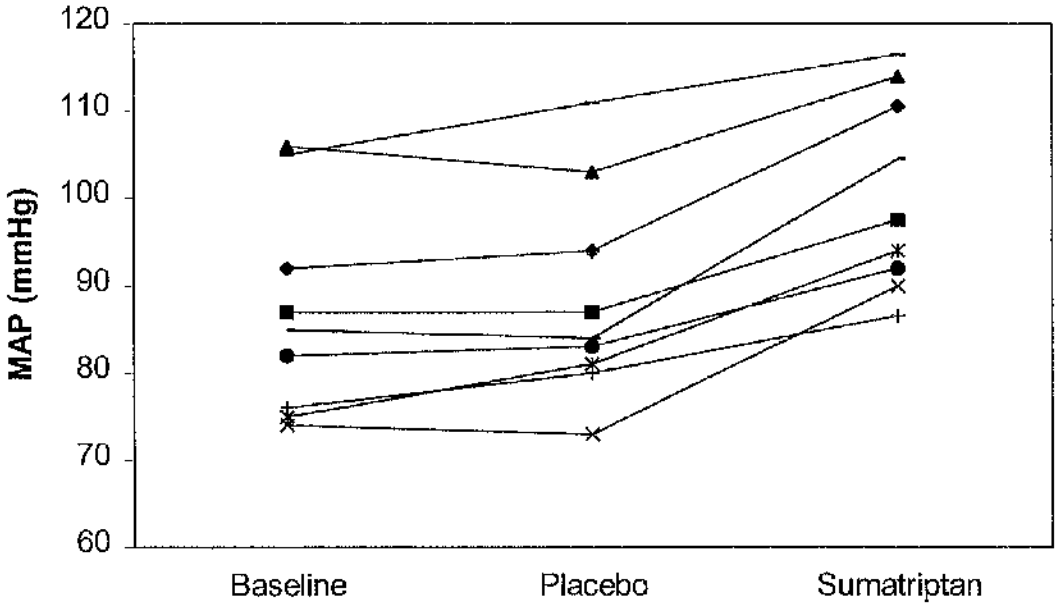


Figure 3.1 Mean arterial pressure (MAP) changes after placebo and sumatriptan injection. Each line represents an individual

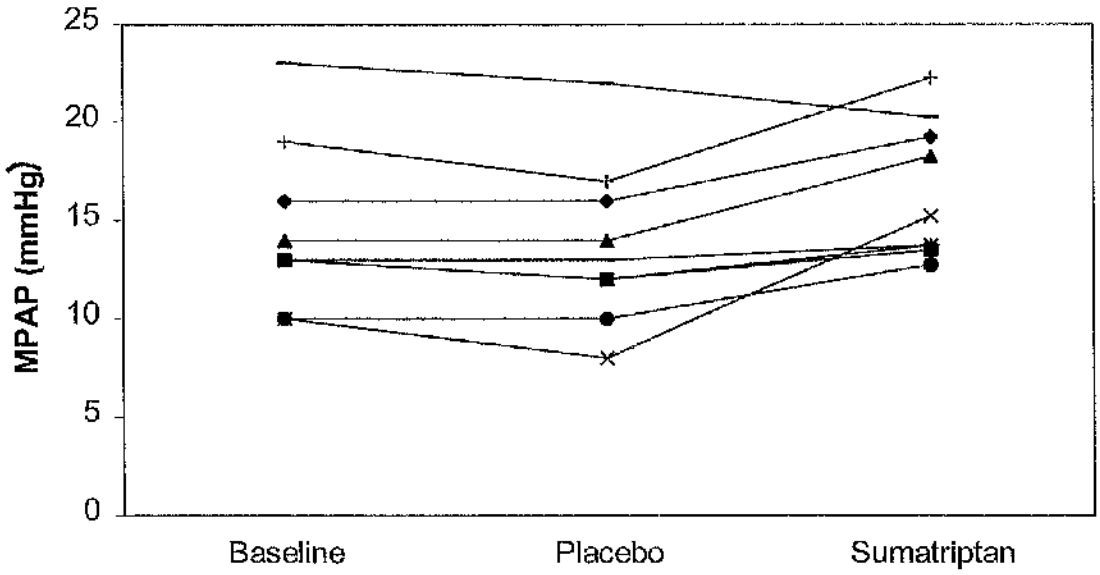


Figure 3.2 Mean pulmonary arterial pressure (MPAP) at baseline and after placebo and sumatriptan injection.Each line represents an individual.

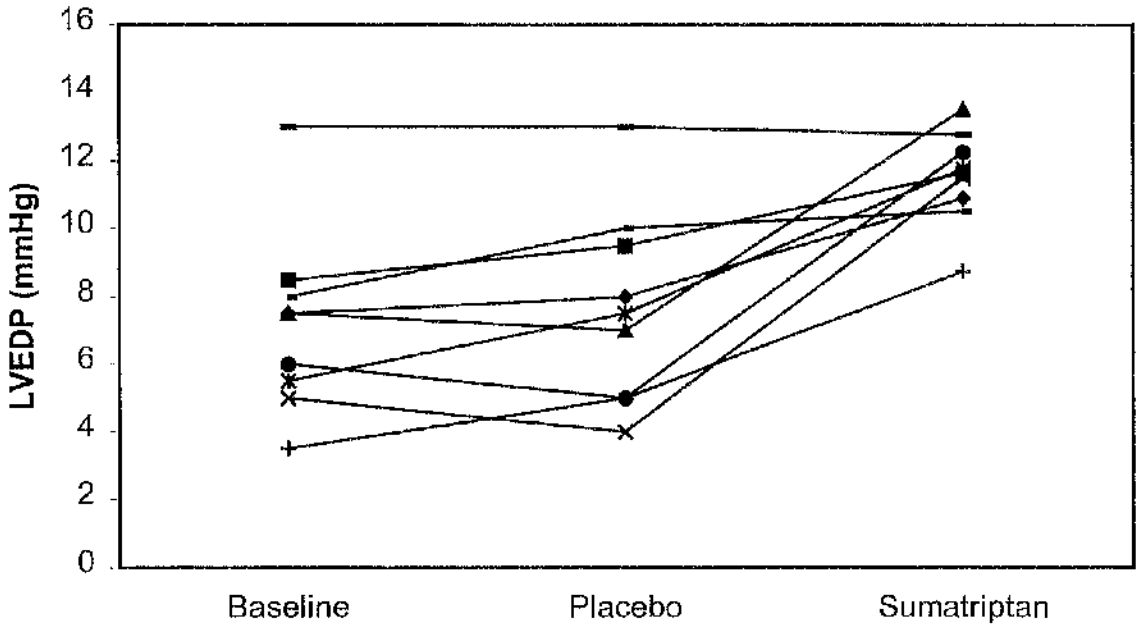


Figure 3.3 Left ventricular end diastolic pressure (LVEDP) at baseline and after placebo and sumatriptan injection. Each line represents an individual.

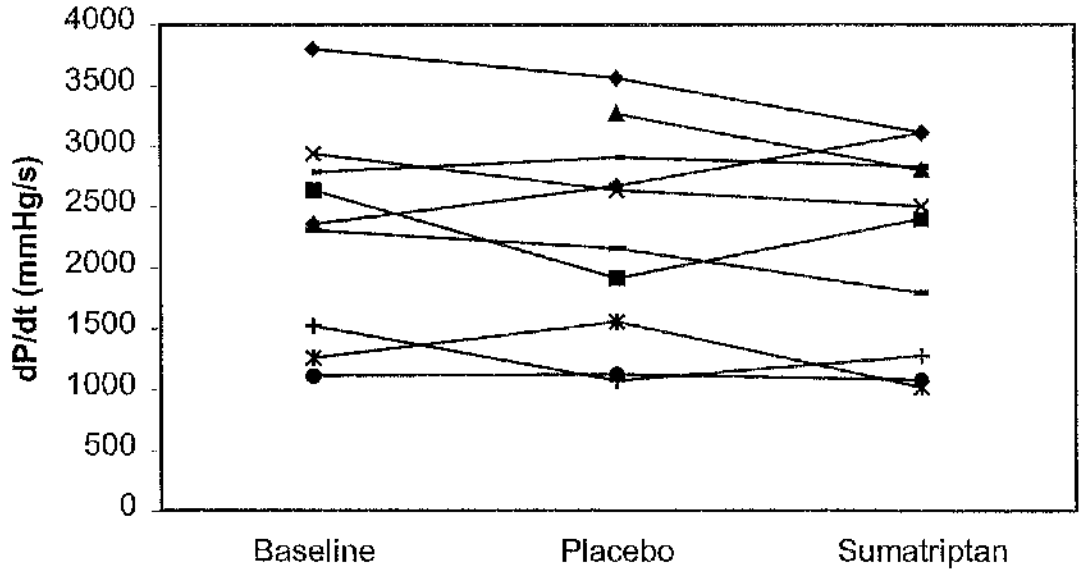


Figure 3.4 Peak rate of left ventricular pressure rise (dP/dt) at baseline and after placebo and sumatriptan. Each line represents an individual.

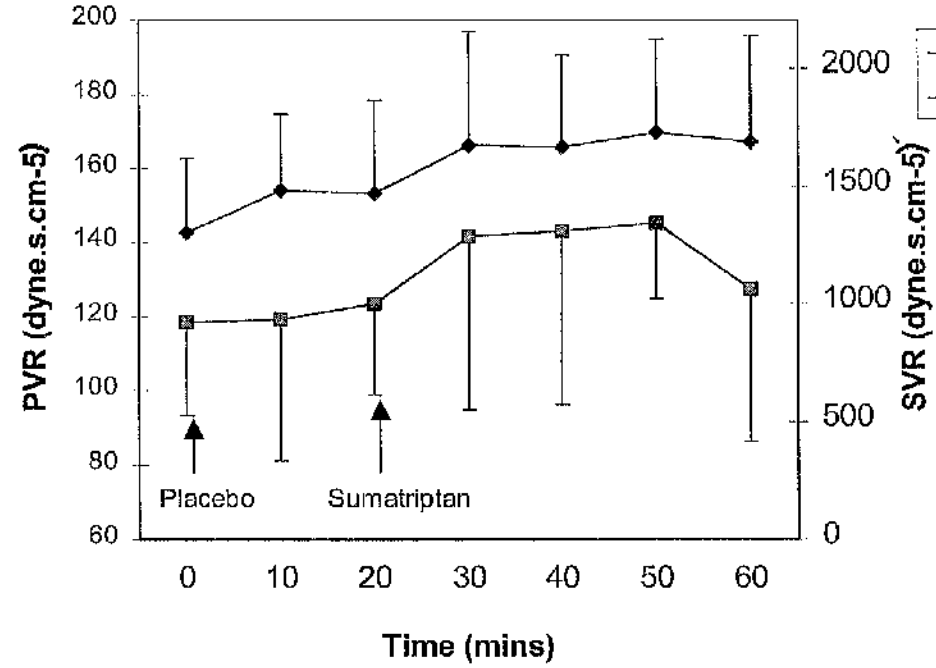


Figure 3.5 Mean (sd) pulmonary (PVR) and systemic (SVR) vascular resistance after placebo and sumatriptan injection.

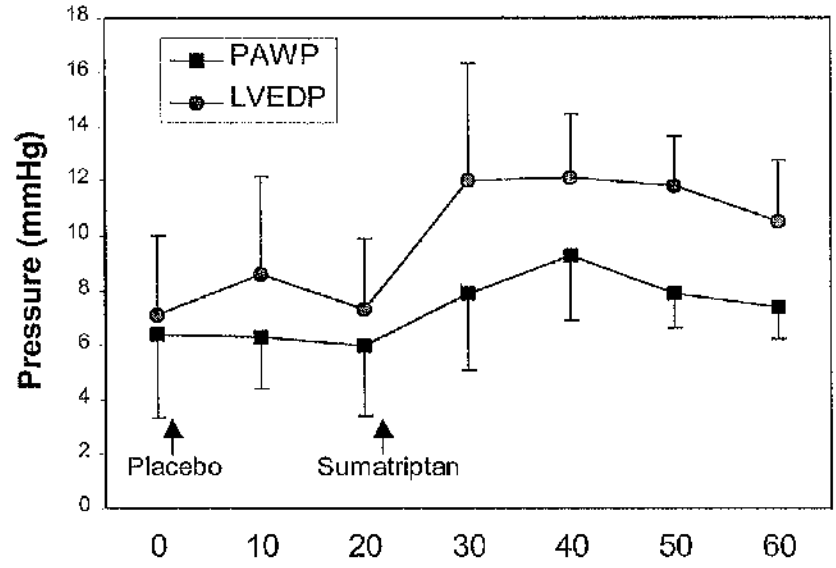


Figure 3.6 Mean (sd) left ventricular end diastolic pressure (LVEDP) and pulmonary artery wedge pressure (PAWP) after placebo and sumatriptan injection.

CHAPTER 4

RELATIVE EFFECTS OF SUMATRIPTAN ON THE PULMONARY AND SYSTEMIC CIRCULATIONS.

INTRODUCTION

Early studies addressing the pharmacodynamics of sumatriptan suggested a selective vasoconstrictive effect on the intracranial vasculature. In isolated vascular preparations, it was shown to cause constriction of dog and primate basilar arteries.[38,39,196] Human isolated basilar artery rings were also found to constrict in response to sumatriptan.[40] In addition to *in vitro* constriction of cranial vessels, *in vivo* experiments also confirmed vasoconstriction. In anaesthetised intact animals, it caused vasoconstriction of feline[193] and canine[254] carotid arterial circulations. When given intravenously to anaesthetised beagle dogs, sumatriptan produced a dose dependent increase in carotid vascular resistance with little or no effect on heart rate or blood pressure thus implying a selective vasoconstrictor action on the canine carotid circulation.[254] Vasoconstrictor responses were resistant to blockade by ketanserin (5-HT_2 antagonist), MDL72222 (5-HT_3 antagonist) and phentolamine (α-adrenoceptor antagonist) but were sensitive to methiothepin (5-HT_1 and 5-HT_2 antagonist) which confirmed a 5-HT_1 mediated response.

Similarly, sumatriptan caused constriction of feline carotid vessels.[193] In this study, both sumatriptan and ergotamine caused dose-dependent reduction in the proportion of cardiac output passing through arteriovenous anastomoses (AVA's). Ergotamine, unlike sumatriptan however, was also associated with increases in diastolic blood pressure and total peripheral resistance suggesting that the effect of sumatriptan on the carotid vasculature was selective. Further evidence of a selective vasoconstriction was also obtained in this study. Using 15μm radiolabelled microspheres the vasoconstrictor effect was shown to be highly localised within the carotid circulation, in particular to carotid

arteriovenous anastomoses. In addition to a selective vasoconstrictor action in animals, Neilsen et al in 1989, assessed toe-arm systolic gradients in man using strain gauge plethysmography and concluded that intravenous sumatriptan had no effect on peripheral arteries in humans.[267]

Subsequently however, evidence arose to suggest that 5-HT_1 receptors were not, as first thought, confined to the cranial circulation. A non-invasive study demonstrated that sumatriptan could cause femoral artery vasoconstriction in humans.[253] Direct invasive evidence of sumatriptan's extracranial activity was confirmed in three studies which revealed both systemic and pulmonary vasoconstrictor actions.[241,242,268] These studies measured arterial pressure changes in response to sumatriptan in patients undergoing diagnostic cardiac catheterisation. Systemic arterial pressure rose by 16-20% with pulmonary artery pressure rising by 40-58%. This relative 2-3 fold percentage increase in pulmonary artery pressure compared to the rise in systemic arterial pressure raised the possibility of a more pronounced effect on the pulmonary circulation. The inference therefore followed that 5-HT_1 receptors were more densely distributed in the pulmonary circulation.[242]

There is a school of thought which considers 5-HT, released from pulmonary neuroendocrine cells and platelets, to be implicated in pulmonary hypertension secondary to hypoxia.[269] This being the case, 5-HT_1 receptors may have a pathophysiological role in pulmonary hypertension and therefore information on the density of 5-HT_1 receptors within the pulmonary circulation would be noteworthy.

The relative effects of sumatriptan on the systemic and pulmonary circulation were therefore more closely re-examined in 28 patients whose results have been reported in 3 previous trials with similar study protocols.[241,242,266] The intention was to obtain more information on the density and clinical relevance of 5-HT_1 receptors in the respective circulations.

METHODS

The protocol was as described in chapter 2 (pages 49-50) In brief, 28 patients were studied with mean arterial pressure (MAP) and mean pulmonary artery pressure (MPAP) being recorded at baseline, following placebo injection and at 10 minute intervals following sumatriptan administration. Twenty patients received 6 mg sumatriptan by subcutaneous injection and 8 received intravenous sumatriptan (maximum dose 48 μ/kg). The ratio of MPAP:MAP was calculated for each time point.

STATISTICS

For each individual, a baseline value was obtained for MAP and MPAP by averaging two baseline observations. As any response occurred within 10 minutes and persisted for the 40 minute study period, the average of the haemodynamic recordings at 10, 20, 30 and 40 minutes after sumatriptan injection were used to summarise the response to sumatriptan. Post-placebo and post-sumatriptan results were then compared with baseline measurements using a paired t-test/confidence interval as the data appeared normally distributed.

RESULTS

Table 4.1 shows the MAP and MPAP for each individual at baseline and after sumatriptan injection.

Mean arterial pressure (MAP)

Baseline mean (sd) MAP was 96 (13)mmHg. Mean (95%C.I.) increase after placebo injection was 0.36mmHg (-1.9 to 2.6, p=0.74). After sumatriptan injection, the mean (95% C.I.) rise in MAP was 11mmHg (7.5-15, p<0.0001).

Mean pulmonary arterial pressure (MPAP)

Baseline mean (sd) MPAP was 16 (6.6) mmHg. After placebo injection, the mean (95% C.I.) change in MPAP was –0.4mmHg (-1.5 to 0.7, p=0.43). Mean increase in MPAP following sumatriptan injection was 4.2mmHg (2.5-5.9, p<0.0001)

Ratio of MPAP:MAP

Baseline mean MPAP:MAP ratio was 0.171 (0.07). No significant change was observed after placebo injection [mean change –0.005 (-0.02 to 0.005, p=0.3)]. Sumatriptan caused a significant increase in MPAP:MAP with a mean (95%C.I.) rise of 0.02 (0.008-0.03, p=0.0023)

DISCUSSION

The observed rise in both mean systemic and pulmonary arterial pressures following sumatriptan administration was to be expected as these observations had been noted in all 3 individual studies.[241,242,268] Overall an 11% increase in mean systemic arterial pressure occurred after sumatriptan injection. Mean pulmonary arterial pressure rose by 26% however, consistent with the previous

observation of an approximate 2- fold relative percentage increase in pulmonary arterial pressure compared to the increase seen in the systemic circulation after sumatriptan.

The significant increase (12%) in the ratio of MPAP:MAP suggests a greater effect of sumatriptan on the pulmonary in comparison to the systemic circulation. It is assumed that the effects of sumatriptan are mediated via 5-HT_1 agonism and it could therefore be inferred that 5-HT_1 receptors exist in greater density in the pulmonary circulation. Alternatively, 5-HT_1 may play a functionally more important role in the pulmonary circulation in humans but confirmatory evidence of this is lacking in the scientific literature.

Studies in bovine species have demonstrated that sumatriptan constricts pulmonary but not mesenteric arteries, implying a lack of functional 5-HT_1 receptors in the bovine mesenteric circulation.[270] The physiology and pathophysiology of the bovine pulmonary vasculature is poorly understood. However, there is evidence that 5-HT induced pulmonary vasoconstriction is mediated via 5-HT_2 receptors.[271] Under conditions of basal tone, ketanserin (5-HT_2 antagonist) competitively inhibited 5-HT induced vasoconstriction in isolated bovine pulmonary artery rings.[272] This observation, together with the fact that sumatriptan displayed little or no agonist activity under these basal conditions suggested a 5-HT_2 receptor mediated response.[272]

When precontracted with the thromboxane mimetic U46619 (10nm) however, sumatriptan was able to induce further contraction which was competitively antagonised by methiothepin (5-HT_1 and 5-HT_2 antagonist) but was resistant to

ketanserin.[272] These results suggest that, in the absence of vascular tone, serotonin induced constriction is mediated by 5-HT_2 receptors whilst with tone present, 5-HT_1 receptor mediated vasoconstriction becomes evident in bovine pulmonary arteries. Both 5-HT_1 and 5-HT_2 receptors may therefore be implicated in pulmonary hypertension. Interestingly the response to sumatriptan was augmented by L-NAME and endothelial removal, manoeuvres which remove the influence of nitric oxide (NO).[272] Responses to 5-HT however, were not augmented by L-NAME or endothelial removal. This indicates that, in bovine pulmonary arteries, the potentiating effect of removal of NO influence is specific to 5-HT_1 receptor activation. Similar facilitatory effects have been reported between α-adrenoceptors and endothelial removal in rabbit pulmonary arteries.[273]

The clinical relevance of these findings is unclear, however. Endothelial abnormalities and dysfunction have been observed in patients with congenital heart disease and pulmonary hypertension[274] and in chronic hypoxic pulmonary hypertension.[275] It may be that there is hypersensitivity to 5-HT_1 receptor stimulation in patients with pulmonary hypertension and endothelial dysfunction. In such cases serotonin–induced vasoconstriction could play a role in the pathogenesis of the disease and there is additional supportive evidence to suggest a role for serotonin. Increased plasma concentrations and turnover of serotonin have been observed in primary pulmonary hypertension[276] and in children with pulmonary hypertension secondary to congenital heart disease.[277] In addition, appetite suppressant drugs with serotonergic activity such as dexfenfluramine and fenfluramine have been associated with an increased risk of pulmonary hypertension.[278]

Ketanserin, a 5-HT_2 antagonist, has been shown to reduce pulmonary vascular resistance following cardiac surgery [279-281] and in a study of 14 patients with pulmonary hypertension secondary to systemic sclerosis.[282] Its use in the treatment of protamine induced pulmonary hypertension has also been reported[283], implicating a potential role for serotonin and 5-HT_2 receptors in the pathogenesis of pulmonary hypertension. In another study of 20 patients with primary pulmonary hypertension, however, ketanserin had only modest pulmonary vasodilator effects of doubtful clinical significance and of similar magnitude to conventional vasodilators.[284] In 7 patients with hypoxic pulmonary hypertension, ketanserin had no effect on pulmonary artery pressure.[285] These contradictory results prevent firm conclusions being drawn over the role of serotonin, particularly 5-HT_2 receptors, in the pathogenesis of pulmonary hypertension.

It must be recognised that pulmonary hypertension is not a single disease entity but has multiple aetiologies, the pathogenesis of which may be equally varied. The finding that sumatriptan appears to have a greater pulmonary than systemic vasoconstrictor effect implies that 5-HT_1 receptors are perhaps more densely distributed in the pulmonary circulation. Whether these 5-HT_1 receptors have a pathogenetic role in pulmonary hypertension is unclear but the aforementioned discussion has highlighted the potentiating effects of endothelial function on 5-HT_1 receptor responses. Unfortunately there is a paucity of selective 5-HT_1 antagonists and no agent is currently suitable for clinical use. If and when a 5-HT_1 antagonist becomes clinically available, its effects on the pulmonary vasculature will have to be established, particularly in individuals with elevated pulmonary artery pressures.

	BASELINE				POST SUMATRIPTAN		
Subject	**MAP**	**MPAP**	**MPAP: MAP ratio**		**MAP**	**MPAP**	**MPAP: MAP ratio**
1	116	18	0.155		126	30	0.238
2	90	9	0.1		105	13	0.123
3	110	35	0.318		118	39	0.331
4	100	19	0.19		100	23	0.230
5	100	14	0.140		102	15	0.147
6	110	16	0.145		121	19	0.157
7	100	14	0.140		120	18	0.150
8	100	14	0.140		145	24	0.166
9	90	30	0.333		95	34	0.358
10	75	13	0.173		94	14	0.150
11	106	14	0.132		114	18	0.158
12	92	16	0.174		111	19	0.171
13	82	10	0.122		92	13	0.141
14	76	19	0.250		87	22	0.253
15	87	13	0.149		98	14	0.143
16	74	10	0.135		90	15	0.167
17	85	13	0.153		105	14	0.133
18	105	23	0.219		117	20	0.171
19	100	18	0.180		106	21	0.198
20	100	17	0.170		92	14	0.152
21	88	12	0.136		87	14	0.161
22	100	10	0.10		110	14	0.127
23	108	12	0.111		136	30	0.221
24	80	16	0.20		92	20	0.217
25	107	11	0.103		119	18	0.151
26	128	14	0.109		141	21	0.149
27	88	14	0.159		93	18	0.194
28	95	33	0.347		100	44	0.440

Table 4.1. Mean arterial pressure (MAP), mean pulmonary arterial pressure (MPAP) and MPAP:MAP ratio at baseline and following sumatriptan injection

Haemodynamic parameter	Mean (sd) baseline value	Mean (95%C.I.) change after placebo	P value	Mean (95%C.I) after sumatriptan	P value
MAP (mmHg)	96 (13)	0.36 (-1.9 to 2.6)	0.74	11 (7.5 to15)	<0.0001
MPAP (mmHg)	16 (6.6)	-0.4 (-1.5 to 0.7)	0.43	4.2 (2.5 to 5.9)	<0.0001
MPAP:MAP	0.171 (0.07)	-0.005 (-0.02 to 0.0005)	0.3	0.02 (0.008 to 0.03)	0.0023

Table 4.2 Haemodynamic parameters at baseline and following placebo and sumatriptan injection. In each case comparisons are made with baseline values.

