VASCULAR ß-ADRENOCEPTORS AND PRESSOR RESPONSE TO ß-ADRENOCEPTOR ANTAGONISTS

By

ALY MOHAMED OMAR ABDELRAHMAN

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Department of Pharmacology

The University of British Columbia
Vancouver, Canada

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Abstract

ß-adrenoceptors have been classified into two subtypes, a $ß_1$-subtype found in the heart and a $ß_2$-subtype found in the vasculature. However, there is evidence that $ß_1$-adrenoceptors may also be present in the vasculature. We have examined the role of ß-adrenoceptors in the vasculature, first in the resistance blood vessels and second in the venous system. Firstly, we studied the effects of isoprenaline (mixed ß), isoprenaline plus ICI 118,551 ($ß_1$), isoprenaline plus atenolol ($ß_2$) and isoprenaline plus both ICI 118,551 and atenolol on haemodynamics in pentobarbital-anaesthetized rats using the radioactive microsphere technique. This study showed that $ß_2$- but not $ß_1$-adrenoceptor stimulation reduced total peripheral resistance (TPR) and mean arterial pressure (MAP). Both $ß_1$- and $ß_2$-adrenoceptor stimulation increased coronary and skeletal muscle vascular conductances. Secondly, we examined the effect of isoprenaline on MAP, heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious rats. Isoprenaline was infused into intact, hexamethonium-pretreated or noradrenaline-pretreated rats. Our results show that under normal conditions, isoprenaline decreased MAP and increased HR and MCFP. Hexamethonium pretreatment did not affect the tachycardic and hypotensive effects of isoprenaline but it abolished the increase in MCFP indicating that this increase was due to reflex venoconstriction. Under conditions of high venous tone, isoprenaline decreased MAP and MCFP and

increased HR. Therefore, our results show that ß-adrenoceptor stimulation mediates direct venodilatation in the presence of a high venous tone and reflex-mediated venoconstriction under normal conditions.

Paradoxical pressor responses to ß-adrenoceptor antagonists have been reported in some clinical and experimental conditions. The mechanisms underlying this phenomenon are not known. We examined the conditions under which ß-adrenoceptor antagonists produced a pressor response. Firstly, we examined the haemodynamic changes which occur during α-adrenoceptor blockade by phentolamine, and after the development of the pressor response to ß-adrenoceptor antagonists in urethane-anaesthetized rats and in conscious rats. In urethane-anaesthetized rats, propranolol reversed the increase in conductance induced by phentolamine in skeletal muscle and skin and it also decreased renal vascular conductance. In conscious rats, propranolol or atenolol reversed the increase in conductance induced by phentolamine in skeletal muscle and in addition, it decreased conductance in the intestinal, renal and cutaneous vasculature. The inhibition of angiotensin converting enzyme by captopril attenuated the ß-adrenoceptor antagonist-induced pressor response demonstrating the importance of the renin-angiotensin system in the production of this response. Secondly, experiments were done to investigate the effects of anaesthetic agents on pressor response to ß-adrenoceptor antagonists. Our results show

that anaesthetic agents have variable effects on ß-adrenoceptor antagonist-induced pressor responses. Urethane did not alter the pressor response to ß-adrenoceptor antagonists. Halothane and ketamine, on the other hand, attenuated the pressor response while ß-adrenoceptor antagonists did not produce a pressor response with pentobarbital, amobarbital or chloralose. It was further shown in pentobarbital-anaesthetized rats that phentolamine increased arteriovenous conductance and reduced MAP with no effects on other vascular beds and propranolol then did not have any effects on vascular conductance, TPR or MAP. An infusion of adrenaline partially restored the pressor response to both propranolol and atenolol showing the importance of adrenaline in the production of a pressor response. Thirdly, dose-response curves for propranolol, atenolol or ICI 118,551 in the presence of both noradrenaline and phentolamine were constructed in the isolated rat pulmonary artery. All three ß-adrenoceptor antagonists completely restored the phentolamine-induced relaxation response. Therefore, our in vitro and in vivo results are in accordance with a possible interaction of α- and ß-adrenoceptor antagonists which leads to subsequent stimulation of the α-adrenoceptors in the presence of adrenaline. The mechanism of this interaction is not clear. The results of in vivo studies show that additional factors such as the renin-angiotensin system may also be involved in this pressor response.

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LIST OF ABBREVIATIONS

Adrenaline	A
Cardiac output	CO
Count per minute	cpm
Heart rate	HR
Hour(s)	h
International Unit	I.U.
Intravenous	I.V.
Mean arterial pressure	MAP
Mean circulatory filling pressure	MCFP
Molecular weight	Mr
Minute	min
Noradrenaline	NA
Seconds	s
Standard error	SE
Subcutaneous	sc
Total peripheral resistance	TPR
Venous plateau pressure	VPP

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GENERAL OVERVIEW

The cardiovascular system plays a special role in living animals and in man. It is the transport system for the delivery of oxygen and removal of carbon dioxide. It delivers nutrients from the gastrointestinal tract to all the body parts, carries waste products of cellular metabolism to the kidney and other excretory organs, transports electrolytes and hormones, maintains body temperature and transports cells and immune substances. The cardiovascular system is mainly under the control of the sympathetic nervous system, the renin angiotensin system and the vasopressin system.

Stimulation of the sympathetic nervous system involves the release of catecholamines from the nerve terminals and from the adrenal medulla. Catecholamines stimulate either α- or ß-adrenoceptors in blood vessels causing either contraction or relaxation, respectively.

The aim of this study is to define the role of ß-adrenoceptors in the different arterial beds in the rat. In addition, we studied the role of ß-adrenoceptors in the venous system. The use of ß-blockers in the presence of phentolamine was associated with a pressor response in animals and in humans. We also examined the conditions under which this pressor response was produced.

1. INTRODUCTION

1.1. The sympathetic nervous system

The general anatomical features of the autonomic chain have been known since the end of the seventeenth century. The ganglia, communicating filaments with the spinal cord, and its branches to the visceral organs were described by Willis (1644). However, at that time, the ganglionated chain was thought to arise from the cranial nerves. In the eighteenth century, the sympathetic ganglia was considered to arise from the spinal cord and this peripheral system was thought to be the site of involuntary action and associated with anabolic and metabolic aspects of digestion and circulation. In the nineteenth century, Gaskell (1886) outlined the anatomy of the peripheral autonomic nervous system and was able to construct the concept of bulbar, thoracolumbar and sacral outflows of involuntary nerves to the ganglia. The concept of a chemical substance being released from the nerve endings was first proposed by Dubois-Reymond (1860). In 1895, Oliver and Schafer showed that extracts from the suprarenal glands produced striking physiological effects upon the heart and arteries. This was followed by studies which showed the similarities in responses between the injections of suprarenal extracts and stimulation of the sympathetic nervous system (Lewandowsky, 1898; Langley, 1901). The active substance of the suprarenal gland was first isolated by Abel and Crawford (1897) and was termed "epinephrin". Later, Takamine (1902)

isolated this substance in a more purified form and he named it "adrenalin". In 1905, Elliot proposed that stimulation of sympathetic nerves resulted in the release of minute amounts of an adrenaline-like substance. The concept of a receptive substance was introduced in 1905 by Langley who proposed that effector cells have excitatory and inhibitory receptive substances and that the response to nerve stimulation depended on the type of substance present. In 1921, Loewi demonstrated that nerve endings, when stimulated, release a chemical substance which acts on the target tissue to produce a response. In the same year, Cannon and Uridil (1921) showed that stimulation of the sympathetic hepatic nerves released a substance which was adrenaline-like and they called it "sympathin". Later in 1933, Cannon and Rosenblueth proposed that "sympathin" is present in two forms, an excitatory form (sympathin E) and an inhibitory form (sympathin I). Extracts from the spleen and heart were found to contain a substance which resembled noradrenaline (Euler, 1946 a, b). In 1949, Peart showed that noradrenaline was the substance released by sympathetic nerve stimulation.

Preganglionic fibres of the sympathetic nervous system arise from the intermediolateral columns of the spinal cord of all the thoracic and the upper three lumbar segments. These fibres synapse with the postganglionic fibres in the ganglia which are found at three locations; paravertebral, prevertebral and terminal. The postganglionic fibres

innervate the various tissues and organs (Lefkowitz et al., 1990).

Blaschko (1939) proposed the synthetic steps of noradrenaline and adrenaline from tyrosine which involve the hydroxylation of tyrosine to DOPA by the enzyme tyrosine hydroxylase, followed by decarboxylation of DOPA to dopamine by the decarboxylase enzyme. These two steps occur in the cytoplasm and are followed by the active transport of dopamine into the adrenergic vesicles and its conversion to noradrenaline by the enzyme dopamine ß-hydroxylase. In the adrenal medulla, noradrenaline is methylated to adrenaline by the enzyme phenylethanolamine-N-methyl transferase. Catecholamines are stored at the terminals of the adrenergic nerve endings in vesicles together with ATP in the ratio of 4:1 as well as the chromogranins. The actions of catecholamines are terminated by either uptake or metabolic transformation. Uptake is divided into neuronal uptake or uptake-1 and extraneuronal uptake or uptake-2. Metabolic transformations of catecholamines involve two enzymes, monoamine oxidase (MAO) and catechol-O-methyltransferase (Lefkowitz et al., 1990).

1.2. Classification of adrenoceptors

Dale (1906) introduced the use of the receptor concept in connection with the sympathetic nervous system when he studied the sympatholytic action of ergot alkaloids. Adrenotropic receptors were considered to be of two classes, those which predominantly mediate excitatory actions and

others which predominantly mediate inhibitory actions. He showed that many of the excitatory actions of adrenaline, but not the cardioaccelerator effects were blocked by ergot alkaloids while the inhibitory effects were not. In 1948, Ahlquist showed that adrenoceptors cannot be simply classified as excitatory and inhibitory. He classified adrenoceptors by ranking the order of potency of six sympathomimetic agonists on different functions. He found that there were only two ranks of potencies for these sympathomimetic amines and concluded that there were two subtypes of adrenoceptors. The one which is predominantly excitatory except in the intestine, was named alpha and the other which is predominantly inhibitory, except in the heart, was named beta (Ahlquist, 1948).

1.3. ß-adrenoceptors

1.3.1. Classification

Lands et al. (1967) classified ß-adrenoceptors into two subtypes, a $ß_1$-subtype found in the heart and adipose tissue and a $ß_2$-subtype found in the vasculature, bronchial and other smooth muscles. This classification was based on the relative potency of a series of sympathomimetic amines. The $ß_1$-adrenoceptor subtype has the following relative sensitivity of, ISO > A = NA while the $ß_2$-adrenoceptor subtype has that of ISO > A >> NA. Pharmacological studies using selective antagonists for both subtypes and ligand binding studies have confirmed this classification. A third subtype, $ß_3$, was cloned and is postulated to be the receptor

which mediates catecholamine actions on metabolic rate (Emorine et al., 1989).

1.3.2. Identification and characterization of ß-adrenoceptors

1.3.2.1. Binding studies

The first successful direct radioligand experiments for ß-adrenoceptors date back to 1974 with the ligands (-)[^{3}H] dihydroalprenolol (DHA) (Lefkowitz et al., 1974), (±)[^{125}I] hydroxybenzylpindolol (IHYP) (Aurbach et al., 1974) and (±)[^{3}H] propranolol (Levitzki et al., 1974). Tritiated compounds have the disadvantage of having low specific radioactivity and therefore requiring high amounts of proteins for binding (Engel et al., 1981). Sporn and Molinoff (1976) reported that IHYP has highly nonspecific binding properties and binds to both ß- and α-adrenoceptors in the rat cortical membrane. IHYP was also shown to bind to serotonin binding sites in rat cortex (Dickinson et al., 1981). This shows that new compounds which are more selective and with higher specific radioactivity were needed. Engel et al. (1981) introduced (±)[^{125}Iodo] cyanopindolol which had no affinity for α-adrenoceptors nor serotonin receptors. In addition to the radioligand ß-adrenoceptor antagonists, the agonists (±)[^{3}H] hydroxybenzylisoprenaline (Lefkowitz and Williams, 1977, Williams and Lefkowitz, 1977) and (±)[^{3}H] isoprenaline (Malchoff and Marinetti, 1976) were used. Recently, more selective radioligand binding antagonists [^{3}H]-ICI 118, 551

(Lemoine et al., 1985) and (-)[^{3}H] bisprolol (Kaumann and Lemoine, 1985), were used for the study of β_2- and β_1-adrenoceptors, respectively. Binding studies were used to examine ß-adrenoceptor subtypes in different tissues via the determination of the relative potencies of the agonists isoprenaline, adrenaline and noradrenaline in competing with nonselective radioligand antagonists such as [^{3}H] DHA and [^{125}I] HYP. Binding studies are also useful in estimating the relative distribution of β_1- and β_2-adrenoceptors in different tissues. Tissues used for binding studies included intact chicken erythrocytes and erythrocyte ghosts (Malchoff and Marinetti, 1975), frog erythrocytes (Mukherjee et al., 1975), rat lung membranes (Barnett et al., 1978), rabbit lung membranes (Rugg et al., 1978; Brodde, 1986), kitten heart (Kaumann and Lemoine, 1985), guinea pig lungs, left ventricular and rat cortical membranes (Engel et al., 1981), rat liver, cat soleus muscle and left ventricle (Minneman et al., 1979a), human heart membranes (Waelbroeck et al., 1983; Heitz et al., 1983), guinea pig trachea, dog heart and lung membranes (Manalan et al., 1981) and rat brain regions which included cortex, caudate, cerebellum, hippocampus and diencephalon (Minneman et al., 1979b).

1.3.2.2. Purification and isolation

Two techniques were used to try to purify and characterize adrenoceptors: affinity or photoaffinity labeling and affinity chromatography. It is to be noted

that these techniques purify only the ß-adrenergic binding site which is not necessarily identical with the receptor.

Various values for the molecular masses (Mr) were shown to exist in different species. Purification of the frog erythrocyte ß-adrenergic binding sites was performed in 1981 They were shown to be composed of a polypeptide of Mr = 58,000d (Shorr et al., 1981). The turkey erythrocyte ß-adrenergic binding site was shown by affinity chromatography to have two peptides with Mr of 40,000 and 45,000 in the ratio of 4:1 (Shorr et al., 1982). Affinity labeling showed that in rat reticulocytes and in frog and turkey erythrocytes, predominant peptides with Mr = 65,000 + 53,000, 58,000 and 45,000 + 39,000, were present, respectively (Lavin et al., 1982). In duck erythyrocytes two polypeptides with Mr = 45,000 + 48,500 were photolabeled in a ratio of 4 : 1 (Rashidbaigi and Ruoho, 1981) while in pigeon erythrocytes there were three photolabeled binding units of Mr = 53,000 + 46,000 + 45,000, in the ratio of 5 (53,000): 2(46,000 + 45,000) (Rashidbaigi and Ruoho, 1982). $ß_2$-adrenergic binding sites purified from lung membranes of hamster, guinea pig and rat contain a peptide of Mr = 64,000 (Benovic et al., 1984). $ß_2$-adrenergic binding sites of guinea pig lung have a photolabeled peptide of Mr = 67,000 (Burgermeister et al., 1983), while those of canine lung membranes have a peptide of Mr = 52,000-53,000 (Homcy et al., 1983). Rat hepatic $ß_2$-adrenergic binding site have a peptide of Mr = 67,000 (Graziano et al., 1985). $ß_1$-

adrenoceptors from human, canine, porcine and rat left ventricle has a binding subunit with Mr = 62,000 (Stiles et al., 1983). Rat fat cells considered to contain β_1-adrenoceptor binding subunits have a peptide of Mr = 67,000 (Cubero and Malbon, 1984). Venter (1987) concluded that both β_1- and β_2-adrenoceptors share many structural features in common including a molecular mass of 68,000 and an isoelectric point of 5. The results of target size analysis show that β_1- and β_2-adrenoceptors have a molecular mass of 140,000 and 120,000, respectively.

1.3.2.3. Cloning and sequencing

ß-adrenoceptors belong to the family of guanine nucleotide binding proteins [(G)-linked receptors]. The genes encoding human (Frielle et al., 1987) and turkey (Yarden et al., 1986) β_1-adrenoceptors and human (Kobilka et al., 1987a) and hamster (Dixon et al., 1986) β_2-adrenoceptors have been cloned and sequenced. In addition, the gene for human β_3 adrenoceptor has been recently cloned and sequenced (Emorine et al., 1989). All ß-adrenoceptor subtypes have seven hydrophobic α-helical membrane spanning domains of 20-28 amino acid residues. These are connected to an amino terminal which is located extracellularly and to an intracellular carboxyl end. Three hydrophilic extracellular and three intracellular loops connect these α-helices. The ß-adrenoceptor also has two sites of N-linked glycosylation near the amino terminus. The human β_1, β_2 and β_3 adrenoceptors consist of 477, 413 (Frielle et al., 1989) and

402 (Emorine et al., 1989) amino acids, respectively. The percentage of sequence homology between β_1- and β_2-adrenoceptors is 54% (Frielle et al., 1989), while sequence homology between β_3- and β_1- or β_2-adrenoceptors is 50.7% and 45.5%, respectively (Emorine et al., 1989). Similarities between the different ß-adrenoceptor subtypes are highest within the transmembrane domains. In comparing β_1- and β_2-adrenoceptors, the amino and carboxy termini of the receptors are less conserved. The first two cytoplasmic loops are conserved while the extracellular loops are not. The third cytoplasmic loop of β_1-adrenoceptors is 27 amino acid residues longer and contains 16 additional proline residues (Frielle et al., 1989). Chimeric receptor construction suggests that the amino acid residues within the α-helix IV-VII especially helix IV determine ß-adrenoceptor agonist subtype specificity while amino acid residues in other helices are less important. Amino acid residues within helices II-VII may be involved in determining β_1- vs β_2-adrenoceptor antagonist specificity (Frielle et al., 1989).

Strader et al., (1989) presented a model for the ligand binding domain of the receptor: (i) the ligand binding pocket lies within the transmembrane domain, (ii) the ligand is attached to the receptor by an ionic interaction between the carboxylate side chain of Asp 113 on helix III and the amino group of the ligand, a hydrogen bond between helix V serine 204 and 207 residues and the catechol hydroxyl groups

and hydrophobic interactions between the helix VI Phe 290 and the aromatic portion of the ligand. It has also been suggested that cysteine residues within the extracellular loops may stabilize the ligand binding pocket via disulfide linkages (Raymond et al., 1990). The amine and carboxyl ends of the third intracellular loop, especially the amino residues (222-229) and (258-270), were demonstrated to be important in the coupling of ß-adrenoceptor to G protein (Strader et al., 1989). The potential sites of phosphorylation by cAMP dependent kinase have been suggested to be present in the third intracellular loop and in the carboxyl terminus while the potential site of phosphorylation by ß-adrenoceptor kinase is a serine and threonine-rich region near the carboxyl terminus (Deblasi, 1989).

1.3.3. Cellular signalling mechanisms

The role of cAMP in the mediation of the effects of adrenaline was first suggested by Rall and Sutherland (1958) who showed that adrenaline increased accumulation of cAMP in fractions of liver homogenates and in particular preparations from heart and skeletal muscle. This was followed by studying different tissues where accumulation of cAMP was increased by adrenaline, including the brain, especially the cerebellum, the lungs, spleen, epididymal fat and erythrocytes (Klainer et al., 1962). It is now generally agreed that the secondary messenger of ß-adrenoceptors in all tissues is cAMP.

The ß-adrenoceptor is coupled to a guanine nucleotide binding protein (G_s) which activates adenyl cyclase (Stiles, 1989). The G_s protein is a heterotrimer formed of a GTP-binding and hydrolysing unit which is the α-subunit, plus a ß and a γ subunit. The G protein is present in three conformational states. The first is the G protein bound to GDP (inactive form). This is followed by the release of GDP converting the inactive form into a quite transient (empty) state. GTP then enters this empty state and an active form of the G protein is produced which returns back to the inactive state when GTP is hydrolysed (Bourne et al., 1991). G protein is normally present tightly bound to GDP. ß-adrenoceptor stimulation speeds the G activation cycle and GDP is replaced with GTP. GTP causes dissociation of the G protein to an α-subunit and to the ßγ subunits. The former will activate adenyl cyclase which in turn increases the level of cAMP (Gilman, 1990). In addition, the α-subunit has a GTPase activity that permits slow hydrolysis of bound GTP to GDP (Weiss et al., 1988). Although it appears that ßγ subunits do not activate adenyl cyclase, they are proposed to be important for several reasons. First, they can stabilize the alpha GDP form, thereby allowing GDP to dissociate slowly at a rate of 100 fold less than in the presence of α-subunit alone. This means that the ßγ subunits dampen signal transmission in the resting state and act as "noise" suppressors (Bourne et al., 1991). Second, their dissociation allows the receptor to act as a catalyst

(Birnbaumer, 1990). Third, their presence allows activation of G proteins, since receptors do not recognize the GDP-α complex alone (Birnbaumer, 1990). Another view is that although association of βγ subunits with α-subunits is important for G protein activation (i.e catalysis of GTP binding), α-subunits alone can also interact with the receptor but G protein activation then is inefficient (Weiss et al., 1988). G protein acts as an amplifier of the ligand effect since a few receptors are capable of activating many G protein molecules. G proteins also allow reversal of the action of the ligand as they have an internal turnoff mechanism whereby the α-subunit hydrolyzes GTP to GDP (Birnbaumer, 1990).

The cAMP protein kinase consists of two different types of subunits, a regulatory (R) subunit which is the binding site for cAMP and a catalytic (C) subunit. The enzyme usually exists as an inactive tetramer, R_2C_2. cAMP binds with high affinity to the R subunit which decreases the affinity of the R subunit for the C subunit and leads to the dissociation of an $R_2.(cAMP)_4$ dimer and two free C subunits that are catalytically active (Taylor et al., 1988).

1.3.4. Localization of ß-adrenoceptors in the Heart.

Lands et al. (1967) originally classified the ß-adrenoceptors in the heart as $ß_1$-adrenoceptors. The $ß_1$-adrenoceptors were proposed to mediate increases in heart rate, contractility and conduction velocity in the atrioventricular node, His Purkinje system and the

ventricles (Lefkowitz et al., 1990). However, results from pharmacological and binding studies suggest that ß-adrenoceptors in the heart are not homogeneous in nature.

Pharmacological studies using cat hearts in vivo and in vitro showed that it was possible that both $ß_1$- and $ß_2$-adrenoceptors are both present and mediate chronotropic responses (Carlsson et al., 1972). ß-adrenoceptor subtypes were proposed to mediate different degrees of chronotropic and inotropic responses. In anaesthetized cats, the $ß_1$-adrenoceptor agonist H 80/62 produced equal inotropic and chronotropic effects at a given dose while the $ß_2$-adrenoceptor agonist, terbutaline, produced significantly greater chronotropic than inotropic response. This was interpreted to indicate that both $ß_1$- and $ß_2$-adrenoceptors are present in the sinoatrial node, and in the myocardium of the ventricles, but with different relative distributions. The $ß_1$-adrenoceptors are the predominant type in both regions with $ß_1$: $ß_2$ concentration ratio higher in ventricle than in sinoatrial node (Carlsson et al., 1977). In the isolated rat atria, salbutamol showed a relatively greater effect on rate than on contractile force (Farmer et al., 1970). Soterenol, another $ß_2$-adrenoceptor selective agonist was shown to have similar effects, as salbutamol, on rate and contractile force of guinea pig atrial strips (Brittain et al., 1970). Using pA_2 values for the selective $ß_1$-adrenoceptor antagonist atenolol and the selective $ß_2$-adrenoceptor antagonist α-methylpropranolol, it was shown

that β_2-adrenoceptors are present in addition to β_1-adrenoceptors in cat but not guinea pig atrium (O'Donnell and Wanstall, 1979a). However, employing the β_2-selective agonist procaterol, a minor population of β_2-adrenoceptors was revealed in the guinea pig (Johansson and Persson, 1983; O'Donnell and Wanstall, 1985) but not rabbit atrium (Costin et al., 1983). Bryan et al. (1981) showed that only β_1-adrenoceptors are involved in mediation of chronotropic effects in the rat atrium. However O'Donnell and Wanstall (1985) showed variable results within the same species in which some rat atria contain a small population of β_2-adrenoceptors while others did not. They concluded that the comparative importance of β_2-adrenoceptors in chronotropic responses in atria are cat > guinea pig > rat > rabbit.

Binding studies showed that the right atria of cat and guinea pig hearts contained both β_1- and β_2-adrenergic binding sites in the ratio of 75 : 25 while the ventricles contained only β_1-type (Hedberg et al., 1980; Engel et al., 1981). In the rabbit, the ratio of β_1 to β_2 for the right and left atrium is 72 : 28 and 82 : 18, respectively, while the ventricles contain mainly β_1-adrenoceptor subtype (Brodde et al., 1982). In humans, there is a mixture of both β_1- and β_2-adrenergic binding sites in both the atrium and ventricle in the ratio of 65 : 35 (Heitz et al., 1983), while in the rat the ratio of β_1 to β_2 is 83 : 17 (Minneman et al., 1979b). In mammals, the role of β_1-adrenoceptors is the same for different regions in the heart while that for

$ß_2$-adrenoceptors is sinoatrial node > atrium > ventricle (Kaumann et al., 1989). The role of $ß_2$-adrenoceptors in mediating increased atrial force is enhanced in patients chronically treated with selective $ß_1$-adrenoceptor antagonists (Hall et al., 1988). Recently, $ß_3$-adrenoceptors have been suggested to be present in the heart; their role is also more important in the sinoatrial node, less in the atrium, and even less in the ventricle. The proposal for the existence of $ß_3$-adrenoceptors in the heart is based on the results of studies using partial agonists such as (-) pindolol and CGP-12177 (Kaumann et al., 1989). (-)Pindolol causes chronotropic effects in the guinea pig atrium at concentrations much higher than those causing ß-adrenoceptor blockade. This chronotropic effect is composed of a highly sensitive component which is apparently mediated by $ß_1$- and $ß_2$-adrenoceptors, and a lower sensitivity component which is not blocked by propranolol or selective $ß_1$- and $ß_2$-adrenoceptor antagonists, but is blocked by bupranolol (Walter et al., 1984).

1.3.5. Beta-adrenoceptor agonists

Adrenaline and noradrenaline are phenylethanolamine derivatives. Noradrenaline is considered to be an α- and a selective $ß_1$-adrenoceptor agonist (Vatner et al., 1985). Noradrenaline is 5-10 times more active for $ß_1$- than $ß_2$-adrenoceptors (Malta et al., 1985a). Isoprenaline, a compound with isopropyl substitution on the amine group, is considered to be the prototype of nonselective ß-

adrenoceptor agonists. Substitution on the amine group of phenylethanolamine is important for ß-adrenoceptor stimulatory effect. The greater the substituent size, the greater the ß-adrenoceptor agonist activity of the compound. There are $ß_2$-selective agonists with tert-butyl substitution, such as salbutamol and terbutaline, and those with an aromatic ring on the N-linked alkyl chain such as ritodrine, salmefamol and fenoterol. The latter compounds are believed to display increased ß-agonist potency and longer duration of action (Phillips, 1980). However, procaterol has an isopropyl substitution on the amine group as well as an ethyl group substituted at the α carbon and an amide nitrogen in a fused ring replacing the metaphenol substituent (Yoshizaki et al., 1976). Procaterol, a partial $ß_2$-agonist with a selectivity for $ß_2$: $ß_1$ between 100 and 1000, has been used to detect a minor population of $ß_2$-adrenoceptors in different tissues (O'Donnell and Wanstall, 1985). The $ß_2$-agonist fenoterol has a $ß_2$: $ß_1$ selectivity of 20 : 1 (O'Donnell and Wanstall, 1981b). Many $ß_2$-adrenoceptor agonists are used clinically as bronchodilators while ritodrine is used as a uterine relaxant to arrest premature labour under certain conditions (Weiner, 1985).

There is a limited availability of selective $ß_1$-adrenoceptor agonists. Dobutamine was classified as a $ß_1$-adrenoceptor selective agonist (Malta et al., 1985a) but was also shown to have agonistic activity at α-adrenoceptors and no appreciable selectivity for either $ß_1$- or $ß_2$- adrenoceptors

(Ruffolo et al., 1984). Prenalterol was suggested from whole animal and human studies to be a selective β_1-adrenoceptor agonist (Carlsson et al., 1977; Jennings et al., 1983). However, prenalterol's β_1 : β_2 adrenoceptor selectivity is dependent on tissue. It is now believed that prenalterol is a nonselective ß-adrenoceptor agonist and the observed cardiovascular selectivity is due to the greater ability of the cardiac tissue, in comparison to the vascular tissue, to respond (Malta et al., 1985a). Recently, McCaffery et al. (1990) showed that prenalterol appears to act at both β_1- and β_2-adrenoceptors in humans. Other available β_1-adrenoceptor agonists include xamoterol, RO363 and OM-isoprenaline. Xamoterol is a partial ß-adrenoceptor agonist (Nuttall and Snow, 1982; Malta et al., 1985b) which possesses a 100-fold selective affinity for β_1-adrenoceptors (Malta et al., 1985a). RO363 is also a partial agonist with β_1-adrenoceptor selectivity (McPherson et al., 1980). It is 2-3 times and 100-350 times less active than isoprenaline at β_1- and β_2-adrenoceptor sites, respectively (Iakovidis et al., 1980). Prenalterol, xamoterol and RO363 are phenoxypropanolamines where an oxymethylene group is inserted between the ring and the ethanolamine side chain. This oxymethylene group was suggested to increase β_1-adrenoceptor activity (Raper et al., 1980). ß-adrenoceptor agonists which are selective for rat adipocyte lipolytic response have been developed. These include BRL 28410, BRL 35113 and BRL 35135 (Wilson et al., 1984).

1.3.6. ß-adrenoceptor antagonists

1.3.6.1. Introduction

The development of ß-adrenoceptor antagonists dates back to the fifties when dichloroisoprenaline (DCI), the first ß-adrenoceptor antagonist was synthesized (Powell and Slater, 1958). Dichloroisoprenaline blocked the stimulatory effects of adrenoceptor agents on the heart as well as peripheral vasodilatation produced by sympathomimetic amines, but did not antagonize adrenoceptor mediated vasoconstriction (Moran and Perkins, 1958). The synthesis of DCI was followed by the synthesis of pronethalol which, in contrast to dichloroisoprenaline had only minor intrinsic sympathomimetic activity (ISA) (Black and Stephenson, 1962). Pronethalol was shown to produce side effects in man such as lightheadedness and slight incoordination followed by nausea and vomiting (Black et al., 1964). It also produced lymphosarcoma and reticulum cell sarcoma in mice (Paget, 1963). Black et al. (1964) later reported the synthesis of propranolol which demonstrated a better therapeutic ratio. Since the introduction of propranolol many other ß-adrenoceptor antagonists have been synthesized.

1.3.6.2. Classifications

Fitzgerald (1969) originally classified ß-adrenoceptor antagonists into five groups:

Group 1: ß-adrenoceptor antagonists with membrane stabilizing activity and ISA and this included two subgroups

depending on the degree of ISA; group 1A has high degree of ISA, agents in this group include dichloroisoprenaline; group 1B, represented by pronethalol, alprenolol, KO 592 and oxprenolol, has less ISA.

Group 2: ß-adrenoceptor antagonists with membrane activity but no ISA; propranolol is a prototype of this group.

Group 3: ß-adrenoceptor antagonists with ISA but no membrane activity; representative drugs include INPEA (1-(4-nitrophenyl)-2-isopropylamine-ethanol.

Group 4: ß-adrenoceptor antagonists with neither membrane activity nor ISA; sotalol is an example of this group.

Group 5: ß-adrenoceptor antagonists having a greater activity on ß-adrenoceptors in some tissues than in others, representative drugs include practolol and butoxamine.

Prichard (1978) reclassified ß-adrenoceptor antagonists into three divisions:

Division I: Nonselective ß-adrenoceptor antagonists and these included four groups: Group I, with both ISA and membrane activity, such as alprenolol and oxprenolol; Group II, with membrane actions but no ISA, such as propranolol; Group III, with ISA but no membrane actions, such as pindolol and Group IV, has neither ISA nor membrane activity, such as sotalol and timolol.

Division II: Cardioselective ß-adrenoceptor antagonists which in turn are divided into four groups and include acebutalol in Group I, practolol in Group III and atenolol in Group IV.

Division III: ß-adrenoceptor antagonists with α-adrenoceptor blocking properties, e.g., labetolol.

1.3.6.3. Chemical structure

ß-adrenoceptor antagonists belong to two main chemical series arylethanolamines and aryloxypropanolamines. Those which are aryloxypropanolamine derivatives are clinically more important (Labrid et al., 1989). The following lists the basic structural features of ß-adrenoceptor antagonists:

1. The amino group: an amine substituent, which is a branched-chain alkyl group, is required for ß-adrenoceptor antagonistic activity of aryloxypropanolamine (Labrid et al., 1989). A high degree of cardioselectivity is obtained by the attachment of 3, 4 dimethoxy-phenylethyl side chain (Hoefle et al., 1975) and 4-amide substituted phenoxyethyl to the amino group, as with tolamolol (Augstein et al., 1973).

2. The aromatic ring: may be a benzenoid as in alprenolol, oxprenolol, practolol, atenolol and tolamolol, a bicyclic aromatic ring as in propranolol and nadolol or heterocyclic ring as in pindolol and timolol. Para substitution by a rigid substituent of at least three atoms in size in the the phenoxy ring renders all compounds cardioselective. This group of compounds include practolol (Dunlop and Shanks,

1968), metoprolol (Ablad et al., 1973) and atenolol (Barret et al., 1973). The introduction of a combination of ortho and para substitution led to the development of very selective $ß_1$-adrenoceptor antagonists such as acebutalol (Phillips, 1980). ISA is partly related to aromatic ring substitution with electron-withdrawing or polar groups which can be an N atom in the case of pindolol, methylamide group in the case of practolol and propylamide in the case of acebutalol (Labrid et al., 1989).

3. The side chain: any substitution in the alpha, beta or gamma position, or in the hydroxyl groups of the oxypropanolamine side chain, causes marked reduction in ß-adrenoceptor activity. Extension of the side chain by one carbon, replacement of the ether oxygen by methylene, sulfur or nitrogen atom and methylation of the beta carbon will lead to partial or complete loss of activity (Philip, 1980). Methylation of the α-carbon generally produces selective $ß_2$-adrenoceptor antagonists such as butoxamine (Levy, 1966) and ICI 118,551 (Biliski et al., 1980).

1.3.6.4. Selective ß-adrenoceptor antagonists

1.3.6.4.1. Selective $ß_2$-adrenoceptor antagonist

The first selective $ß_2$-adrenoceptor antagonist to be synthesized was butoxamine (Burns and Lemberger, 1965). Butoxamine reduced the vasodilator effect of isoprenaline and reversed the vasodilator response of ethylnorepinephrine but had no effect on the chronotropic and inotropic effects of adrenaline and noradrenaline (Levy, 1966). Subsequent $ß_2$-

adrenoceptor antagonists developed include H 35/25 (Levi, 1967), IPS 339 (Imbs et al., 1977), α-methylpropranolol (Fitzgerald and O'Donnell, 1978) and ICI 118,551 (Bilski et al., 1980). The pA_2 values for butoxamine in the trachea were 5.2-6.4, depending on the agonist, while in the atria they were 5.2-5.3. Its $ß_2$: $ß_1$ selectivity was 17 : 1 (O'Donnell and Wanstall, 1979b). The $ß_2$: $ß_1$ selectivity for H 35/25 and α-methylpropranolol was 13.5 : 1 and 11 : 1, respectively, with pA_2 values of 5.4-6.6 and 7.4-8.5, respectively (O'Donnell and Wanstall, 1979b). The low pA_2 values for butoxamine and H 35/25 show that these compounds lack potency. Different values of $ß_2$: $ß_1$ selectivity have been reported for IPS 339. Imbs et al. (1977) reported pA_2 values for IPS 339 of 6.0-9.2 and selectivity values of 155 : 1 in in vitro and 23-26 : 1 in in vivo experiments. On the other hand, O'Donnell and Walduck (1980) reported pA_2 values of 7.3-8.0 and a selectivity value of only 3.3 : 1 for IPS 339. ICI 118,551 has a high degree of selectivity and specificity for $ß_2$-adrenoceptors with a selectivity value of 123 : 1 and a pA_2 value of 9.3 and 7.2 in the uterus and the atrium, respectively (Biliski et al., 1983). Less selectivity $ß_2$: $ß_1$ of 53.7 : 1 and lower pA_2 values of 8.7 and 7.0 were reported for this compound in the trachea and atria, respectively (O'Donnell and Wanstall, 1980). ICI 118,551 has no partial agonist activity but has membrane stabilizing actions (Biliski et al., 1983).

1.3.6.4.2. Selective $ß_1$-adrenoceptor antagonists

Practolol was the first selective β_1-adrenoceptor antagonist to be synthesized. It has 1/4-1/3 the effects of propranolol in blocking the chronotropic and inotropic effects of isoprenaline in anaesthetized dogs and 1/150 the activity of propranolol in blocking adrenaline-induced relaxation of isolated guinea pig tracheal chain (Dunlop and Shanks, 1968). Practolol is a partial agonist with a pA_2 of 6.9. Its selectivity values are 69 : 1 when measured by comparison of pA_2 values from guinea pig atria and trachea (Leclerc et al., 1984), 8.7 : 1 when comparisons of chronotropic action and the peripheral resistance in dogs were made or 9 : 1 from binding studies (Leclerc et al., 1984). Unlike practolol, atenolol does not have partial agonistic properties. It has a pA_2 value of 7.3 (Barrett et al., 1973). Atenolol has a β_1 : β_2 selectivity ratio of 5.5 : 1 by comparing the potency ratio for antagonism of atrial versus vascular actions of isoprenaline (Biliski et al., 1983) and 4 : 1 as measured by binding studies (Leclerc et al., 1984). Celiprolol is also a β_1-blocker with ISA and it has a 100-fold greater ability to antagonize β_1- than β_2-adrenergic stimulation (Jackson et al., 1987). Celiprolol, however, has β_2-adrenoceptor agonist activity (Taylor, 1988). Betaxolol has a pA_2 value of 8.3 and a selectivity ratio of 224 : 1. Metoprolol, on the other hand has a pA_2 value of 7.5 and a selectivity ratio of 32 : 1 (Boudot et al., 1979) while tolamolol has a pA_2 value of 8.3 and β_1 : β_2 ratio of 9.3 : 1 (Adam et al., 1974). Biliski et

al. (1983) explained that the possible reasons that drug selectivity ratios vary with different studies were due to differences in tissues, agonists or methodologies used for pA_2 determinations.

