VASODILATORS AND REGIONAL BLOOD FLOW

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Aan mijn ouders,
voor Toos, Paul en Eva

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CHAPTER 1:
INTRODUCTION

Nowadays many different antihypertensive drugs are available which lower the blood pressure through various mechanisms of action. Accordingly, the haemodynamic changes that accompany the fall in blood pressure vary considerably depending upon the respective site of action of the drug and the more or less pronounced contribution of cardiovascular reflex mechanisms in the overall haemodynamic response.

The aim of the studies described in the present thesis is to characterize and compare the haemodynamic profiles of various types of antihypertensive drugs with special emphasis on the drug induced changes in regional blood flows. For this purpose we have used the radioactive microsphere technique to study the systemic and regional haemodynamic effects of a series of "direct" and "indirect" vasodilators in conscious hypertensive rabbits.

DETERMINANTS OF BLOOD PRESSURE

The perfusion pressure is defined by the pressure differential across the circulation (arterial pressure - central venous pressure). Because of the relatively low value and small fluctuations in the central venous pressure, the latter value may be disregarded. A distinction is made between the systolic pressure, provided by the contracting ventricle, and the diastolic pressure, measured in the period prior to ventricular contraction. The mean blood pressure is obtained by integrating the arterial pulse wave or, alternatively, by adding one-third of the pulse pressure to the diastolic pressure.

In symplified terms the arterial blood pressure is dependent upon two fundamental variables: the total systemic blood flow (cardiac output) and the resistance offered by the blood vessels to this forward flow (total peripheral resistance) (Bowman and Rand, 1980a; Tarazi, 1983). The relation between arterial pressure, blood flow and resistance to this flow is derived from Pois-

seuille's formula which describes the rate of laminar flow ($\dot{Q}$) through a cylindric tube as

$$\dot{Q} = (P_1 - P_2)\frac{r^4}{l.v}\,k \qquad (1)$$

where (P_1-P_2) is the pressure differential at the two ends of the tube, r and l are radius and length of the tube, respectively, v is the viscosity of the fluid and k is a constant factor. When applied to the circulation, assuming unchanging vascular length,

$$CO = \frac{MBP}{TPR} \quad \text{or} \quad MBP = CO \times TPR \qquad (2)$$

where cardiac output (CO) is equivalent to $\dot{Q}$, mean arterial blood pressure (MBP) is equivalent to the pressure differential (P_1-P_2) and total peripheral resistance (TPR) is determined by the viscosity of the blood and the radius of the blood vessels. For the whole circulation total peripheral resistance is equal to the sum of all vascular resistances in the various organs and tissues arranged in a parallel circuit:

$$\frac{1}{TPR} = \frac{1}{R_1} + \frac{1}{R_2} + \ldots\ldots + \frac{1}{R_n} \qquad (3)$$

Because the radius is magnified to the fourth power in equation (1), resistance may be markedly affected by relatively small changes in vessel diameter. On the other hand, it will be clear from equation (3) that changes in vessel diameter in one vascular bed can be counteracted by opposing changes in other beds and, thus, may not necessarily affect total peripheral resistance. In each vascular bed about 70 % of the resistance offered to the arterial blood flow is accounted for by the arterioles. In contrast, postcapillary vessels serve primarily as capacitance ves-

sels. Approximately 70 % of the total blood volume is found on the venous side of the circulation. Thus, variations in diameter of the venous capacitance vessels cause large shifts in the circulating blood volume and may acutely alter the venous return of blood to the heart (Bowman and Rand, 1980a; Miller et al., 1982).

CONTROL OF BLOOD PRESSURE

The basic goal of the circulation is to provide blood flow to each tissue as needed to meet its requirements (Guyton, 1981). This is achieved by a combination of local reactions of the blood vessels in the tissues as well as simultaneous gross adjustments of large portions of the circulation. The overall system for circulatory control can be divided into two parts; i) those controls that are intrinsic to the haemodynamic complex itself and ii) those controls that are extrinsic to the haemodynamic components (Guyton, 1976). Examples of intrinsic controls are autoregulatory mechanisms for acute and long term adjustments of local blood flows in response to changes in metabolic demands of the tissues, regulation of cardiac output by venous return according to the Frank-Starling law and long term control of pressure and blood volume by the kidney (pressure diuresis). The extrinsic controls include the nervous reflexes, which are especially important for acute modifications of the circulatory function, and hormonal systems such as the renin-angiotensin aldosteron system (figure 1).

The purpose of pressure control is to keep the pressure high enough to ensure adequate blood flow to all tissues, even during acute stresses (Guyton, 1981). The autonomic nervous system plays a dominant role in the acute regulation of the arterial blood pressure (figure 1). Afferent information is obtained by the central nervous system via several receptor systems of which the arterial baroreceptors, located in the aortic arch and carotid bifurcation, are best described. Additional information is received from other receptor systems such as the cardiopulmonary "low pressor receptors", primarily situated in the atria and pulmonary arteries, chemoreceptors in aorta, carotid arteries, heart and lungs and receptors in the central nervous system that react upon acute falls in cerebral blood flow. The afferent receptor systems

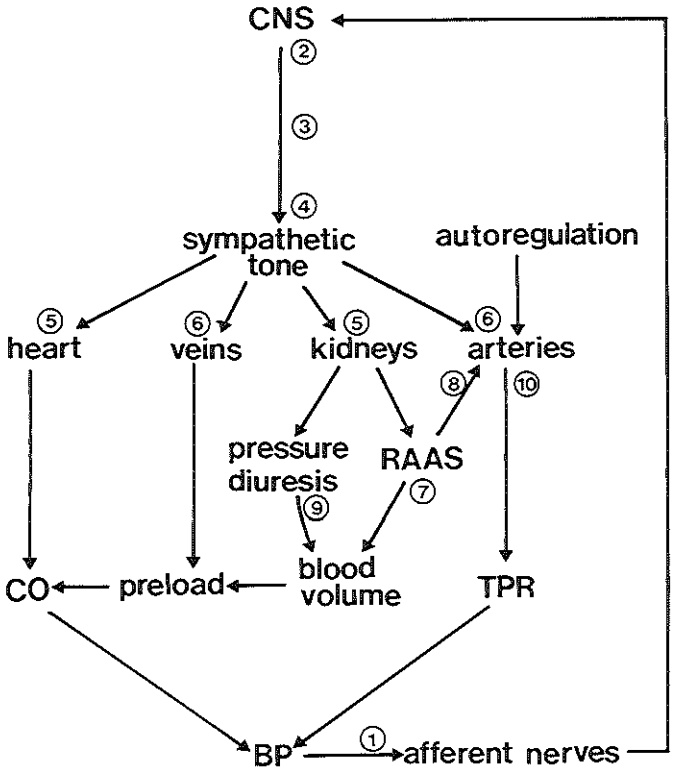


Figure 1. Schematic representation of blood pressure control mechanisms. The numbers in the circles refer to sites of action of antihypertensive drugs (table 1).

transmit several stimuli to the medullary vasomotor centers in the brain which are also influenced by higher integrative centers located in the hypothalamus, the limbic system and cortex (Shephard, 1982; Kuchel, 1983; Struyker-Boudier, 1984).

The integration of all influences results in a continuous readjustment of the circulation by way of autonomic efferents having a sympathetic and parasympathetic component (figure 1). Although changes in vagal activity play an important role in the regulation of the heart rate, the sympathetic system predominantly determines cardiovascular control via efferent nerves to the heart and to all vascular beds. In addition, sympathetic fibers are responsible for the secretion of catecholamines from the adrenal medulla. An

TABLE 1. Classification and some examples of antihypertensive drugs.

Site of action	Class	Examples
Autonomic nervous system		
	Veratrum alkaloids (1)	
		Protoveratrine
	Centrally acting drugs (2)	
		Clonidine
		α-methyldopa
	Ganglion blocking drugs (3)	
		Hexamethonium
	Postganglionic adrenergic depressants (4)	
		Reserpine
		Guanethidine
	β-adrenoceptor antagonists (5)	
		Atenolol (β_1)
		Propranolol (β_1, β_2)
		Pindolol (β_1, β_2, ISA)
	α-adrenoceptor antagonists (6)	
		Prazosin (α_1)
		Phentolamine (α_1, α_2)
Renin-angiotensin system		
	Converting-enzyme inhibitors (7)	
		Captopril
	Angiotensin II antagonists (8)	
		Saralasin
Kidney tubulus		
	Loop diuretics (9)	
		Furosemide
	Thiazide diuretics (9)	
		Chlorothiazide
	Potassium sparing diuretics (9)	
		Spironolactone
Vascular smooth muscle		
	Calcium antagonists (10)	
		Nifedipine
		Felodipine
	Others (10)	
		Hydralazine
		Minoxidil
		Diazoxide
		Nitroprusside

Numbers refer to site of action of the drugs as represented in figure 1. Information, given within brackets, refers to primary pharmacological actions. ISA, intrinsic sympathomimetic activity.

increase in sympathetic activity causes cardiac stimulation via β_1-adrenoceptors resulting in an enhanced cardiac output and constriction of the arterioles via α-adrenoceptors causing an increase in total peripheral resistance (figure 1). In addition, constriction of venous capacitance vessels, also mediated via

α-adrenoceptors, enhances venous return to the heart thereby increasing the cardiac output as a result of an increased filling pressure. Activation of vascular β_2-adrenoceptors causes vasodilatation particularly in the blood vessels to the voluntary muscles (Bowman and Rand, 1980a; Miller et al., 1982).

The autonomic nervous system and hormonal systems are interrelated to each other (figure 1). Sympathetic nervous activity is one of the most important factors in the regulation of renin release, while, on the other hand, an augmented release of noradrenaline from synaptic nerve terminals contributes to the vasoconstrictor action of angiotensin II. Sodium balance is another important factor modifying both sympathetic and renin-angiotensin responses. Other substances interfering with catecholamine release and action are prostaglandines, products of the kallikrein-bradykinin system, histamine and 5-hydroxytryptamine. In addition, noradrenaline regulates its own release from nerve terminals by a negative feedback system mediated via presynaptic α_2-receptors (Kuchel, 1983; Shephard, 1982). Finally, the recent discovery of the atrial natriuretic factor, a vasoactive peptide that counteracts the effects of noradrenaline and angiotensin II and causes natriuresis, illustrates that our knowledge of the various factors involved in blood pressure regulation is probably still far from complete (Kleinert et al., 1984).

ANTIHYPERTENSIVE TREATMENT

Arterial pressure is controlled at a fixed average by several reflex mechanisms that assure a relatively constant pressure. In hypertensive individuals the set point for the arterial pressure is abnormally high. Hypertension is a prime risk factor predisposing to cardiovascular disorders which may lead to cerebral accidents, myocardial infarction, congestive heart failure and renovascular sclerosis terminating in renal failure. Control of hypertension has been shown to have a great potential for reducing morbidity and mortality (Staessen et al., 1983).

The treatment of hypertension is mainly based upon the administration of drugs although non-specific forms of treatment, such as loss of weight, restriction of salt intake, psychotherapy

and elimination of additional risk factors and, in special cases, surgery, may lead to normalization of the blood pressure. The multifactorial nature of blood pressure control has permitted the development of drugs affecting various aspects of the circulatory control (figure 1). Antihypertensive drugs are generally classified, in accordance with their main site of action, into four groups (table 1): i) drugs that interfere with the autonomic nervous system, ii) inhibitors of the renin-angiotensin system, iii) diuretic agents and, iv) "direct" vasodilators (Gerber and Nies, 1983; Van Zwieten, 1984a).

Interference with the autonomic nervous system is possible at various levels. Veratrum alkaloids lower the blood pressure by increasing the excitability of chemoreceptors in heart, lungs and great vessels. Stimulation of these receptors leads to bradycardia and dilatation of resistance vessels due to an increase in vagal tone and a reduction in sympathetic tone. Because of serious side-effects, these drugs are no longer used in hypertensive therapy (Bowman and Rand, 1980). Clonidine and α-methyldopa are examples of centrally acting antihypertensive drugs which owe their activity to the stimulation of α_2-adrenoceptors in the pontomedullary region of the brain. These compounds lower the arterial blood pressure by reducing, both, the cardiac output and total peripheral resistance due to a decrease in peripheral sympathetic tone (Gerber and Nies, 1983; Timmermans, 1984). Ganglion blocking agents inhibit the transmission across the autonomic ganglia thereby reducing sympathetic as well as parasympathetic tone. Side-effects, caused by the combined blockade of both branches of the autonomic nervous system, are widespread and severe, for which reason these drugs are no longer used for chronic therapy (Gerber and Nies, 1983). Postganglionic adrenergic depressants, such as reserpine and guanethidine, inhibit sympathetic nerve activity by interference with the uptake, storage and/or release of noradrenaline at the adrenergic nerve endings. The blood pressure is lowered due to an inhibition of the sympathetic tone in the heart and blood vessels (Gerber and Nies, 1983; Boura and Green, 1984).

β-Adrenoceptor antagonists have become some of the most commonly used drugs in the treatment of hypertension. Due to blockade of cardiac β_1-adrenoceptors, these compounds reduce the heart rate and cardiac output. In addition β-blockers inhibit the renin

release. Due to partial agonism, a vasodilator response mediated via β_2-adrenoceptors may be produced by drugs with intrinsic sympathomimetic activity (ISA) such as pindolol. The exact mechanism by which β-adrenoceptor antagonists exert their antihypertensive action is unknown. After acute administration the fall in cardiac output is counteracted by a reflex-mediated increase in total peripheral resistance. Only after a few hours or days the blood pressure begins to decrease in parallel with a return of total peripheral resistance to pretreatment levels. Nowadays, many different β-adrenoceptor antagonists are available which vary with respect to their kinetic characteristics, their selectivity for β_1-receptors, the presence or absence of intrinsic sympathomimetic activity, membrane selectivity and lipid solubility (Gerber and Nies, 1983; Fitzgerald, 1984).

α-Adrenoceptor antagonists decrease total peripheral resistance by antagonizing α-adrenoceptor-mediated contraction of vascular smooth muscles. The application of aselective α-blockers, like phentolamine, is limited because of lack of sustained efficacy and unwanted side-effects such as palpitations. Selective blockade of α_1-adrenoceptors for instance with prazosin , which leaves the negative feedback on noradrenaline release mediated via presynaptic α_2-receptors intact thereby causing a less pronounced reflex activation of the heart, has proven to be more successful (Cavero and Roach, 1980; Gerber and Nies, 1983; Timmermans and Van Zwieten, 1984).

Drugs that interfere with the renin-angiotensin system reduce the blood pressure by inhibiting the formation (converting-enzyme inhibitors) or by antagonizing the action (angiotensin II antagonists) of the pressor hormone angiotensin II. Apart from a direct action on vascular smooth muscle, angiotensin II also has an excitatory influence on sympathetic nervous activity. Captopril, which inhibits the converting enzyme responsible for the formation of angiotensin II is already successfully used in the treatment of hypertension (Ondetti and Cushman, 1980; Sweet and Blain, 1984).

Diuretic agents, besides β-adrenoceptor antagonists, are also regarded as drugs of first choice although they have a modest blood pressure lowering effect that may not be sufficient in severe hypertension. The exact mechanism by which these agents lower the blood pressure is not certain. The compounds act primarily on

the kidney tubulus to increase salt and water excretion. The initial haemodynamic response is volume retraction and a decrease in cardiac output. However, over a period of days or weeks, cardiac output and plasma volume return close to pretreatment levels and the hypotensive effect is maintained by a decrease in peripheral vascular resistance. A wide variety of mechanisms has been suggested to be involved in the long-term hypotensive action of diuretics including the inhibitory effects of sodium depletion on pressor responses induced by the sympathetic nervous system and angiotensin II (Gerber and Nies, 1983; Struyker-Boudier, 1983; Greven and Heidenreich, 1984).

The last group consists of compounds with a "direct" action on vascular smooth muscle. To this group belong the calcium antagonists which share the common ability to relax vascular smooth muscle, impair pacemaker activity in the heart and reduce cardiac contractility by decreasing the availability of calcium in the cells (Fleckenstein, 1977). Because their hypotensive efficacy is based upon the reduction in total peripheral resistance, compounds have been developed which show selectivity towards vascular smooth muscle. (Van Zwieten, 1984b). Other vascular smooth muscle relaxants used in antihypertensive therapy are hydralazine, minoxidil, diazoxide and nitroprusside (Gerber and Nies, 1983; Van Zwieten, 1984c). The exact mechanism by which these agents cause relaxation of the vascular smooth muscles is unknown. Vasodilator drugs can be differentiated with respect to their arteriolar and venous sites of attack. For antihypertensive drugs the therapeutic effect is predominantly based upon a reduction in total peripheral resistance due to a relaxation of precapillary arterioles. Hydralazine and minoxidil selectively dilate arterial resistance vessels. Diazoxide, calcium antagonists, and also the "indirect" vasodilators, captopril and prazosin, affect predominantly the arterial side of the circulation whereas nitroprusside dilates both arteriolar and venous vessels (Van Zwieten, 1980).

The classification of antihypertensive drugs (table 1) is rather arbitrary and may change in the future. This is for instance illustrated by the fact that prazosin, in the first edition of "Hypertension" (Goldberg et al., 1977) listed as a direct vasodilator, is now recognized as a postsynaptic α_1-adrenoceptor antagonist and is classified as such in the second edition of "Hyper-

tension" (Gerber and Nies, 1983). However, in addition to postsynaptic α_1-adrenoceptor blockade, a central action causing a reduction in sympathetic outflow has also been suggested to contribute to the hypotensive action of prazosin (McCall and Humphrey, 1981). Similarly, the action of the "direct" vasodilator hydralazine appears, at least in part, to be dependent upon an intact endothelium suggesting that an "indirect" action (the release of an, as yet unknown, natural vasodilator substance from the endothelium) contributes to the blood pressure lowering effects of the drug (Spokas et al., 1984). In addition, new compounds have been developed with multiple sites of action such as labetolol which antagonizes both, α- and β-adrenoceptors (Fitzgerald, 1984) and ketanserin with $5HT_2$-receptor and α_1-adrenoceptor blocking properties (Van Nueten et al., 1981).

DRUG-INDUCED INHIBITION AND ACTIVATION OF CARDIOVASCULAR REFLEX MECHANISMS

An important implication of the multifactorial nature of blood pressure regulation is that, in response to the fall in blood presure produced by antihypertensive drugs, cardiovascular reflex mechanisms become activated which oppose the initial hypotensive action (Reid, 1981; Struyker-Boudier, 1984). For instance, the use of direct vasodilators as a sole agent in hypertensive therapy is rather limited because of the reflex-mediated increase in heart rate and cardiac output due to the hypotension-induced activation of the baroreceptor reflex. The consequent enhancement in myocardial oxygen requirements are particularly undesirable in patients with inadequate coronary perfusion. In addition, both, the fall in renal perfusion pressure and the reflex-mediated increased sympathetic tone activate the renin-angiotensin system leading to angiotensin II formation and salt and water retention. These disadvantages can be largely overcome by using vasodilator drugs in combination with β-adrenoceptor antagonists, to attenuate the sympathetic nervous tone, and, when fluid retention is significant, diuretics. On the other hand, due to pharmacological interference with cardiovascular reflex mechanisms, blood pressure control may become greatly

impaired leading to undesirable side-effects. Thus, symptoms such as postural and exercise hypotension are common side-effects of drugs that cause an aselectve inhibition of the sympathetic vasomotor tone (Gerber and Nies, 1983).

AIM OF THE THESIS

At present many antihypertensive drugs are available with various mechanisms of action. Consequently, the changes in haemodynamic variables that accompany the fall in blood pressure differ accordingly. Although the systemic haemodynamic effects of antihypertensive drugs are rather well studied, less is known about the regional haemodynamic changes during antihypertensive treatment. The studies described in the present thesis were designed to investigate and compare the haemodynamic profiles of a series of "direct" and "indirect" vasodilators with special emphasis on the drug-induced changes in regional blood flows. Direct vasodilator drugs have gained renewed interest now that the most common problems experienced with these drugs, reflex-mediated cardiac stimulation and salt and water retention, can be counteracted by the concomitant administration of β-adrenoceptor antagonists and diuretic agents (Gerber and Nies, 1983). The development of new compounds in this group, such as calcium antagonists, have contributed to the revival of interest in vasodilator drugs. In addition, vasodilators with an indirect action, such as converting-enzyme inhibitors (Ondetti and Cushman, 1980) and selective α_1-adrenoceptor antagonists (Cavero and Roach, 1980), appear to cause a less pronounced activation of cardiovascular reflex mechanisms. Finally, because the dominant haemodynamic disturbance in hypertension is an increase in total peripheral resistance, vasodilator drugs are a rational approach to treatment (Reid, 1980; Van Zwieten, 1984c).

According to Lund-Johansen (1982), "The vast majority of physicians treating hypertensive patients rarely think about the haemodynamic mechanisms underlying the increased blood pressure nor do they care much about how the blood pressure is reduced as long as it is brought near to "normal" during rest and side-effects are acceptable". A better understanding of the haemodynamic profiles

of antihypertensive drugs may contribute to a more rational choice of a certain drug tailored to the possible needs of a patient.

METHODOLOGY

Studies have been performed on conscious hypertensive rabbits. By using hypertensive animals, the obtained data have a higher clinical relevance. Moreover, the hypotensive potency of drugs can be determined more easily when the blood pressure is increased (Zandberg, 1984).

Systemic and regional haemodynamic variables were measured with the radioactive microsphere technique (Heymann et al., 1977). Although only a restricted number of measurements can be made in one animal, the method has the advantage that cardiac output and its complete distribution can be determined in one animal. Moreover, by using chronic catheters for the microsphere administrations, studies can be performed on conscious animals. Apart from a direct effect on haemodynamic variables, anaesthetic agents also inhibit autonomic nervous activity and thus interfere with cardiovascular reflex mechanisms (Bowman and Rand, 1980b). The use of conscious animals guarantees a normal function of the reflex mechanisms which play an important role in the haemodynamic profiles of antihypertensive drugs. The application of the radioactive microsphere technique in conscious rabbits is the subject of the second chapter of this thesis.

Due to their docile nature and size, rabbits are very suitable for studies of the circulation in conscious animals using the radioactive microsphere technique. Hypertension can be produced in these animals rather easily and with relatively low costs by wrapping both kidneys with cellophane to induce cellophane perinephritis hypertension (Page, 1939). A characterization of the changes in systemic and regional haemodynamic variables involved in this experimental form of hypertension is included in the studies (chapter 3).

The drugs used to study the haemodynamic profiles are the converting-enzyme inhibitor captopril (chapter 4), the arterial vasodilator hydralazine, alone and in combination with the β-adrenoceptor antagonists atenolol (chapter 5), the calcium anta-

gonist felodipine (chapter 6), the α_1-adrenoceptor antagonist prazosin (chapter 7) and the $5HT_2$ receptor antagonist ketanserin (chapter 8). A comparison of the haemodynamic profiles of the various drugs used is given in chapter 9.

REFERENCES

Boura, A.L.A., Green, A.P. (1984) Depressants of peripheral sympathetic nerve function, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 194-238.

Bowman, W.C., Rand, M.J. (1980a) The cardiovascular system and drugs affecting the circulation, in "Textbook of Pharmacology" (Blackwell Scientific Publications, Oxford), chapter 23: 1-68.

Bowman, W.C., Rand, M.J. (1980b) Anaesthesia and general anaesthetics, in "Textbook of Pharmacology" (see Bowman and Rand, 1980a), chapter 7: 1-15.

Cavero, I., Roach, A.G. (1980) The pharmacology of prazosin, a novel antihypertensive drug. Life Sci. 27: 1525-1540.

Fitzgerald, A. (1984) β-adrenoceptor antagonists, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 249-306.

Pleckenstein, A. (1977) Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Ann. Rev. Pharmacol. Toxicol. 17: 149-160.

Gerber, J.G., Nies, A.S. (1983) Pharmacology of antihypertensive drugs, in "Hypertension" (see Tarazi, 1983), 1093-1127.

Goldberg, L.I., Rick, J.H., Oparil, S. (1977) Pharmacology of antihypertensive agents, In "Hypertension" (Eds. Genest, J., Koiw, E., Kuchel, O., McGraw-Hill Book Company, New York), 990-1024.

Greven, J., Heidenreich, O. (1984) Diuretic drugs in hypertension, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 66101.

Guyton, A.C., Cowley, A.W., Young, D.B., Coleman, T.G., Hall, J.E., DeClue, J.W. (1976) Integration and control of circulatory function, in Int. Rev. Pharmacol., volume 9: Cardiovascular Physiology II (Eds. Guyton, A.C., Cowley, A.W., University Park Press, Baltimore), 341-385.

Guyton, A.C. (1981) The relationship of cardiac output and pressure control. Circulation 84: 1079-1088.

Kleinert, H.D., Maack, T., Atlas, S.A., Januszewicz, A., Sealley, J.E., Laragh, J.H. (1984) Atrial natriuretic factor inhibits angiotensin-, norepinephrine-, and potassium-induced vascular contractility. Hypertension 6 (suppl 1): 143-147.

Kuchel, O. (1983) The autonomic nervous system and blood pressure regulation in human hypertension, in "Hypertension" (see Tarazi, 1983), 140-160.