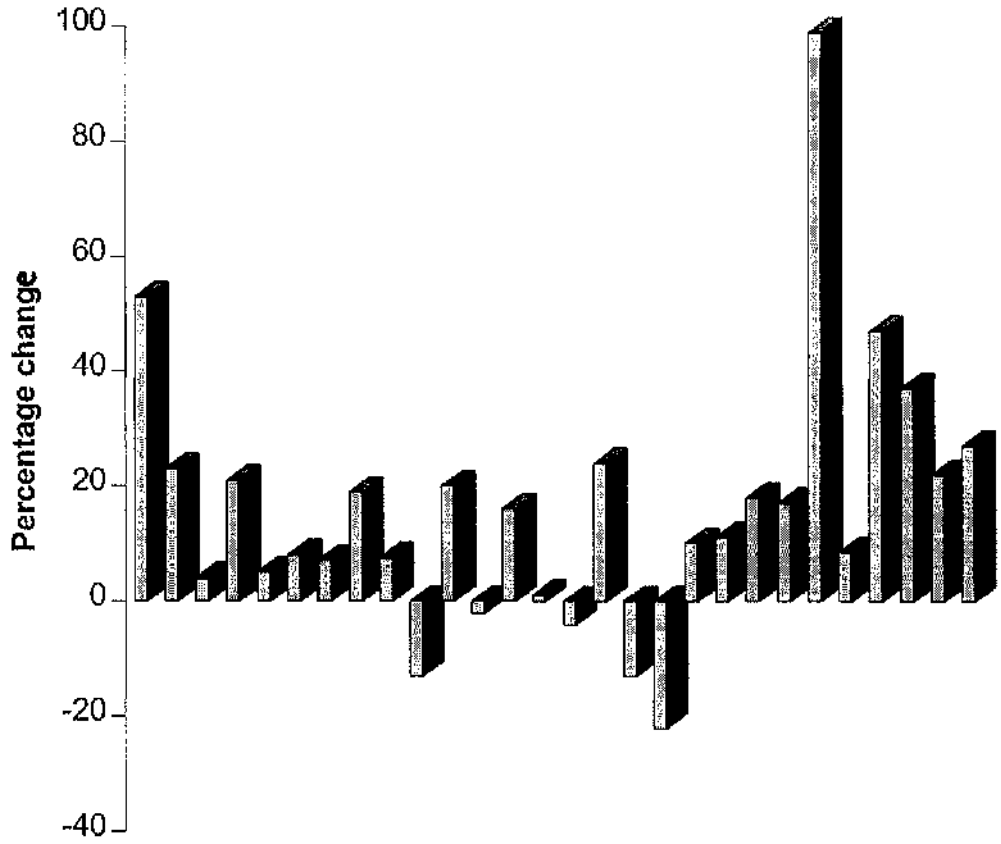


Figure 4.1 Percentage change in MPAP:MAP ratio for each subject after sumatriptan injection

CHAPTER 5

THE EFFECT OF SUMATRIPTAN ON SYSTOLIC TIME INTERVALS

INTRODUCTION

HISTORICAL PERSPECTIVE

In the mid nineteenth century Ludwig studied arterial and venous pulse contours and how they varied in differing disease states.[286] Garrod in 1874 noted the inverse relationship between heart rate and the duration of left ventricular ejection.[287] Later attempts were then made to elucidate the relationship between duration of systole and cycle length and to apply this measurement as a surrogate of cardiac performance in man.[288,289] By the 1920's Wiggers began to realise the full potential of systolic time intervals (STI's) when he was able to more clearly delineate the phases of systole.[290,291] Katz and Feil then applied electronic recordings to determine the phases of systole in the presence of LV diseases.[292-294] In 1942, Blumberger reported extensive studies on the systolic intervals in a wide variety of cardiac disorders.

With the subsequent development of direct measures of LV function, the significance of systolic time intervals in man could be related to ventricular performance on a more quantitative basis and the measurement of STI's became more popular during the 60's and 70's. More recently, rapidly advancing, non-invasive ultrasound techniques have allowed greater appreciation of cardiac performance in disease states, but the measurement of STI's still represents a sensitive and valid method of assessing cardiovascular function.

Measurement of STI's: Methodology

Ventricular performance can be assessed by the ability of the ventricle to pump blood (stroke volume, cardiac output), the capacity to generate force (ie the

maximal rate of left ventricular pressure rise [maximal $\delta P/\delta T$]), the ability to shorten with each contraction (ejection fraction), the temporal relationships of contraction (systolic time intervals) and combinations of these variables. Most tests of ventricular function deal with stroke volume-cardiac output or force. The systolic time intervals are the only tests for which the sole measure of ventricular function is time.

The three basic STI's are the pre-ejection period (PEP), the left ventricular ejection time (LVET) and the total electromechanical systole (EMS or QS_2). There are several methods of measuring STI's but regardless of methodology, meticulous detail must be applied to recording techniques to avoid misinterpretation. In the most widely employed technique, STI's are obtained from simultaneous, fast lead recordings (100 mm/sec) of the electrocardiogram, the phonocardiogram and the carotid pulsation. The ECG lead which most clearly displays the onset of ventricular depolarisation is selected. The phonocardiogram is placed over the upper precordium in an area which provides a clear view of the initial high frequency aortic components of the second heart sound (A_2). A filter system should be employed to eradicate lower frequency (< 100 Hz) sounds. Carotid arterial pulsation is recorded with a funnel shaped pick up attached by polythene tubing to a pressure transducer. Again it is crucial that the rapid upstroke and pointed, single incisural notch on the carotid pulse tracings are clearly discernible. Failure to obtain a sharp incisural notch is a common source of error and is likely to occur if carotid pulse tracings are small.

The QS_2 interval is measured from the onset of ventricular depolarisation to the first high frequency vibration of A_2. The LVET is measured from the beginning upstroke to the trough of the incisural notch of the carotid pulse tracing (Figure 2.1). The PEP is that interval from the beginning of ventricular depolarisation to the beginning of LV ejection. Subtracting the LVET from QS_2 therefore derives the PEP. The PEP consists of the electromechanical interval plus the isovolumetric contraction time. Except in the presence of left ventricular conduction delay, (e.g. left bundle branch block) the electromechanical interval is relatively constant. Therefore, variations in PEP usually reflect changes in isovolumetric contraction time. Measurement of the isovolumetric contraction time itself is subject to variability and is dependent on where the first high frequency components of the second heart sound are found.

STI's can also be derived from simultaneous fast speed ECG and haemodynamic pressure recordings obtained using fluid filled catheters in the aorta. In this situation, the incisural notch of the aortic pressure tracing corresponds exactly with the initial vibrations of A_2.[295] Using this technique, the incisural notch can be readily identified and the difficulty in identifying the variable first components of A_2 can be circumvented. In addition, the incisural notch is often more difficult to identify in small carotid pulse wave tracings obtained externally, a problem not encountered when aortic pressure recordings are invasively obtained. There are however problems using fluid filled catheters to obtain aortic pressure tracings, namely the inherent transmission delays through the catheters. In this situation the absolute values of the STI's are slightly longer than those obtained externally.

In applying STI measurements, corrections must be made for heart rate. When corrected for heart rate, the STI's are suffixed with the letter I (hence PEP becomes PEP_I etc). The linear regression equations for this are detailed in table 5.1. At rates below 110/min, the PEP and LVET shorten proportionately as heart rate increases. In the normal range of heart rates, the ratio of PEP/LVET is often employed as a measure of LV function when either the PEP or LVET may both be within normal limits.

Systolic Time Intervals in Clinical Pharmacology

To evaluate the effect of drugs on the CVS requires sensitive, exact and valid methods, which preferentially should be non-invasive. STI fit these criteria and have been widely employed in the investigation of pharmacological agents. Changes in inotropy, preload and afterload produce characteristic alterations in the STI's (see table 5.2).[296-299]

Positive inotropic drugs, which cause little or no change in afterload, will shorten isovolumic contraction time and hence will also shorten the PEP. In this situation LVET shortens and, as a result, QS_2 will also decrease. Conversely, negative inotropic drugs, which prolong isovolumic contraction time, will prolong the PEP.[296-299] LVET is generally shortened by both negative and positive inotropes.[296,297] In the presence of negative inotropes there are directionally opposite changes in the PEP (increases) and LVET (decreases), and the duration of systole (QS_2) remains relatively constant. QS_2 is therefore an insensitive marker of a negative inotropic action. Because positive inotropes shorten both PEP and LVET, a reduction in QS_2 is the most sensitive STI marker in detecting positive inotropic stimulation. A positive inotropic effect is

therefore suggested by a shortening of QS_2. Since negative inotropes will prolong PEP but shorten LVET, an increase in the PEP:LVET ratio is indicative of a negative inotropic effect.[296,297] STI's can therefore be used to non-invasively assess the inotropic effects of cardiovascular agents.

Caution must however be exercised in the interpretation of STI's, particularly when acute changes in afterload or preload occur.[300] Acute increases in afterload prolong the PEP but have no effect or slightly prolong the LVET. Overall, drugs which acutely increase afterload, will therefore also increase the PEP:LVET ratio (as do negative inotropes). Negatively inotropic drugs can be distinguished from pressor agents on account of their differing effects on the LVET.

Augmented ventricular filling (increased pre-load) results in an increase in stroke volume. This is accompanied by a prolongation of the LVET and an abbreviation of the PEP, hence the PEP:LVET ratio decreases. A decrease in pre-load (reduced ventricular filling) will produce opposite changes in PEP, LVET and PEP:LVET, namely increases in PEP and PEP:LVET ratio with a reduction in LVET. Systolic time intervals are therefore ideally suited for studying the cardiovascular actions of pharmacologic agents being able to distinguish negative inotropy from positive inotropy and acute changes in afterload from those occurring when preload is altered.

Sumatriptan and systolic time intervals

As discussed above, systolic time interval estimation can be used to non-invasively assess the cardiovascular activity of drugs. Invasive haemodynamic

studies have obvious limitations, restricting the potential for clinical studies. A reliable non-invasive tool to evaluate the effects of 5-HT_1 agonists would therefore be welcome. This would open up the possibility of performing serial studies. The aim of the study was therefore to assess the effects of sumatriptan on the duration of the systolic time intervals and to relate these alterations to changes in haemodynamic variables measured invasively.

PATIENTS AND METHODS

Haemodynamic tracings were analysable for twenty-six patients (14M, 12F, age 49±10 yrs) who had participated in three previous sumatriptan haemodynamic studies.[241,242, chapter 3] Eighteen patients received subcutaneous placebo followed by subcutaneous sumatriptan 6mg. Eight patients received a 10minute intravenous infusion of placebo followed by a 10minute intravenous infusion of sumatriptan to a total maximum dose of 48$\mu g.kg.min^{-1}$. Aortic pressure tracings were recorded at baseline, following placebo and at 10minute intervals for 40minutes. Systolic time intervals were calculated for each timepoint as described in chapter 2 (page 51).

DATA ANALYSIS

For each individual, a baseline value was obtained from two averaged observations. As any response occurred within 10 minutes and persisted for the 40 minute study period, the average of the recordings at 10, 20, 30 and 40 minutes after sumatriptan injection was used to summarise the response to sumatriptan.

Statistics

Post-placebo and post-sumatriptan results were compared with baseline measurements using a paired t-test/confidence interval as the data appeared normally distributed.

RESULTS

Results are shown in figure 5.1 and in Table 5.3. Figures given in parentheses represent the 95% confidence intervals for the mean change from baseline.

ELECTROCARDIOGRAPHY

There were no changes in ECG morphology as assessed from the hard copies of six lead ECG's taken at 10 min intervals throughout the study.

HEART RATE

Baseline heart rate was 68 (SD13.5) per minute. After sumatriptan, heart rate decreased by 4.6 bpm (1.3 -8, p = 0.0094).

SYSTEMIC ARTERIAL PRESSURES

Mean baseline values for systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) were 124, 73 and 94mmHg, respectively. There was no significant change between baseline and placebo values. After sumatriptan, systolic arterial pressure rose by 19 mmHg (14.5 - 23.6, p=0.0001). Diastolic arterial pressure rose by 6.9 mmHg (3.9 - 9.8, p=0.0001). Mean arterial pressure increased by 11.5 mmHg (8.6 – 14.3, p = 0.0001).

SYSTOLIC TIME INTERVALS

There were no significant differences between baseline and placebo values. Mean baseline (heart rate corrected) values for the systolic time intervals were; total electromechanical systole (EMS) 585ms, pre-ejection period (PEP) 163ms and left ventricular ejection time (LVET) 423ms. Mean baseline PEP/LVET ratio

was 0.387. After sumatriptan administration, EMS rose significantly by 25ms (17-33, p=0.0001), PEP by 14ms (8-21, p=0.0002) and LVET by 11ms (5-16, p=0.0006). PEP/LVET ratio rose after sumatriptan by 0.023 (0.007-0.04, p=0.009). See figure 5.1.

DISCUSSION

The present study assessed the measurement of systolic time intervals as a potential non-invasive marker for the effects of sumatriptan on the cardiovascular system. Conventionally, STI's are measured from simultaneous, fast recording, (i.e. paper speed > 100 mm/sec) of the electrocardiogram, phonocardiogram and carotid arterial pulsation. In this study STI's were calculated using high quality, fast paper speed arterial tracings and ECG recordings. A phonocardiogram was not recorded as STI measurements were based on invasive pressure recordings. The onset of the second heart sound (A_2) was therefore not recorded in this set up and so total electromechanical systole cannot be denoted by the conventional nomenclature (QS_2) but for purposes of this discussion will be referred to as EMS. The advantage of employing the sharp inscription of the incisural notch of the aortic pressure tracing as a marker of A_2 in this protocol is that the difficulty in identifying the variable first high frequency components of A_2 are not encountered.

The absolute duration of the systolic intervals in this study is longer than in those protocols utilising phonocardiograms as there is an inherent conduction delay in fluid filled catheters which will lead to a prolongation of the pre-ejection period and total electromechanical systole. The PEP consists of two sub intervals; electromechanical delay and isovolumic contraction time (ICT). In the absence of conduction disturbances (QRS < 90ms) the ICT and PEP are closely correlated.[293] In this study no sequential changes in ECG morphology

were noted and it is therefore assumed that the change in PEP reflects prolongation of ICT.

This study demonstrates that sumatriptan, in clinically relevant doses, significantly prolongs EMS, PEP and LVET with a consequent rise in the PEP/LVET ratio. . The observed increase in PEP and PEP:LVET in response to sumatriptan may have occurred secondary to a negative inotropic effect or due to an acute rise in afterload. Analysis of the overall change in all STI's allows the precise mode of action to be established. Negative inotropes would be expected to decrease LVET without significant effect on the EMS, a pattern observed by Boudoulas et al when studying the negative inotropic effects of lidocaine.[301] Acute increases in afterload however prolong the EMS and LVET as well as the PEP and PEP:LVET and this pattern resembles closely the change in STI's following sumatriptan injection. Also against a negative inotropic action is the lack of a previous invasive study to show a significant change in the peak rate of rise of LV pressure ($\delta P/\delta t$) following sumatriptan.[266] These STI changes are therefore consistent with a sumatriptan-induced increase in afterload as witnessed by the rise in systemic arterial pressures.

Increases in afterload induced by intravenous injection of methoxamine are, like sumatriptan, accompanied by a lengthening of the PEP, QS_2 and LVET.[301] Shaver et al maintained heart rate constant with atrial pacing during methoxamine infusion, thereby preventing reflex bradycardia and noted an approximate 1 ms increase in LVET for each mmHg rise in systolic arterial pressure.[302] Angiotensin infusion, an agent with almost exclusive changes in afterload however, causes increases in QS_2 and PEP, but with a reduction in LVET.[303] Increases in PEP:LVET are seen with acute increases in afterload and in response to drugs with negative inotropic effects.[297]

The effect of sumatriptan on pre-load is less clear. Increases in left ventricular end-diastolic pressure (LVEDP) and pulmonary artery wedge pressure (PAWP) may be accounted for by increases in pre-load and afterload.[266] A prolongation of EMS following sumatriptan is also in keeping with an increase in pre-load but the PEP would normally shorten as pre-load increases. It is therefore assumed that, although sumatriptan may augment pre-load, the over-riding effect seems to be an increase in afterload.

Invasive haemodynamic studies of serotonergic compounds have obvious limitations and therefore the use of systolic time intervals as a non-invasive marker of haemodynamics has appeal. For example, the duration of action of serotonergic compounds can be assessed utilising the STI changes as physiological markers of the pharmacological activity. In this study the STI changes were evident within 10 mins, in keeping with a sumatriptan t_{max} of 5-20 mins[261] and a 15 min response rate of 74% in cluster headache.[257] The duration of the response in this study was maintained to at least 40 mins, consistent with sumatriptan's 1 hour anti-migraine response rate of 77%.[258]

In conclusion, it has been shown that sumatriptan induces changes in STI's consistent with its effect on afterload, and that the measurement of systolic time intervals may represent an accurate, non-invasive method of investigating the influence of serotonergic compounds on the cardiovascular system. This requires confirmation in a protocol that measures STI using a phonocardiogram, carotid arterial waveforms and the electrocardiogram.

Systolic interval	Sex	STI-Index	Standard deviation
QS_2	Male	QS_{2I} = -2.1HR + 546	14
	Female	QS_{2I} = -2.0 HR + 549	14
PEP	Male	PEP_I = -0.4 HR + 131	10
	Female	PEP_I = -0.4 HR + 133	11
LVET	Male	$LVET_I$ = -1.7 HR + 413	10
	Female	$LVET_I$ = -1.6 HR + 418	10

Table 5.1 Linear regression equations for systolic time intervals in both sexes. QS_2 = Total electromechanical systole, PEP = pre-ejection period, LVET = Left ventricular ejection time, HR = Heart rate.

Systolic Interval	Increase	Decrease
PEP	↑Afterload	↓Afterload
	↓Preload	↑Preload
	Negative inotropes	Positive inotropes
LVET	↑Preload	↓Preload
	↑Afterload	Positive inotropes
	Aortic stenosis	Negative inotropes
QS_2	↑Afterload	Positive inotropes
	LBBB	
PEP:LVET	Negative inotropes	Positive inotropes
	LV dysfunction	

Table 5.2 Factors affecting the STI's. LBBB = Left bundle branch block

	Mean Baseline (SD)	Mean change after placebo (95% C.I.)	P value	Mean change after sumatriptan (95% C.I.)	P value
HR (bpm)	68 (14)	-2 (-4.5 to 0.2)	0.069	-4.6 (-8 to –1.3)	0.0094
SAP(mmHg)	124 (18)	2 (-0.9 to 4.5)	0.17	19 (14.5 to 23.6)	0.0001
DAP(mmHg)	73 (11)	0.7 (-1.4 to 2.7)	0.5	6.9 (3.9 to 9.8)	0.0001
MAP(mmHg)	94 (14)	0.6 (-1.6 to 2.8)	0.58	11.5 (8.6 to 14.3)	0.0001
EMS(ms)	585 (38)	3 (-4 to10)	0.43	25 (17 to 33)	0.0001
PEP(ms)	163 (32)	-0.7 (-6.8 to 5.4)	0.81	14 (8 to 21)	0.0002
LVET(ms)	423 (29)	3.5 (-1.5 to 8.5)	0.16	11 (5 to16)	0.0006
PEP/LVET	0.39 (084)	-0.006 (-0.02to 0.01)	0.48	0.02 (0.01to 0.04)	0.0088

TABLE 5.3: Arterial pressure and systolic time interval changes following placebo and sumatriptan. Baseline values are expressed as mean (SD). Figures given in parentheses represent 95% confidence intervals for the mean change from baseline.

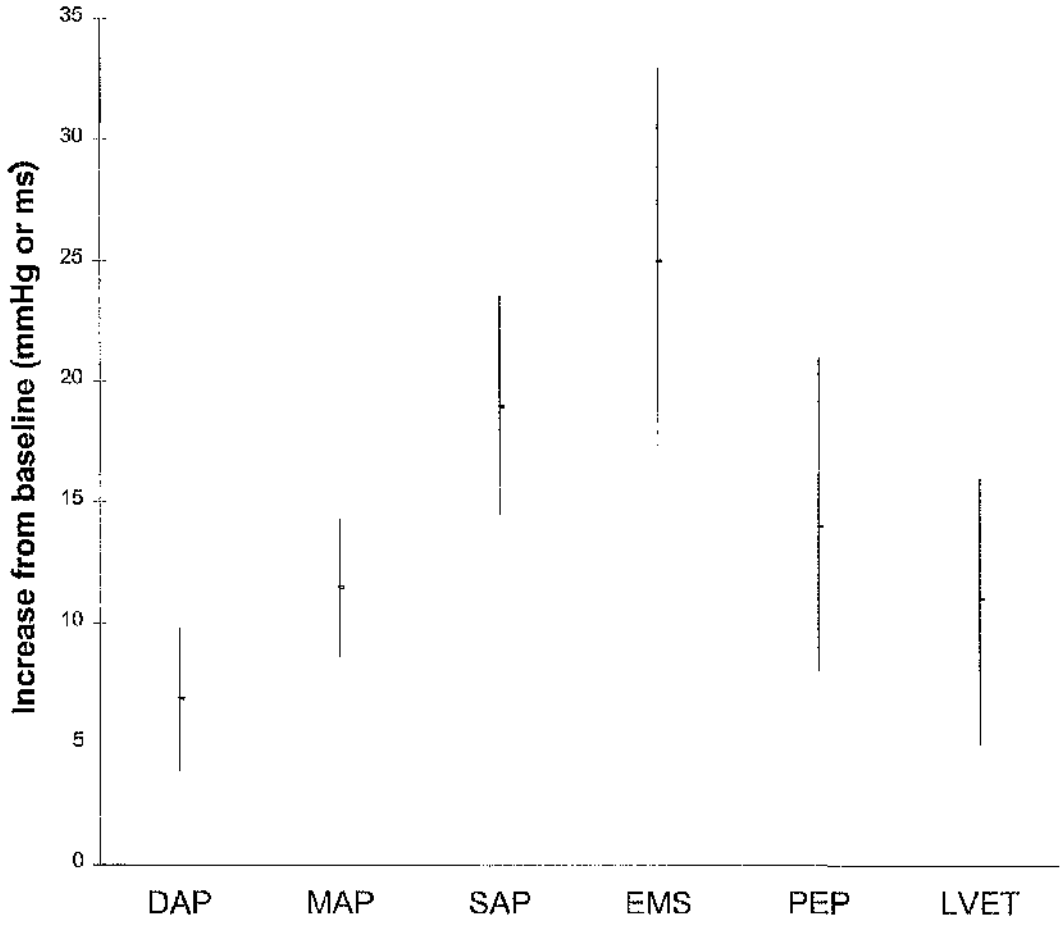


Figure 5.1 Mean (95%C.I.) increases in systemic arterial pressure and systolic time intervals after sumatriptan injection.

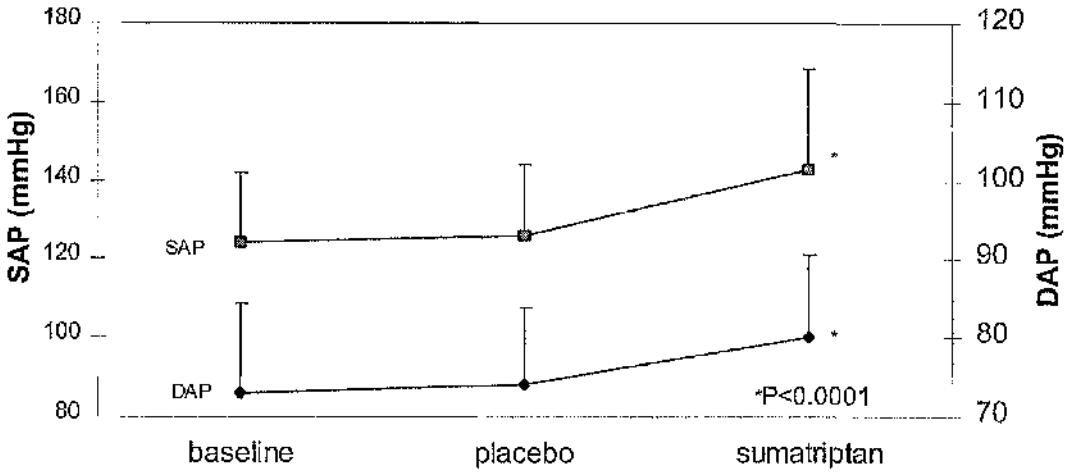


Figure 5.2 Systolic (SAP) and diastolic (DAP) arterial pressures (s.d.) following placebo and sumatriptan

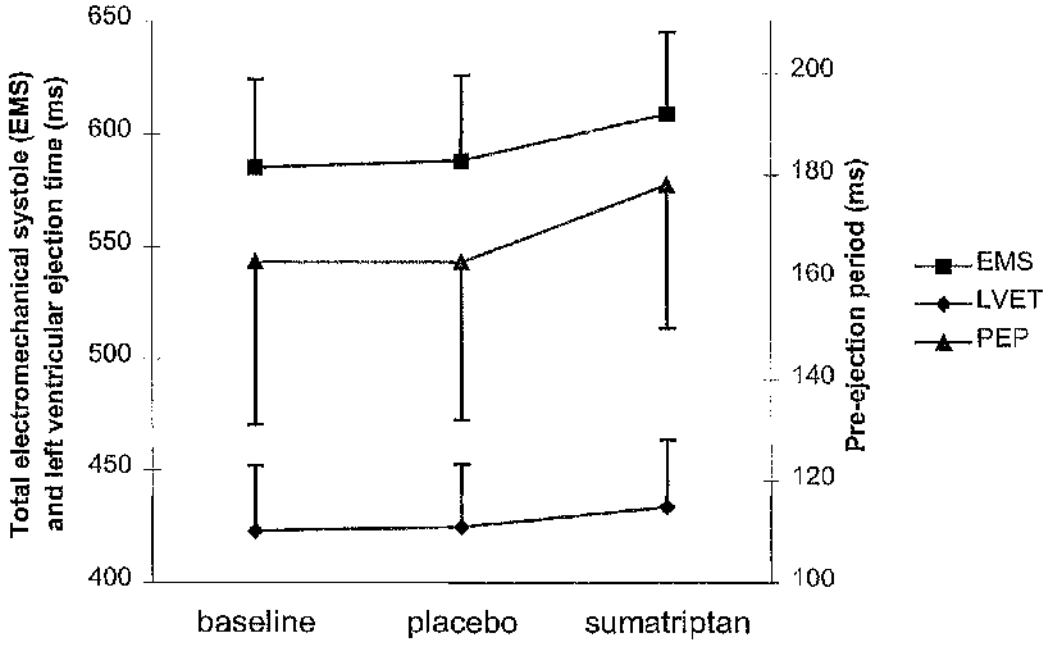


Figure 5.3 Systolic time interval changes (s.d.) after placebo and sumatriptan

CHAPTER 6

THE EFFECT OF SUBCUTANEOUS NARATRIPTAN, A 5-$HT_{1B/D}$ AGONIST ON THE SYSTEMIC, PULMONARY AND THE CORONARY CIRCULATIONS

INTRODUCTION

Naratriptan, an analogue of sumatriptan, is a selective 5-$HT_{1B/1D}$ agonist. Although the efficacy of sumatriptan in acute migraine is well established,[255,256,259] poor oral bioavailability (14%) and short duration of action ($t_{½}$ = 2 hrs) are recognised limitations.[304] Naratriptan, however, has high oral bioavailability (60-70%) and a longer duration of action ($t_{½}$ = 6 hrs), making it a potentially more attractive anti-migraine compound.[304]

The biological pre-clinical profile of naratriptan has been established in a number of carefully controlled *in vitro* trials reported in the one paper.[305] Naratriptan has high affinity for human recombinant 5-HT_{1B} and 5-HT_{1D} receptors, having six fold and three fold higher affinity respectively than sumatriptan at these two receptors.[305] Affinity for 5-HT_{1A} and 5-HT_3 receptors is low. In isolated dog basilar and middle cerebral artery, naratriptan was approximately equipotent with serotonin, but roughly three fold more potent than sumatriptan. Maximal contraction was similar for all 3 agonists, however. In dog isolated basilar artery, methiothepin, but not ketanserin, antagonised naratriptan-induced contractions, indicating a 5-HT_1 mediated effect. In human isolated coronary arteries, naratriptan produced a maximum contractile effect one-third that of serotonin, but was four times more potent than sumatriptan, although the maximum contraction elicited was similar for sumatriptan and naratriptan.

In vivo experiments in anaesthetised dogs, naratriptan caused dose-dependent long-lasting increases in carotid vascular resistance with parallel reductions in carotid blood flow.[305] Although a 92% increase in carotid vascular resistance and 44% decrease in carotid blood flow were elicited by naratriptan, no significant

change in blood pressure was observed. This suggests that, like sumatriptan, naratriptan causes a selective vasoconstriction of the carotid vascular bed in dogs.[254] Naratriptan, was again more potent (approximately two fold) than sumatriptan.