1.4. Alpha adrenoceptors

1.4.1. Classification

Brown and Gillespie (1956; 1957) showed that in the perfused cat spleen, the presence of an irreversible alpha-adrenoceptor antagonist increased noradrenaline overflow with nerve stimulation. These investigators attributed this action to the blockade of postjunctional receptors resulting in higher levels of noradrenaline. The concept of a presynaptic receptor regulation of noradrenaline release was introduced by four independent groups (Farnebo and Hamberger, 1971; Kirpekar and Puig, 1971; Langer et al., 1971; Starke, 1971). Starke (1972) showed that, in the rabbit heart, the relative potencies of phenylephrine, oxymetazoline and naphazoline at the presynaptic receptors did not agree with those at the postsynaptic receptors. In the cat spleen, phenoxybenzamine was more potent in blocking postsynaptic rather than presynaptic receptors (Langer, 1973). This led to the classification of α-adrenoceptors into α_1- and α_2-subtypes based on anatomical distribution of receptors. Prejunctional adrenoceptors mediating inhibition of the release of noradrenaline were termed α_2 while postjunctional adrenoceptors mediating contraction were termed α_1 (Langer, 1974). Subsequently, compounds that are

selective for subtypes of adrenoceptors became available and α-adrenoceptors were classified on the basis of their differential selectivity for various adrenoceptor agonists and antagonists (McGrath, 1982; Timmermans and Van Zweiten, 1982). Starke (1981) defined α_1- and α_2- adrenoceptors as α_1- with antagonist affinity of prazosin >> corynanthine = yohimbine > rauwolscine while α_2 with affinity of rauwolscine = yohimbine >> corynanthine = prazosin. In addition to their existence in prejunctional sites, α_2-adrenoceptors were also found to exist postsynaptically in vascular smooth muscles, platelets, pancreas, the central nervous system and other tissues (Timmermans and Van Zweiten, 1982). Further subclassifications of α-adrenoceptors have been made:

1.4.1.1. α_1-adrenoceptors

In 1982, two different classifications of α_1-adrenoceptors were proposed. One classification recognized a new subtype termed α_{1S} which was stimulated by SGD 101/75 and blocked by phenoxybenzamine. The other subtype was sensitive to noradrenaline but not SGD 101/75 (Coates et al., 1982). The second classification differentiated α_1-adrenoceptors into α_{1a} which was sensitive to phenylethanolamines and imidazolines and α_{1b} which was only sensitive to phenylethanolamines (McGrath, 1982). In 1986, α_1-adrenoceptors were again classified into α_{1A} and α_{1B} according their affinities to phentolamine and WB 4101. The site with higher affinity for phentolamine was termed α_{1A}

while that with lower affinity was termed α_{1B}. WB4101 labeled only α_{1A} subtype while prazosin had equal affinity at both subtypes. The prazosin : phentolamine potency ratio for α_{1A} and α_{1B} is 3.5 : 1 and 80 : 1 respectively (Morrow and Greese, 1986). In the same year, α_1 adrenoceptors were also classified into α_{1H} and α_{1L} according to their high or low affinity for prazosin, respectively (Flavahan and Vanhoute, 1986). α_1-adrenoceptors were divided into α_{1a} which has higher affinity for WB 4101 and allows influx of calcium through dihydropyridine sensitive channels and α_{1b} which has lower affinity for WB 4101 and stimulates inositol phospholipid hydrolysis (Han et al., 1987). Chlorethylclonidine inactivated α_{1b} but did not affect α_{1a} (Minneman et al., 1988). Muramatsu et al. (1990) showed that α_1-adrenoceptors could be classified into three subtypes α_{1H}, α_{1L} and α_{1N} by antagonist affinity; α_{1H} with affinity prazosin > HV723 = WB4101 > yohimbine, α_{1L} where prazosin = HV723 = WB4101 > yohimbine and α_{1N} where HV723 > WB4101 > prazosin > yohimbine. α_{1H} is sensitive while α_{1L} and α_{1N} are insensitive to chlorethylclonidine.

1.4.1.2. <u>α_2-adrenoceptors</u>

There is evidence that the antagonist SK&F 104078 is more selective for post- than presynaptic α_2-adrenoceptors which suggest that there may be two different subtypes of α_2-adrenoceptors (Hieble et al., 1988). Postjunctional α_2-adrenoceptors were proposed to be classified into α_{2A}- and α_{2B}-adrenoceptors according to functional (Turner et al.,

1984) and binding studies (Bylund et al., 1988). Alpha-2A has low affinity for prazosin whereas α_{2B} has high affinity for prazosin. A third, α_{2C} has been proposed to be present in the opossum kidney OK cell line (Murphy and Bylund, 1988). In the rat brain, quantitative autoradiography showed that there are two classes of receptors, α_{2s} which is rauwolscine sensitive and α_{2i} which is insensitive to rauwolscine (Boyajian et al., 1987). Results from binding studies are also consistent with this classification (Boyajian and Leslie, 1987). $Alpha_{2B}$ and α_{2s} have been localized to the caudate nucleus and this suggests that they are similar while α_{2A} is considered to be equivalent to α_{2i} (Regan et al., 1988). α_{2A} has a selective ligand oxymetazoline while α_{2B} has ARC-239 and chlorpromazine as selective ligands. α_{2A} and α_{2B} have a dissociation constant ratio (prazosin to yohimbine) of 240-570 and 5.4, respectively (Murphy and Bylund, 1988). α_{2C} has a high affinity for prazosin and a prazosin to yohimbine dissociation constant ratio of of 40 (Murphy and Bylund, 1988).

1.4.2. Identification and characterization

1.4.2.1. Binding studies

[^{3}H]-dihydroergocryptine which labels both α_1- and α_2-adrenoceptor subtypes with equal affinity was the first ligand used to study α-adrenoceptors (Miach et al., 1978). The proportions of α_1- to α_2-adrenoceptors were estimated by the analysis of biphasic displacement curves generated by selective agents (Hoffman et al., 1979). Subsequently, α_1-

adrenoceptors were identified by the antagonists, [^{3}H]-WB 4101 and [^{3}H]-prazosin (Morrow and Greese, 1986). α_2-adrenoceptors were identified with [^{3}H]-clonidine, [^{3}H] aminoclonidine, [^{3}H] yohimbine, [^{3}H] rauwolscine, and [^{3}H] idazoxan. Binding studies have been performed in a variety of tissues including rat brain (Cheung et al., 1982; Morrow and Greese, 1986), vas deferens (U'Prichard and Snyder, 1979), guinea pig lung (Barnes et al., 1979), calf brain membranes (U'Prichard and Synder, 1977), rabbit and human bladder (Levin et al., 1988), rabbit uterine smooth muscle (Williams et al., 1976), human platelets (Cheung et al., 1982), human spleen, colon and kidney (Dickinson et al., 1986), rat tail artery (Cheung and Triggle, 1988), bovine retinal blood vessels (Forster et al., 1987) and rat mesenteric artery (Agrawal and Daniel, 1985).

1.4.2.2. Purification and isolation

Different molecular masses for α_1-adrenoceptor binding sites have been reported: 45,000 (Guellaen et al., 1982), 59,000 (Graham et al., 1982) and 85,000 (Venter, 1987). The proteins of lower molecular mass may be protolytic fragments of the intact 85,000 α_1-adrenoceptor binding site. Target size analysis showed that α_1-adrenoceptor binding site has a Mr of 160,000 indicating that they are present in the membrane in the form of dimers (Venter et al., 1984). The hepatic α_1-adrenoceptor has a tertiary structure and is stabilized by disulfide and strong hydrophobic bonds (Parini et al., 1987).

Several studies have shown that α_2-adrenoceptor binding sites have a Mr of 64,000 - 65,000 including binding sites purified from platelets and porcine brain (Regan et al., 1986; Repaske et al., 1987), [^{3}H]phenoxybenzamine affinity labeled human platelets (Regan et al., 1986) and [^{3}H] parazidoclonidine photolabelled adrenal cortical α_2-adrenoceptor binding sites (Jaiswal and Sharma, 1985). Other studies showed that α_2-adrenoceptor binding sites have a Mr of 85,000 and it was concluded that rat liver α_1- and platelet α_2-adrenoceptors are isoreceptors and dimers with the same molecular mass 85,000 and target size 160,000 (Shreeve et al., 1985; Venter, 1987).

1.4.2.3. Cloning and sequencing

1.4.2.3.1. α_1-adrenoceptors

The cloning of the cDNA which encodes the hamster α_1-adrenoceptor was reported. The pharmacological properties of this receptor, namely, the source (a vas deferens derived cell line), low affinity for WB4101, inhibition by chlorethylclonidine and its coupling to inositol phospholipid metabolism, resembled those described for the α_{1B}-adrenoceptor subtype (Cotecchia et al., 1988). A second α_1-adrenoceptor was cloned from the bovine brain cDNA library. This receptor showed pharmacological properties proposed for α_{1A} but unlike the α_{1A} adrenoceptor, it was sensitive to inhibition by chlorethylclonidine and was not expressed in tissues such as rat vas deferens and hippocampus where the α_{1A}-adrenoceptors have been previously found (Schwinn et

al., 1990). Recently a third cDNA clone from rat brain has been identified with high affinities for prazosin and WB4101 (Harrison et al., 1991). The hamster α_1-adrenoceptor has an amino acid sequence of 515 residues. It has seven membrane spanning domains of 20-25 hydrophobic residues connected by three extracytoplasmic and three cytoplasmic loops with an extracellular NH_2 terminus and a COOH intracellular terminus (Gottechia et al., 1988). The bovine brain α_1-adrenoceptor has a structure similar to that of the hamster α_1-adrenoceptor. Both have 72% identity within the membrane spanning domain which is consistent with the presence of a very similar ligand binding domain. Sequence conservation extends to those regions of the third cytoplasmic loop and carboxyl-terminal cytoplasmic tail which are presumed to lie closest to the plasma membrane. This might suggest similar effector functions of these receptors (Schwinn et al., 1990).

1.4.2.3.2. α_2-adrenoceptors

Three different genes for α_2-adrenoceptors have been cloned. The first is from human platelets and has been termed α_2C_{10} (Kobilka et al., 1987b). The second is from human kidney and resides on chromosome 4 (α_2C_4) (Regan et al., 1988) and the third resides on chromosome 2 (α_2C_2) (Lomasney et al., 1990). Kobilka et al. (1987b) proposed that α_2C_{10} is similar to α_{2A}. α_2C_4, was suggested to be equivalent to α_{2B} receptor (Regan et al., 1988), however Lorenz et al. (1990) proposed that it represents both α_2B

and α_2C. In contrast, Harrison et al. (1991) concluded that α_2C_4 could correspond to the α_{2C} subtype in the opossum kidney cell OK line. The α_2C_2 was found to be α_{2B}- like (Harrison et al., 1991). As with any G-protein linked receptor, α_2-adrenoceptors are formed of seven hydrophobic membrane spanning domains with three extracellular and three intracellular loops. α_2C_{10}-, α_2C_4- and α_2C_2-adrenoceptors are formed of 450, 461 and 451 residues, respectively (Lomasney et al., 1990). The greatest similarity between α_2C_{10} and $_2C_4$ (75%) (Regan et al., 1988), and between α_2C_2 and α_2C_4 and α_2C_2(75% & 74%) are in the transmembrane regions. The amino terminus, the carboxyl terminus and the third cytoplasmic loop represent the most divergent domains (Lomasney et al., 1990). Employing chimeric receptors it was shown that the seventh membrane spanning domain of α_2-adrenoceptors may contain the major determinant of ligand specificity (Kobilka et al., 1988).

1.4.3. Molecular mechanisms

1.4.3.1. α_1-adrenoceptors

Hokin and Sherwin (1957) were the first to report that α-adrenoceptors activate phosphatidyl inositol turnover. They showed that adrenaline stimulated phosphatidyl inositol turnover in salivary gland slices and that this response was blocked by dibenamine and ergotamine. The effect of adrenaline on phosphatidyl inositol labeling in hepatocytes was 1000 times more sensitive to blockade by prazosin than by yohimbine. This demonstrated that the effect is mediated

via the activation of α_1-adrenoceptors (Tolbert et al., 1980). It is now widely accepted that α_1-adrenoceptors are coupled to phosphoinositide turnover (Garcia-Sainz, 1987). The initial step after agonist induced receptor activation involves the activation of guanine nucleotide regulatory protein (Gx). Evidence for Gx involvement came from binding studies in which G_x decreased the affinity of α_1 adrenoceptors for agonists (Lynch et al., 1985). Wallace and Fain (1985) also showed that G_x stimulates phosphoinositide breakdown in isolated liver membranes. Garcia Sainz (1987) suggested that G_x appears to be insensitive to pertusis toxin and is different from adenyl cyclase coupled G_i and G_s. Guanine nucleotide activates phospholipase C enzyme which in turn leads to the hydrolysis of phosphatidylinositol-(4,5)-biphosphate to inositol-(1,4,5)-triphosphate [Ins(1,4,5)P_3] and diacylglycerol. Ins(1,4,5)P_3 acts as a secondary messenger activating the release of calcium from endoplasmic reticulum and calciosomes thereby increasing intracellular calcium concentration. Ins(1,4,5)P_3 is metabolized to either the inactive Ins(1,4)P_2 or the potentially active Ins(1,3,4,5)P_4. The latter is proposed to facilitate calcium entry across the plasma membrane and promote calcium movement between various intracellular non-mitochondrial stores (Nahorski, 1990). Diacylglycerol activates protein kinase C which may be involved in the propagation of the hormonal signal and is part of a feedback system through phosphorylating α_1-adrenoceptors (Garcia-

Sainz, 1985). Han et al. (1987) provided evidence for the existence of two α_1-adrenoceptor subtypes with two biochemical responses; α_{1b}- involves the hydrolysis of inositol phospholipid while α_{1a}- involves the activation of dihydropyridine sensitive calcium channels. This is in accordance with results from rat aorta experiments which show that the partial agonist Sgd 101/75 produced contraction by facilitating extracellular calcium entry which is sensitive to blockade by nifedipine while agonists with higher intrinsic activity facilitate phosphatidyl-inositol turnover (Chiu et al., 1987).

1.4.3.2. α_2-adrenoceptors

Alpha-2 adrenoceptors are coupled to adenyl cyclase via G_i (Limbird, 1988). G_i is a heterotrimic protein with α, ß and γ subunits and is activated in the presence of GTP. It is possible that α_2-adrenoceptor agonists attenuate the activity of adenyl cyclase by dissociating the α and ß subunits of G_i. The dissociated ß-subunit interacts with the α-subunit of G_x thereby preventing it from stimulating adenyl cyclase (Homcy and Graham, 1985). Limbird (1988) reviewed other α_2-adrenoceptor mediated pathways that lead to secretion or contraction responses. One such pathway is the acceleration of Na^+/H^+ exchange which plays an important role in adrenaline-evoked dense granule release from human platelets. This pathway involves the increase of cellular pH which sensitizes phospholipase A_2 resulting in the activation of arachidonic acid metabolism and the generation

of various cyclooxygenase derivatives which leads to inositol triphospate and diacylglycerol formation, increase in intracellular calcium, activation of protein kinase and finally, dense granule release. Isom et al. (1987) reported the existence of this pathway in neuroblastoma X Glioma cells. North et al. (1987) showed that the activation of α_2-adrenoceptors leads to an increase in membrane potassium conductance in guinea pig submucous plexus, rat locus coeruleus and substantia gelatinosa. Potassium channel activation leads to hyperpolarization which depresses neurotransmitter and hormonal release. This activatation possesses properties of an inward rectifier current which may involve GTP binding protein (Limbird, 1988). Inhibition of selected voltage-dependent calcium channels was also proposed to be one of the pathways involved in the inhibition of neurotransmitter and hormonal release (Limbird, 1988).

1.4.4. <u>α-adrenoceptor antagonists</u>

Ergotoxin was the first α-adrenoceptor antagonist to be described (Dale, 1906). α-adrenoceptor antagonists can be divided into four groups: ß-haloethylamine alkylating agents, imidazoline analogs, piperazinyl quinazolines and indole derivatives (Hoffman and Lefkowitz, 1990). The best known haloalkylamine derivatives are dibenamine and phenoxybenzamine. Phenoxybenzamine forms a reactive ethyleniminium or aziridinium ion and is covalently conjugated with α-adrenoceptors leading to irreversible

blockade of the receptor. It has a slight selectivity for α_1-adrenoceptors and it inhibits both neuronal and extraneuronal tissue uptake of catecholamines. In addition, it has antimuscarinic, antihistaminic and antiserotoninergic actions (Hoffman and Lefkowitz, 1990).

Meier and Yonkman (1949) were the first to report the adrenolytic properties of the immidazoline derivative, phentolamine. Roberts et al. (1952) showed that phentolamine increased femoral arterial flow, decreased peripheral resistance and blocked the constrictor response of adrenaline in the innervated hindlimb of the dog. Phentolamine is a nonselective α-adrenoceptor antagonist which also blocks serotonin receptors and release histamine from mast cells (Hoffman and Lefkowitz, 1990). McPherson and Angus (1989) reported that phentolamine, at concentrations higher than those required to block α-adrenoceptors, antagonized the vascular action of the potassium channel opener, cromakalim. The reported pA_2 values for phentolamine are 7.1 and 7.9 for the rat mesenteric artery (McPherson et al., 1984) and thoracic aorta (Digges and Summer, 1983), respectively. Phentolamine was shown to release insulin from isolated mouse islets (Schulz and Hasselblatt, 1989).

The prototype for piprazinylquinazolines is prazosin; other members of this group include terazosin, doxazosin and trimazosin. All derivatives of piprazinylquinazolines are selective antagonists of α_1-adrenoceptors (DeJonge et al.,

1986). Prazosin is also an inhibitor of cyclic nucleotide phosphodiesterase (Hess, 1975) and it causes vasodilatation but little reflex tachycardia (Hoffman and Lefkowitz, 1990). Other selective α_1-adrenoceptor antagonists include corynanthine, WB 4101, YM12617 (De Marini et al., 1987). The pA_2 values for prazosin, WB 4101, YM12617 and corynanthine are 8.9 (Honda et al., 1985), 8.8 (Melchiorri et al., 1984), 10.1 (Honda et al., 1985) and 6.6 (Weitzell et al., 1979), respectively.

The first preferentially selective α_2-adrenoceptor antagonist to be reported was yohimbine (Starke et al., 1975a). Rauwolscine is one of the yohimbine diastereomers which is more selective for α_2-adrenoceptors than yohimbine (Starke, 1981). Lattimar et al. (1984) showed that some benzoquinolizines (WY25309, WY26392 and WY26703) were α_2-selective antagonists and these compounds are more potent and selective than yohimbine. Chapleo et al. (1983) reported a series of benzodioxan analogs with α_2-adrenoceptor antagonist selectivity of which idazoxan is the prototype.

1.4.5. Vascular α-adrenoceptors

Prejunctional α-adrenoceptors have been identified in many vascular tissues for example, the rabbit pulmonary artery (Starke et al., 1975b), rabbit ear artery (Drew, 1979), rabbit and cat autoperfused hindlimb (Steppeler et al., 1978; Pichler and Kobinger, 1978). The existence of postjunctional α_1- and α_2-adrenoceptors was first

demonstrated by the inability of prazosin to completely antagonize noradrenaline-induced contractions of the isolated human palmar digital arteries (Moulds and Jauernig, 1977), while it was able to antagonize noradrenaline-induced contractions of human visceral arteries (Jauernig et al., 1978). This indicated that there were prazosin-resistant and prazosin-sensitive vasoconstrictor α-adrenoceptors in human vascular smooth muscle. Later, the existence of postsynaptic α_1- and α_2-adrenoceptors was shown in rats (Timmermans et al., 1979; Drew and Whiting, 1979), rabbits (Hamilton and Reid, 1982), dogs (Langer et al., 1981) and cats (Timmermans, 1981).

1.5. Aim of the thesis

1.5.1. Role of ß-adrenoceptors in the vasculature

The ß-adrenoceptors of peripheral vascular smooth muscles were originally considered to be of the $ß_2$-adrenoceptor subtype (Lands et al., 1967). There is now growing evidence that $ß_1$-adrenoceptors may also be present in the vascular smooth muscles of different beds such as the canine renal vasculature (Taira et al., 1977), rat pulmonary artery (O'Donnell and Wanstall, 1981a), cat cerebral vessels (Edvinsson and Owman, 1974), rat jugular vein (Cohen and Wiley, 1978), rat aorta (O'Donnell and Wanstall, 1984a), rat femoral and mesenteric arteries (Fujimoto et al., 1988) and the vasculature supplying the adipose tissue (Belfrage, 1978).

The subclassification of ß-adrenoceptors in the coronary vasculature remains controversial. Most *in vitro* studies showed that canine (O'Donnell and Wanstall, 1984b; Nakane et al., 1988; Toda and Okamura, 1990), porcine (Drew and Levy, 1972; Johansson, 1973), rabbit (Delande et al., 1974), bovine (Purdy et al., 1988), rat (Nyborg and Mikkelsen, 1985), human and monkey (Toda and Okamura, 1990) and sheep (Brine et al., 1979) coronary arteries contain only $ß_1$-adrenoceptors. Binding studies showed that porcine (Schwartz and Velly, 1983), bovine (Vatner et al., 1986) and canine (Nakane et al., 1988) coronary arteries contain both $ß_1$- and $ß_2$-adrenoceptors with a ratio of $ß_1$ to $ß_2$ of 65 : 35, 1.5 - 2 : 1 and 74 - 77 : 23 - 26 respectively. In an extensive review, Feigl (1983) concluded that most *in vivo* studies showed that $ß_2$-adrenoceptors predominate in the coronary vasculature. By the use of electromagnetic flow measurements, Lucchesi and Hodgeman (1971) showed that ß-adrenoceptors in the canine circumflex coronary artery were of the $ß_1$-subtype. Recently, *in vivo* studies showed that both $ß_1$- and $ß_2$-adrenoceptors are present in the canine (Jackson et al., 1987; Trivella et al., 1990) and bovine (Vatner et al., 1986) coronary vasculatures.

The aim of the present work was to determine the functional distribution of subtypes of ß-adrenoceptors in the resistance blood vessels of pentobarbital-anaesthetized rats. The effects of atenolol and ICI 118,551, selective $ß_1$- and $ß_2$-adrenoceptor antagonists, respectively, on

cardiac and vasodilator response of isoprenaline were investigated by means of the dual radiolabeled microsphere technique.

The exact role of ß-adrenoceptors in mediating contraction or relaxation of the venous system is still controversial. Beta-adrenoceptors have been shown to be present in the human saphenous veins (Coupar, 1970), rat jugular vein (Dukles and Hurlbert, 1986), rabbit facial vein (Pegram et al., 1976), rabbit portal vein (Sutter, 1965; Hughes and Vane, 1967) and lateral saphenous vein (Guimaraes and Osswald, 1968). They mediated venodilatation to a degree which depends on the pre-existing tone and segment of vein studied. However, at high doses isoprenaline caused contractile responses which were abolished by α-adrenoceptor blockade (Sutter, 1965; Coupar, 1970). In humans, isoprenaline injection caused venoconstriction (Eckstein and Hamilton, 1959) or venodilatation (Beck et al., 1970) of the forearm vein. Leenen and Reeves (1987) showed that both $ß_1$- and $ß_2$-adrenoceptors were involved in the augmentation of venous return. In anaesthetized dogs, isoprenaline increased venous return and this effect was neither abolished by hexamethonium (Kaiser et al., 1964) nor carotid sinus denervation (Imai et al., 1978). In conscious dogs with cardiac output maintained constant, isoprenaline reduced MAP and increased central venous pressure (Bennett et al., 1984). In sedated dogs treated with hexamethonium, the selective $ß_2$-adrenoceptor agonist terbutaline did not

have any effect on MCFP, an index of body venous tone (Guyton et al., 1973; Pang and Tabrizchi, 1986), but reduced venous compliance (Lee et al., 1987). In anaesthetized open-chest dogs, a single dose of isoprenaline did not produce any change in MCFP at normal tone but it caused venodilatation after venous tone was increased with angiotensin II (Hirakawa et al., 1984). Rothe et al. (1990) demonstrated that isoprenaline has little influence on the MCFP in anaesthetized mongrel dogs.

The aim of this study was to determine the dose-response effects of isoprenaline on MCFP in conscious rats. MCFP is the equilibrium pressure which would occur throughout the circulation if all the pressures were brought to an equilibrium (Guyton, 1955). MCFP was shown experimentally to be directly proportional to venous return (Guyton, 1955) and mathematically to be inversely related to venous compliance (Grodins, 1959).

The mathematical bases of MCFP was formulated by Grodins (1959).

$$Q = (P_a - P_v)/R \quad (a)$$

$$P_a = BV_a/C_a \quad (b)$$

$$P_v = BV_v/C_v \quad (c)$$

$$BV = BV_a + BV_v \quad (d)$$

Where Q = cardiac output during steady state; P_a and P_v = arterial and venous pressures, respectively; R = systemic vascular resistance; C_a and C_v = arterial and venous compliances, respectively; BV_a and BV_v = arterial and venous

blood volumes. Equations (e) and (f) are obtained by rearranging these equations:

$$P_a = BV/(C_a + C_v) + C_vRQ/(C_a + C_v) \quad (e).$$

$$P_v = BV/(C_a + C_v) - C_aRQ/(C_a + CV) \quad (f).$$

When the circulation is stopped, i.e., ($Q = 0$), $P_a = P_v = BV/(C_a + C_v)$, at that time an equilibrium pressure can be obtained throughout the circulation. This pressure is called the MCFP.

1.5.2. Pressor responses to ß-adrenoceptor antagonists

Paradoxical pressor responses to ß-adrenoceptor antagonists have been reported in humans in certain clinical conditions such as insulin-induced hypoglycemia (McMurty, 1974; Lioyd-Mostyn and Oram, 1975), pheochromocytoma (Prichard and Ross, 1966) and patients treated with methyldopa (Nies and Shand, 1973). Different conditions of mental and physical stress also led to a pressor response to ß-adrenoceptor antagonist. Andren et al. (1981) showed that an increase in noise level during the administration of propranolol was associated with a pressor response. Waal-Manning (1974) showed that propranolol produced an increase in diastolic pressure during hand grip and mental arithmetic stress. Drayer et al. (1976) reported that propranolol produced a pressor response in 11% of patients. In another group of patients with psychosis, 50% of the patients developed hypertension after propranolol administration and most of them had increased catecholamine levels in the urine (Atsmon et al., 1972). In all these conditions there was an

increase in the activity of the sympathetic nervous system (Cleophas et al., 1988). Zahir (1971) reported that in young hypertensive patients, propranolol pretreatment markedly attenuated the hypotensive effect of phentolamine. Phentolamine-induced orthostatic hypotension was also prevented by ß-blockade (Majid et al., 1974).

In anaesthetized dogs, propranolol did not increase systemic arterial pressure but caused an increase in the femoral perfusion pressure (Nakane and Kusakari, 1966). It also produced sustained vasoconstriction in the denervated autoperfused hind limbs of dogs (Kayaalp and Kiran, 1966). The vasoconstrictor response was still present after blockade of α-adrenoceptors by phentolamine (Kayaalp and Turker, 1967).

The first report of a pressor response in rats was in 1969, where Dasgupta reported that propranolol produced an increased MAP in urethane-anaesthetized rats. This pressor response was blocked by pretreatment with reserpine, but not by pretreatment with hexamethonium nor phenoxybenzamine. These results were confirmed by Yamamoto and Sekiya (1969) who showed that either pronethalol or propranolol was capable of producing a sustained rise in MAP. This pressor response was markedly reduced by adrenalectomy or ganglionic blockade but markedly potentiated by α-adrenoceptor blockade. A pressor response to a ß-adrenoceptor antagonist was also reported by other investigators (Regoli, 1970;

Sugawara et al., 1980; Himori et al., 1984; Himori and Ishimori, 1988).

Propranolol administered either orally (Kato et al., 1976) or i.v. (Nakao et al., 1975) produced a pressor response in both conscious, spontaneously hypertensive and renal hypertensive rats. In conscious normotensive rats pretreated with phentolamine, d, l and dl forms of propranolol, as well as practolol and YB-2, produced dose-dependent pressor responses. The maximal effects were similar for different isomers of propranolol but the threshold dose for the pressor effect of the d-isomer was higher than that of l-isomer (Nakao et al., 1975). Gomes et al. (1978) also showed that in conscious rats pretreated with phentolamine, propranolol restored MAP to almost the control levels. Tabrizchi et al. (1988) showed that propranolol, atenolol and ICI 118,551 produced a dose-dependent increase in MAP in phentolamine-treated conscious rats. The pressor response to propranolol was present only in rats rendered hypotensive by phentolamine but not in those treated with methacholine nor sodium nitroprusside. These results suggest that a pressor response to propranolol requires the presence of an α-adrenoceptor blockade (Tabrizchi and Pang, 1989).

We aimed to study the conditions under which a pressor response to a ß-adrenoceptor antagonist occurs. We decided to study haemodynamic changes during infusion of phentolamine and after selective and nonselective blockade of ß-

adrenoceptors by atenolol and propranolol, respectively, in both conscious rats and urethane-anaesthetized rats. We also examined whether or not pressor responses to ß-adrenoceptor antagonists occured in phentolamine-treated rats under conditions where the renin-angiotensin system had been inhibited. This was done in order to demonstrate the importance of the latter system.

Propranolol failed to produce a pressor response in pentobarbital-anaesthetized rats in our preliminary studies, and so we also investigated the effects of various anaesthetic agents on the pressor response to ß-adrenoceptor antagonists. In order to investigate the reason for the absence of a pressor response to propranolol in pentobarbital-anaesthetized rats, we determined regional flow distribution in anaesthetized rats during phentolamine-infusion and again after the injection of propranolol in the presence of phentolamine-infusion. In another group of pentobarbital-anaesthetized rats adrenaline was also infused prior to infusion of phentolamine and ß-adrenoceptor antagonist injection in an attempt to clarify if absence of adrenaline inhibits ß-adrenoceptor antagonist pressor responses. Lastly, we determined whether ß-adrenoceptor antagonists reverse the effect of α-adrenoceptor blockade in an <u>in vitro</u> vascular smooth muscle preparation where experimental conditions could be rigidly controlled.

2. MATERIALS AND METHODS

2.1. Preparation of the rats

2.1.1. In vivo experiments

2.1.1.1. Measurements of MAP and HR

Male Sprague-Dawley rats from Charles river (300 - 400 g) were used in all experiments. The right femoral artery and both femoral veins of the anaesthetized rats were cannulated for the measurement of MAP by a pressure transducer (P23DB, Gould Statham, CA, USA), and for injection of drugs, respectively. All cannulae were filled with heparinized saline (25 I.U./ml). HR was determined electronically from the upstroke of the arterial pulse pressure using a tachograph (Grass, Model 7P4G). Different anaesthetic agents were used in various studies, namely, halothane (4% in air for induction and 1.5% in air for maintenance), urethane (1 g/kg, i.p.), pentobarbital (65 mg/kg, i.p.), amobarbital (100 mg/kg i.p.), ketamine (125 mg/kg, i.p.) and chloralose (90 mg/kg, i.p.). In conscious rat experiments, halothane was used to briefly anaesthetize the rats so as to allow cannulations of the femoral arteries and veins. The cannulae were tunneled s.c. to the back of the neck, exteriorized and secured. Rats were allowed 4 h to recover from the effects of surgery and anaesthesia before further use.

2.1.1.2. Measurements of the MCFP

MCFP measurements in conscious rats were determined by the method of Yamamoto et al. (1980). Male Sprague Dawley rats were anaesthetized with halothane (4% in air for induction and 1.5% for maintenance). Catheters were placed in the femoral artery for the measurement of the MAP and HR, in the right femoral vein for the infusion of drugs and in the inferior vena cava for the measurement of the CVP. A saline-filled-balloon-tipped catheter was inserted into the right atrium via the right external jugular vein. The proper location of the balloon was tested by inflation of the balloon to stop the circulation completely. This was shown by a simultaneous decrease in MAP to less than 25 mmHg and an increase in CVP. All cannulae were filled with heparinized saline (25 I.U./ml) and tunneled to the back of the neck, exteriorized and secured. The rats were allowed 24 h to recover from the effects of surgery and anaesthesia before further use.

2.1.1.3. Measurements of CO and BF

Rats were prepared as described for the measurement of MAP and HR. In addition, a cannula was inserted into the left ventricle via the right carotid artery for the injection of microspheres and another cannula was inserted into the left femoral artery for the withdrawal of blood.

2.1.2. In vitro experiments

Sprague-Dawley rats (250 - 300 g) were killed by a blow on the head followed by cervical dislocation. A small ring segment of the main pulmonary artery was immediately removed, mounted over two horizontal stainless steel rods and placed inside a 20 ml organ bath filled with Kreb's solution bubbled with 95% O_2 and 5% CO_2 at 37 °C and containing desipramine HCl (10^{-5} M) and corticosterone HCl (10^{-5} M) to prevent neuronal and tissue uptake of noradrenaline, respectively. One of the rods was attached to a force-displacement transducer (Grass FT03) for isometric recording on a Grass Polygraph (Model 79D). The preparation was adjusted and maintained at a passive force of 10 mN and equilibrated for 1 h before further use.

2.2. Experimental protocol

2.2.1. Selectivity of atenolol and ICI 118,551

Six groups of experiments (I-VI, n = 4 in each group) were performed in pentobarbital-anaesthetized rats to test the $ß_1$ and $ß_2$ selectivity of atenolol (100 µg/kg) and ICI 118,551 (30 µg/kg), respectively. In groups I and II, dose-chronotropic response curves for dobutamine (0.5 to 128 µg/kg) were obtained 10 min prior to and 5 min after i.v. injection of atenolol and ICI 118,551, respectively. In groups III and IV, dose-depressor response curves for salbutamol (0.05 -6.4 µg/kg) were determined 10 min prior to and 5 min after i.v. injection of atenolol and ICI 118,551, respectively. In group V, the chronotropic effect of

isoprenaline (32 ng/kg/min) was investigated 10 min before and 5 min after i.v. injection of atenolol. In group VI, the vasodepressor effect of the same dose of isoprenaline was investigated 10 min before and 5 min after i.v. injection of ICI 118,551.

2.2.2. β_1 and β_2-adrenoceptor stimulation on haemodynamics in pentobarbital-anaesthetized rats

Five groups of rats (n = 8 in each group) were used to investigate the effects of vehicle, mixed ß-stimulation, β_1-stimulation, β_2-stimulation and mixed ß-blockade in groups VII - XI, respectively on MAP, HR, CO, TPR and blood flows. In groups VII and VIII, microspheres were injected into the left ventricle 30 min after surgery. After 5 min, normal saline (0.9% $NaCl_2$, 0.013 ml/min/rat) or isoprenaline (32 ng/kg/min), was infused into groups VII and VIII, respectively. Microspheres were injected a second time 10 min after the start of vehicle or isoprenaline infusion. Groups IX, X and XI were treated similar to group VIII except that ICI 118,551 (30 µg/kg), atenolol, (100 µg/kg) or both of these blockers, respectively, were i.v. injected immediately after the first injection of microspheres and isoprenaline infusion was started 5 min after the injection of a ß-adrenoceptor antagonist. MAP and HR recordings during the first and second injections of microspheres were used to indicate responses during control conditions and drug treatments, respectively.

2.2.3. Effect of isoprenaline on MCFP in conscious rats

Rats were divided into six groups (n = 6 in each group) in a complete random design. In group XII, dose-response curves of isoprenaline on MAP, HR and MCFP were constructed. Individual doses for isoprenaline were infused (2.5×10^{-10} - 8×10^{-9} mol/kg /min) for 5 min; each dose was followed by a recovery period of 10 min. In group XIII, normal saline was infused at the same rate as isoprenaline and this group served as the time control for group XII. In group XIV, hexamethonium ($4.6 - 7.6 \times 10^{-7}$ mol/kg/min) was continuously infused and at 10 min after the start of the infusion a dose-response curve to isoprenaline was constructed. Group XV which served as a control for group XIV, was treated similarly to group XIV except that, instead of isoprenaline, normal saline was infused at the same rate as isoprenaline. In each experiment in group XIV and XV, the lowest dose of hexamethonium producing > 50% inhibition of the tachycardic response to acetylcholine (20 μg/kg) was used. In group XVI, noradrenaline was continuously infused (7.1×10^{-8} mol/kg/min) and at 20 min after the start of the infusion, individual doses of isoprenaline were infused (5×10^{-10} - 4×10^{-9} mol/kg/min). Group XVII rats, the controls for group XVI, were treated similarly to group XVI except that normal saline was infused in place of isoprenaline.

2.2.4. Pressor response to ß-adrenoceptor antagonists in phentolamine-treated rats

2.2.4.1. Haemodynamic changes in urethane-anaesthetized rats

Four groups of rats (n = 8 in each group) were used to investigate the effects of i.v. infusions of normal saline (group XVIII), phentolamine (group XIX), propranolol in rats given phentolamine (group XX) and saline in rats given phentolamine (group XXI) on MAP, HR, CO, blood flows and vascular conductances. In groups XVIII and XIX, the first injection of radioactively-labelled microspheres was conducted 30 min after surgery and this was followed immediately by the infusion of saline (0.026 ml/min/rat) or phentolamine (300 µg/kg/min), respectively. Ten min later, a second set of microspheres was injected. In group XX, phentolamine infusion was started 30 min after surgery and this was followed, 10 min later, by the injection of the first set of microspheres. After another 10 min, propranolol (100 µg/kg) was injected i.v. This was followed by the injection of a second set of microspheres 1 min after the injection of propranolol. The same protocol as in group XX was followed in group XXI except that instead of propranolol, saline (0.1 ml/rat) was injected.