Lund-Johansen, P. (1982) Hemodynamics of hypertension with and without treatment, in "Cardiology", volume 1: Hypertension (Eds. Sleight, P., Preis, E., Butterworth Scientific, London), 173-182.

McCall, R.B., Humphrey, S.J. (1981) Evidence for a central depressor action of postsynaptic α_1-adrenergic antagonists. J. Auton. Nerv. Syst. 3: 9-23.

Miller, R.R., Fennel, W.H., Young, J.B., Palamo, A.R., Quinones, M.A. (1982) Differential systemic arterial and venous action and consequent cardiac effects of vasodilator drugs. Progr. Cardiovasc. Dis. 24: 353-374.

Ondetti, M.A., Cushman, D.W. (1980) Inhibition of the renin-angiotensin system; a new approach to the therapy of hypertension. J. Med. Chem. 24: 355-361.

Page, I.H. (1939) Production of persistant hypertension by cellophane perinephritis. J. Am. Med. Ass. 113: 2046-2048.
Reid, J.L. (1980) Vasodilators in the management of hypertension. Progr. Pharmacol. 3/4: 77-82.
Shephard, J.T. (1982) Reflex control of arterial blood pressure. Cardiovasc. Res. 16: 357-383.
Spokas, E.G., Folco, B., Quilly, J., Chander, P., McGiff, J.C. (1984) Endothelial mechanism in the vascular action of hydralazine. Hypertension 5(suppl I): 107-111.
Staessen, J., Cattaert, A., Fagard, R., Lijnen, P., Vanhees, L., Amery, A. (1983) Epidemiology of treated compared to untreated hypertension, in "Hypertension" (see Tarazi, 1983), 1069-1093.
Struyker-Boudier, H.A.J., Smits, J.F.M., Kleinjans, J.C.S., Van Essen, H. (1983) Hemodynamics of diuretic agents. Clin. Exp. Hypertension A5: 209-223.
Struyker-Boudier, H.A.J. (1984) The interaction of antihypertensive drugs with mechanisms of blood pressure, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 46-65.
Sweet, C.S., Blain, E.H. (1984) Angiotensin-converting enzyme inhibitors, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 343-363.
Tarazi, R.C. (1983) Haemodynamics, salt, and water, in "Hypertension" (Eds. Genest, J., Kuchel, O., Hamet, P., Cantin, M., McGraw-Hill Book Company, New York), 15-42.
Timmermans, P.B.M.W.M. (1984) Centrally acting drugs, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 102-153.
Timmermans, P.B.M.W.M., Van Zwieten, P.A. (1984) α-adrenoceptor antagonists, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 249-306.
Van Nueten, J.M., Janssen, P.A.J., Van Beek, J., Xhonneux, R., Verbeuren, T.J., Vanhoutte, P.M. (1981) Vascular effects of ketanserin (R 41468), a novel antagonist of 5-HT_2 serotonergic receptors. J. Pharmacol. Exp. Ther. 218: 217-230.
Van Zwieten, P.A. (1980) Vasodilator drugs. Progr. Pharmacol. 3/4: 69-75.
Van Zwieten, P.A. (1984a) General introduction: The classification of antihypertensive drugs, in "Handbook of Hypertension", volume 3: Pharmacology of Antihypertensive Drugs (Ed. Van Zwieten, P.A., Elsevier Science Publishers B.V., Amsterdam), 1-6.
Van Zwieten, P.A. (1984b) Vasodilator drugs with direct action on vascular smooth muscle, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 307-330.
Van Zwieten, P.A. (1984c) Calcium entry blockers (calcium antagonists), in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 331-342.
Zandberg, P. (1984) Animal models in experimental hypertension: Relevance to drug testing and discovery, in "Pharmacology of Antihypertensive Drugs" (see Van Zwieten, 1984a), 6-45.

CHAPTER 2:
APPLICATION OF THE RADIOACTIVE MICROSPHERE TECHNIQUE FOR STUDIES OF THE CIRCULATION IN CONSCIOUS RABBITS

In order to test the suitability of the radioactive microsphere technique for successive measurements of systemic and regional haemodynamic variables in conscious animals, the technique was applied to determine baseline values of cardiac output and regional blood flows and the changes in these variables observed 15 minutes after three successive injections of physiological saline (0.1 ml/kg each, i.v.) administered at 20 minutes intervals in conscious New Zealand White rabbits (n=8). The four batches of microspheres, with a diameter of 15 μm and labeled with different isotopes, were injected directly into the left atrium via a catheter implanted about 10 days before the experiment. Cardiac output was determined using an arterial reference blood sample withdrawn during microsphere infusions from a catheter inserted into the left carotid artery at least 4 days prior to the experiment.

The arterial blood pressure, heart rate, cardiac output and total peripheral resistance were not significantly altered by the 3 saline injections. Also no consistent changes were observed in blood flows to any of the regional vascular beds studied.

A close correlation (r_s=0.99, $p < 0.001$) was found between blood flows to the left and right kidney which suggests that the microspheres were adequately mixed with the arterial blood. In addition, the blood flows to the left and right cerebral hemisphere were also significantly correlated (r_s=0.68, $p < 0.001$) and not significantly different from each other indicating that, despite ligation of the left carotid artery, collateral circulation was sufficient to permit even distribution of cerebral blood flow.

In conclusion, the radioactive microsphere technique is a suitable method for the study of systemic and regional haemodynamics in conscious rabbits. At least four different measurements can be made in a period of one hour.

The principle of the radioactive microsphere technique is that when microspheres, labeled with a radioactive isotope, are injected into the left side of the heart, they mix with the arter-

ial blood and are distributed to the tissues in the same proportion as the blood. However, in contrast to the blood cells, the spheres, being rigid and having a larger diameter (15 μm), become trapped in the capillary beds. The numbers of microspheres impacted in the various tissues are assessed by counting the radioactivity present. The fraction of the cardiac output distributed to a tissue can be derived by dividing the tissue radioactivity by the sum of radioactivity in all body tissues. If one imposes an artificial organ of which the flow is already known (e.g. a pump withdrawing blood), the radioactivity of this "organ" can be utilized to calculate cardiac output and the absolute tissue blood flows. Several measurements can be made by injecting various batches of microspheres labeled with different isotopes.

The radioactive microsphere technique can be applied to study cardiac output and its distribution in animals (Hales, 1974; Heymann et al., 1977). Using a chronic catheter, implanted in the left atrium several days before the experiment, it is possible to perform measurements in conscious animals (Warren and Ledingham, 1972). Thus the technique appears to be useful for investigating the haemodynamic profiles of vasoactive drugs, the aim of the present thesis.

The various applications and the limitations of the radioactive microsphere technique has been the subject of many articles (Neutze et al., 1968; Hales, 1974; Warren and Ledingham, 1974; Heymann et al., 1977; Hof and Hof, 1981). However, information on the possible changes in haemodynamic variables when several batches of microspheres are administered in conscious animals is still limited. Therefore, it was decided to perform a control study with radioactive microspheres to ascertain the magnitude of the effects of three successive injections of physiological saline on systemic and regional haemodynamic variables in conscious male New Zealand White rabbits. Some of the results obtained in this study have been published elsewhere (Bolt and Saxena, 1984).

METHODS

Animals and surgical procedures

Experiments were performed on eight male New Zealand White

rabbits (body weight, 3.0 ± 0.1 kg). Nine to fourteen days before the experiment a left-sided thoracotomy was performed under anaesthesia, initiated with Hypnorm, 0.5 ml, i.m. (equivalent to 15 mg fluonison and 0.3 mg fentanyl) and maintained with a mixture of N_2O-halothane, in order to cannulate the left atrial appendage with a nylon catheter (0.75 mm i.d.), constructed as described by Warren and Ledingham (1972), for the administration of microspheres. Subsequently, four to eight days before the experiment the left carotid artery was cannulated under light Hypnorm anaesthesia for the measurement of blood pressure and heart rate and for withdrawing arterial blood samples. The catheter was passed along the carotid artery until the tip of the catheter was situated in the aortic arch. Atrial and carotid catheters were tunneled subcutaneously and exteriorized at the dorsal side of the neck. The catheters were flushed with a heparin solution (Thromboliquine, 5000 I.U./ml) every two or three days to prevent blood clotting. The operations were performed under sterile conditions and the animals were injected with a long-acting penicillin preparation post-operatively.

Measurements of haemodynamic variables

On the day of the experiment the animals were placed in a rabbit restrainer box. A Statham P23Dc pressure transducer was connected to the carotid catheter to record the arterial blood pressure and heart rate on a Grass model 7 polygraph. Mean arterial blood pressure was obtained by electronically damping the pressure signals.

Cardiac output and regional blood flows were measured with the radioactive microsphere technique using the reference blood sample method (Hales, 1974; Heymann et al., 1977). About 100,000 microspheres (Nen-Trac) with a nominal diameter of 15 μm and labeled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc were suspended in 0.5 ml saline containing a trace of Tween 80. At each measurement the suspension was shaken vigourously using a Vortex mixer and injected (flushed with 1.5 ml saline) into the left atrial catheter over a 20 seconds period. A reference arterial blood sample was withdrawn from the carotid catheter at a rate of 1.85 ml/min, beginning about 5 seconds before and continuing for about 60 seconds after the injection of microspheres. The amount of

withdrawn blood was replaced with one-third volume of human serum albumen (Albumen merieux 20%).

At the end of the experiment, the animals were killed with an overdose of sodium pentobarbital. All organs and tissues were removed, weighed, cut into small pieces and placed into plastic vials. The radioactivity (cpm) in the vials was counted with a γ-scintillation counter (Packard, model 5986), attached to a multichannel analyser (Conrac) to discriminate isotope energies. With the exception of the skin, skeletal muscles and bones, for which an aliquot of at least 30% was taken, all organs were counted in their entirety.

Experimental protocol

The marginal ear vein was cannulated under local anaesthesia (Lidocaine, 2 %) for the administration of physiological saline. After a stabilization period of at least 30 minutes, 4 different batches of microspheres, with a nominal diameter of 15 μm, were injected at 20 minutes intervals. Fifteen minutes before the 2nd, 3rd and 4th microsphere administration an injection of saline (0.1 ml/kg, i.v.) was given. Just before each microsphere injection an arterial blood sample (0.3 ml) was obtained to measure pH, P_{O_2} and P_{CO_2} using an ABL-2 (Radiometer, Copenhagen).

Calculations and statistical evaluation

Data were analysed on a PDP-11/70 computer, using a set of computer programs especially designed for the radioactive microsphere technique (Saxena et al., 1980). The fraction of cardiac output, received by the tissues, was calculated as the ratio of tissue radioactivity (I_{tis}) and total body radioactivity (I_{tot}); the latter was determined by adding together the radioactivity in all body parts and in the withdrawn blood sample (I_{art}). Cardiac output (CO) was calculated as:

$$CO\ (ml/min) = (I_{tot}/I_{art}) \times \dot{Q}_{art}\ (ml/min)$$

where $\dot{Q}_{art}$ is the rate of withdrawal of the arterial blood sample (1.85 ml/min). Blood flows to individual organs and tissues

($\dot{Q}_{tis}$) were calculated in a similar manner:

$$\dot{Q}_{tis}\ (ml/min) = (I_{tis}/I_{art}) \times \dot{Q}_{art}\ (ml/min)$$

All data have been expressed as mean (±SEM) in the text. Due to skewed distribution and lack of homogeneity of variances in some variables, non-parametric tests (Siegel, 1956) were used for the statistical evaluation. Initially, the Friedman's two way analysis of variance was applied to establish whether the samples represented different populations. The Wilcoxon matched-pairs signed ranks test was used to test the significance of the changes in haemodynamic variables from baseline values and for the comparison of blood flow values in different organs. Differences were considered significant for $p < 0.05$ (2-tailed). Correlations were determined using the Spearman Rank test.

RESULTS

Blood gases

The pH-, P_{CO_2} - and P_{O_2} -values at baseline and after the three saline injections were: pH, 7.39 ± 0.01, 7.41 ± 0.02, 7.43 ± 0.01 and 7.41 ± 0.02; P_{CO_2}, 39 ± 3, 39 ± 2, 39 ± 3 and 38 ± 3 mmHg; and P_{O_2}, 85 ± 2, 91 ± 3, 87 ± 3 and 87 ± 3 mmHg, respectively. The values after saline administration were not significantly different from those at baseline period.

Systemic haemodynamic variables

Table 1 shows the changes in systolic, diastolic and mean arterial blood pressure, heart rate, cardiac output, stroke volume and total peripheral resistance before and after the three subsequent saline injections in the conscious rabbits. Blood pressure and heart rate, recorded directly from the pulse signals in the aortic arch, were not significantly altered by the successive administrations of saline. Also no consistent changes were observed in the cardiac output, measured with the radioactive microsphere technique, nor in the derived variables, stroke volume and total

Table 1. Effects of 3 successive injections of saline (0.1 ml/kg) on systemic haemodynamic variables in normotensive rabbits (n=8).

	Baseline	Saline		
		0.1 ml/kg	0.1 ml/kg	0.1 ml/kg
ARTERIAL BLOOD PRESSURE (mmHg) :				
Systolic	91 ± 4	93 ± 4	94 ± 4	91 ± 5
Diastolic	73 ± 4	71 ± 4	73 ± 4	71 ± 4
Mean	81 ± 4	81 ± 4	83 ± 5	81 ± 4
HEART RATE (beats/min)	210 ± 7	220 ±11	216 ± 10	217 ± 13
CARDIAC OUTPUT (ml/min)	513 ± 35	483 ± 35	480 ± 38	490 ± 38
STROKE VOLUME (ml)	2.4 ± 0.2	2.2 ± 0.1	2.3 ± 0.2	2.3 ± 0.2
TOTAL PERIPHERAL RESISTANCE (mmHg/l.min^{-1})	163 ± 13	172 ± 10	179 ± 14	170 ± 12

peripheral resistance.

Regional haemodynamic variables

Figure 1 shows the changes in regional blood flows after the subsequent saline injections in the conscious animals. No consistent changes could be noticed in any of the vascular beds. Because 15 μm microspheres reach the lungs via, both, peripheral arteriovenous anastomoses and bronchial arteries, the values denoted for the lungs represent the combination of arteriovenous anastomotic and bronchial flows.

A close correlation was found between the blood flows to the left and right kidney (r_s = 0.99; $p < 0.001$, figure 2A). The mean blood flow values before and after the subsequent saline injections were for the left kidney, 41 ± 6, 39 ± 6, 40 ± 5 and 39 ± 5 ml/min, respectively, and for the right kidney, 41 ± 6, 39 ± 6, 40 ± 5 and 39 ± 5 ml/min, respectively. The deviation between blood flows to the left and right cerebral haemisphere was larger,

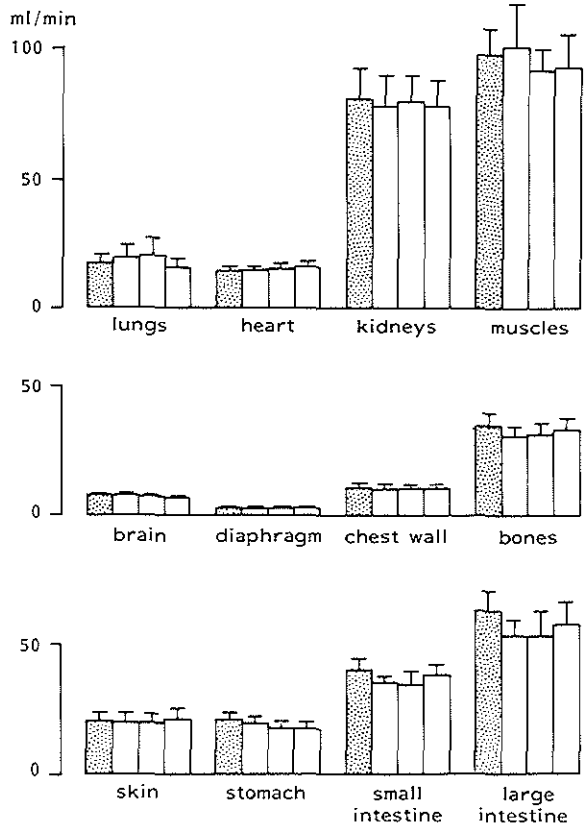


Figure 1. Effects of 3 successive injections of physiological saline (0.1 ml/kg, i.v.) on regional blood flows (ml/min) in conscious rabbits (n=8). Dotted bars, baseline values; open bars, 15 min after successive saline injections.

but still a higly significant correlation was found (r_s = 0.68; $p < 0.001$, figure 2B). The blood flow values, before and after the three administrations of saline, for the left cerebral hemisphere (2.7 ± 0.3, 2.2 ± 0.3, 2.2 ± 0.3 and 2.1 ± 0.3 ml/min, respectively) were not significantly different from those for the right cerebral haemisphere (2.3 ± 0.3, 2.1 ± 0.3, 2.1 ± 0.3 and 2.1 ± 0.2 ml/min, respectively). The saline injections did not significantly affect the blood flows.

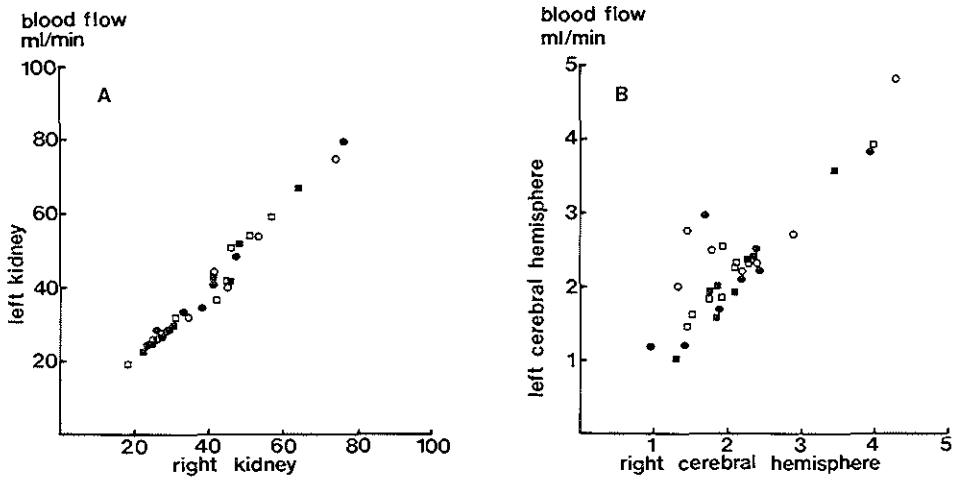


Figure 2. Comparison of absolute blood flows to left and right kidney (A) and to left and right cerebral hemisphere (B) in conscious rabbits (n=8). Measurements were made before (open circles) and 15 minutes after three subsequent administrations of 0.1 ml/kg saline (filled circles, open squares and filled squares, respectively).

DISCUSSION

The radioactive microsphere technique was used to study the effects of three successive administrations of 0.1 ml of saline on systemic and regional haemodynamic variables in conscious male New Zealand White rabbits. The baseline values of the various variables obtained in the present study agreed reasonably well with those previously reported for conscious rabbits (Neutze et al., 1968; Warren and Ledingham, 1974; Van Boom and Saxena, 1980; Hof and Hof, 1981, table 2). In addition, because no consistent changes in systemic variables as well as regional blood flows were produced by the successive saline injections, the method can be succesfully employed to monitor drug-induced changes in these variables on at least four different points in time. This is also compatible with the reported total amount of microspheres with a diameter of 15 μm that can be administered before haemodynamic disorders are observed ($> 6 \times 10^5$, Warren and Ledingham, 1974; Hales, 1974, Heymann et al., 1977).

An important implication of the radioactive microsphere tech-

Table 2. Cardiac output and percentage of cardiac output distributed to various organs and tissues measured with the radioactive microsphere technique in conscious normotensive rabbits.

Reference:	Bolt[a]	Van Boom[b]	Hof[c]	Warren[d]			Neutze[e]
Diameter of microspheres	15	15	15	15	25	50	50
Cardiac output (ml/min.kg)	172	183	–	–	–	–	215
Heart	3.0	3.3	2.4	3.3	3.7	3.8	3.1
Brain	1.5	1.2	1.7	–	1.1	1.0	1.2
Kidneys	15.4	13.2	16.6	13.6	13.9	14.9	16.2
GIT	23.3	14.5	25.3	17.3	19.3	20.1	24.2
Muscles	20.5	20.1	–	–	–	–	17.2
Bones	6.4	9.2	–	–	–	–	–
Skin	4.7	7.3	–	5.7	5.5	7.4*	7.4
Lungs	3.7	–	7.6	12.6	13.4	3.2*	2.6

a, present study; b, Van Boom and Saxena, 1980, cardiac output measured with electromagnetic flow probes; c, Hof and Hof, 1981; d, Warren and Ledingham, 1974; e, Neutze et al., 1968, cardiac output determined with dye dilution. *, value significantly different from respective values obtained with 25 µm spheres; –, not measured; GIT, gastro-intestinal tract.

niques is that after left atrial injection some of the 15 µm spheres bypass the peripheral vascular beds via arteriovenous anastomoses (AVAs) with a diameter larger than that of the spheres. Because AVAs are not present in the lungs (Hof and Hof, 1981), the shunted spheres are recovered in these organs. After the simultaneous injection of microspheres of two different sizes into the left atrium of conscious rabbits, Warren and Ledingham (1974) found that the proportion of microspheres lodged in the lungs decreased from 12.9 ± 1.8 % for 25 µm spheres to 3.2 ± 3.2 % for 50 µm spheres (table 2). Although the measured values for 25 µm spheres were rather high when compared with the values obtained in the present study, these investigators clearly demonstrated that arteriovenous shunting can play an important role in the deposition of microspheres in the lungs. In contrast to the lungs, the

amount of microspheres trapped in the skin increased when larger spheres were used (table 2), indicating the presence of AVAs in the cutaneous circulation (Saxena and Verdouw, 1985). Even when 50 μm spheres were used, recovery of microspheres in the lungs could not be fully ascribed to bronchial artery flow since significant shunting of these spheres was still demonstrated in the ear and hindleg of rabbits. This is in agreement with histological observations showing that AVAs have a diameter up to 150 μm (Heymann et al., 1977). The exact contribution of bronchial artery flow remains to be established, but it has been estimated to be less than 2% of cardiac output (Baille et al., 1982; Saxena and Verdouw, 1982).

A comparison of blood flows to paired organs is generally considered as a check on streaming effects of microspheres (Neutze, 1968). The correlations between the blood flows to the left and right kidney and to the left and right cerebral hemisphere were highly significant which indicated an adequate dispersion of microspheres throughout the blood stream. Ligation of the left carotid artery for insertion of a catheter for blood pressure measurements and the withdrawal of blood samples, could possibly affect the cerebral circulation. A comparison of the blood flows to the left and right cerebral hemispheres showed no consistent difference between the blood flows to these areas, that on the left side sometimes being higher and sometimes being lower than that of the opposite site. Thus, collateral circulation appears to be sufficient to permit an even distribution of the cerebral blood flow (Neutze et al., 1968).

In conclusion, the radioactive microsphere technique appears to be suitable to measure cardiac output and regional blood flows in conscious rabbits. By using various batches of microspheres, labeled with different isotopes, at least four successive measurements can be made reliably in a period of one hour.

REFERENCES

Baille, E.M., Nelems, J.M.B., Schulzer, M., Parc, P.D. (1982) Measurement of regional blood flow and bronchovascular resistance in dogs. J. Appl. Physiol. Respir. Environ. Exer. Physiol. 53: 1044-1049.

Bolt, G.R., Saxena, P.R. (1984) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205-213.

Hales, J.R.S. (1974) Radioactive microsphere technique for studies of the circulation. Clin. Exp. Pharmacol. Physiol. suppl. 1: 31-46.

Heymann, M.A., Payne, B.D., Hoffman, J.I.E., Rudolph, A.M. (1977) Blood flow measurements with radionuclide-labeled particles. Progr. Cardiovasc. Dis. 20: 55-79.

Hof, R.P., Hof, A. (1981) Very small microspheres are useful for the determination of cardiac output but not organ blood flow in conscious rabbits. Experientia 37: 438-439.

Neutze, J.M., Wyler, F., Rudolph, A.M. (1968) Use of radioactive microspheres to assess distribution of cardiac output in rabbits. Amer. J. Physiol. 215: 486-495.

Saxena, P.R., Schamhardt, H.C., Forsyth, R.P., Loeve, J. (1980) Computer programs for the radioactive microsphere technique. Computer Programs Biomed. 12: 63-84.

Saxena, P.R., Verdouw, P.D. (1982) Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic flow. J. Physiol. 332: 501-520.

Saxena, P.R., Verdouw, P.D. (1985) Tissue blood flow and localization of arteriovenous anastomoses in pigs via microspheres of four different sizes. Pflügers Archiv. Eur. J. Physiol., in press.

Siegel, S. (1956) Non-parametric statistics for behavioral sciences. (McGraw-Hill Kogakusha, Tokyo).

Van Boom, M., Saxena, P.R. (1980) Systemic and regional haemodynamics in experimental renal hypertension in conscious rabbits. Clin. Exp. Pharmacol. Physiol. 7: 627-634.