In summary, naratriptan has a pre-clinical profile similar to that of sumatriptan, but is approximately three to six fold more potent at 5-HT_{1B} and 5-HT_{1D} receptors. It is obviously impossible to accurately extrapolate results from *in vitro* experiments and from other species to the clinical situation, hence the need for controlled clinical trials. In humans, sumatriptan has been shown to cause vasopressor responses in the systemic and pulmonary circulation, with an additional 14-16% reduction in coronary artery diameter.[241,242] Given the pharmacodynamic pre-clinical similarities between sumatriptan and naratriptan, one might also expect naratriptan to induce systemic, pulmonary and coronary vasoconstriction.

The effect of naratriptan (1.5 mg s.c.) on cardiac haemodynamics and coronary circulation was therefore assessed in 10 patients undergoing diagnostic cardiac catheterisation. The experimental model was based on that previously applied to sumatriptan.

METHODS

The study protocol is summarised in table 6.1. Ten subjects (2 M, 8 F, age range 44-69 yrs) undergoing diagnostic cardiac catheterisation were studied. Exclusion criteria are documented in chapter 2. Haemodynamics were measured in an identical manner to that described for sumatriptan in chapter 2 (pages 49-50)

TIME (mins)	-20	-10	0	10	20	30	40
Coronary angiogram	*	*	*	*	*	*	*
Baseline measurements	*						
Placebo injection		*					
Naratriptan 1.5mg s.c.			*				
6 lead ECG	*	*	*	*	*	*	*
Cardiac Haemodynamics	*	*	*	*	*	*	*

Table 6.1 Study protocol. The haemodynamic effects of naratriptan.

Quantitative Coronary Angiography

Quantitative coronary angiography was performed on 2 right and 8 left coronary arteries. The procedure has been described in chapter 2 (page 51). Ten proximal (one from each patient), 8 mid and 9 distal sites were identified. Two blinded observers measured coronary artery diameter at these sites using the automated edge detection programme.

Data analysis

A baseline value was obtained for each haemodynamic parameter by averaging two observations. As any response occurred within 10 minutes and persisted for the 40 minute study period, the average of the haemodynamic recordings at 10, 20, 30 and 40 minutes after naratriptan injection were used to summarise the response to naratriptan. Similarly when assessing coronary artery diameter responses to naratriptan, the average of the artery diameter at 10, 20 and 30 minutes was used as a summary measure of the response.

Statistics

Post-placebo and post-naratriptan results were compared with baseline measurements using a paired t-test/confidence interval as the data appeared normally distributed.

RESULTS

Results are summarised in Tables 6.2 and 6.3. Figures given in parenthesis represent 95% confidence intervals for the mean change from baseline.

Heart Rate and Cardiac Output

No significant change in mean heart rate (95%C.I. –2.5 to 3.8 bpm, p=0.64) was observed with a base-line rate of 67(S.D.8) and post-naratriptan rate of 68 (S.D.8) beats/min. Cardiac output was 5.62 (S.D.0.9) L/min at baseline and 5.69 (S.D.1.0) L/min post-naratriptan (95%C.I. –0.33 to 0.47, p=0.70).

Systemic Arterial Pressure

Mean baseline values for systemic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) were 135 mmHg, 70 mmHg and 96 mmHg respectively. No significant change from baseline was observed after placebo injection. Following naratriptan injection, SAP, DAP and MAP had a mean (95%C.I.) rise of 14.8 (7.6 to 22.0, p= 0.0015), 6.3 (3.9 to 8.7,p=0.0002) and 12.4 (7.0 to 17.8,p=0.0007) mmHg respectively.

Systemic Vascular Resistance

Mean baseline SVR was 1314 dyne.s.cm^{-5}. No significant change was observed after placebo but SVR rose on average by 163 dyne s.cm^{-5} (95%C.I. 74 to 253, p=0.003) after naratriptan.

Pulmonary Arterial Pressure

Mean baseline values for pulmonary arterial systolic pressure (PASP), pulmonary arterial diastolic pressure (PADP) and mean pulmonary arterial pressure (MPAP) were 23.7 mmHg, 8.2 mmHg and 14.1mmHg respectively. No significant change was observed after placebo injection. Following naratriptan injection PASP, PADP and MPAP rose by 4.4 mmHg (95%C.I. 2.0 to 6.9,p=0.003), 2.6 mmHg (95%C.I. 0.7 to 4.4, p=0.012) and 3.5 mmHg (95%C.I. 2.4 to 4.7, p=0.0001) respectively.

Pulmonary Vascular Resistance (PVR)

Mean baseline PVR was 97 dyne.s.cm^{-5}. No significant change was observed after placebo but PVR rose by 19 dyne.s.cm^{-5} (95%C.I. 3 to 34, p=0.025) after naratriptan.

Pulmonary Artery Wedge Pressure (PAWP)

Mean baseline PAWP was 7.3 mmHg. No significant change occurred after placebo, but PAWP rose on average by 2.1mmHg (95%C.I. 1.9 to 2.4, p=0.009) after naratriptan.

Right Atrial Pressure (RAP)

Mean baseline RAP was 6.2 mmHg. No significant change occurred after placebo but following naratriptan injection, RAP rose on average by 0.8mmHg (95% C.I. 0.2 to 1.3, p=0.01).

Coronary Artery Dimensions

Coronary arteries were divided into proximal, middle and distal segments. One artery measurement from each individual was used for baseline comparisons. No significant differences were observed between naratriptan and placebo or baseline values (See Table 6.2 and Figure 6.3). Mean baseline proximal coronary artery diameter was 3.70cm. After naratriptan, mean change in artery diameter was –0.08cm (95%C.I. –0.27-0.11, p=0.37).

Plasma Drug Concentrations

Mean plasma naratriptan concentrations at 10 and 20 mins post dosage were 17.15 (S.D. 5.9) ng/ml and 17.83 (S.D. 2.05) ng/ml respectively.

Adverse Events

Adverse events were reported by 5 subjects (50%). Four subjects reported chest discomfort, which occurred within 5 minutes of naratriptan injection. These events were described as mild or moderate in severity and spontaneously resolved after 10 minutes. One subject experienced a headache classified as serious and the patient was therefore detained in hospital overnight. This headache commenced 170 mins after naratriptan injection and resolved 9 hrs later. Whether the headache was related to naratriptan or not is uncertain. No patients had an elevated creatinine kinase following naratriptan injection.

DISCUSSION

These results show a clinically non-significant, vasopressor response in the systemic and pulmonary circulation after subcutaneous naratriptan administration. The response occurred within 10 mins of injection and persisted for the 40 min study period. The rise in total systemic and pulmonary vascular resistance without a change in heart rate and cardiac output suggests that the pressure changes are consequent upon a direct vasoconstrictor effect. It is assumed that the haemodynamic effects are drug related although procedure related effects cannot be excluded.

These responses were to be expected as naratriptan exhibits similar pharmacological properties to sumatriptan.[305-308] Subcutaneous administration of naratriptan up to a dose of 10 mg has been associated with statistically significant increases in mean and systolic blood pressure up to 13 and 15 mmHg respectively (Glaxo Group Research). The rise in mean systemic arterial pressure in this study (12%) was of lesser magnitude than the 20% increase observed with sumatriptan 6 mg subcutaneously.[242] Pulmonary artery pressure changes following naratriptan injection were of a similar magnitude to sumatriptan-induced changes, with an approximate 30-40% rise accompanying injection of either agent.[242] As seen with sumatriptan, the relative percentage increase (32%) in pulmonary artery pressure following naratriptan was greater than systemic pressure changes (12%), again suggesting the possibility of a greater density of 5-HT_1 receptors in the pulmonary circulation. Naratriptan has high affinity for human cloned 5-HT_{1B} and 5-HT_{1D} receptors with approximately 6 and 3 fold higher affinity than sumatriptan, respectively for these subtypes.[305-308] In anaesthetised beagle dogs, naratriptan displayed a similar profile of action to sumatriptan, but was about two fold more

potent at vascular 5-HT_1 receptors.[306] In a range of isolated vascular tissue preparations used to study 5-HT_1 receptor mediated effects, naratriptan was found to be four times more potent than sumatriptan.[307] More recently, naratriptan has displayed greater potency compared to sumatriptan at recombinant rabbit saphenous vein 5-HT_{1B} receptors.[308] Human isolated coronary arteries contract in response to both naratriptan and sumatriptan, but naratriptan is approximately four times more potent than sumatriptan in this respect, although the maximum contraction is similar.[305]

In vivo therapeutic doses of sumatriptan have previously been noted to cause a 14-16% reduction in coronary artery diameter[241,242] raising concerns that this may be associated with rare serious cardiac adverse events.[238,239] In this small study, naratriptan in a therapeutic dose of 1.5 mg subcutaneously, had an insignificant effect on the coronary arteries similar to that of placebo. The reason for this is unclear, but may relate to a relative dose difference or reflect minor differences in receptor subtype interaction between the two drugs.

The functional state of the endothelium may be important, as the presence of atherosclerosis, resulting in dysfunctional endothelium, has been shown to influence coronary artery responses to serotonergic agonists.[55,229,233,235,309-312] The patients in this study and in the previous sumatriptan studies,[241,242] had normal or near normal coronary arteries but may still have had occult endothelial dysfunction which contributed to the observed differences between the two drugs. Interestingly, a recent *in vitro* study found sumatriptan less potent than other triptans in contracting isolated human coronary arteries with functional endothelium.[313]

The effect of naratriptan and other triptans on diseased coronary artery segments would be of interest but presently all triptans are contraindicated in patients with, or at risk of, coronary artery disease. Assessment of coronary artery blood flow would also be desirable, but was not part of the present study. Preliminary evidence in migraineurs, however, suggests that naratriptan induced changes in myocardial perfusion are substantially less than those seen with ergotamine.[314]

Four of the patients reported sensations of chest heaviness or tightness without significant ECG changes, rise in creatinine kinase or reduction in coronary artery diameter. One individual's ECG showed inverted T waves in leads 1 and AVL at baseline. This normalised at 30 mins post naratriptan in lead 1 but had reverted to the baseline state the following day and was not considered to be of clinical concern. These non-specific findings are similar to experience with sumatriptan.

In summary, naratriptan, like sumatriptan and other 5-HT_1agonists, has been shown to cause vasoconstrictor responses in the systemic and pulmonary circulation, presumably via direct 5-HT_1 receptor agonism. Unlike sumatriptan, however, no significant effect on coronary artery dimensions was observed in this study. This may relate to relative dose or potency differences of the two drugs or may be a reflection of differing endothelial function among subjects. These findings, however, could have therapeutic importance and may widen the clinical application of this group of compounds depending on the results of future studies and clinical observation.

	Mean baseline value (S.D.)	Mean change after placebo (95% C.I.)	P Value	Mean change after sumatriptan (95% C.I.)	P value
HR (bpm)	67 (8)	-1.9 (-4.0 to 0.16)	0.59	0.67 (-2.5 to 3.8)	0.64
CO ($l.min^{-1}$)	5.62 (0.9)	0.14 (-0.19 to 0.48)	0.35	0.07 (-0.33 to 0.47)	0.7
SAP (mmHg)	135 (31)	-0.44 (-10.7 to 9.8)	0.92	14.8 (7.6 to 22.0)*	0.0015
DAP (mmHg)	70 (13)	-0.11 (-3.2 to 3.0)	0.94	6.3 (3.9 to 8.7)*	0.0002
MAP (mmHg)	96 (18)	1.1 (-3.6 to 5.8)	0.6	12.4 (7.0 to 17.8)*	0.0007
SVR ($dyne.s.cm^{-5}$)	1314 (330)	-16 (-94 to 62)	0.65	163 (74 to 253)*	0.003
PASP (mmHg)	23.7 (7.1)	0 (-1.7 to 1.7)	1.0	4.4 (2.0 to 6.9)*	0.003
PADP (mmHg)	8.2 (3.6)	0.4 (-1.5 to 2.3)	0.65	2.6 (0.7 to 4.4)*	0.012
MPAP (mmHg)	14.1 (4.6)	0.2 (-1.2 to 1.5)	0.78	3.5 (2.4 to 4.7)*	0.0001
PVR ($dyne.s.cm^{-5}$)	97 (38)	0.2 (-21 to 22)	0.98	19 (3 to 34)*	0.025
PAWP (mmHg)	7.3 (3.9)	0.1 (-0.7 to 0.9)	0.75	2.1 (1.9 to 2.4)*	0.009
RAP (mmHg)	6.2 (2.9)	0.3 (-0.4 to 1.1)	0.35	0.8 (0.2 to 1.3)*	0.01

Table 6.2 Cardiac haemodynamics at baseline and changes after placebo and naratriptan injection. In each case comparisons are made with baseline values.

	Mean (S.D.) baseline diameter (mm)	Mean change after placebo (95%C.I.)	P value	Mean change after naratriptan (95%C.I.)	P value
Proximal segments (n=10)	3.70 (0.63)	-0.06(-0.15 to 0.02)	0.12	-0.08(-0.27 to 0.11)	0.37
Middle segments (n=8)	2.63 (0.73)	-0.15(-0.38 to 0.07)	0.15	-0.10(-0.29 to 0.08)	0.23
Distal segments (n=9)	1.65 (0.37)	-0.04(-0.12 to 0.04)	0.3	-0.15(-0.36 to 0.04)	0.09

Table 6.3 Mean coronary artery diameter (mm) at baseline and following placebo and naratriptan injection. In each case comparisons are made with baseline values.

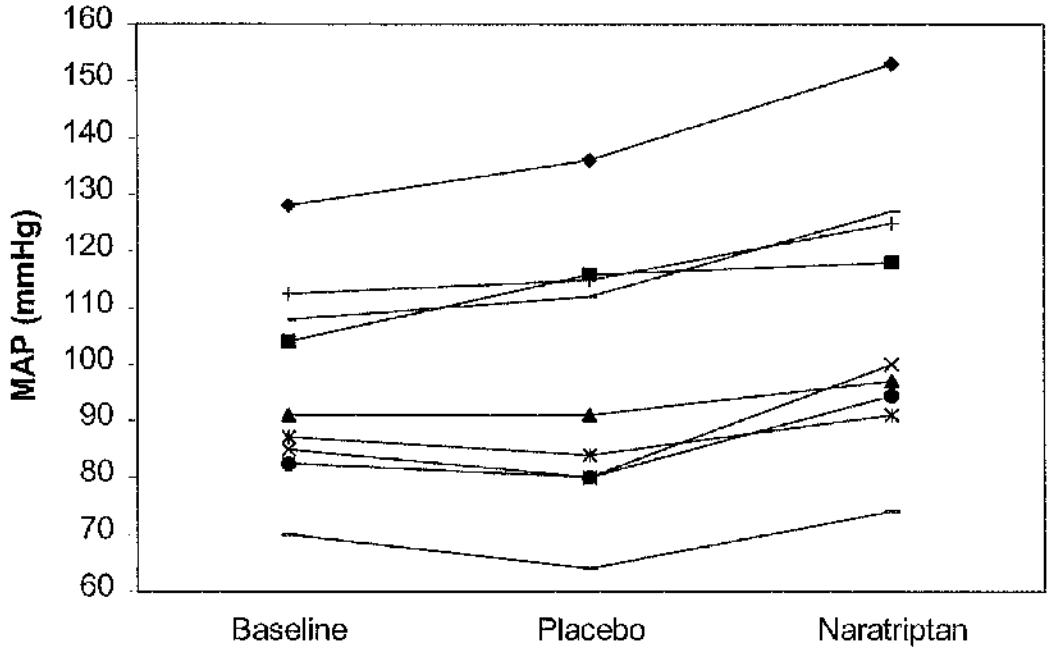


Figure 6.1 Mean arterial pressure (MAP) at baseline and after placebo and naratriptan injection. Each line represents an individual.

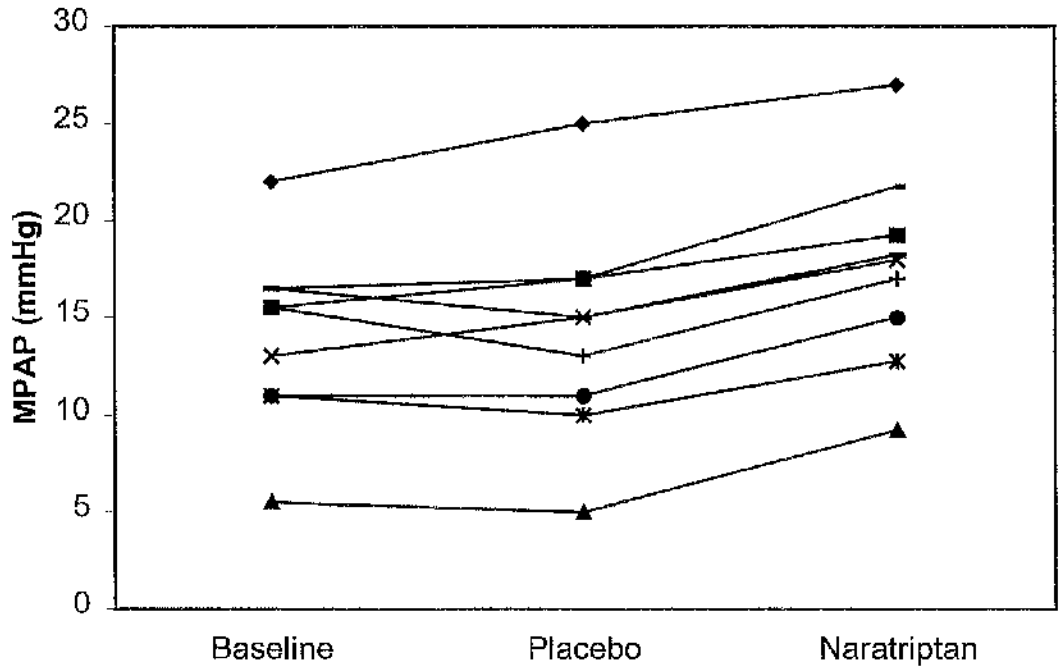


Figure 6.2 Mean pulmonary artery pressure (MPAP) at baseline and after placebo and naratriptan injection. Each line represents an individual.

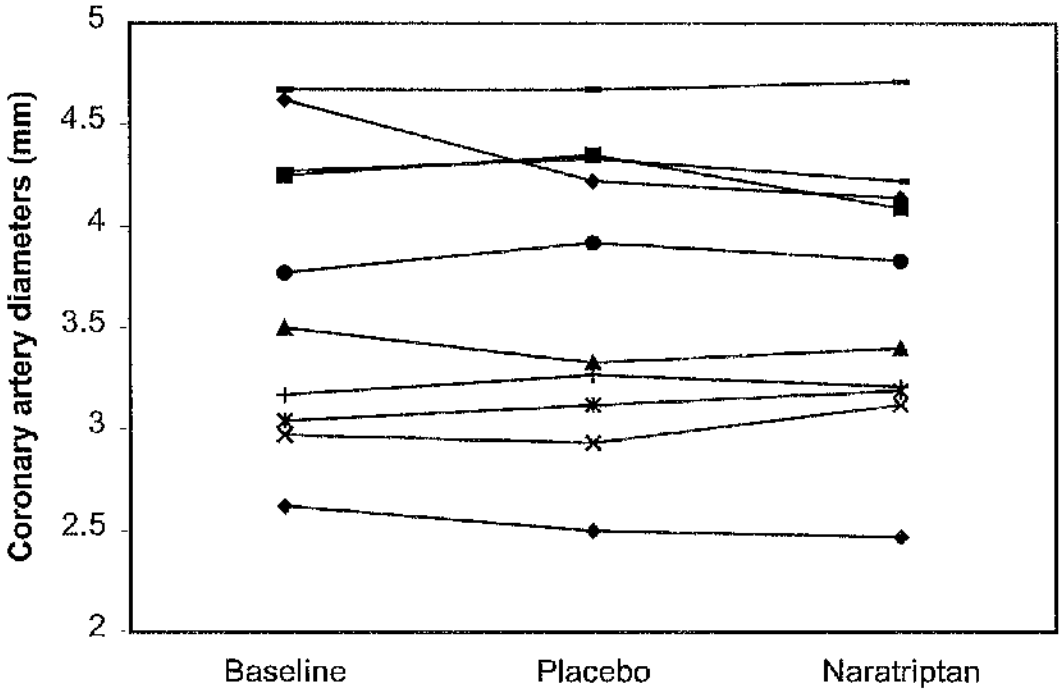


Figure 6.3 Proximal coronary artery diameters at baseline and after placebo and naratriptan injection. Each line represents an individual.

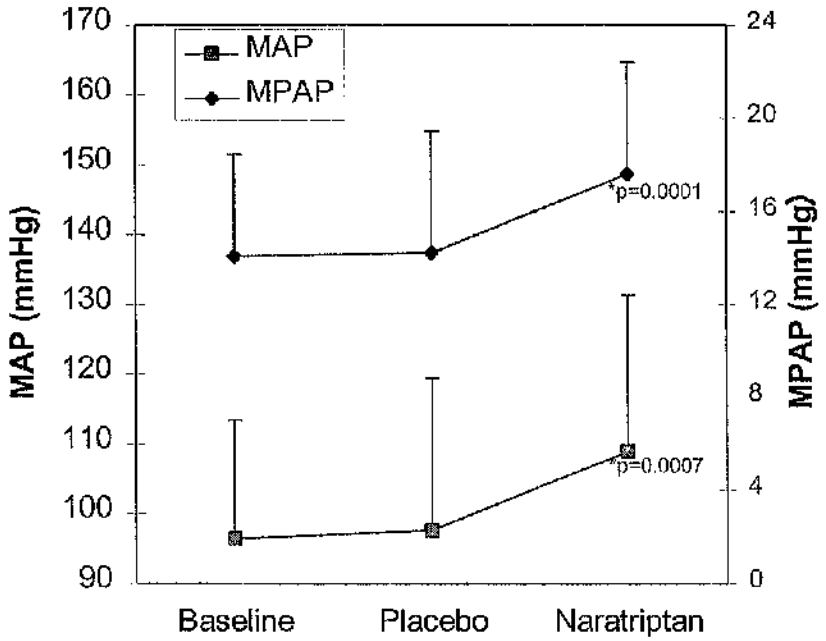


Figure 6.4 Mean arterial (MAP) and mean pulmonary arterial pressure (MPAP) changes after placebo and naratriptan injection.

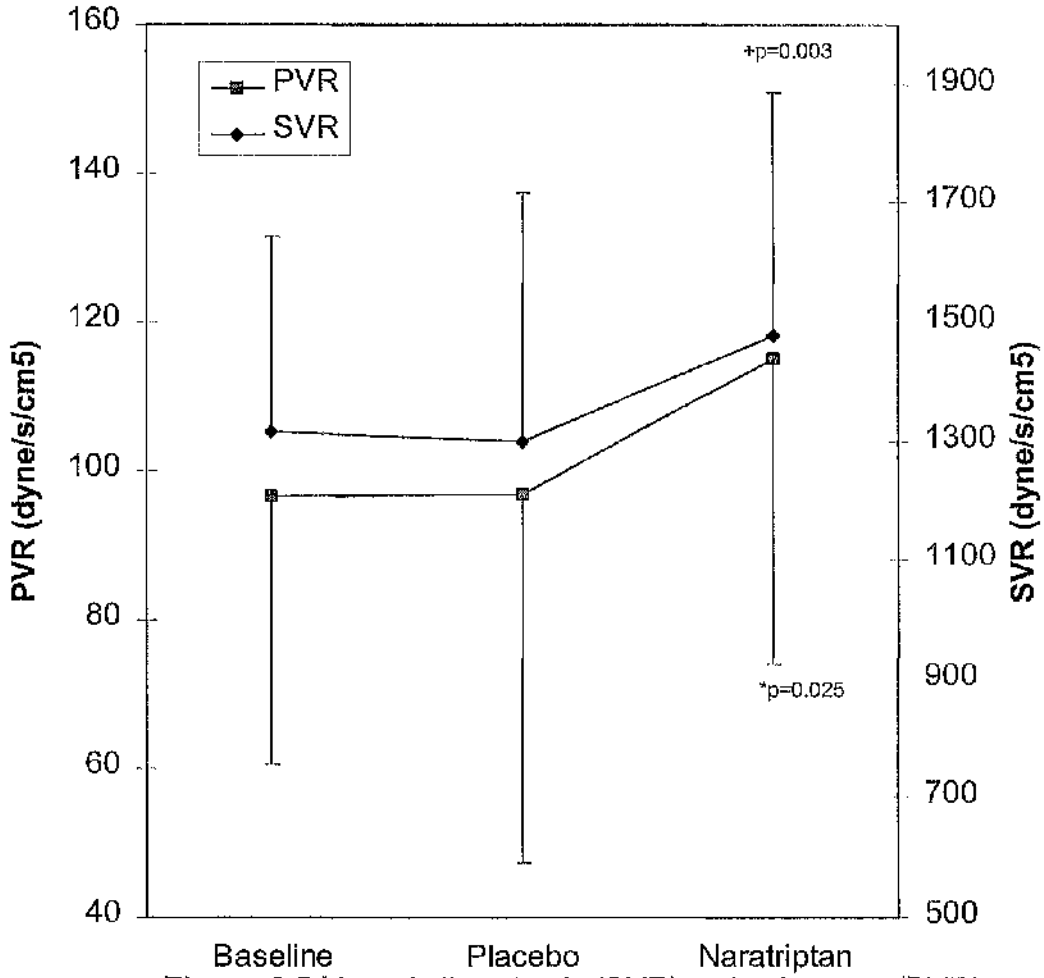


Figure 6.5 Mean (sd) systemic (SVR) and pulmonary (PVR) vascular resistance changes after placebo and naratriptan injection.

CHAPTER 7

THE EFFECT OF ELETRIPTAN, A 5-HT_{1D} AGONIST, ON THE SYSTEMIC, PULMONARY AND CORONARY CIRCULATIONS.

INTRODUCTION

Symptomatic relief is gained in 70-80% of acute migraine attacks treated with sumatriptan, the prototype and first clinically available 5-HT_1 agonist.[255,256] However, as discussed earlier in this thesis, its use is complicated by limited oral bio-availability and by side effects which include chest tightness in approximately 3-5% of patients.[243] There is conclusive evidence from both *in vitro*[42,48] and *in vivo*[241,242] studies, that the cardiovascular effects of sumatriptan are not confined to the cranial circulation as was originally thought. These extra-cranial vascular actions may be responsible for some of the more serious adverse reactions to sumatriptan.[238-240]

Eletriptan is a "second generation" 5-HT_1 receptor agonist. It is a potent and selective agonist at the 5-HT_{1D} receptor subtype and is currently in clinical development for the acute treatment of migraine. There is some evidence that eletriptan has greater cranioselectivity than sumatriptan and this is of potential interest in terms of cardiovascular safety. In both *in vitro* and *in vivo* pre-clinical studies, eletriptan has shown lower potency than sumatriptan to cause coronary artery vasoconstriction but remains equipotent in tissues believed to reflect anti migraine activity (Pfizer Central Research data). For example, both sumatriptan and eletriptan are potent constrictors of isolated canine saphenous veins but eletriptan was approximately 2 fold more selective for saphenous vein compared to the coronary artery. On the contrary, sumatriptan displayed 1.7 fold selectivity for the coronary artery compared to the saphenous vein.