2.2.4.2. Haemodynamic changes in conscious rats

Four groups of rats (XXII - XXV, n = 6 in each group) were used to investigate the effects of normal saline

(XXII), phentolamine (XXIII), propranolol in rats given phentolamine (XXIV) and atenolol in rats given phentolamine (XXV) on MAP, HR, CO, TPR, blood flows and vascular conductances. After injecting the first set of microspheres, normal saline (0.026 ml/min/rat) or phentolamine (300 μg/kg/min) was infused i.v. into groups XXII and XXIII, respectively. Fifteen min after the start of saline or phentolamine infusion, a second set of microspheres was injected. In group XXIV, phentolamine was continuously infused followed 10 min later by the injection of the first set of microspheres. After another 5 min, propranolol (100 μg/kg) was i.v. injected. This was followed by the injection of a second set of microspheres 30 s after the injection of propranolol. The same protocol as XXIV was followed in group XXV except that instead of propranolol, atenolol (100 μg/kg) was i.v. injected.

2.2.4.3. Effects of anaesthetic agents

2.2.4.3.1. Effects of urethane, pentobarbital and halothane on dose-response curves to propranolol, atenolol and ICI 118,551

Rats were divided into nine groups: groups XXVI, XXVII, and XXVIII (n = 6) were anaesthetized with urethane: groups XXIX (n = 5), XXX (n = 6) and XXXI (n = 6) were anaesthetized with pentobarbital; Groups XXXII (n = 8), XXXIII (n = 6) and XXXIV (n = 6) were anaesthetized with halothane. All rats were continuously i.v. infused with

phentolamine (300 μg/kg/min). After 10 min of infusion, a dose-response curve to i.v. bolus injections of a ß-adrenoceptor antagonist was constructed in each group of rats: propranolol (3×10^{-9} - 1.92×10^{-7} mol/kg) in groups XXVI, XXIX and XXXII; ICI 118,551 (2×10^{-9} - 1.28×10^{-7} mol/kg) in groups XXVII, XXX and XXXIII; atenolol (3×10^{-9} - 3.84×10^{-7} mol/kg) in groups XXVIII, XXXI and XXXIV. MAP recordings were noted at 1 min after the injection of each dose of a ß-adrenoceptor antagonist.

2.2.4.3.2. Effects of pentobarbital, amobarbital, ketamine and chloralose on i.v. bolus of propranolol

Rats were divided into four groups (n = 5-6 in each group): Groups XXXV, XXXVI, XXXVII and XXXVIII were anaesthetized with pentobarbital, amobarbital, ketamine and chloralose, respectively. All rats were continuously infused with phentolamine (300 μg/kg/min). Ten min after phentolamine infusion, propranolol (3×10^{-7} mol/kg, i.e. 100 μg/kg) was i.v. injected into each rat. MAP was noted 10 min after phentolamine infusion and 1 min after the injection of propranolol.

2.2.4.3.3. Effects of i.v. infusion of adrenaline on i.v.. bolus propranolol and atenolol in pentobarbital-anaesthetized rats

Two groups of pentobarbital-anaesthetized rats (n = 6 in each group) were used. Groups XXXIX and XL were given

continuous i.v. infusion of adrenaline (300 ng/kg/min) followed 10 min later by continuous i.v. infusion of phentolamine (300 µg/kg/min). After another 10 min, propranolol (3×10^{-7} mol/kg) and atenolol (3×10^{-7} mol/kg, i.e. 100 µg/kg) were i.v. injected into groups XXXIX and XL, respectively. MAP was noted 10 min after adrenaline and phentolamine infusions and 1 min after the injection of a ß-adrenoceptor antagonist.

2.2.4.3.4. Haemodynamic changes in pentobarbital-anaesthetized rats

Two groups of rats were used to investigate the haemodynamic effects of normal saline (group XLI, n = 6) and propranolol (group XLII, n = 8). After injecting the first set of microspheres, normal saline (0.026 ml/min/rat) or phentolamine (300 µg/kg/min) was i.v. infused into group XLI and XLII, respectively. Ten min after the start of saline or phentolamine infusion, a second set of microspheres was injected. In group XLI and XLII, 15 min after the start of infusion of saline or phentolamine, respectively, saline or propranolol (100 µg/kg) was i.v. injected followed 30 s later by the injection of a third set of microspheres.

2.2.4.4. Effects of captopril

Rats were divided in three groups (XLIII - XLV, n = 6 in each group). Captopril (5 mg/kg) was injected as an i.v. bolus into rats in all three groups. Ten min later,

phentolamine was continuously infused (300 μg/kg/min). Ten min after the start of phentolamine infusion, propranolol (100 μg/kg), atenolol (100 μg/kg) and ICI 118,551 (30 μg/kg) were i.v. injected in groups XLIII, XLIV and XLV, respectively.

2.2.5. In vitro cumulative dose-response curves of β-adrenoceptor antagonists

Isolated rat pulmonary arteries were divided into three groups (XLVI, XLVII and XLVIII n = 6 in each group). They were contracted with noradrenaline (10^{-6} M) and at the plateau of the contractile response, phentolamine (10^{-6} M) was added and left in the bath for 50 min. The tissues were then washed and allowed 1 h to recover. Afterwards, the tissues were again contracted with noradrenaline (10^{-6}M) and relaxed with phentolamine (10^{-6} M) for 20 min. Then, in the presence of noradrenaline and phentolamine, cumulative dose-response curves for propranolol (10^{-9} - 10^{-6} M), ICI 118,551 (10^{-9} - 10^{-5} M) and atenolol (10^{-9} to 3 X 10^{-5} M), were constructed in groups XLVI, XLVII and XLVIII, respectively. The maximum force and EC_{50} values were obtained from individual dose-response curves.

2.3. The microsphere technique

2.3.1. Method

CO, blood flow and vascular conductance were determined by the reference sample method (Malick et al., 1976; Pang,

1983). Radioactively labeled microspheres, ^{57}Co, ^{113}Sn and ^{51}Cr (15 μm diameter, Du Pont, Canada) were used. They were suspended in Ficoll 70 (10% in chlorbutanol) (Sigma Chemical Co., St. Louis, MO, USA) and Tween 80. Ten seconds before the injection of microspheres, blood was withdrawn (Harvard infusion/withdrawal pump) from the femoral artery into a heparinized syringe at a rate of 0.35 ml/min for 1 min. A 0.15 ml sample of a vigorously vortexed precounted microsphere suspension (containing 20,000 - 40,000 microspheres) labeled with either ^{57}Co, ^{113}Sn or ^{51}Cr was then injected and flushed with (0.2 ml) saline over 10 s into the left ventricle. In half of the dual-isotope studies, ^{57}Co was given first and ^{113}Sn second. In the other half of the experiments, the order of administration of isotopes was reversed. In triple isotope studies, attempts were made to alter as much as possible the sequence in which the isotopes were given. This was done to avoid a possibility of a variation in the distribution between microspheres labeled with different isotopes and to avoid variations due to different counting efficiencies for the different isotopes. At the end of the experiments, the animals were killed by an overdose of pentobarbital. Whole organs (lungs, heart, liver, stomach, intestine, caecum, colon, kidneys, spleen, testis and brain), as well as representative samples from skeletal muscle (30 - 40 g) and skin (30 - 40 g) were removed, weighed and loaded into vials for counting radioactivity. The samples of skin were

obtained from the dorsal and ventral areas and muscle samples were taken from the chest, abdomen and back. Large organs were cut into small pieces and loaded into several vials to a level less than 3 cm from the base. When blood flow to the left kidney differed more than 20% from that of the right kidney, the experiment was rejected, as it was assumed that the mixing of the microspheres was not adequate. Blood samples, tissue samples, syringes used for the injection and flushing of the microsphere suspension and for the collection of blood, and test tubes used for holding the microsphere samples were counted for radioactivity using a Searle 1185 series dual channel automatic gamma counting system (Nuclear-Chicago, Illinois, USA). In the dual isotope experiments, correction of Co counts was made by substracting Sn spillover (5 - 8%) from Co counts. In the triple isotope experiments, there was correction for the Co counts by substracting the Sn and Cr spillovers (8% each) and there was also correction for the Cr counts by substracting the Sn spillover (11%).

2.3.2. Calculations

$$\text{CO (ml/min)} = \frac{\text{Blood withdrawal rate (ml/min) x total injected cpm}}{\text{cpm in withdrawn blood}}$$

$$\text{TPR (mmHg.min/ml)} = \frac{\text{MAP (mmHg)}}{\text{CO (ml/min)}}$$

$$\text{Tissue BF (ml/min)} = \frac{\text{Blood withdrawal rate (ml/min) x tissue cpm}}{\text{cpm in withdrawn blood}}$$

$$\text{Tissue conductance (ml/mmHg.min)} = \frac{\text{blood flow (ml/min)}}{\text{MAP (mmHg)}}$$

Total amount of radioactivity (cpm) injected was obtained by subtracting the amount of radioactivity left in the tube, injecting syringe, and flushing syringe from the amount of radioactivity originally present in the tube. Radioactivity in the blood was obtained by adding the amount of radioactivity in the blood sample, in the cannula and in the syringe used for collecting blood.

2.4. Measurements of MCFP

MCFP measurements were made in conscious, unrestrained rats after temporarily stopping the circulation by means of inflating the balloon previously inserted into the right atrium. Within 5 s following inflation of the balloon with the injection of saline, MAP decreased while CVP increased to a plateau value. The difference between steady state CVP, measured within 5 s of circulatory arrest, and baseline CVP, before the inflation of the balloon is referred here as VPP. Samar and Coleman (1978) reported that there was incomplete equilibration of arterial and venous pressures. To correct for this, MCFP was calculated from the following equation (Yamamoto et al., 1980):

$$\text{MCFP (mmHg)} = \text{VPP} + 1/60(\text{FAP}-\text{VPP}).$$

FAP represents the final arterial pressure (mmHg) obtained within 5 s following circulatory arrest.

2.5. Statistical analysis

All results were analysed by analysis of variance (ANOVA). In some experiments, data were logarithmically transformed before statistical analysis to obtain normal distribution. Duncan's multiple-range test was used to compare group means. A probability of error $p < 0.05$ was preselected as the criterion for statistical significance.

2.6. Drugs

In vivo: isoprenaline HCl, atenolol, dl propranolol HCl, norepinephrine bitartrate, adrenaline (Sigma Chemical Co., St. Louis, MO, USA), phentolamine HCl (Ciba Pharmaceuticals, N.J., USA) and hexamethonium bromide (K and K Lab., CA, USA) were dissolved in normal saline. ICI 118,551 HCl (Imperial Chemical, Macclesfield, Cheshire, England) and dobutamine HCl (Eli Lilly Canada, Toronto, Ontario) were dissolved in distilled water. Salbutamol sulphate vials (5 mg/10ml) were obtained from Allen & Hanburys (Toronto, Montreal) and the drug solution was diluted with normal saline. The following anaesthetic agents were used: halothane (Ayerst Lab., Montreal, Canada), α-chloralose (BDH Chemical Ltd., Poole, England), urethane (ethyl carbamate) and ketamine HCl (Sigma Chemical Co., MO, USA), sodium pentobarbital (M.T.C. Pharmaceuticals, Ontario, Canada) and amobarbital (Eli Lilly & Co., Ontario, Canada).

In vitro: Drugs used were desipramine HCl, corticosterone (Sigma Chemical Co., St Louis, USA),

norepinephrine bitartrate, propranolol, atenolol, ICI 118,551 and phentolamine HCl. With the exception of noradrenaline which was made up in 0.01 N HCl, all other stock solutions were made up in distilled water. Dilution of drugs were made with Kreb's solution which has the following composition (mM): NaCl, 112; KCl, 4.5; $NaHCO_3$, 26.2; KH_2PO_4, 1.2; $MgCl_2$, 1.2; EDTA, 0.026; Glucose, 11.1 and $CaCl_2$, 2.5.

3. RESULTS

3.1. Selectivity of atenolol and ICI 118,551

The ED_{50} values for dobutamine chronotropic responses and salbutamol vasodepressor responses with and without the presence of a ß-adrenoceptor antagonist (I - IV) are shown in Table 1. The injection of dobutamine in group I caused a dose-dependent increase in HR, from 345 ± 10 to a maximum of 503 ± 8 beats/min. After i.v. injection of atenolol in group I, dobutamine also caused a dose-dependent increase in HR, from 348 ± 9 to a maximum of 483 ± 11 beats/min, but the second curve was shifted to the right, with a significant increase in the ED_{50} value. In group II, dobutamine also caused a dose-dependent increase in HR, from 328 ± 27 to a maximum of 455 ± 24 beats/min in the control condition. After i.v. injection of ICI 118,551 in group II, dobutamine had a similar dose response curve, with HR increased from 342 ± 18 to a maximum of 470 ± 20 beats/min and the ED_{50} value unchanged. In group III, salbutamol caused a dose-dependent decrease of MAP, from 98 ± 4 to 50 ± 6 mmHg. After treatment with atenolol, the dose response curve of salbutamol was similar to that in the control condition, with MAP decreased from 95 ± 2 to 48 ± 4 mmHg and the ED_{50} value unchanged. In group IV, salbutamol also caused a dose-dependent decrease in MAP from 98 ± 3 to 40 ± 3 mmHg in the control condition. ICI 118,551 caused a parallel shift to the right of the salbutamol vasodepressor curve with MAP decreased from 97 ± 1 to 40 ± 4 mmHg and a significant

Table 1. Effect of atenolol (100 μg/kg) and ICI 118,551 (30 μg/kg) on the ED_{50} values of the chronotropic effect of dobutamine and vasodepressor effect of salbutamol in pentobarbital-anaesthetized rats (groups I - IV, n = 4 per group).

Drugs	Dobutamine ED_{50} (μg/kg)		Salbutamol ED_{50} (μg/kg)	
	Control	Treatment	Control	Treatment
Atenolol	5.8 ± 0.8	23.2 ± 3.5[a]	0.19 ± 0.07	0.18 ± 0.07
ICI 118,551	5.9 ± 0.7	5.6 ± 0.7	0.20 ± 0.10	0.84 ± 0.32[a]

Values represent mean ± S.E.
[a]Significantly different from control values ($p < 0.05$).

increase in the ED_{50} value. In group V, infusion of isoprenaline increased HR from 320 ± 12 to 427 ± 20 beats/min, whereas in group VI isoprenaline decreased MAP from 100 ± 3 to 88 ± 4 mmHg. Atenolol completely abolished this chronotropic effect in group V, while ICI 118,551 completely abolished the vasodepressor effect of isoprenaline in group VI.

3.2. Effects of $ß_1$- and $ß_2$-adrenoceptor stimulation

3.2.1. Effects on MAP, TPR, CO and HR

The effects of vehicle, mixed ß-stimulation, $ß_1$-stimulation, $ß_2$-stimulation and mixed ß-blockade in groups VII - XI, respectively, on cardiovascular functions are shown in Figures 1 (MAP and TPR) and 2 (CO and HR). Mixed ß-, $ß_1$- and $ß_2$-stimulation, and mixed ß-blockade were attained by i.v. infusion of, isoprenaline alone, isoprenaline in rats pretreated with ICI 118,551, isoprenaline in rats pretreated with atenolol and isoprenaline in rats treated with both atenolol and ICI 118,551, respectively. The infusion of normal saline (group VII) did not significantly affect MAP, TPR, CO or HR. Isoprenaline (group VIII) caused a significant increase in HR (Fig 2) but did not significantly alter MAP, TPR or CO. Isoprenaline in rats given ICI 118,551 (group IX) did not significantly alter MAP, TPR or CO, but caused a significant increase in HR (Fig 2). Isoprenaline in rats pretreated with atenolol (group X) altered neither HR nor CO, but significantly decreased MAP and TPR.

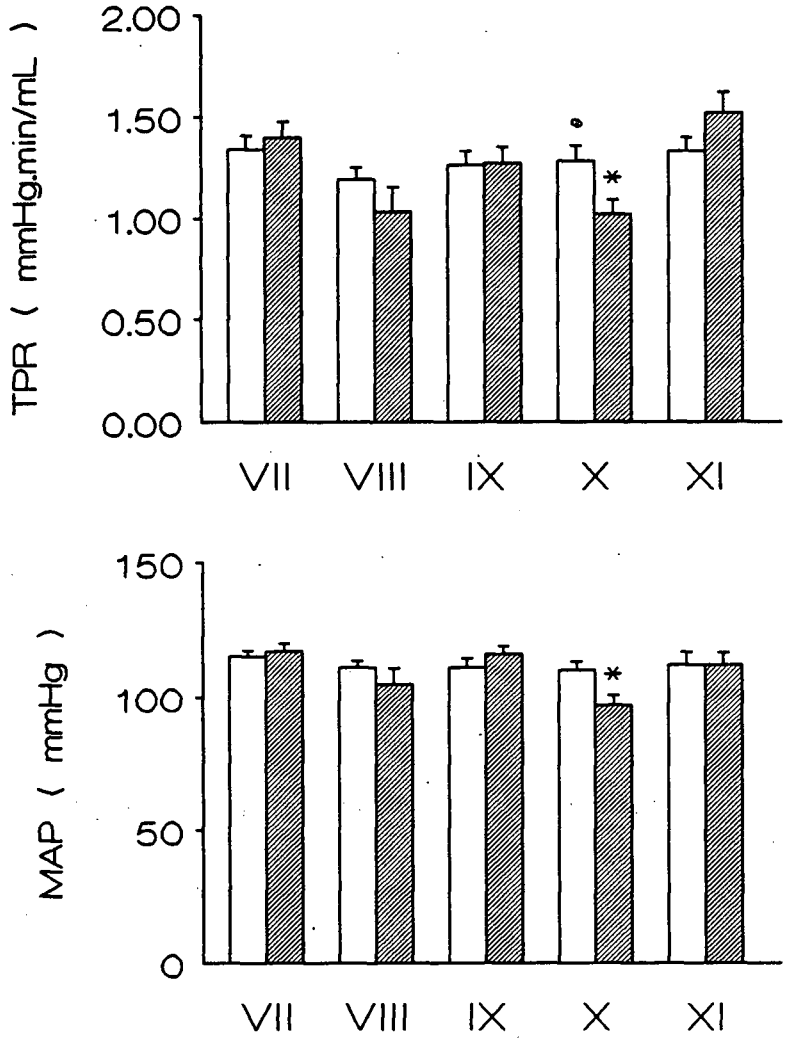


Fig. 1. Effects of normal saline (VII), isoprenaline (32 ng/kg/min, VIII), ICI 118,551 (30 μg/kg) with isoprenaline (IX), atenolol (100 μg/kg) with isoprenaline (X) and, ICI 118,551 and atenolol with isoprenaline (XI) on total peripheral resistance (TPR) and mean arterial pressure (MAP) in five groups (n = 8 each) of pentobarbital-anaesthetized rats. Open bars denote pretreatment and hatched bars denote post-treatment values. Values are mean ± S.E. *Significantly different from control values ($p < 0.05$).

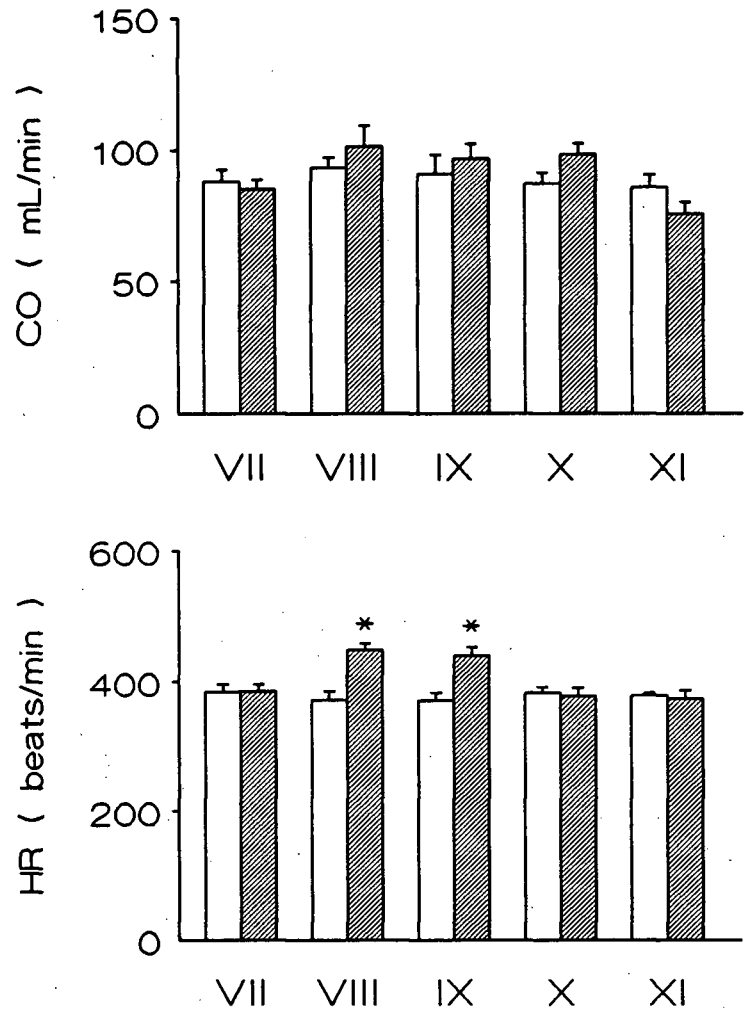


Fig. 2. Effects of normal saline (VII), isoprenaline (32 ng/kg/min, VIII), ICI 118,551 (30 μg/kg) with isoprenaline (IX), atenolol (100 μg/kg) with isoprenaline (X) and, ICI 118,551 and atenolol with isoprenaline (XI) on heart rate (HR) and cardiac output (CO) in five groups (n = 8 each) of pentobarbital-anaesthetized rats. Open bars denote pretreatment and hatched bars denote post-treatment values. Values are mean ± S.E. *Significantly different from control ($p < 0.05$).

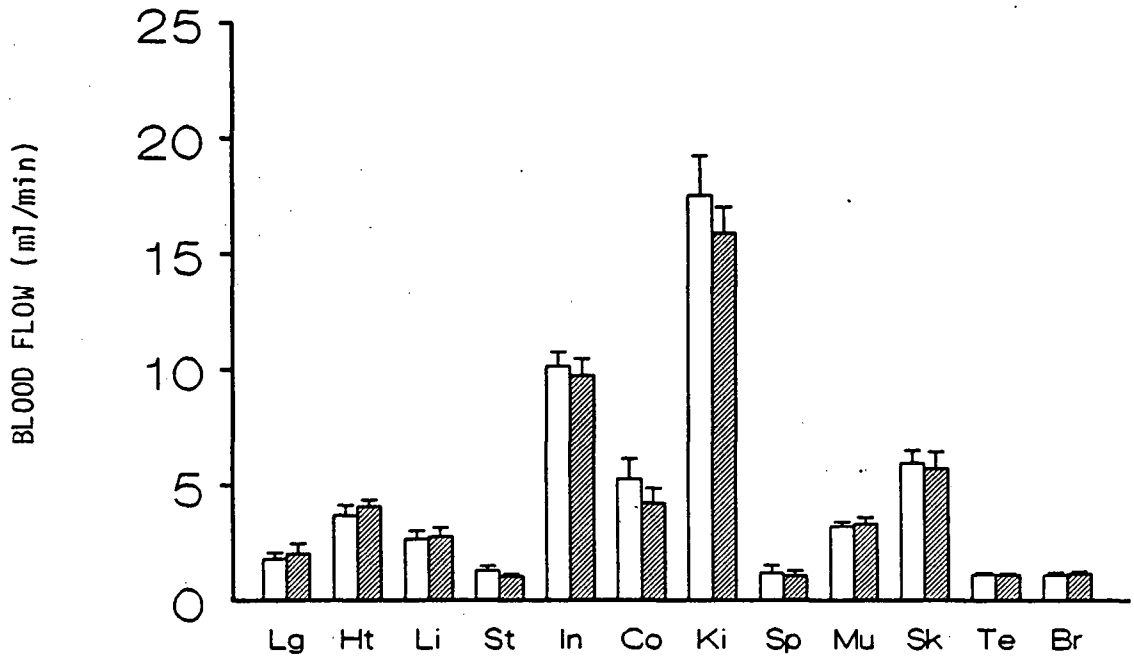
Isoprenaline in rats pretreated with both ß-adrenoceptor antagonists (group XI) did not significantly affect MAP, TPR, CO or HR, although there was a tendency for TPR to increase.

3.2.2. Effects on blood flow and vascular conductances

The infusion of normal saline (group VII) altered neither tissue blood flow nor vascular conductance in any organs or tissues (Fig. 3). The infusion of isoprenaline (group VIII) caused a slight but not significant increase in coronary blood flow but a large and a significant increase in muscle blood flow (Fig. 4a). When flow was normalized for variations in arterial pressure to give conductance values, isoprenaline was found to cause a significant increase (by 50%) in coronary arterial conductance and a large increase (three times control value) in skeletal muscle vascular conductance (Fig. 4b). Flows and vascular conductances in other organs and tissues were not significantly affected. The infusion of isoprenaline in rats treated with ICI 118,551 to reveal $ß_1$-stimulation (group IX) caused significant increases in coronary and muscle blood flow and conductances (Fig. 5a and 5b). Flows and vascular conductances in other organs and tissues were not significantly affected by this treatment. The increase in muscle vascular conductance (by 40%) in group IX was significantly less than that in group VIII. $ß_2$-stimulation by isoprenaline in rats treated

Fig. 3. Effects of normal saline on the distribution of blood flow (a) and vascular conductance (b) in pentobarbital-anaesthetized rats (group VII, n = 8). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk), testis (Te) and brain (Br). Control (open bars); Saline (hatched bars).

(a)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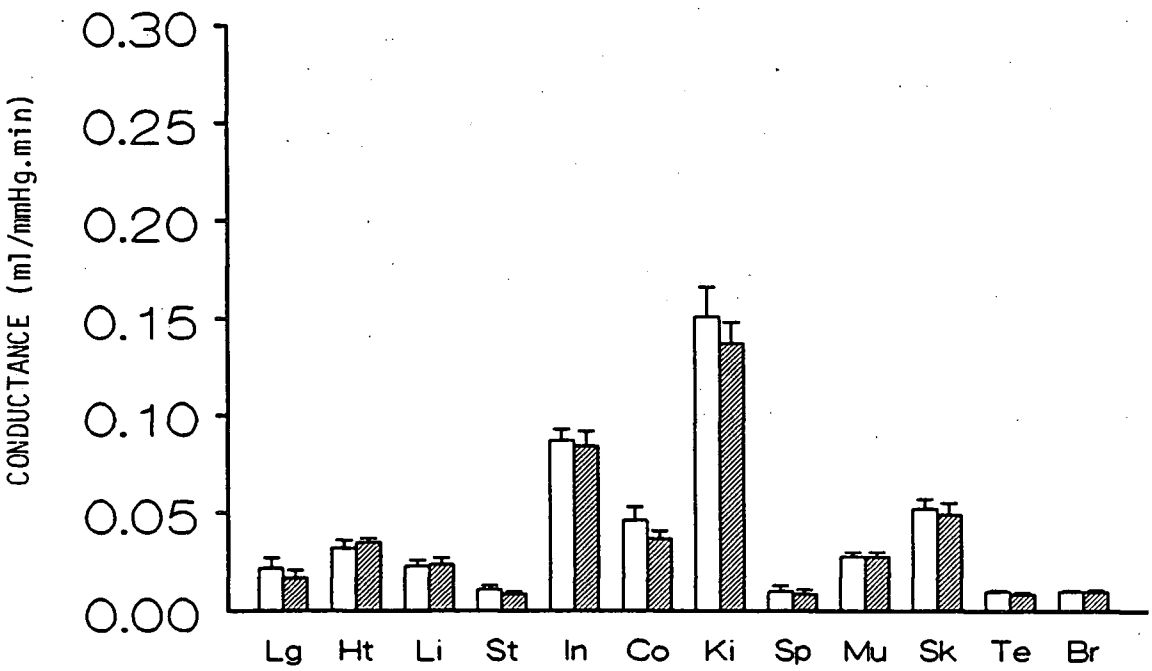
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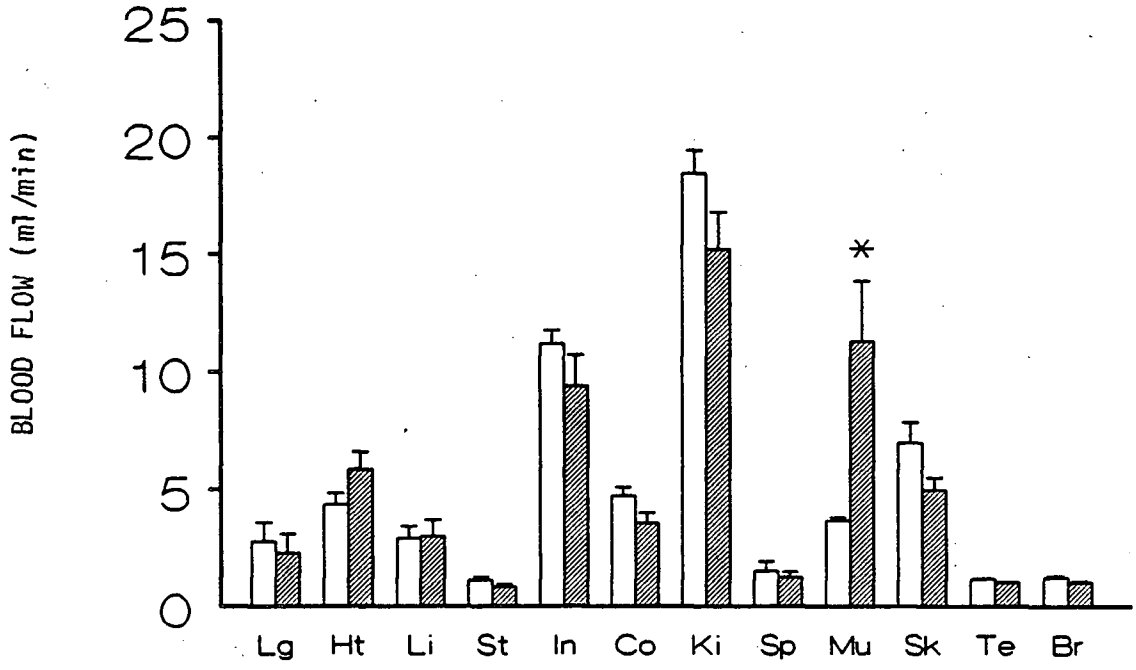
Fig. 4. Effects of isoprenaline (32 ng/kg/min) on the distribution of blood flow (a) and vascular conductance (b) in pentobarbital-anaesthetized rats (group VIII, n = 8). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk), testis (Te) and brain (Br). Control (open bars); isoprenaline (hatched bars). *Significantly different from control ($p < 0.05$).

(a)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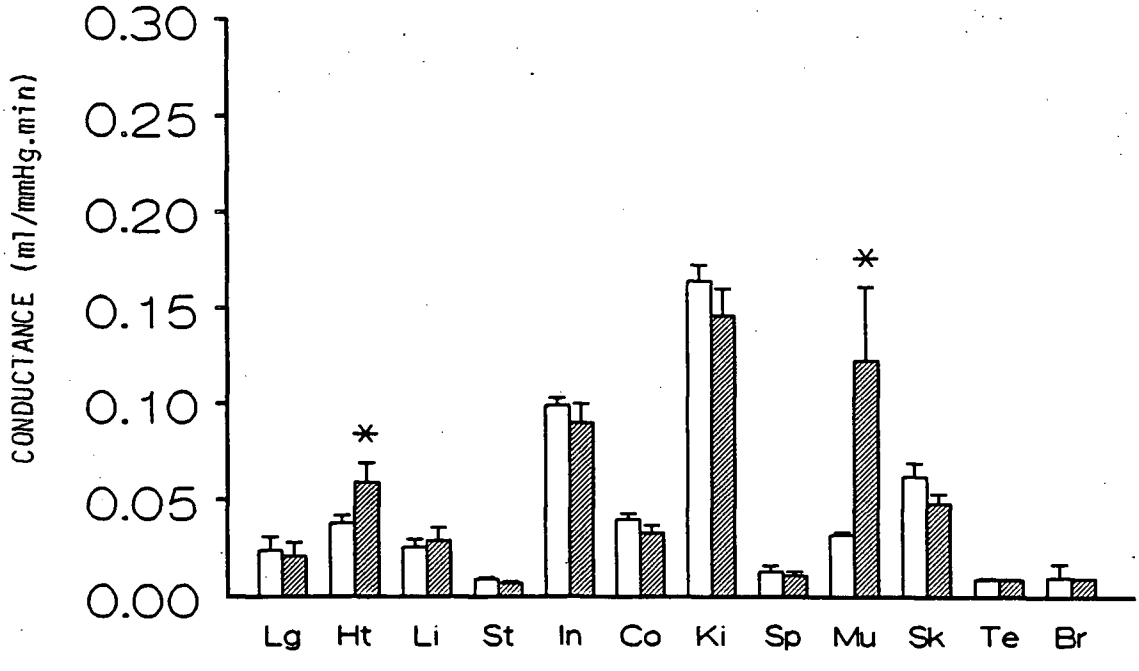
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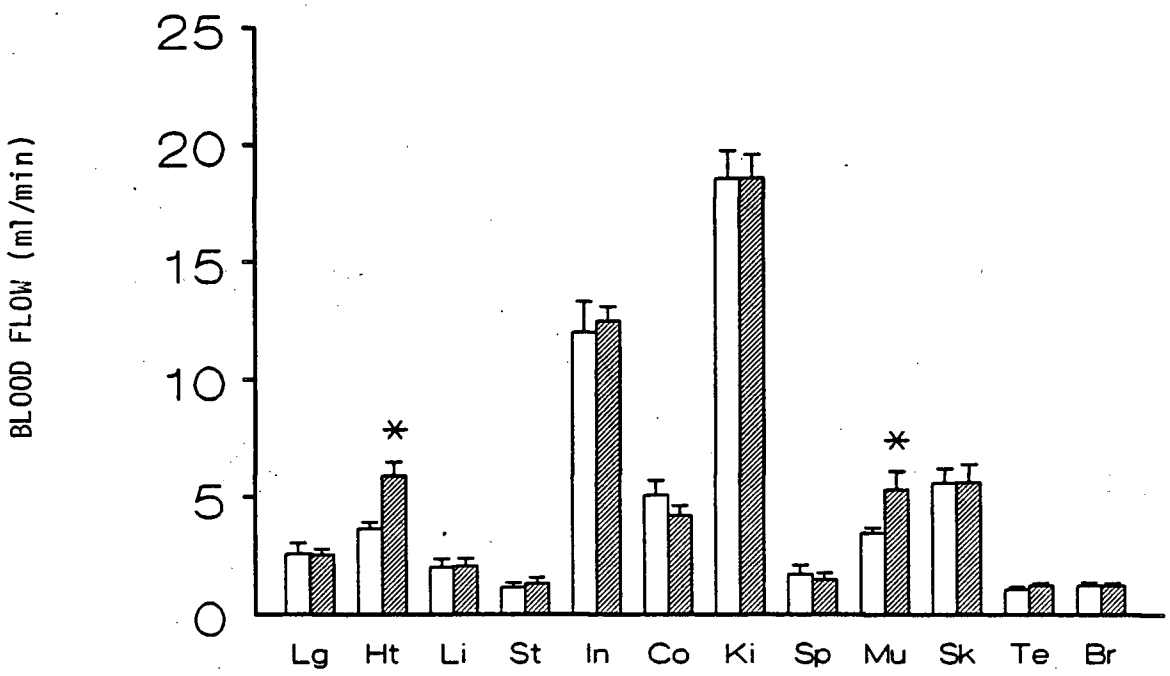
Fig. 5. Effects of isoprenaline (32 ng/kg/min) on the distribution of blood flow (a) and vascular conductance (b) in pentobarbital-anaesthetized rats (group IX, n = 8) pretreated with ICI 118,551 (30 μg/kg). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk), testis (Te) and brain (Br). Control (open bars); isoprenaline in the presence of ICI 118,551 (hatched bars). *Significantly different from control ($p < 0.05$).