Warren, D.J., Ledingham, J.G.G. (1972) Chronic left atrial catheterization in the rabbit. Plug Arch. 335: 246-251.

Warren, D.J., Ledingham, J.G.G. (1974) Measurement of cardiac output distribution using microspheres. Some practical and theoretical considerations. Cardiovasc. Res. 8: 570-581.

CHAPTER 3:
SYSTEMIC AND REGIONAL HAEMODYNAMIC CHARACTERISTICS OF BILATERAL CELLOPHANE PERINEPHRITIS HYPERTENSION IN CONSCIOUS RABBITS

Using the radioactive microsphere technique, systemic and regional haemodynamic variables were measured in normotensive rabbits and in rabbits with bilateral cellophane perinephritis hypertension about 6 weeks after encapsulation of the kidneys. An average decrease in cardiac output of 18 percent was measured in the hypertensive rabbits as a result of a reduction in stroke volume; heart rate remained unchanged. Thus, in the established phase hypertension was maintained by the elevated total peripheral resistance. A redistribution of cardiac output was observed in the hypertensive rabbits; a significantly higher fraction was received by the brain, small intestine and heart. The weight normalized blood flow to the kidneys, spleen, skeletal muscles, bones and fat was significantly decreased whereas an increase in vascular resistance was noticed in the hypertensive rabbits in all the organs investigated. A negative correlation existed between the weight of the left ventricle and the blood flow to the left ventricle in hypertensive rabbits, suggesting inadequate coronary perfusion as myocardial hypertrophy becomes more pronounced.

Experimental forms of hypertension serve as a model for human hypertension and by investigating these models relevant information may be obtained concerning haemodynamic changes involved in human hypertension (Zandberg, 1984). Recent studies in our laboratory have shown that hypertension induced by unilateral cellophane wrapping of one kidney with contralateral nephrectomy in rabbits is characterized by a rather generalized increase in regional vascular resistances except for some splanchnic organs (Van Boom and Saxena, 1980). The enormous increase in total peripheral resistance in these animals is accompanied by a decrease in heart rate and stroke volume, resulting in a large reduction (> 35%) in cardiac output (Ichikawa et al., 1977; Van Boom and Saxena,1980).

The severe impairment of the performance of the heart in unilateral cellophane perinephritis and the reduction in renal mass due to nephrectomy are rather exceptional phenomena. Bilateral cellophane perinephritis hypertension, on the other hand, might be a more relevant model for human hypertension. Indeed, a less severe reduction in cardiac output has been observed in rabbits with bilateral cellophane perinephritis (Fletcher et al., 1976) and the more moderate increases in blood pressure and total peripheral resistance (Campbell et al., 1973; Fletcher et al., 1976) indicate that regional haemodynamics are affected in a different way, qualitatively and/or quantitatively. Therefore, we have investigated systemic and regional haemodynamics in rabbits with bilateral cellophane perinephritis to characterize the cardiovascular changes involved in this experimental form of hypertension. The results obtained in this study have been published elsewhere (Bolt and Saxena, 1983).

METHODS

Animals and surgical procedures

Experiments were performed on 8 normotensive and 16 hypertensive male New Zealand White rabbits. Hypertension was induced by bilateral cellophane wrapping of the kidneys, according to the method of Page (Page, 1939; Page et al., 1955). Under Hypnorm anaesthesia (0.5 ml/kg, i.m.; equivalent to 15 mg fluonison and 0.3 mg fentanyl per kg) both kidneys were exposed through flank incisions and wrapped in cellophane. Experiments were performed 6 to 7 weeks later. Nine to fourteen days before the experiment, a left sided thoracotomy was performed in order to cannulate the left atrial appendage with a nylon catheter for the injection of microspheres (Warren and Ledingham, 1972). Subsequently, 4 to 8 days before the experiment the left carotid artery was cannulated under light Hypnorm anaesthesia for the measurement of arterial blood pressure and heart rate and for the withdrawal of arterial blood samples during the experiments. The various surgical procedures have been described in detail elsewhere (Bolt and Saxena, 1983; Bolt and Saxena, 1984; chapter 2 of this thesis).

Measurement of haemodynamic variables

On the day of the experiment the rabbits were placed in a rabbit restrainer box. A Statham P23Dc pressure transducer was connected to the carotid catheter to measure the systolic and diastolic arterial blood pressure. These variables were recorded continuously on a Grass model 7 polygraph. Mean arterial blood pressure was obtained by electronically damping the above signal, while heart rate was counted from the pulsatile blood pressure signal. After a stabilization period of about 40 min an arterial blood sample (0.4 ml) was obtained for the analyses of blood gases using an ABL-2 (Radiometer). Cardiac output and regional haemodynamic variables were measured with the radioactive microsphere technique using the reference blood sample method (Hales, 1974; Heymann et al., 1977). Nen Trac microspheres with a nominal diameter of 15 μm and labeled with either ^{141}Ce, ^{113}Sn, ^{103}Ru or ^{95}Nb were used. After measuring baseline values normotensive animals received three subsequent injections of microspheres at 20 min interfalls, 15 min after an i.v. injection of saline. No significant changes could be detected between the values of haemodynamic variables, obtained with the four different microsphere injections (see chapter 2). Subsequent injections of microspheres were also administered in hypertensive rabbits after treatment with antihypertensive drugs. Details of the microsphere technique and the formulae used for the calculation of cardiac output ($ml.min^{-1}.kg^{-1}$), its distribution to the various organs and tissues (% of cardiac output) and regional blood flows ($ml.min^{-1}.100g^{-1}$) are given in chapter 2 (Saxena et al., 1980). Peripheral vascular resistances were calculated by dividing mean arterial blood pressure (mmHg) by respective tissue blood flows ($ml.min^{-1}.100g^{-1}$).

Statistical evaluation

For normotensive rabbits the mean values of the variables measured during the first and second microsphere injection were used for comparison with the values of the baseline variables in hypertensive rabbits. Due to skewed distribution and lack of homogeneity of variances in some variables the non-parametric Mann-Whitney U test was used to compare the data in normotensive and hypertensive groups (Siegel, 1956). Differences were

considered significant for $p < 0.05$ (2-tailed). Correlations were determined using the Spearman Rank test. All data have been expressed as mean (± S.E.M.) in the text.

RESULTS

Blood gases, body- and organ weights

Blood gases, measured in normotensive and hypertensive rabbits, did not differ significantly (table 1). Also shown in table 1 are body- and organ- (kidneys, heart and left ventricle) weights measured in the two groups of animals. Total kidney mass was somewhat higher in hypertensive animals but the difference did not achieve significance. The weights of heart and left ventricle were increased significantly in the hypertensive group. There was no significant difference in the weight of other tissues in the two groups.

Table 1. Blood gases, body- and organ weights measured in 8 normotensive and 16 hypertensive rabbits.

		Normotensive	Hypertensive
Blood chemistry:@			
pH		7.38 ± 0.02	7.38 ± 0.02
P_{CO_2}	(mmHg)	35.0 ± 1.54	40.6 ± 3.10
P_{O_2}	(mmHg)	87.8 ± 1.95	90.0 ± 1.60
Body- and organ weights:			
Body weight	(kg)	3.0 ± 0.1	3.0 ± 0.1
Kidneys	(g)	19.7 ± 0.8	21.4 ± 0.7
	(% body weight)	0.66± 0.029	0.72± 0.018
Heart	(g)	6.2 ± 0.4	8.0 ± 0.3*
	(% body weight)	0.21± 0.006	0.27± 0.006*
Left ventricle	(g)	2.6 ± 0.3	3.3 ± 0.2*
	(% body weight)	0.09± 0.006	0.11± 0.005*

*, significantly different ($p < 0.05$) from the values in normotensive rabbits.
@, n=14 in hypertensive rabbits.

Systemic haemodynamics

Table 2 shows the values of the systemic haemodynamic variables measured in the normotensive and hypertensive rabbits. Systolic, diastolic and mean arterial blood pressure were significantly increased in rabbits with bilateral cellophane perinephritis. The pulse pressure in the hypertensive rabbits, calculated as the difference between systolic and diastolic pressure, was significantly higher than in the normotensive rabbits, indicating an increased aortic compliance in the hypertensive group. Cardiac index was decreased in hypertensive rabbits due to a decrease in stroke volume as heart rate did not differ significantly in the two groups. The calculated total peripheral resistance was substantially higher in hypertensive rabbits.

Table 2. Systemic haemodynamic variables measured in 8 normotensive and 16 hypertensive rabbits.

		Normotensive	Hypertensive
Arterial blood pressure			
systolic	(mmHg)	97 ± 3	148 ± 4*
diastolic	(mmHg)	76 ± 3	117 ± 3*
mean	(mmHg)	86 ± 3	131 ± 3*
Pulse pressure	(mmHg)	21 ± 1	31 ± 2*
Heart rate	(beats.min^{-1})	226 ± 9	242 ± 8
Stroke volume	(ml)	2.43 ± 0.19	1.86 ± 0.07*
Cardiac index	(ml.min^{-1}.kg^{-1})	182 ± 10	150 ± 5*
Total peripheral resistance	(mmHg/l.min^{-1}.kg^{-1})	495 ± 39	889 ± 42*

*, significantly different ($p < 0.05$) from the values in normotensive rabbits.

Regional haemodynamics

Table 3 shows the values of the regional haemodynamic

Table 3. Distribution of cardiac output, regional blood flows and vascular resistances in 8 normotensive and 16 hypertensive rabbits.

	Cardiac output distribution (%)		Blood flow ($ml.min^{-1}.100g^{-1}$)		Resistance ($mmHg/ml.min^{-1}.100g^{-1}$)	
	Normotensive	Hypertensive	Normotensive	Hypertensive	Normotensive	Hypertensive
Heart	3,0± 0,20	3,9± 0,18*	272± 26	215± 11	0,35± 0,04	0,63± 0,03*
Brain	1,5± 0,11	1,9± 0,12*	82± 5	84± 5	1,10± 0,10	1,64± 0,10*
Kidneys	15,4± 1,38	13,5± 0,62	463± 63	284± 14*	0,22± 0,02	0,48± 0,03*
Stomach	4,2± 0,59	4,9± 0,42	70± 8	70± 7	1,42± 0,23	2,11± 0,20*
Small intestine	7,6± 0,59	10,1± 0,60*	104± 8	103± 6	0,88± 0,09	1,32± 0,08*
Large intestine	11,5± 1,11	12,0± 0,53	70± 7	60± 3	1,23± 0,10	2,32± 0,17*
Spleen	1,6± 0,28	1,3± 0,15	684± 137	372± 44*	0,17± 0,03	0,45± 0,07*
Mesentery and pancreas	1,8± 0,13	2,0± 0,15	56± 6	44± 4	1,66± 0,17	3,40± 0,34*
Liver	1,5± 0,47	1,0± 0,13	8± 3	4± 1	24,80± 7,33	42,17± 4,74*
Skin	4,7± 0,76	4,3± 0,37	9± 1	7± 1	10,95± 1,37	21,10± 1,48*
Skeletal muscle	20,5± 1,32	19,3± 1,13	10± 0,4	8± 1*	8,93± 0,41	18,62± 1,39*
Bones	6,4± 0,55	5,9± 0,33*	16± 2	12± 1*	5,71± 0,45	11,62± 0,79*
Fat	2,8± 0,50	1,2± 0,20*	44± 5	32± 5*	2,59± 0,60	5,21± 0,63*

*, significantly different ($p < 0,05$) from the values in normotensive rabbits.
@, hepatic artery flow.

variables. In the hypertensive rabbits a significantly larger percentage of the cardiac output is received by the heart, brain and small intestine: a smaller fraction is received by the fat. Compared with the normotensive group, the blood flow ($ml.min^{-1}.100g^{-1}$) to the kidneys, spleen, skeletal muscles, bones and fat was significantly decreased in the hypertensive group. Regional vascular resistances ($mmHg/ml.min^{-1}.100g^{-1}$) were significantly higher in the rabbits with bilateral cellophane perinephritis in all the organs investigated, with the exception of the liver where the increase in vascular resistance was not significant. However, this could be attributed to the large deviation in the measured values of the vascular resistance in the liver.

In figure 1 are shown the tissue blood flow values in hypertensive rabbits expressed as percentage change from the mean values in normotensive rabbits. Despite the lower cardiac output

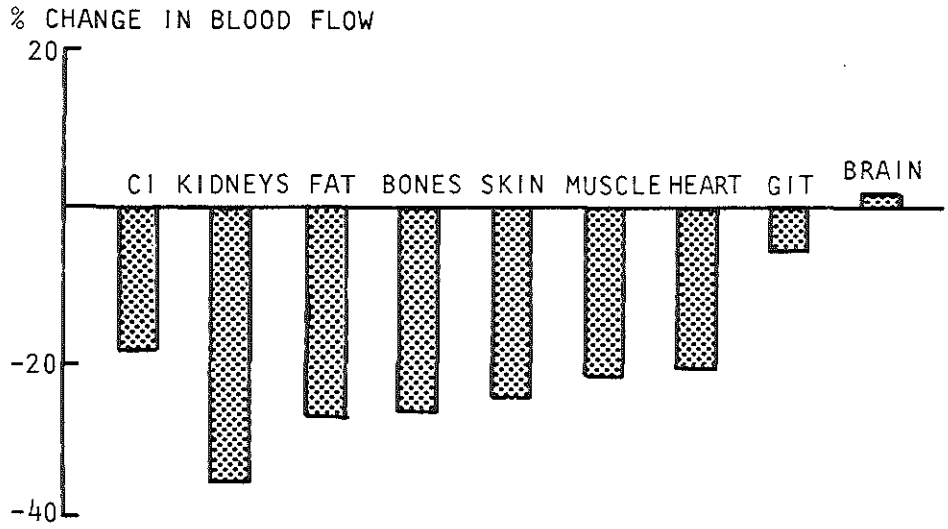


Figure 1. Mean values of cardiac index (CI) and regional blood flows in hypertensive rabbits (n=16) were compared with corresponding values in normotensive rabbits (n=8). In the figure are shown blood flows in hypertensive rabbits expressed as percentage change from mean values in normotensive rabbits. GIT, gastro-intestinal tract. In hypertensive animals the regional blood flow ($ml.min^{-1}.100g^{-1}$) was significantly decreased in the kidneys, fat, bones and skeletal muscles ($p < 0.05$).

in hypertensive animals, the blood flow to the brain was maintained at a normal level. No change in blood flow could be detected in cerebral hemispheres, cerebellum or brainstem of the hypertensive rabbits when compared with the values obtained in normotensive rabbits. Similarly, the blood flow to the stomach and small intestine was normal in hypertensive rabbits. A decrease in the blood flow to the large intestine caused the small reduction in blood flow calculated for the gastro-intestinal tract. The decrease in blood flow was most pronounced in the kidneys where it exceeded the reduction in cardiac index.

Although there was no significant reduction in blood flow to the heart a negative correlation existed between weight of the left ventricle and the weight normalized blood flow to left

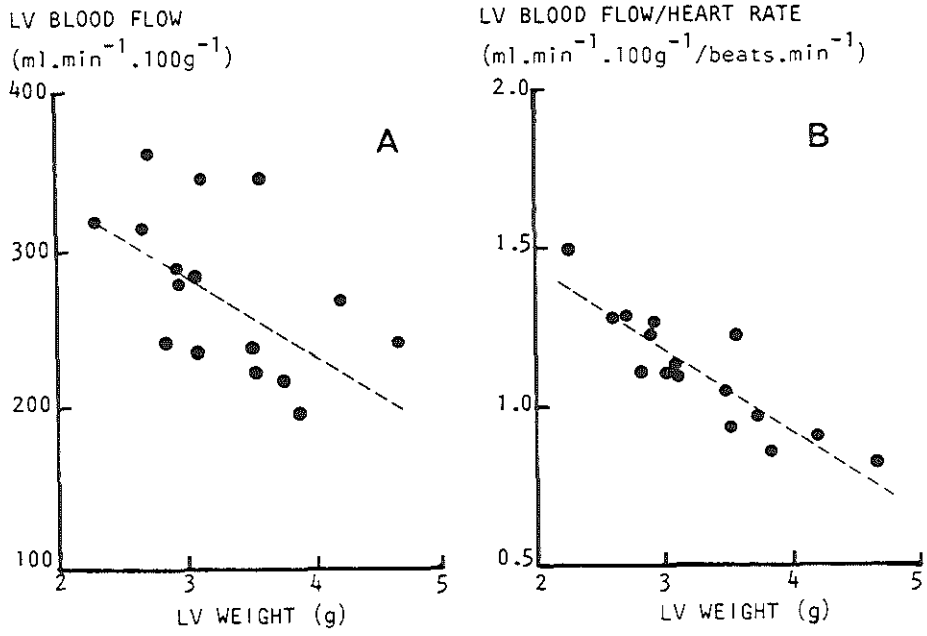


Figure 2. A negative correlation existed between the weight (g) of the left ventricle (LV) and the blood flow to the left ventricle (ml.min^{-1}.100g^{-1}) in the hypertensive rabbits (r_s=0.51, $p < 0.05$, figure 2A). This correlation became even more obvious, when the left ventricle blood flow was divided by the heart rate to normalize for the momentary changes in metabolic activity of the heart (r_s=0.86, $p < 0.05$, figure 2B).

ventricle in the hypertensive rabbits (figure 2A, r_s=0.51, $p < 0.05$). This correlation became even more obvious when the blood flow to the left ventricle was divided by the heart rate to normalize for the metabolic activity of the heart (figure 2B, r_s=0.86, $p < 0.05$).

Figure 3 shows total peripheral and regional vascular resistances in hypertensive rabbits expressed as percentage change from the mean values in normotensive rabbits. The elevation was most prominent in the kidneys, skeletal muscles, bones, fat and skin where the increase in resistance was higher in comparison with the increase in total peripheral resistance. The elevation in vascular resistance in the brain and gastro-intestinal tract was less than the increase in total peripheral resistance.

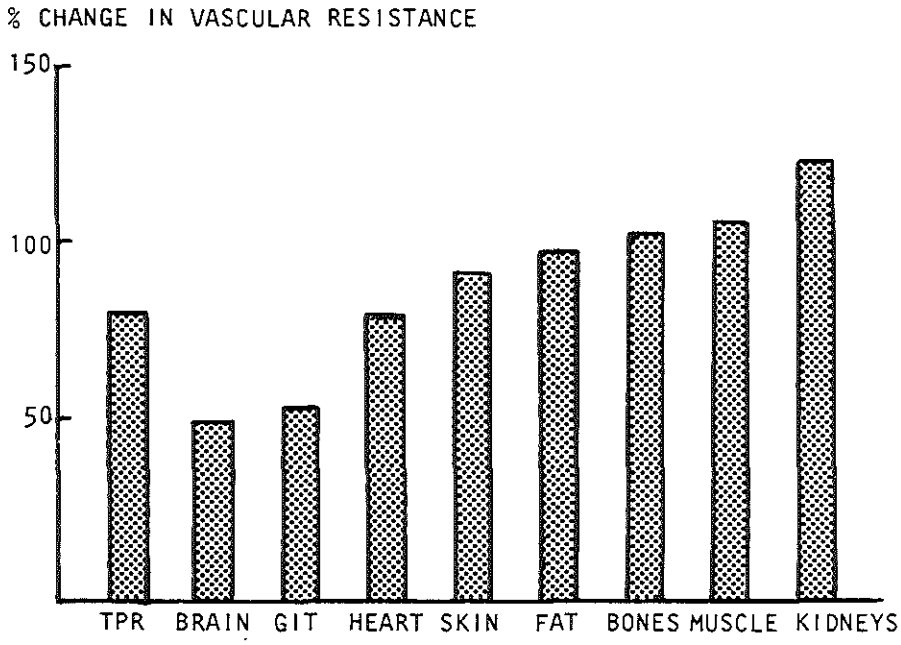


Figure 3. Mean values of total peripheral resistance (TPR) and regional vascular resistances in hypertensive rabbits were compared with corresponding values in normotensive rabbits. Vascular resistances are expressed as percentage change from mean values in normotensive animals. GIT, gastro-intestinal tract. In hypertensive animals vascular resistances were significantly increased in all the organs investigated ($p < 0.05$).

DISCUSSION

The results indicate that in the established phase of bilateral cellophane perinephritis hypertension in rabbits the increase in blood pressure is due to an elevated total peripheral resistance. Cardiac output is decreased as a consequence of a reduction in stroke volume. The fall in cardiac output by nearly 20% with unchanged heart rate has been reported previously in rabbits with bilateral cellophane perinephritis (Fletcher et al., 1976). However, in rabbits with unilateral cellophane perinephritis and contralateral nephrectomy a more severe reduction in cardiac output (> 35%) has been measured as a result of a decrease in both, stroke volume and heart rate (Ichikawa et al., 1977; Van Boom and Saxena, 1980). Thus one might conclude that in comparison with the one-kidney perinephritis, the cardiac function is impaired less in the two-kidney form.

The total peripheral resistance in rabbits with one-kidney perinephritis hypertension is more than doubled (Ichikawa et al., 1977; Van Boom and Saxena, 1980) whereas, in our present experiments, an increase of only 80% was observed. This is in agreement with the values reported by Fletcher et al. (1976) and it indicates qualitative and/or quantitative differences in regional haemodynamics between the two forms of experimental hypertension in the same species. A redistribution of the cardiac output is observed in the present study in rabbits with bilateral cellophane perinephritis hypertension. A larger fraction of the cardiac output is received by the brain, heart and small intestine, which results in normal blood flow values to the brain and gastro-intestinal tract. The finding, that hind limb blood flow in rabbits with bilateral cellophane perinephritis hypertension is decreased (West et al., 1975; Angus et al., 1976), is in accordance with our observations of a decreased blood flow to skeletal muscles and bones in the hypertensive rabbits. Regional blood flows are also significantly reduced in the kidneys, spleen and fat. The same distribution pattern can be observed in rabbits with unilateral cellophane perinephritis hypertension, except for a significantly reduced blood flow to the brain in these animals and, of course, a decrease in percentage cardiac output received by the kidneys due to nephrectomy (Van

Boom and Saxena, 1980). The reduction in blood supply to the brain demonstrates again the more severe consequences of unilateral cellophane wrapping with contralateral nephrectomy on cardiovascular variables. Although different vascular beds can be affected in experimental and clinical forms of hypertension (Lund-Johansen, 1980), usually the brain succeeds in maintaining blood supply at a normal level, as has been demonstrated in renal and spontaneously hypertensive rats (Flohr et al, 1976; Ferrone et al., 1979) and in salt loading hypertension in the dog (Liard, 1981). However, Gavras et al. (1980) also noticed a decrease in blood flow to the brain after acute renal artery occlusion in the dog.

The increase in percentage of cardiac output received by the heart in rabbits with bilateral cellophane perinephritis hypertension, as indeed in the unilateral cellophane perinephritis hypertension (Van Boom and Saxena, 1980), can be attributed to hypertrophy of the heart. In rats with chronic renal hypertension a two-fold increase in myocardial hydroxyproline content has been found, indicating increased collagen synthesis (Averill et al., 1976). This increase in non-contractile fibrous tissue in the hypertrophied heart may be a potent factor in altering cardiac structure leading to a depression in cardiac performance by increasing ventricular compliance (Ferrario et al., 1976). Usually, it is found that the weight normalized myocardial blood flow in animals with left ventricular hypertrophy is within normal limits (Mueller et al., 1978; Marcus et al., 1979; Yamori et al., 1979; Breisch et al., 1980). This is in accordance with our observations in rabbits with bilateral cellophane perinephritis hypertension, where cardiac blood flow is not significantly reduced. However, there are several indications of an inadequate myocardial perfusion in human and experimental hypertension with cardiac hypertrophy, usually indicated by a decrease in coronary reserve (Marcus et al., 1979; Breisch et al., 1980). Mueller et al. (1978) observed a failure of the cross-sectional area of the coronary bed to increase commensurate with the degree of hypertrophy in dogs with renal hypertension. In young spontaneously hypertensive rats an inverse correlation between heart weight and the lumen to transectional area ratios of coronary arteries has been found (Yamori et al., 1979). These

latter findings are compatible with our observation of a negative correlation between blood flow to the left ventricle and the weight of the left ventricle in the hypertensive rabbits. This correlation is even more convincing when the blood flow to left ventricle is divided by the heart rate. An increase in myocardial blood flow, as a result of coronary autoregulation in response to elevated metabolic requirements, can be observed in stress situations and during exercise. These temporary changes in myocardial blood flow can be predicted by the heart rate. Indeed, a close linear relationship between these variables has been observed during exercise in normal dogs and in dogs with left ventricular hypertrophy due to perinephritic hypertension (Bache and Vrobel, 1979). Therefore, by using the ratio of blood flow to the left ventricle and heart rate, a correction is made for momentary changes in myocardial blood flow during the experiments. A decrease in myocardial blood flow as ventricular weight increases may be an indication for inadequate coronary perfusion in the pressure overloaded hypertrophied heart. On the other hand, it is possible that the non-contractile tissue, becoming a more important component in the hypertrophied heart (Averill et al., 1976), requires less blood flow.