In vivo, in the anaesthetised dog, eletriptan demonstrated a statistically significant 5-fold selectivity for reduction in carotid artery blood flow relative to

the reduction in left circumflex coronary artery diameter. This compared favourably with sumatriptan which demonstrated a non-significant 2-fold selectivity for carotid blood flow reduction (Pfizer Central Research data). These data infer that, in the anaesthetised dog, for a given reduction in carotid artery blood flow (a surrogate marker of potential anti-migraine efficacy), less coronary artery constriction is induced by eletriptan than sumatriptan. This suggests a potential increase in the selectivity of eletriptan over sumatriptan for the target vascular territory but the vasoactive effects of serotonergic compounds are notoriously species dependent and these results cannot be extrapolated to the clinical setting.

Within the proposed clinical dosing range (16.7 –102 mcg/kg i.v and 1.5- 30mg p.o.) eletriptan displays approximately linear increases in plasma concentration with dosing. Eletriptan has been evaluated in over 100 volunteers and over 200 migraine patients. Preliminary studies revealed that intravenous doses of 50-102 mcg/kg (over 15 minutes) and oral doses of 5,20 and 30mg were well tolerated and efficacious in acute migraine. Only 2 episodes of chest pain were reported, one by a 68 year old woman 2 hours after dosing with 20mg orally and after prolonged and severe vomiting. The other episode of chest pain occurred in a volunteer after dosing with 120mg orally (Pfizer Central Research data).

Bearing in mind the favourable pre-clinical profile of eletriptan, the effects on the human vasculature require to be established *in vivo* to ascertain whether theoretical advantages might be translated into a potentially safer anti-migraine compound. In this chapter the effects of intravenous eletriptan on the systemic,

pulmonary and coronary circulation were therefore established in a similar clinical model to that applied to sumatriptan and naratriptan.

METHODS

The study protocol is summarised in table 7.1. Ten patients (2M:8F; mean age 51, range 39-75) were studied. Exclusion criteria were as discussed in chapter 2 (page 49). After initial baseline readings had been taken, a placebo infusion of 16ml of 0.9% saline was administered via an antecubital vein at 96ml/hr for 10 minutes. Five minutes after completing the placebo infusion, eletriptan was infused through the same cannula at a rate of 3.33mcg/kg/min for 15 minutes (total dose 50mcg/kg). This concentration was chosen in order to achieve plasma levels similar to those found after a 40mg oral dose of eletriptan. Measurements continued for a 30 minute "wash out" period after cessation of the eletriptan infusion. Haemodynamic pressure recordings and quantitative coronary angiography were performed in a manner identical to that described in chapter 2 (pages 49-51).

Statistical Analysis

A mean placebo value for each parameter was calculated from the average of 3 recordings during the placebo infusion. Similarly, a mean eletriptan value for each parameter was calculated from the haemodynamic recordings during eletriptan infusion. During the wash out phase, a mean wash out value was also established for each haemodynamic variable. Measurements during placebo infusion, during eletriptan infusion and after eletriptan infusion were then compared to baseline values by t-test. All results are expressed as 95% confidence intervals and significance levels are stated.

RESULTS

Haemodynamic Variables

There were no significant differences between baseline and placebo values for any of the haemodynamic variables. Results are summarised in table 7.2.

Heart Rate

Mean (s.d.) heart rate at baseline was 69 (9.5) bpm. After eletriptan infusion, the mean change in heart rate was –1 bpm (95%C.I. –1.6 to 3.6, p=0.6)

Cardiac output (C.O.)

Mean (s.d.) baseline C.O. was 5.8 (1.3) $l.min^{-1}$. After eletriptan infusion C.O. fell significantly by an average of 1.0 $l.min^{-1}$ (95%C.I. 0.4 to 1.6, p=0.018).

Systemic Arterial Pressure

Mean (s.d.) baseline values for systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) were 131 (25.7)mmHg, 69 (12.3)mmHg and 91 (14.8) mmHg respectively. Mean (95%C.I.) changes following eletriptan infusion were: SAP 4.3mmHg (–5.8 to 14.4, p=0.5) : DAP 4.6mmHg (–0.7 to 9.9, p=0.1) : MAP 7mmHg (-0.7 to 9.9, p=0.09).

Pulmonary Arterial Pressure

Mean (s.d.) baseline values for pulmonary artery systolic pressure (PASP), pulmonary artery diastolic pressure (PADP) and mean pulmonary artery pressure (MPAP) were 21 (4)mmHg, 8 (2.1)mmHg and 14 (2.7)mmHg respectively. Mean (95%C.I.) changes following eletriptan infusion were: PASP

1.1mmHg (-1.5 to 3.7, p=0.5): PADP 3.4mmHg (-1.2 to 8.0, p=0.2) : MPAP 0.6mmHg (-2.1 to 3.1, p=0.7).

Pulmonary Artery Wedge Pressure (PAWP)

Mean (s.d.) baseline PAWP was 8.1 (3) mmHg. Mean (95%C.I.) change after eletriptan infusion was 0.6mmHg (-1.0 to 2.2, p=0.5).

Right Atrial Pressure (RAP)

Mean (s.d.) baseline RAP was 5.0 (2.1) mmHg. Mean (95%C.I.) change after eletriptan was 0.9mmHg (-0.3 to 2.1, p=0.2)

Systemic Vascular Resistance (SVR)

Mean (s.d.) baseline SVR was 1221 (232) dyne.s.cm^{-5}. After eletriptan infusion, SVR rose significantly by an average of 298 dyne.s.cm^{-5} (95%C.I. 161-435, p=0.004).

Pulmonary Vascular Resistance (PVR)

Mean (s.d.) baseline PVR was 70 (33) dyne.s.cm^{-5}. Eletriptan infusion was associated with a significant increase in PVR. The mean maximum rise was 31 dyne.s.cm^{-5} (95%C.I. 9-53, p=0.03).

Coronary effects

During eletriptan infusion, one subject developed a segmental constriction in the proximal right coronary which was accompanied by mild chest tightness but no ECG changes. The constriction persisted to a lesser degree after the end of the eletriptan infusion, as did the chest tightness, which was later relieved by sub-lingual nitrates. Two experienced independent cardiologists

felt that catheter induced spasm could not be excluded as a cause of the segmental constriction. Statistical analysis of the coronary dimensions was performed including this subject.

Mean (s.d.) baseline coronary artery diameters were 2.96 (0.59)mm, 2.40 (0.81) mm and 2.20 (0.58)mm for proximal, middle and distal coronary artery segments respectively. No significant changes occurred during or after eletriptan infusion. Mean (95%C.I.) change in proximal coronary segments was –0.11mm (-0.44 to 0.22, p=0.5). Corresponding figures for mid and distal segments were 0.16mm (-0.16 to 0.32, p=0.38) and –0.09mm (-0.29 to 0.11, p=0.45) respectively.

DISCUSSION

Unlike sumatriptan and naratriptan, eletriptan failed to elicit significant increases in systemic or pulmonary arterial blood pressure in this small group of patients. If vasoconstrictive effects are a class action of the triptan group of 5-HT_1 agonists, then increases in arterial and pulmonary pressures might have been expected following the administration of eletriptan. It is unclear therefore why no such effect was seen with eletriptan. The patients in this study came from an identical clinical substrate to those in the sumatriptan and naratriptan studies and different patient characteristics seem an unlikely explanation.

Relative dose differences between the respective drugs could provide an explanation. The dose of eletriptan in this study (total dose 50mcg/kg i.v.) was toward the lower end of the proposed clinical dosing range (16.7 –102 mcg/kg) and it may be that in relatively low doses, eletriptan has little effect on the

pulmonary or systemic circulation whilst maintaining anti-migraine efficacy. This possibility has been discussed with regard to the differing actions of naratriptan and sumatriptan on coronary artery dimensions (see chapter 6).

There is evidence that the anti-migraine activity of sumatriptan relates not only to vascular receptor properties but also on an ability to modulate release of certain neuropeptides responsible for neurogenic inflammation (plasma protein extravasation and vasodilatation).[260,261] The dose response curves for these two properties may differ such that sumatriptan induced inhibition of neurogenic inflammation occurs at a lower dose than that required to produce vasoconstriction. Interestingly, sumatriptan selectively inhibited plasma extravasation in the dura mater of the rat and guinea pig at doses that did not increase systemic arterial pressure.[261]

Although no effect was seen on systemic or pulmonary arterial pressure, both systemic and pulmonary vascular resistance increased after eletriptan infusion. SVR rose by 24% from baseline with a 44% rise in PVR. As seen with both sumatriptan and naratriptan, the percentage rise in PVR exceeded that for SVR in keeping with the possibility that 5-HT_1 receptors are more densely distributed within the pulmonary circulation. Eletriptan however, was associated with a statistically, but not clinically, significant 17% decrease in cardiac output. It is possible that the observed increases in both SVR and PVR are a reflection of this reduction in cardiac output as cardiac output is the denominator in the formulae required to calculate SVR and PVR.

In the absence of changes in heart rate, the reduction in cardiac output seen after eletriptan could be attributed to a reduction in stroke volume. Negative inotropic effects of 5-HT_1 agonists have not been reported however. Sumatriptan (6mg s.c.) had no significant effect on maximal rate of left ventricular pressure rise ($\delta P/ \delta t$) as seen in chapter 3. Assessment of STI responses has however shown an increase in the PEP:LVET ratio after sumatriptan (see chapter 5) but this is more likely to reflect an acute increase in afterload rather than a negative inotropic effect.

Sumatriptan has vasoconstrictor properties on the coronary circulation as evident from the 14-16% reduction in coronary artery diameter seen after both intravenous[241] and subcutaneous[242] injection. Neither naratriptan nor eletriptan have been observed to share this property. Possible reasons for the disparity between naratriptan and sumatriptan in this regard have previously been discussed. Similar arguments may explain the difference between eletriptan and sumatriptan.

Eletriptan is a potent 5-HT_{1D} agonist, which in animal models shows coronary constriction, but at a dose four times higher than sumatriptan. Higher selectivity for non-coronary vascular beds is seen in animal studies and this is compatible with the lack of coronary effects in the present study. These findings may be explained by eletriptan's 13-fold selectivity for the 5-HT_{1D} receptor subtype over the 5-HT_{1B} subtype (Pfizer Central Research). Although receptor subtypes in the human coronary artery are not well delineated, there is evidence that the 5-HT_{1B} but not the 5-HT_{1D} subtype is expressed in canine coronary arteries and saphenous veins, both vessels being commonly used as

models for vascular responses in human coronaries.[315] Recent evidence also suggests that coronary artery spasm in variant angina may be mediated by the 5-HT_{1B} receptor subgroup.[316]

Clearly one subject experienced a marked segmental constriction of the right coronary artery during eletriptan infusion which could either be due to a direct drug effect or due to catheter related spasm. Catheter induced spasm is often seen in the proximal right coronary artery, although in this case the timing of the constriction seems to correlate well with the drug infusion. However, segmental constriction of this type has not been described previously with 5-HT_1 agonists.

Current 5-HT_1 agonists are contra-indicated in patients with known ischaemic heart disease and are recommended to be used with caution in those with conditions predisposing to coronary artery disease. This data and receptor selectivity studies provide theoretical evidence to suggest that eletriptan may be the 5-HT_1 agonist of choice in patients with coronary risk factors but no overt coronary artery disease. It must not be overlooked however that the patients in this study had normal or near normal coronary arteries.

It is well recognised that, *in vivo,* the effects of serotonin agonism on human coronary arteries is dependent on the presence or absence of atherosclerosis.[233, 234] In one study, serotonin acted as a vasodilator and increased coronary artery blood flow in patients with normal coronary arteries.[233] In another study, intracoronary serotonin caused vasodilatation of angiographically normal coronary arteries at low doses but vasoconstricted at

higher doses.[234] In subjects with atherosclerosis however, intracoronary 5-HT caused only vasoconstriction. In another study in patients with stable angina, ketanserin (5-HT_2 antagonist) was unable to attenuate serotonin induced coronary artery spasm indicating that 5-HT_1 receptor activation may be implicated.[235] A similar study assessing the effect of eletriptan on diseased coronary arteries would therefore be required to confirm claims that eletriptan has potentially enhanced cardiovascular safety.

In summary, eletriptan, unlike sumatriptan, did not cause significant vasoconstriction in the systemic, pulmonary or coronary vasculature. Whether this relates to relative differences in 5-HT_{1B} and 5-HT_{1D} receptor subtype selectivity between the two drugs is unclear.

	Base line	0	5	10	15	20	25	30	35	40	45	50	55	60
6 Lead ECG	*		*	*	*	*	*	*	*	*	*	*	*	*
Placebo infusion		*	*	*										
Eletriptan infusion					*	*	*							
Cardiac output	*				*			*			*			*
Haemo-dynamics	*		*	*	*	*	*	*	*	*	*	*	*	*
Coronary angio	*				*			*			*			*

Table 7.1 Study protocol: The haemodynamic effects of eletriptan

Variable	Baseline (S.D.)	Mean change (95% C.I.) after placebo	P value	Mean change (95% C.I.) after eletriptan	P value
HR (bpm)	69 (9.5)	-2 (-5.6 to 1.6)	0.91	- 1 (-1.6 to 3.6)	0.6
C.O. ($l.min^{-1}$)	5.8 (1.3)	-0.24 (-0.49 to 0.01)	0.13	-1.0 (-0.4 to –1.6)*	0.018
SAP (mmHg)	131 (25.7)	-7.8 (-15.3 to – 0.3)	0.1	4.3 (-5.8 to 14.4)	0.5
MAP (mmHg)	91 (14.8)	0.6 (-5.9 to 7.1)	0.9	7.0 (-0.7 to 9.9)	0.09
DAP (mmHg)	69 (12.3)	0.6 (-3.8 to 5.0)	0.8	4.6 (-0.7 to 9.9)	0.1
PASP (mmHg)	21 (4)	-0.9 (-3.5 to 1.7)	0.5	1.1 (-1.5 to 3.7)	0.5
MPAP (mmHg)	14 (2.7)	-0.8 (-3.3 to 1.7)	0.6	0.6 (-2.1 to 3.1)	0.7
PADP (mmHg)	8.0 (2.1)	-0.1 (-1.9 to 1.7)	0.9	3.4 (-1.2 to 8.0)	0.2
PAWP (mmHg)	8.1 (3.0)	0.1 (-2.0 to 2.2)	0.9	0.6 (-1.0 to 2.2)	0.5
RAP (mmHg)	5.0 (2.1)	0.07 (-1.19 to 1.33)	0.68	0.9 (-0.3 to 2.1)	0.2
SVR ($dyne.s.cm^{-5}$)	1221 (232)	38 (-115 to 192)	0.66	298 (161 to 435)*	0.004
PVR ($dyne.s.cm^{-5}$)	70 (33)	14 (-12 to 40)	0.4	31 (9 to 53)*	0.03

Table 7.2. Haemodynamic variables at baseline, after placebo and maximal change after eletriptan infusion.

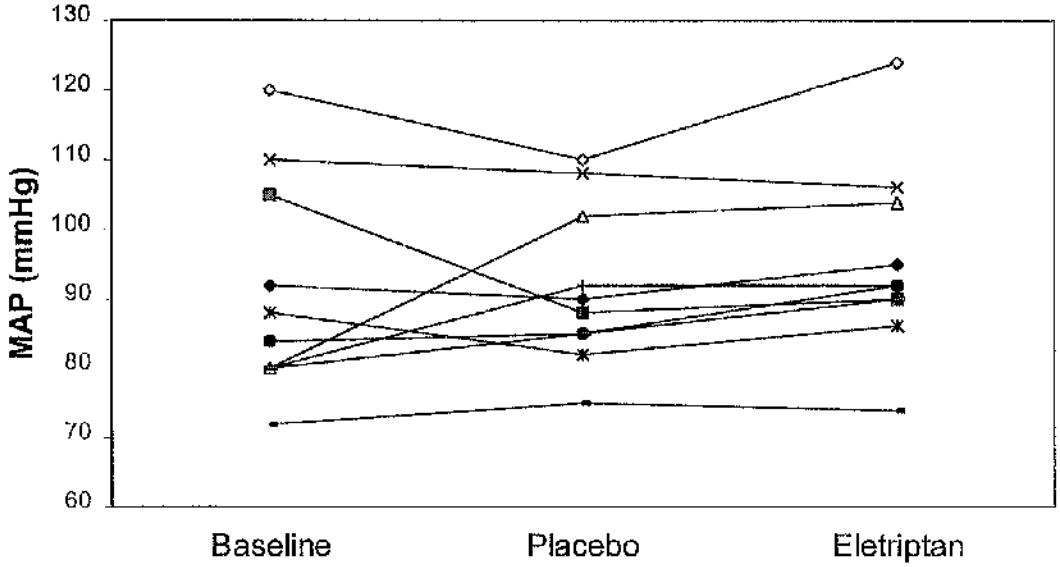


Figure 7.1 Mean arterial pressures (MAP) at baseline and after placebo and eletriptan infusion. Each line represents an individual.

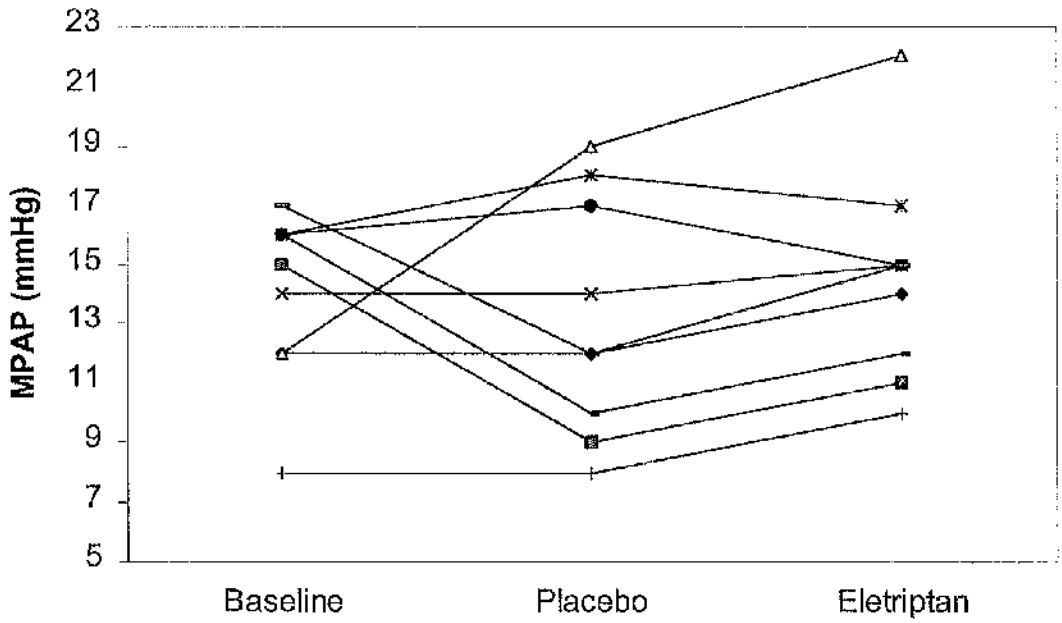


Figure 7.2 Mean pulmonary artery pressures (MPAP) at baseline and after placebo and eletriptan infusion. Each line represents an individual.

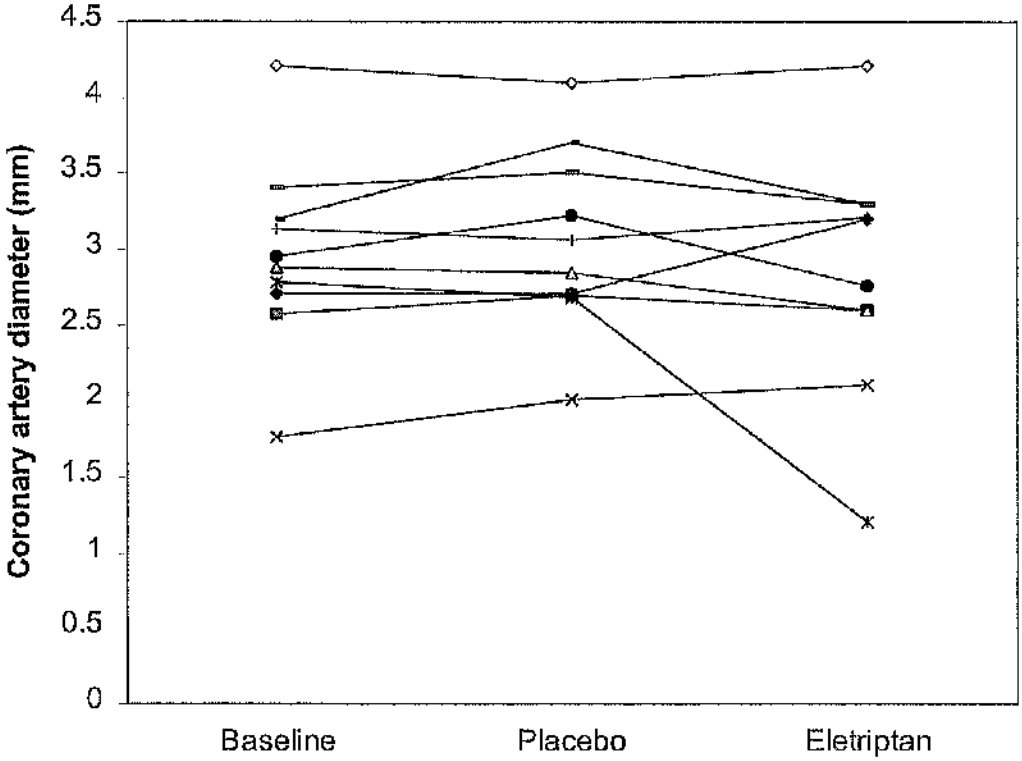


Figure 7.3 Proximal coronary artery diameters at baseline and after placebo and eletriptan infusion. Each line represents an individual.

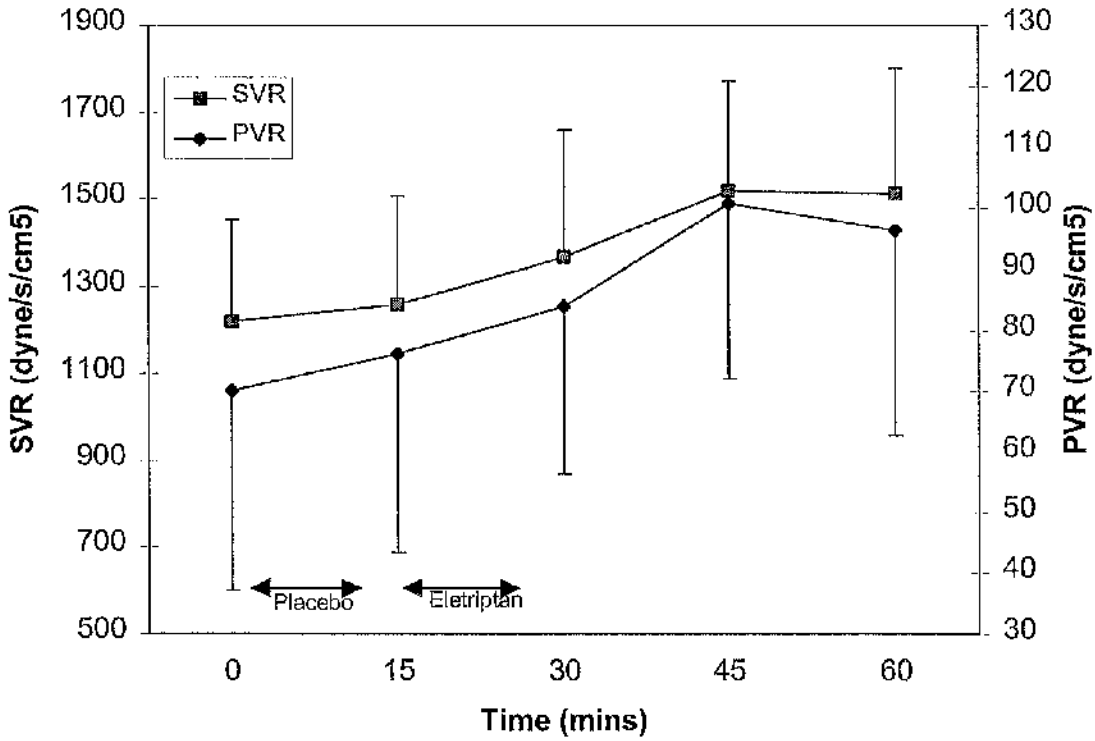


Figure 7.4 Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) responses to placebo and eletriptan infusion.

CHAPTER 8

THE EFFECT OF KETANSERIN, A 5-HT_2 ANTAGONIST, ON THE SYSTEMIC, PULMONARY AND CORONARY CIRCULATIONS.

INTRODUCTION

Clinical Pharmacology of Ketanserin:

Systemic Circulation

Ketanserin is a selective 5-HT_2 antagonist with additional, though weaker antagonist activity at α-adrenoreceptors.[317] This 5-HT_2 receptor is present in most systemic arterial smooth muscle and ketanserin can competitively inhibit serotonin induced smooth muscle contraction.[318] In isolated mesenteric arterial strips from hypertensive, compared to normotensive rats, the response to serotonin is augmented.[319,320] Increased responsiveness to serotonin has also been consistently reported in patients with essential hypertension[318] and Mehta & Mehta in 1981 suggested this may arise on account of increased platelet activation in such patients.[321]Stott et al in 1988 also noticed enhanced serotonin induced platelet aggregation in untreated patients with essential hypertension compared to a normotensive control group.[322] It remains uncertain whether this observed increase in platelet aggregation in hypertensives is a contributing factor to, or a consequence of the disease process. Nonetheless, there is evidence for a pathophysiological role of serotonin in hypertension.

Further support implicating serotonin in hypertension is the documented hypotensive effects of ketanserin in hypertensive patients. Given intravenously to hypertensive patients, ketanserin rapidly reduced systemic arterial blood pressure as measured non-invasively.[279,280,323,324] Typically, a 10mg intravenous bolus of ketanserin caused an approximate 20mmHg drop in blood pressure. The hypotensive action was age-related with greater blood pressure drops in the older (> 60 year old) population.[325] For example in patients under

60 years old, a 10% decrease in blood pressure was noted, the corresponding figure in over 60 year oids being 20-25%.[325] Given orally in small studies, ketanserin has also been shown to have hypotensive properties similar to thiazides, metoprolol or captopril.[326-328] Given long term (2-3 months) ketanserin 20-40 mg bd reduced diastolic blood pressure by 13-18mmHg.[327,328]

Pulmonary Circulation

Not surprisingly, serotonin also has effects on the pulmonary vasculature. The isolated human pulmonary artery contracts when exposed to serotonin[329] and this has led to speculation that serotonin may be implicated in some forms of pulmonary hypertension. For example, in adult respiratory distress syndrome there was a correlation between the serum concentration of serotonin and the pulmonary vascular pressure gradient.[330] In experimental pulmonary embolism, rises in pulmonary arterial pressure have been accompanied by increased plasma serotonin concentrations and ketanserin[331] and methysergide[332] could inhibit the rise in pulmonary arterial pressure. Ketanserin has also been shown to reduce pulmonary vascular resistance in 14 patients with pulmonary hypertension secondary to systemic sclerosis.[282] In two other small studies however, ketanserin had only modest or no pulmonary vasodilator effects in patients with primary[284] or hypoxic[285] pulmonary hypertension. The role, if any, of serotonin in pulmonary hypertension therefore remains unclear.