(a)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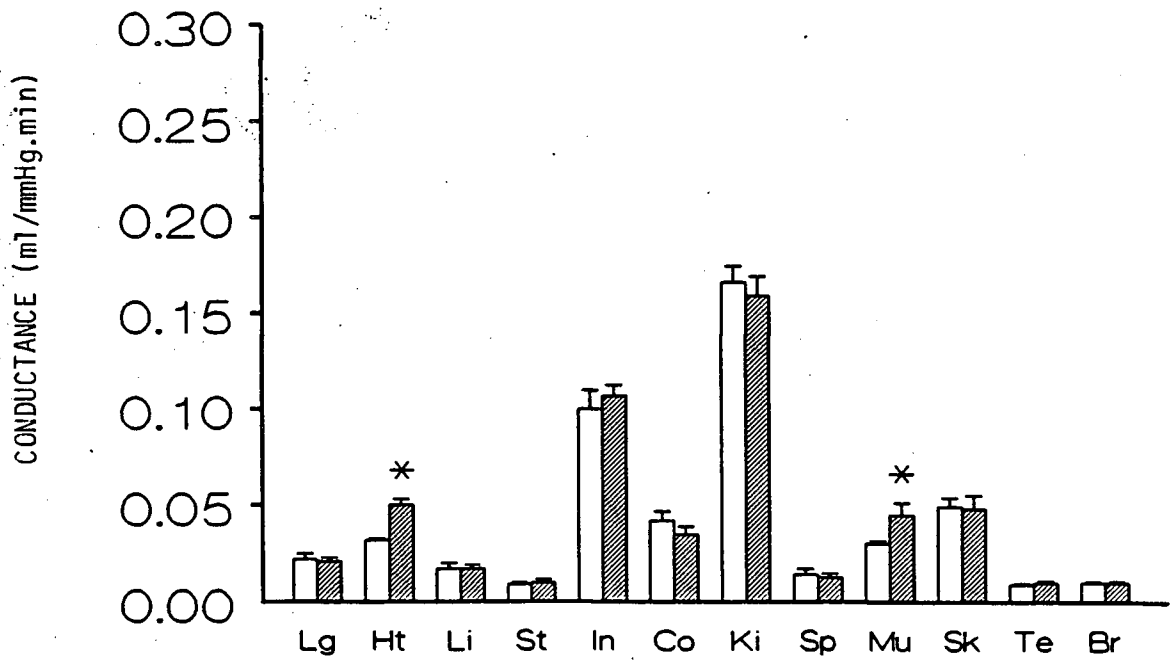
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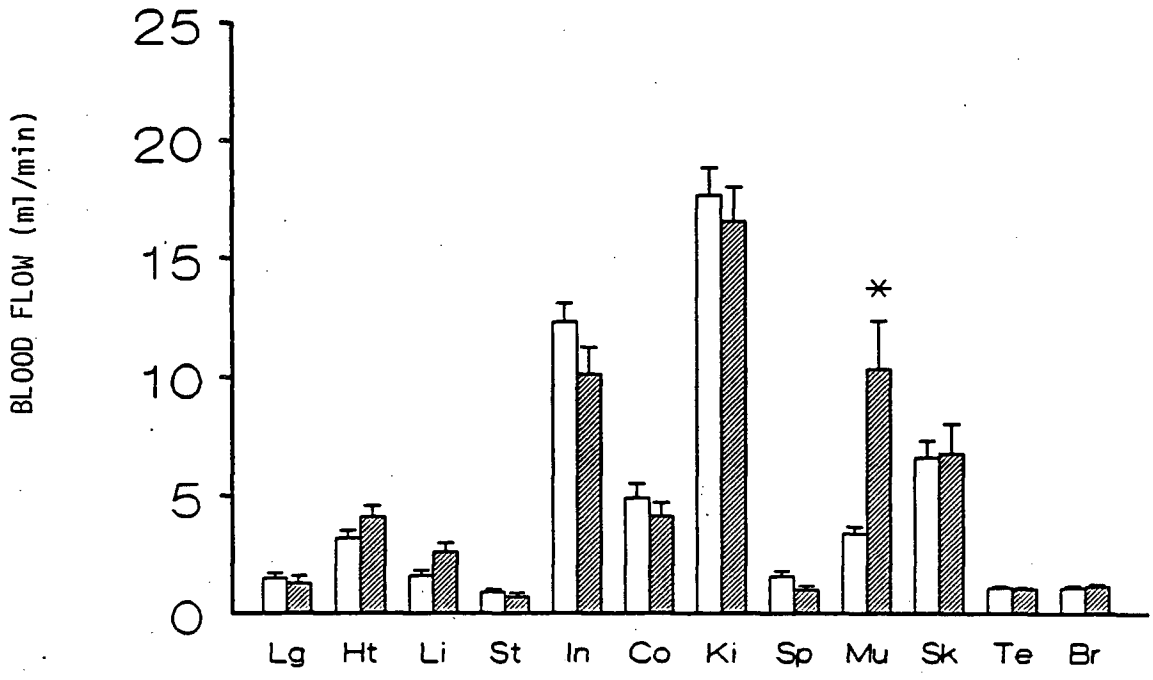
Fig. 6. Effects of isoprenaline (32 ng/kg/min) on the distribution of blood flow (a) and vascular conductance (b) in pentobarbital-anaesthetized rats (group X, n = 8) pretreated with atenolol (100 μg/kg). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk), testis (Te) and brain (Br). Control (open bars); isoprenaline in the presence of atenolol (hatched bars). *Significantly different from control ($p < 0.05$).

(a)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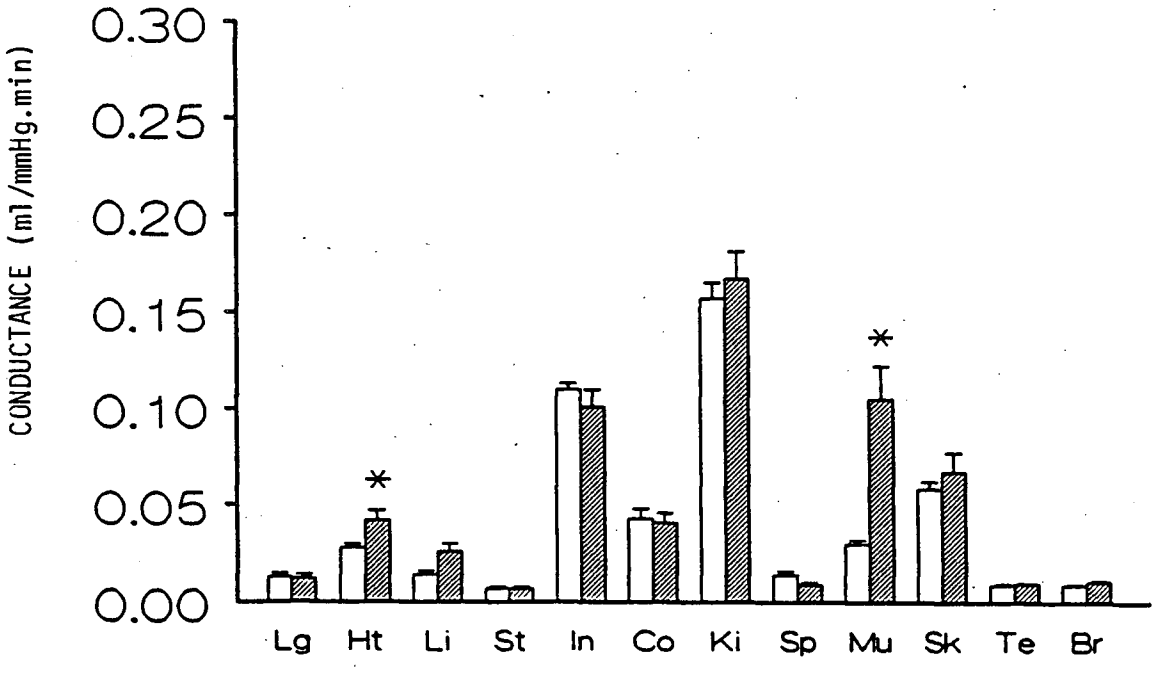
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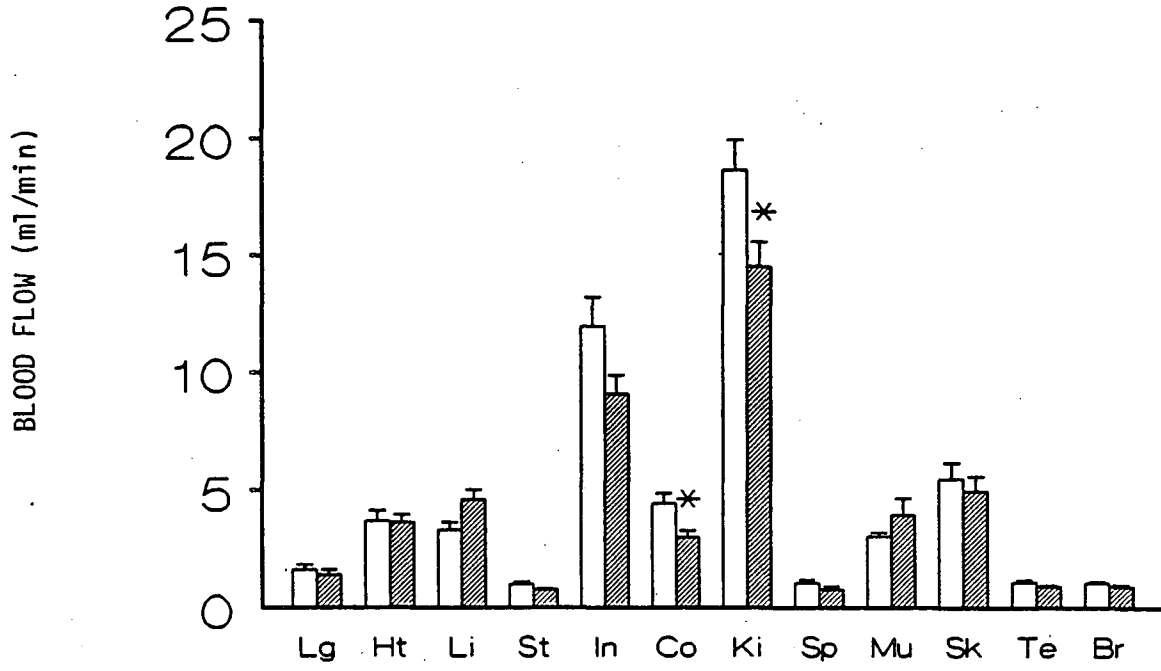
Fig. 7. Effects of isoprenaline (32 ng/kg/min) on the distribution of blood flow (a) and vascular conductance (b) in pentobarbital-anaesthetized rats (group XI, n = 8) pretreated with ICI 118,551 (30 μg/kg) and atenolol (100 μg/kg). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk), testis (Te) and brain (Br). Control (open bars); isoprenaline in the presence of ICI 118,551 and atenolol (hatched bars). *Significantly different from control ($p < 0.05$).

(a)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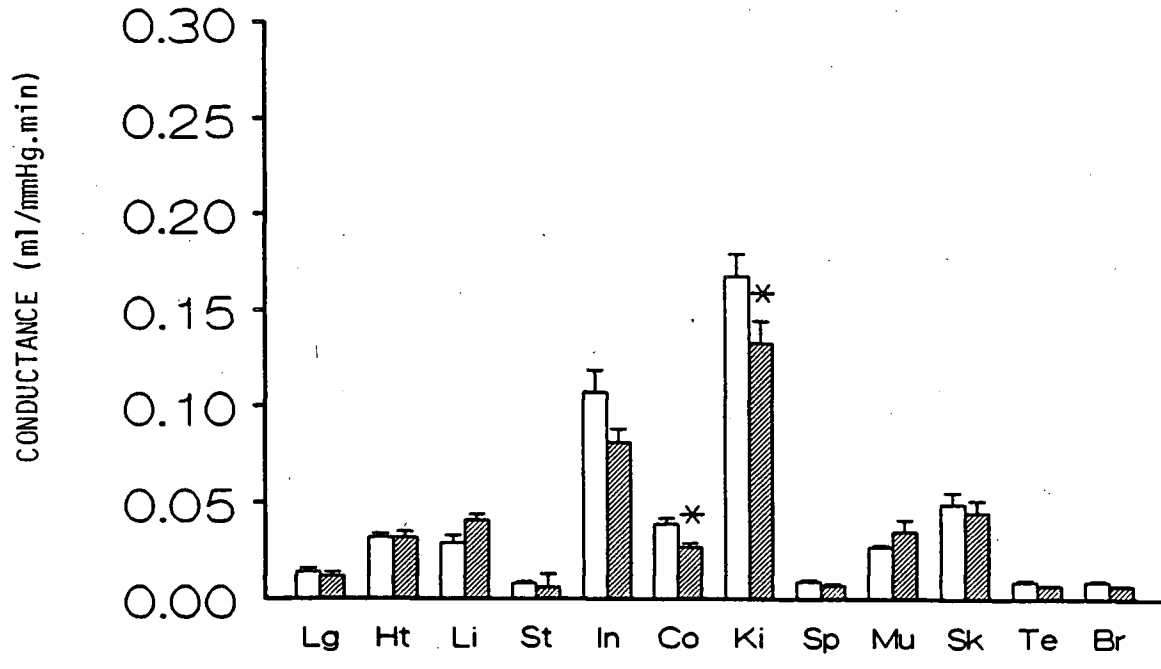
*

(b)


CONDUCTANCE (ml/mmHg.min)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Te
Br
*
*

with atenolol (group X) caused a large increase in muscle blood flow (Fig. 6a) similar to that in group VIII which received isoprenaline only (Fig. 4a); vascular conductances in the heart and muscle were also similarly, significantly increased (Fig. 6b). Flows and conductances in other tissues and organs were also not significantly altered by isoprenaline in rats pretreated with atenolol. The infusion of isoprenaline in rats treated with both adrenoceptor blockers (group XI) caused significant decreases in blood flows and conductances in kidneys, colon and caecum. Flows and vascular conductances in other beds were not significantly affected by this treatment (Fig. 7a and 7b).

3.3. Effects of isoprenaline on MCFP in conscious rats

Table 2 includes the control values of MAP, HR and MCFP for the six groups (XII - XVII). There were no significant differences in control haemodynamic values among the groups. Mean baseline CVP in all groups was 2.9 ± 0.6 mmHg (n = 36) prior to the inflation of the atrial balloon or any drug (or vehicle) treatment. None of the treatments significantly altered values of baseline CVP.

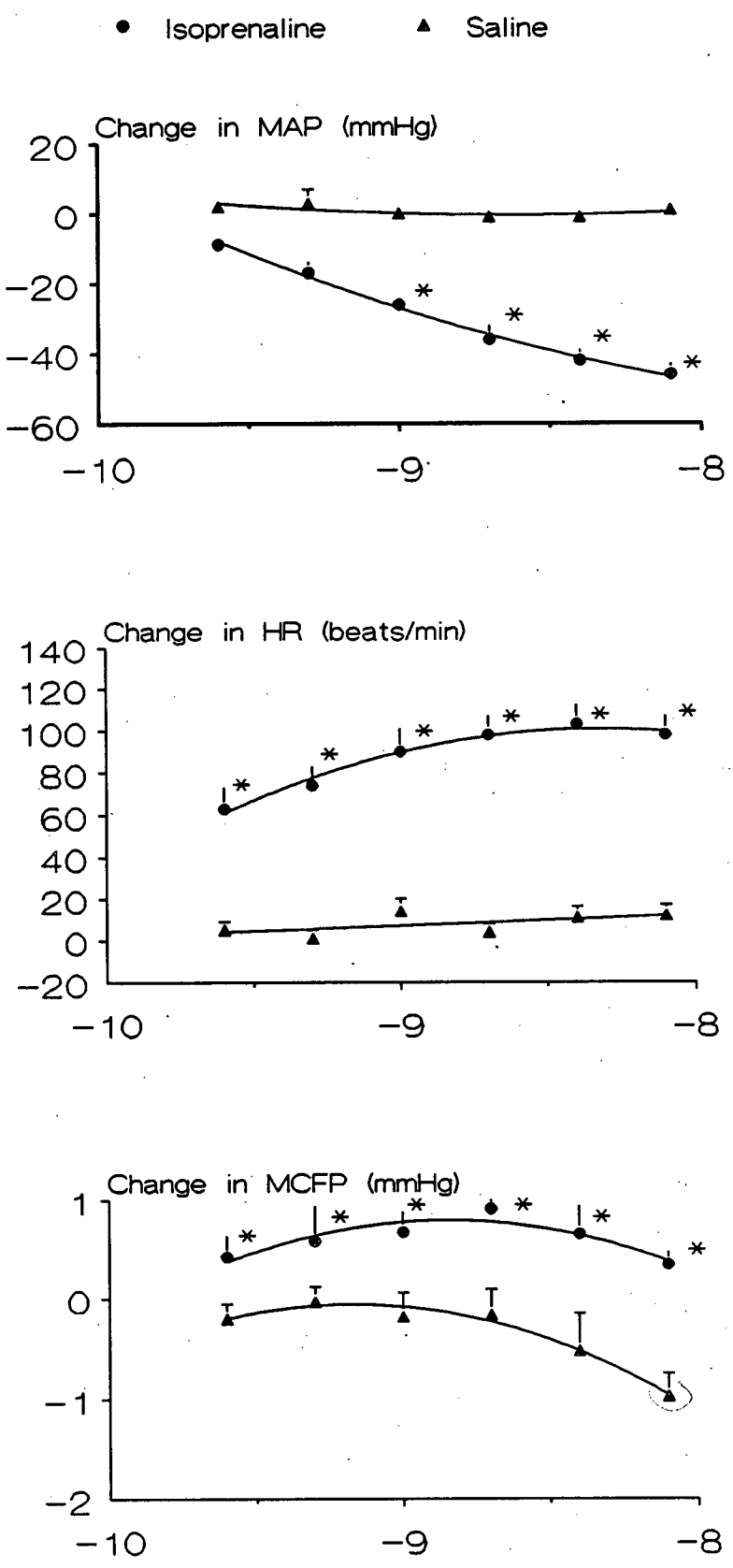
The infusion of saline in group XIII caused no changes in MAP and HR but it produced a small and gradual decrease in MCFP with time which reached statistical significance at the last dose (Fig. 8). In group XII, isoprenaline dose-dependently increased HR and decreased MAP, accompanied by a small increase in MCFP which, when compared with the predrug

Table 2. Control values (means ± S.E.) of mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious rats (groups XII - XVII, n = 6 per group).

	MAP (mmHg)	HR (beats/min)	MCFP (mmHg)
Group XII	110 ± 4	416 ± 9	5.5 ± 0.3
Group XIII	108 ± 3	377 ± 14	5.4 ± 0.1
Group XIV			
No hexamethonium	112 ± 3	395 ± 11	5.4 ± 0.1
Hexamethonium	98 ± 3[a]	386 ± 14	4.7 ± 0.1[a]
Group XV			
No hexamethonium	111 ± 4	368 ± 14	5.7 ± 0.2
Hexamethonium	100 ± 6[a]	365 ± 16	5.0 ± 0.1[a]
Group XVI			
No noradrenaline	112 ± 5	386 ± 11	5.2 ± 0.1
Noradrenaline	156 ± 2[a]	369 ± 14	7.9 ± 0.2[a]
Group XVII			
No noradrenaline	108 ± 4	375 ± 13	5.4 ± 0.2
Noradrenaline	143 ± 7[a]	352 ± 12	7.1 ± 0.3[a]

[a]Significantly different from values before the administration of a drug ($p < 0.05$).

Fig. 8. Dose-response curves for the effects (represented as change from control values) of isoprenaline (group XII) or saline (group XIII) on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious, intact rats. Each point represents the mean ± S.E. (n = 6 each). *Significantly different from the normal saline group ($p < 0.05$).


● Isoprenaline ▲ Saline
Change in MAP (mmHg)
20
0
−20
−40
−60
−10
−9
−8
Change in HR (beats/min)
140
120
100
80
60
40
20
0
−20
−10
−9
−8
Change in MCFP (mmHg)
1
0
−1
−2
−10
−9
−8
Log (mol/kg/min)

control value prior to the infusion of isoprenaline, reached statistical significance at the fourth infused dose and when, compared with the corresponding MCFP value in the time-control group (XIII), reached statistical significance at all doses (Fig. 8). In both groups XIV and XV, hexamethonium caused similar decreases in MAP and MCFP but it had no significant effect on HR (Table 2). Saline infusion in group XV affected neither MAP nor HR but caused a small and gradual but insignificant decline in MCFP (Fig. 9). Isoprenaline in group XIV increased HR and decreased MAP but it had no significant effect on MCFP when compared with either the predrug control value within the same group or the corresponding readings in the saline group (XV) (Fig. 9). In groups XVI and XVII, noradrenaline caused similar increases in MAP and MCFP and small but insignificant reductions in HR (Table 2). The infusion of saline in group XVII did not significantly affect MAP. There was a tendency for HR to gradually increase and MCFP to decrease with the passage of time but these changes are not statistically significant (Fig. 10). Isoprenaline decreased MAP and MCFP and increased HR when compared with the corresponding predrug control values within the same group or with the corresponding MAP, MCFP and HR readings in the time-control group. Isoprenaline, however, did not decrease MCFP back to the control level prior to the infusion of noradrenaline (Fig. 10).

3.4. Pressor response to ß-adrenoceptor antagonists

Fig. 9. Dose-response curves for the effects (represented as change from control values) of isoprenaline (group XIV) or saline (group XV) on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious, hexamethonium-treated rats. Each point represents the mean ± S.E. (n = 6 each). *Significantly different from the normal saline group ($p < 0.05$)

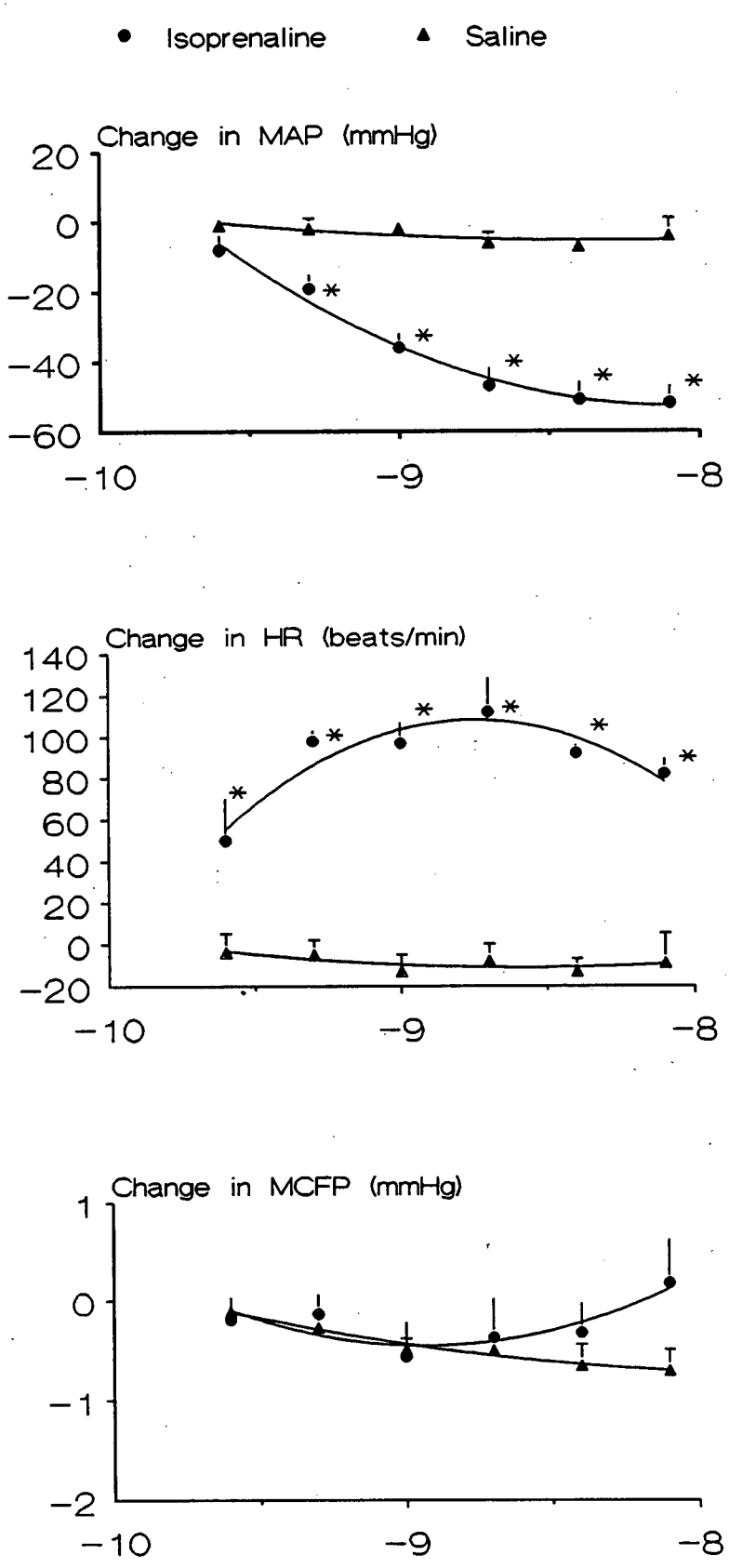
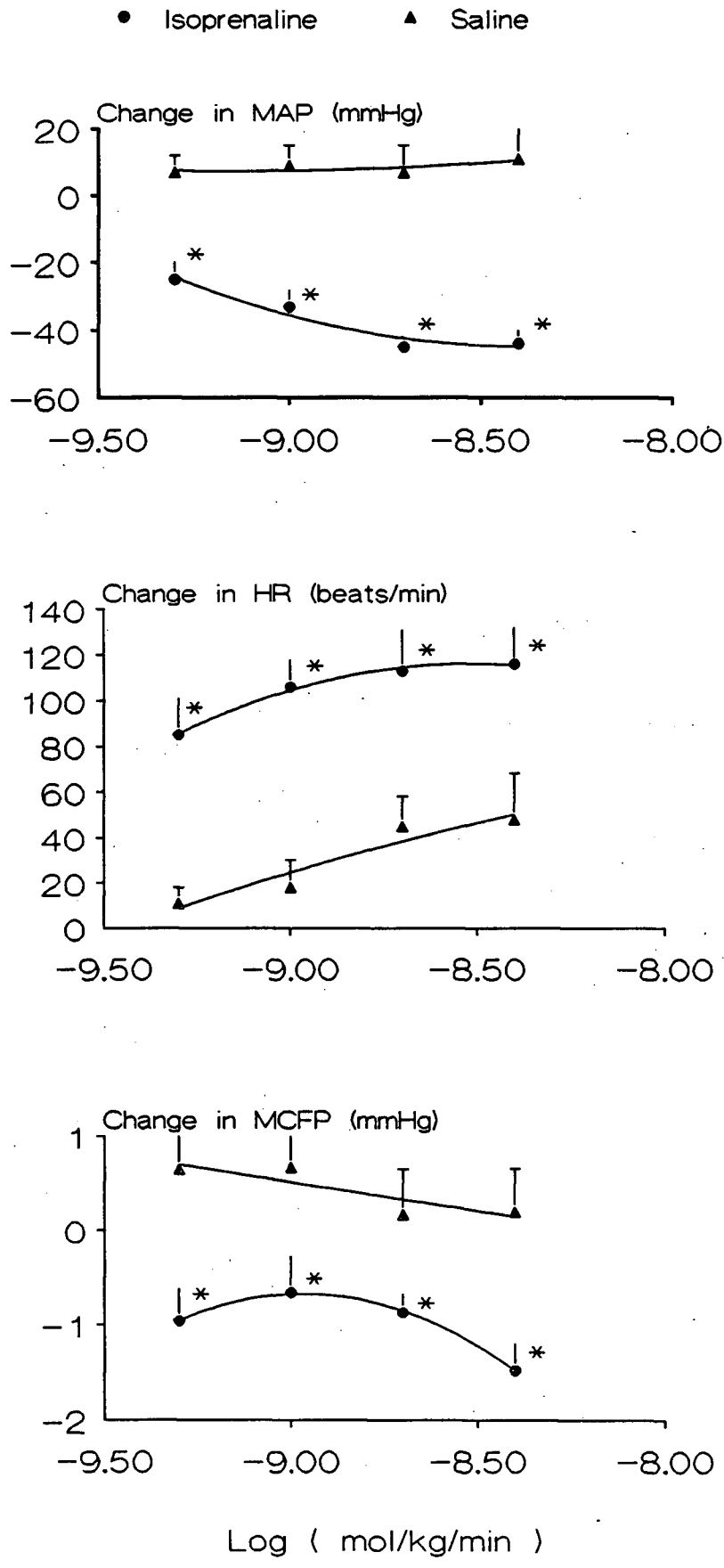

● Isoprenaline
▲ Saline
Change in MAP (mmHg)
20
0
−20
−40
−60
−10
−9
−8
Change in HR (beats/min)
140
120
100
80
60
40
20
0
−20
−10
−9
−8
Change in MCFP (mmHg)
1
0
−1
−2
−10
−9
−8
Log (mol/kg/min)

Fig. 10. Dose-response curves for the effects (represented as change from control values) of isoprenaline (group XVI) or saline (group XVII) on mean arterial pressure (MAP), heart rate (HR) and mean circulatory filling pressure (MCFP) in conscious, noradrenaline-treated rats. Each point represents the mean ± S.E. (n = 6 each). *Significantly different from the normal saline group ($p < 0.05$).


● Isoprenaline ▲ Saline
Change in MAP (mmHg)
20
0
-20
-40
-60
-9.50
-9.00
-8.50
-8.00
Change in HR (beats/min)
140
120
100
80
60
40
20
0
-9.50
-9.00
-8.50
-8.00
Change in MCFP (mmHg)
1
0
-1
-2
-9.50
-9.00
-8.50
-8.00
Log (mol/kg/min)

3.4.1. Haemodynamic changes in urethane-anaesthetized rats

3.4.1.1. Effects on MAP, TPR, CO and HR

The effects of saline (group XVIII), phentolamine (XIX), propranolol in phentolamine-treated rats (group XX) and saline (group XXI) in phentolamine-treated rats on cardiovascular functions are shown in figures 11 (MAP and TPR) and 12 (CO and HR). Groups XVIII and XXI are time controls for groups XIX and XX, respectively. MAP and HR readings were taken at the time of injection of the microspheres. The infusion of normal saline in group XVIII did not produce any significant effects on MAP and HR. There was a tendency for CO to increase and TPR to decrease in the 10 min period between the injections of the two sets of microspheres, but these changes were not statistically significant. The infusion of phentolamine in group XIX significantly decreased MAP by reducing TPR but did not alter HR. CO was slightly but not significantly decreased by phentolamine. The infusion of phentolamine in groups XX and XXI caused similar decreases in MAP as in group XIX, from 84 ± 2 to 50 ± 2 and from 94 ± 4 to 57 ± 2 mmHg, respectively. The injection of propranolol in rats given phentolamine in group XX significantly increased MAP to a level (90 mmHg) which is slightly, but not significantly, higher than control MAP (84 mmHg) prior to the infusion of phentolamine, and it raised TPR but altered neither HR nor CO. The pressor response to propranolol reached a peak within 1 min of injection of the drug and was sustained at approximately 90% of the peak response

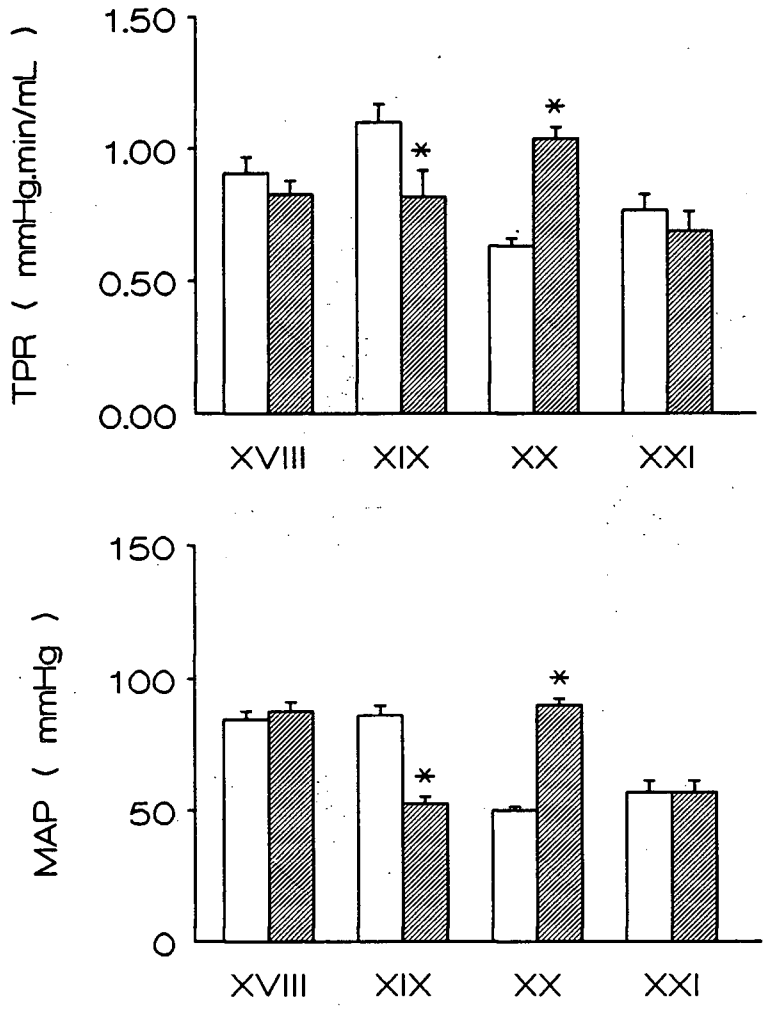


Fig. 11. Effects of normal saline (XVIII), phentolamine (300 μg/kg/min, XIX), propranolol (100 μg/kg) in the presence of phentolamine (XX), and saline in the presence of phentolamine (XXI), on total peripheral resistance (TPR) and mean arterial pressure (MAP) in four groups (n = 8 each) of urethane-anaesthetized rats. Open bars denote pretreatment and hatched bars denote post-treatment values. Values are mean ± S.E. *Significantly different from pretreatment ($p < 0.05$).

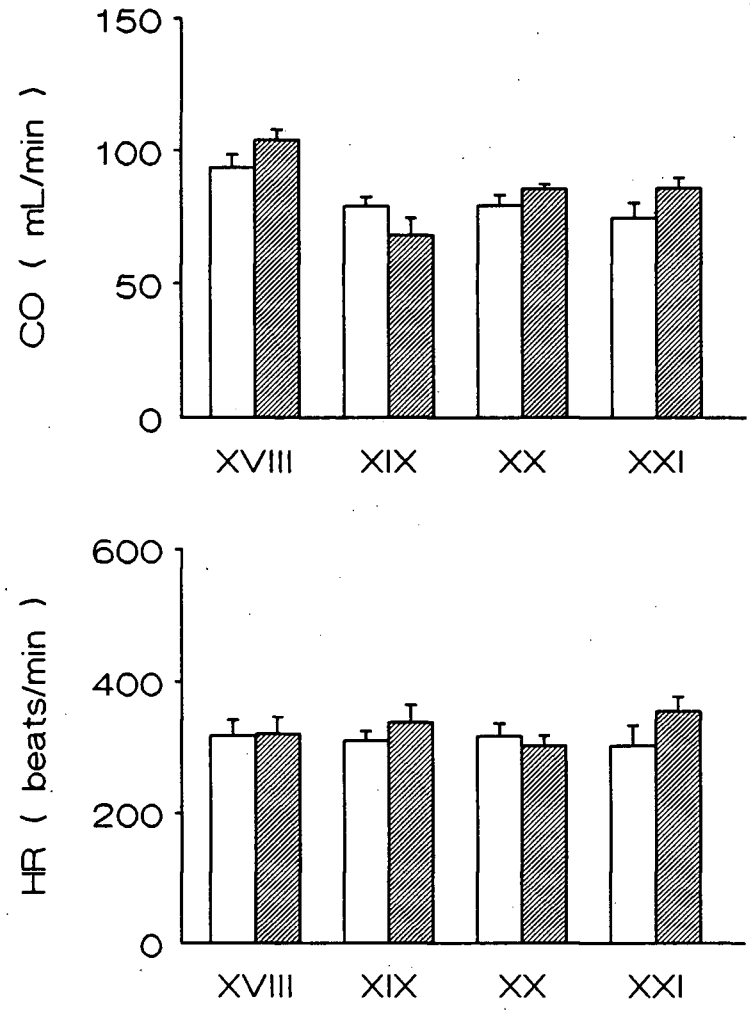


Fig. 12. Effects of normal saline (XVIII), phentolamine (300 μg/kg/min, XIX), propranolol (100 μg/kg) in the presence of phentolamine (XX), and saline in the presence of phentolamine (XXI), on heart rate (HR) and cardiac output (CO) in four groups (n = 8 each) of urethane-anaesthetized rats. Open bars denote pretreatment and hatched bars denote post-treatment values. Values are mean ± S.E. *Significantly different from pretreatment ($p < 0.05$).

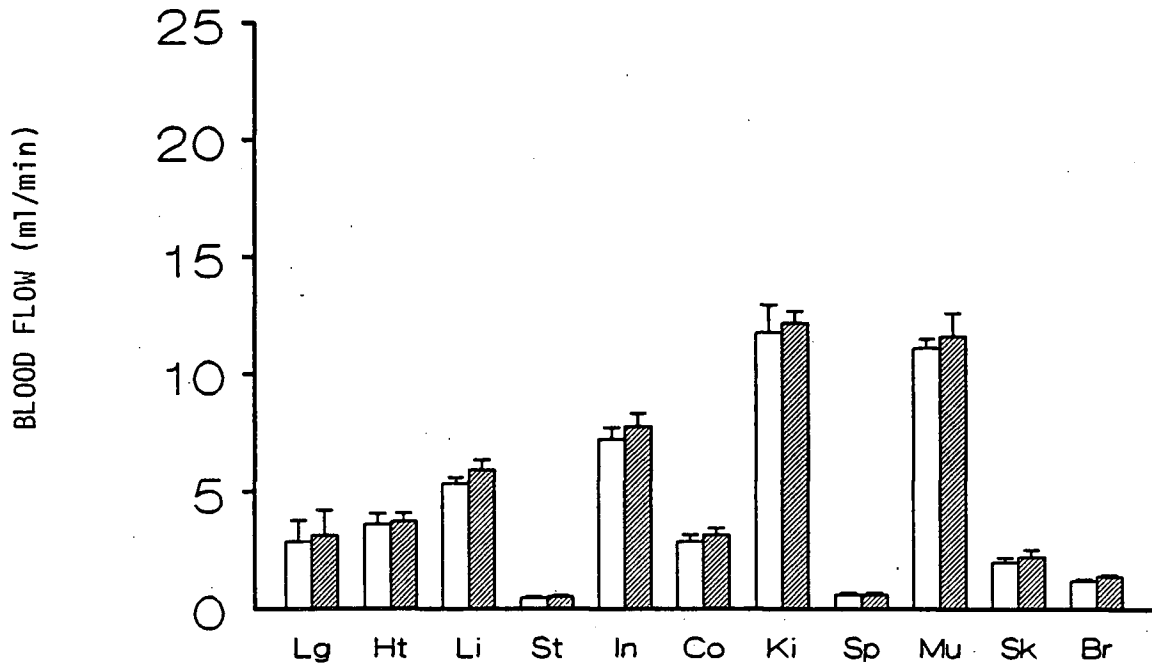
10 min later, at the time of termination of the experiments. The injection of saline in rats given phentolamine in group XXI altered neither MAP nor HR. There was again a tendency for TPR to decrease and CO to increase with the passage of time but these changes were not statistically significant.

3.4.1.2. Effects on blood flows and vascular conductances:

The infusion of saline in group XVIII neither affected blood flow nor vascular conductance in any organs or tissues (Fig. 13). The infusion of phentolamine in group XIX significantly decreased flows in the liver, stomach, colon and caecum, and kidneys but did not affect flows in any other organs or tissues (Fig. 14a). Phentolamine significantly increased vascular conductances in the muscle and skin beds but did not affect conductance in other beds (Fig. 14b). In group XX rats previously treated with phentolamine, propranolol reduced muscle flow but increased flows to the lungs, heart, liver, intestine, colon and caecum, kidneys and spleen. Flows in the other organs or tissues were not affected (Fig. 15a). When flow was normalized for MAP (conductance), propranolol caused significant decreases in arterial conductances in the muscle, skin and kidneys but did not affect conductances in other beds (Fig. 15b). The injection of saline in group XXI did not produce any significant change in flow or arterial conductance in any tissue or organ (Fig. 16).