In dogs with one-kidney perinephritic hypertension an increase in venous compliance has been described (Simon et al., 1976). The pulse pressure increased significantly in the rabbits with bilateral cellophane perineprhitis hypertension, which may be a reflection of a more generalized decrease in vascular compliance involving the arterial part of the circulation as well. The finding that in cellophane perinephritis hypertension in dogs the systolic blood pressure begins to rise earlier than the diastolic pressure also indicates lowering of arterial compliance (Castro-Tavaras et al., 1981). A decrease in vascular distensibility has been postulated to result from an increase in the wall to lumen ratio of the blood vessels (Folkow, 1978). Besides these structural vascular changes, a decrease in vascularity may also contribute to the enhanced peripheral resistance (West et al., 1975; Angus et al., 1976). The changes in vascular resistance observed in the present study in rabbits with bilateral cellophane perinephritis hypertension are most prominent in the kidneys, where the increase exceeds the

enhancement in total peripheral resistance. A marked elevation of the renal vascular resistance, together with a fall in renal blood flow, appears to be a common characteristic of many experimental and clinical forms of hypertension (Flohr et al., 1976; Ferrone et al., 1979; Gavras and Liang, 1980; Lund-Johansen, 1980; Van Boom and Saxena, 1980; Liard, 1981; Zandberg, 1984). In the skeletal muscles, bones, fat, skin and heart, the increase in vascular resistance is more or less equal to the elevation in total peripheral resistance, whereas the changes in the brain and gastro-intestinal tract are less pronounced but still significant.

In conclusion, the established phase of hypertension in rabbits with bilateral cellophane perinephritis is maintained by an increase in total peripheral resistance. Although cardiac output is reduced in the hypertensive rabbits, blood flow remains unchanged in the brain and gastro-intestinal tract. A significant reduction in blood flow is measured in the kidneys, spleen, skeletal muscles, bones and fat. In comparison with hypertension induced by unilateral cellophane perinephritis (Van Boom and Saxena, 1980), the haemodynamic consequences of bilateral cellophane perinephritis are less severe. This is demonstrated most saliently by a larger impairment of cardiac performance, a reduced blood flow to the brain and, of course, a more pronounced reduction in renal blood flow due to nephrectomy in the one-kidney form.

REFERENCES

Angus, J.A., West, M.J., Korner, P.I. (1976) Assessment of autonomic and non-autonomic components of resting hindlimb vascular resistance and reactivity to pressor substances in renal hypertensive rabbits. Clin. Sci. Mol. Med. 51: 57s-59s.

Averill, D.B., Ferrario, C.M., Tarazi, R.C., Sen, S., Bajbus, R. (1976) Cardiac performance in rats with renal hypertension. Circulation Res. 38: 280-288.

Bache, R.J., Vrobel, T.R. (1979) Effects of exercise on blood flow in the hypertrophied heart. Am. J. Cardiol. 44: 1029-1033.

Breisch, E.A., Houser, S.R., Carey, R.A., Spann, J.F., Bove, A.A. (1980) Myocardial blood flow and capillary density in chronic pressure overload of the felic left ventricle. Cardiovascular Res. 14: 469-475.

Bolt, G.R., Saxena, P.R. (1983) Systemic and regional hemodynamic characteristics of bilateral cellophane perinephritis hypertension in conscious rabbits. Clin. Exp. Hypertension A5: 885-901.

Bolt, G.R., Saxena, P.R. (1984) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205-213.

Campbell, D.J., Skinner, S.H., Day, A.J. (1973) Cellophane perinephritis hypertension and its reversal in rabbits; Effect on plasma renin, renin substrate, and renal mass. Circulation Res. 33: 105–112.

Castro-Tavaras, J., Garrett, J., Goncalves, V. (1981) Cellophane perinephritic hypertension in the dog; Some biochemical, haemodynamic and pathological characteristics. Blood Vessels 18: 67–74.

Ferrario, C.M., Kocoglov, A., Bajbus, R., Nadzam, G.R. (1976) Ventricular performance after onset of renal hypertension. Clin. Sci. Mol. Med. 51: 141s–143s.

Ferrone, R.A., Walsh, G.M., Tsuchiya, M., Frohlich, E.D. (1979) Comparison of haemodynamics in conscious spontaneous and renal hypertensive rats. Am. J. Physiol. 235: F174–F179.

Fletcher, P.J., Korner, P.I., Angus, J.A., Oliver, J.R. (1976) Changes in cardiac output and total peripheral resistance during development of renal hypertension in the rabbit; lack of confirmity with the autoregulation theory. Circulation Res. 39: 633–639.

Flohr, H., Bruell, W., Dahners, H.W., Redel, D., Conradi, H., Stoepel, K. (1976) Regional distribution of vascular resistance in two models of experimental renovascular hypertension. Pflüg. Arch. 362: 157–164.

Folkow, B. (1978) Cardiovascular structural adaptation; its role in the initiation and maintenance of primary hypertension. Clin. Sci. Mol. Med. 55: 3s–22s.

Gavras, H., Liang, C.S. (1980) Acute renovascular hypertension in conscious dogs. Interaction of the renin-angiotensin system and sympathetic nervous system in systemic hemodynamic and regional blood flow responses, Circulation Res. 47: 358–365.

Hales, J.R.S. (1974) Radioactive microsphere techniques for studies of the circulation. Clin. Exp. Pharmacol. Physiol. 15: 31–46.

Ichikawa, S., Johnson, J.A., Stanton, M.W., Payne, C.G., Keitzer, W.P. (1977) Hemodynamic effects of an angiotensin II antagonist in rabbits with perinephritis hypertension. Proc. Soc. Exp. Biol. Med. 155: 259–263.

Liard, J. (1981) Regional blood flows in salt loading hypertension in the dog. Am. J. Physiol. 240: H361–H367.

Lund-Johansen, P. (1980) Haemodynamics in essential hypertension. Clin. Sci. 59: 343s–354s.

Marcus, M.L., Mueller, T.M., Gascho, J.A, Kerber, R.E. (1979) Effects of cardiac hypertrophy secundary to hypertension on the coronary circulation. Am. J. Cardiol. 44: 1023–1028.

Mueller, T.M., Marcus, M.L., Kerber, R.E., Young, J.A., Barnes, R.W., Abboud, F.M. (1978) Effect of renal hypertension and left ventricular hypertrophy on the coronary circulation in dogs. Circulation Res. 42: 543–549.

Page, I.H., Salmoiraghi, G.C., McGubbin, J.W. (1955) Observations on the method of producing perinephric experimental hypertension. J. Lab. Clin. Med. 46: 914–916.

Page, I.H. (1939) The production of persistant arterial hypertension by cellophane perinephritis. J. Am. Med. Ass. 113: 2046–2048.

Saxena, P.R., Schamhardt, H.C., Forsyth, R.P., Loeve, J. (1980) Computer programs for the radioactive microsphere technique: Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. Computer Programs Biomed. 12: 63–84.

Siegel, S. (1956) Non parametric statistics for the behavioural sciences, McGraw-Hill Kogakusha, Tokyo.

Simon, G., Pamnani, M.B., Overbeck, H.W. (1976) Decreased venous compliance in dogs with chronic renal hypertension. Proc. Soc. Exp. Biol. Med. 152: 122–125.

Van Boom, M, Saxena, P.R. (1980) Systemic and regional haemodynamics in experimental renal hypertension in conscious rabbits. Clin. Exp. Pharmacol. Physiol. 7: 627–634.

Warren, D.J., Ledingham, J.G.G. (1972) Chronic left atrial catheterization in the rabbit. Pflüg. Arch. 335: 167–172.

West, M.J., Angus, J.A., Korner, P.I. (1975) Estimation of non-autonomic and autonomic components of iliac bed vascular resistance in renal hypertensive rabbits. Cardiovascular Res. 9: 697–706.

Yamori, Y., Mori, C., Nishio, T., Ooshima, A., Horie, T., Ohtaka, M., Soeda, T., Saito, M., Abe, K., Nara, Y., Nakao, Y., Kihara, M. (1979) Cardiac hypertrophy in early hypertension. Am. J. Cardiol. 44: 964–969.

Zandberg, P. (1984) Animal models in experimental hypertension: Relevance to drug testing and discovery, in "Handbook of Hypertension", volume 3: Pharmacology of Antihypertensive drugs (Ed. Van Zwieten, P.A., Elsevier Science Publishers B.V., Amsterdam) 6–45.

CHAPTER 4:
HAEMODYNAMIC PROFILE OF CAPTOPRIL IN CONSCIOUS HYPERTENSIVE RABBITS

The radioactive microsphere technique was used to study the systemic and regional haemodynamic effects of the converting enzyme inhibitor captopril (0.1, 0.3 and 1.0 mg/kg) ten minutes after *i.v.* administration in conscious rabbits with bilateral cellophane perinephritis hypertension, an experimental model of hypertension associated with normal plasma renin levels.

Captopril lowered the arterial blood pressure as a result of a dose dependent decrease in total peripheral resistance. The fall in blood pressure was accompanied by an increase in cardiac output after the second and third dose of captopril; the heart rate was not significantly altered. Captopril produced a generalized peripheral vasodilatation, the changes in vascular conductance being most pronounced in the kidneys, intestines and skin which resulted in a significant increase in blood flow to these vascular beds.

The effective antihypertensive properties of captopril in this "low plasma renin" model of hypertension and the uniform increase in vascular conductances produced by captopril, which antagonizes the generalized increase in vascular resistances that characterizes cellophane perinephritis hypertension, may indicate the involvement of an increased activity of the renin-angiotensin system in tissues such as the vascular wall and brain in the maintenance of the elevated blood pressure in this hypertensive form.

Inhibitors of the renin-angiotensin system (RAS) effectively lower the arterial blood pressure in clinical and experimental forms of hypertension associated with high plasma renin levels (Ondetti and Cushman, 1981). In addition, converting enzyme inhibitors (CEIs) are also capable of reducing the blood pressure in hypertensive forms with low or normal plasma renin activity, a property which is not shared by angiotensin (Ang) II receptor antagonists or Ang antibodies (Antonaccio and Cushman, 1981; Loyke, 1981). Since the angiotensin converting enzyme is the same enzyme as kininase II, it is possible that accumulation of the vasodepressor peptides kallidin and bradykinin serves as an

additional mechanism in the hypotensive action of CEIs (Mimran et al., 1980). However, the evidence presented thus far denies an important contribution of the increase in plasma kinin levels in the hypotensive mechanism of CEIs (Unger et al., 1983).

Recently it has been demonstrated that a local generation of Ang II in tissues such as the vascular wall and brain may play an important role in the maintenance of blood pressure in certain models of hypertension (Swales, 1979). An increased renin content has been found in the arterial wall of spontaneously hypertensive rats with normal plasma renin levels (Asaad and Antonaccio, 1982). Moreover, an interference of CEIs with the local generation of Ang has been demonstrated (Cohen and Kurz, 1982; Unger et al., 1984) which has led to the suggestion that the differences in hypotensive activity of CEIs and Ang II antagonists in certain models of hypertension are related to their relative abilities to penetrate these local sites (Antonaccio et al., 1980). In order to confirm the effectiveness of CEIs in lowering blood pressure in "low plasma renin" hypertension (Vollmer et al., 1981) we studied the hypotensive effects of captopril in conscious rabbits with bilateral cellophane perinephritis hypertension. In addition, we measured the captopril-induced changes in regional blood flows and vascular conductances using the radioactive microsphere technique. This has been investigated because the rather selective vasodilatation after converting enzyme inhibition in "high plasma renin" models (Gavras et al., 1978; Gavras and Liang, 1980) and the more generalized vasodilatation produced by captopril in spontaneously hypertensive rats with normal plasma renin levels (Antonaccio and Cushman, 1981; Richer et al., 1983) indicates that the regional haemodynamic profile of CEIs may differ depending on the experimental form of hypertension used. The results obtained in this study will be published elsewhere (Bolt and Saxena, 1985).

METHODS

Animals and surgical procedures

Experiments were performed on eight male hypertensive New Zealand White rabbits (3.3 ± 0.1 kg). Hypertension was induced by

bilateral cellophane wrapping of the kidneys (Page, 1939), six to ten weeks before the experiment. Nine to fourteen days before the experiment a left-sided thoracotomy was performed in order to cannulate the left atrial appendage with a nylon catheter for the administration of microspheres (Warren and Ledingham, 1972). Subsequently, four to eight days before the experiment the left carotid artery was cannulated for the measurement of blood pressure and heart rate during the experiments and the withdrawal of arterial blood samples. The different surgical procedures together with the systemic and regional haemodynamic characteristics of bilateral cellophane perinephritic hypertension in rabbits have been described in detail elsewhere (Bolt and Saxena, 1983; Bolt and Saxena, 1984a; chapter 2 and 3).

Measurement of haemodynamic variables

On the day of the experiment the animals were placed in a rabbit restrainer box. A Statham P23Dc pressure transducer was connected to the carotid catheter for the recording of the arterial blood pressure and the heart rate using a Grass model 7 polygraph. Mean blood pressure was obtained by electronically damping of the blood pressure signals. Cardiac output and regional blood flows were measured with the radioactive microsphere technique, using the reference blood sample method (Hales, 1974; Heymann et al., 1977; Bolt and Saxena, 1984a). Nen-Trac microspheres were used with a diameter of 15 μm and labeled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc. Cardiac output (ml/min) and regional blood flows (ml/min) were calculated using a set of computer programs especially designed for the radioactive microsphere technique (Saxena et al., 1980). Details are given in chapter 2. Peripheral vascular conductances were obtained by dividing respective tissue blood flows (ml/min) by mean arterial blood pressure (mmHg).

Experimental protocol

The marginal ear vein was cannulated under local anaesthesia (lidocaine 2%) for the administration of captopril HCl (Squibb Inc., Princetown, USA) dissolved in physiological saline. After a stabilization period of at least 30 min the first batch of microspheres was injected to determine baseline values of cardiac

output and regional haemodynamic variables. Heart rate and arterial blood pressure values were recorded continuously. After measuring baseline values each animal received three cumulative doses (0.1, 0.3 and 1.0 mg/kg) of captopril at 15 min intervals. A batch of microspheres was injected 10 min after each dose of captopril at which time point the maximal fall in blood pressure was observed. An arterial blood sample (0.3 ml) was withdrawn immediatly after each microsphere injection to measure pH-, P_{CO_2}- and P_{O_2}-values, using an ABL-2 (Radiometer, Copenhagen).

Statistical evaluation

All data have been expressed as mean (±SEM) in the text. Due to skewed distribution and lack of homogeneity of variances in some variables, non-parametric tests were used for the statistical evaluation (Siegel, 1956). Initially, the Friedman's two way analysis of variance was used to establish whether the samples represented different populations. The Wilcoxon matched-pairs signed ranks test was applied to test the "significance" ($p < 0.05$, two-tailed) of the changes in haemodynamic variables from baseline values.

RESULTS

Blood gases

Values of arterial blood gases and pH were not affected by captopril. Before and 10 min after the successive doses of captopril (0.1, 0.3 and 1.0 mg/kg) the respective values were: pH, 7.40 ± 0.01, 7.36 ± 0.03, 7.38 ± 0.02 and 7.40 ± 0.02; P_{CO_2}, 32 ± 2, 29 ± 1, 28 ± 2 and 27 ± 1 mmHg; and P_{O_2}, 95 ± 2, 98 ± 1, 97 ± 2 and 97 ± 2 mmHg.

Systemic haemodynamic variables

Table 1 shows the effects of captopril, 0.1, 0.3 and 1.0 mg/kg, on systemic haemodynamic variables 10 minutes after the administration in the conscious rabbits with bilateral cellophane perinephritis hypertension. A fall in blood pressure was observed after the successive doses of captopril which could be attributed to a dose-dependent reduction in total peripheral resistance.

Table 1. Effects of captopril, 0.1, 0.3 and 1.0 mg/kg, on systemic haemodynamic variables 10 minutes after *i.v.* administration in conscious hypertensive rabbits (n=8).

		captopril		
	baseline	0.1 mg/kg	0.3 mg/kg	1.0 mg/kg
Arterial blood pressure (mmHg):				
Systolic	142 ± 6	126 ± 4*	112 ± 7*	103 ± 9*
Diastolic	106 ± 6	89 ± 5*	79 ± 6*	72 ± 8*
Mean	121 ± 6	105 ± 5*	91 ± 6*	84 ± 8*
Heart rate (beats/min)	259 ± 16	264 ± 14	274 ± 11	279 ± 8
Cardiac output (ml/min)	433 ± 31	486 ± 39	549 ± 39*	531 ± 41*
Stroke volume (ml)	1.7 ± 0.1	1.9 ± 0.2	2.0 ± 0.2	1.9 ± 0.2
Total peripheral resistance (mmHg/l.min^{-1})	293 ± 33	224 ± 19*	171 ± 16*	160 ± 15*

*, significantly different from baseline values ($p < 0.05$).

Although the changes in heart rate and stroke volume were not significant, both variables tended to increase which led to a moderate but significant rise in cardiac output after the second and third dose of captopril.

Regional haemodynamic variables

Figure 1 shows the changes in regional blood flows to the different organs and tissues measured ten minutes after the successive administrations of captopril (0.1, 0.3 and 1.0 mg/kg) in the conscious hypertensive rabbits. The compound increased the blood flow to the intestines, kidneys and skin, but did not significantly affect that to the stomach, skeletal muscles, brain, heart, bones and "lungs". As the lungs receive 15 μm microspheres via peripheral arteriovenous anastomoses as well as via bronchial arteries, the measured blood flow values represent the combination of arteriovenous anastomotic and bronchial flows. Though total coronary flow did not change, the blood flow to the right ventricle increased significantly after the second and third dose of captopril (+29 ± 8 and +34 ± 7%, respectively). The converting enzyme inhibitor did not affect the weight-normalized left ventricular endocardial/epicardial blood flow ratio; the values

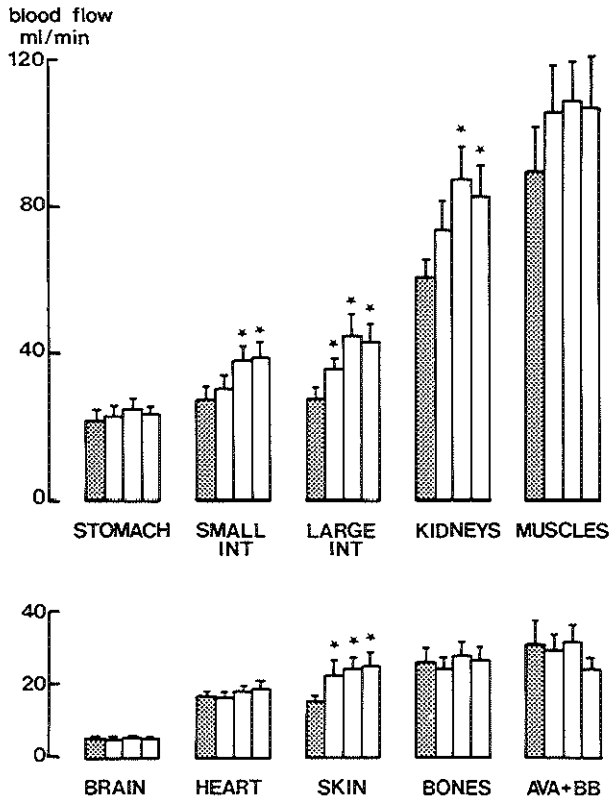


*Figure 1. Effects of captopril on regional blood flows (ml/min) in conscious hypertensive rabbits (n=8). Dotted bars, baseline values; open bars, values 10 min after the i.v. administration of captopril, 0.1, 0.3 and 1.0 mg/kg, respectively; AVA + BB, arteriovenous anastomoses + bronchial vascular bed; *, significant change from baseline values ($p < 0.05$).*

being 1.2 ± 0.1, 1.1 ± 0.1, 1.1 ± 0.1 and 1.1 ± 0.1 before and after the successive doses of captopril, respectively, indicating a normal regional distribution of blood in the left ventricular wall after captopril administration. The compound had also no effect on the blood flows to the different regions of the brain (cerebral hemispheres, cerebellum, brainstem).

Figure 2 shows that captopril produced a rather generalized vasodilatation (increase in vascular conductance) in the different vascular beds. The changes after the successive doses of captopril were most pronounced in the intestines, the kidneys, skeletal muscles and skin. More moderate changes were observed in

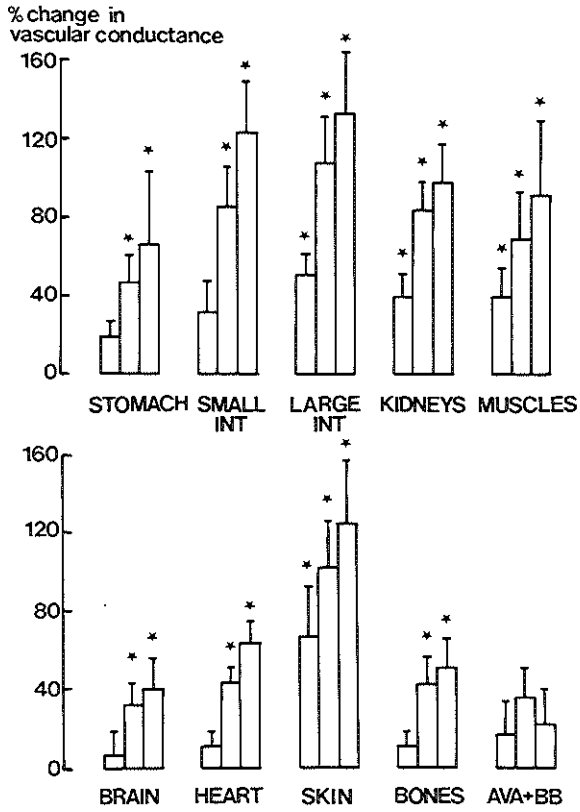


*Figure 2. Effects of captopril on regional vascular conductances in percentage change from baseline values 10 min after the i.v. administration of captopril, 0.1, 0.3 and 1.0 mg/kg, respectively, in conscious hypertensive rabbits (n=8). AVA + BB, arteriovenous anastomoses + bronchial vascular bed; *, significant change from baseline values ($p < 0.05$).*

the stomach, brain, heart and bones whereas the mean vascular conductance of the combination of the arteriovenous anastomoses and bronchial vascular bed was not significantly affected. In the heart the changes were most pronounced in the right ventricle where the vascular conductance increased by 28 ± 10, 74 ± 14 and 104 ± 22%, respectively, after the three successive doses of captopril. The respective changes in vascular conductance in the left ventricle were +13 ± 9, +56 ± 13 and +73 ± 11%, in the intra-ventricular septum +16 ± 10, +46 ± 10 and +71 ± 14%, and in the atria -4 ± 7, +46 ± 21 and +56 ± 23%. In the cerebral vascular bed the vascular conductance increased after the

successive doses of captopril in the cerebral hemispheres by 5 ± 11, 36 ± 15 and 43 ± 18%, in the cerebellum by 10 ± 13, 48 ± 15 and 55 ± 16% and in the brainstem by 29 ± 19, 49 ± 15 and 71 ± 23%, respectively.

DISCUSSION

This study describes the systemic and regional haemodynamic effects of captopril in conscious rabbits with bilateral cellophane perinephritis hypertension. The two-kidney perinephritis model of hypertension produced in rabbits and dogs is associated with normal plasma renin levels (Page, 1939; Campbell et al., 1973). In addition, infusion of the Ang II antagonist saralasin into rabbits and dogs with cellophane perinephritis hypertension fails to lower the arterial blood pressure (Ichikawa et al., 1977). Despite these facts which suggest that the RAS is not involved in maintaining the elevated blood pressure in this hypertensive model (Ichikawa et al., 1977), a pronounced fall in blood pressure is observed in the present study after captopril administration in conscious perinephritic rabbits. The CEI captopril has also been found capable of reducing the blood pressure in dogs with cellophane perinephritic hypertension, implicating a greater role for the RAS in this hypertensive model than suggested in previous investigations using other pharmacological blockers of the RAS (Vollmer et al., 1981). Recently it has been demonstrated that CEIs interfere not only with the plasma RAS but also with the local Ang II generation in tissues such as the vascular wall and brain (Asaad and Antonaccio, 1982; Cohen and Kurz, 1982; Unger et al., 1984). Thus the differences in the hypotensive activity of Ang II antagonists and CEIs may be explained in part by their relative abilities to penetrate these local sites (Antonaccio et al., 1980). Further support for this contention is provided by the finding that the fall in blood pressure produced by CEIs is closely correlated with the degree of converting enzyme inhibition in tissues whereas the antihypertensive potencies of these compounds are neither reflected by changes in converting enzyme activity in the plasma or lung vascular endothelium, nor by reductions in circulating Ang

II (Cohen and Kurz, 1982; Unger et al., 1984).