Ketanserin has consistently been shown, however, to have hypotensive effects on the pulmonary circulation following coronary artery bypass grafting.[279] Van

der Starre et al for example, noted reductions in pulmonary artery systolic pressure (-9%), pulmonary artery diastolic pressure (-27%) and pulmonary vascular resistance (-32%) in 20 patients whose pre-treatment pulmonary artery pressures and pulmonary vascular resistance were within normal limits.[280] This suggests that serotonin influences resting tone within the pulmonary circulation as well as in some conditions with pathologically increased pulmonary vascular tone.

Coronary Circulation

Serotonin may play an important pathophysiological role in acute coronary syndromes such as unstable angina or myocardial infarction. In patients with complex coronary artery lesions, elevated serotonin levels have been observed by sampling blood from the coronary sinus.[226] Blood samples from the coronary sinus are also capable of inducing canine coronary artery vasoconstriction *in vitro* - an action which can be attenuated with serotonin antagonists.[227] Serotonin constricts coronary artery rings from explanted human hearts, an action mediated by both 5-HT_1 and 5-HT_2 receptors.[43,44,232]

As a 5-HT_2 antagonist, ketanserin may therefore have the ability to influence *in vivo* coronary artery responses. Two recent studies have confirmed an important role for 5-HT_2 receptors in mediating coronary artery vasoconstriction during percutaneous transluminal coronary angioplasty (PTCA).[236,237] During angioplasty, the degree of coronary artery vasoconstriction was related to the quantity of serotonin liberated[236] and was attenuated, at least partly, by ketanserin.

During angioplasty, it is assumed that endothelial injury induces platelet aggregation with subsequent serotonin release resulting in vasoconstriction. Under resting, stable conditions, however, levels of serotonin are likely to be low such that serotonin may have little influence on coronary artery tone. Whereas ketanserin has been shown to be effective at times of serotonin release[236,237], its *in vivo* effects on human coronary arteries under resting conditions remain unknown.

In this chapter, ketanserin was used as a pharmacological agent to investigate the role of 5-HT_2 receptors on the coronary circulation under resting, stable conditions. The effect of ketanserin on the systemic and pulmonary circulation was also examined. Haemodynamic assessments were made using direct invasive recordings of aortic and pulmonary arterial pressures. With regard to the systemic circulation in particular, this technique obviates the methodological difficulties of measuring blood pressure by cuff sphygmomanometer (eg Korotkoff sounds, digit preference).

METHODS

Invasive investigations were performed in 10 patients (7 male, 3 female, mean age 57 $\pm$ 6 yrs). The protocol is summarised in table 8.1. Exclusion criteria and quantitative coronary angiography were as described in chapter 2 (pages 49-51). Coronary angiograms and haemodynamic tracings were analysed by a blinded observer.

TIME (mins)	-20	-10	0	5	15
Coronary angiogram	*	*	*	*	*
Baseline measurements	*				
Placebo injection		*			
Ketanserin 10mg i.v.			*		
6 lead ECG	*	*	*	*	*
Cardiac Haemodynamics	*	*	*	*	*

Table 8.1 Study protocol: The haemodynamic effects of ketanserin.

Statistics

Data are expressed as mean (s.d.). The effect of placebo and ketanserin i.v. bolus on central haemodynamics was analysed for each haemodynamic response using a paired t-test/confidence interval analysis. No significant differences were identified between baseline and post-placebo values. Significant differences between post-placebo values and post-ketanserin values ($p < 0.05$) are highlighted by an asterisk.

RESULTS

See tables 8.2 and 8.3 and figures 8.1, 8.2 and 8.3.

Heart Rate and Electrocardiography

There was no significant change in ECG morphology following ketanserin injection. Mean heart rate was 68 (16) bpm after placebo injection. Five minutes after ketanserin, heart rate rose by a mean of 2.7 (95%C.I. 0.6-5.4, p=0.06)

Cardiac output

No significant differences were observed between baseline and placebo values nor between the two post ketanserin values. Mean (s.d.) cardiac output was 5.4 (1.1) L/min after placebo. Fifteen minutes post ketanserin the mean change in C.O was 0.3 (95%C.I. –0.2 to 0.8, p = 0.42).

Systemic arterial pressure

No significant change was evident between baseline and placebo values Mean (s.d.) systolic arterial pressure (SAP), diastolic arterial pressure (DAP) and mean arterial pressure (MAP) following placebo were 143 (17), 74 (11) and 101 (12) mmHg respectively. Five minutes after ketanserin, SAP had fallen by a mean of 21mmHg (95%C.I. 14.5-27.5, p=0.002). Similarly, DAP fell significantly by an average of 8 mmHg (5.8-10.2. p=0.0001). Five minutes after ketanserin, MAP had fallen by 12mmHg (7.9-16.1, p=0.0006).

Pulmonary artery pressures

No significant changes were observed between baseline and placebo values. Mean (s.d.) pulmonary artery systolic pressure (PASP), pulmonary artery diastolic pressure (PADP) and mean pulmonary artery pressure (MPAP) were 22.3 (3.5), 8.2 (3.4) and 14.3(2.8) mmHg respectively after placebo injection. Significant decreases in PADP and MPAP, but not PASP were observed fifteen minutes after ketanserin. PADP fell by 1.5mmHg (95% C.I. 0.3-2.7, p=0.04). MPAP fell by 2.4mmHg (95% C.I. 0.7-4.1, p=0.028). PASP fell by a mean of 2.2mmHg (95% C.I. –0.4 to 4.8, p=0.1).

Pulmonary artery wedge pressure

There was no significant change between baseline and placebo values. Mean pulmonary artery wedge pressure (PAWP) was 7.5 (10) mmHg after placebo and had fallen significantly 15 minutes after ketanserin by an average of 2 mmHg (95% C.I. 1.0 - 3.2, p=0.011).

Right atrial pressure

There was no significant difference between placebo and baseline values. Right atrial pressure (RAP) was 5.4 (2.4) mmHg following placebo injection and mean change after ketanserin was 0.7mmHg (95% C.I. –0.6 to 2.0, p= 0.35).

Systemic vascular resistance

There was no significant difference between placebo and baseline values. Mean systemic vascular resistance (SVR) was1463 (256) dyne s cm^{-5} after placebo and fell by 233 (95% C.I. 88-378, p=0.018) dyne s cm^{-5} five minutes after ketanserin.

Pulmonary vascular resistance

There was no significant difference between placebo and baseline values. Pulmonary vascular resistance (PVR) fell significantly from a baseline of 111 (37) dyne.s.cm^{-5} by a mean of 33 (95%C.I. 14-52, p=0.009) dyne.s.cm^{-5} five minutes after ketanserin.

CORONARY ARTERY DIMENSIONS

No significant change in mean proximal, mid or distal coronary artery dimensions was observed between baseline and placebo values. Neither was there a change in the mean stenotic index between the baseline value and after placebo injection.

Proximal coronary artery segments

Mean (s.d.) diameter of the proximal coronary artery segments after placebo was 4.14 (0.7) mm. Five minutes after ketanserin injection, mean change in coronary artery diameter was -0.06mm (95%C.I. –0.1 to 0.22, p=0.45).

Middle coronary artery segments

Mean diameter of the mid coronary artery segments after placebo injection was 2.39 (0.42)mm. Five minutes after ketanserin injection, mean change in coronary artery diameter was 0.06mm (95%C.I. –0.25 to 0.13, p=0.56).

Distal coronary artery segments

Mean diameter of the distal coronary artery segments after placebo injection was 1.33 (0.34)mm. Five minutes after ketanserin injection, mean change in coronary artery diameter was -0.02mm (95%C.I. –0.12 to 0.16, p=0.84).

Coronary artery stenotic index

Mean coronary artery stenosis after placebo injection was 78 (13)%. Five minutes after ketanserin injection, mean change in coronary artery stenotic index was -2% (95%C.I. –6 to 2, p=0.37).

DISCUSSION

Ketanserin 10 mg intravenously has been shown to have a vasodilator response in the systemic and pulmonary circulations. In the absence of changes in heart rate or cardiac output, the fall in systemic and pulmonary arterial pressures and in systemic and pulmonary vascular resistances can be attributed to a direct vasodilator effect. In general, the effects of ketanserin occurred early after intravenous injection and had often resolved within 15 minutes. This study identified a 10-15% reduction in systemic arterial pressures, with changes of similar magnitude in the pulmonary circulation. These results are similar to previously reported haemodynamic changes following intravenous ketanserin.[279,282] It is assumed that these effects are mediated via 5-HT_2 and/or α-adrenergic receptor antagonism although non drug induced changes (eg. ionic contrast media) cannot be fully excluded.

There is evidence that the anti-hypertensive effect of ketanserin relies on both of these mechanisms. Ritanserin, a 5-HT_2 antagonist with much weaker α-receptor properties fails to reduce blood pressure when given intravenously to hypertensive patients, whereas ketanserin does.[333] On the contrary, the pressor response to the α-agonist phenylephrine is not reduced by the i.v. administration of hypotensive doses of ketanserin, although it was markedly reduced by equivalent doses of the potent α-antagonist prazosin.[334] The observation that ritanserin greatly enhances the anti-hypertensive effect of small doses of prazosin on the spontaneously hypertensive rat supports the theory that ketanserin produces an anti-hypertensive effect due to combined antagonism of both 5-HT_2 and α-receptors.[335,336] A central mechanism of action seems less likely as the hypotensive effect of ketanserin is not

accompanied by reduced noradrenaline levels, unlike clonidine and methyldopa.[337] Interestingly, the fall in systemic arterial pressure was not associated with a reflex increase in heart rate, a finding consistent with previous reports.[279,280]

Ketanserin has been noted to have pulmonary vasodilatory effects in hypertensive patients undergoing coronary artery bypass surgery. Van den Broucke and colleagues observed a significant 5-6% reduction in pulmonary artery pressure, 5% reduction in pulmonary artery wedge pressure and an 8% decrease in pulmonary vascular resistance following successive i.v. bolus doses of ketanserin to a maximum of 30mg.[281] Van der Starre et al reported a 13% decrease in systolic arterial pressure and a 9% decrease in pulmonary artery systolic pressure after an i.v. bolus of 10mg ketanserin followed by a low dose infusion.[280] Corresponding falls in total systemic (17.5%) and pulmonary vascular resistances (32%) were noted. Richter et al reported a 35% reduction in systemic vascular resistance, but interestingly there was no significant change in pulmonary vascular resistance following 10mg intravenous ketanserin.[279]

This study demonstrated approximately equipotent vasodilator responses on the pulmonary and systemic circulation, the magnitude of the responses being comparable to previous findings.[279,281] This suggests that 5-HT_2 receptors are equally distributed in the systemic and pulmonary circulations in humans.

Coronary artery responses

This study revealed no significant effect of ketanserin on coronary artery dimensions. Both 5-HT_1-like and 5-HT_2 receptors exist in coronary arteries, although the role of these two receptors on the coronary circulation is complex and not fully understood. *In vitro* responses using human epicardial coronary artery rings from explanted hearts have shown that serotonin causes contraction.[43,44] As ketanserin partially attenuated these responses, it was assumed that 5-HT_2 receptors were functionally more important with a residual contraction (30% of the maximum effect) being 5-HT_1-like mediated.[44] Kaumann et al, however, have reported the predominance of 5-HT_1-like over 5-HT_2 receptors in mediating serotonin-evoked contractions.[232] *In vivo* serotonin displays divergent effects on the coronary arteries, acting as a vasodilator in normal arteries and a vasoconstrictor in the presence of coronary atherosclerosis.[233] Serotonin evoked vasoconstriction in diseased coronary arteries is ketanserin-sensitive, suggesting that vasoconstriction is a 5-HT_2-receptor mediated phenomenon.[233] Two more recent studies have confirmed an important role for 5-HT_2 receptors when vasoconstriction is observed during percutaneous transluminal coronary angioplasty (PTCA).[236,237] Following balloon dilatation, vasoconstriction distal to the angioplasty site is frequently noted. The degree of vasoconstriction is related to the amount of serotonin released into the circulation[236] and in both of the above studies ketanserin attenuated, at least partly, this vasoconstriction.

In theory, therefore, one might have expected a significant change in coronary artery dimensions in the present study. There are a number of reasons, however, why such an effect was not observed. In the absence of platelet

aggregation and in a group of patients with stable symptoms, circulating levels of serotonin are likely to be low and, consequently, 5-HT_2 receptors may exhibit minimal influence on resting coronary artery tone. Furthermore, all patients in this group were taking aspirin which may have modified platelet function. An identical study in patients with unstable angina (and presumably higher plasma serotonin levels), may have demonstrated a significant vasodilator response to ketanserin. Nonetheless, patients with coronary artery atherosclerosis have locally elevated serotonin concentrations and ketanserin could theoretically have interacted.[226] The route of administration or dose of ketanserin employed in the present study (10 mg i.v.) may have been insufficient to effect a response. Golino et al [233,236] used a higher dose of ketanserin (0.25 mg/kg) to inhibit coronary artery vasospasm following PTCA. In the other study investigating the effects of 5-HT_2 antagonism during coronary angioplasty, ketanserin was administered by the intracoronary route.[237]

The observed non-significant reduction in proximal coronary artery dimensions is probably a reflection of the decrease in afterload following ketanserin administration. A direct vasoconstrictive action of ketanserin seems very unlikely in view of its known pharmacological properties and that of 5-HT_2 receptors. Attenuation of vasodilator tone could explain the minor reduction in coronary artery diameter, but this is not a strong possibility. Serotonin can influence vascular tone indirectly by stimulating release of endothelium derived relaxant factors (EDRF). This action, however, appears to be mediated by a 5-HT_1-like mechanism as 5-HT_2 (ketanserin) and 5-HT_3 (ondansetron) receptor

antagonists failed to block these responses, whereas methysergide and methiothepin (non-selective 5-HT antagonists) are potent antagonists.[84,105] In summary, equipotent vasodilator responses were seen in the systemic and pulmonary arterial circulations following an intravenous dose of 10 mg ketanserin. No significant change in coronary artery diameter or coronary artery stenotic index was observed after ketanserin. Further studies assessing the effects of ketanserin on coronary artery tone at times of platelet aggregation are indicated. Such agents may have therapeutic applications in the treatment of myocardial infarction and unstable angina or during PTCA.

	BASELINE	PLACEBO	Maximum change after ketanserin (95%C.I.)	P value
HR (bpm)	68.8±16.6	68±16.3	-2.7 (-0.3 to –5.1)	0.06
SAP (mmHg)	137±17.3	143±16.6	-21(-14.5 to –27.5)	0.002*
DAP (mmHg)	71.6±9.9	73.6±10.5	-8 (-5.8 to –10.2)	0.0001*
MAP(mmHg)	98±10.9	101±11.7	-12 (-7.9 to 16.1)	0.0006*
PASP (mmHg)	22.7±2.95	22.3±3.5	-2.2 (-4.8 to 0.4)	0.1
PADP (mmHg)	8.1±2.98	8.2±3.35	-1.5 (-0.3 to –2.7)	0.04*
MPAP(mmHg)	14.3±3.28	14.3±2.78	-2.4 (-0.7 to –4.1)	0.028*
SVR(dyne.s.c.m^{-5})	1394±239	1463±256	-233 (-88 to –378)	0.018*
PVR(dyne.s.c.m^{-5})	111±37	99±30	-33 (-14 to –52)	0.009*
PAWP(mmHg)	6.8±3.1	7.5±2.3	-2 (-1.0 to –3.2)	0.011*
RAP(mmHg)	4.6±2.2	5.4±2.4	0.7 (-0.6 to 2.0)	0.35
CO(L/min^{-1})	5.54±1.09	5.41±1.09	0.3 (-0.2 to 0.8)	0.42

Table 8.2 Mean haemodynamic values at baseline and after placebo and ketanserin injections. Baseline and placebo figures are expressed as mean ± S.D. * =statistically significant compared to placebo.

	Proximal segments (n = 12)	Mid segments (n = 8)	Distal segments (n = 6)	Stenotic segments (n = 6)
Baseline	4.16 (0.79)	2.55 (0.43)	1.45 (0.26)	77.5 (13.4)%
Placebo	4.14 (0.74)	2.39 (0.42)	1.33 (0.34)	77.7 (12.5)%
5 mins post ketanserin	4.08 (0.68)	2.45 (0.37)	1.32 (0.30)	75.3 (12.1)%

Table 8.3 Mean (s.d.) coronary artery dimensions at baseline and after placebo and ketanserin injection. No significant differences were identified.

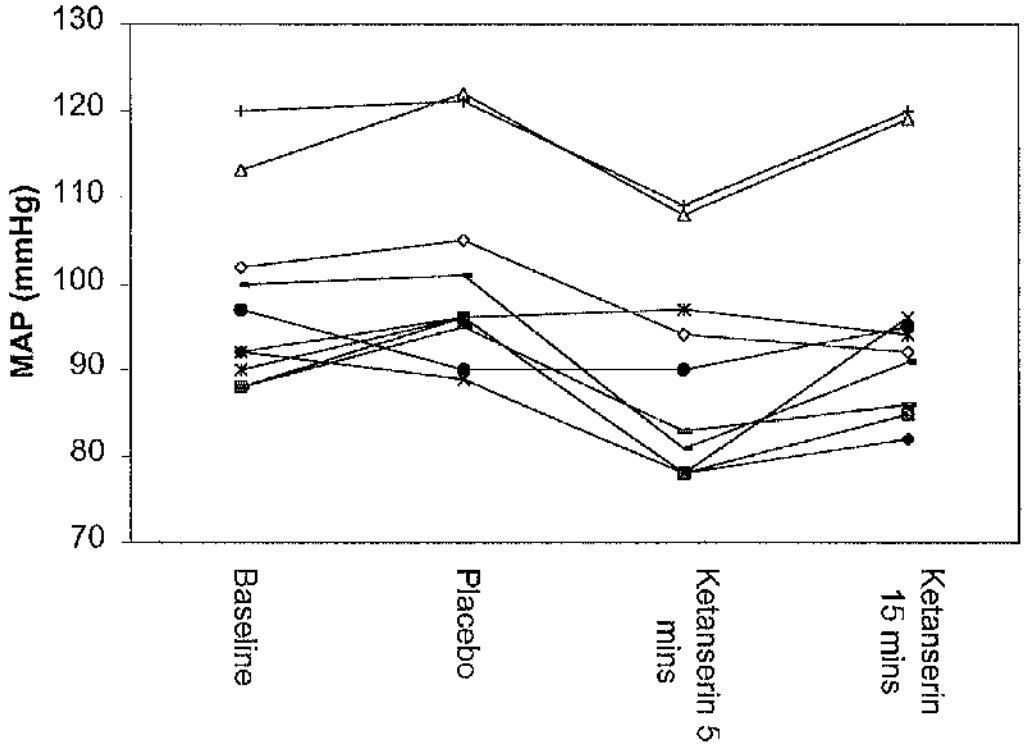


Figure 8.1 Mean arterial pressure (MAP) at baseline and after placebo and ketanserin injection. Each line represents an individual.

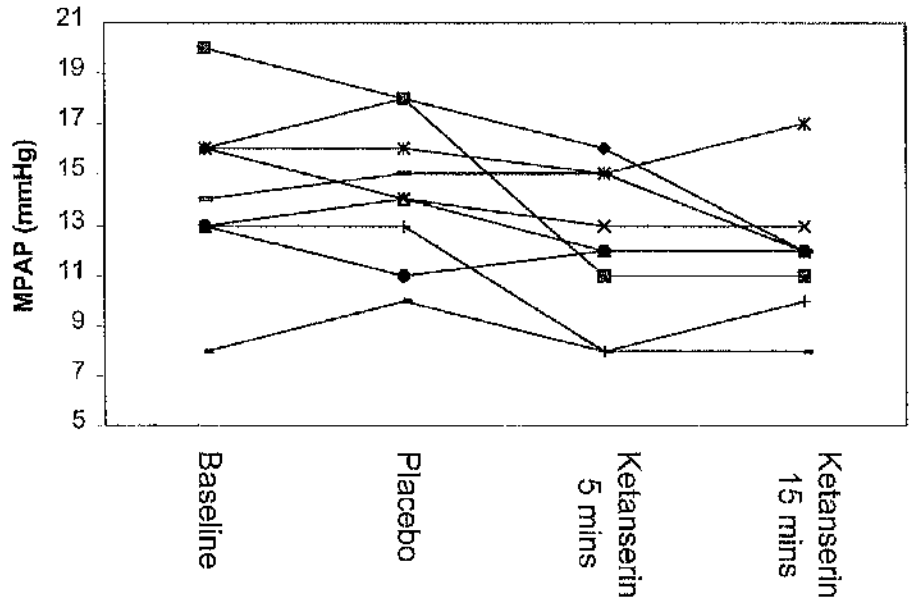


Figure 8.2 Mean pulmonary artery pressure (MPAP) at baseline and after placebo and ketanserin injection. Each line represents an individual.

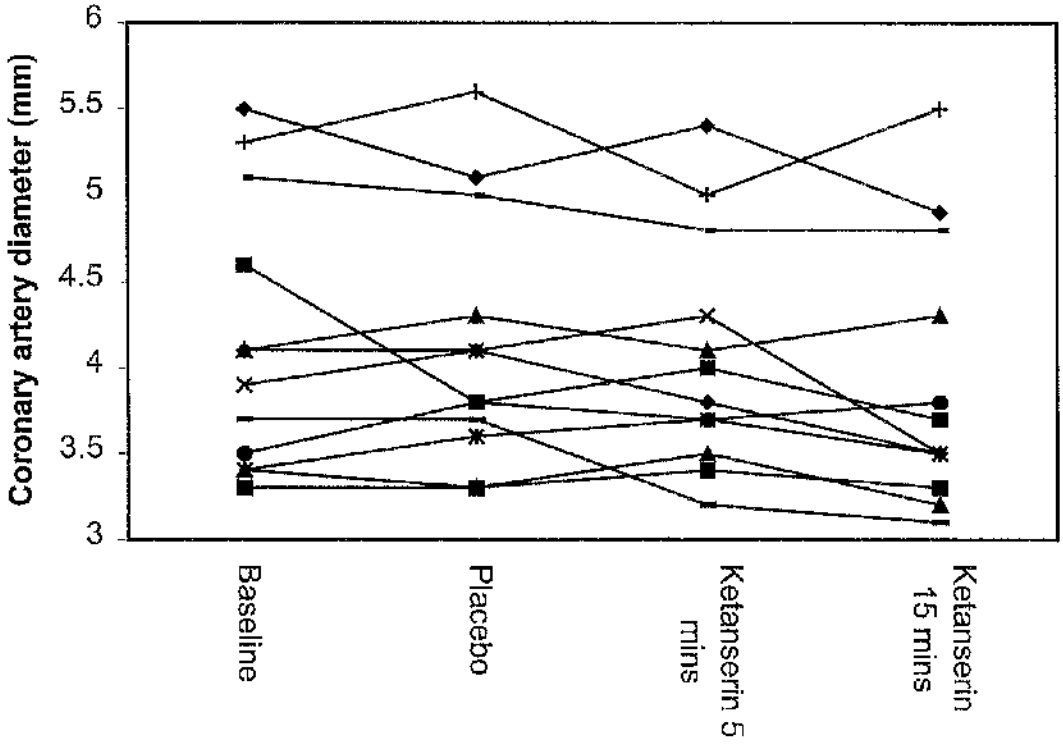


Figure 8.3 . Coronary artery responses at baseline and after placebo and ketanserin injections. Each line represents a measured point in a proximal coronary artery.

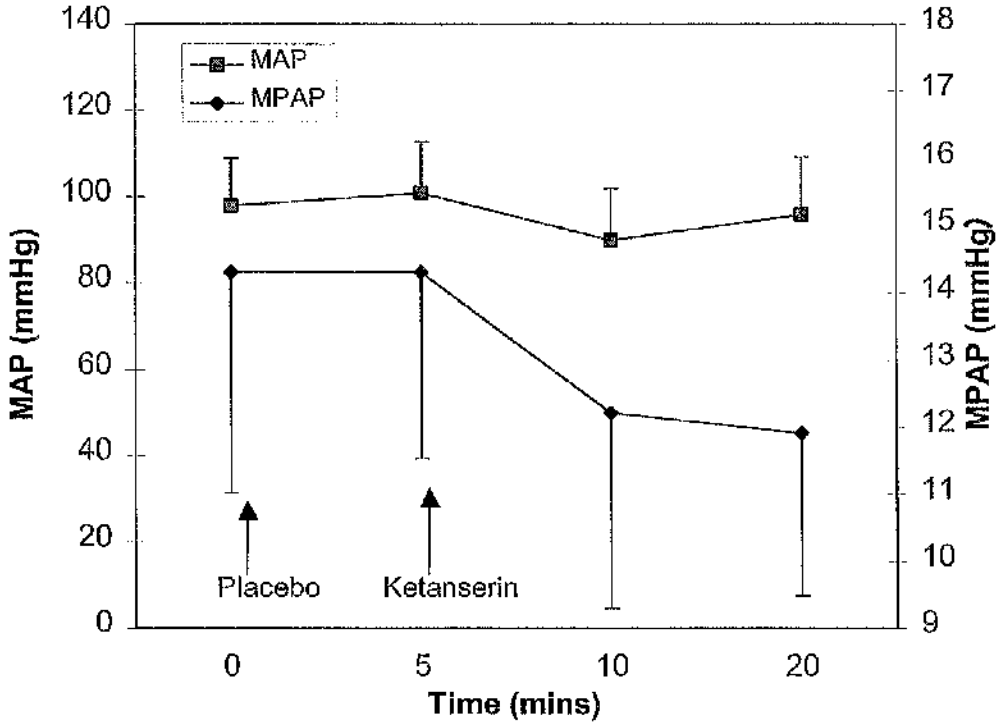


Figure 8.4 Mean arterial pressure (MAP) and mean pulmonary arterial pressure (MPAP) responses to placebo and ketanserin.