Fig. 13. Effects of normal saline infusion on the distribution of blood flow (a) and vascular conductance (b) in urethane-anaesthetized rats (group XVIII, n = 8). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk) and brain (Br). Control (open bars); normal saline (hatched bars).

(a)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15
10
5
0
Lg
Ht
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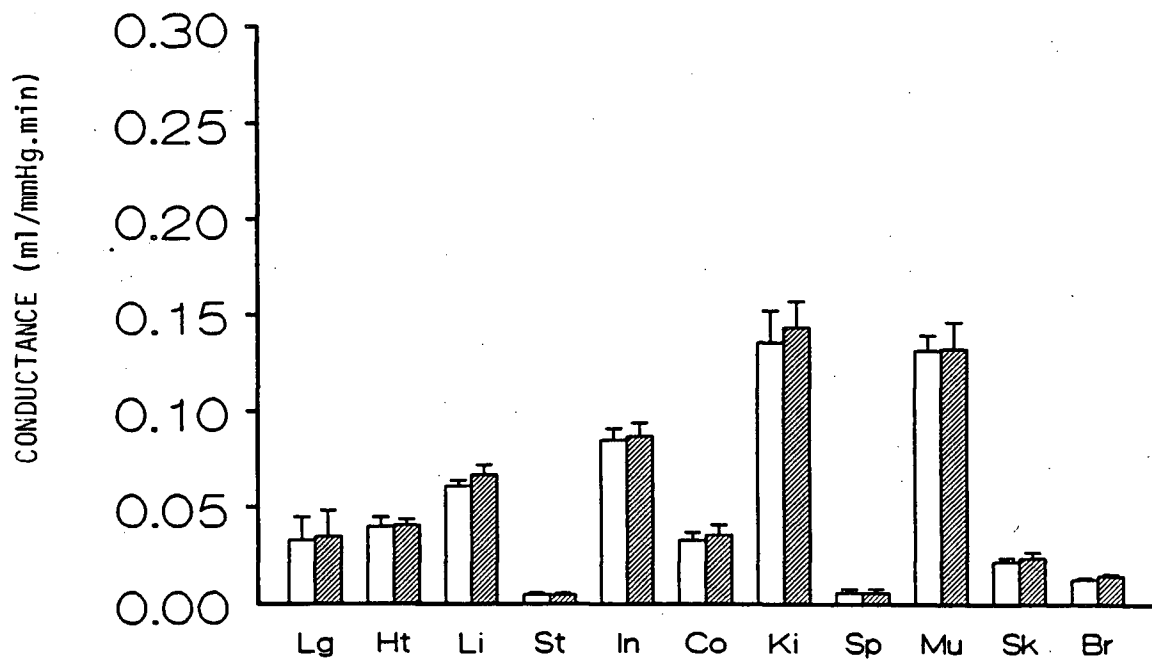
St
In
Co
Ki
Sp
Mu
Sk
Br

(b)


CONDUCTANCE (ml/mmHg.min)
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0.25
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0.15
0.10
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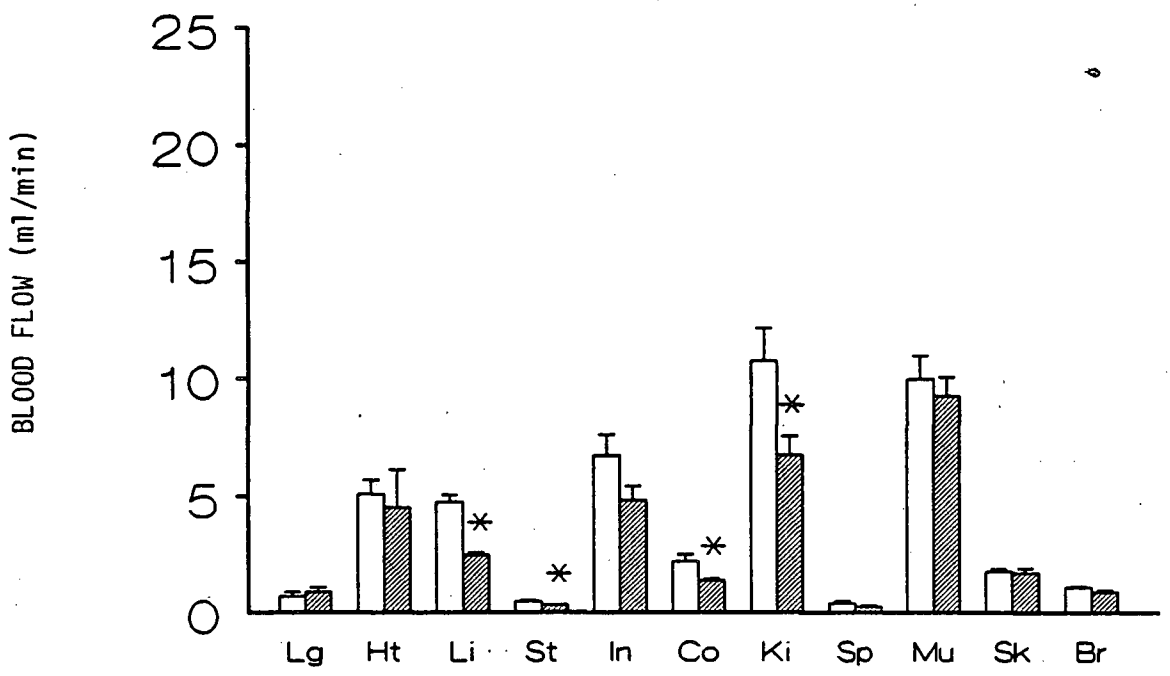
0.00
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Br

Fig. 14. Effects of phentolamine infusion (300 μg/kg/min) on the distribution of blood flow (a) and vascular conductance (b) in urethane-anaesthetized rats (group XIX, n = 8). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk) and brain (Br). Control (open bars); phentolamine (hatched bars). *Significantly different from control ($p < 0.05$).

(a)


BLOOD FLOW (ml/min)
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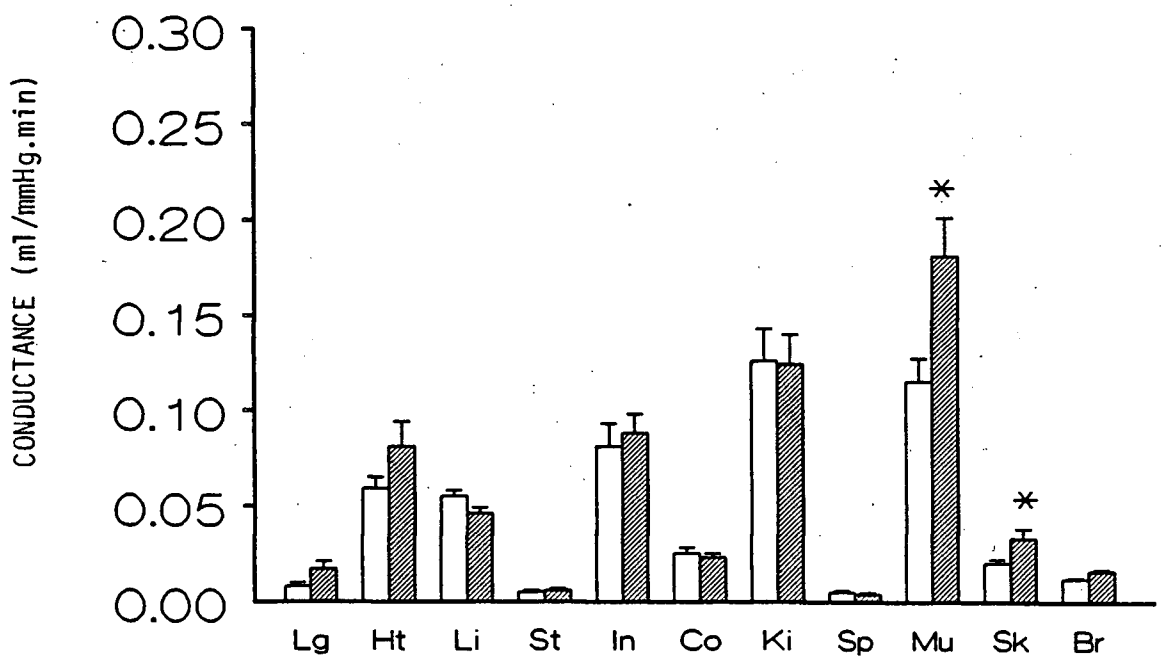
20
15
10
5
0
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Br

(b)


CONDUCTANCE (ml/mmHg.min)
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0.25
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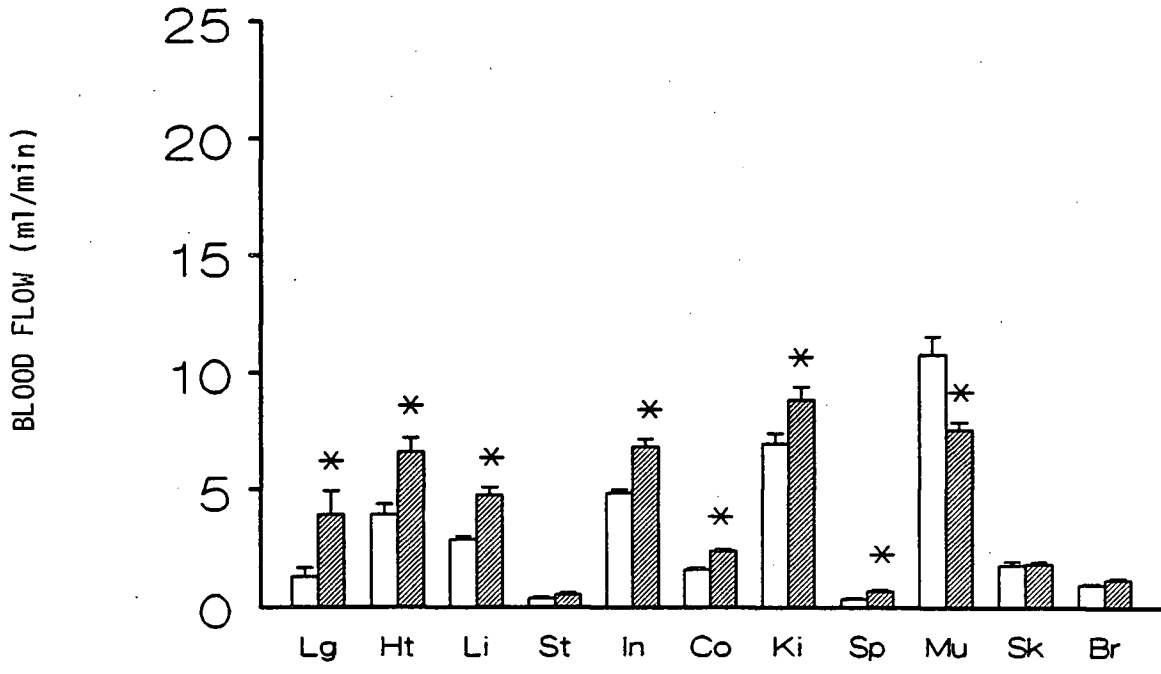
0.10
0.05
0.00
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Br

Fig. 15. Effects of propranolol (100 μg/kg) on the distribution of blood flow (a) and vascular conductance (b) in phentolamine-treated (300 μg/kg/min), urethane-anaesthetized rats (group XX, n = 8). Values are mean ± S.E. Organs or tissue samples are: Lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk) and brain (Br). Phentolamine treatment (open bars); propranolol in the presence of phentolamine treatment (hatched bars). *Significantly different from phentolamine treatment ($p < 0.05$).

(a)


BLOOD FLOW (ml/min)
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Lg
Ht
Li
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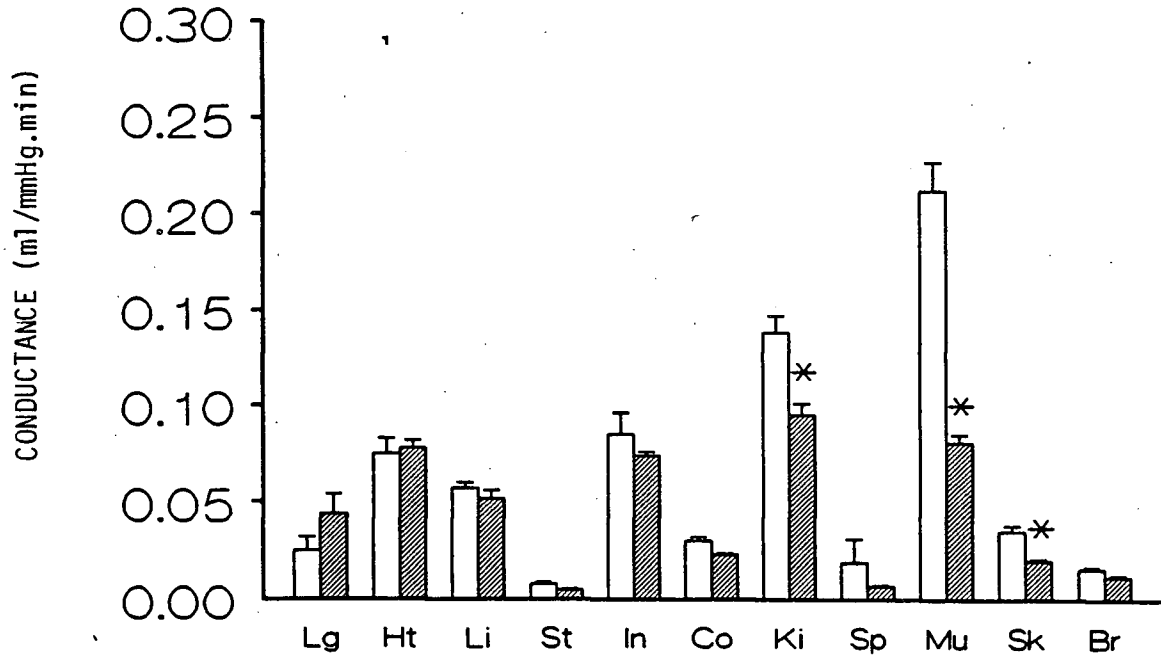
In
Co
Ki
Sp
Mu
Sk
Br

(b)


CONDUCTANCE (ml/mmHg.min)
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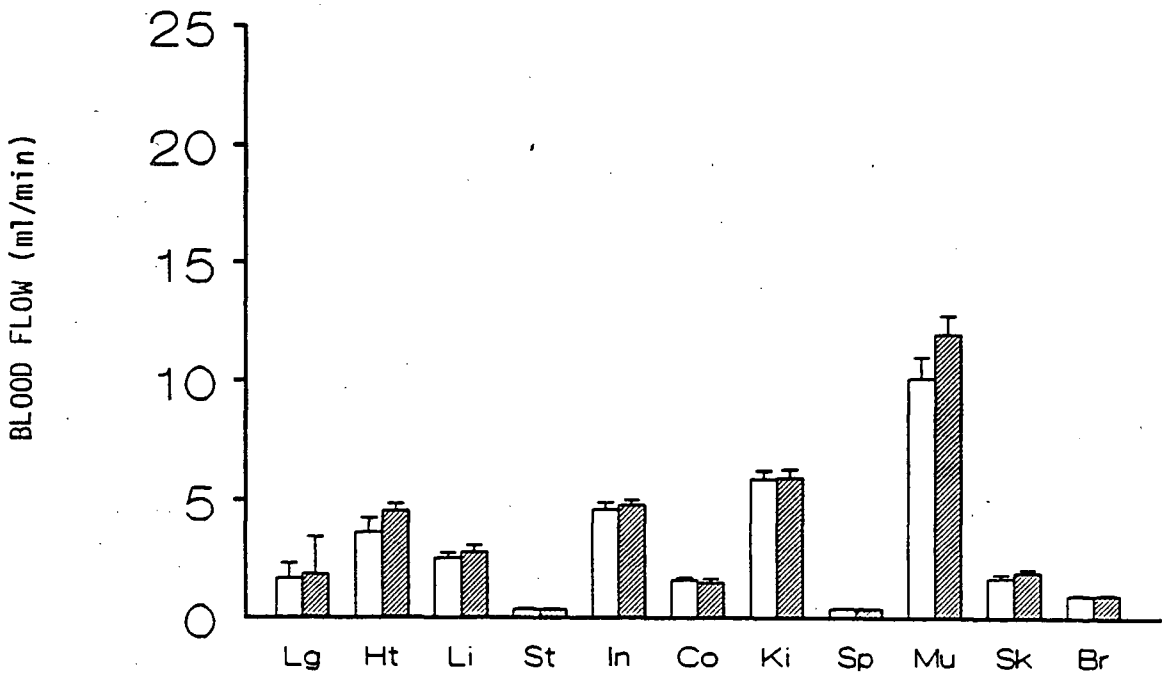
0.15
0.10
0.05
0.00
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Br

Fig. 16. Effets of normal saline on the distribution of blood flow (a) and vascular conductance (b) in phentolamine-treated (300 μg/kg/min), urethane-anaesthetized rats (group XXI, n = 8). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 40 g of skeletal muscle (Mu), 40 g of skin (Sk) and brain (Br). Phentolamine treatment (open bars); saline in the presence of phentolamine (hatched bars).

(a)


BLOOD FLOW (ml/min)
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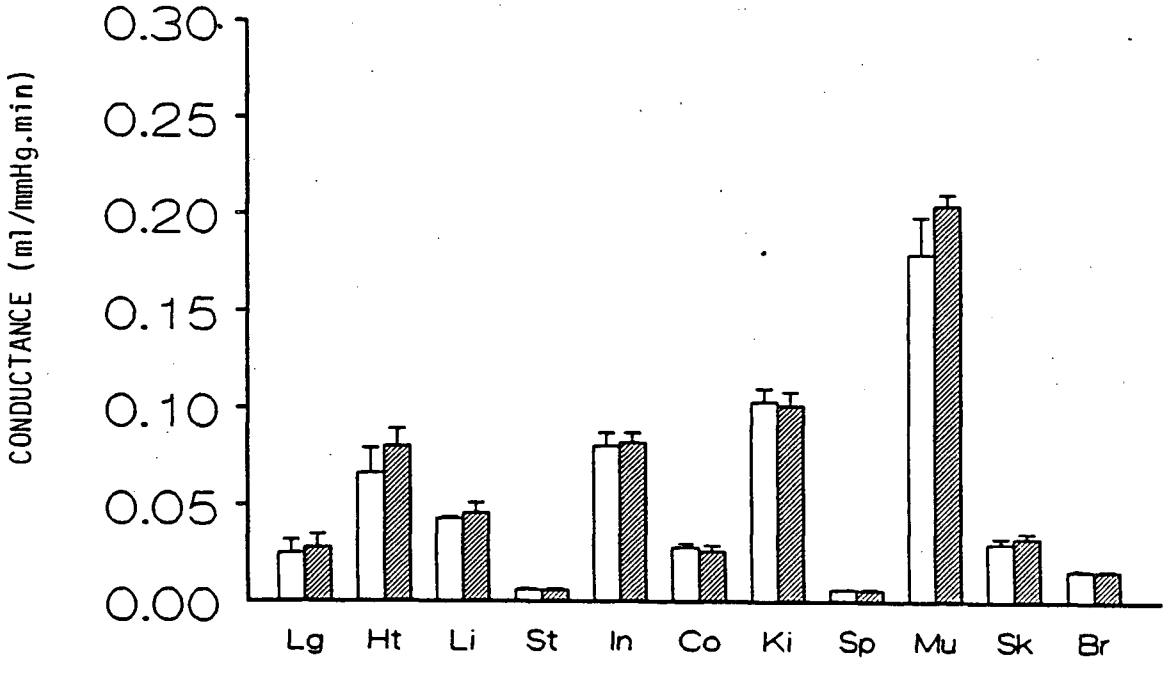
20
15
10
5
0
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Br

(b)


CONDUCTANCE (ml/mmHg.min)
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0.20
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0.10
0.05
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Lg
Ht
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In
Co
Ki
Sp
Mu
Sk
Br

3.4.2. Haemodynamic changes in conscious rats

3.4.2.1. Effects on MAP, TPR, CO and HR

Figure 17 shows the changes of MAP and TPR, while figure 18 shows the effects on CO and HR after the administrations of normal saline, phentolamine, propranolol in phentolamine-treated rats and atenolol in phentolamine-treated rats in groups XXII, XXIII, XXIV and XXV, respectively. The infusion of normal saline in group XXII did not significantly affect MAP, TPR, CO or HR. The infusion of phentolamine in group XXIII significantly increased HR and decreased MAP by reducing TPR since CO was not altered. Phentolamine in groups XXIV and XXV caused similar decreases in MAP as that in group XXIII, from 110 ± 4 to 73 ± 3 mmHg and from 111 ± 2 to 71 ± 3 mmHg, respectively. HR was also increased from 365 ± 16 to 450 ± 15 and from 368 ± 12 to 471 ± 8 beats/min in group XXIV and XXV, respectively. The subsequent injection of propranolol (group XXIV) and atenolol (group XXV) in rats given phentolamine significantly increased MAP back to control levels (114 ± 3 and 112 ± 5 mmHg, respectively). The pressor effects of propranolol and atenolol were accompanied by an increase in TPR, no change in CO and reduced HR.

3.4.2.2. Effects on blood flows and vascular conductances:

The infusion of normal saline in group XXII affected neither blood flow nor vascular conductance in any organ or tissue (Fig. 19). The infusion of phentolamine in

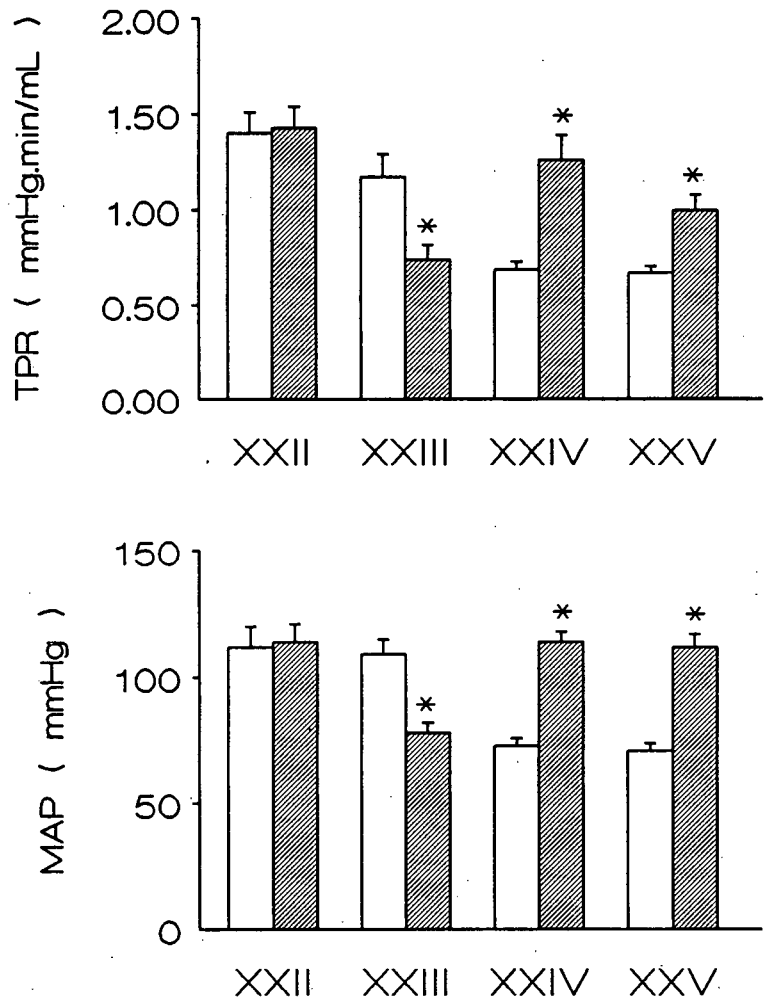


Fig. 17. Effects of normal saline (XXII), phentolamine (300 μg/kg/min, XXIII), propranolol (100 μg/kg) in the presence of phentolamine (XXIV) and, atenolol (100 μg/kg) in the presence of phentolamine (XXV), on total peripheral resistance (TPR) and mean arterial pressure (MAP) in four groups (n = 6 each) of conscious rats. Pretreatment (open bars); post-treatment (hatched bars). Values are mean ± S.E. *Significantly different from pretreatment ($P < 0.05$).

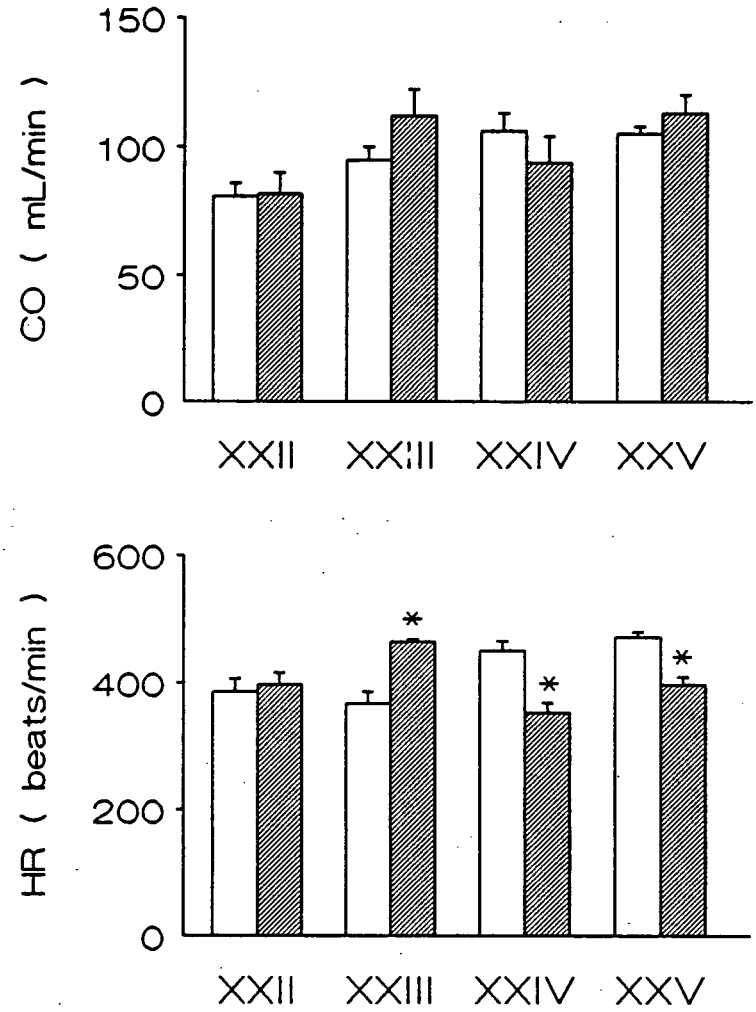
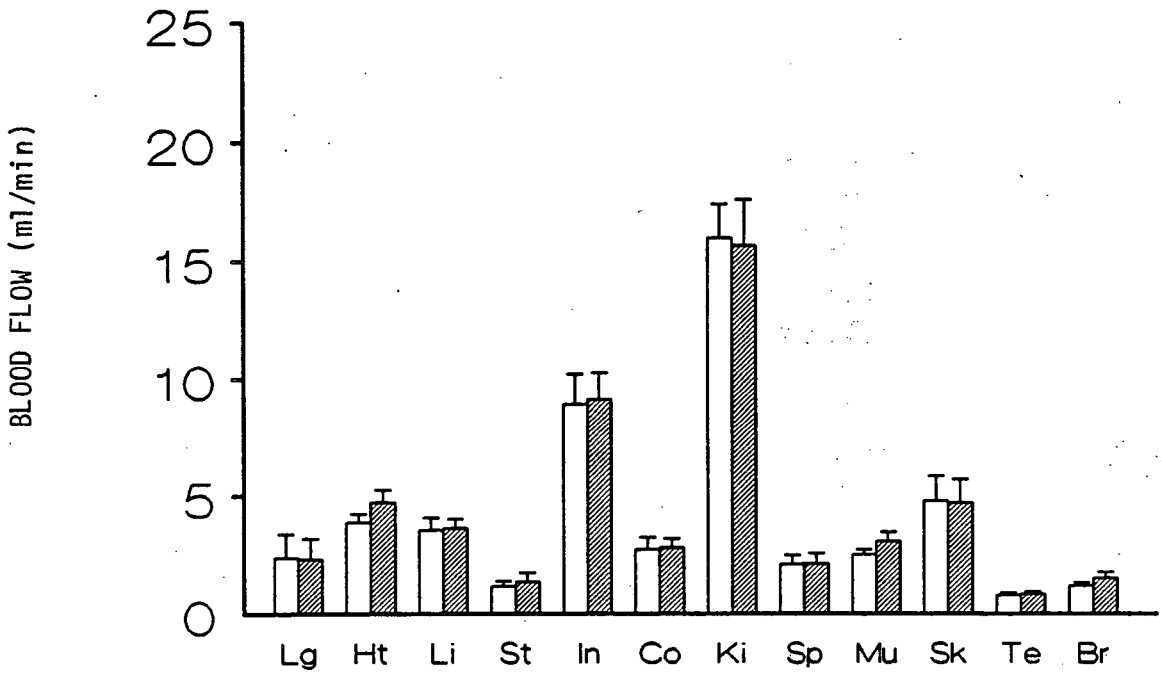


Fig. 18. Effects of normal saline (XXII), phentolamine (300 μg/kg/min, XXIII), propranolol (100 μg/kg) in the presence of phentolamine (XXIV) and, atenolol (100 μg/kg) in the presence of phentolamine (XXV), on heart rate (HR) and cardiac output (CO) in four groups (n = 6 each) of conscious rats. Pretreatment (open bars); post-treatment (hatched bars). Values are mean ± S.E. *Significantly different from pretreatment ($p < 0.05$).

Fig. 19. Effects of normal saline infusion on the distribution of blood flow (a) and vascular conductance (b) in conscious rats (group XXII, n = 6). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 30 g of skeletal muscle (Mu), 30 g of skin (Sk), testis (Te) and brain (Br). Control (open bars); normal saline (hatched bars).

(a)


BLOOD FLOW (ml/min)
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15
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5
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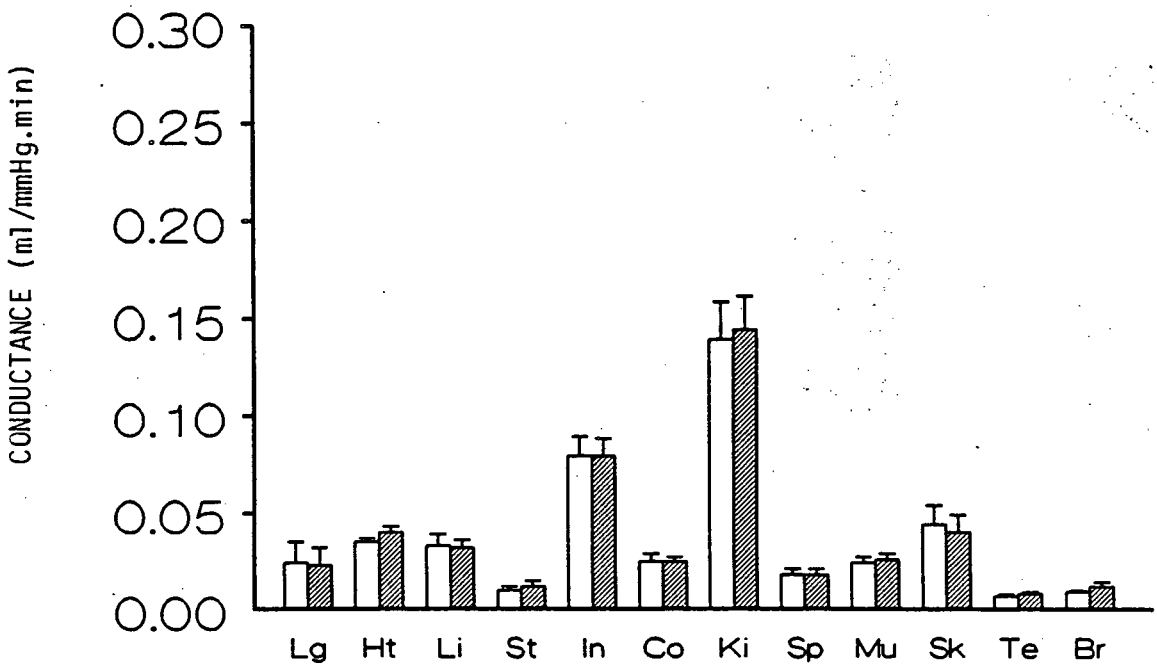
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Te
Br

(b)


CONDUCTANCE (ml/mmHg.min)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Te
Br

group XXIII significantly increased flows in the lungs, heart and skeletal muscle, but decreased flows in the stomach, kidneys and spleen (Fig. 20a). Phentolamine significantly increased vascular conductances in the lungs, heart and skeletal muscle but it did not affect conductances in other vascular beds (Fig. 20b). In group XXIV rats previously treated with phentolamine, propranolol reduced muscle flow, increased flow to the lungs but did not affect flows in other organs or tissues (Fig. 21a). Propranolol caused significant decreases in vascular conductances in the heart, intestine, kidneys, skeletal muscle and skin (Fig. 21b). The injection of atenolol in group XXV decreased blood flow to the skeletal muscle and increased blood flows to the lungs, intestine, caecum and colon, spleen, testis and brain (Fig. 22a). Atenolol reduced vascular conductances in the intestine, kidneys, skeletal muscle and skin (Fig. 22b).

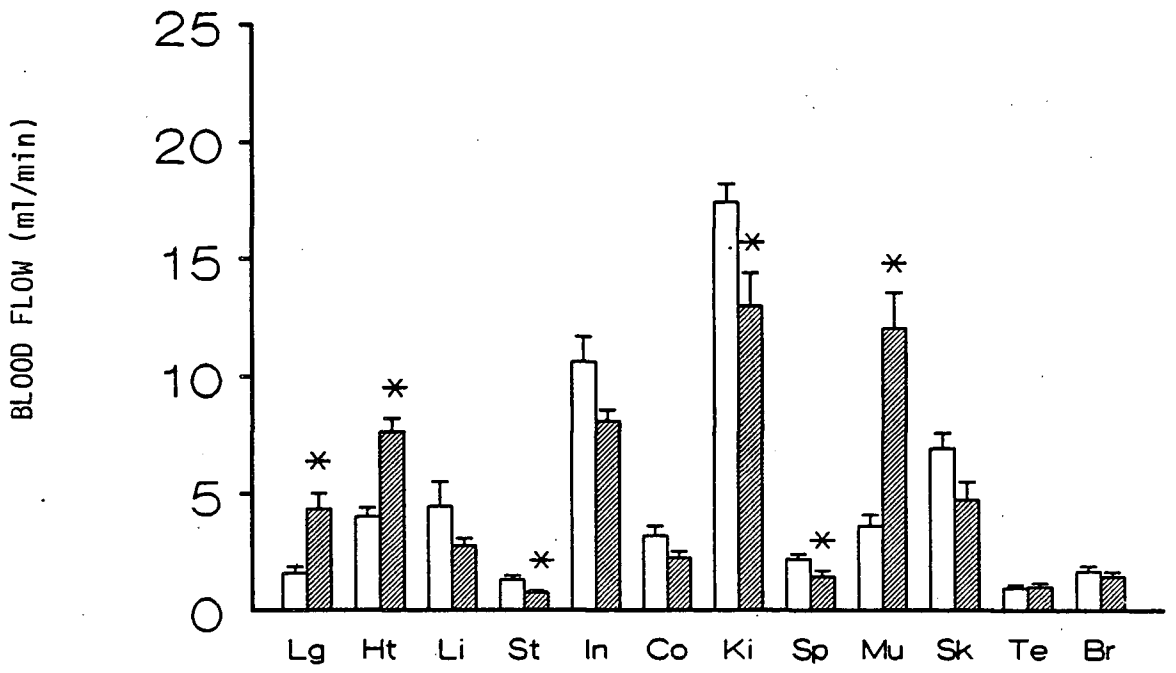
3.4.3. Effects of anaesthetic agents

3.4.3.1. Effects of urethane, pentobarbital and halothane on dose-response curves to ß-blockers

Baseline MAP (pooled values) in rats anaesthetized with pentobarbital is significantly higher than MAP in rats anaesthetized with urethane or halothane (Table 3). Phentolamine reduced MAP in all nine groups (XXVI - XXXIV) of rats (Table 3). An i. v. bolus of propranolol, ICI 118 ,551 or atenolol dose-dependently increased MAP in rats

Fig. 20. Effects of phentolamine infusion (300 μg/kg/min) on the distribution of blood flow (a) and vascular conductance (b) in conscious rats (group XXIII, n = 6). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 30 g of skeletal muscle (Mu), 30 g of skin (Sk), testis (Te) and brain (Br). Control (open bars); phentolamine (hatched bars). *Significantly different from control ($p < 0.05$).