The decrease in blood pressure after the successive doses of captopril in conscious hypertensive rabbits is due to a dose-related fall in the total peripheral resistance which illustrates the arterial vasodilator properties of the CEI. A pronounced reflex tachycardia, which usually accompanies the fall in blood pressure after vasodilator treatment in conscious animals due to the hypotension induced activation of the baroreceptor reflex, (Bolt and Saxena, 1984a,b,c) was not observed in the present study after captopril administration. This has been attributed to the ability of captopril to alter the set point of the baroreceptors (Hatton et al., 1981). In addition, because endogenous Ang II has a facilitory effect on the autonomic nervous activity (Lumbers and Potter, 1982; Heyndrickx et al., 1976), its inhibition by captopril may blunt the reflex response secondary to the fall in blood pressure. Since a reflex stimulation of the heart was not observed in the present study, the elevated cardiac output after captopril, 0.3 and 1.0 mg/kg, may be due to the pronounced fall in afterload resulting from the reduction in total peripheral resistance (Liang et al., 1978). The increase in cardiac output also suggests that the CEI predominantly affects the arterial site of the circulation since an unchanged or even reduced cardiac output is expected after dilatation of venous capacitance vessels and the subsequent reduction in venous return (Tarazi et al., 1976). Similar changes in cardiac output have been reported after captopril administration in spontaneously hypertensive rats which also have normal plasma renin levels (Antonaccio and Cushman, 1981).

A rather generalized peripheral vasodilatation is observed after the administration of captopril in the conscious rabbits with cellophane perinephritis hypertension. This is not expected from the differential pattern of vasoconstriction produced by exogenous Ang II with the greatest effects on the renal and coronary bed and less intense effects on the mesenteric and iliac bed (Mark et al., 1972; Heyndrickx et al., 1976). Compatible with the response of exogenous Ang II is the rather selective vasodilatation observed after converting enzyme inhibition in hypertensive dogs submitted to renal artery occlusion (Gavras and Liang, 1980) and in normotensive sodium depleted dogs (Gavras et

al., 1978), with significant falls in vascular resistance in the heart, brain and kidneys and no effects on muscles and skin. However, in contrast to perinephritic hypertension, the models mentioned above are both associated with high plasma renin levels. A more generalized vasodilatation, comparable with the captopril-induced changes noticed in the present study, has been observed after captopril administration in spontaneously hypertensive rats (Antonaccio and Cushman, 1981; Richer et al., 1983), which have normal plasma renin levels but an elevated arterial vessel wall renin content (Asaad and Antonaccio, 1982). Thus it is possible that the regional haemodynamic profile of CEIs varies in different models of hypertension depending on the relative contribution of plasma and tissue renin in the maintenance of the elevated blood pressure. However, differences in the haemodynamic profile of captopril as a result of the use of different species cannot be excluded.

The increase in vascular conductance produced by captopril in the present study is less pronounced in the heart and brain, both organs known to posses a strong autoregulatory mechanism which maintains the arterial blood supply in accordance with the respective metabolic demands. The changes in myocardial activity can be predicted, at least in part, by the product of heart rate and systolic blood pressure (Weber and Janicki, 1979). This variable decreased after the successive captopril doses by 9 ± 3, 15 ± 5 and 19 ± 7%, respectively. Nevertheless, the vascular conductance increased in the coronary vascular bed and a significant increase in blood flow to the right ventricle was measured indicating a pronounced direct vasodilator action of captopril in this vascular bed.

A large increase in vascular conductance is observed after captopril administration in the kidneys and intestines, which resulted in an increased blood supply to these vascular beds. A pronounced vasodilatation in the renal and splanchnical bed has been reported in previous investigations using low as well as high plasma renin models of hypertension (Gavras at al., 1978; Gavras and Liang, 1980; Antonaccio and Cushman, 1981; Richer et al., 1983; Suzuki et al., 1984). The increase in vascular conductance in the cutaneous and muscular vascular bed has also been found in spontaneously hypertensive rats (Antonaccio and Cushman, 1981).

Especially the vasodilatation and increase in blood flow in the skin is remarkable considering the relative inactivity of vasodilators of different types to affect the cutaneous vascular bed (Bolt and Saxena, 1984a,b,c). In view of the known vasoactive properties of kinins in the vasculature of the skin (Rowell, 1981), a possible contribution of the captopril-induced inhibition of the kinin degradation must be considered.

Finally, the regional haemodynamic profile obtained with captopril completely counteracts the generalized increase in peripheral vascular resistances with, quantitatively, the most pronounced changes in kidneys, muscles and skin and more moderate changes in the brain measured in conscious rabbits with bilateral cellophane perinephritis hypertension (Bolt and Saxena, 1983). This may indicate an important role for the RAS in tissues such as the vascular wall and/or brain, in the maintenance of the elevated blood pressure in this experimental form of hypertension.

REFERENCES

Antonaccio, M.J., Cushman, D.W. (1981) Drugs inhibiting the renin-angiotensin system. Fed. Proc. 40: 2275–2284.

Antonaccio, M.J., Rubin, B., Horovitz, Z.P. (1980) Cardiovascular pharmacology of captopril, an orally active inhibitor of angiotensin converting enzyme. Drugs Exp. Clin. Res. 6: 519–532.

Asaad, M.M., Antonaccio, M.J. (1982) Vascular wall renin in spontaneously hypertensive rats. Hypertension 4: 487–493.

Bolt, G.R., Saxena, P.R. (1983) Systemic and regional hemodynamic characteristics of bilateral cellophane perinephritis hypertension in conscious rabbits. Clin. Exp. Hypertension A5: 885–901.

Bolt, G.R., Saxena, P.R. (1984a) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205–213.

Bolt, G.R., Saxena, P.R. (1984b) Acute systemic and regional hemodynamic effects of felodipine, a new calcium antagonist, in conscious renal hypertensive rabbits. J. Cardiovasc. Pharmacol. 6: 707–712.

Bolt, G.R., Saxena, P.R. (1984c) Acute systemic and regional haemodynamic profile of prazosin in conscious hypertensive rabbits. Eur. J. Pharmacol. 104: 151–158.

Bolt, G.R., Saxena, P.R. (1985) Acute systemic and regional haemodynamic profile of captopril in conscious rabbits with bilateral cellophane perinephritis hypertension. Accepted for publication in Clin. Exp. Pharmacol. Physiol.

Campbell, D.J., Skinner, S.L., Day, A.J. (1973) Cellophane perinephritis hypertension and its reversal in rabbits. Circ. Res. 33: 105–112.

Cohen, M.L., Kurz, K.D. (1982) Angiotensin converting enzyme inhibition in tissues from spontaneously hypertensive rats after treatment with captopril or MK-421. J. Pharmacol. Exp. Ther. 220: 63-69.

Gavras, H., Liang, C.S., Brunner, H.R. (1978) Redistribution of regional blood flow after inhibition of the angiotensin-converting enzyme. Circ. Res. 43(suppl. 1): 59-63.

Gavras, H., Liang, C.S. (1980) Acute renovascular hypertension in conscious dogs. Circ. Res. 47: 356-365.

Hales, J.R.S. (1974) Radioactive microsphere technique for studies of the circulation. Clin. Exp. Pharmacol. Physiol. Suppl 1: 31-46.

Hatton, R., Clough, D., Faulkner, K., Conway, J. (1981) Angiotensin converting enzyme inhibitor resets baroreceptor reflexes in conscious dogs. Hypertension 3: 676-681.

Heymann, M.A., Payne, B.D., Hoffman, J.I.E., Rudolph, A.M. (1977) Blood flow measurements with radionuclide labelled particles. Progr. Cardiovasc. Dis. 20: 55-79.

Heyndrickx, G.R., Boettcher, D.H., Vatner, S.F. (1976) Effects of angiotensin, vasopressin, and methoxamine on cardiac function and blood flow distribution in conscious dogs. Am. J. Physiol. 231: 1579-1587.

Ichikawa, S., Johnson, J.A., Stanton, M.W., Payne, C.G., Keitzer, W.F. (1977) Hemodynamic effects of an angiotensin II antagonist in rabbits with perinephritis hypertension. Proc. Soc. Exp. Biol. Med. 155:259-263.

Liang, C.S., Gavras, H., Hood, W.B. (1978) Renin-angiotensin system inhibition in conscious sodium-depleted dogs. J. Clin. Invest. 61: 874-883.

Loyke, H.F. (1981) Effect of converting enzyme and angiotensin II inhibitors in chronic one kidney bipolar ligated hypertension. Pharmacol. Res. Commun. 13: 501-509.

Lumbers, E.R., Potter, E.K. (1982) The effects of vasoactive peptides on the carotid cardiac baroreflex. Clin. Exp. Pharmacol. Physiol. Suppl. 7: 45-49.

Mark, A.L., Abboud, F.M., Schmid, P.G. Heistad, D.D., Mayer, H.E. (1972) Differences in direct effects of adrenergic stimuli on coronary, cutaneous, and muscular vessels. J. Clin. Invest. 51: 279-287.

Mimran, A., Targhetta, R., Laroche, B. (1980) The antihypertensive effect of captopril. Hypertension 2: 732-737.

Ondetti, M.A., Cushman, D.W. (1981) Inhibition of the renin-angiotensin system. A new approach to the therapy of hypertension. J. Med. Chem. 24: 355-361.

Page, I.H. (1939) The production of persistent hypertension by cellophane perinephritis. J. Am. Med. Ass. 113: 2046-2048.

Richer, C., Doussau, M.P., Giudicelli, J.F. (1983) Effects of captopril and enalapril on regional vascular resistance and reactivity in spontaneously hypertensive rats. Hypertension 5: 312-320.

Rowell, L.B. (1981) Active neurogenic vasodilatation in man. In: Vasodilatation (Eds. P.M. Vanhoutte, I. Leusen, Raven Press, New York): 1-12.

Saxena, P.R., Schamhardt, H.C., Forsyth, R.P., Loeve, J. (1980) Computer programs for the radioactive microsphere technique. Computer Programs Biomed. 12: 63-84.

Siegel, S. (1956) Non-Parametric Statistics for Behavioural Sciences. (McGraw-Hill Kogakusha, Tokyo).

Suzuki, M., Ohyama, Y., Satoh, S. (1984) Conversion of angiotensin I to angiotensin II and inactivation of bradykinin in canine peripheral vascular beds. J. Cardiovasc. Pharmacol. 6: 244-250.

Swales, J.D. (1979) Arterial wall or plasma renin in hypertension? Clin. Sci. 56: 293-298.

Tarazi, R.C., Dustan, H.P., Bravo, E.L., Niarchos, A.P. (1976) Vasodilating drugs: Contrasting haemodynamic effects. Clin. Sci. Mol. Med. 51: 575s-578s.

Unger, T., Ganten, D., Lang, R.E. (1983) Converting enzyme inhibitors: Antihypertensive drugs with unexpected mechanisms. Trends Pharmacol. Sci, 4: 514-519.

Unger, T., Ganten, D., Lang, R.E., Schölkens, B.A. (1984) Is tissue converting enzyme inhibition a determinant of the antihypertensive efficacy of converting enzyme inhibitors ? Studies with two different compounds, HOE498 and MK421, in spontaneously hypertensive rats. J. Cardiovasc. Pharmacol. 6: 872-880.

Vollmer, R.R., Boccagno, J.A., Steinbacher, T.E., Horovitz, Z.P., Murthy, V.S. (1981) Antihypertensive activity of captopril (SQ 14,225), an orally active inhibitor of angiotensin converting enzyme in conscious two-kidney perinephritic hypertensive dogs. J. Pharmacol. Exp. Ther. 216: 225-231.

Warren, D.J., Ledingham, J.G.G. (1972) Chronic left atrial catheterization in the rabbit. Pflüg. Arch. 335: 167-172.

Weber, K.T., Janicki, J.S. (1979) The metabolic demand and oxygen supply of the heart: Physiologic and clinical considerations. Am. J. Cardiol. 44: 722-729.

CHAPTER 5:
INTERACTION OF ATENOLOL WITH THE HAEMODYNAMIC PROFILE OF HYDRALAZINE IN CONSCIOUS HYPERTENSIVE RABBITS

The radioactive microsphere technique was used to characterize the acute haemodynamic profile of hydralazine, alone and in combination with atenolol, in conscious hypertensive rabbits. Hydralazine, 0.3 mg/kg, *i.v.*, increased the heart rate, stroke volume and cardiac output and decreased total peripheral resistance. A fall in arterial blood pressure was observed only at higher doses (1.0 and 3.0 mg/kg, *i.v.*) due to a further reduction in total peripheral resistance. The drug caused vasodilatation in the heart, brain, kidneys, diaphragm, chest wall and large intestine. In contrast, a, probably reflex-mediated, vasoconstriction was noticed in the skin, stomach and small intestine. In the heart hydralazine preferentially increased blood flow to the outer layers of the left ventricular wall, which resulted in a significant decrease in the endocardial/epicardial blood flow ratio. Hydralazine also greatly enhanced the percentage of 15 μm microspheres distributed to the lungs, indicating an increased arteriovenous anastomotic flow.

Atenolol (1 mg/kg, *i.v.*) elicited bradycardia and moderately reduced blood pressure due to a decrease in cardiac output. In combination with atenolol, hydralazine 0.3 mg/kg caused a less pronounced cardiac stimulation and, now, a fall in blood pressure was produced by the low dose of hydralazine. In contrast, with the high hydralazine dose (3.0 mg/kg) the synergistic effect on blood pressure response disappeared due to an increase in cardiac output, despite effective β-adrenoceptor blockade. In addition, atenolol interfered with the vasodilator response of hydralazine in the heart, skeletal muscles and the arteriovenous anastomoses. The β-adrenoceptor antagonist increased the endocardial/epicardial blood flow ratio and thereby abolished the negative effect of hydralazine on this parameter.

In conclusion, the antihypertensive drugs acted synergistically only at a low hydralazine dose. A better haemodynamic profile was obtained after combined treatment as atenolol protected against the undesirable changes in regional myocardial blood flow distribution observed with hydralazine alone.

The use of arterial vasodilators in hypertensive therapy has gained renewed interest, now that the reflex tachycardia induced by these agents can be blocked effectively by the simultaneous administration of a β-adrenoceptor antagonist (Koch-Weser, 1978). In addition, vasodilators counteract the increase in vascular tone, which is commonly observed after treatment with β-adrenoceptor antagonists alone (Man in 't Veld and Schalekamp, 1982). Although, for the reasons mentioned above, a synergistic effect on blood pressure after combined treatment with these antihypertensive drugs might be expected, contradictory results have been obtained in clinical (Gutkin et al., 1977) and experimental (Provost et al., 1981; Kubo et al., 1981) studies. This has led to speculations on an interference of β-adrenoceptor antagonists with the peripheral effects of vasodilators, possibly by an accentuation of the reflex-mediated vasoconstrictor response due to "unopposed" α-adrenoceptor stimulation (Gutkin et al., 1977). In order to obtain additional information concerning the interaction of the direct- and reflex-mediated effects of vasodilators and β-adrenoceptor antagonists, we used the radioactive microsphere technique to investigate the acute effects of hydralazine and atenolol on systemic as well as regional haemodynamic variables in conscious rabbits with bilateral cellophane perinephritis hypertension. The cardioselective β_1-adrenoceptor antagonist was chosen to minimize the blockade of vascular β_2 adrenoceptors (Barrett, 1977). The data presented in this chapter have been published elsewhere (Bolt and Saxena, 1984).

METHODS

Animals and surgical procedures

Experiments were performed on conscious male New Zealand White rabbits with bilateral cellophane perinephritis hypertension (Page, 1939). Nine to fourteen days before the experiment a left-sided thoracotomy was performed in order to cannulate the left atrial appendage with a nylon catheter for the administration of microspheres (Warren and Ledingham, 1972). Subsequently, four to eight days before the experiment the left carotid artery was

cannulated for the measurement of blood pressure and heart rate during the experiments and the withdrawal of arterial blood samples. The different surgical procedures have been described in detail elsewhere (Bolt and Saxena, 1983; chapter 2 and 3).

Measurement of haemodynamic variables

On the day of the experiment the animals were placed in a rabbit restrainer box. A Statham P23Dc pressure transducer was connected to the carotid catheter for the recording of the arterial blood pressure and heart rate using a Grass model 7 polygraph. Mean arterial blood pressure was obtained by electronically damping the pressure signals. Cardiac output and regional blood flows were measured with the radioactive microsphere technique, using the reference blood sample method (Hales, 1974; Heymann et al., 1977). Nen-Trac microspheres were used with a diameter of 15 μm and labeled with either ^{141}Ce, ^{113}Sn, ^{103}Ru or ^{95}Nb. Cardiac output (ml/min) and regional blood flows (ml/min) were calculated using a set of computer programs, especially designed for the radioactive microsphere technique (Saxena et al., 1980; details are given in the second chapter of this thesis). Peripheral vascular conductances were obtained by dividing the respective tissue blood flows (ml/min) by the mean arterial blood pressure (mmHg).

Experimental protocol

The effects of hydralazine (Apresoline, Ciba Farmaca bv, Arnhem, The Netherlands) and atenolol (Tenormin, ICI Farma bv, Rotterdam, The Netherlands) were investigated in two groups of ten conscious hypertensive rabbits. The marginal ear vein was cannulated under local anaesthesia (Lidocaine 2%) for the administration of drugs. After a stabilization period of at least 30 minutes, the first batch of microspheres was injected to determine baseline values of cardiac output and regional haemodynamic variables. Subsequently, the first group of rabbits (body weight: 3.07 $\pm$ 0.06 kg) received 3 cumulative doses of hydralazine (0.3, 1.0 and 3.0 mg/kg, i.v.) at 20 minutes intervals (figure 1A). Fifteen minutes after each hydralazine dose a batch of microspheres was injected to measure the hydralazine-induced changes in cardiac output and regional haemodynamics.

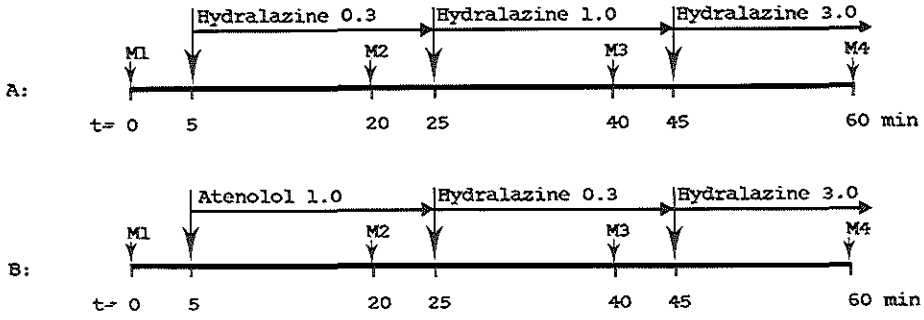


Figure 1. Schematic representation of the time scedules used to study the effects of hydralazine (0.3, 1.0 and 3.0 mg/kg) (A) and of atenolol (1.0 mg/kg) in combination with hydralazine (0.3 and 3.0 mg/kg) (B). Four successive badges of microspheres (M1 to M4) were injected at 20 min intervals, 5 min before and 15 min after the subsequent administrations of the drugs.

In preliminary experiments it was shown that at about 15 minutes after each hydralazine administration the maximal change in blood pressure was reached after which period the response stabilized. Just before each microsphere injection, values of heart rate and blood pressure were obtained and an arterial blood sample (0.3 ml) was taken to measure pH, P_{CO_2} and P_{O_2} using an ABL-2 (Radiometer, Copenhagen). The second group of rabbits (body weight: 2.84 ± 0.10 kg) received atenolol, 1.0 mg/kg *i.v.* and, subsequently, hydralazine 0.3 and 3.0 mg/kg (cumulative doses) using the same experimental protocol (figure 1B). The dose of atenolol was selected on basis of preliminary experiments, in which the β-adrenoceptor blocking effect of atenolol was ascertained using the heart rate responses to isoprenaline in 2 conscious rabbits (6 observations). Atenolol, 1 mg/kg, caused a shift in the dose-response curve of 1.4 ± 0.04 log unit. The blocking effect remained stable for at least 55 minutes. The interaction of atenolol with only two doses of hydralazine could be studied, because of the availability of four batches of microspheres.

Statistical evaluation

All data have been expressed as mean (±SEM) in the text. Due to skewed distribution and lack of homogeneity of variances in some variables, non-parametric tests (Siegel, 1956) were used for the statistical evaluation. The changes in haemodynamic variables from the baseline values were calculated in each experiment seperately. Initially the Friedman's two-way analysis of variance was used to establish whether the samples represented different populations. For the comparison of data in one group of animals the Wilcoxon matched-pairs signed ranks test was employed. The Mann Whitney U-test was chosen for the comparison of the drug-induced changes in the two different groups of animals. Differences were considered significant for $p < 0.05$ (2-tailed). Correlations were determined using the Spearman Rank test.

RESULTS

Effects of hydralazine

Blood gases

Significant changes in arterial blood gases were observed after the administration of hydralazine, 0.3, 1.0 and 3.0 mg/kg. P_{O_2} increased from 88 ± 2 to 101 ± 2, 101 ± 2 and 104 ± 3 mmHg and P_{CO_2} decreased from 38 ± 2 to 30 ± 2, 30 ± 2 and 28 ± 1 mmHg after the successive hydralazine injections. The pH value increased from 7.32 ± 0.02 to 7.50 ± 0.03, 7.52 ± 0.03 and 7.53 ± 0.03.

Systemic haemodynamics

The effects of hydralazine (0.3, 1.0 and 3.0 mg/kg) on systemic haemodynamic variables in hypertensive rabbits are shown in table 1. The lowest hydralazine dose significantly increased the heart rate, cardiac output and stroke volume and decreased total peripheral resistance. These effects were observed in the absence of any hypotensive response with this dose. Instead, a small but significant rise in systolic and mean blood pressure was noticed. Only after hydralazine 1.0 and 3.0 mg/kg blood pressure decreased, accompanied by a further increase in heart rate and decrease in total peripheral resistance.

Table 1. Effects of hydralazine, 0.3, 1.0 and 3.0 mg/kg, on systemic haemodynamic variables in hypertensive rabbits (n=10).

	Baseline	Hydralazine		
		0.3 mg/kg	1.0 mg/kg	3.0 mg/kg
ARTERIAL BLOOD PRESSURE :				
Systolic (mmHg)	151 ± 5	158 ± 5[a]	142 ± 5	124 ± 5[a]
Diastolic (mmHg)	121 ± 4	123 ± 4	109 ± 3[a]	94 ± 4[a]
Mean (mmHg)	134 ± 4	139 ± 4[a]	122 ± 5[a]	106 ± 5[a]
HEART RATE (beats/min)	242 ± 8	280 ± 14[a]	307 ± 9[a]	309 ± 11[a]
CARDIAC OUTPUT (ml/min)	483 ± 30	665 ± 46[a]	641 ± 68[a]	636 ± 63[a]
STROKE VOLUME (ml)	2.0 ± 0.2	2.4 ± 0.2[a]	2.1 ± 0.3	2.1 ± 0.3
TOTAL PERIPHERAL RESISTANCE (mmHg/l.min^{-1})	287 ± 23	217 ± 17[a]	207 ± 20[a]	179 ± 19[a]

a, significantly different from baseline values ($p < 0.05$).

Tissue blood flow

The effects of hydralazine, 0.3, 1.0 and 3.0 mg/kg, on the total amount of blood received by the different organs in hypertensive rabbits are shown in figure 2. As can be deduced from this figure, an important part of the increase in cardiac output after hydralazine was received by the lungs, heart and kidneys. The proportion of 15 μm microspheres trapped in the lungs increased from 3.6 ± 0.8% to 6.7 ± 1.2%, 9.2 ± 1.3% and 8.2 ± 1.4%, respectively, after the three successive hydralazine doses. Considering the small contribution of the bronchial vascular bed in the total circulation (Warren and Ledingham, 1974), the large changes in blood supply to the lungs observed after hydralazine administration probably indicated an increased shunting of blood from the arterial to the venous side of the circulation via arteriovenous anastomoses (AVAs). Also in the brain, diaphragm and chest wall a significant increase in blood supply was measured, however, only a small amount of the total cardiac output is received by these organs. Blood flowes to skeletal muscles and bones were not significantly altered by hydralazine although, especially at the lowest dose of the drug, the blood supply to the muscular bed tended to increase. The arterial vasodilator reduced the blood flow to the skin, stomach

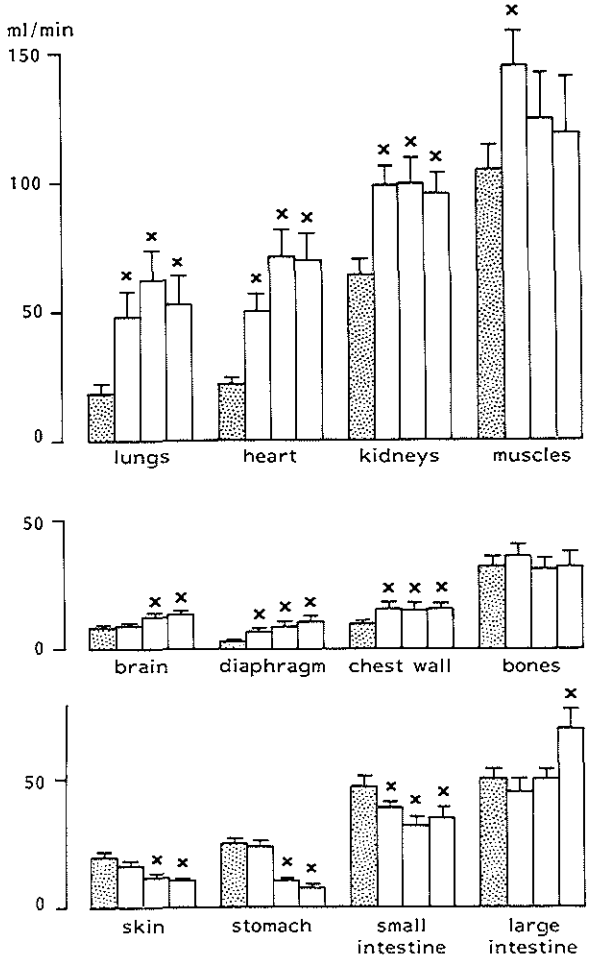


Figure 2: Effects of hydralazine on regional blood flow (ml/min) in conscious renal hypertensive rabbits (n=10). Dotted bars, baseline values; open bars, 15 min after hydralazine, 0.3, 1.0 and 3.0 mg/kg, respectively; x, significantly different from baseline values ($p < 0.05$).

and small intestine. In the large intestine the blood flow was significantly raised after the third hydralazine dose.