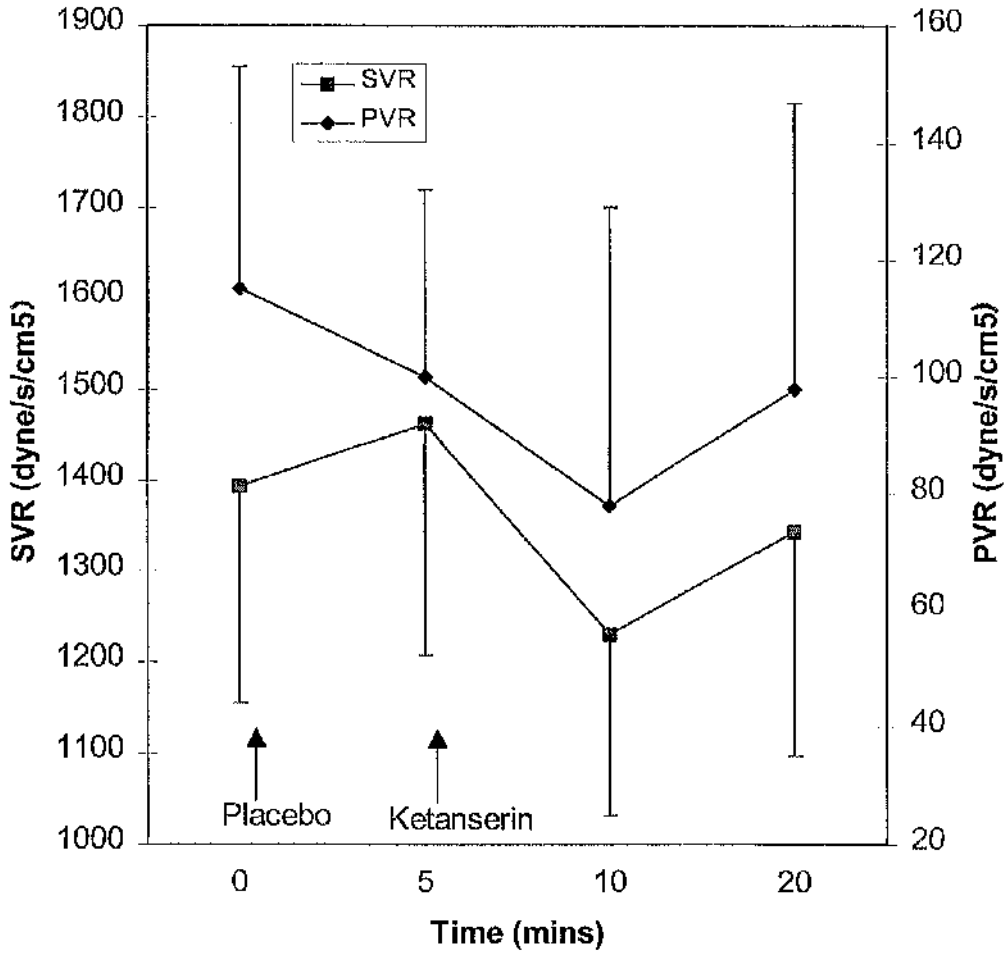


Figure 8.5 Systemic vascular resistance (SVR) and pulmonary vascular resistance (PVR) responses to placebo and ketanserin.

CHAPTER 9

THE EFFECT OF SUMATRIPTAN ON FOREARM BLOOD FLOW

INTRODUCTION

As previously discussed in chapter 1, serotonin influences the vascular system in a complex manner. It can cause both vasodilatation and vasoconstriction depending on the dose, species, type of blood vessel and on the presence or absence of several physiological or pathological features[185,338-341]. Initial studies of the effect of serotonin on the cardiovascular system were performed *in vitro* or on animal preparations. In the last decade, however, Blauw and colleagues have assessed the role of serotonin in the human forearm vascular bed [342-344]. Employing the technique of venous occlusion plethysmography (see below) they found that 5-HT induced a biphasic vascular response [344] Within a few minutes of commencement of serotonin infusion, a transient, rapid vasorelaxation was observed. This was succeeded by a sustained vasodilatation with lower doses (< 10 ng/kg/min) and a vasoconstriction at the highest dose of 5-HT (80 ng/kg/min). Maximal vasodilatation was 60-70% with a maximum constriction of 34%.

The initial response was elicited in a dose-independent fashion and was not blocked by propranolol or ketanserin, suggesting that neither the beta-adrenoreceptor nor the 5-HT_2 receptor was involved. 5-HT-induced release of EDRF could have been implicated, but a dose-dependent response would have been expected in that case.[55,345,346] Alternatively an interaction with the cholinergic nervous system could have occurred [347,348]. A third potential mechanism involved in the 5-HT-induced vasorelaxation is the inhibition of noradrenaline release by an action of pre-synaptic 5-HT receptors on the sympathetic nerve terminals [194,217]. The sustained vasodilator response was also resistant to 5-HT_2 receptor blockade with ketanserin, indicating that 5-HT_2

receptors were not involved in the vasodilator response to serotonin. At vasoconstrictive doses of serotonin, however, ketanserin was an effective antagonist. Taken together, these results suggest that serotonin-evoked vasoconstriction via 5-HT_2 receptor agonism, whilst vasodilatation ensued through an agonist action at some other unknown 5-HT receptor. Interestingly, the arterial effects of serotonin were not age related [344] (c.f. the anti-hypertensive effects of ketanserin).

Initially it was thought that serotonin induced vasodilatation was mediated by a 5-HT_1-like receptor. As new 5-HT receptors were identified in the early 1990's, however, evidence pointed to a 5-HT_3 or 5-HT_4 receptor-mediated phenomenon, as ICS205-930 (a mixed 5-HT_3/5-HT_4 antagonist) attenuated this vasodilator response[120]. The serotonin-induced vasodilatation was later challenged with ondansetron and granisetron (5-HT_3 receptor antagonists) without effect, thereby implying that the 5-HT_4 receptor exerted a significant role in the human forearm vascular bed [349]. Subsequent work by the same authors revealed that the serotonin-induced vasodilatation was mediated by the nitric oxide (NO) pathway on account that N-monomethyl-L-arginine (NMMA), an inhibitor of nitric oxide synthase, abolished the response[350]. A functional role for the 5-HT_{1A} receptor was excluded as flesinoxan, a 5-HT_{1A} receptor agonist, decreased forearm vascular resistance only slightly, and at high doses[351].

It appears that 5-HT_1-like receptors on the vascular endothelium mediate vasodilatation in the human forearm via the "NO pathway"[351,352] but the precise nature of the 5-HT receptor subtype involved is uncertain. The effect of

sumatriptan, a 5-$HT_{1D/1B}$ agonist on forearm blood flow is therefore of interest in elucidating which 5-HT_1 receptor subtype(s) influence forearm vascular resistance in the human.

MEASUREMENT OF FOREARM BLOOD FLOW USING MERCURY STRAIN GAUGE PLETHYSMOGRAPHY

Mercury strain gauge plethysmography has been used to measure volume changes in human limbs for over 40 years. Blood flow can be measured on cylindrical parts such as the calf or forearm, using venous occlusion plethysmography. Measurements are made in a draught-free room at a constant temperature (usually 25°C). With the patient relaxed in a supine position, a venous occlusion cuff is wrapped around the arm above the elbow, another pneumatic cuff is wrapped around the wrist. A mercury-in-silastic strain gauge is positioned so that it encircles the forearm at its widest part. The gauge should then be applied with approximately 10g tension. Excess tension in the gauge retards forearm expansion and should therefore be avoided. In practical terms, the gauge should be approximately 2 cms shorter than the forearm circumference.

Once the plethysmograph has been balanced and a stable base-line is noted on the recorder, the wrist cuff is inflated well above the systolic blood pressure. At this point the base-line usually requires adjusting and after 30-45 seconds, recordings can then be made. Whilst recording, the arm cuffs are rapidly inflated to approximately 40 mmHg (higher than venous pressure, but below arterial diastolic pressure). This produces a slope deflection above the base-line (see figure 9.1) which can later be quantified. Several recordings should

be made at each time point by repetitive inflation and deflation of the arm cuff. After recordings have been completed, the wrist cuffs are deflated. A rest period of several minutes is required to allow hyperaemia to subside before subsequent recording.

CALCULATION OF BLOOD FLOW

Blood flow can be calculated from the venous occlusion tracings by determining the slope of the flow curve. Provided that an electric calibration using the number of divisions corresponding to a X% resistant change is known, blood flow can be calculated using the undernoted formula.

$$\text{Flow (ml/100ml tissue/min)} = \frac{\text{slope (divisions per mm)}}{\text{calibration (divisions per X\% volume change)}}$$

Problems with reproducibility of measurement of forearm blood flow have been described, but coefficients of variation of approximately 15% have been reported. [247,248]

METHODS

Ten healthy male volunteers attended on two study days. In conventional venous occlusion plethysmography studies, active drug is given intra-arterially and the contralateral arm is used as a control for forearm blood flow. In this study, however, sumatriptan was administered subcutaneously and therefore two study days were required. On one occasion the subject received subcutaneous placebo injection and on the other 6 mg subcutaneous sumatriptan on a double-blind basis. Studies were performed at the same time

of the day, under fasted conditions and at a constant room temperature between 24 and 26^0C.

During a 30 minute stabilisation period, forearm blood flow and blood pressure were measured at 10 min intervals. Two blood flow measurements were then taken and the mean of these was taken as the baseline value. A subcutaneous injection of placebo or sumatriptan was then administered on a double-blind basis. Forearm blood flow (a mean of 5 sequential measurements) and blood pressure were then measured at approximately 7min intervals for 35 mins. Blood sampling for catecholamines were taken through an i.v. cannula in the right antecubital fossa at base-line and at all time intervals thereafter. The study protocol is summarised in table 9.1.

TIME (mins)	-10	0	7	14	21	28	35
Baseline measurements	*						
Placebo/sumatriptan injection		*					
Forearm blood flow measurement	*	*	*	*	*	*	*
Blood pressure	*	*	*	*	*	*	*

Table 9.1 Study protocol: The effects of sumatriptan on forearm blood flow

Data analysis and statistics

A baseline value was obtained for forearm blood flow and blood pressure by averaging two observations. The response to placebo and sumatriptan was taken as the average of the forearm blood flow and BP recordings at 7,14,21,28 35 minutes after injection. The effect of placebo and subcutaneous

sumatriptan on forearm blood flow was then analysed using a paired t-test/confidence interval analysis in the same manner as in the other studies.

RESULTS

A summary of the results is displayed in table 9.2.

Heart Rate

Mean (sd) baseline heart rate on the placebo treatment day was 64 (9) bpm and on the sumatriptan treatment day was 66 (9) bpm. Mean change in heart rate was -2 bpm (95% CI -4 to 0, p=0.032) after placebo. On the sumatriptan treatment day, mean change in heart rate after sumatriptan was -2 bpm (95% CI -4 to 1, p=0.08).

Blood pressure

On the placebo treatment day, mean (sd) baseline systolic blood pressure (SBP) and diastolic blood pressure (DBP) were 118 (6) mmHg and 62 (10) mmHg respectively. Mean changes in SBP and DBP after placebo injection were 1mmHg (95%CI -2 to 4, p=0.47) and 0 mmHg (95%CI -2 to 3, p=0.85) respectively. On the sumatriptan treatment day, the corresponding baseline values of SBP and DBP were 122 (5) and 61(10) mmHg. Mean changes in SBP and DBP after sumatriptan injection were 6 mmHg (95% CI 2 to 10, p=0.01) and 6 mmHg (95% CI 3 to 9, p=0.001) respectively.

Change in forearm blood flow

On the placebo treatment day the mean percentage change (from baseline) in forearm blood flow in the right arm was –1% (95% CI –9 to 6, p=0.68) and in the left arm was 4% (95%CI –4 to 11, p=0.28). On the sumatriptan treatment

day, mean percentage change (from baseline) in forearm blood flow in the right arm was 18% (95% CI –5 to 41, p=0.11) and in the left arm was 7% (95% CI –24 to 38, p=0.63).

Plasma noradrenaline levels

On the placebo treatment day, mean (sd) baseline plasma noradrenaline was 0.69 (0.2) nmol.l^{-1}. Mean change in plasma noradrenaline after placebo injection was 0.004 nmol.l^{-1} (95%CI –0.05 to 0.06, p=0.87). On the sumatriptan study day, mean (sd) baseline plasma noradrenaline was 0.64 (0.17) nmol.l^{-1}. Mean change in plasma noradrenaline after sumatriptan injection was –0.16 nmol.l^{-1} (95%CI –0.25 to –0.08, p=0.0027).

Discussion

Sumatriptan (6mg subcutaneously) produced expected changes in blood pressure. The blood pressure increases seen were of a smaller magnitude than that seen in chapters 3,4 and 5 presumably because, in those chapters, arterial pressure was measured invasively rather than by an automated cuff method. Although statistically significant, these blood pressure changes were not of clinical concern.

No clinically or statistically significant change in forearm blood flow was observed. This may have been due to a lack of functional vasoactive 5-HT$_1$ receptors within the forearm and there is supportive evidence for this. Naratriptan, given subcutaneously, at both therapeutic and supratherapeutic doses, had no significant effect on forearm blood flow in an almost identical protocol.[353] On the contrary however, intra-arterial sumatriptan reduced

forearm blood flow in a dose dependent fashion indicating the presence of sumatriptan-sensitive 5-$HT_{1B/D}$ receptors in the human forearm.[354]

The lack of effect on forearm blood flow in this protocol may reflect the route of drug administration. Sumatriptan was administered subcutaneously in this study to maintain consistency and allow comparison throughout the whole thesis. In addition, initial advice from Glaxo Group Research indicated that sumatriptan was unsuitable for intra-arterial administration. Intra-arterial administration has the obvious advantage of isolating the forearm circulation from systemic effects. In addition, the contralateral arm can be employed as a control and therefore only one study day is required. Administering sumatriptan subcutaneously means that systemic endocrine or hormonal effects could ensue which in turn may influence vascular tone in the forearm. Two study days are also required and biological variation may arise despite efforts to maintain standard experimental conditions.

Sumatriptan injection caused no significant change in forearm blood flow but was associated with a reduction in plasma noradrenaline levels. As previously stated, intra-arterial sumatriptan causes dose dependent reductions in forearm blood flow [354] so a similar effect following subcutaneous sumatriptan might have been expected. A reduction in plasma noradrenaline levels (as seen here) could however result in a vasodilator response with subsequent negation of a direct 5-$HT_{1B/D}$ mediated vasoconstrictor effect. This may explain the apparent lack of effect of sumatriptan on forearm blood flow in this study. Interestingly, inhibition of noradrenaline release by an action on pre-synaptic 5-

HT receptors on sympathetic nerve terminals has previously been proposed as a mechanism of 5-HT induced vasodilatation[194,217].

An inhibitory effect of 5-HT and related drugs on transmitter release from post ganglionic sympathetic nerves has been confirmed in a number of various organs in different species (see Chapter 1- Inhibition of noradrenergic neurons, page 35). This effect appears 5-HT_1 receptor mediated as suggested by the high agonist potency of 5-HT and 5-CT[222,223] and antagonism by methiothepin, metergoline and methysergide[158,219,222,224] but not by ketanserin, LY53857 or metoclopramide.[158,219,222]

Interestingly, one individual (myself, SH) had a pronounced vasodilator response to sumatriptan (see figure 9.2). Unfortunately plasma noradrenaline levels were not obtained in this individual so it is unclear whether an exaggerated fall in plasma noradrenaline occurred. Blood pressure increased in this individual from a baseline of 114/79 mmHg to a peak of 130/85 seven minutes after sumatriptan administration but this rise was not exceptional. Chest and neck pressure was experienced after sumatriptan by this subject but 4 other subjects also experienced this typical side effect of sumatriptan.

It is impossible to comment on whether sumatriptan-induced effects on the venous circulation occurred in this study. It is possible to measure total venous capacity and venous outflow (emptying rate) using this apparatus but this was not part of the present study. This is a potential area of future research.

In summary, sumatriptan 6mg s.c. increased blood pressure slightly but had no effect on heart rate or forearm blood flow. Sumatriptan injection was however associated with a reduction in plasma noradrenaline levels and this may have counteracted a direct 5-$HT_{1B/D}$ receptor mediated effect on the forearm vasculature.

	Mean change from baseline after placebo (95% C.I.)	P value	Mean change from baseline after sumatriptan (95% C.I.)	P value
HR (bpm)	-2 (-4 to 0)	0.032*	-2 (-4 to 1)	0.08
SBP(mmHg)	1 (–2 to 4)	0.47	6 (2 to 10)	0.01*
DBP(mmHg)	0 (-2 to 3)	0.85	6 (3 to 9)	0.001*
Percentage change in right forearm flow	-1 (-9 to 6)	0.68	18 (-5 to41)	0.11
Percentage change in left forearm flow	4 (-4 to 11)	0.28	7 (-24 to 38)	0.63
Plasma noradrenaline (nmol/l)	0.004 (-0.05 to 0.06)	0.87	-0.16 (-0.25 to – 0.08)	0.0027*

Table 9.2 Mean changes in forearm blood flow, heart rate, blood pressure and plasma noradrenaline after placebo and sumatriptan injection. * = significant compared to baseline.

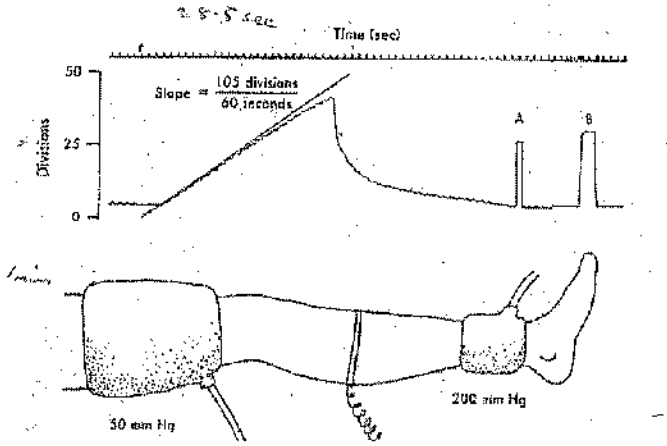


Figure 9.1 Diagram of limb blood flow assessment using mercury strain gauge plethysmography.

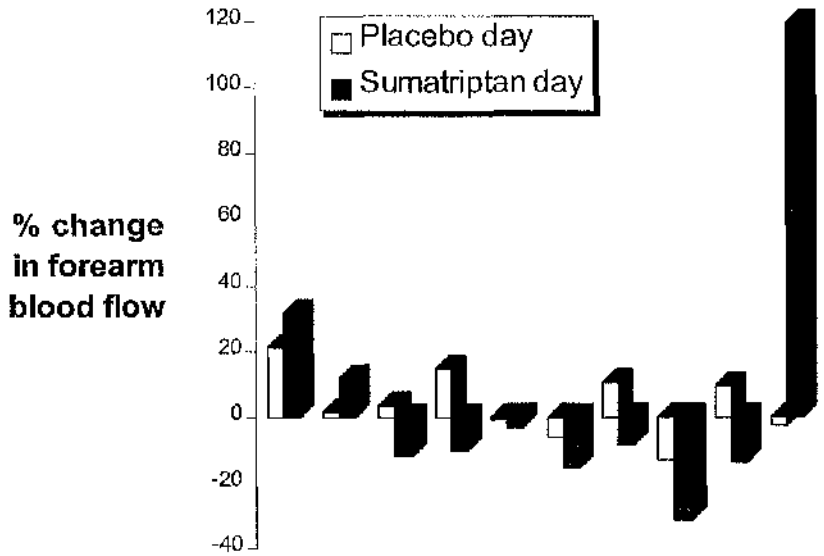


Figure 9.2 Mean percentage change in forearm blood flow (from baseline) after placebo and sumatriptan injection. Each individual is represented by adjacent columns.

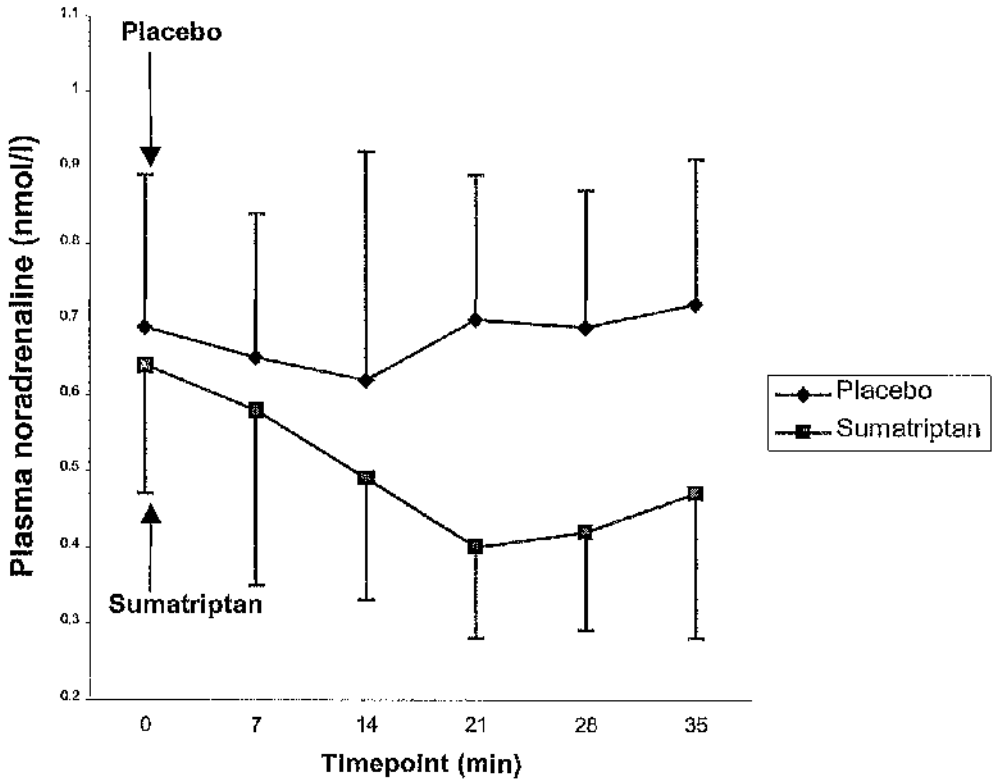


Figure 9.3 Mean (SD) plasma noradrenaline levels after placebo and sumatriptan injection.

CHAPTER 10

DISCUSSION

Serotonin was first recognised over 50 years ago. Initially thought to have purely vasoconstrictor properties, serotonin is now known to have important physiological and pathophysiological roles in the cardiovascular system, platelet function, gastrointestinal motility, mood, appetite regulation and in acute migraine.

Knowledge of serotonin receptor function is rapidly changing and updating with current consensus recognising 7 main receptor subtypes, many of which themselves have subclassifications.

The effect of serotonin on any tissue is species dependent and may differ between in vitro and in vivo preparations. Hence it is impossible to extrapolate results from laboratory animals or isolated human artery preparations to the clinical situation. The need for detailed in vivo human studies is therefore apparent.

In humans, the 5-HT_1 and 5-HT_2 receptor subtypes have greatest direct influence on the cardiovascular system. Additional neurally mediated effects on the cardiovascular system are influenced by 5-HT_3 receptors. This thesis looked predominantly at the in vivo role of the 5-HT_1 and 5-HT_2 receptors in the human cardiovascular system. As this involved predominantly invasive techniques, studies had to be limited to small numbers and therefore are open to criticism. Nonetheless, the findings of the present studies provide important insights into the role of 5-HT_1 and 5-HT_2 receptors in humans.

SUMATRIPTAN

Sumatriptan, a 5-HT_1 agonist with greatest activity at the 5-$HT_{1B/D}$ receptor subtypes, has been seen to have generalised vasoconstrictor properties. Cerebral vasoconstriction is thought to be an important part of the mechanism by which sumatriptan relieves acute migraine pain and initial reports suggested that this drug was a selective vasoconstrictor in the cranial circulation. This thesis has conclusively disproved this line of thought. Sumatriptan induced rises in systemic and pulmonary arterial pressure are rarely of clinical concern but the known coronary vasoconstrictive properties require cautious prescription of this drug to patients who may be at risk of coronary artery disease.

Previous invasive haemodynamic assessment of sumatriptan's actions also suggested a greater magnitude of vasoconstriction in the pulmonary compared to the systemic circulation. This possibility has been looked at more closely in this thesis and, by including the results of previously reported studies with present results, there is some evidence to suggest that sumatriptan is a more potent vasoconstrictor in the pulmonary circulation. Whether this reflects a greater density of 5-HT_1 receptors in the pulmonary circulation remains open to question. It may be that the systemic arterial circulation is exposed to additional hormonal or central nervous system influence which is not present within the pulmonary circulation.

Sumatriptan has also been previously noted to cause increases in pulmonary artery wedge pressure raising the possibility of a negative isotropic effect or of a venoconstrictive action. This thesis looked at the effect of sumatriptan on left

ventricular end diastolic pressure (LVEDP) and ventricular contractility (rate of change of left ventricular pressure, dP/dt). No evidence of negative inotropism was observed and it is assumed that the observed rise in LVEDP is consequent upon an acute increase in afterload. No confirmatory evidence of a venoconstrictive action has been observed.

SYSTOLIC TIME INTERVALS

As stated earlier, one of the limitations in cardiovascular pharmacology is the need for invasive haemodynamic assessments. The possibility of performing non-invasive haemodynamic studies is an attractive option. Measurement of systolic time intervals represents an established method of assessing the effects of cardioactive drugs. This study measured systolic time intervals from haemodynamic traces. This confirmed the acute increase in afterload after sumatriptan administration with significant increases in the pre-ejection period, left ventricular ejection time and total electro-mechanical systole. Although there was no direct correlation between the rise in any of the STI's and systemic blood pressure, the results suggest that measurement of STI's may be a suitable non invasive method of assessing the haemodynamic effects of serotonergic agents.

NARATRIPTAN

Naratriptan is a newer 5-HT_1 agonist which, like sumatriptan, is effective in the treatment of acute migraine. It has been seen to have a similar pharmacological profile to sumatriptan and not surprisingly displays similar haemodynamic effects with acute increases in both systemic and pulmonary arterial pressures following administration. Naratriptan 1.5mg subcutaneously

caused an approximate 11-12% increase in systemic arterial pressure which is less than the 20% augmentation seen after sumatriptan 6mg s.c. Interestingly this therapeutic dose of naratriptan was not associated with significant coronary artery vasoconstriction unlike previous findings with a therapeutic dose of 6mg s.c. sumatriptan. Why naratriptan and sumatriptan should have differing effects on the coronary circulation is unclear. The difference may simply reflect dose differences or arise from subtle as yet unknown differences in receptor interaction between the two drugs. Regardless, these observations could have therapeutic importance.

ELETRIPTAN

Eletriptan is a "second generation" 5-HT_1 receptor agonist. It is a potent and selective agonist at the 5-HT_{1D} receptor subtype with 13 fold selectivity for the 5-HT_{1D} receptor subtype over the 5-HT_{1B} subtype. Eletriptan infusion produced no significant rise in either systemic or pulmonary arterial pressures unlike sumatriptan and naratriptan. Total systemic and pulmonary vascular resistance increased following eletriptan administration implying vasoconstriction in the respective circulations. Cardiac output, however, fell significantly after eletriptan and, as this parameter is used as the denominator in the formulae for the calculation of SVR and PVR, it may be that the apparent rise in SVR and PVR reflects the change in cardiac output. It is difficult to explain why eletriptan should reduce cardiac output as 5-HT receptors are not known to have inotropic actions.

The lack of coronary vasoconstriction after eletriptan is consistent with evidence suggesting that eletriptan has greater cranioselectivity than

sumatriptan. In animal models, eletriptan-mediated coronary constriction occurs at a dose four times higher than sumatriptan. It is possible that the apparent differences in the pharmacological profile of eletriptan compared to sumatriptan and naratriptan result from its enhanced selectivity for the 5-HT_{1D} receptor subtype. If the 5-HT_{1D} receptor is selectively distributed within the cranial circulation, eletriptan may potentially have a "safer" cardiovascular profile but this awaits confirmation.