(a)


BLOOD FLOW (ml/min)
25
20
15
10
5
0
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Te
Br

(b)

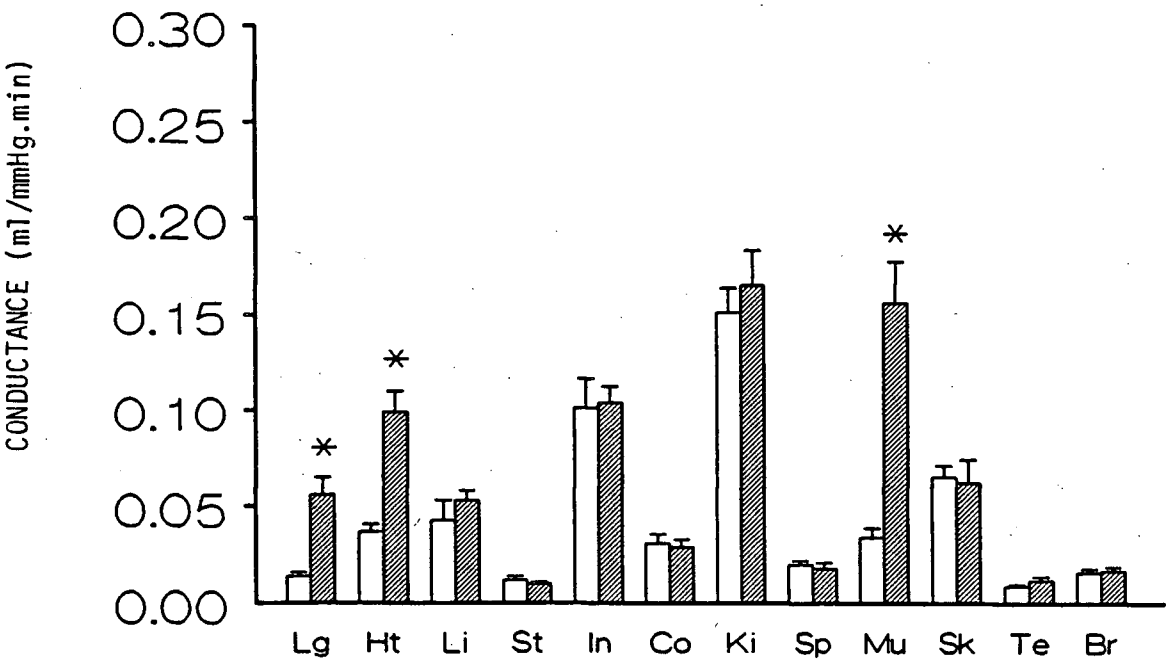
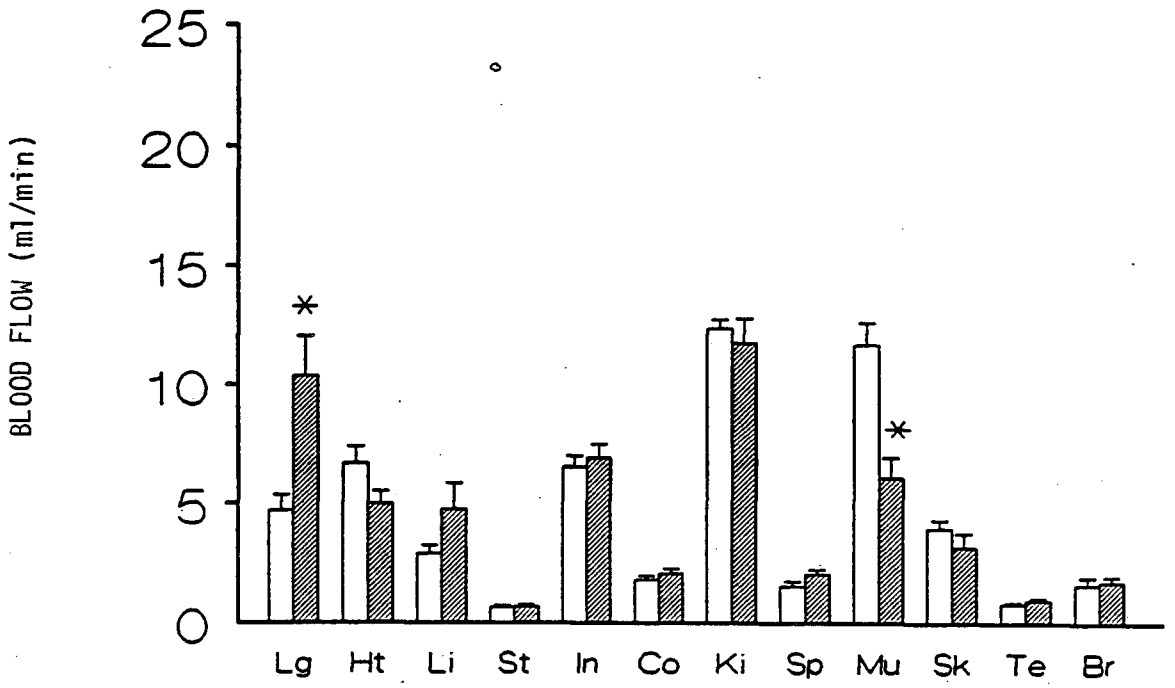

CONDUCTANCE (ml/mmHg.min)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Te
Br

Fig. 21. Effects of propranolol (100 μg/kg) on the distribution of blood flow (a) and vascular conductance (b) in phentolamine-treated (300 μg/kg/min) conscious rats (group XXIV, n = 6). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 30 g of skeletal muscle (Mu), 30 g of skin (Sk), testis (Te) and brain (Br). Phentolamine-treated (open bars); propranolol after phentolamine treatment (hatched bars). *Significantly different from phentolamine treatment ($p < 0.05$).

(a)


BLOOD FLOW (ml/min)
25
20
15
10
5
0
Lg
Ht
Li
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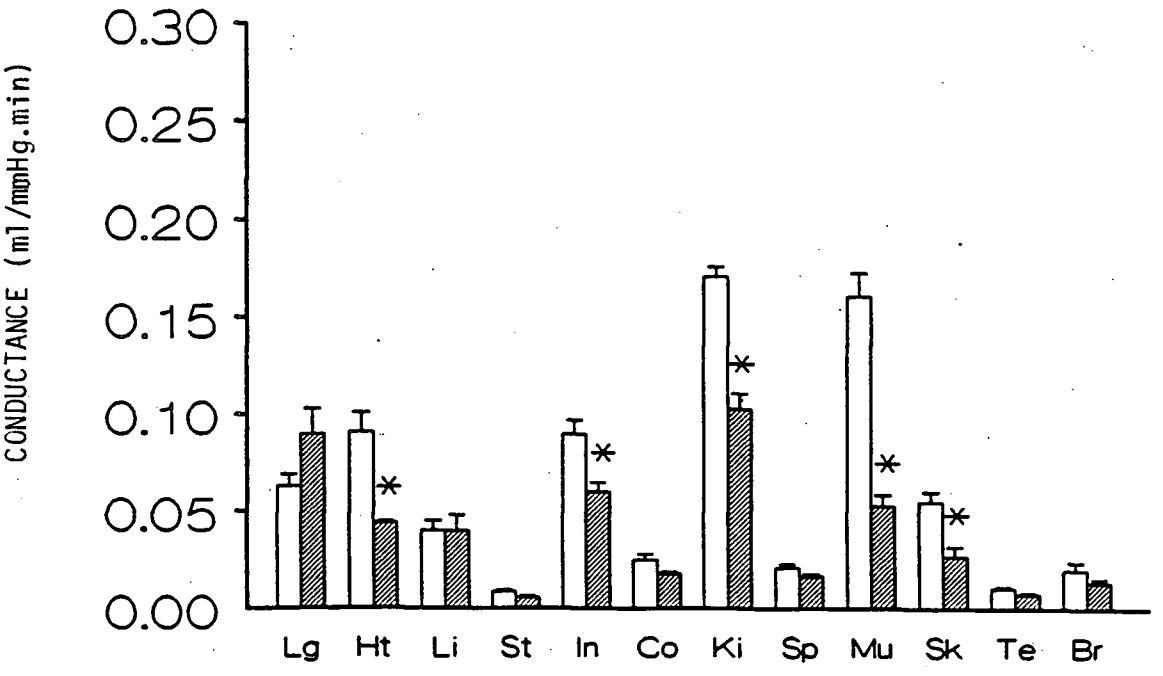
In
Co
Ki
Sp
Mu
Sk
Te
Br

(b)


CONDUCTANCE (ml/mmHg.min)
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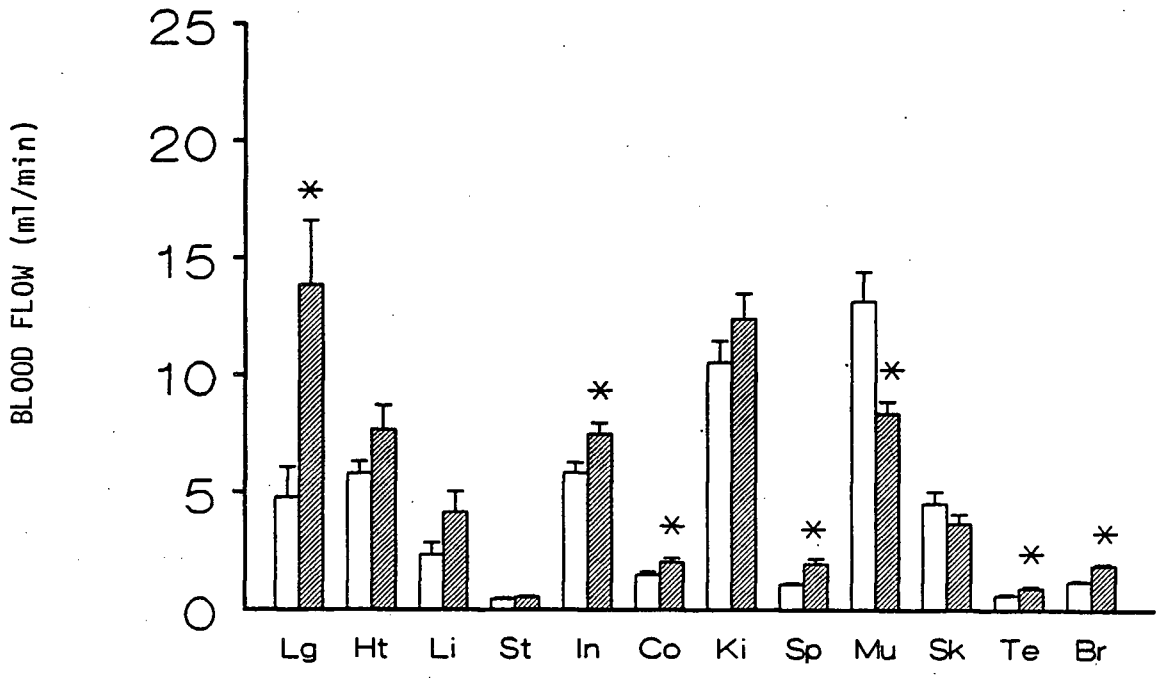
0.25
0.20
0.15
0.10
0.05
0.00
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Te
Br

Fig. 22. Effects of atenolol (100 μg/kg) on the distribution of blood flow (a) and vascular conductance (b) in phentolamine-treated (300 μg/kg/min), conscious rats (group XXV, n = 6). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 30 g of skeletal muscle (Mu), 30 g of skin (Sk), testis (Te) and brain (Br). Phentolamine-treated (open bars); atenolol after phentolamine treatment (hatched bars). *Signifcantly different from phentolamine treatment ($p < 0.05$).

(a)


BLOOD FLOW (ml/min)
25
20
15
10
5
0
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Te
Br

(b)

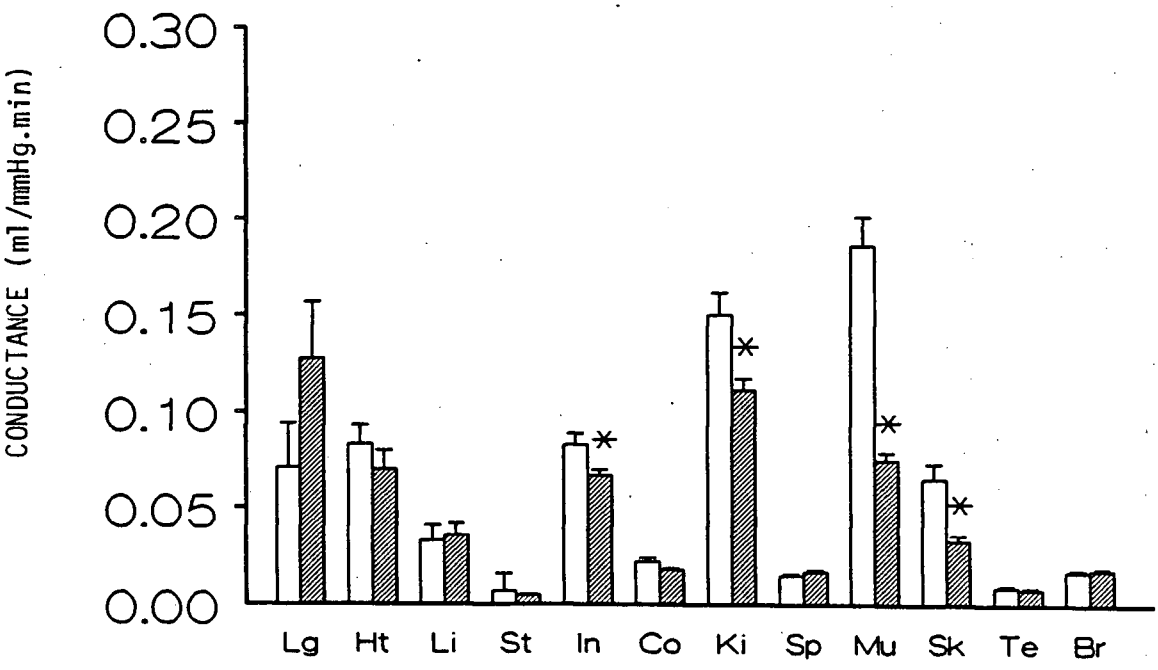

CONDUCTANCE (ml/mmHg.min)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Lg
Ht
Li
St
In
Co
Ki
Sp
Mu
Sk
Te
Br

Table 3. Mean arterial pressure (means ± S.E.) prior to and 10 min after the infusion of phentolamine (300 μg/kg/min) in rats anaesthetized with urethane (Groups XXVI - XXVIII), pentobarbital (Groups XXIX - XXXI) or halothane (Groups XXXII - XXXIV).

	n	Control	Phentolamine
Urethane:			
Group XXVI	6	92 ± 7	56 ± 6[a]
Group XXVII	6	84 ± 2	48 ± 6[a]
Group XXVIII	6	92 ± 7	47 ± 2[a]
Pooled	18	89 ± 3	51 ± 3[a]
Pentobarbital:			
Group XXIX	5	105 ± 3	66 ± 6[a]
Group XXX	6	103 ± 7	77 ± 4[a]
Group XXXI	6	100 ± 3	80 ± 2[a]
Pooled	17	102 ± 2[b]	75 ± 3[a]
Halothane:			
Group XXXII	8	98 ± 1	69 ± 3[a]
Group XXXIII	6	84 ± 1	47 ± 2[a]
Group XXXIV	6	83 ± 3	61 ± 2[a]
Pooled	20	89 ± 2	63 ± 2[a]

[a]Significantly different from control values ($p < 0.05$).
[b]Significantly different from pooled values in rats anaesthetized with urethane or halothane ($p < 0.05$).

anaesthetized with urethane but not pentobarbital (Fig. 23, 24 and 25). In rats anaesthetized with halothane, ICI 118,551, but neither propranolol nor atenolol, caused a small dose-dependent increase in MAP. The maximal increase in MAP in response to the highest dose of ICI 118,551 under the influence of halothane (group XXXIII) was approximately 25% of the corresponding MAP value under the influence of urethane (group XXVII) even when baseline MAPs prior to and after the infusion of phentolamine were similar in the two groups (Table 3).

3.4.3.2. Effects of pentobarbital, amobarbital, ketamine and chloralose on i.v. bolus of propranolol

In groups rats anaesthetized with pentobarbital (XXXV), amobarbital (XXXVI) and chloralose (XXXVIII), phentolamine reduced MAP while propranolol did not produce any significant effect on MAP (Fig. 26). In ketamine anaesthetized rats (XXXVII), MAP was higher than in rats anaesthetized with pentobarbital, amobarbital and chloralose and phentolamine caused a greater reduction in MAP. The injection of propranolol partially restored MAP to a level which is lower than baseline MAP (Fig. 26).

3.4.3.3. Effect of adrenaline infusion on i.v. bolus propranolol and atenolol in pentobarbital-anaesthetized rats

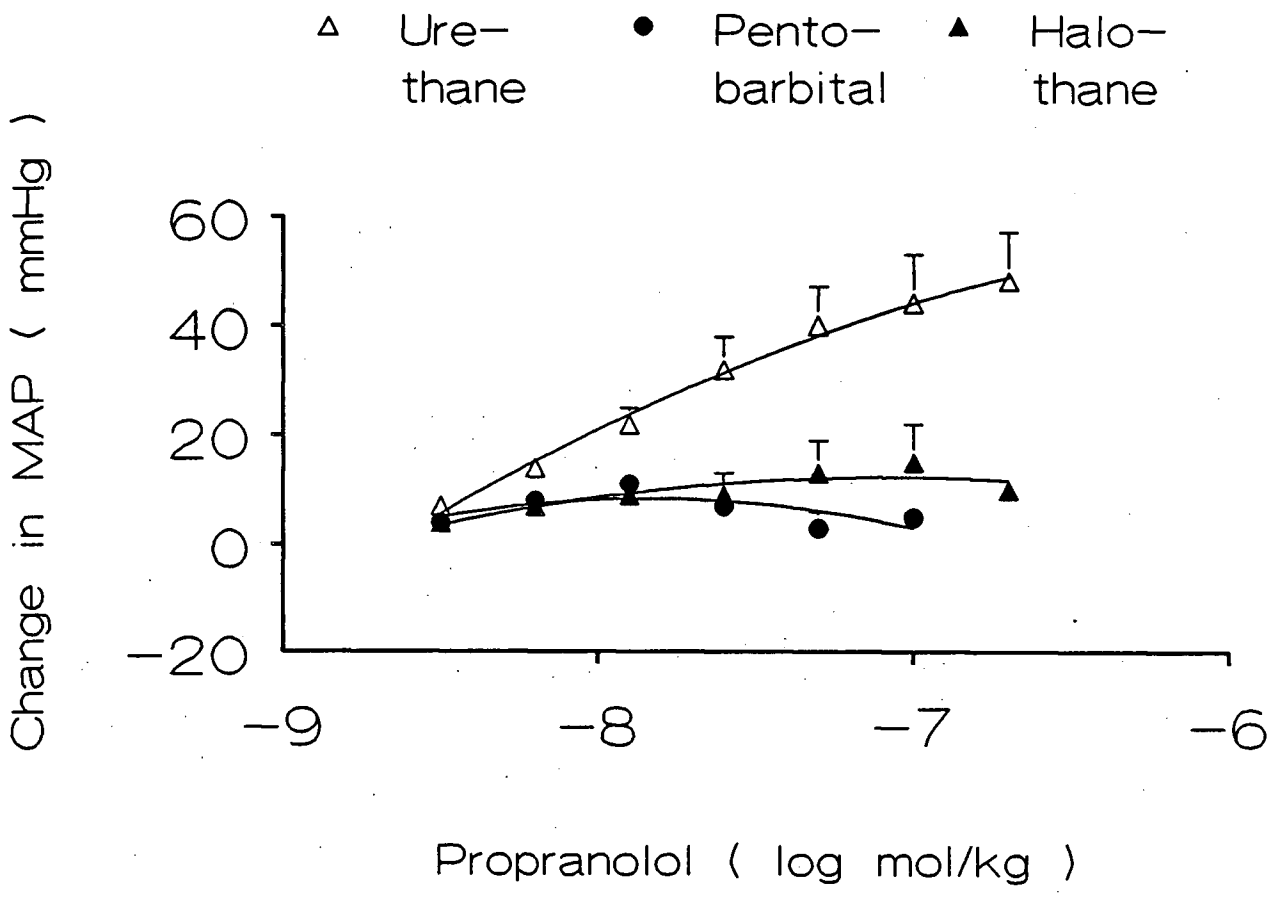


Fig. 23. Dose-response curves for propranolol on mean arterial pressure (MAP) in groups of urethane (XXVI), pentobarbital (XXIX) and halothane (XXXII) anaesthetized rats pretreated with phentolamine (300 μg/kg/min). Each point represents the mean ± S.E. (n = 5-8 each).

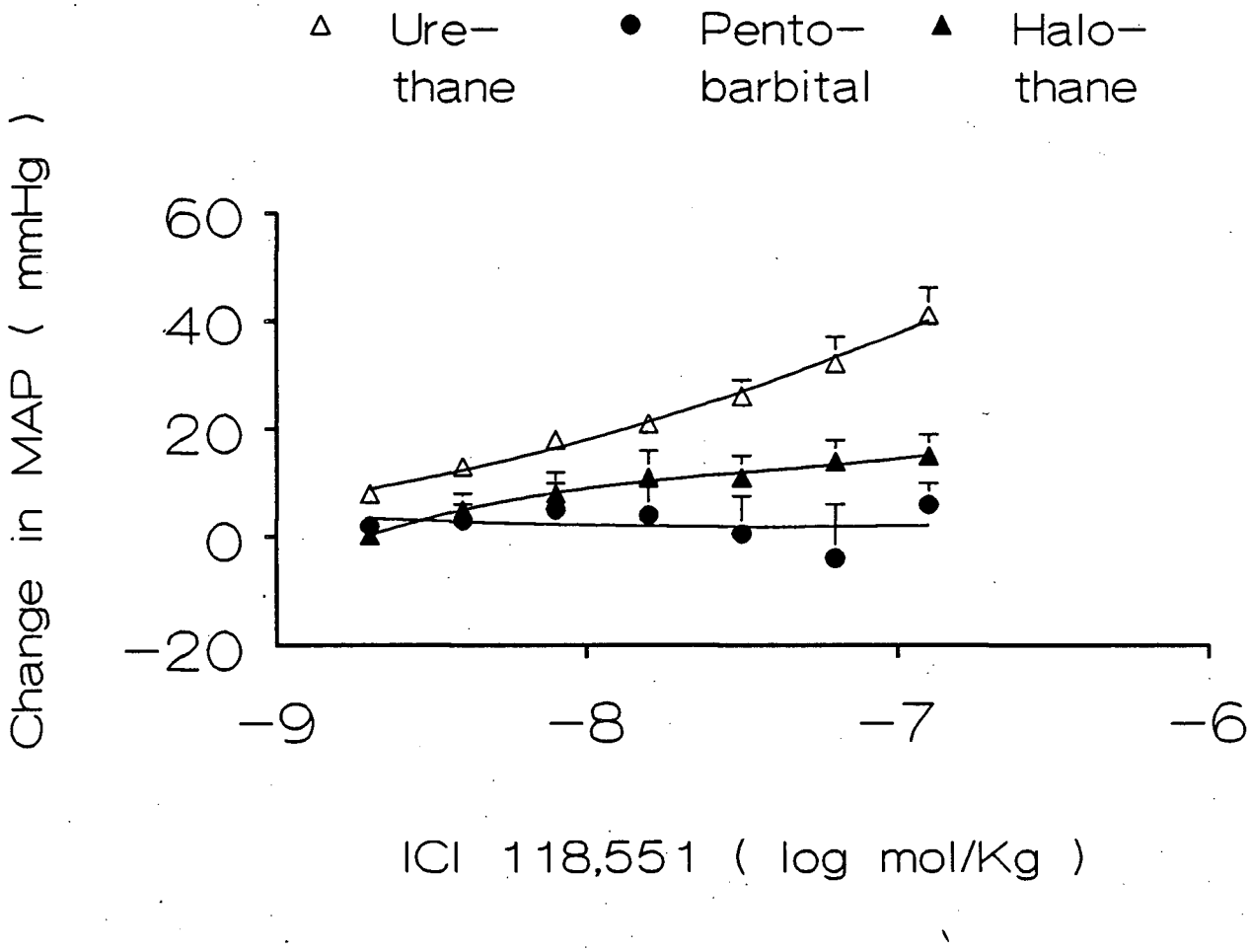


Fig. 24. Dose-response curves for ICI 118,551 on mean arterial pressure (MAP) in groups of urethane (XXVII), pentobarbital (XXX) and halothane (XXXIII) anaesthetized rats pretreated with phentolamine (300 μg/kg/min). Each point represents the mean ± S.E. (n = 6 each).

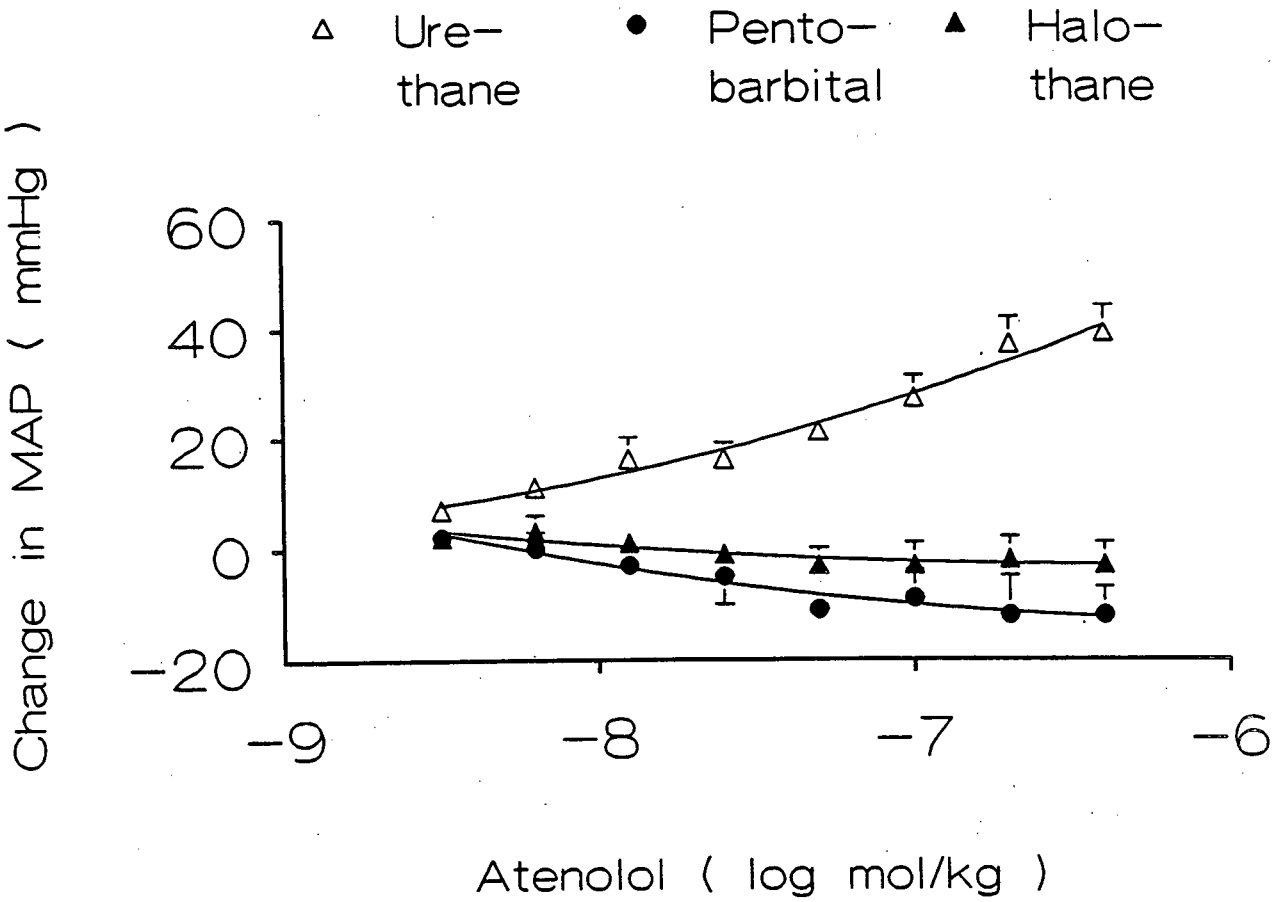


Fig. 25. Dose-response curves for atenolol on mean arterial pressure (MAP) in groups of urethane (XXVIII), pentobarbital (XXXI) and halothane (XXXIV) anaesthetized rats pretreated with phentolamine (300 μg/kg/min). Each point represents the mean ± S.E. (n = 6 each).

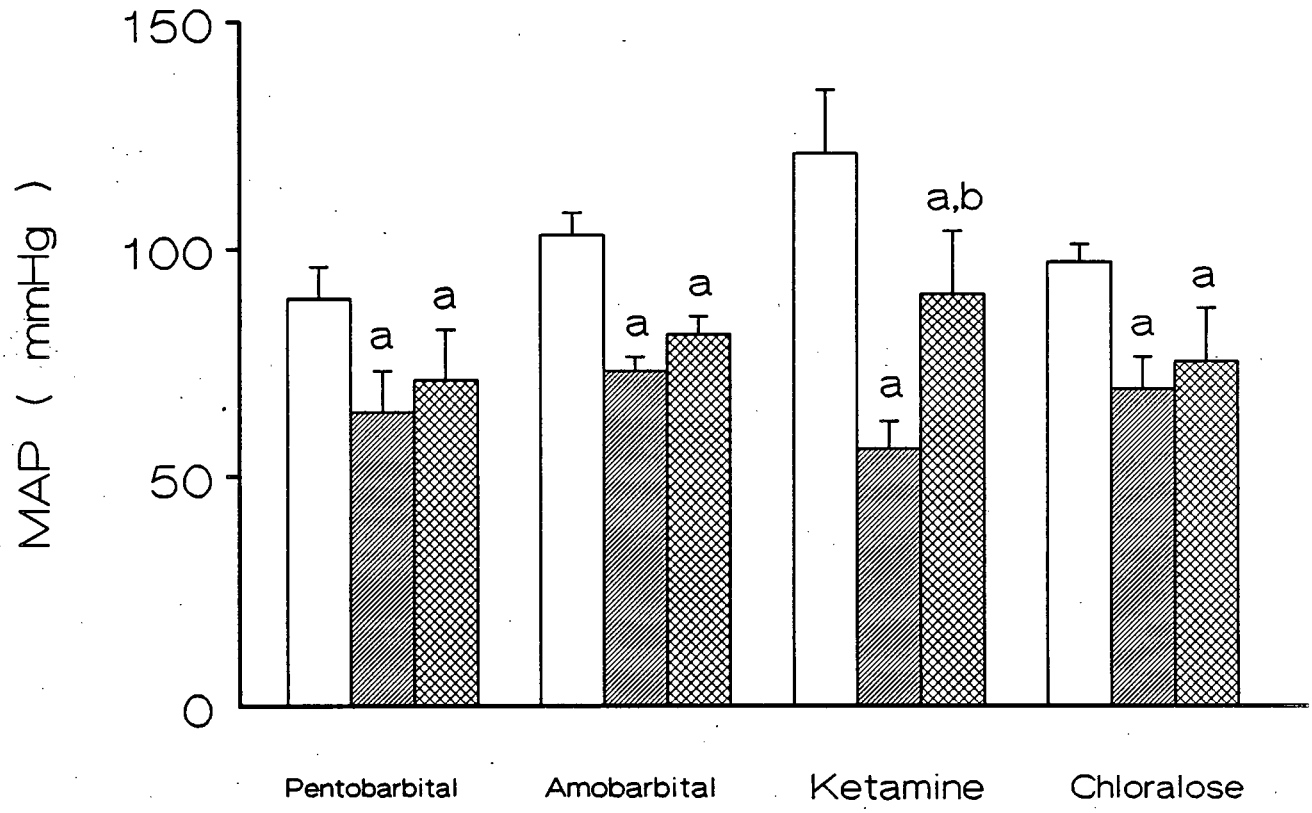


Fig. 26. Mean arterial pressure (MAP) in groups of rats anaesthetized with pentobarbital, amobarbital, ketamine or chloralose (n = 5-6 each) during control conditions (open bars), 10 min after the start of a continuous infusion of phentolamine (300 μg/kg/min) (hatched bars) and 1 min after the injection of propranolol (100 μg/kg) during the infusion of phentolamine (cross-hatched bars). [a]Significantly different from control ($p < 0.05$); [b]Significantly different from phentolamine treatment ($p < 0.05$).

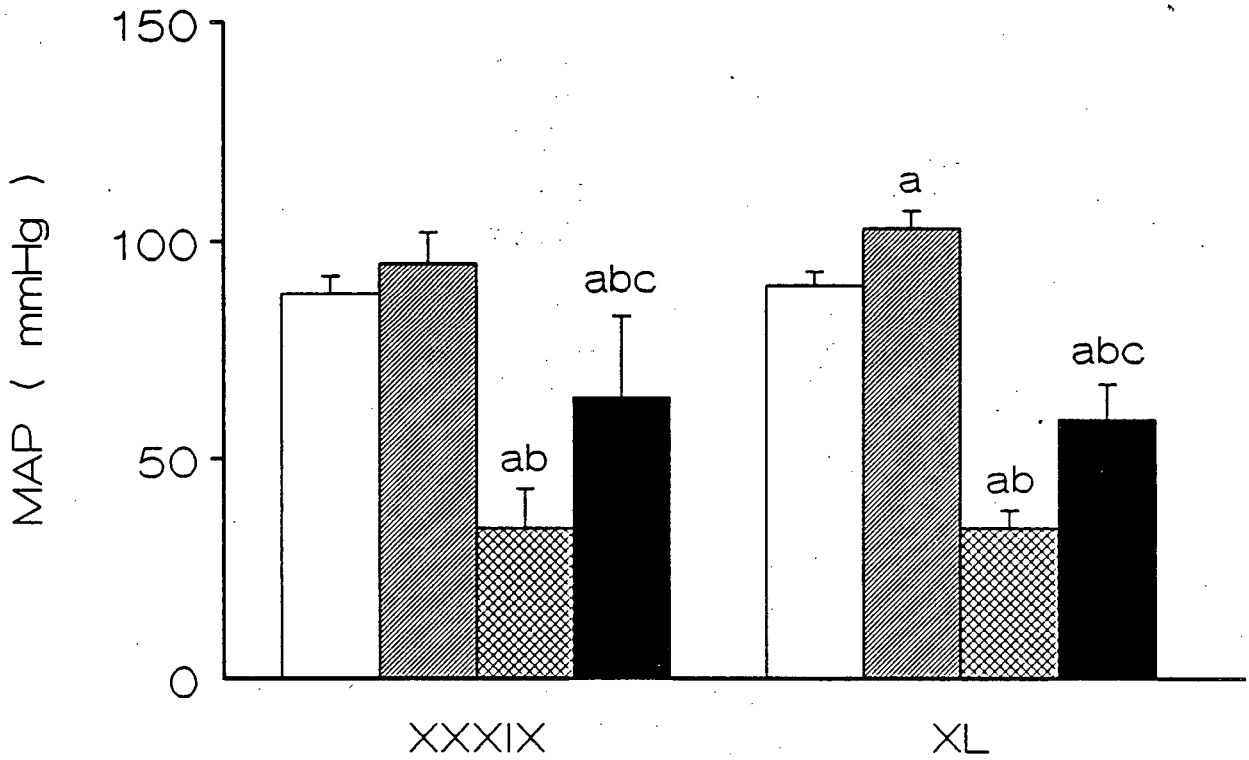


Fig. 27. Mean arterial pressure (MAP) in pentobarbital-anaesthetized rats (n = 6 each) during control conditions (open bars), 10 min after the start of a continuous adrenaline (300 ng/kg/min) infusion (hatched bars), 10 min after the start of phentolamine (300 µg/kg/min) infusion in the presence of adrenaline (cross-hatched bars) and 1 min after the injection of propranolol (100 µg/kg, group XXXIX) or atenolol (100 µg/kg, group XL) during the infusions of adrenaline and phentolamine (closed bars). [a]Significantly different from control ($p < 0.05$); [b]Significantly different from adrenaline ($p < 0.05$); [c]Significantly different from phentolamine ($p < 0.05$).

In groups XXXIX and XL, the infusion of adrenaline caused a small increase in MAP which was significant in group XL but not in XXXIX. The infusion of phentolamine reduced MAP in both groups. An i.v. bolus of propranolol and atenolol partially restored MAP to levels lower than MAP attained after the infusion of adrenaline (Fig. 27).

3.4.3.4 Haemodynamic changes in pentobarbital-anaesthetized rats

The infusion of saline (XLI) showed that time alone did not cause any changes in MAP, TPR (Fig. 28), CO, HR (Fig. 29) blood flow or vascular conductances (Fig. 30, 31). In group XLII, the infusion of phentolamine reduced MAP and TPR (Fig. 28), CO and HR (Fig. 29), however, only the reduction in MAP reached statistical significance. Propranolol injection in the presence of phentolamine did not have any significant effects on MAP, TPR, CO or HR (Fig. 28, 29). Phentolamine infusion increased blood flow to the lungs and reduced flows to the skin and, caecum and colon. When BF was normalized by MAP, vascular conductance was increased only in the lungs. Subsequent propranolol injection did not have any significant effects on blood flow or vascular conductances in any of the organs or tissues (Fig. 32, 33).

3.4.4. Effects of captopril

The control MAPs in the three groups, XLIII, XLIV and

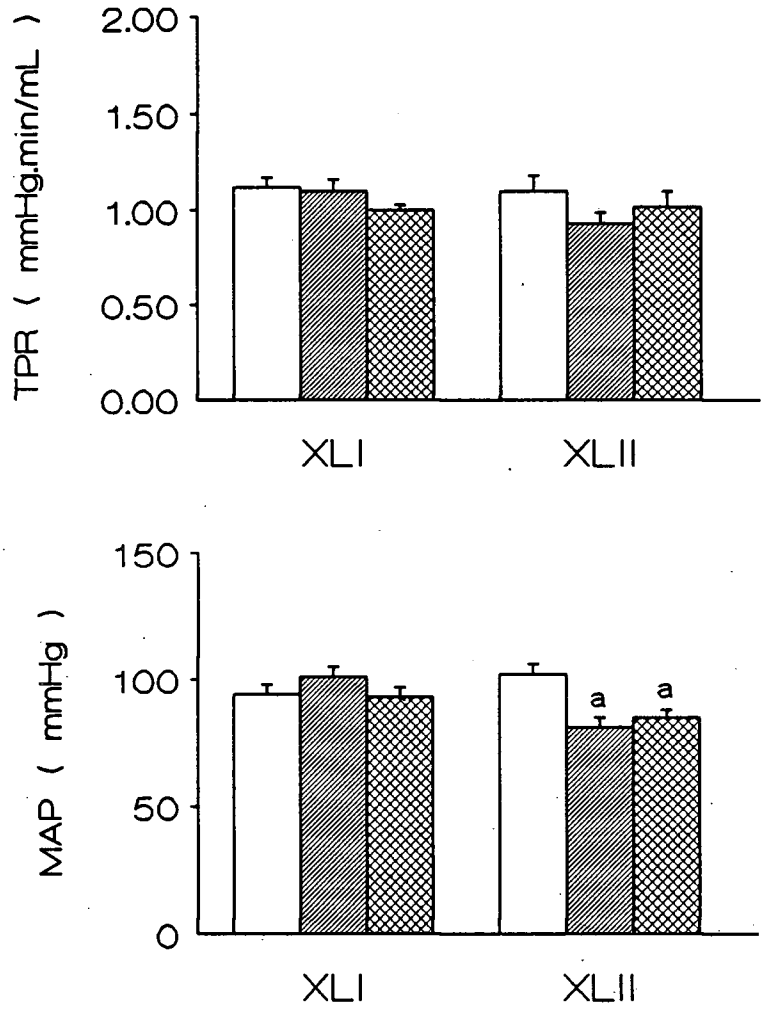


Fig. 28. Total peripheral resistance (TPR) and mean arterial pressure (MAP) in two groups of rats. Group (XLI, n = 6) during control conditions, 10 min after the start of saline infusion and 1 min after saline injection in the presence of saline infusion. Group (XLII, n = 8) during control conditions, 10 min after phentolamine infusion (300 μg/kg/min) and 1 min after propranlol (100 μg/kg) injection in the presence of phentolamine infusion. Control (open bars), after first treatment (hatched bars) and after second treatment (cross-hatched bars). [a]Significantly different from control ($p < 0.05$).