Figure 3 shows the effects of hydralazine on the blood supply to the different regions of the brain and the left ventricular wall. In the brain an increase in blood flow was measured in cerebellum and brain stem, while in the cerebral hemispheres the change in blood supply was not significant. In the left

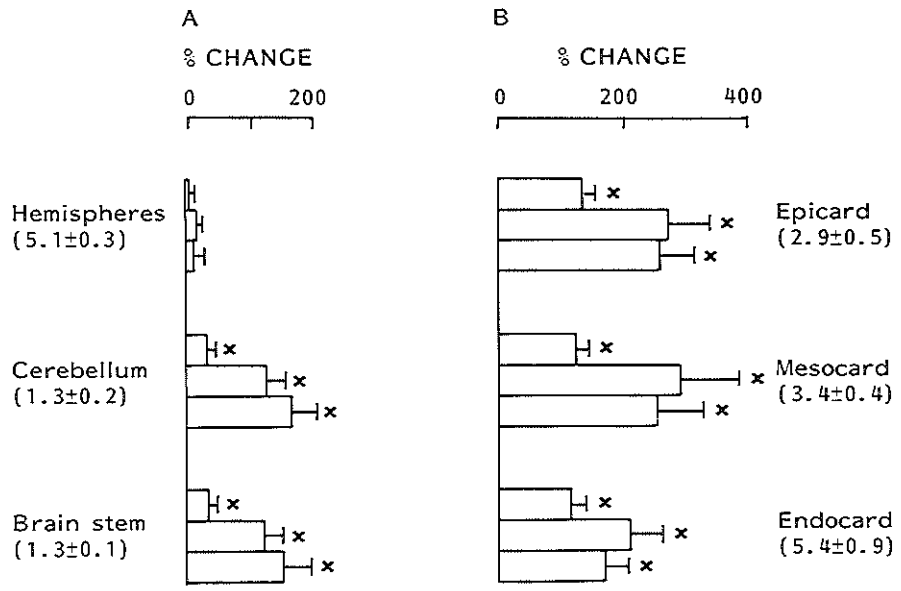


Figure 3: Effects of hydralazine (0.3, 1.0 and 3.0 mg/kg, i.v.) on blood flow to different regions of the brain (A) and the left ventricular wall (B) in percentage change from baseline values (n=10). Between brackets: baseline values in ml/min; x, significant change from baseline values ($p < 0.05$).

ventricular wall the increase in blood flow to epicard and mesocard was larger than in endocard. This resulted in a significant reduction in the endocardial/epicardial blood flow ratio, which was lowered by the three successive hydralazine doses from 1.34 ± 0.06 to 1.20 ± 0.05, 1.10 ± 0.04 and 0.99 ± 0.05, respectively.

Vascular conductance

Dose dependent increases in vascular conductances were observed after hydralazine 0.3, 1.0 and 3.0 mg/kg in the combination of AVAs and bronchial bed, in the diaphragm, heart, brain, kidneys and chest wall (figure 4). In the skeletal muscles and bones the vascular conductance tended to increase although significant levels were not reached. A vasoconstriction was measured in the skin, stomach and small intestine after the administration of the 3 successive doses of the vasodilator, while in the large intestine a significant increase in vascular conductance was noticed after hydralazine 3.0 mg/kg.

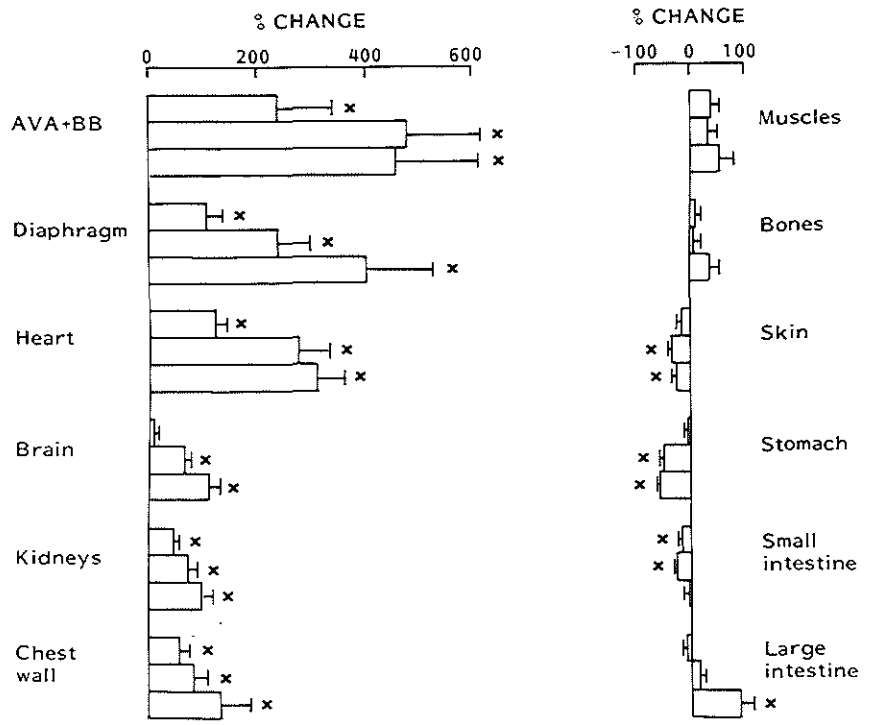


Figure 4: Effects of hydralazine (0.3, 1.0 and 3.0 mg/kg, i.v.) on vascular conductances in percentage change from baseline values (n=10). x, Significant change from baseline values ($p < 0.05$); AVA+BB, arteriovenous anastomoses + bronchial vascular bed.

Effects of atenolol and hydralazine

Blood gases

Atenolol (1.0 mg/kg) had no effects on arterial blood gases nor did the drug modify the significant changes in these variables produced by hydralazine. When hydralazine, 0.3 and 3.0 mg/kg, was given, P_{O_2} increased from 90 ± 2 and 91 ± 2 mmHg (before and after atenolol, respectively) to 102 ± 2 and 103 ± 3 mmHg, respectively, P_{CO_2} decreased from 36 ± 3 and 35 ± 4 mmHg to 27 ± 3 and 26 ± 3 mmHg, and the pH value increased from 7.45 ± 0.01 and 7.45 ± 0.02 to 7.56 ± 0.02 and 7.56 ± 0.02.

Systemic haemodynamics

After the administration of atenolol (1.0 mg/kg) a small decrease in blood pressure was measured. Heart rate and cardiac

Table 2. Effects of atenolol, 1.0 mg/kg, alone and in combination with hydralazine, 0.3 and 3.0 mg/kg, on systemic haemodynamic variables in hypertensive rabbits (n=10).

	Baseline	Atenolol	Hydralazine	
		1.0 mg/kg	0.3 mg/kg	3.0 mg/kg
ARTERIAL BLOOD PRESSURE :				
Systolic (mmHg)	140 ± 6	132 ± 6[a]	126 ± 5[a,b]	113 ± 5[a]
Diastolic (mmHg)	111 ± 4	103 ± 4[a]	95 ± 5[a,b]	86 ± 5[a]
Mean (mmHg)	124 ± 4	116 ± 5[a]	110 ± 5[a,b]	99 ± 5[a]
HEART RATE (beats/min)	231 ± 16	190 ± 11[a]	210 ± 11[a,b]	235 ± 10[b]
CARDIAC OUTPUT (ml/min)	482 ± 59	405 ± 35[a]	440 ± 36[b]	551 ± 63[a]
STROKE VOLUME (ml)	2.1 ± 0.2	2.2 ± 0.2	2.2 ± 0.2	2.4 ± 0.2
TOTAL PERIPHERAL RESISTANCE ($mmHg/l.min^{-1}$)	286 ± 31	319 ± 44	273 ± 34	191 ± 17[a]

a, significantly different from baseline values ($p < 0.05$); b, combined response of atenolol and hydralazine significantly different from response after treatment with hydralazine alone (see table 1)($p < 0.05$).

output were reduced, while stroke volume and total peripheral resistance did not change significantly (table 2). After pretreatment with atenolol, hydralazine (0.3 and 3.0 mg/kg) further reduced blood pressure. Heart rate increased, but baseline values were not exceeded significantly after combined treatment. Cardiac output increased and total peripheral resistance decreased after the administration of hydralazine 0.3 and 3.0 mg/kg. The combined response of atenolol and the low hydralazine dose on systolic, diastolic and mean blood pressure, heart rate and cardiac output (table 2) differed significantly from the response of hydralazine, 0.3 mg/kg, alone (table 1). The decrease in total peripheral resistance also seemed to be smaller after combined treatment, however, the difference with the response after single treatment was not significant. The synergistic effect on the blood pressure response was not observed with the higher dose of hydralazine due to the large increase in cardiac output after hydralazine, 3.0 mg/kg, despite effective β-adrenoceptor blockade. Only the combined response on the heart rate was still significantly different from the response after single treatment with hydralazine, 3.0 mg/kg.

Tissue blood flow

In figure 5 are shown the effects of atenolol (1.0 mg/kg), alone and in combination with hydralazine (0.3 and 3.0 mg/kg), on the amount of blood received by the different organs and tissues in the hypertensive rabbits. An overall tendency for blood flow

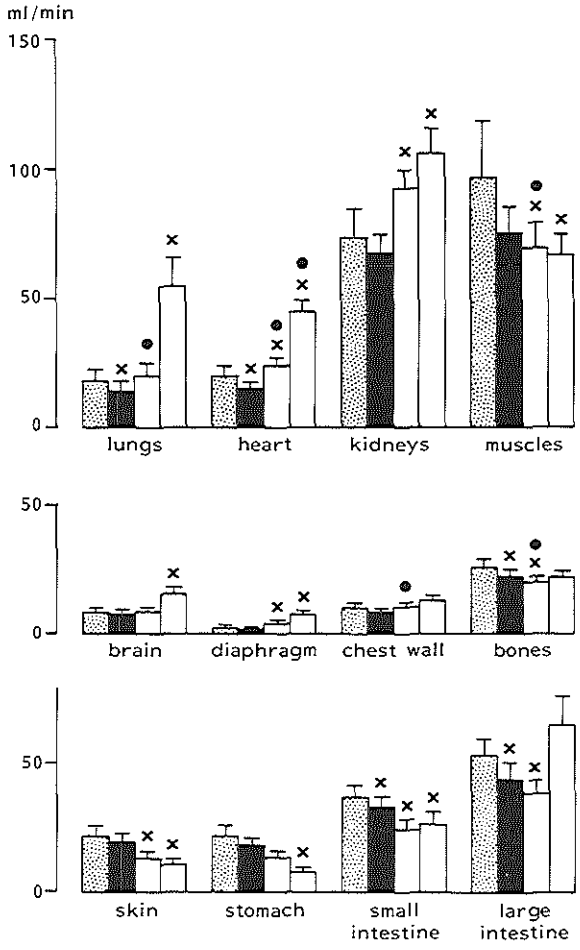


Figure 5: Effects of atenolol (1.0 mg/kg, i.v.) alone or in combination with hydralazine (0.3 and 3.0 mg/kg, i.v.) on regional blood flow (ml/min) in conscious renal hypertensive rabbits (n=10). Dotted bars, baseline values; filled bars, atenolol (1.0 mg/kg); open bars, hydralazine, 0.3 and 3.0 mg/kg respectively; x, significantly different from baseline values; ●, combined response of atenolol and hydralazine significantly different from response of hydralazine alone ($p < 0.05$).

to decrease was noticed after the administration of the β-adrenoceptor antagonist. Significant reductions in blood supply were measured in the lungs (arteriovenous anastomotic + bronchial flow), heart, bones, small intestine and large intestine. After atenolol pretreatment, hydralazine increased blood flow to the lungs, heart, kidneys, brain and diaphragm, while a decrease in blood supply to the skin, stomach and small intestine was observed. Atenolol decreased the proportion of microspheres distributed to the lungs from 4.0 ± 1.4% to 3.7 ± 1.4%, while again after hydralazine the percentage increased up to 4.6 ± 1.2% and 9.5 ± 1.5%, respectively. However, in combination with atenolol, hydralazine, 0.3 mg/kg, caused a significantly smaller increase in blood flow to the lungs, when compared with the changes induced by hydralazine alone, which indicated an inhibition by atenolol of the hydralazine-induced increase in arteriovenous anastomotic flow. Together with atenolol, the hydralazine-induced increase in blood flow to the heart was significantly smaller for both hydralazine doses. In skeletal muscles and bones a significant reduction in arterial blood supply was measured and also in these vascular beds the combined response of atenolol with the low hydralazine dose differed significantly from the response of hydralazine, 0.3 mg/kg, alone. Combined treatment with atenolol also reduced the hydralazine-induced increase in blood flow to the chest wall.

The β-adrenoceptor antagonist had no effect on the blood supply to the different regions of the brain (figure 6). After combined treatment with atenolol, again the changes in blood flow induced by hydralazine were most pronounced in cerebellum and brain stem, and not significantly different from the effects after single treatment with hydralazine. In the left ventricular wall atenolol, 1.0 mg/kg, decreased blood flow in epicard by 24 ± 5%, in mesocard by 18 ± 6% and in endocard by 15 ± 4% (figure 6). Consequently, there was a significant increase in the endocardial/epicardial blood flow ratio from 1.51 ± 0.06 to 1.69 ± 0.07. The favourable blood flow redistribution towards the endocard after atenolol compensated for the negative effect of hydralazine on the endocardial/epicardial blood flow ratio as is shown in figure 6 where the combined effects of atenolol (1.0 mg/kg) and hydralazine (0.3 and 3.0 mg/kg) on the blood flow to

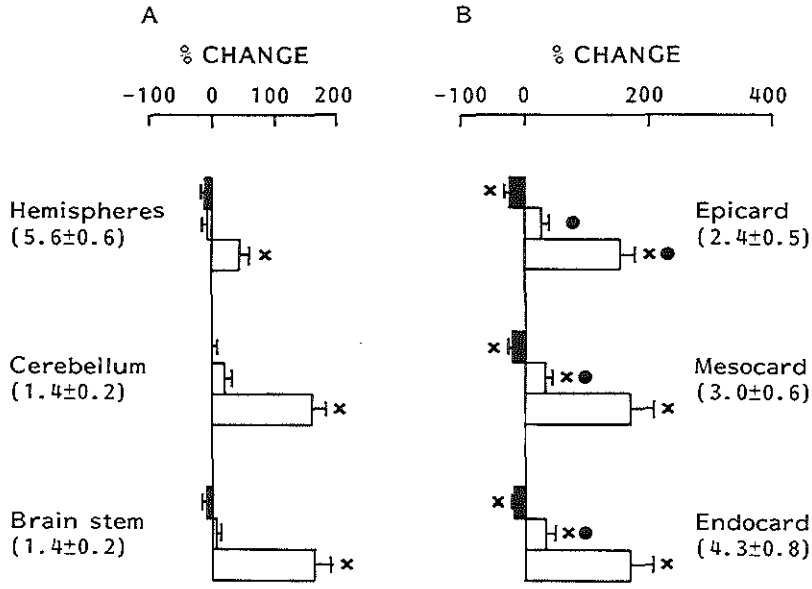


Figure 6: Effects of atenolol (1.0 mg/kg) alone and in combination with hydralazine (0.3 and 3.0 mg/kg) on blood flow to different regions of the brain (A) and the left ventricular wall (B) in percentage change from baseline values (n=10). Between brackets: baseline values in ml/min; filled bars, atenolol; open bars, hydralazine, 0.3 and 3.0 mg/kg respectively; x, significant change from baseline values; ●, combined response of atenolol and hydralazine significantly different from response of hydralazine alone (p < 0.05).

epi-, meso- and endocard appeared to be similar. After hydralazine, 0.3 and 3.0 mg/kg, the endocardial/epicardial blood flow ratio decreased to 1.59 ± 0.07 and 1.57 ± 0.04, respectively, but the values were not lower than that at baseline level (1.51 ± 0.06). In addition, only the combination of atenolol with the low hydralazine dose resulted in a smaller increase in blood flow to the endocard, while in the epicard the combined response of atenolol with both hydralazine doses was significantly different from the response of hydralazine alone.

Vascular conductance

Only small changes in regional vascular conductances were observed in the hypertensive rabbits after the administration of atenolol, 1.0 mg/kg (figure 7). A significant decrease was measured in the combination of AVAs and bronchial vascular bed, in the heart and in the large intestine. In the presence of atenolol, hydralazine again caused vasodilatation in the

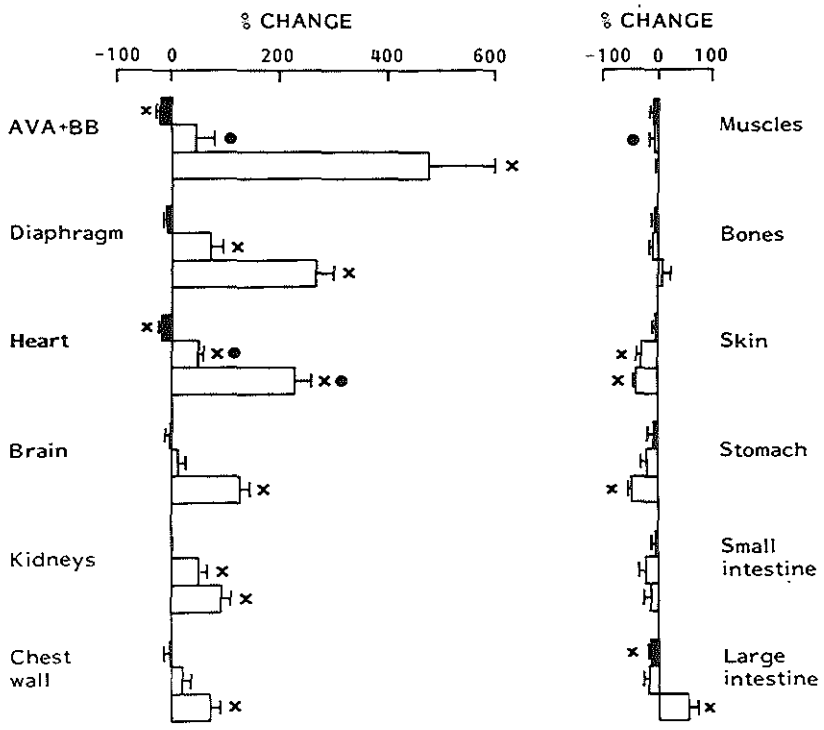


Figure 7: Effects of atenolol (1.0 mg/kg) alone and in combination with hydralazine (0.3 and 3.0 mg/kg) on vascular conductances in percentage change from baseline values (n=10). Filled bars, atenolol; open bars, hydralazine, 0.3 and 3.0 mg/kg respectively; x, significant change from baseline values; ●, combined response of atenolol and hydralazine significantly different from response of hydralazine alone ($p < 0.05$); AVA+BB, arteriovenous anastomoses + bronchial vascular bed.

combination of AVAs and bronchial bed. However, the combined response of atenolol and the low hydralazine dose was significantly smaller, when compared with the response of hydralazine, 0.3 mg/kg, alone. Also in the heart and the skeletal muscles the combined effects of atenolol and the low hydralazine dose were significantly different from the changes observed after single treatment with hydralazine, 0.3 mg/kg. The increase in vascular conductance in the heart was reduced, while in the skeletal muscles no vasodilatation could be observed after combined treatment. Only in the heart a significantly smaller increase in vascular conductance was observed after combined

treatment with atenolol and the high hydralazine dose. Atenolol did not significantly modify the hydralazine-induced vasodilatation in diaphragm, brain, kidneys, chest wall and large intestine nor did the $\beta_1$adrenoceptor antagonist interfere with the vasoconstrictor response after hydralazine, 0.3 and 3.0 mg/kg in the skin, stomach and small intestine.

DISCUSSION

Systemic effects of hydralazine and atenolol

Using a low dose of hydralazine (0.3 mg/kg) an increase in heart rate, stroke volume and cardiac output and a decrease in total peripheral resistance was noticed in the conscious renal hypertensive rabbits, while no hypotensive effect could be measured. Considerable cardiac stimulation with hydralazine in the presence of an unchanged or even elevated arterial blood pressure has been reported earlier (Spokas and Wang, 1980; Vidrio and Tena, 1980; Lin et al.,1983). Spokas and Wang (1980) have suggested that, in addition to baroreceptor activation, the augmentation of heart rate and cardiac output after hydralazine results from an increase in venous return which activates atrial stretch receptors (Bainbridge reflex). In fact, it is generally agreed that drugs, which cause arterial vasodilatation without affecting venous tone, elicit a greater increase in heart rate and cardiac output than vasodilators which dilate both resistance and capacitance vessels (Tarazi et al., 1976). When higher doses of hydralazine were administered, a reduction in blood pressure was observed. Thus, the increased cardiac output no longer compensated for the further decrease in total peripheral resistance.

In the renal hypertensive rabbits atenolol (1.0 mg/kg), like propranolol (Van Boom and Saxena, 1983), caused a small reduction in blood pressure which was accompanied by a decrease in heart rate and cardiac output. After atenolol there was a tendency for total peripheral resistance to increase and a clear negative correlation existed between the changes in cardiac output and total peripheral resistance (r_s = 0.927, $p < 0.05$, figure 8). This suggests that a large fall in arterial blood pressure after

atenolol, as a result of the lowered cardiac output, is prevented by reflex-mediated peripheral vasoconstriction (Man in 't Veld and Schalekamp, 1982).

In contrast with the effects of hydralazine alone, the low hydralazine dose (0.3 mg/kg) immediately caused a further decrease in blood pressure after atenolol pretreatment because of an inhibition of the hydralazine-induced cardiac stimulation. In agreement with other studies on the interaction of β-adrenoceptor antagonists and arterial vasodilators (Gutkin et al., 1977; Provost et al., 1981; Kubo et al., 1981; Man in 't Veld et al., 1978) a synergistic effect on blood pressure was no longer observed with the high hydralazine dose (3.0 mg/kg). In part this could be explained by the increase in cardiac output after hydralazine (3.0 mg/kg) despite β_1-adrenoceptor blockade. In addition, a tendency was observed for total peripheral resistance to decrease less after atenolol pretreatment, indicating a possible interference of atenolol with the peripheral effects of hydralazine. Although mean heart rate values after combined

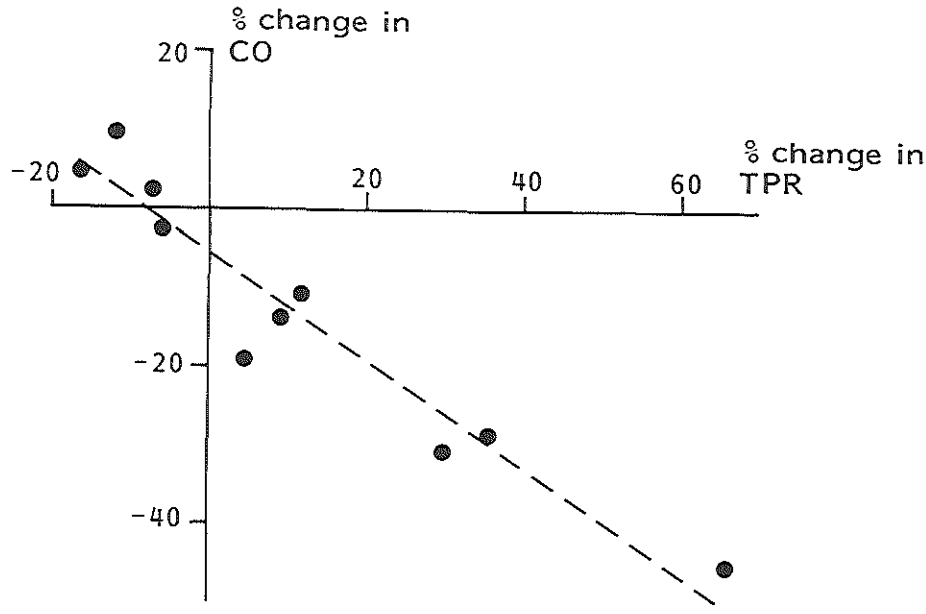


Figure 8: A significant negative correlation existed between the atenolol (1.0 mg/kg)-induced changes in cardiac output (CO) and total peripheral resistance (TPR) in conscious renal hypertensive rabbits (r_s=0.927, $p < 0.05$).

treatment did not exceed baseline values, the hydralazine-induced reflex tachycardia was still present after β-adrenoceptor blockade. Because the blockade of the isoprenaline-induced tachycardia by atenolol does nor wear off during the course of these experiments (see Methods), it appears that a withdrawal of the parasympathetic tone is mainly responsible for the vasodilator-induced reflex tachycardia (Man in 't Veld et al., 1978; Reid, 1979). However, a possible direct myocardial stimulatory action of hydralazine cannot be excluded (Songkittiguna and Rand, 1982).