KETANSERIN

Ketanserin is a 5-HT_2 antagonist with additional alpha adrenoceptor antagonist properties. It has well documented vasodilator properties in the pulmonary and systemic circulation. More recently, ketanserin has been shown to prevent coronary artery vasoconstriction following percutaneous transluminal coronary angioplasty (P.T.C.A.). This thesis assessed the effect of intravenous ketanserin on coronary artery dimensions in patients with stable angina but identified no coronary vasodilator properties. It is assumed that, in patients with stable symptoms, circulating levels of serotonin are low and consequently 5-HT_2 receptor antagonists may exhibit minimal influence on resting coronary artery tone. Following PTCA however, local serotonin levels rise and cause vasoconstriction. Ketanserin can obviously inhibit this serotonin efflux and prevent coronary artery vasoconstriction. Whether ketanserin or other 5-HT_2 antagonists may have therapeutic applications in the treatment of myocardial infarction or unstable angina remains to be established.

SUMATRIPTAN AND FOREARM BLOOD FLOW

Subcutaneous sumatriptan was found to have no effect on forearm blood flow as assessed by strain gauge plethysmography. This might have been expected as naratriptan also had no effect on forearm blood flow when administered by the subcutaneous route. Intra-arterial administration of sumatriptan has however been associated with dose dependent reductions in forearm blood flow. Intra-arterial administration has the obvious advantage of isolating the forearm circulation from systemic effects. In this study, systemic effects may have occurred and in particular, noradrenaline levels fell after sumatriptan administration. It is possible that a direct 5-HT_1-mediated vasoconstriction followed sumatriptan administration but that this was negated by the reduction in plasma noradrenaline levels which caused a vasodilator response.

SUMMARY

This thesis has shown that 5-HT_1 and 5-HT_2 receptors have important modulatory roles in the systemic, pulmonary and coronary circulations. Sumatriptan, naratriptan and eletriptan, 5-HT_1 agonists, are systemic and pulmonary vasoconstrictors whereas ketanserin, a 5-HT_2 antagonist acts as a systemic and pulmonary vasodilator. There is some evidence that 5-HT_1 receptors may be more densely distributed in the pulmonary compared to the systemic circulation. The potential role of systolic time intervals as a non-invasive method of assessing the cardiovascular effects of serotonergic compounds has been highlighted.

FUTURE WORK

Further studies of the effects of serotonergic agents on the coronary circulation would be of interest. Many subjects in this thesis had normal epicardial coronary arteries and it could be presumed they had intact endothelial function. A number of other factors predisposing to endothelial dysfunction were prevalent in the patient population however and the functional capacity of the endothelium for each patient was not known. It would be advantageous to know if the effects of the various serotonergic agents on the coronary circulation are dependent on endothelial function.

This could be assessed in similar quantitative coronary angiographic studies. Normal endothelial function could be confirmed by determining whether acetylcholine produces a coronary vasodilator response. Thereafter the various 5-HT agonists and antagonists could be administered and the coronary responses established. These responses could then be compared to those of patients with documented dysfunctional endothelium (eg. vasoconstrictor response to acetylcholine) or in patients with normally functioning endothelium who were pre-treated with L-NAME or NMMA (which would inhibit nitric oxide synthase). This would particularly help establish the interaction between the 5-HT_1 receptor and EDRF release in vivo.

In addition to performing quantitative coronary angiography, the concomitant use of intravascular ultrasound and flow wires in these protocols would allow coronary artery responses to 5-HT_1 agonists and 5-HT_2 antagonists to be assessed with heightened accuracy. Intravascular ultrasound and flow wire usage would however significantly increase research costs.

The issue of differing 5-HT_1 receptor density/function between the systemic and pulmonary circulations requires further examination. This could be difficult to demonstrate in vivo in the absence of clinically available, highly selective 5-HT_1 receptor antagonists. One possible in vitro study would be to take specimens, from "heart beating" donors, of both the systemic and pulmonary arterial circulation and construct dose response curves to each of the triptans in a conventional organ bath set up with an isometric transducer. The maximum contractile response (E_{max}) and the concentration eliciting 50% of E_{max} (EC_{50}) could then be established for each agonist in the respective circulations to determine rank order of potency (EC_{50}) and efficacy (E_{max}). It would be important to perform these studies initially in the presence of an intact endothelium and then repeating the experiments after denuding the endothelium and washing the preparations. This would confirm firstly, whether the triptans have greater efficacy and potency on the pulmonary circulation and would also clarify the in vitro interaction between 5-HT_1 receptors and endothelial derived relaxing factor in the human systemic and pulmonary circulation.

Should selective 5-HT_1 antagonists become available, their haemodynamic and coronary vasomotor properties could be established using experimental protocols similar to those performed for the 5-HT_1 agonists. Again the studies would need to be performed in patients with documented normally functioning endothelium and also after inhibition of NO synthase. These 5-HT_1 antagonists could be used to challenge the effects of the triptans studied in this thesis. This again would involve similar protocols but would require the patients to be pre-treated with various doses of the 5- HT_1 antagonist. If the dose of 5-HT_1

antagonist required to inhibit the vasoconstrictor response of the triptan differed between the pulmonary and systemic circulation, this might add to the evidence suggesting differential 5-HT_1 receptor density and function.

Systolic time intervals were calculated from invasively obtained arterial pressure tracings in this thesis. The potential use of STI's as a non-invasive marker of the haemodynamic effects of 5-HT_1 agonists has been discussed. This needs to be evaluated further with the conventional technique of measuring STI's using the electrocardiogram, phonocardiogram and carotid arterial pulse waveforms. Repeated measurement of the STI's (using the conventional non-invasive technique described in chapter 5) in healthy volunteers could establish the reproducibility of this procedure. The effect of sumatriptan on the STI's measured non-invasively could then be established using a randomised, placebo controlled trial. A power calculation to estimate the required number of patients would be required.

It is impossible to comment on whether sumatriptan-induced effects on the venous circulation occurred in this study. It is possible to measure total venous capacity and venous outflow (emptying rate) using the strain gauge plethysmography technique but this was not part of the present study. This is a potential area of future research and could be assessed using the protocol employed in chapter 9. Sumatriptan would however be administered intra-arterially and this would obviate the need for two study days.

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Appendix 1

Publications arising from this work:

Hood S, Birnie D, MacIntyre PD, Hillis WS.

Sumatriptan induced chest pain (letter).

Lancet 1994 **344**(8935):1500-01

Hood S, Birnie D, Hillis WS

The haemodynamic effects of subcutaneous sumatriptan, a $5HT_1$ receptor agonist.

Br J Clin Pharmacol 1997; **43**:327-28.

Hood S, Birnie D, Nasser A, Hillis WS

The effect of ketanserin on central haemodynamics and coronary circulation.

Journal of Cardiovascular Pharmacology 1998;**32(6)**:983-87.

Hood S, Birnie D, Swan L, Murray LS, Whitehouse H, Hillis WS.

Effects of subcutaneous naratriptan on systemic and pulmonary haemodynamics and coronary artery diameter in humans.

Journal of Cardiovascular Pharmacology 1999;**34(1)**:89-94

Hood S, Birnie D, Murray LS, MacIntyre PD, Hillis WS.

Changes in systolic time intervals-A non invasive marker for the haemodynamic effects of sumatriptan.

Br J Clin Pharmacol 1999;**48**:331-35

Appendix 3a. Sumatriptan haemodynamic data.
Placebo was administered at time = 0mins
Sumatriptan was administered at time = 20 mins

Heart Rate			Timepoint (mins)					
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	65	68	66	64	57	56	67	68
E0293	56	57	56	50	55	52	52	50
E0294	74	70	69	69	69	62	63	60
E0295		78		72	66	65	66	65
E0296	64	55	65	60	58	64	58	57
E0297	55	57	57	50	57	54	46	46
E0298	71	71	72	73	78	71	70	74
E0299	59	58	54	75	68	63	70	72
E0300	74	78	75	76	70	74	67	72
Cardiac output								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	5.33	5.18	5.11	4.56	4.67	4.49	4.52	5.02
E0293	5.1	6.5	4.8	5	5.1	5.1	4.4	4.5
E0294		4.7	5	4.9	5	4.7	4.8	4.5
E0295		5.3		4.6	5	4.9	5.1	5.2
E0296	4.5	4.3	4.2	4.5	4.2	4.4	4.3	3.9
E0297	3.6	3.5	3.3	3.1	3	3.2	3.3	3
E0298	7.3	6.8	7	7.7	7.6	6.9	6.8	7.4
E0299	4.8	4.4	5	4.3	4.1	4.2	3.7	4.1
E0300	5	5	4.8	4.6	4.4	4.6	4.2	4.1
Systolic arterial pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	120	128	126	134	162	152	160	163
E0293	136	138	139	142	166	166	158	150
E0294	142		145	133	166	160	164	156
E0295		94		90	120	126	118	113
E0296	92	100	105	105	110	124	130	130
E0297	110	117	108	112	137	134	126	132
E0298	98	102	106	104	108	120	121	118
E0299	100	120	116	120	140	140	140	156
E0300	154	140	156	152	174	180	156	144
Diastolic arterial pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	60	68	64	72	82	76	81	79
E0293	60	58	56	60	68	68	68	60
E0294	80		80	74	84	76	82	79
E0295		56		56	68	74	68	63
E0296	54	58	64	64	64	70	70	70
E0297	58	62	60	68	72	68	68	66
E0298	59	56	60	58	60	62	64	66
E0299	58	60	60	66	78	80	80	92
E0300	80	75	82	84	88	92	82	79
Mean arterial pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	92	92	92	96	116	106	108	112
E0293	85	88	86	88	100	100	100	90
E0294	106		106	100	116	112	116	112
E0295		74		72	92	96	88	84
E0296	71	78	82	80	84	90	112	90
E0297	79	84	80	85	96	94	88	90
E0298	76	76	80	80	84	88	88	86
E0299	78	92	80	88	100	104	104	110
E0300	110	100	110	112	122	126	112	106

Appendix 3a. Sumatriptan haemodynamic data.
Placebo was administered at time = 0mins
Sumatriptan was administered at time = 20 mins

Pulmonary artery systolic pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	24	25	23	23	26	27	29	30
E0293	24	23	24	22	22	23	23	20
E0294	23	24	21	24	30	33	29	32
E0295		17		15	20	28	26	22
E0296	23	20	22	23	20	23	27	24
E0297	16	20	17	18	22	23	19	17
E0298	28	30	31	31	34	37	35	35
E0299	20	26	20	24	28	24	24	24
E0300	35	39	37	37	44	42	30	31
Pulmonary artery diastolic pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	10	10	10	9	11	11	12	13
E0293	7	6	6	8	6	10	10	9
E0294	4	8	4	7	7	9	9	11
E0295		4		3	8	11	9	8
E0296	8	6	8	8	8	8	10	8
E0297	6	7	5	6	10	10	8	7
E0298	8	16	15	10	16	18	17	17
E0299	8	14	8	10	12	10	10	8
E0300	14	14	12	11	15	14	10	7
Mean pulmonary artery pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	15	16	16	16	18	18	20	21
E0293	12	13	12	12	11	16	15	12
E0294	13	14	13	15	18	19	17	19
E0295		10		8	12	18	17	14
E0296	14	12	11	12	12	13	16	14
E0297	9	11	9	10	14	14	13	10
E0298	18	20	18	16	22	23	22	22
E0299	10	16	12	14	16	14	13	12
E0300	22	23	22	21	25	25	16	15
Right atrial pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	6	7.5	7	7.5	7	9	8	11
E0293	4	3	5	5	3	5	6	4
E0294	7	8	7	7	7	8	7	8
E0295		0.5		0.2	3	6	5	4
E0296	3	3	2	3	3	5	6	5
E0297	3	2	2	2	4	4	3	3
E0298	8	2	2	3	5	4	6	3
E0299	4	8	5	6	6	10	10	6
E0300	8	8	6	8	8	8	9	9
Pulmonary artery wedge pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	7	8	7.5	8	12	12	12	15
E0293	4	6	6	5	4	7	6	6
E0294	6	5	5	7	10	10	8	8
E0295		4		3	6	12	10	8
E0296	7	7	6	5	5	7	8	8
E0297	5	6	4	4	9	10	7	6
E0298	6	3	7	5	7	7	8	9
E0299	2	10	6	8	10	8	7	8
E0300	14	13	10	11	12	13	9	6

Appendix 3a. Sumatriptan haemodynamic data.
Placebo was administered at time = 0mins
Sumatriptan was administered at time = 20 mins

Left ventricular end diastolic pressure								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	8	7	8	8	9	11	11	12.5
E0293	8	9	10	9	12	12.5	12	10
E0294	10	5	7	7	19	13	11	11
E0295		5		4	10	14	12	10
E0296	7	4	8	7	7	9	16	15
E0297	6	6	6	4	14	14	11	10
E0298	3	4	4	6	8	9	10	8
E0299	7	9	10	10	9	10	11	12
E0300	14	12	15	11	17	15	11	8
Peak rate of LV pressure rise (dP/dt)								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291		2360	2915	2429	3401	3262	3158	2637
E0292		2640	1740	2100	2400			
E0293			4140	2400	3240	2640	2460	2880
E0294	2640	3240	2640	2640	3180	2400	2520	1920
E0295		1260		1560	1200	840	720	1320
E0296	1100	1125	1000	1250	1250	1065	1000	1000
E0297	1500	1550	950	1200	1375	1100	1225	1400
E0298	2640	2940	3000	2820	2940	3480	2460	2460
E0299	2280	2340	2100	2220	2280	1680	1680	1560
E0300	3952	3640	4004	3120	3120	2496	3952	2912
Pulmonary vascular resistance								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	120	124	133	140	103	107	142	96
E0293	125	86	100	112	110	141	163	106
E0294		102	96	130	176	187	167	195
E0295		91		87	96	98	110	92
E0296	124	93	95	124	133	109	149	123
E0297	89	114	121	129	107	100	145	107
E0298	131	200	126	114	158	186	165	141
E0299	133	109	96	112	117	114	130	78
E0300	128	160	200	174	236	209	133	176
Systemic vascular resistance								
Subject	**-10**	**0**	**10**	**20**	**30**	**40**	**50**	**60**
E0291	1291	1305	1330	1552	1867	1728	1770	1610
E0293	1270	1046	1317	1328	1521	1490	1709	1529
E0294			1622	1506	1699	1732	1807	1841
E0295		1109		1249	1424	1469	1302	1230
E0296	1209	1395	1524	1369	1543	1545	1972	1744
E0297	1689	1874	1891	2142	2453	2250	2060	2320
E0298	745	870	891	800	831	974	965	897
E0299	1233	1527	1360	1525	1834	1790	2032	2029
E0300	1632	1472	1733	1809	2073	2052	1962	1893

Appendix 5a. The effect of sumtriptan on systolic time intervals.

	Heart Rate			Timepoint (mins)		
SUBJECT	Baseline	Placebo	10	20	30	40
E0296	55	60	57	57	56	59
E0297	54	56	55	44	46	43
E0294	69	72	68	62	63	
E0299	54	53	61	59	65	71
E0300	71	73	75	71	68	66
E0293	67	51	59	55	52	48
E0298	77	72	71	71	73	72
E0295	71	72	64	65	66	65
E001	57	56	53	57	51	55
E002	62	59	56	56	52	65
E003		74	73	69	68	70
E004	80	87	94	89	83	96
E005		74	70	72	74	72
E006	69	63	60	53	53	62
E007		64	67	65	61	60
E008		58	60	62	59	66
E009	82	81	78	84	73	75
E010	85	79	73	74		
E011	81	77	85	79		
E012	66	67	70	71		
E013	59	55	57	57		
E014	63	66	58	56		
E015	66	64	56	60		
E016	49	43	40	40		
E017	107	97	86	82		
E018	53	47	43	46		
Systolic arterial pressure						
	Baseline	Placebo	10	20	30	40
SUBJECT	96	105	110	124	130	130
E0296	114	110	137	134	126	132
E0297	142	139	166	160	164	
E0294	110	118	140	140	140	156
E0299	147	154	174	180	156	144
E0300	137	141	166	166	158	150
E0293	100	105	108	120	121	118
E0298	94	90	120	126	118	113
E0295	124	134	124	128	128	122
E001	120	118	132	120	130	120
E002		150	188	164	170	160
E003	134	134	136	162	150	128
E004		120	144	120	110	120
E005	118	120	148	130	132	144
E006		154	220	210	200	200
E007		156	196	188	190	190
E008	102	110	120	124	120	128
E009	133	132	150	145		
E010	146	144	190	176		
E011	158	143	160	168		
E012	129	133	164	150		
E013	116	114	120	118		
E014	120	120	150	128		
E015	150	161	194	181		
E016	120	121	140	130		
E017	119	123	144	134		
E018						

Appendix 5a. The effect of sumtriptan on systolic time intervals.

Diastolic arterial pressure						
SUBJECT	Baseline	Placebo	10	20	30	40
E0296	56	64	64	70	70	70
E0297	60	64	72	68	68	66
E0294	80	77	84	76	82	
E0299	59	63	78	80	80	92
E0300	78	83	88	92	82	79
E0293	59	58	68	68	68	60
E0298	58	59	60	62	64	66
E0295	56	56	68	74	68	64
E001	80	82	78	80	80	78
E002	70	70	80	80	70	76
E003		80	90	80	80	80
E004	82	82	100	96	100	96
E005		80	90	84	78	80
E006	80	80	90	78	78	80
E007		88	108	100	100	100
E008		60	80	80	80	80
E009	70	80	80	80	78	78
E010	83	82	90	85		
E011	82	83	108	94		
E012	88	81	92	96		
E013	75	79	86	80		
E014	75	73	76	76		
E015	72	75	80	76		
E016	94	82	103	77		
E017	80	80	80	80		
E018	62	61	64	64		
Mean arterial pressure						
SUBJECT	Baseline	Placebo	10	20	30	40
E0296	75	81	84	90	112	90
E0297	82	83	96	94	88	90
E0294	106	103	116	112	116	
E0299	85	84	100	104	104	110
E0300	105	111	122	126	112	106
E0293	87	87	100	100	90	90
E0298	76	80	84	88	88	86
E0295	74	72	92	96	88	84
E001	100	102	98	98	102	100
E002	90	80	100	100	90	90
E003		110	128	118	120	116
E004	110	104	120	122	120	110
E005		100	116	100	92	100
E006	90	100	108	100	100	110
E007		116	148	146	136	140
E008		96	120	120	118	120
E009	88	96	100	100	94	96
E010	103	105	110	110		
E011	108	109	144	128		
E012	111	103	116	120		
E013	95	99	118	108		
E014	90	90	96	90		
E015	90	91	110	100		
E016	128	123	161	126		
E017	93	93	100	100		
E018	80	83	94	90		

Appendix 5a. The effect of sumtriptan on systolic time intervals.

Electromechanical systole (EMS)						
SUBJECT	Baseline	Placebo	10	20	30	40
E0296	580	601	582	608	615	626
E0297	558	559	572	588	628	580
E0294	603	608	618	614	620	
E0299	535	537	548	546	548	536
E0300	577	583	596	588	566	566
E0293	558	575	590	592	582	576
E0298	613	612	621	629	653	629
E0295	626	652	666	658	654	652
E001	557	576	559	576	626	616
E002	592	595	608	591	583	618
E003		580	626	661	632	611
E004	543	583	592	571	580	586
E005		596	624	645	602	627
E006	618	587	617	632	622	625
E007		555	615	610	579	588
E008		644	626	622	627	628
E009	483	461	488	509	496	551
E010	613	604	651	660		
E011	641	626	633	651		
E012	597	586	634	612		
E013	595	599	628	652		
E014	552	556	596	578		
E015	621	607	629	620		
E016	587	593	673	658		
E017	649	650	683	656		
E018	584		604	593		
Left ventricular ejection time(LVET)						
SUBJECT	Baseline	Placebo	10	20	30	40
E0296	431	441	423	449	457	464
E0297	440	425	429	434	430	429
E0294	428	436	453	451	441	
E0299	441	445	450	436	432	418
E0300	410	410	410	404	396	396
E0293	447	457	433	464	441	443
E0298	438	429	439	435	450	436
E0295	424	425	444	458	444	444
E001	440	455	453	458	448	454
E002	445	438	450		447	446
E003		461	486	504	490	464
E004	382	418	410	398	411	388
E005		413	411	432	409	404
E006	433	424	421	434	456	441
E007		429	461	456	446	448
E008		480	478	473	476	460
E009	316	318	326	335	327	320
E010	411	421	440	420		
E011	440	439	459	460		
E012	435	440	457	458		
E013	422	429	442	432		
E014	407	414	416	404		
E015	421	429	439	432		
E016	414	418	462	451		
E017	446	429	460	443		
E018		436	434	436		

Appendix 5a. The effect of sumtriptan on systolic time intervals.

Pre-ejection period (PEP)						
SUBJECT	Baseline	Placebo	10	20	30	40
E0296	149	160	159	159	159	162
E0297	118	134	143	154	198	151
E0294	175	172	165	163	179	
E0299	94	92	98	110	116	118
E0300	167	173	186	184	170	170
E0293	111	118	157	128	141	133
E0298	175	183	182	194	203	193
E0295	202	227	222	200	210	208
E001	117	121	106	118	178	162
E002	147	157	158	591	136	172
E003		119	140	157	142	147
E004	161	165	182	173	169	198
E005		183	213	213	193	223
E006	185	163	196	198	166	184
E007		126	154	154	133	140
E008		164	148	149	151	168
E009	167	143	162	174	169	231
E010	202	183	211	240		
E011	201	187	174	191		
E012	162	146	177	154		
E013	173	170	186	220		
E014	145	142	180	174		
E015	200	178	190	168		
E016	173	175	211	207		
E017	203	221	223	213		
E018	148		170	157		
					179	193
PEP:LVET Ratio						
SUBJECT	Baseline	Placebo	10	20	30	40
E0296	0.346	0.363	0.376	0.354	0.348	0.349
E0297	0.268	0.315	0.333	0.355	0.460	0.352
E0294	0.409	0.394	0.364	0.361	0.406	
E0299	0.213	0.207	0.218	0.252	0.269	0.282
E0300	0.407	0.422	0.454	0.455	0.429	0.429
E0293	0.248	0.258	0.363	0.276	0.320	0.300
E0298	0.400	0.427	0.415	0.446	0.451	0.443
E0295	0.476	0.534	0.500	0.437	0.473	0.468
E001	0.266	0.266	0.234	0.258	0.397	0.357
E002	0.330	0.358	0.351	#DIV/0!	0.304	0.386
E003		0.258	0.288	0.312	0.290	0.317
E004	0.421	0.395	0.444	0.435	0.411	0.510
E005		0.443	0.518	0.493	0.472	0.552
E006	0.427	0.384	0.466	0.456	0.364	0.417
E007		0.294	0.334	0.338	0.298	0.313
E008		0.342	0.310	0.315	0.317	0.365
E009	0.528	0.450	0.497	0.519	0.517	0.722
E010	0.491	0.435	0.480	0.571		
E011	0.457	0.426	0.379	0.415		
E012	0.372	0.332	0.387	0.336		
E013	0.410	0.396	0.421	0.509		
E014	0.356	0.343	0.433	0.431		
E015	0.475	0.415	0.433	0.435		
E016	0.418	0.419	0.457	0.459		
E017	0.455	0.515	0.485	0.481		
E018	0.339		0.392	0.360		

Appendix 6a. Naratriptan Haemodynamic data
Placebo was administered at time = -10mins.
Naratriptan was administered at time= 0mins

Heart Rate				Timepoint (mins)			
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	64	64	65	67	67	68	71
1415	75	74	76	80	76	76	76
1416	75	72	70	70	72	68	73
1417	64	62	62	61	65	70	68
1418	77	83	74	79	79	68	83
1419	74	70	66	68	68	63	66
1420	64	62	62	64	70	68	70
1421	65	62	63	66	75	70	63
1425	54	50	50	49	53	48	48
Cardiac Output							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	4.8	4.6	4.6	4.7	5	4.6	4.8
1415	6.6	6.5	7.8	7.9	8.5	7.3	7.5
1416	4.7	4.8	4.7	4.5	4.2	4.3	4.2
1417	6.3	5.9	6	5.9	6.2	6.5	6.6
1418	5.4	5.6	5.4	5.2	5.1	5.1	5.1
1419	6.1	5.9	6.2	6.1	5.7	5.6	5.6
1420	7.3	7.3	7.5	7.7	7.6	7.2	6.7
1421	5.3	5	5	5.7	6.2	5.1	5
1425	4.6	4.5	4.7	4.5	4.4	4.6	4
Systolic arterial pressure							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	169	195	187	216	216	208	200
1415	128	126	149	160	153	154	153
1416	130	128	122	132	133	132	132
1417	120	115	120	125	130	130	136
1418	104	106	98	116	120	114	118
1419	130	124	102	122	132	140	126
1420	150	165	170	178	180	180	180
1421	180	180	178	196	204	190	184
1425	94	94	89	104	103	106	106
Diastolic arterial pressure							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	89	100	98	104	110	104	100
1415	80	82	86	92	88	88	88
1416	67	66	64	70	70	68	74
1417	60	60	60	65	70	70	70
1418	66	64	60	70	72	70	64
1419	60	62	60	64	68	68	66
1420	80	80	80	82	90	80	90
1421	70	72	76	82	88	80	74
1425	54	54	48	52	55	56	57
Mean arterial pressure							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	120	136	136	156	160	148	148
1415	104	104	116	120	116	120	116
1416	90	92	91	96	97	96	99
1417	80	90	80	100	100	100	100
1418	90	84	84	90	94	90	90
1419	80	85	80	90	96	100	92
1420	110	115	115	130	130	120	120
1421	108	108	112	128	140	120	120
1425	70	70	64	72	74	76	74

Appendix 6a. Naratriptan Haemodynamic data
Placebo was administered at time = -10mins.
Naratriptan was administered at time= 0mins

Pulmonary artery systolic pressure							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	32	35	36	41	42	40	41
1415	23	23	23	28	27	27	28
1416	10	10	9	17	19	19	16
1417	25	26	28	28	29	29	31
1418	20	20	22	22	22	20	21
1419	18	17	16	26	25	22	23
1420	29	30	26	31	28	28	28
1421	27	30	30	38	40	35	32
1425	25	26	23	29	27	26	27
Pulmonary artery diastolic pressure							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	10	14	14	18	16	17	15
1415	10	7	12	15	15	14	15
1416	0	0	1	4	6	6	3
1417	8	9	11	10	13	11	12
1418	8	4	7	10	8	8	8
1419	9	6	4	8	8	5	6
1420	11	9	7	12	9	10	8
1421	12	9	12	15	14	9	13
1425	10	11	9	8	14	12	11
Mean pulmonary arterial pressure							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	20	24	25	28	26	28	26
1415	17	14	17	21	19	18	19
1416	5	6	5	9	10	10	8
1417	12	14	15	16	18	18	20
1418	12	10	10	14	13	12	12
1419	12	10	11	16	16	14	14
1420	15	16	13	19	17	16	16
1421	17	16	17	24	25	20	18
1425	16	17	15	18	20	17	18
Right atrial pressure							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	6	8	8	10	8	8	6
1415	9	7	8	9	9	9	9
1416	0	0	0	1	1	2	0
1417	9	8	8	8	8	8	9
1418	3	4	5	4	5	4	5
1419	6	6	8	6	6	6	6
1420	9	10	9	9	10	10	9
1421	6	5	6	8	8	7	6
1425	7	9	7	9	9	10	10
Pulmonary artery wedge pressure							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	12	15	14	15	20	15	12
1415	10	6	10	11	10	11	10
1416	3	2	2	6	4	5	4
1417	10	8	8	8	11	11	12
1418	3	4	4	6	6	6	6
1419	3	5	3	8	8	5	4
1420	6	8	8	9	12	8	6
1421	7	5	6	10	12	7	5
1425	12	13	12	15	15	14	14