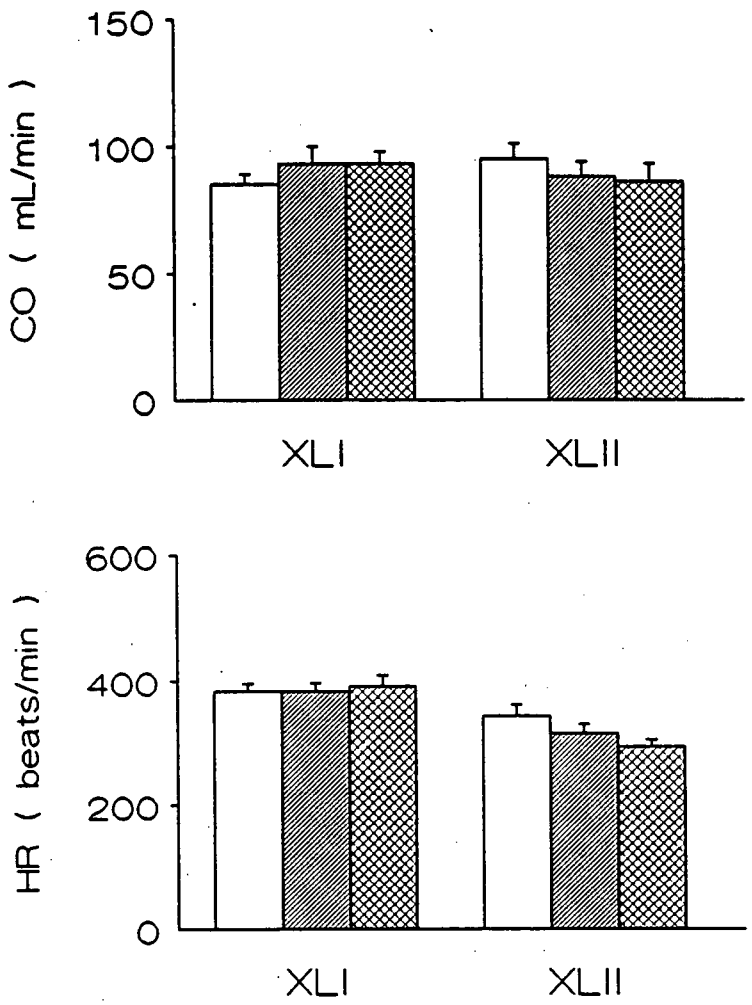
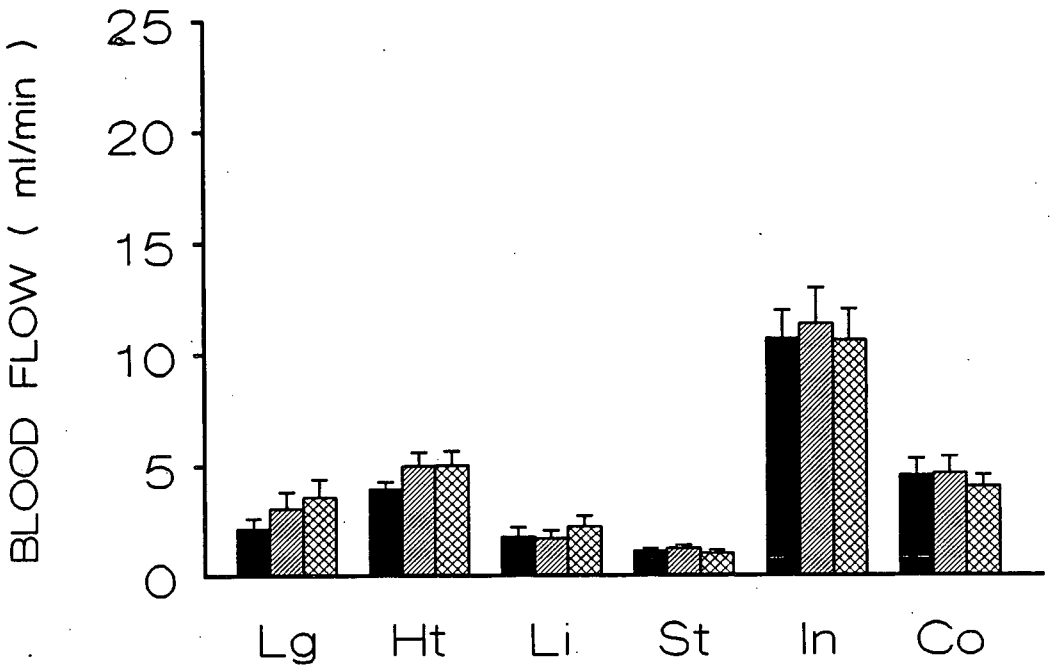


Fig. 29. Cardiac output (CO) and heart rate (HR) in two groups of rats. Group (XLI, n = 6) during control conditions, 10 min after the start of saline infusion and 1 min after saline injection in the presence of saline infusion. Group (XLII, n = 8) during control conditions, 10 min after phentolamine infusion (300 μg/kg/min) and 1 min after propranolol (100 μg/kg) injection in the presence of phentolamine infusion. Control (open bars), after first treatment (hatched bars) and after second treatment (cross-hatched bars).

Fig. 30. Distribution of blood flow in pentobarbital-anaesthetized rats (group XLI, n = 6), during control conditions (closed bars), 10 min after the start of normal saline infusion (hatched bars) and 1 min after normal saline injection during the continuous infusion of normal saline (cross-hatched bars). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 30 g of skeletal muscle (Mu), 30 g of skin (Sk), testis (Te) and brain (Br).


BLOOD FLOW (ml/min)
25
20
15
10
5
0
Lg
Ht
Li
St
In
Co

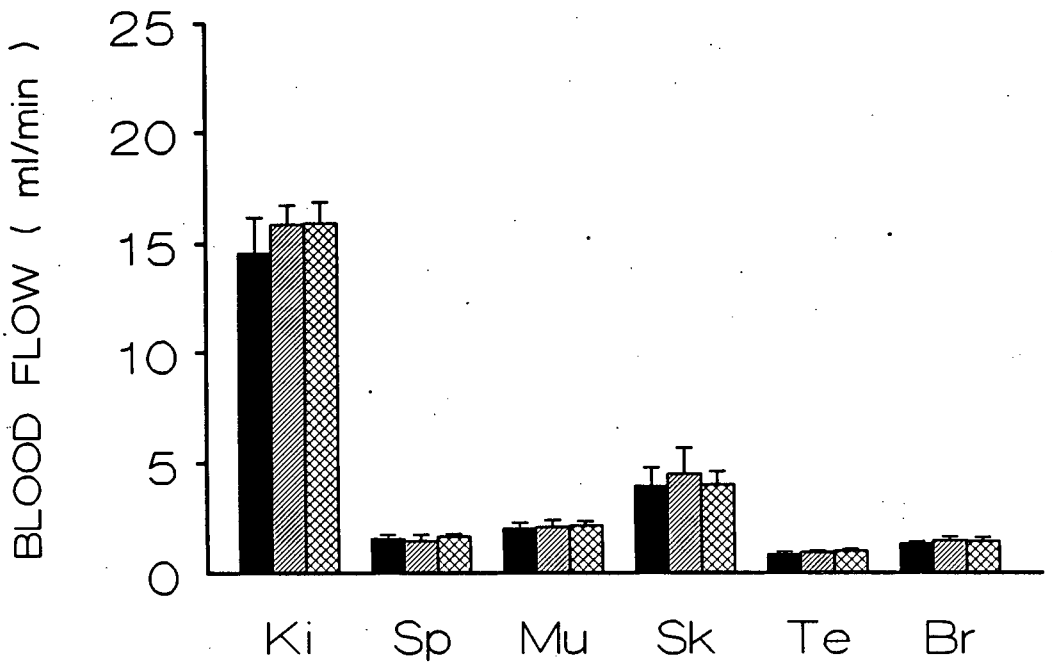
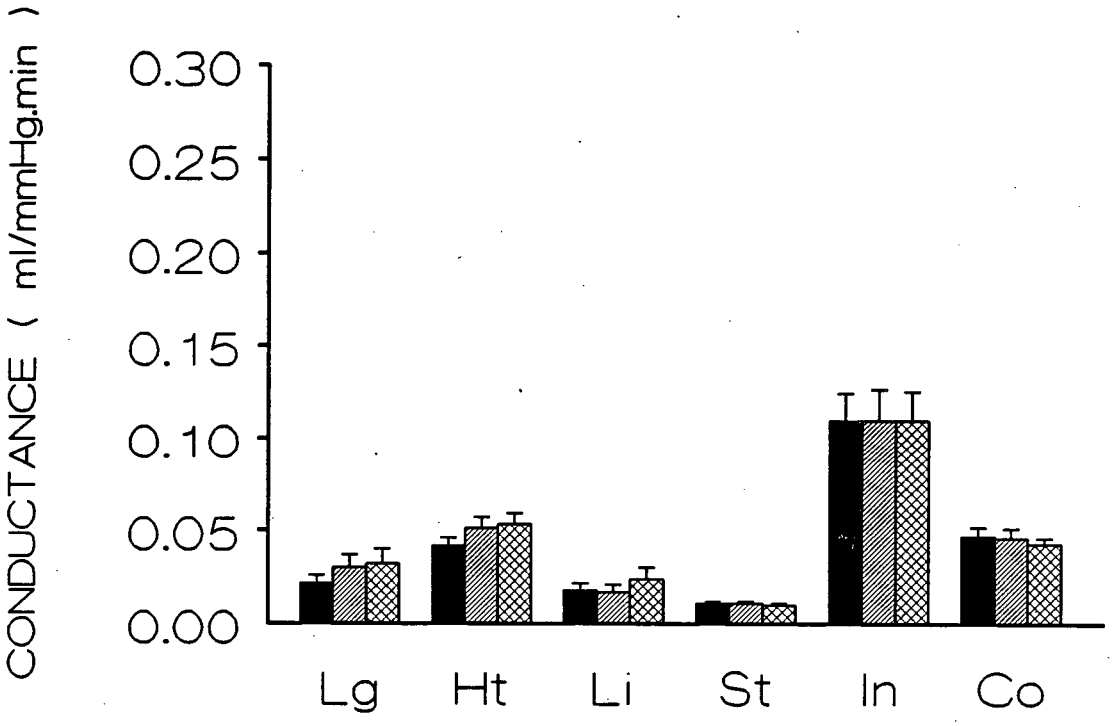

BLOOD FLOW (ml/min)
25
20
15
10
5
0
Ki
Sp
Mu
Sk
Te
Br

Fig. 31. Vascular conductance in pentobarbital-anaesthetized rats (group XLI, n = 6), during control conditions (closed bars), 10 min after the start of normal saline infusion (hatched bars) and 1 min after normal saline injection during the continuous infusion of normal saline (cross-hatched bars). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 30 g of skeletal muscle (Mu), 30 g of skin (Sk), testis (Te) and brain (Br).


CONDUCTANCE (ml/mmHg.min)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Lg
Ht
Li
St
In
Co

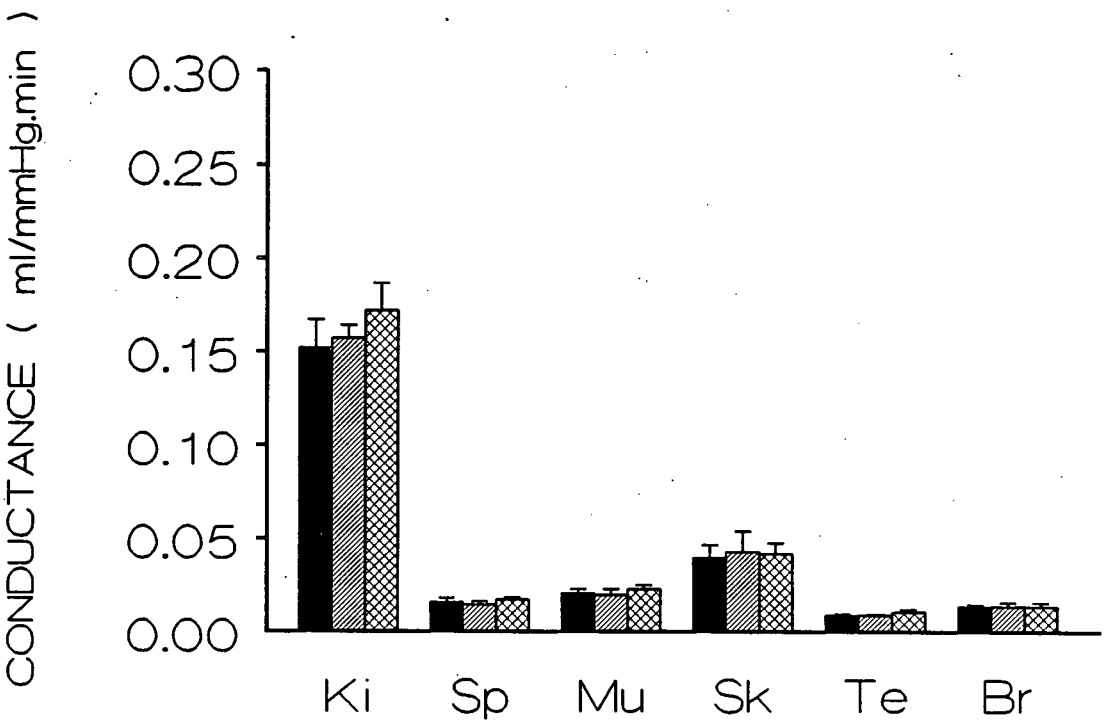
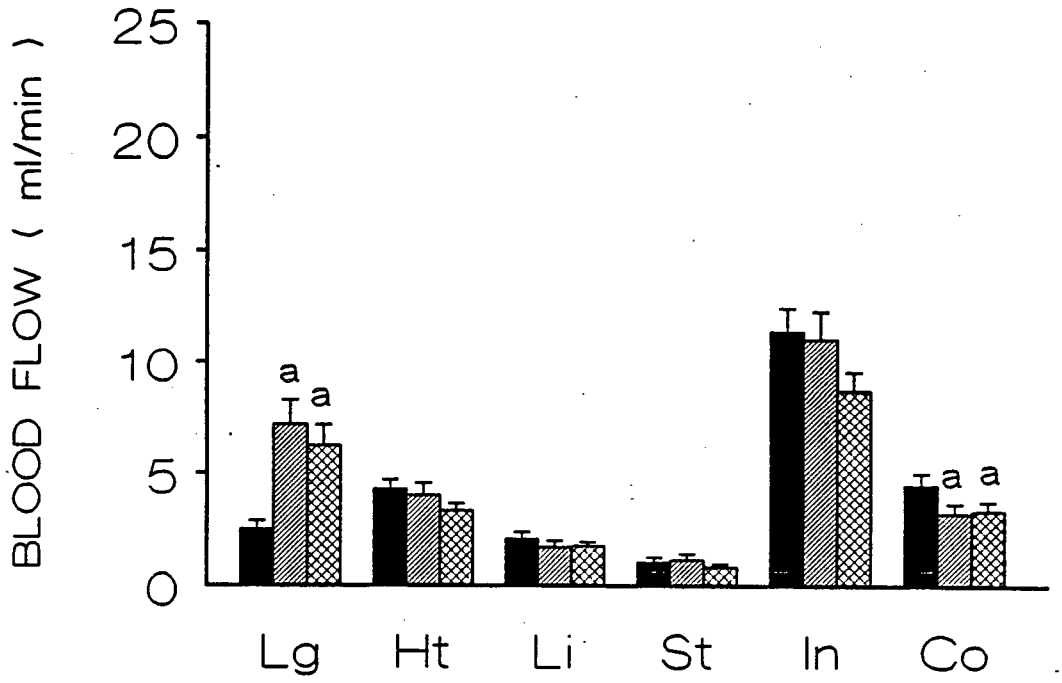

CONDUCTANCE (ml/mmHg.min)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Ki
Sp
Mu
Sk
Te
Br

Fig. 32. Distribution of blood flow in pentobarbital-anaesthetized rats (group XLII, n = 8), during control conditions (closed bars), 10 min after the start of phentolamine (300 μg/kg/min) infusion (hatched bars) and 1 min after propranolol (100 μg/kg) injection during the continuous infusion of phentolamine (cross-hatched bars). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 30 g of skeletal muscle (Mu), 30 g of skin (Sk), testis (Te) and brain (Br). [a]Significantly different from control ($p < 0.05$).


BLOOD FLOW (ml/min)
25
20
15
10
5
0
a
a
a
a
Lg
Ht
Li
St
In
Co

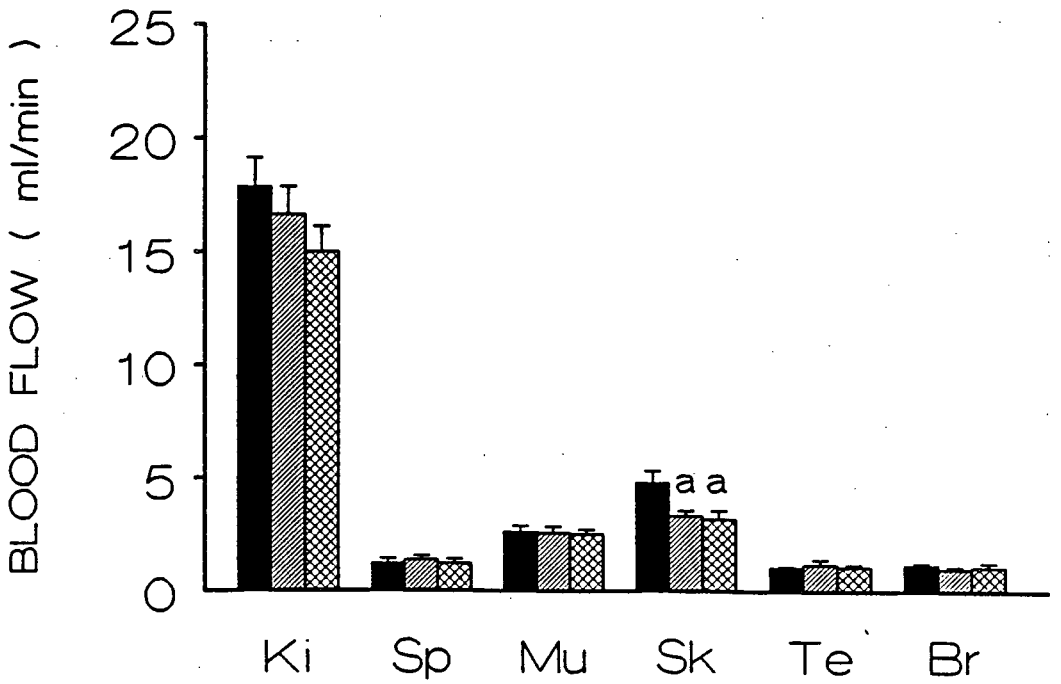
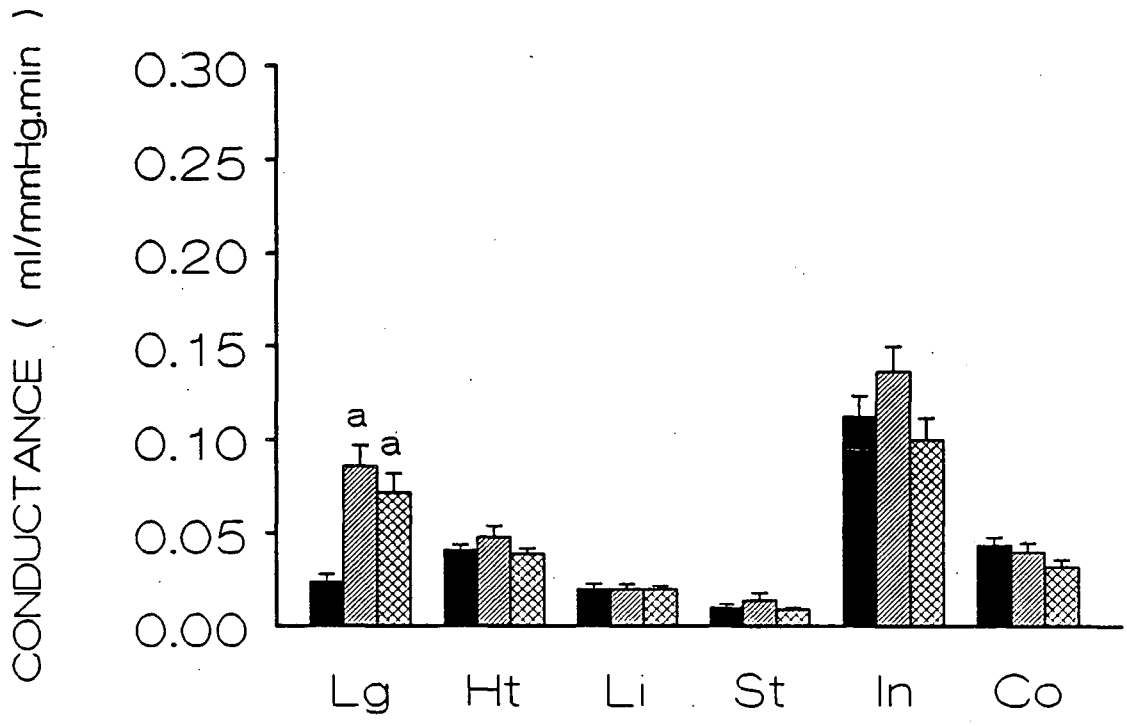
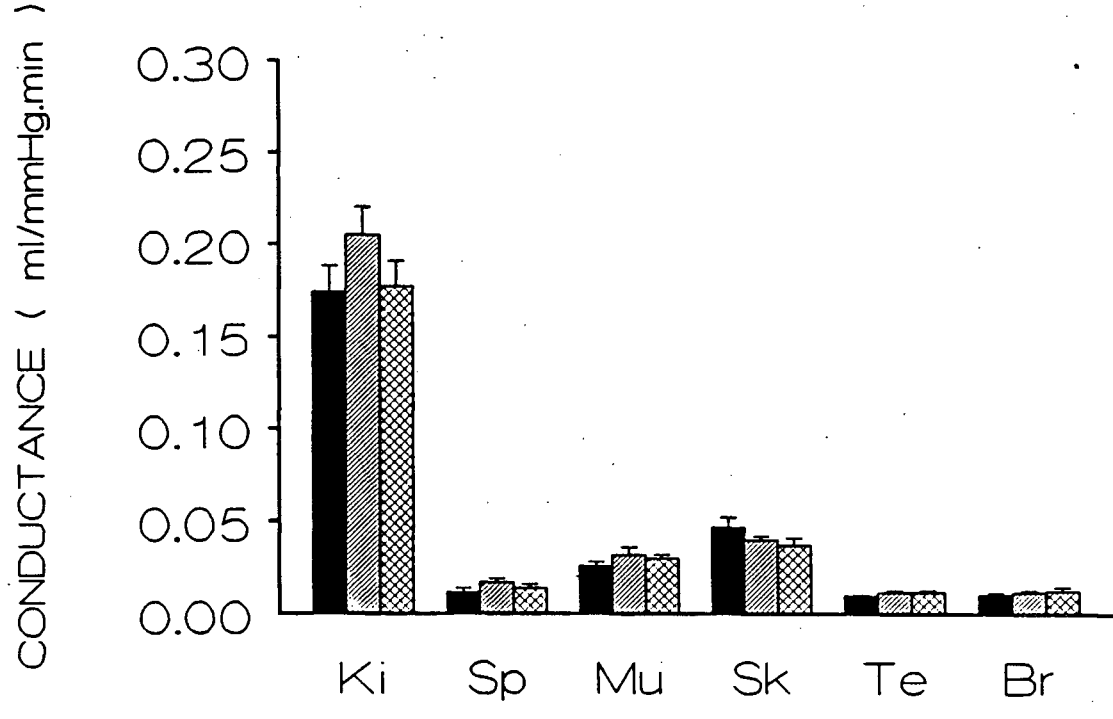

BLOOD FLOW (ml/min)
25
20
15
10
5
0
a
a
Ki
Sp
Mu
Sk
Te
Br

Fig. 33. Vascular conductance in pentobarbital-anaesthetized rats (group XLII, n = 8), during control conditions (closed bars), 10 min after the start of phentolamine (300 μg/kg/min) infusion (hatched bars) and 1 min after propranolol (100 μg/kg) injection during the continuous infusion of phentolamine (cross-hatched bars). Values are mean ± S.E. Organs or tissue samples are: lungs (Lg), heart (Ht), liver (Li), stomach (St), intestine (In), colon and caecum (Co), kidneys (Ki), spleen (Sp), 30 g of skeletal muscle (Mu), 30 g of skin (Sk), testis (Te) and brain (Br). [a]Significantly different from control ($p < 0.05$).


CONDUCTANCE (ml/mmHg.min)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
a
a
Lg
Ht
Li
St
In
Co


CONDUCTANCE (ml/mmHg.min)
0.30
0.25
0.20
0.15
0.10
0.05
0.00
Ki
Sp
Mu
Sk
Te
Br

XLV were, 106 ± 4, 105 ± 1 and 107 ± 2 mmHg, respectively. Captopril reduced MAP in all three groups, however, only the decrease in the second group reached statistical significance. Phentolamine reduced MAP in the three groups while subsequent injections of propranolol, atenolol and ICI 118,551 increased MAP; however, MAP in the three groups were not restored back to control levels prior to the infusion of phentolamine (Fig. 34). The pressor response to atenolol lasted for only 1 min after which MAP fell back to the phentolamine baseline value while those to propranolol and ICI 118,551 were sustained for the 20 min observation time before the termination of the experiment.

3.5. In vitro cumulative dose response curves of ß-adrenoceptor antagonists

In the three groups (XLVI, XLVII and XLVIII) of isolated rat pulmonary arteries, noradrenaline (10^{-6}M) caused an increase in force which was subsequently reduced by phentolamine and maintained for 50 min. After a full recovery of the response, noradrenaline again caused a similar increase in force which was similarly reduced by phentolamine. The subsequent addition of propranolol, ICI 118,551 and atenolol dose-dependently increased force (Fig. 36) with EC_{50} values of $4.1 \pm 0.3 \times 10^{-8}$, $1.2 \pm 0.3 \times 10^{-7}$ and $3.8 \pm 1 \times 10^{-7}$ M, respectively. Maximum forces developed in response to the ß-adrenoceptor antagonists were not significantly different from force produced by noradrenaline (Fig. 35).

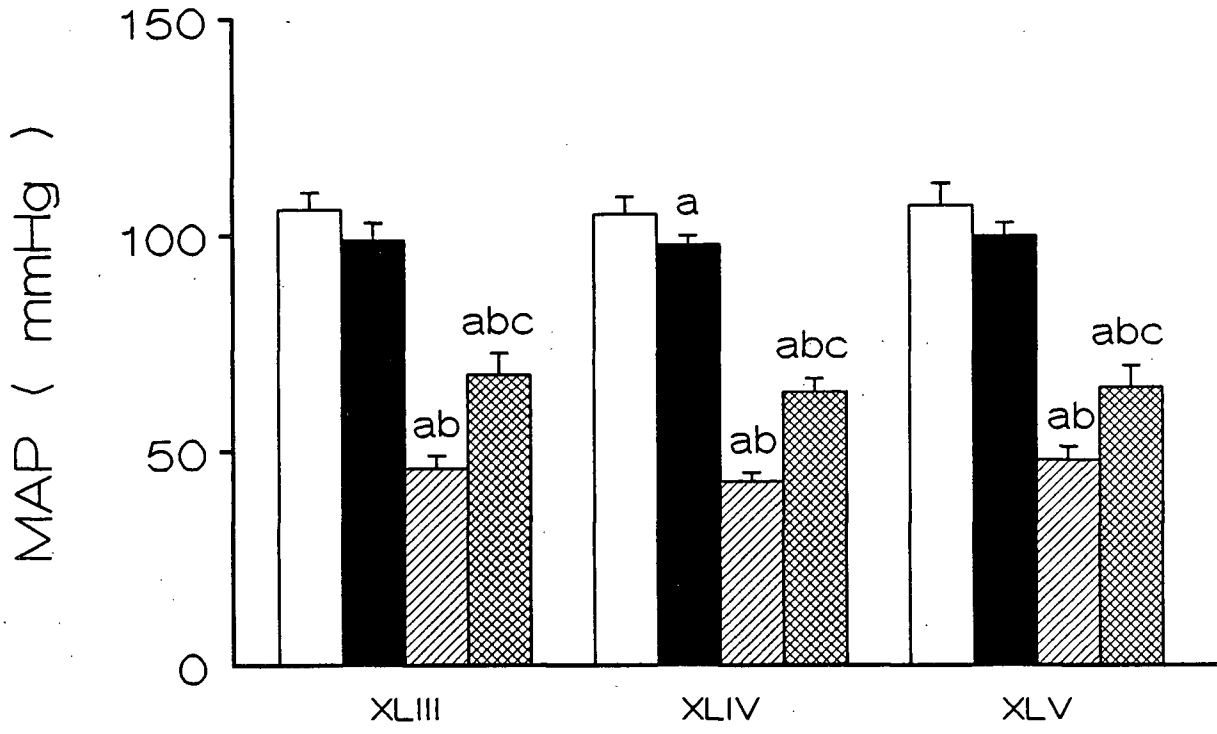


Fig. 34. Mean arterial pressure (MAP) in groups of conscious rats (n = 6 each) during control conditions (open bars), 10 min after the injection of captopril (5 mg/kg) (closed bars), 10 min after the start of phentolamine infusion (300 μg/kg/min) (hatched bars) and 1 min after the injection of propranolol (100 μg/kg), atenolol (100 μg/kg) or ICI 118,551 (30 μg/kg) during the infusion of phentolamine (cross-hatched bars) in groups XLIII, XLIV and XLV, respectively. [a]Significantly different from control ($p < 0.05$); [b]Significantly different from captopril ($p < 0.05$); [c]significantly different from phentolamine after captopril injection ($p < 0.05$).

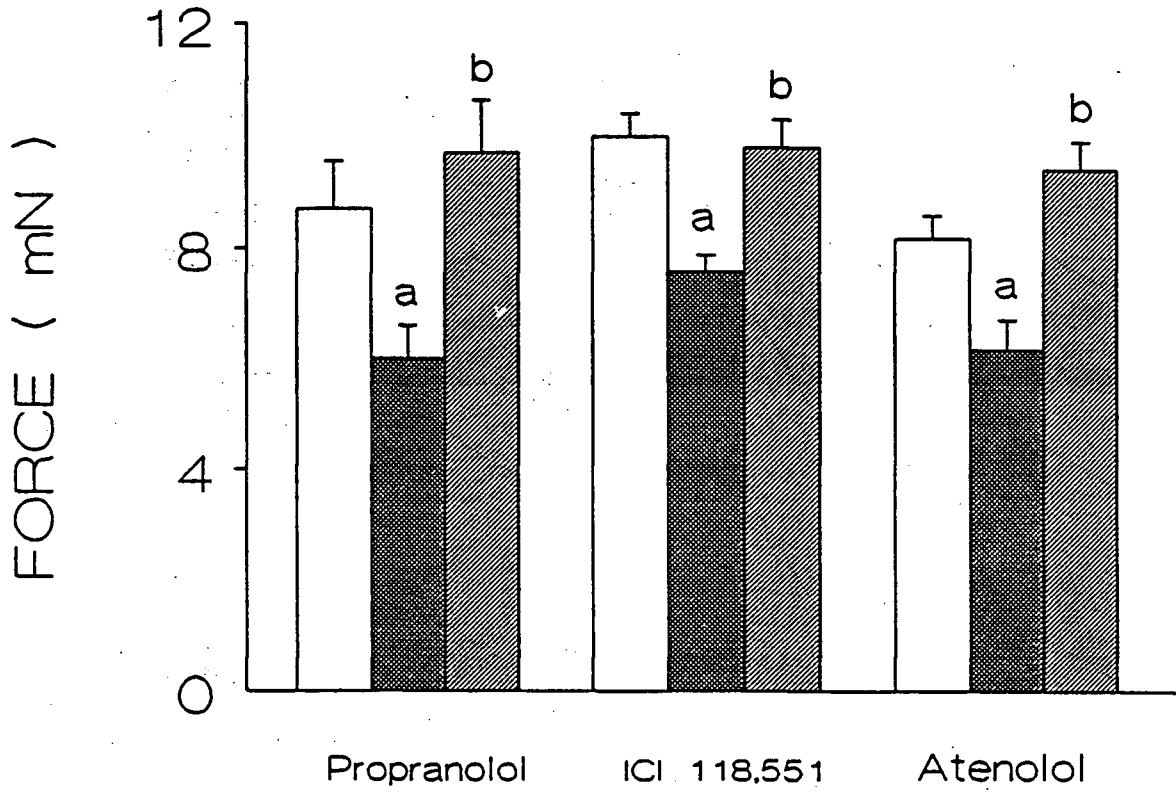


Fig. 35. Maximum developed force in isolated rat pulmonary artery (n = 6 each) in response to noradrenaline (10^{-6} M) (open bars), in the presence of phentolamine (10^{-6} M) (cross-hatched bars) and both phentolamine and ß-blockers (hatched bars). [a]Significantly different from control ($p < 0.05$); [b]Significantly different from phentolamine and noradrenaline ($p < 0.05$).

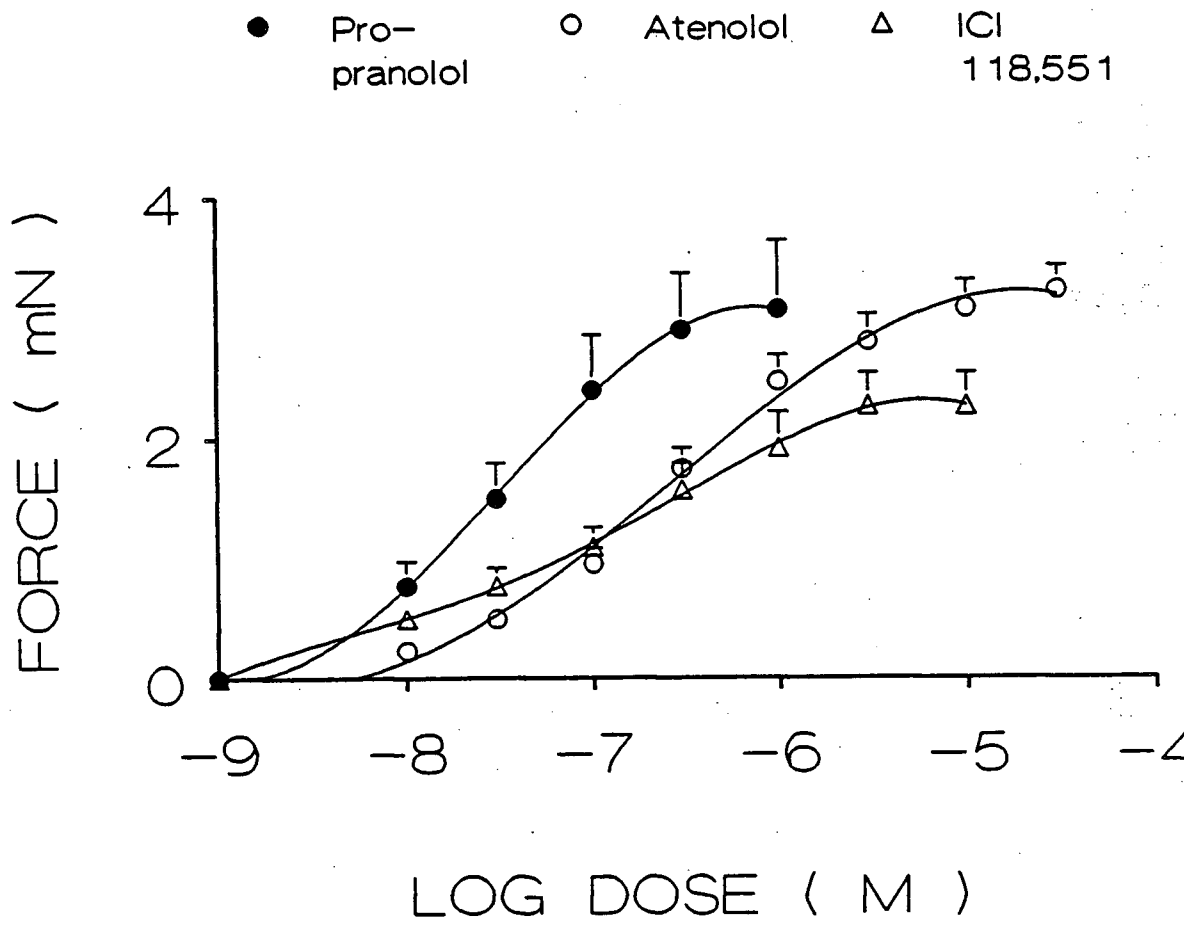


Fig. 36. Cumulative dose-response curves for propranolol, ICI 118,551 and atenolol on the isolated rat pulmonary artery in the presence of noradrenaline (10^{-6} M) and phentolamine (10^{-6} M).