Regional effects of hydralazine and atenolol

Considering the small contribution of the bronchial vascular bed in the total circulation (Warren and Ledingham, 1974), the large increase in microsphere content of the lungs after hydralazine must be attributed for a major part to an increase in the arteriovenous anastomotic blood flow. Similarly, the decrease in the amount of microspheres trapped in the lungs after atenolol can be explained by a constriction of AVAs due to reflex-mediated α-adrenoceptor stimulation. Indeed, Spence et al. (1972) have demonstrated that AVAs are under α-adrenergic control. However, the apparently very large vasodilatation of AVAs after hydralazine without any sign of opposing reflex-mediated vasoconstriction suggests that, compared to the adrenergic influence, these non-nutritional vessels are more effectively regulated by tissue autoregulatory mechanisms. Thus, if the <u>overall</u> metabolic need of the body remains essentially unchanged and the cardiac output increases (as after hydralazine) or decreases (as after atenolol), there should be parallel changes in arteriovenous anastomotic flow. Accordingly, as atenolol reduces the hydralazine-induced increase in cardiac output, a smaller enhancement of the arteriovenous anastomotic flow is expected and observed after combined treatment.

An adaptation of the myocardial blood flow to the enhanced metabolic demands resulting from the hydralazine-induced cardiac stimulation obviously contributed to the large increase in blood flow to the heart observed after hydralazine administration. Similarly, a reduction in metabolic activity after β_1-adrenoceptor blockade probably determines for a major part the coronary

vasoconstriction observed in the present study after atenolol administration. The favourable blood flow redistribution towards the endocardium after β-adrenoceptor blockade has been reported earlier (Saxena, 1983). An increase in diastolic perfusion time and a decrease in myocardial wall stress after atenolol are possible explanations for this phenomenon (Thomas et al., 1981; Saxena, 1983). Moreover, the increase in the endocardial/epicardial blood flow ratio after atenolol completely abolished the negative effect of hydralazine on this parameter. This important observation indicates that β-adrenoceptor antagonists not only oppose the hydralazine-induced cardiac stimulation, but may also prevent the potentially injurious effects of hydralazine on regional myocardial blood supply which has been associated with the precipitation of myocardial ischaemia after treatment with arterial vasodilators.

Pronounced vasodilatation after hydralazine was also observed in the brain, kidneys, diaphragm, chest wall and large intestine. Because a strong cerebral autoregulation has been demonstrated, it is possible that an increase in metabolic activity in certain regions of the brain, for instance in the cardio-acceleration and/or vasomotor centres in medulla and pons, contributed to the large increase in blood flow to cerebellum and brainstem after hydralazine. Autoregulatory mechanisms possibly also participated in the hydralazine-induced vasodilatation in diaphragm and chest wall, because an increase in ventilation after hydralazine has been reported previously (Johnston, 1975) and is also indicated in the present study by the rise in P_{O_2} and fall in P_{CO_2} observed after hydralazine administration.

The complete absence of the vasodilator actions of hydralazine in skin, stomach and small intestine may be attributed to the activation of cardiovascular reflex mechanisms. Reflex-mediated vasoconstrictor responses, due to an enhanced sympathetic vascular tone and circulating catecholamines, oppose the direct vasodilator actions of hydralazine. In addition, it has been shown that even in acute experiments the activation of the renin-angiotensin system contributes to the reflex-mediated changes in vascular tone (Gutkin et al., 1977). A decrease in blood flow to the skin and stomach after hydralazine has also been observed in anaesthetized rabbits (Johnston, 1975) and in

conscious spontaneously hypertensive rats (Pegram et al., 1982). In contrast, a generalized vasodilator response after hydralazine administration which included the femoral and mesenteric vascular beds has been reported in studies on blood prefused circulations isolated from the autonomic nervous system in rats (Sakai et al., 1980).

A tendency for vascular conductance to increase was observed after hydralazine administration in the skeletal muscles and bones. Spokas and Wang (1980), observing a moderate vasodilatation in the femoral vascular bed of conscious dogs only at low, non-hypotensive, doses of hydralazine, suggested that with hypotension reflex-mediated vasoconstriction might off-set the direct femoral vasodilating action of hydralazine. In contrast with the effects of hydralazine alone, the low hydralazine dose caused an immediate further reduction in blood pressure when given after atenolol in the hypertensive rabbits. Hence, a more pronounced reflex-mediated vasoconstrictor respons can be expected which is compatible with the significantly different response in the skeletal muscles after combined treatment with atenolol and hydralazine. In addition, although atenolol is known as a β_1 selective adrenoceptor antagonist, a partial blockade of vascular β_2 receptors in skeletal muscles resulting in an accentuation of the reflex-mediated vasoconstrictor response, due to "unopposed" α-adrenoceptor stimulation, cannot be excluded (Gutkin et al., 1977) and may contribute to the complete absence of the vasodilator response in the skeletal muscles after combined treatment.

In conclusion, cardiovascular reflex mechanisms and tissue autoregulation play an important role in the observed systemic and regional haemodynamic effects of hydralazine and atenolol in conscious renal hypertensive rabbits. The cardioselective β-adrenoceptor antagonist inhibited the hydralazine-induced cardiac stimulation and thereby accentuated the hypotensive effect of the arterial vasodilator. With higher hydralazine doses the synergistic effect on the blood pressure response disappeared, due to an increase in cardiac output, despite effective β-adrenoceptor blockade. Moreover, atenolol indirectly reduced the peripheral vasodilator effects of hydralazine in certain vascular beds.

Finally, atenolol abolished the potentially injurious effects of hydralazine on regional blood flow distribution in the left ventricular wall.

REFERENCES

Barrett, A.M. (1977) The pharmacology of atenolol. Postgrad. Med. J. 53(suppl. 3): 58–64.

Bolt, G.R., Saxena, P.R. (1983) Systemic and regional hemodynamic characteristics of bilateral cellophane perinephritis hypertension in conscious rabbits. Clin. Exp. Hypertension 5A: 885–901.

Bolt, G.R., Saxena, P.R. (1984) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205–213.

Gutkin, M., Das, B., Chin, B.K., Mezey, K., Modlinger, R.S. (1977) Synergistic effects of hydralazine and alpha- or beta-adrenergic blockers: The role of plasma renin activity. J. Clin. Pharmacol. 17: 509–518.

Hales, J.R.S. (1974) Radioactive microsphere technique for studies of the circulation. Clin. Exp. Pharmacol. Physiol. suppl. 1: 31–46.

Heymann, M.A., Payne, B.D., Hoffman, J.I.E., Rudolph, A.M. (1977) Blood flow measurements with radionuclide-labelled particles. Progr. Cardiovasc. Dis. 20: 55–79.

Johnston, B.M. (1975) The use of radioactive microspheres to compare the effects of hydralazine, guanethidine and SKF 24260 on the redistribution of cardiac output in anaesthetized rabbits. Brit. J. Pharmacol. 55: 393–402.

Koch-Weser, J. (1978) The comeback of hydralazine. Amer. Heart J. 95: 1–3, 1978.

Kubo, K., Yamashita, M., Misu, Y. (1981) Effects of β-blocking agents on the cardiovascular action of hydralazine in conscious rats. Japan J. Pharmacol. 31: 1087–1090.

Lin, M.S., McNay, J.L., Shepherd, A.M.M., Musgrave, G.E., Kent, K.T. (1983) Increased plasma norepinephrine accompanies persistant tachycardia after hydralazine. Hypertension 5: 257–263.

Man in 't Veld, A.J., Wenting, G.J., Verhoeven, R.P., Schalekamp, M.A.D.H. (1978) Sympathetic and parasympathetic components of cardiostimulation during vasodilator treatment of hypertension. Clin. Sci. Mol. Med. 55: 329s–332s.

Man in 't Veld, A.J., Schalekamp, M.A.D.H. (1982) How intrinsic sympathomimetic activity modulates the haemodynamic response to β-adrenoceptor antagonists. A clue to the nature of their antihypertensive mechanism. Brit. J. Clin. Pharmacol. 13: 245s–257s.

Page, I.H. (1939) The production of persistent hypertension by cellophane perinephritis. J. Amer. Med. Ass. 113: 2046–2048.

Pegram, B.L., Ishise, S., Frohlich, E.D. (1982) Effect of methyldopa, clonidine and hydralazine on cardiac mass and haemodynamics in Wistar Kyoto and spontaneously hypertensive rats. Cardiovasc. Res. 16: 40–46, 1982.

Provost, Y., Lindenbaum, A., Cohen, Y., Wepierre, J. (1981) Cardiovascular effects of dihydralazine, diazoxide and minoxidil alone or associated with propranolol in renal hypertensive rats. Arch. Int. Pharmacodyn. 251: 103–115.

Reid, J.L. (1979) Acute and chronic β-receptor blockade with propranolol and the cardiovascular response to intravenous sodium nitroprusside in the conscious rabbit. J. Cardiovasc. Pharmacol. 1: 403–414.

Sakai, K., Akima, M. Adachi, J. (1980) Pharmacological features of the coronary, renal, mesenteric, and femoral vascular beds of rats revealed by intra-arterial administration of drugs. J. Cardiovasc. Res. 2: 453–470.

Saxena, P.R., Schamhardt, H.C., Forsyth, R.P., Loeve, J. (1980) Computer programs for the radioactive microsphere technique. Computer Programs Biomed. 12: 63–84.

Saxena, P.R. (1983) Beta-adrenoceptor antagonists and blood flow to the jeopardized myocardium. Eur. Heart J. 4s: 101–108.

Siegel, S. (1956) Non-parametric statistics for behavioral sciences, Tokyo: McGraw-Hill Kogakusha.

Songkittiguna, P., Rand, M.J. (1982) The mechanisms of initropic and chronotropic actions of hydralazine on rat isolated atria. Arch. Int. Pharmacodyn. 255: 268–280.

Spence, R.J., Rhodes, B.A., Wagner, H.N. (1972) Regulation of arteriovenous anastomotic and capillary blood flow in the dog leg. Amer. J. Physiol. 222: 326–332.

Spokas, E.G., Wang, H.H. (1980) Regional blood flow and cardiac response to hydralazine. J. Pharmacol. Exp. Ther. 212: 294–303.

Tarazi, R.C., Dustam, H.P., Bravo, E.L., Niarchos, A.P. (1976) Vasodilating drugs: contrasting haemodynamic effects. Clin. Sci. Mol. Med. 51: 575s–578s.

Thomas, M., Gabe, I.T., Kimber, H., Mills, J., Sweeting, T.J. (1981) Does β-blockade directly reduce myocardial oxygen consumption? Cardiovasc. Res. 15: 254–261.

Van Boom, M.P., Saxena, P.R. (1983) Tissue blood flow changes induced by propranolol infusion in conscious normotensive and renal hypertensive rabbits. Arch. Int. Pharmacodyn. Ther. 264: 96–109.

Vidrio, H., Tena, I. (1980) Hydralazine tachycardia and sympathetic cardiovascular reactivity in normal subjects. Clin. Pharmacol. Ther. 28: 587–591.

Warren, D.J., Ledingham, J.G.G. (1972) Chronic left atrial catheterization in the rabbit. Plug Arch. 335: 246–251.

Warren, D.J., Ledingham, J.G.G. (1974) Measurement of cardiac output distribution using microspheres. Some practical and theoretical considerations. Cardiovasc. Res. 8: 570–581.

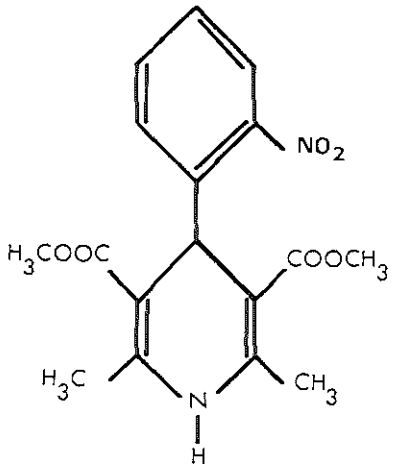
CHAPTER 6:
HAEMODYNAMIC PROFILE OF FELODIPINE, A NEW CALCIUM ANTAGONIST, IN CONSCIOUS HYPERTENSIVE RABBITS

The radioactive microsphere technique was used to study the acute systemic and regional haemodynamic effects of felodipine (3, 10 and 30 μg/kg, *i.v.*) in conscious rabbits with bilateral cellophane perinephritis hypertension. A dose-dependent decrease in arterial blood pressure was observed after felodipine administration, accompanied by tachycardia. Cardiac output increased significantly after the third felodipine dose. Thus, the hypotensive effect of the drug resulted from a reduction in total peripheral resistance.

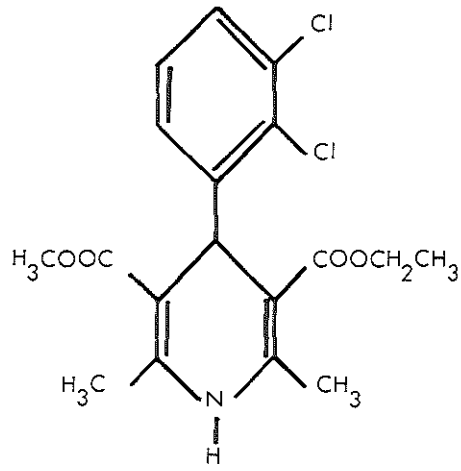
Felodipine increased the blood flow to the heart. However, probably secondary to the reduction in diastolic perfusion time, a decrease in the endocardial/epicardial blood flow ratio was noticed in the left ventricular wall. The drug also enhanced the blood supply to the brain, the gastro-intestinal tract and, at higher doses, to the skeletal muscles while that to the kidneys and the bones remained unchanged. After the highest dose of felodipine a significant decrease in the blood flow to the skin was measured. With the exception of the cutaneous vascular bed, felodipine caused a rather generalized peripheral vasodilatation.

In conclusion, felodipine appears to be a very effective antihypertensive agent. However, the stimulation of the heart and the unfavourable regional blood flow distribution in the left ventricular wall illustrate the negative aspects of single treatment with this arterial vasodilator.

Calcium entry blockers are a heterogeneous group of compounds which share the common ability to relax vascular smooth muscle, decrease pacemaker activity and reduce cardiac muscle force development (Fleckenstein, 1977; Naylor, 1983; Saini, 1984). Because the antihypertensive property of calcium entry blockers is based upon the decrease in total peripheral resistance induced by these agents, efforts have been made to synthesize new compounds, which show selectivity for vascular smooth muscle. This has led to the development of felodipine, a new dihydropyridine derivative



Nifedipine



Felodipine

Figure 1. Structural formulae of nifedipine and felodipine.

with a structure resembling that of nifedipine (figure 1). The vasodilator potency of the compound appears to be 100 times greater than its negative inotropic potency as was shown in a comparative study on the isolated rat portal vein and rat papillary muscle (Ljung, 1980). Since considerable heterogeneity in the affinity of different vascular smooth muscle preparations for calcium entry blockers has been demonstrated (Vanhoutte, 1982; Naylor, 1983), we have studied the systemic as well as regional haemodynamic effects of felodipine in conscious renal hypertensive rabbits using the radioactive microsphere technique. The data presented in this chapter have been published elsewhere (Bolt and Saxena, 1983a; Bolt and Saxena, 1984a).

METHODS

Animals and surgical procedures

Experiments were performed on 10 male hypertensive New Zealand White rabbits (2.9 ± 0.1 kg). Hypertension was induced by bilateral cellophane wrapping of the kidneys according to the

method of Page (1939), 6-10 weeks before the experiment. Nine to forteen days before the experiment a left-sided thoracotomy was performed in order to cannulate the left atrial appendage with a nylon catheter for the administration of microspheres (Warren and Ledingham, 1972). Subsequently, 4-8 days before the experiment the left carotid artery was cannulated for the measurement of blood pressure and heart rate during the experiments and the withdrawal of arterial blood samples. The different surgical procedures have been described in detail elsewhere (Bolt and Saxena, 1983b; Bolt and Saxena, 1984b; chapter 2 and 3).

Measurement of haemodynamic variables

On the day of the experiment the animals were placed in a rabbit restrainer box. A Statham P23Dc pressure transducer was connected to the carotid catheter for the recording of the arterial blood pressure and the heart rate, using a Grass model 7 polygraph. Mean blood pressure was obtained by electronically damping of the blood pressure signals.

Cardiac output and regional blood flows were measured with the radioactive microsphere technique, using the reference blood sample method (Hales, 1974; Heymann et al., 1977). Nen-Trac microspheres were used with a nominal diameter of 15 μm and labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc. Cardiac output (ml/min) and regional blood flows (ml/min) were calculated using a set of computer programs especially designed for the radioactive microsphere technique (Saxena et al., 1980; relevant information is given in chapter 2). Peripheral vascular conductances were obtained by dividing respective tissue blood flows (ml/min) by mean arterial blood pressure (mmHg).

Experimental protocol

The marginal ear vein was cannulated under local anaesthesia (lidocaine 2%) for the administration of felodipine (Astra Pharmaceuticals AB, Molndal, Sweden). After a stabilization period of at least 30 minutes, the first batch of microspheres was injected to determine baseline values of cardiac output and regional haemodynamic variables. Heart rate and blood pressure values were recorded continuously. After measuring baseline values each animal received 3 cumulative doses (3, 10 and 30

μg/kg) of felodipine dissolved in 10% polyethyleneglycol. Ten minutes after each dose a batch of microspheres was injected. An arterial blood sample (0.3 ml) was withdrawn just before each microsphere injection to measure pH-, P_{CO_2}- and P_{O_2}-values, using an ABL-2 (Radiometer, Copenhagen).

Statistical evaluation

All data have been expressed as mean (±SEM) in the text. Due to skewed distribution and lack of homogeneity of variances in some variables, we used non-parametric tests (Siegel, 1956). Initially, the Friedman's two way analysis of variance was used to establish whether the samples represented different populations. The changes in haemodynamic variables from the baseline values were calculated separately in each experiment and the "significance" ($p < 0.05$, two-tailed) of these changes was determined by using the Wilcoxon matched-pairs signed-ranks test.

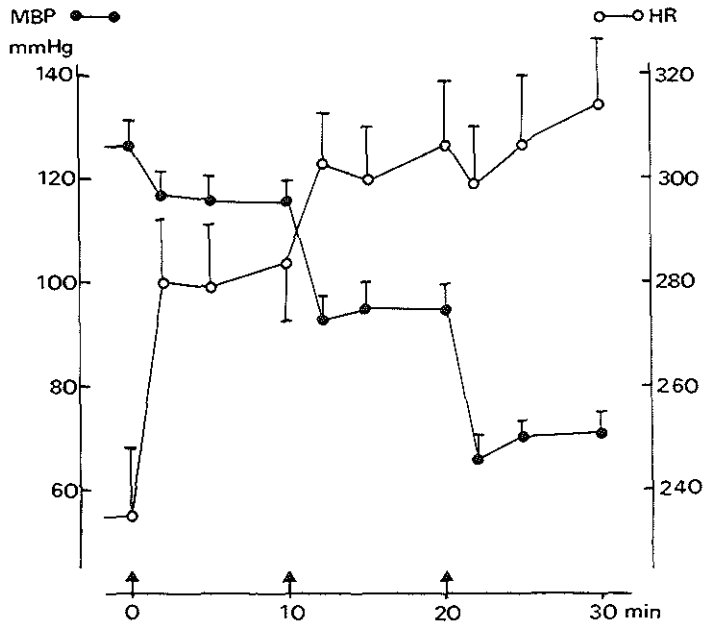


Figure 2. Effects of felodipine (3, 10 and 30 μg/kg, i.v.) on mean arterial blood pressure (MBP) and heart rate (HR) in conscious renal hypertensive rabbits (n=10).

RESULTS

Blood gases

The effects of felodipine, 3, 10 and 30 μg/kg, on pH-, P_{CO_2}- and P_{O_2}-values were measured 10 minutes after the i.v. administration of the drug in the conscious renal hypertensive rabbits. Mean values were for pH: 7.44 ± 0.04, 7.45 ± 0.03, 7.45 ± 0.04 and 7.45 ± 0.04, for P_{CO_2}: 43 ± 2, 42 ± 4, 42 ± 3 and 39 ± 3 mmHg and for P_{O_2}: 83 ± 3, 85 ± 3, 87 ± 3 and 94 ± 4 mmHg before and after the 3 successive doses, respectively. Only the P_{O_2}-value was significantly increased after the highest felodipine dose.

Systemic haemodynamic variables

Figure 2 illustrates that within 2 minutes after the i.v. administration of felodipine in conscious renal hypertensive rabbits the maximum effect on blood pressure was reached. The hypotensive effect remained stable for at least 10 minutes and was accompanied by an increase in heart rate.

Table 1 shows the changes in systolic and diastolic blood

TABLE 1. Effects of felodipine on systemic haemodynamics 10 minutes after i.v. administration in conscious renal hypertensive rabbits (n=8).

	Baseline	Felodipine (μg/kg):		
		3	10	30
ARTERIAL BLOOD PRESSURE				
systolic (mmHg)	139 ± 5	133 ± 4	112 ± 7*	91 ± 6*
diastolic (mmHg)	113 ± 5	108 ± 6	89 ± 6*	66 ± 4*
HEART RATE (beats/min)	231 ± 14	277 ± 11*	300 ± 13*	304 ± 14*
CARDIAC OUTPUT (ml/min)	477 ± 56	514 ± 51	590 ± 66	696 ± 99*
STROKE VOLUME (ml)	2.1 ± 0.2	1.8 ± 0.2	2.0 ± 0.2	2.2 ± 0.3
TOTAL PERIPHERAL RESISTANCE ($mmHg/l.min^{-1}$)	290 ± 33	248 ± 25	187 ± 26*	128 ± 19*

*, significantly different from baseline values ($p < 0.05$).

pressure, heart rate, cardiac output, stroke volume and total peripheral resistance, 10 minutes after the administration of the 3 successive felodipine doses. The hypotensive effect of the drug resulted from a dose-dependent decrease in total peripheral resistance. After the highest felodipine dose a significant increase in cardiac output was measured; stroke volume did not change significantly.

Regional haemodynamic variables

Figure 3 shows the effects of felodipine on regional blood flows measured ten minutes after the administration of the drug in

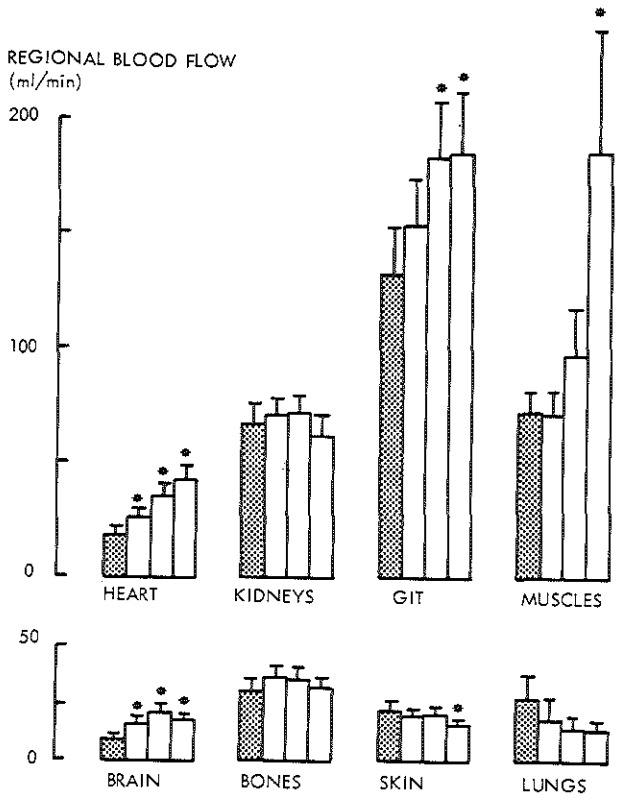


*Figure 3. Effects of felodipine (3, 10 and 30 µg/kg, i.v.) on regional blood flows (ml/min) in conscious renal hypertensive rabbits (n=8). Dotted bars, baseline values; open bars, values 10 minutes after the successive felodipine doses; *, significantly different from baseline values ($p < 0.05$); GIT, gastro-intestinal tract; AVA+BB, arteriovenous anastomoses + bronchial vascular bed.*

the conscious animals. A large increase in blood flow was observed in the heart, the gastro-intestinal tract and, after the highest dose, in the skeletal muscles. Felodipine also increased blood supply to the brain, although this organ received only a small part of total cardiac output. Blood flows to the kidneys and the bones were not effected by the drug. In the skin a significant reduction in blood flow was measured after felodipine 30 µg/kg. As the lungs receive microspheres via bronchial arteries as well as peripheral arteriovenous anastomoses, the blood flow values represent a combination of bronchial and arteriovenous anastomotic flow. The decrease in this variable observed with felodipine did not reach a significant level.