Appendix 6a. Naratriptan Haemodynamic data
Placebo was administered at time = -10mins.
Naratriptan was administered at time= 0mins

Pulmonary vascular resistance							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	133	157	191	170	96	226	233
1415	85	98	72	101	85	77	96
1416	34	67	51	53	114	102	86
1417	25	81	86	108	90	86	97
1418	133	86	89	123	110	94	94
1419	118	68	103	105	112	129	143
1420	99	88	53	104	53	89	119
1421	151	176	176	196	168	204	208
1425	70	71	51	53	91	52	80
Systemic vascular resistance							
Subjec	**-20**	**-10**	**0**	**10**	**20**	**30**	**40**
1414	1900	2226	2226	2519	2432	2434	2366
1415	1152	1194	1108	1124	1007	1216	1141
1416	1531	1533	1548	1688	1828	1748	1885
1417	902	1112	886	1247	1187	1132	1103
1418	1288	1143	1170	1323	1396	1349	1333
1419	970	1071	929	1102	1263	1342	1229
1420	1107	1151	1131	1257	1263	1222	1325
1421	1540	1648	1712	1684	1703	1772	1824
1425	1096	1084	970	1120	1182	1148	1280

Appendix 6b. Naratriptan coronary artery data

PROXIMAL ARTERIES						
ARTERY	SUBJECT	BASELIN	PLACEB	MIN 10	MIN 20	MIN 30
Cx	1414	2.09	1.92	1.73	2.26	1.85
LAD	1414	3.15	3.07	2.95	3.23	2.78
LMS	1415	5.36	5.6	5.27	5	5.27
Cx	1415	3.38	3.21	3.29	3.06	3
LAD	1415	4	4.24	4.66	3.55	4
LMS	1416	3.8	3.8	3.8	4.1	4
Cx	1416	3.1	2.7	2.8	2.7	2.5
LAD	1416	3.6	3.5	3.6	3.4	3.2
RCA	1417	2.97	2.93	2.92	3.36	3.07
LMS	1418	3.5	3.54	3.79	3.8	3.32
Cx	1418	2.33	2.47	2.8	2.68	2.58
LAD	1418	3.28	3.35	3.24	3.24	3.3
LMS	1419	4.6	4.77	4.6	4.5	4.77
Cx	1419	3.16	3.33	3.45	2.91	3.31
LAD	1419	3.55	3.66	3.82	3.55	3.55
RCA	1420	3.17	3.27	3.17	3.25	
LMS	1421	5.63	5.7	5.66	5.64	5.64
Cx	1421	3.16	3.11	3.09	3	3.07
LAD	1421	4.02	4.18	4.15	3.85	3.89
LMS	1425	5.33	5.33	5.33	5.31	5.41
Cx	1425	3.67	3.67	3.75	3.68	3.73
LAD	1425	5	5	5.17	4.94	5.07
LMS	1423	5.94	5.33	5.32	5.33	5.24
Cx	1423	3.66	3.68	3.48	3.66	3.66
LAD	1423	4.27	3.66	3.48	3.38	3.68
MID ARTERIES						
Cx	1414	1.89	1.32	1.79	1.51	1.35
OM	1414	1.73	1.78	1.69	1.87	1.6
LAD	1414	1.93	2.06	1.98	2.09	1.95
Cx	1415	2.4	2.86	2.57	2.88	2.56
LAD	1415	3.48	3.92	3.39	3.22	3.21
LAD	1416	2.2	2.2	2	1.9	2
OM	1416	2.2	2	1.9	1.7	1.9
RCA	1417	2.71	2.71	2.71	3.06	2.74
PDA	1417	2.4	2.55	2.53	2.6	2.4
Cx	1419	1.45	1.49	1.54	1.55	1.46
OM	1419	2.05	2	2.18	2.26	1.94
RCA	1420	3.23	3.17	2.87	2.63	
Cx	1421	2.92	3.07	2.97	2.86	3.06
LAD	1421	1.9	2.13	2.23	1.75	2
OM	1421	1.9	2	2.05	1.75	2
Cx	1425	2.67	2.33	2.33	2.31	2.3
LAD	1425	3.62	3.3	3.3	3.3	3.51
OM	1425	3	2.87	2.41	2.38	2.42
Cx	1423	2.33	2.01	1.9	2	2.14
LAD	1423	1.94	1.93	1.9	2	2.1
OM	1423	1.77	1.7	1.73	1.69	1.74

Appendix 6b. Naratriptan coronary artery data

DISTAL ARTERIES						
ARTERY	SUBJECT	BASELIN	PLACEB	MIN 10	MIN 20	MIN 30
Cx	1414	1.49	1.18	1.22	1.11	
LAD	1414	1.96	1.48	1.81	1.27	1.39
Cx	1415	1.5	1.33	1.34	1.57	1.25
LAD	1415	1.58	1.69	1.71	2.02	1.62
OM	1415		1.69	1.71	2.02	1.62
LAD	1416	2.1	1.6	1.8	1.6	1.5
RCA	1417	1.8	1.73	1.7	1.49	1.46
Cx	1419	1.36	1.33	1.31	1.29	1.4
LAD	1419	1.21	1.2	1.28	1.17	1.29
PDA	1420	2.2	2.13	1.88	1.95	
LAD	1421	1.26	1.27	1.11	1.18	1.2
Cx	1425	1.94	1.67	1.94	1.74	1.82
LAD	1425	1.37	1.41	1.41	1.4	1.35
Cx	1423	1.4	1.2	1.41	1.33	1.42
LAD	1423	1.39	1.35	1.34	1.33	1.36
LAD	Left anterior descending					
RCA	Right coronary artery					
Cx	Circumflex					
PDA	Posterior descending artery					
LMS	Left main stem					
OM	Obtuse marginal					

Appendix 7a. Eletriptan haemodynamic data.
Placebo infusion was administered between times 0- 10 mins
Eletriptan infusion was administered between times 15-25 mins.

	Heart Rate												
Subject	0	5	10	15	20	25	30	35	40	45	50	55	60
001	75	80	76	74		75	75	75	80	80	79	75	70
002	75	66	66	71	71	72	72	60	58	58	58	58	58
003	75	75	75	75	78	75	75	70	72	75	70		75
004	62	57	67	63	64	68	66	61	60	65	61	70	68
005	60	60	58	58	60	60	60	60	60	60	60	60	58
006	62	72	67	65	70	72	65	65	70	66	70	64	66
007	74	60	64	60	62	60	74	60	72	70	75	60	60
008	50	50	50	50	50	50	50	50	50	54	50	56	50
009	72	75	70	60	70	75	60	70	75	72	60	75	75
010	84	90	94	94	80	80	80	84	80	75	75	75	75
	Systolic arterial pressure												
Subject	0	5	10	15	20	25	30	35	40	45	50	55	60
001	120	120	120	120		120	130	134	124	126	124	130	120
002	150	123	120	122	130	141	138	138	122	132	130	125	128
003	114	120	124	126	124		136	128	130	134	128		128
004	178	172	164	158	186	172	170	174	178	168	166	152	170
005	122	102	116	116	120	120	122	130	130	128	134	132	136
006	109	100	116	104	118	120	122	134	148	154	145	134	142
007	110	114	120	124	128	130	128	126	126	122	140	140	134
008	100	96	100	94	96	93	100	103	102	104	104	104	110
009	140	110	118	116	118	120	120	126	116	122	120	120	140
010	170	166	148	156	166	170	190	170	168	152	164	160	182
	Diastolic arterial pressure												
Subject	0	5	10	15	20	25	30	35	40	45	50	55	60
001	70	72	72	70		74	74	78	76	74	74	76	70
002	60	60	60	58	58	56	60	60	56	60	58	58	58
003	58	74	74	78	78		82	78	80	80	78		80
004	84	82	78	77	84	82	80	81	80	80	81	78	80
005	64	57	62	60	62	66	64	64	68	64	70	68	70
006	64	60	64	60	66	68	70	80	82	82	82	75	80
007	60	68	68	68	70	68	68	88	86	84	74	76	70
008	52	53	54	56	56	50	53	57	60	58	58	60	57
009	90	80	80	80	80	80	78	82	78	80	76	74	84
010	84	86	80	90	88	90	92	90	84	86	86	90	94
	Pulmonary artery systolic pressure												
Subject	0	5	10	15	20	25	30	35	40	45	50	55	60
001	19	20	21	20		23	22	22	22	20	22	22	22
002	28	18	17	15	16	20	21	22	18	19	22	20	20
003	24	28	26	30	30		36	30	31	31	30		28
004													
005	19	21	19	18	17	18	20	22	23	22	24	22	22
006	23	24	23	25	26	26	27	28	27	27	28	27	27
007	24	24	23	21	22	23	19	22	23	20	22	20	24
008	14	14	13	12	14	13	17	18	16	16	16	16	15
009	18	16	16	16	18	17	17	18	18	18	18	20	18
010	24	28	26	24	24	26	26	28	23	22	24	25	26
	Pulmonary artery diastolic pressure												
Subject	0	5	10	15	20	25	30	35	40	45	50	55	60
001	6	7	7	7		12	12	10	10	8	8	8	8
002	12	6	6	5	6	8	7	8	7	7	10	8	8
003	8	10	10	12	10		36	30	31	31	30		28
004													
005	9	7	8	9	10	10	11	11	12	10	11	10	10
006	8	10	10	11	13	11	12	13	14	13	17	15	13
007	10	12	12	12	12	12	10	11	11	10	12	9	11
008	4	5	4	6	6	4	6	5	6	4	5	3	3
009	8	8	8	8	8	9	8	8	8	8	8	8	7
010	8	8	4	4	8	8	9	8	4	8	8	7	12
	Pulmonary artery wedge pressure												
Subject	0	5	10	15	20	25	30	35	40	45	50	55	60
001	3	10	7	7		8	8	7	8	7	8	7	8
002	8	6	4	4	6	7	6	8	6	6	7	6	7
003	7	10	10	10	11		11	10	9	10	9		8
004													
005	11	9	10	10	10	11	11	12	11	12	10	10	10
006	10	11	12	13	11	12	13	15	14	13	15	13	13
007	14	16	12	12	13	13	11	12	11	9	13	12	12
008	7	6	4	7	5	7	6	7	6	5	5	6	5
009	6	5	5	5	7	7	7	7	7	7	7	6	6
010	7	4	3	3	3	5	8	7	5	3	5	5	6
	Right atrial pressure												
Subject	0	5	10	15	20	25	30	35	40	45	50	55	60
001	2	2	2	3		3	3	4	4	4	5	4	4
002	3	2.5	1.5	1	2	3	3	3.5	3	2.5	3	3	3
003	6	4	4	5	6		7	6	6	6	6		6
004													
005	9	5	6	6	6	6	7	9	7	6	7	6	7
006	6	8	8	9	9	9	9	10	9	9	11	10	10
007	7	8	6	8	8	8	7	8	8	7	7	6	8
008	4	3	4	2	2	4	2	4	2	2	3	4	2
009	4		7	7	7	7	7	7	6	7	6	5	5
010	4	9	7	7	7	9	6	7	6	6	6	6	6

Appendix 7a. Eletriptan haemodynamic data.
Placebo infusion was administered between times 0- 10 mins
Eletriptan infusion was administered between times 15-25 mins.

	Systemic vascular resistance						Proximal coronary artery segments					
Subject	0	15	30	45	60	Subject	0	15	30	45	60
001	1145	1208	1177	1443	1317	001	2.7	2.7	2.9	3.2	3
002	987	842	1114	1344	1310	002	2.67	2.69	2.6	2.69	2.76
003	967	1308	1299	1434	1322	003	2.88	2.84	2.96	2.6	2.75
004						004	1.76	2.01	2.27	2.1	2.27
005	1681	1451	1760	1915	1843	005	2.78	2.66	0.95	1.21	2.57
006	1000	1026	1038	1440	1393	006	2.95	3.22	3.16	2.75	3
007	1112	1531	1553	1555	1756	007	3.14	3.06	2.76	3.21	3.26
008	1298	1486	1504	1656	1660	008	3.2	3.7	3.4	3.3	3.2
009	1291	959	1029	1031	1048	009	3.4	3.5	3.6	3.3	3.4
010	1499	1520	1844	1850	1971	010	4.2	4.1	3.9	4.2	3.6
						Distal coronary artery segments					
Subject	0	15	30	45	60	Subject	0	15	30	45	60
001	114	67	76.8	141	71.6	001	1.9	1.6	2.3	1.8	2.1
002	67.7	48.4	64	78.6	63.1	002	2.08	2.03	1.97	2.17	1.78
003	65.4	121	147	141	120	003	1.44	1.88	2.41	1.84	2.01
004						004	1.49	1.74	2.6	1.9	1.95
005	84	76	89	91	130	005	2.6	2.7	2.66	2.13	2.35
006	77	67	50	87	89	006	1.8	1.9	1.91	1.81	1.81
007	31	91	73	112	80	007	2.78	2.29	2.11	2.21	2.16
008	19.1	20.4	83.6	66	86.3	008	2.7	2.5	2.5	2.8	2.4
009	63.3	61.4	62	49.7	60.2	009	2.9	2.7	3	3	3.4
010	129.2	132.8	109.4	119.9	167	010	4.3	4.1	4.1	4.2	4.3
						Middle coronary artery segments					
Subject	0	15	30	45	60	Subject	0	15	30	45	60
001	6.29	5.62	6.25	5.1	5.59	001	2.1	2.2	2.2	2.2	2.2
002	6.27	6.27	6.25	5.06	5.07	002	1.64	1.85	1.78	1.76	1.53
003	6.12	5.93	5.97	5.09	5.99	003	2.02	1.73	1.9	1.9	2.05
004						004	1.34	1.91	1.95	1.69	1.9
005	3.76	4.19	3.59	3.61	3.69	005	2.84	2.84	2.16	2.13	2.11
006	6.2	6	6.4	6.5	5.4	006	1.44	1.24	1.53	1.45	1.34
007	5.25	4.39	4.38	4.27	4.01	007	2.69	2.79	2.23	2.69	2.69
008	4.19	3.93	3.83	3.72	3.71	008	2.5	2.7	2.5	2.5	2.4
009	6.32	6.51	6.45	6.44	6.64	009	2.9	2.6	2.8	2.4	2.7
010	5.19	5.42	5.12	4.87	4.79	010	2.7	2.6	2.9	2.6	2.7

Appendix 8a. Ketanserin haemodynamic data.

	Systolic arterial pressure					Diastolic arterial pressure			
Subject	Baseline	Placebo	Ket 5min	Ket 15min		Baseline	Placebo	Ket 5min	Ket 15min
EL	140	140	108	110		66	56	64	56
JF	115	131	100	108		70	62	66	60
RC	154	171	147	162		97	97	92	89
WL	145	139	120	142		59	60	62	51
DM	114	129	125	122		73	61	66	73
GC	121	123	120	124		71	73	74	67
KW	168	173	149	160		84	88	85	80
AC	143	147	112	124		68	61	69	57
RMcW	128	138	117	124		71	61	65	61
WR	138	141	125	122		77	67	74	70

	Mean arterial pressure					Pulmonary artery systolic pressure			
Subject	Baseline	Placebo	Ket 5min	Ket 15min		Baseline	Placebo	Ket 5min	Ket 15min
EL	92	96	78	82					
JF	88	96	78	85		24	24	28	20.5
RC	113	122	108	119		27	14	24	16
WL	92	89	78	96		20.5	20	20	19
DM	90	96	97	94		23	20	22	20
GC	97	90	90	95		25	25	23	28
KW	120	121	109	120		20	18	18	19
AC	100	101	81	91		20	17	20	15
RMcW	88	95	83	86		19	19	19	18
WR	102	105	94	92		26	25	27	25

	Pulmonary artery diastolic pressure					Mean pulmonary artery pressure			
Subject	Baseline	Placebo	Ket 5min	Ket 15min		Baseline	Placebo	Ket 5min	Ket 15min
EL									
JF	10	7	12	9		16	18	16	12
RC	8.5	8	12	13.5		20	18	11	11
WL	4	5	8	6		13	14	12	12
DM	8	8	8	8		16	14	13	13
GC	10	12	12	11		16	16	15	17
KW	7	6	7	5		13	11	12	12
AC	4	6	6	9		13	13	8	10
RMcW	3	2	2	4		8	10	8	8
WR	8	6	7	7		14	15	15	12

	Pulmonary artery wedge pressure					Cardiac output			
Subject	Baseline	Placebo	Ket 5min	Ket 15min		Baseline	Placebo	Ket 5min	Ket 15min
EL									
JF	7	4	7.5	5		5.4	6.03	5.62	5.48
RC	8	7	7	13		6.76	6.82	6.76	7.36
WL	9	8	11	10		6.8	4.18	6.52	4.55
DM	10	7.5	8	7		6.2	5.7	5.9	6.1
GC	4	4	8	6		5.4	5.05	5.05	4.87
KW	6	6	6	5		5.9	5.94	5.82	6.08
AC	3	3	7	6		4.29	4.15	4.69	4.39
RMcW	6	4	6	2		4.09	4.06	4.3	4.34
WR						6.25	6.75	6.5	6.7

Appendix 8a. Ketanserin haemodynamic data.

	Systemic vascular resistance				Pulmonary vascular resistance			
Sublect	Baseline	Placebo	Ket 5min	Ket 15min	Baseline	Placebo	Ket 5min	Ket 15min
EL								
JF	1039	1211	1170	1201	128	119	139	161
RC	1183	1136	1310	1310	36	47	129	76
WL	908	1538	1094	1627	37	47	57	53
DM	1274	1154	1174	1305	41	84	84	118
GC	1299	1528	1289	1365	174	193	127	164
KW	1457	1526	1572	1562	82	81	67	105
AC	1364	1768	1760	1889	85	137	116	128
RMcW	1488	1567	1623	1754	37	78	79	111
WR	1058	1122	1101	1156				

	Heart Rate			
	Baseline	Placebo	Ket 5min	Ket 15min
Sublect	54	53	58	59
EL	75	75	78	72
JF	91	97	98	98
RC	49	49	51	51
WL	60	52	54	54
DM	67	70	72	71
GC	94	87	87	90
KW	68	65	66	66
AC	48	54	52	49
RMcW	82	78	91	79
WR				

Appendix 8b. Ketanserin coronary artery data.

Proximal coronary artery segments			
Baseline	**Placebo**	**Ket 5min**	**Ket 15min**
5.5	5.1	5.4	4.9
4.6	3.8	4	3.7
4.1	4.3	4.1	4.3
3.9	4.1	4.3	3.5
3.4	3.6	3.7	3.5
3.5	3.8	3.7	3.8
5.3	5.6	5	5.5
5.1	5	4.8	4.8
3.7	3.7	3.2	3.1
4.1	4.1	3.8	3.5
3.3	3.3	3.4	3.3
3.4	3.3	3.5	3.2
Middle coronary artery segments			
Baseline	**Placebo**	**Ket 5mln**	**Ket 15min**
3	2.7	2.7	2.7
2.2	1.9	2	2
2.8	2.9	3.1	3.2
2.6	2.6	2.2	2.1
2.1	2	2.2	2.3
2	2.3	2.2	3.3
2.5	1.9	2.5	2.4
3.2	2.8	2.7	2.8
Distal coronary artery segments			
Baseline	**Placebo**	**Ket 5min**	**Ket 15min**
1.8	1.6	1.4	1.5
1.5	1.6	1.6	1.4
1.6	1.5	1.7	1.8
1.5	1.5	1.2	1.3
1.1	0.9	1	1
1.2	0.9	1	0.7

Appendix 9a. Sumatriptan forearm blood flow data.

	Plasma noradrenaline (nmol/l)				Placebo day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
TB	0.91	0.9	0.94	1.03	0.91	0.97
NC	0.72	0.78	0.76	0.72	0.64	0.68
DC	0.6	0.49	0	0.55	0.52	0.45
MJ	0.47	0.46	0.37	0.43	0.5	0.73
PM	0.72	0.62	0.66	0.72	0.74	0.75
JM	1.04	1	0.92	0.85	0.95	0.98
SM	0.52	0.68	0.63	0.62	0.65	0.63
DR	0.47	0.47	0.41	0.49	0.47	0.46
SW	0.8	0.95	0.89	0.86	0.87	0.84
SH						
	Plasma noradrenaline (nmol/l)				Sumatriptan day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
TB	0.82	0.63	0.51	0.43	0.4	0.48
NC	0.56	0.65	0.67	0.41	0.58	0.65
DC	0.9	1.04	0.75	0.51	0.55	0.64
MJ	0.34	0.29	0.27	0.19	0.24	0.23
PM	0.69	0.65	0.41	0.51	0.41	0.36
JM	0.51	0.46	0.4	0.38	0.33	0.32
SM	0.69	0.4	0.43	0.37	0.36	0.44
DR	0.48	0.36	0.33	0.26	0.33	0.31
SW	0.74	0.77	0.64	0.58	0.6	0.81
SH						
	Systolic BP (mmHg)				Placebo day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
NC	117	120	127	119	119	118
DR	115	122	129	129	121	128
SM	121	126	124	116	121	118
SH	116	118	121	116	118	121
DC	124	124	124	117	125	118
SW	120	116	116	117	117	111
MJ	121	124	129	127	125	123
PM	114	109	108	115	114	113
TB	107	106	110	107	111	107
JM	128	127	127	127	127	118
	Systolic BP (mmHg)				Sumatriptan day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
NC	120	132	130	127	125	124
DR	120	130	112	129	128	120
SM	127	130	123	131	131	131
SH	114	130	124	121	123	121
DC	127	129	128	128	120	128
SW	122	138	137	137	135	136
MJ	133	131	130	137	136	136
PM	118	127	132	129	119	124
TB	121	120	119	127	111	119
JM	119	132	131	131	130	129

Appendix 9a. Sumatriptan forearm blood flow data.

	Diastolic BP (mmHg)				Placebo day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
NC	62	62	61	64	59	68
DR	63	64	63	67	67	61
SM	45	48	46	48	51	39
SH	82	81	76	74	79	81
DC	54	57	56	57	58	54
SW	54	49	50	50	56	52
MJ	64	61	61	63	67	63
PM	64	65	57	64	67	62
TB	57	64	69	65	66	61
JM	70	73	60	64	68	68
	Diastolic BP (mmHg)				Sumatriptan day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
NC	71	84	79	73	71	69
DR	64	67	64	71	70	69
SM	42	40	44	50	41	57
SH	79	85	83	83	87	85
DC	54	65	66	58	58	67
SW	59	63	72	64	60	71
MJ	64	65	68	71	66	66
PM	66	75	75	71	72	79
TB	54	57	52	48	59	53
JM	54	72	69	64	68	67
	Heart Rate (bpm)				Placebo day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
NC	71	67	72	67	68	67
DR	52	50	51	54	50	44
SM	57	59	58	60	60	60
SH	74	73	75	72	76	75
DC	60	58	56	64	64	65
SW	53	47	49	52	49	49
MJ	64	60	62	58	57	53
PM	61	56	57	65	55	56
TB	80	76	77	76	80	77
JM	68	66	64	61	57	61
	Heart Rate (bpm)				Sumatriptan day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
NC	64	63	64	57	60	67
DR	53	54	47	55	58	55
SM	58	56	61	56	55	62
SH	78	88	87	82	78	76
DC	63	58	57	57	60	66
SW	63	56	63	60	53	55
MJ	63	57	62	64	62	59
PM	73	67	74	74	70	70
TB	77	77	76	75	69	71
JM	65	70	63	58	62	61

Appendix 9a. Sumatriptan forearm blood flow data.

	Right forearm blood flow (ml/100ml/min				Placebo day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
TB	2.02	2.52	2.13	2.3	2.31	1.73
NC	6.9	7.46	7.48	7.3	7.29	6.86
DC	2.41	2.37	2.11	2.41	2.62	2.7
MJ	2.69	2.81	2.58	2.55	2.55	2.65
PM	3.45	3.23	3.25	3.48	3.87	3.33
JM	2.85	2.98	2.73	1.87	2.15	1.74
SM	4.29	4.89	4.5	4.02	4.17	4.11
DR	4.69	4.24	4.16	3.99	3.98	3.67
SW	2.21	2.65	2.9	2.38	2.38	2.45
SH	3.72	3.74	2.71	3.06	2.81	4.17
	Right forearm blood flow (ml/100ml/min				Sumatriptan day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
TB	2.36	3.88	4.2	3.75	3.51	3.38
NC	3.71	6.66	5.75	5.05	4.74	4.85
DC	2.44	2.88	2.89	3.23	3.36	3.11
MJ	3.92	4.38	4.15	3.94	3.66	3.99
PM	4.13	3.72	5.63	5.06	4.8	4.29
JM	1.56	2.01	1.43	1.48	1.57	1.39
SM	4.27	4.89	4.5	4.02	4.17	4.11
DR	3.18	3.11	2.78	3.02	3.53	3.09
SW	4.5	3.37	3.71	3.72	3.65	3.45
SH	3.14	5.22	5.33	5.46	5.59	5.28
	Left forearm blood flow				Placebo day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
TB	2.86	3.52	3.02	3.38	3.66	3.74
NC	11.21	12.43	11.73	11.27	11.5	9.96
DC	4.31	4.21	4.53	4.45	4.4	4.6
MJ	3.64	4.06	4.07	3.99	4.43	4.24
PM	3.41	3.17	3.23	3.49	3.74	3.26
JM	2.76	2.7	3.91	2.07	2.27	2.13
SM	4.84	6.12	5.44	4.88	5.1	5.12
DR	5.33	5.12	4.58	4.74	4.27	4.37
SW	2.63	2.84	2.75	3.13	2.72	2.93
SH	3.41	3.33	2.74	2.97	3.23	4.34
	Left forearm blood flow				Sumatriptan day	
Subject	Baseline	7 mins	14 mins	21 mins	28 mins	35 mins
TB	3.64	5.2	5.17	4.89	4.3	4.37
NC	5.53	7.06	6.29	6.01	5.57	5.84
DC	3.22	2.79	2.86	2.76	2.87	2.92
MJ	3.59	3.71	3.4	3.24	2.77	3.01
PM	4.19	3.09	5.05	4.76	3.96	3.46
JM	2.41	2.58	1.85	2.1	1.83	1.8
SM	4.84	6.12	5.44	4.88	5.1	5.12
DR	4.95	3.81	3.14	3.2	3.61	3.25
SW	5.02	3.77	4.55	4.61	4.3	4.44
SH	4.3	9.03	9	9.62	9.67	9.89