4 DISCUSSION

4.1. Selectivity of atenolol and ICI 118,551

ICI 118,551 (30 μg/kg) shifted the dose-response curve for the vasodepressor effect to salbutamol but had no effect on the chronotropic response to dobutamine. Atenolol (100 μg/kg), on the other hand, shifted the dose-response curve for the chronotropic effect of dobutamine and had no effect on the vasodepressor action of salbutamol. Furthermore, ICI 118,551 completely abolished the vasodepressor and atenolol completely abolished the chronotropic effect of isoprenaline, indicating the effectiveness of blockade of $ß_2$- and $ß_1$-adrenoceptors by ICI 118,551 and atenolol, respectively.

4.2. Role of $ß_1$- and $ß_2$-adrenoceptors in the vasculature

Infusion of the saline did not affect haemodynamics, indicating the reproducibility of the dual-isotope microsphere technique used in these experimental conditions.

Infusion of the low dose of isoprenaline significantly increased HR and tended to decrease MAP and TPR and increase CO; however the latter changes were small and not statistically significant. Although CO and TPR were not significantly affected, distribution of regional blood flow was altered, with flow markedly increased in the muscle bed and slightly but not significantly increased in the coronary bed. Vascular conductances in both the muscle and coronary beds were significantly elevated suggesting the relative

importance of ß-adrenoceptors in the skeletal muscle and coronary vasculature. Vascular conductance was not significantly changed in the lungs, liver, gastrointestinal tract, testis and brain suggesting the relative lack of ß-adrenoceptors in the vascular beds in these sites. There was a tendency for conductance in the kidneys and skin to decrease, perhaps as a result of a compensatory increase in vasomotor influence to maintain MAP.

When isoprenaline was infused into rats pretreated with ICI 118,551 to obtund $ß_1$-adrenoceptor stimulation, HR was increased to an extent similar to that in rats that received isoprenaline only and MAP, CO and TPR also were not affected. Blood flows and conductances in coronary and muscle beds were increased. The increase in muscle vascular conductance was considerably less than that in rats that received isoprenaline only, suggesting that $ß_1$-adrenoceptor stimulation played a minor role in the vasodilator effect of isoprenaline in the muscle bed. Since $ß_1$-adrenoceptor stimulation caused both chronotropic and coronary dilator effects, it is not clear whether the increase in coronary conductance was entirely secondary to increased metabolic requirements or, in addition, there was a direct vasodilator effect on $ß_1$-adrenoceptors in the coronary vasculature. Flow and conductances in other vascular beds were not affected by $ß_1$-adrenoceptor stimulation.

The infusion of isoprenaline in in the presence of ateolol to obtund $ß_2$-adrenoceptor stimulation decreased MAP

and TPR but did not affect HR and CO. Since the previous infusion of isoprenaline alone at the same rate did not significantly reduce MAP and TPR, this suggests that blockade of the cardiostimulatory effect of isoprenaline by atenolol unmasked the depressor effect of isoprenaline. In the atenolol-treated rats that received isoprenaline, there was increased blood flow and conductance in the muscle beds, whereas only conductance was increased in the coronary bed; the magnitudes of these increases were similar to those in rats that received isoprenaline only. Flow and conductance in other organs and tissues were not significantly affected. Therefore, our results suggest that only $ß_2$-adrenoceptors play a major role in the vasodilator effect of isoprenaline.

The infusion of isoprenaline in rats that received both ICI 118,551 and atenolol did not significantly affect MAP, TPR, CO and HR. There was, however, a tendency for CO to decrease and TPR to increase. The slight increase in vasomotor tone after nonselective blockade of ß-adrenoceptors is possibly due to the antagonism of the effects of endogenously-released catecholamines on ß-adrenoceptors. There were reductions in blood flows and vascular conductances in the kidneys, colon and caecum. Increase in flow and conductances in the coronary or skeletal muscle beds were no longer evident. This confirms that the slight increase in skeletal muscle blood flow after the infusion of isoprenaline in the presence of ICI 118,551 described previously was most probably due to the activation

of β_1-adrenoceptors and not due to an unblocked residual β_2-adrenoceptor stimulation.

To summarize, the vascular beds most sensitive to the actions of isoprenaline are those of the coronary and skeletal muscle. The vasodilator effects of isoprenaline in these two beds are mediated via the activation of both β_1- and β_2-adrenoceptors.

Other studies have shown the presence of β_1-adrenoceptors in the vascular smooth muscles of different beds such as the canine renal vasculature (Taira et al., 1977), rat pulmonary artery (O'Donnell and Wanstall, 1981a), cat cerebral vessels (Edvinsson and Owman, 1974), rat jugular vein (Cohen and Wiley, 1978), rat aorta (O'Donnell and Wanstall, 1984a), rat femoral and mesenteric arteries (Fujimoto et al., 1988) and the vasculature supplying the adipose tissue (Belfrage, 1978).

In anaesthetized cats treated with phentolamine, vasodilatatory responses to noradrenaline as well as to the selective β_1-adrenoceptor agonists oxymethyleneisoprenaline and RO 363 were blocked by atenolol while vasodilator responses to adrenaline were blocked by butoxamine. This shows that in the cat, activation of either β_1- or β_2-adrenoceptors mediates vasodilatation (McPherson et al., 1981). In phentolamine-treated conscious dogs, the administration of noradrenaline or endogenously-released noradrenaline elicited peripheral vasodilatation which was blocked by

atenolol suggesting that β_1-adrenoceptors mediate vasodilatation under these conditions (Vatner et al., 1985).

In the coronary vasculature, most in vitro experiments show the presence of β_1- but not β_2-adrenoceptors (O'Donnell and Wanstall, 1984b; Toda and Okamura, 1990; Baron et al., 1972; Drew and Levy, 1972; Nakane et al., 1988; Johansson, 1973; Delande et al., 1974; Purdy et al., 1988). Since large coronary arteries were used in all these studies, the results may not be indicative of responses in resistance blood vessels. Nyborg and Mickkelson (1985) studied ß-adrenoceptor subtypes in isolated rat intramyocardial resistance arteries (i.d. = 200 μm) and they concluded that β_1-adrenoceptors were present in these vessels. Binding studies (Schwartz and Velly, 1983; Vatner et al., 1986; Nakane et al., 1988) suggested the presence of both subtypes of ß-adrenoceptive sites in the coronary vasculature.

Most in vivo studies suggested the presence of β_2-adrenoceptors in the coronary vasculature (Feigl, 1983). However, there is a technical problem with the use of beating hearts for these studies since they were incapable of demonstrating the presence of coronary β_1-adrenoceptors, because β_1-adrenoceptor stimulation increases myocardial work thereby causing metabolic coronary vasodilatation. Lucchesi and Hodgeman (1971) used both isoprenaline and calcium chloride to increase coronary blood flow and they attributed the difference between the increases in coronary

blood flow induced by the two agents to be the result of direct stimulation of coronary ß-adrenoceptors by isoprenaline. Since the selective $ß_1$-adrenoceptor blocker practolol abolished the isoprenaline effect, the authors concluded that $ß_1$-adrenoceptors were present only in the coronary vasculature. However, Jackson et al. (1987), using the same method in which calcium chloride is compared to a ß-adrenoceptor agonist (but with a more selective $ß_1$-blocker celiprolol) showed that the isoprenaline-mediated increase in coronary blood flow was attenuated but not completely blocked by celiprolol while that of noradrenaline was completely blocked by celiprolol. Although, celiprorol is known to have $ß_2$-agonistic effect (Taylor, 1988), the authors used a dose (0.3 mg/kg) which was shown to have no effect on zinoterol (selective $ß_2$-agonist)-induced increase in hindlimb blood flow indicating that this dose of celiprolol has no effect on $ß_2$-adrenoceptors. The authors proposed the presence of both $ß_1$- and $ß_2$-adrenoceptors in the coronary vasculature. The use of calcium chloride as an inotropic agent was criticized as it has direct vasoconstrictor in addition to inotropic effects (Mark et al., 1972). Arrested heart preparations were used to separate the increase in coronary blood flow due to metabolic effects from that due to direct stimulation of coronary ß-adrenoceptors. However, there is a drawback of this preparation due to its unphysiological condition. It was concluded from the result of one study using potassium

chloride arrested hearts that β_2-adrenoceptors predominate in the coronary vasculature (Gross and Feigl, 1975). Trivella et al. (1990), using a canine nonbeating atrioventricular-blocked cardiac preparation to determine coronary blood flow during prolonged asystoles after stopping cardiac pacing, concluded that both β_1- and β_2-adrenoceptors are present in the coronary vasculature.

Discrepancies in experimental findings between different laboratories may have resulted from the use of different species of animals and different methodologies, with *in vitro* and *in vivo* results indicative of responses in large arteries and small resistance vessels, respectively. Because small vessels are important determinants in blood flow distribution it may not be valid to use results from *in vitro* studies which use large arteries to indicate responses in resistance blood vessels. In addition, *in vitro* results may depend on whether or not care was taken to preserve the intimal surface of the vasculature during preparation, as evidence shows that the arterial endothelium may contribute to ß-adrenoceptor-mediated relaxation response (Rubanyi and Vanhoutte, 1985).

4.3. Role of ß-adrenoceptors in the venous system

We examined the effect of isoprenaline under different experimental conditions, namely, basal conditions, after ganglionic blockade with hexamethonium, and after the elevation of vascular tone with the infusion of

noradrenaline. In the three control groups, there was a consistent but small gradual decline of MCFP. This has been observed previously (Waite et al., 1988; D'Oyley et al., 1989). In the intact rat, isoprenaline decreased MAP, and increased both HR and MCFP. The increase in MCFP was more prominent when the readings were compared with those of the time control group. To determine if the venoconstrictor effect was mediated by a direct effect, or via reflex sympathetic activation, isoprenaline was also given to rats previously treated with hexamethonium so as to attenuate autonomic nerve activity. We selected to use a dose of hexamethonium which inhibited the tachycardic response to acetylcholine by >50%. This regimen was chosen to allow for a degree of venous tone in order that further venodilatation could be seen. In accordance with published results (D'Oyley and Pang, 1990), hexamethonium reduced both MAP and MCFP but had no significant effect on HR. Under these conditions, isoprenaline again dose-dependently increased HR and reduced MAP but failed to have any significant effect on MCFP. This indicated that the constrictor effect of isoprenaline on venous tone in the intact rat was indeed a consequence of reflex activation of the sympathetic nervous system. There was, however, a small but insignificant increase in MCFP at the highest isoprenaline dose level possibly as a result of incomplete ganglionic blockade. The infusion of noradrenaline significantly increased MAP and MCFP while slightly but insignificantly reduced HR. It

has been shown that the infusion of noradrenaline into rats pretreated with propranolol caused dose-dependent increases in MAP as well as MCFP and reduced HR (Pang and Tabrizchi, 1986). This suggests that the lack of a significant bradycardic response in the present study was due to the opposing influence of direct β_1-adrenoceptor stimulation. Under the condition of elevated vascular tone, the infusion of isoprenaline increased HR but reduced both MAP and MCFP.

In summary, in intact rats isoprenaline increases MCFP by an indirect effect, i.e., hypotension-induced venoconstriction. The direct effect of isoprenaline is dependent on the initial venous tone so that after ganglionic blockade, isoprenaline had no venodilator effect while in conditions of high venous tone isoprenaline produced venodilatation.

In <u>in vitro</u> venous preparations such as the rabbit portal vein (Sutter, 1965; Hughes and Vane, 1967), rat jugular vein (Duckles and Hurlbert, 1986) and canine saphenous vein (Guimaraes and Osswald, 1969), isoprenaline, at low doses, caused relaxation. Therefore, our <u>in vivo</u> results showing direct venodilator responses to isoprenaline are in accordance with the results of low doses of isoprenaline in <u>in vitro</u> studies.

Our results are also in accordance with studies on perfused vascular beds where the intraarterial injection of isoprenaline caused small dilator responses in the veins of the paw and muscle of the foreleg of dog (Abboud et al.,

1965). The venodilator effects were enhanced when isoprenaline was injected in the presence of venous constriction induced by the intraarterial infusion of noradrenaline. Webb-Peploe and Shepherd (1969), using dog perfused lateral saphenous veins, showed that the effect of isoprenaline is proportional to the initial degree of vein wall tension. This conclusion was based on the findings that after sympathectomy, isoprenaline had no dilator effect, while during venoconstriction produced by either electrical stimulation of the lumbar sympathetic chain or infusion of venoconstrictor drugs as serotonin, potassium chloride, noradrenaline and adrenaline, isoprenaline produced venodilatation.

Our results are not in accordance with the experiments in dogs which showed that isoprenaline increases venous return by stimulating ß-adrenoceptors. Kaiser et al. (1964), using an in vivo preparation consisting of complete cardiopulmonary bypass surgery and a bubble oxygenator, showed that isoprenaline caused a displacement of blood from the vasculature of a dog to the oxygenator which denotes venoconstriction. Since the venoconstriction was blocked by the nonselective ß-adrenoceptor antagonist nethalide, but not by hexamethonium, the authors concluded that ß-adrenoceptors mediated venoconstriction. An alternative explanation for the ß-adrenoceptor increase in venous return was proposed by Green (1977), who suggested that isoprenaline dilated the hepatic outflow vessels thereby

reducing the splanchnic venous time constant and the effective splanchnic back pressure resulting in the release of splanchnic blood volume for redistribution to other areas of the systemic circulation. Our results are also not in agreement with those of Imai et al. (1978) who used an open-loop method in dogs with cardiac output held constant. In these experiments, isoprenaline increased the venous return; the increase was not blocked by the interference of sinoaortic baroreceptor reflex but was completely abolished by propranolol. They concluded that ß-adrenoceptors mediated the increase in venous return by decreasing venous resistance.

The drawback of experiments using the open loop reservoir system was reviewed by Greenway (1982) who suggested that drug induced-changes in the reservoir volume (unstressed volume) could be due to changes in arterial or venous resistances, heart rate and contractility in addition to changes in venous compliance. Therefore, although it is possible to determine that there is a change in reservoir volume, it is not possible to determine which factor caused the effect. He also warned that extracorpreal circuits may modify drugs effects.

Our results are also not in accordance with the results of another study in conscious dogs with cardiac output maintained constant via the production of atrioventicular block and alteration of ventricular rate to compensate for changes in stroke volume and in which isoprenaline was found

to reduce MAP and increase central venous pressure (Bennett et al., 1984). However, since autonomic function was not interfered with, changes in central venous pressure could be a result of reflex mechanisms. In ganglionic blocked sedated dogs, terbutaline, a selective β_2-agonist, did not affect MCFP but it reduced venous compliance (Lee et al., 1984). The authors concluded that β_2-adrenoceptors mediate venoconstriction.

Hirakawa et al. (1984) showed that in anaesthetized and open-chest dogs, a single dose of isoprenaline did not produce any changes in MCFP under normal conditions but caused venodilatation after venous tone was increased with infusion of angiotensin II. Our observations also illustrate the ability of isoprenaline to dilate veins in conditions of elevated vascular tone. Rothe et al. (1990) also showed that in anaesthetized dogs, isoprenaline had little effect on MCFP. The lack of reflex venoconstrictor effect of isoprenaline at normal tone may be a consequence of modulation by pentobarbital of autonomic reflex mechanisms. Pentobarbital has been shown to decrease plasma catecholamine levels, rectal temperature, MAP and HR in dogs suggesting that the drug suppresses activities of the sympathetic nervous system (Baum et al., 1985). When given i.p. into conscious rats, pentobarbital lowered MCFP (Samar and Coleman, 1978), suggesting that it reduced sympathetic tone to the venous system. In whole animal experiments, isoprenaline may cause variable venous effects, depending on

the methodologies used for the estimation of venous responses, experimental conditions, presence or absence of anaesthetic agents and species of animals. Therefore, interpretations of results are difficult due to the complexity of cardiovascular control mechanisms in the intact animals.

In humans, when isoprenaline was injected locally in the forearm vein at a dose which did not produce systemic effects, there was venodilatation (Beck et al., 1970). When infused i.v. at doses which produce systemic effects, isoprenaline constricted the human forearm vein (Eckstein and Hamilton, 1959) suggesting that the venoconstrictor response of isoprenaline was likely mediated by reflex mechanisms rather than direct stimulation of ß-adrenoceptors on venous smooth muscle. Venous return changes have been inferred from changes in left-ventricular end-diastolic dimension (LVED) determined by echocardiography. By this method, it was shown that isoprenaline, adrenaline and terbutaline directly while hydralazine indirectly (by increasing endogenous sympathetic nerve activity) increased venous return by stimulation of ß-adrenoceptors in the veins. This study concluded that adrenaline mediates ncreases in venous return mainly by stimulating $ß_2$-adrenoceptors and to a lesser extent by stimulating $ß_1$-adrenoceptors. In contrast, endogenous sympathetic activity appears to increase venous return primarily via activation of $ß_1$-adrenoceptors (Leenen and Reeves, 1987). However, two

other possible mechanisms were given to account for this increase. First, ß-adrenoceptor stimulation may increase LV compliance and this may increase LVED. Secondly, ß-adrenoceptor stimulation may change respiration and intrathoracic pressures thereby increasing venous return. As reviewed by Greenway (1982), venous return (i.e, cardiac output) is affected by venous compliance as well as other factors which include cardiac contractility, HR, arterial and venous resistances. Therefore, it is not valid to use changes in venous return to reflect changes in venous compliance.

4.4. Pressor response to ß-adrenoceptor antagonists in phentolamine-treated rats

4.4.1. Haemodynamic changes

In urethane-anaesthetized rats, baseline blood flow to the skeletal muscle was markedly greater than the corresponding readings in conscious rats, halothane-anaesthetized (Tabrizchi and Pang, 1987) and pentobarbital-anaesthetized rats. This suggests that urethane anaesthesia enhances blood flow to the skeletal muscle bed. Urethane anaesthesia has been shown to block α_2-adrenoceptors (Armstrong et al., 1982). Since it has been shown that either α_1- or α_2-adrenoceptor blockade in the halothane-anaesthetized rats increased the percent distribution of CO to the skeletal muscle bed (Tabrizchi and Pang, 1987), it might be reasoned that urethane increased flow in the

skeletal muscle bed via the blockade of postjunctional α_2-adrenoceptors.

The infusion of phentolamine in urethane-anaesthetized rats decreased MAP primarily by reducing TPR. CO was slightly but not significantly decreased while HR was not altered by phentolamine. Blood flow was decreased in the liver, stomach, colon and caecum, and kidney. This reduction of blood flow was probably a consequence of decreased perfusion pressure caused by phentolamine since conductance was not decreased in any of the named organs. Conductance was significantly increased by phentolamine in skeletal muscle and skin beds suggesting that α-adrenoceptors are important in mediating a resting vasoconstriction in these beds.

In phentolamine-treated rats, propranolol increased MAP by increasing TPR. Blood flow to the lungs, heart, liver, intestine, colon and caecum, kidneys and spleen was increased while flow to the skeletal muscle was decreased. Since MAP was also increased, the increase of flow in some beds may reflect passive changes secondary to the increase in perfusion pressure. Normalization of flows to conductances shows decreases in skeletal muscle, skin and kidneys and no changes in other vascular beds. The decreases in muscle and skin conductances produced by propranolol are in accordance with the reversal of the vasodilator effect of phentolamine in these beds. The decrease in conductance in the kidney bed suggests that

changes in addition to the reversal of α-adrenoceptor blockade also may have taken place.

In conscious rats, phentolamine decreased MAP by reducing TPR. HR was increased probably via hypotension-induced reflex changes in autonomic nerve activities. Blood flows were decreased in the stomach, kidneys and spleen. The reduction of blood flow in these vascular beds was probably a consequence of decreased perfusion pressure since vascular conductance was not decreased in any organ or tissue. Blood flows and vascular conductances were increased in the lungs, heart and skeletal muscle. This indicates that α-adrenoceptors are most important in these vascular beds although the increase in the coronary blood flow was also due to the increase in metabolic demands as a result of the reflex increase in heart rate caused by phentolamine.

The increase in flows and conductances to the lungs and skeletal muscle are in accordance with the effects of phentolamine on the distribution of CO in halothane-anaesthetized rats (Tabrizchi and Pang, 1987). It is important to note that the number of microspheres found in the lungs is the sum of spheres trapped in the bronchial circulation and those reaching the venous side of the circulation through the arteriovenous anastomoses. Bronchial blood flow is very small and large changes in the bronchial flow should not affect greatly the number of microspheres in the lungs (Hof and Hof, 1989). Therefore our results suggest that

phentolamine vasodilates the arteriovenous anastomoses vessels which are in accordance with results in the dog leg showing that phentolamine increased arteriovenous flow in that bed (Spence et al., 1972).

In phentolamine-treated conscious rats, propranolol increased MAP by increasing TPR since CO was not altered. HR was reduced as a result of the blockade of β_1-adrenoceptors. Blood flow to the lungs was increased while flow to the skeletal muscle was decreased. The increase in blood flow to the lungs was related to passive changes secondary to the increase in MAP since vascular conductance in the lungs was not altered. Conductances were decreased in the heart, intestine, kidneys, skeletal muscle and skin beds. In phentolamine-treated rats, atenolol produced a pressor response which was of similar magnitude to that produced by propranolol. MAP was also increased by the elevation of TPR since CO was not altered. HR was reduced due to the blockade of β_1-adrenoceptors. Blood flow to the lungs, intestine, colon and caecum, spleen, testis and brain was increased while skeletal muscle blood flow was reduced. The increase in blood flow was due to passive changes secondary to the increase in MAP since conductances in these beds were not altered. Vascular conductance was reduced by atenolol in the intestine, kidneys, skeletal muscle and skin beds. It is important to note that the dose of atenolol used in the present study is selective for the blockade of β_1-adrenoceptors since it has been shown that atenolol

(100 μg/kg) did not shift the ED_{50} value of salbutamol for lowering MAP in conscious rats (Tabrizchi et al., 1988). Therefore, selective blockade of $ß_1$-adrenoceptors by atenolol and nonselective blockade by propranolol produced pressor responses of similar magnitudes and constrictions of intestine, kidneys, skeletal muscle and skin vascular beds. The failure of both atenolol and propranolol to decrease lung flow and conductance is most likely due to the lack of control of the arteriovenous anastomoses by ß-adrenoceptors (Spence et al., 1972). Coronary conductance was reduced by propranolol but not by atenolol. Our results suggest that $ß_2$-adrenoceptors are important in the mediation of vasodilatation in the coronary vasculature and confirm our previous results on the effects of isoprenaline on coronary conductance. They are in agreement with those of in vivo studies as reviewed by Feigl (1983). In contrast to our results on coronary flow, Vatner and Hintze (1983) reported that propranolol and atenolol produced similar degrees of coronary vasoconstriction in phentolamine-treated dogs. The use of different species of animals, doses of drugs and experimental conditions may account for the different observations on coronary flow with selective $ß_1$- and nonselective ß-blockade.

It has been speculated that the pressor effect caused by a ß-adrenoceptor antagonist was due to the antagonism of $ß_2$-adrenoceptor-mediated vasodilatation (Kayaalp and Turker, 1979; Yamamoto and Sekiya, 1969; Himori et al., 1984;

Himori and Ishimori, 1988). However, previous studies in our laboratory have shown that the injection of very small doses of either atenolol or ICI 118,551 into the conscious rat caused similar pressor response (Tabrizchi et al., 1988). This suggests that the blockade of vasodilator β_2-adrenoceptors is not the mechanism of this pressor response. Our haemodynamic studies in conscious rat confirm that the pressor response to ß-adrenoceptor antagonists is indeed not due to the antagonism of β_2-adrenoceptor-mediated vasodilatation since atenolol and propranolol produced similar haemodynamic effects.

In urethane-anaesthetized rats, propranolol reversed the vasodilator effect of phentolamine in the skin and skeletal muscle and in addition, it vasoconstricted the kidneys. In conscious rats, either propranolol or atenolol reversed the vasodilator effect of phentolamine mainly in the skeletal muscle but in addition, both drugs vasoconstricted the kidneys, intestine and skin. This shows that the skeletal muscle vasculature is the most-affected bed. This may also explain the observation that in urethane-anaesthetized rats where the skeletal muscle blood flow is high, propranolol produced a pressor response with or without the infusion of phentolamine (Yamamoto and Sekiya, 1979; Regoli, 1970). However, in conscious rats, pretreatment with phentolamine is needed to produce a pressor response (Tabrizchi et al., 1988). Tabrizchi and Pang (1989) also showed that propranolol did not produce a pressor response in conscious

rats pretreated with either sodium nitroprusside or the cholinergic agonist methacholine suggesting that prior α-adrenoceptor blockade is needed for the pressor response to a ß-adrenoceptor antagonist. However, it is not clear why conductance was decreased in the kidneys, skin and intestine with the ß-adrenoceptor antagonists although phentolamine did not increase conductance in these beds. It is likely that during the infusion of phentolamine, other endogenous vasopressor agents are released to oppose the vasodilator effect of phentolamine. Phentolamine was shown to increase the secretion of catecholamines (Tabrizchi and Pang, 1987) and renin (Keeton and Campbell, 1981). Activation of the renin-angiotensin system may oppose the vasodilatatory effects of phentolamine (Gardiner and Bennett, 1988). It has been shown that angiotensin II exerts the most prominent vasoconstrictor effects in the kidneys and skin (Pang, 1983). It is possible that phentolamine did vasodilate the kidneys and skin bed, however, these effects were concealed by the vasoconstrictor effects of the endogenously-released angiotensin II. Therefore, it is possible that the vasoconstrictor effect of the ß-adrenoceptor antagonists in the kidneys and skin beds also were due to antagonism of vasodilator effects of phentolamine (presumably due to unopposed vasoconstrictor action of angiotension II in these beds).

To explore the possible role of the renin-angiotensin system in the pressor response to ß-adrenoceptor

antagonists, captopril was injected in three groups of rats before the start of phentolamine infusion and the subsequent injection of ß-adrenoceptor antagonists propranolol, ICI 118,551 and atenolol. Since MAP was reduced more by phentolamine in the presence of captopril than in its absence in all the groups, it suggests that the renin-angiotensin system was involved with opposing the direct vasodilator effect of phentolamine. In the absence of the renin-angiotensin system all three ß-adrenoceptor antagonists produced pressor responses but MAPs were not restored to the initial prephentolamine control value. This confirms the importance of the renin-angiotensin system in the production of the pressor response.

4.4.2. Effects of anaesthetic agents

In urethane-anaesthetized rats pretreated with phentolamine, i.v. bolus doses of propranolol, atenolol or ICI 118,551 each produced a dose-dependent increase in MAP which restored MAP to control values. However, in halothane-anaesthetized rats pretreated with phentolamine, the injection of propranolol or atenolol did not produce a pressor response. On the other hand, ICI 118,551 caused a small dose-dependent increase in MAP. The maximum rise was only 25% of that in conscious rats (Tabrizchi et al., 1988; Tabrizchi and Pang, 1989) and urethane-anaesthetized rats in the present study. It has been shown that phentolamine decreases MAP in halothane-anaesthestized rats by reducing

cardiac output and not total peripheral resistance (Tabrizchi and Pang, 1987). It is therefore conceivable that the lack of pressor response with propranolol and atenolol in phentolamine-treated halothane-anaesthetized rats is related to the possible further reduction of cardiac output via the blockade of β_1-adrenoceptors. In addition, halothane has been reported to attenuate the pressor response to both phenylephrine and azepexole in dogs (Kenny et al., 1990) and to block the pressor response to N^G-nitro-L-arginine in rats (Wang et al., 1991). Halothane also reduced the amplitude of oscillations produced by noradrenaline in the rat isolated mesenteric vein and this was attributed to the inhibition of calcium release from the sarcoplasmic reticulum (Marijic et al., 1990). This anaesthetic agent also reduced phenylephrine-induced contraction in isolated rat aorta (Sprague et al., 1974) and serotonin- and acetylcholine-induced contraction in endothelium free porcine coronary artery and this was explained to be due to the inhibitory effect of halothane on agonist induced-inositol phosphate formation (Ozhan et al., 1990). Su and Zhang (1990) showed that halothane decreased tension development in the intact aortic ring due to combined effects of a depression of Ca^{2+}-induced activation of the contractile proteins and a decrease of sarcoplasmic reticulum Ca^{2+} accumulation leading to reduced Ca^{2+} release for muscle contraction. Therefore, it is possible that non-specific inhibition of Ca^{2+}-release is responsible for the

the inhibition of pressor response to ß-adrenoceptor antagonists.

In pentobarbital-anaesthetized, phentolamine-treated rats, all ß-adrenoceptor antagonists failed to produce a pressor response. Pentobarbital has been shown to decrease plasma catecholamine levels in rats (Farnebo et al., 1979) and dogs (Zimpfer et al., 1982; Baum et al., 1985). Holmes and Schneider (1973) reported that pentobarbital reduced acetylcholine-induced catecholamine release in the isolated bovine chromaffin vesicles. The mechanism may involve the interruption of a link between receptor activation and catecholamine release. In order to examine whether or not catecholamines affect the pressor response to ß-adrenoceptor antagonists, adrenaline was infused into two additional groups of rats. The infusion of adrenaline in rats pretreated with phentolamine caused markedly greater reductions in MAP. The subsequent injections of both propranolol and atenolol partially restored MAP. Since propranolol and atenolol caused similar pressor responses, it is unlikely that the partial reversal involved only the blockade of vasodilatory $ß_2$-adrenoceptors. These results are in agreement with those of Tabrizchi and Pang (1990) which show that adrenaline is required for the partial restoration of the pressor response to a ß-adrenoceptor antagonist in phentolamine-treated rats. The haemodynamic changes in pentobarbital-anaesthetized rats showed that phentolamine increased blood flow only to the lungs while

blood flow was reduced in the caecum, colon and skin. Vascular conductance was increased only in the lungs which indicates that phentolamine is an important vasodilator of arteriovenous anastomoses. In contrast to the situation in conscious rats, blood flow to the skeletal muscle was not altered by phentolamine. Propranolol did not produce changes in MAP, flow or conductance in any vascular bed. This again shows that it is important to have increased blood flow to the skeletal muscle by α-adrenoceptor blockade in order to produce a pressor response to a ß-adrenoceptor antagonist. Further experiments were carried out to examine if other barbiturate anaesthetic agents similarly suppressed the pressor response to propranolol. The results show that propranolol also failed to produce a pressor response in rats anaesthetized with amobarbital.

Under the influence of ketamine, propranolol partially reversed the hypotensive effect of phentolamine. The reason for the partial reversal by ß-adrenoceptor antagonists of phentolamine-induced hypotension in ketamine-anaesthetized rats is not clear at the present time. However, ketamine is known to stimulate the cardiovascular system by centrally-mediated sympathomimetic effects, and to inhibit intraneuronal and extraneuronal catecholamine uptake (Riou et al. 1989). This may explain why MAP was higher in animals anaesthetized with ketamine than with other anaesthetic agents. It has been shown in <u>in vivo</u> studies that, in the absence of autonomic control, ketamine has

direct myocardial depressant properties in dogs (Schwartz and Horwitz, 1975). It is therefore possible that following α- and ß-adrenoceptor blockade, the direct myocardial depressant effect of ketamine attenuated the pressor effect of propranolol. In in vitro studies, ketamine, in doses relevant to those used in surgical induction, inhibited the development of spontaneous mechanical activity and lowered baseline tension of rat aortae and portal veins. In addition, it attenuated agonist-induced contractions of rat aortic strips (Altura et al., 1980). The relaxant effect of ketamine in the rabbit ear artery was proposed to be due to a decrease of Ca^{2+}-influx through the plasma membrane or an interference with the process of signal transduction between receptor occupation on the plasma membrane and Ca^{2+} release from intracellular stores via the inhibition of hydrolysis of phosphatidylinositol 4,5-biphosphate (PIP_2) (Kanamura et al., 1989). Therefore, it is possible that ketamine attenuated the pressor response to ß-adrenoceptor antagonists by its direct relaxant effect.

In chloralose-anaesthesia, pressor response to a ß-adrenoceptor antagonist was completely abolished. Chloralose had a profound negative inotropic effect on the dog heart-lung preparation (Bass and Buckley, 1966). Charney et al. (1970) showed that chloralose anaesthesia in dogs was associated with a transient increase in cardiac output which was abolished by α- and ß-adrenoceptor blockade. The intrinsic depressant effect of chloralose,

like that of ketamine, may have been normally masked by the sympathomimetic effects of the anaesthetic. However, following α- and ß-adrenoceptor blockade, the myocardial depressant effect of chloralose may have been unmasked resulting in the abolition of the pressor response to a ß-adrenoceptor antagonist.

Our results show that anaesthetic agents variably affect the response to a ß-adrenoceptor antagonist. The injection of a ß-adrenoceptor antagonist caused a pressor response in phentolamine-treated conscious rats and urethane-anaesthetized rats. Pressor responses to propranolol were attenuated in phentolamine-treated rats anaesthetized with ketamine or halothane and were abolished in rats anaesthetized with barbiturates, halothane and chloralose. The reasons for the differential effects of anaesthetic agents on the response to ß-adrenoceptor antagonists remain obscure.

4.5. Reversal of α-adrenoceptor blockade by ß-adrenoceptor antagonists in the isolated rat pulmonary artery

The rat pulmonary artery which contains both α- and ß-adrenoceptors was used to test the hypothesis that a ß-adrenoceptor antagonist reverses the effect of α-adrenoceptor blockade. It was shown by Fleish and Hooker (1976) that ß-adrenoceptors mediate relaxation in the rat pulmonary artery. The predominant ß-adrenoceptors in this preparation were reported to be of the $ß_2$-subtype although a

small population of $ß_1$-subtype was also present (O'Donnell and Wanstall, 1981a). In preliminary experiments, noradrenaline produced a maximum contraction of 12.1 ± 1.3 mN indicating that this tissue was sensitive to the drug. Dose-response curves for noradrenaline were repeated four times with no significant changes in either the EC_{50} value or maximum response. This shows that the preparation was stable over the study period. It is to be noted that the pulmonary artery is not a systemic artery. However, the aim of the study was only to show if there is an interaction between ß-blockers and α-blockers at the level of the receptor. The responses from our in vitro preparation may not be representative of the results in in vivo preparations of the interaction of ß-blockers and α-blockers. In in vivo the interaction between α- and ß-blockers likely occurs in small resistance vessels.

Noradrenaline caused a dose-dependent increase in force. Phentolamine partially blocked the effect of noradrenaline and this antagonism was not affected by time indicating that under the experimental conditions, the effect of phentolamine was not overcome by noradrenaline. Propranolol, atenolol and ICI 118,551 were shown to restore completely and in a dose-dependent manner the effect of noradrenaline which was previously antagonized by phentolamine. Since all three ß-adrenoceptor antagonists were effective in reversing the effect of phentolamine, it is most likely that the vasoconstrictor effect of the ß-

adrenoceptor antagonists was primarily due to the reversal of α-adrenoceptor blockade. This confirms that the pressor responses of ß-adrenoceptor antagonists are not due to the blockade of vasodilatory $ß_2$-adrenoceptors.

4.6. Interaction between α- and ß-adrenoceptor antagonists

The mechanism of the pressor response to ß-adrenoceptor antagonists is unknown, however, several hypotheses were proposed: first, ß-adrenoceptor antagonists may raise MAP and vasoconstrict tissue vasculature by antagonizing $ß_2$-adrenoceptor mediated vasodilation (Kayaalp and Turker, 1967; Yamamoto and Sekiya, 1969; Himori et al., 1984; Himori and Ishimori, 1984). Our results are not in agreement with this hypothesis although we cannot exclude the possibility that the blockade of $ß_2$-adrenoceptors may contribute to the pressor response. Second, ß-adrenoceptor antagonist pressor effect is attributed to a centrally mediated release of adrenal catecholamines (Kayaalp and Kiran, 1967; Sugawara et al., 1980). However, Tabrizchi et al. (1989) showed that ß-adrenoceptor antagonists do not increase the levels of plasma catecholamines in conscious rats. Third, ß-adrenoceptor antagonists may reverse the effects of α-adrenoceptor blockade by displacing α-adrenoceptor antagonists by an unknown mechanism (Olivers et al., 1965; Regoli, 1970). Fourth, ß-adrenoceptor antagonists, via the blockade of ß-adrenoceptors, may allow more adrenaline to react with α-adrenoceptors which are not

blocked (Prichard and Ross, 1966; Regoli, 1970). Our results are in accordance with the last two hypotheses that a possible interaction of α- and ß-adrenoceptor antagonists occurs and this may result in subsequent stimulation of the α-adrenoceptors. The mechanism of the interaction is not quite clear. Moreover in in vivo experiments, additional factors such as the renin-angiotensin system may also be involved in the pressor response to ß-adrenoceptor antagonists.

4.7. Conclusions

1. $ß_2$-adrenoceptor stimulation by a small dose of isoprenaline decreased TPR and MAP but $ß_1$-adrenoceptor stimulation affected neither TPR nor MAP. Isoprenaline initiated coronary and skeletal muscle vasodilatation via the activation of $ß_1$- and $ß_2$-adrenoceptors. Vascular conductances in other vascular beds were not affected.
2. Isoprenaline increased venous tone in intact, conscious rats. It had no effect on venous tone in rats pretreated with hexamethonium and it decreased venous tone in rats infused with noradrenaline to produce a high venous tone. Therefore, ß-adrenoceptor stimulation mediated direct venodilatation but a high venous tone is needed.
3. ß-adrenoceptor antagonists produced pressor responses primarily by reversing the effect of phentolamine in the

skeletal muscle bed in conscious rats and urethane-anaesthetized rats. In addition, ß-adrenoceptor antagonists vasoconstricted skin, kidneys and intestinal vascular beds.

4. The renin-angiotensin system had to be intact in order for complete reversal of phentolamine-induced hypotension by ß-adrenoceptor antagonists.
5. Anaesthetic agents had varying effects on the pressor effects of ß-adrenoceptor antagonists in phentolamine-treated rats. This ranged from the absence of influence with urethane, attenuated pressor response with halothane and ketamine and absence of a pressor response with halothane, pentobarbital, amobarbital and chloralose.
6. The haemodynamic changes induced by phentolamine were altered by the presence of anaesthetic agents, and this might have subsequently affected the pressor response to ß-adrenoceptor antagonists.
7. Under <u>in vitro</u> conditions, propranolol, atenolol and ICI 118,551 reversed the α-adrenoceptor blockade effect of phentolamine. This suggests that there is an interaction between an α-adrenoceptor antagonist and a ß-adrenoceptor antagonist resulting in the reversal of the effect of α-adrenoceptor blockade.

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