Figure 4 shows that a redistribution of the myocardial blood flow was observed after felodipine; the increase in blood flow

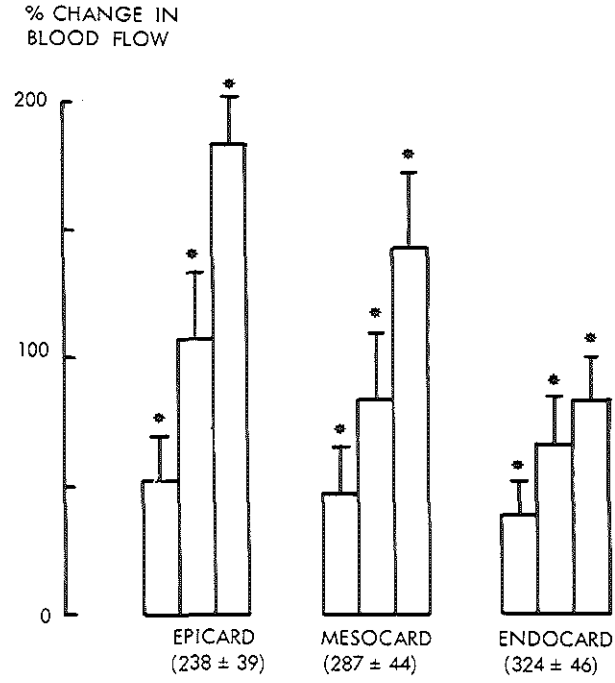


*Figure 4. Effects of felodipine (3, 10 and 30 µg/kg respectively) on blood flows to different regions of the left ventricular wall shown in percentage change from baseline values, measured 10 minutes after the i.v. administration of the drug in conscious renal hypertensive rabbits (n=8). Between brackets: baseline values in ml/min.100g; *, significant change from baseline values ($p < 0.05$).*

was most pronounced in the outer layers of the left ventricular wall. This resulted in a reduction of the weight-normalized endocardial/epicardial blood flow ratio, which decreased from 1.42 ± 0.08 to 1.32 ± 0.10 ($p > 0.05$), 1.15 ± 0.11 ($p < 0.05$) and 0.91 ± 0.07 ($p < 0.05$), respectively, after the 3 successive felodipine doses.

A rather generalized vasodilatation (increased vascular conductance) was observed after the i.v. administration of

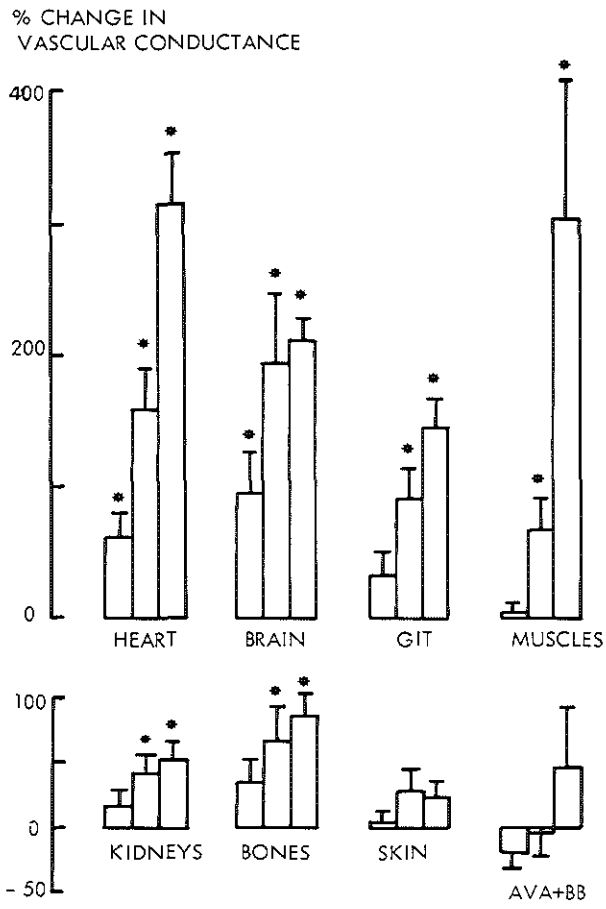


*Figure 5. Effects of felodipine (3, 10 and 30 µg/kg, respectively) on regional vascular conductances shown in percentage change from baseline values and measured 10 minutes after the i.v. administration of the drug in conscious renal hypertensive rabbits (n=8). *, Significant change from baseline values ($p < 0.05$); GIT, gastro-intestinal tract; AVA+BB, arteriovenous anastomoses + bronchial vascular bed.*

felodipine (figure 5). The changes in vascular conductance were most pronounced in the heart, brain, gastro-intestinal tract and skeletal muscles. A relatively smaller but still significant increase was noticed in the kidneys and bones. In the heart and brain a significant increase in vascular conductance was already measured with felodipine, 3 μg/kg, while in the skeletal muscles vasodilatation could only be observed at higher doses. The changes in vascular conductance in the skin and in the combination of bronchial bed and arteriovenous anastomoses were also not significant.

DISCUSSION

Using the radioactive microsphere technique we studied the acute systemic and regional haemodynamic effects of felodipine. A dose-dependent decrease in arterial blood pressure accompanied by an increase in heart rate was measured after the i.v. administration of the drug in the conscious renal hypertensive rabbits. The latter effect was probably due to an increased sympathetic activity and a withdrawal of the vagal tone as a result of the hypotension-induced activation of the baroreceptor reflex (Nakaya et al., 1983; Saini, 1984). Especially at higher doses, felodipine increased the cardiac output. Thus, the hypotensive effect of felodipine resulted from a dose-dependent decrease in total peripheral resistance.

A rather generalized peripheral vasodilatation was observed after the administration of felodipine in the conscious hypertensive rabbits. Because in the intact organism the sympathetic nervous system is the most important determinant of vascular function (Vanhoutte, 1981) it is remarkable that, despite the large reduction in arterial pressure and the resultant reflex-mediated increase in sympathetic tone, no compensatory vasoconstriction could be observed with felodipine in any of the organs selected for flow measurement. This is in contrast to the effects of acute administration of the arterial vasodilator hydralazine in conscious hypertensive rabbits where, in addition to a vasodilator response in most organs and tissues, a vasoconstriction was noticed in the skin, stomach and small

intestine (Bolt and Saxena, 1984b). Thus, when compared with hydralazine, felodipine appears to be a more potent vasodilator. In addition, calcium entry blockers inhibit the contractions evoked by most naturally occurring vasoconstrictor substances (Vanhoutte, 1982), which possibly explains the absence of a reflex-mediated vasoconstrictor response in the present study.

Apart from a direct effect of felodipine on the coronary vasculature, a metabolic autoregulatory vasodilatation, secondary to the cardiac stimulation, possibly contributed to the enhanced blood supply to the heart. However, on the other hand, a decrease in myocardial oxygen consumption is caused by the felodipine-induced reduction in afterload. Moreover, after intracoronary administration of felodipine in anaesthetized pigs, Verdouw et al. (1983) noticed a large decrease in myocardial O_2-extraction despite minimal changes in myocardial O_2-demand, suggesting that a possible direct effect of the drug on myocardial metabolism is involved as well. Felodipine preferentially increased blood flow to the outer layers of the left ventricular wall, which resulted in a reduction in the endocardial/epicardial blood flow ratio. This phenomenon, which has been associated with the precipitation of myocardial ischaemia after treatment with arterial vasodilators especially in patients with limited coronary reserve, is probably related to the decrease in diastolic perfusion time that results from the increase in heart rate (Boudoulais et al., 1979). Indeed, pretreatment with the cardioselective beta-adrenoceptor antagonist atenolol not only inhibited the reflex tachycardia observed after hydralazine administration in conscious hypertensive rabbits, but also prevented the negative effect of this arterial vasodilator on the endocardial/epicardial blood flow ratio in the left ventricular wall (Bolt and Saxena, 1984b).

Because a strong autoregulatory mechanism has also been demonstrated in the brain, it is possible that an increase in cerebral metabolism contributed to the pronounced increase in cerebral blood flow. However, Haws et al. (1983) noticed a similar increase in cerebral blood supply with no change in cerebral O_2-consumption after the acute administration of nimodipine in anaesthetized rabbits, suggesting that the strong vasodilator potency of the dihydropyridine was due to a direct

effect on vascular muscle rather than to cerebral autoregulation. Felodipine did not change renal blood flow and a relatively small vasodilatation was observed in the renal vascular bed. Minor effects on renal blood flow have also been reported for nifedipine and PY 108-068 in anaesthetized cats (Hof et al., 1982), for diltiazem in conscious rats (Flaim and Zelis, 1982) and for nimodipine in conscious rabbits (Haws et al., 1983). Also in the gastro-intestinal tract and the skeletal muscles pronounced vasodilatation was observed, however, especially in the skeletal muscles higher doses were needed to obtain significant changes. This is compatible with the reported differences in affinity of smooth muscles of different vascular beds for calcium entry blockers (Mullett et al., 1983).

Felodipine did not significantly change the vascular conductance in the skin. Moreover, a decrease in arterial blood supply to the skin was measured after the third felodipine dose. The relative insensitivity of the cutaneous vascular bed to calcium entry blockers has been reported previously (Vanhoutte and Rimele, 1982). Similarly, the combined arteriovenous anastomotic and bronchial artery flow decreased after felodipine administration although significant levels were not reached. Considering the small contribution of the bronchial vascular bed in the total circulation (Warren and Ledingham, 1974; Saxena and verdouw; 1982) this may indicate a reduction in the arteriovenous shunting of blood via arteriovenous anastomoses. Similar effects have been reported for nifedipine and PY 108-068 in anaesthetized cats (Hof et al., 1982).

Finally, considering the similarities between the regional haemodynamic profiles of felodipine and that of other, structurally related (nimodipine, PY 108-068 and nifedipine) and unrelated (diltiazem) calcium antagonists, a similar mechanism of action is probably involved in the vascular effects of these agents. In addition to the blockade of the voltage-dependent Ca^{2+} channel (Fleckenstein, 1977), several investigators have suggested an intracellular site of action for some of these compounds. Boström et al. (1980), who demonstrated binding to calmodulin by felodipine, have suggested that the dihydropyridine may exert its vascular effects by inhibiting calmodulin-dependent myosin light chain kinase activity (Herzig, 1984) rather than by a

blockade of calcium influx across the sarcolemma. However, the relatively high concentrations necessary to demonstrate this intracellular action makes its pharmacological significance doubtful (Saida and Van Breemen, 1983). Moreover, it has been shown that calmodulin binding is not necessarily related to inhibition of smooth muscle myosin light chain kinase activity (Silver et al., 1984). Thus, an inhibitory effect on Ca^{2+}-fluxes across the sarcolemma, as proposed by Fleckenstein (1977), is still the most likely mechanism responsible for the vascular smooth muscle relaxing potency of felodipine as well as other calcium antagonists.

In conclusion, felodipine appears to be an effective antihypertensive agent. However, the cardiac stimulation accompanied by the preferential increase in blood flow to the outer layers of the left ventricular wall illustrated the negative aspects of single treatment with this arterial vasodilator.

REFERENCES

Bolt, G.R., Saxena, P.R. (1983a) Cardiovascular profile of felodipine, a new Ca-antagonist, in conscious renal hypertensive rabbits: a comparison with hydralazine. Brit. J. Pharmacol. 1983: 612p.

Bolt, G.R., Saxena, P.R. (1983b) Systemic and regional haemodynamic characteristics of bilateral cellophane perinephritis hypertension in conscious rabbits. Clin. Exp. Hyper. A5: 885–901.

Bolt, G.R., Saxena, P.R. (1984a) Acute systemic and regional hemodynamic effects of felodipine, a new calcium antagonist, in conscious renal hypertensive rabbits. J. Cardiovasc. Pharmacol. 6: 707–712.

Bolt, G.R., Saxena, P.R. (1984b) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious and renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205–213.

Boström, S.L., Ljung, B., Mardh, S., Forsey, S., Thulin, E. (1981) Interaction of the antihypertensive drug felodipine with calmodulin. Nature 292: 777–778.

Boudoulas, H., Rittgers, S.E., Lewis, R.P., Leier, C.V., Weissler, A.M. (1979) Changes in diastolic time with various pharmacologic agents. Implications for myocardial perfusion. Circulation 60: 164–169.

Flaim, S.F., Zelis, R. (1982) Effects of diltiazem on total cardiac output distribution in conscious rats. J. Pharmacol. Exp. Ther. 222: 359–366.

Fleckenstein, A. (1977) Specific pharmacology of calcium in myocardium, cardiac pacemakers, and vascular smooth muscle. Ann. Rev. Pharmacol. Toxicol. 17: 149–166.

Hales, J.R.S. (1974) Radioactive microsphere technique for studies of the circulation. Clin. Exp. Pharmacol. Physiol. suppl 1: 31-46.
Haws, C.W., Gourley, J.K., Heistad, D.D. (1983) Effects of nimodipine on cerebral blood flow. J. Pharmacol. Exp. Ther. 225: 24-28.
Herzig, J.W. (1984) Contractile proteins: possible targets for drug action. Trends Pharmacol. Sci. 5: 296-300.
Heymann, M.A., Payne, B.D., Hoffman, J.I.E., Rudolph, A.M. (1977) Blood flow measurements with radionuclide-labelled particles. Progr. Cardiovasc. Dis. 20: 55-79.
Hof, R.P., Hof, A., Neumann, P. (1982) Effects of PY 108-068, a new calcium antagonist on general hemodynamics and regional blood flows in anesthetized cats: a comparison with nifedipine. J. Cardiovasc. Pharmacol. 4: 352-362.
Ljung, B. (1980) Vascular versus myocardial selectivity in vitro of H 154/82, a new dihydropyridine. Blood Vessels 17: 154.
Mullett, M., Gharaibeh, M., Warltier, D.C., Gross, G.J. (1983) The effect of diltiazem, a calcium channel blocking agent, on vasoconstrictor responses to norepinephrine, serotonin and potassium depolarization in canine coronary and femoral arteries. Gen. Pharmacol. 14: 259-264.
Nakaya, H., Schwartz, A., Millard, R.W. (1983) Reflex chronotropic and inotropic effects of calcium channel-blocking agents in conscious dogs. Circ. Res. 52: 302-311.
Naylor, WG. (1983) The heterogeneity of slow channel blockers (calcium antagonists). Int. J. Cardiol. 3: 391-400.
Page, I.H. (1939) Production of persistent hypertension by cellophane perinephritis. J. Amer. Med. Ass. 113: 2046-2048.
Saida, K, Van Breemen, C. (1983) Mechanism of Ca^{2+} antagonist-induced vasodilatation. Circ. Res. 52: 137-142.
Saini, R.K. (1984) Calcium antagonists, in "Cardiovascular Pharmacology" (Ed. Antonaccio, M., Raven Press, New York): 415-452.
Saxena, P.R., Schamhardt, H.C., Forsyth, R.P., Loeve, J. (1980) Computer programs for the radioactive microsphere technique. Computer Programs Biomed. 12: 63-84.
Saxena, P.R., Verdouw, P.D. (1982) Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic flow. J. Physiol. 332: 501-520.
Siegel, S. (1956) Non-parametric statistics for behavioural sciences. (McGraw-Hill Kogakusha, Tokyo).

Silver, P.J., Dachiw, J. Ambrose, J.M. (1984) Effects of calcium antagonists and vasodilators on arterial myosin phosphorylation and actin-myosin interaction. J. Pharmacol. Exp. Ther. 230: 141-148.
Vanhoutte, P.M. (1981) Introductory remarks: alpha- and beta-adrenergic receptors and the cardiovascular system. J. Cardiovasc. Pharmacol. 3(suppl 1): 1-13.
Vanhoutte, P.M. (1982) Calcium entry blockers and vascular smooth muscle. Circulation 65(suppl 1): 11-19.
Vanhoutte, P.M., Rimele, T.J. (1982) Calcium and alpha-adrenoceptors in activation of vascular smooth muscle. J. Cardiovasc. Pharmacol. 4: S280-S286.
Verdouw, P.D., Wolffenbuttel, B.H.R., Scheffer, M.G. (1983) Cardiovascular actions of the calmodulin inhibitor felodipine. Naunyn-Schmiedeberg's Arch. Pharmacol. 323: 350-354.
Warren, D.J., Ledingham, J.G.G. (1972) Chronic left atrial catheterization in the rabbit. Pflüg. Arch. 335: 246-251.
Warren, D.J., Ledingham, J.G.G. (1974) Measurement of cardiac output and distribution using microspheres. Some practical and theoretical considerations. Cardiovasc. Res. 8: 570-581.

CHAPTER 7:
HAEMODYNAMIC PROFILE OF PRAZOSIN IN CONSCIOUS HYPERTENSIVE RABBITS

The radioactive microsphere technique was used to study the acute systemic and regional haemodynamic effects of prazosin (0.01, 0.03 and 0.10 mg/kg, *i.v.*) in conscious hypertensive rabbits. The α_1-adrenoceptor antagonist decreased the arterial blood pressure dose-dependently and increased the heart rate. A reduction in stroke volume was measured which resulted in a moderate but significant fall in cardiac output at the highest dose of prazosin.

Due to the prazosin-induced vasodilatation in the kidneys, intestines and bones, the blood flow to these tissues was not reduced despite the decrease in cardiac output. In contrast, a reduction in the blood flow to the skeletal muscles, skin and, at the highest dose, to the heart, brain and stomach was measured after prazosin administration.

In conclusion, the selective vasodilatation produced by prazosin causes a redistribution of the cardiac output at the expense of vital organs such as the brain, a finding which may possibly explain the symptoms related to the first dose phenomenon of prazosin in man.

Prazosin is an antihypertensive agent that is believed to act through a blockade of postsynaptic α_1-adrenoceptors (Cavero and Roach, 1980; Stanaszek et al., 1983). When compared with a nonselective α-adrenoceptor antagonist, such as phentolamine, relatively minor changes in heart rate and renin activity are observed at hypotensive doses of prazosin (Massingham and Hayden, 1975; Graham and Pettinger, 1979). This has been associated with the failure of prazosin to block presynaptic α_2-adrenoceptors thereby leaving unaffected the negative feedback mechanism by which noradrenaline regulates its own release from synaptic nerve endings (Cambridge et al., 1977).

Relatively little attention has been paid to the peripheral vascular effects of the α_1-adrenoceptor antagonist. Results obtained from *in-vitro* studies indicate that differences exist in the sensitivity of various vascular beds to the sympatholytic actions of prazosin (Jauernig et al., 1978). Moreover, *in-vivo*

the effects of the α_1-adrenoceptor antagonist may vary in the different organs and tissues depending upon the basal sympathetic tone in the respective vascular beds. The heterogeneity in the vascular response which may result in a redistribution of the cardiac output has been used as a possible explanation for the serious side effects such as dizziness and even loss of consciousness observed after the acute administration of prazosin in man, often referred to as the "first dose phenomenon" (Moulds and Jauernig, 1977). Therefore, we studied the acute effects of prazosin on systemic as well as regional haemodynamic variables in conscious rabbits with bilateral cellophane perinephritis hypertension using the radioactive microsphere technique. The data presented in this chapter have been published elsewhere (Bolt and Saxena, 1984a).

MATERIALS AND METHODS

Animals and surgical procedures

Experiments were performed on ten male hypertensive New Zealand White rabbits (3.4 ± 0.1 kg). Hypertension was induced by bilateral cellophane wrapping of the kidneys, six to ten weeks before the experiment, according to the method of Page (1939). Nine to fourteen days before the experiment a left-sided thoracotomy was performed in order to cannulate the left atrial appendage with a nylon catheter for the administration of microspheres (Warren and Ledingham, 1972). Subsequently, four to eight days before the experiment the left carotid artery was cannulated for the measurement of blood pressure and heart rate during the experiments and the withdrawal of arterial blood samples. The different surgical procedures have been described in detail elsewhere (Bolt and Saxena, 1983; chapter 2 and 3 of this thesis).

Measurement of haemodynamic variables

On the day of the experiment the animals were placed in a rabbit restrainer box. A Statham P23Dc pressure transducer was connected to the carotid catheter for the recording of the arterial blood pressure and the heart rate using a Grass model 7

polygraph. Mean blood pressure was obtained by electronically damping of the blood pressure signals. Cardiac output and regional blood flows were measured with the radioactive microsphere technique, using the reference blood sample method (Hales, 1974; Heymann et al., 1977). Nen-Trac microspheres with a nominal diameter of 15 ± 1 (SD) μm and labeled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc were used. Cardiac output (ml/min) and regional blood flows (ml/min) were calculated using a set of computer programs especially designed for the radioactive microsphere technique (Saxena et al., 1980; relevant information is given in chapter 2). Peripheral vascular conductances were obtained by dividing respective tissue blood flows (ml/min) by mean arterial blood pressure (mmHg).

Experimental protocol

The marginal ear vein was cannulated under local anaesthesia (lidocaine 2%) for the administration of prazosin HCl (Pfizer B.V., Rotterdam). The compound was dissolved in distilled water. The concentrations were such that the maximum injection volume was less than 0.5 ml. After a stabilization period of at least 30 minutes the first batch of microspheres was injected to determine baseline values of cardiac output and regional haemodynamic variables. Heart rate and arterial blood pressure values were recorded continuously. After measuring baseline values each animal received three cumulative doses (0.01, 0.03 and 0.10 mg/kg) of prazosin at 20 minutes intervals. Fifteen minutes after each dose a batch of microspheres was injected. An arterial blood sample (0.3 ml) was withdrawn immediatly after each microsphere injection to measure pH-, P_{CO_2}- and P_{O_2}-values, using an ABL-2 (Radiometer, Copenhagen).

Statistical evaluation

All data have been expressed as mean (±SEM) in the text. Due to skewed distribution and lack of homogeneity of variances in some variables, non-parametric tests were used for the statistical evaluation (Siegel, 1956). Initially, the Friedman's two way analysis of variance was used to establish whether the samples represented different populations. The Wilcoxon matched-pairs signed ranks test was applied to test the "significance"

($P < 0.05$, two-tailed) of the changes in haemodynamic variables from baseline values.

RESULTS

Blood gases

Values of arterial blood gases and pH were not affected by prazosin. Before and 15 minutes after the successive doses of prazosin (0.01, 0.03 and 0.10 mg/kg) the respective values were: pH, 7.44 ± 0.01, 7.46 ± 0.01, 7.46 ± 0.01 and 7.45 ± 0.01; P_{CO_2}, 31 ± 2, 31 ± 2, 34 ± 2 and 33 ± 2 mmHg; and P_{O_2}, 91 ± 2, 91 ± 2, 89 ± 2 and 90 ± 3 mmHg.

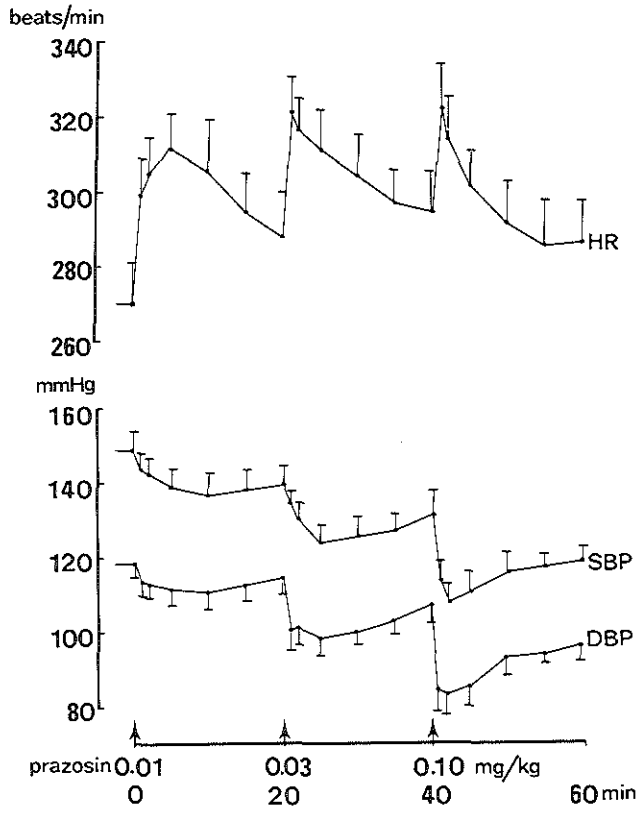


Figure 1. Time course of heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure responses after i.v. administration of prazosin, 0.01, 0.03 and 0.10 mg/kg, in conscious hypertensive rabbits (n=10).

Systemic haemodynamic variables

Figure 1 shows the time course of the prazosin-induced changes in heart rate, systolic and diastolic arterial blood pressures. A dose dependent decrease in blood pressure accompanied by an increase in heart rate was observed with prazosin. The latter effect reached a maximum within the first five minutes after each prazosin administration (+19 ± 3%, +21 ± 3% and +20 ±3% above baseline level, respectively) and then gradually declined. Figure 2 shows the changes in systemic haemodynamic variables 15 minutes after the successive doses of prazosin (0.01, 0.03 and 0.10 mg/kg). Despite the increase in heart rate a significant reduction in cardiac output was measured at the highest prazosin dose due to a decrease in stroke volume. The α_1-adrenoceptor antagonist decreased the total peripheral resistance dose-dependently.

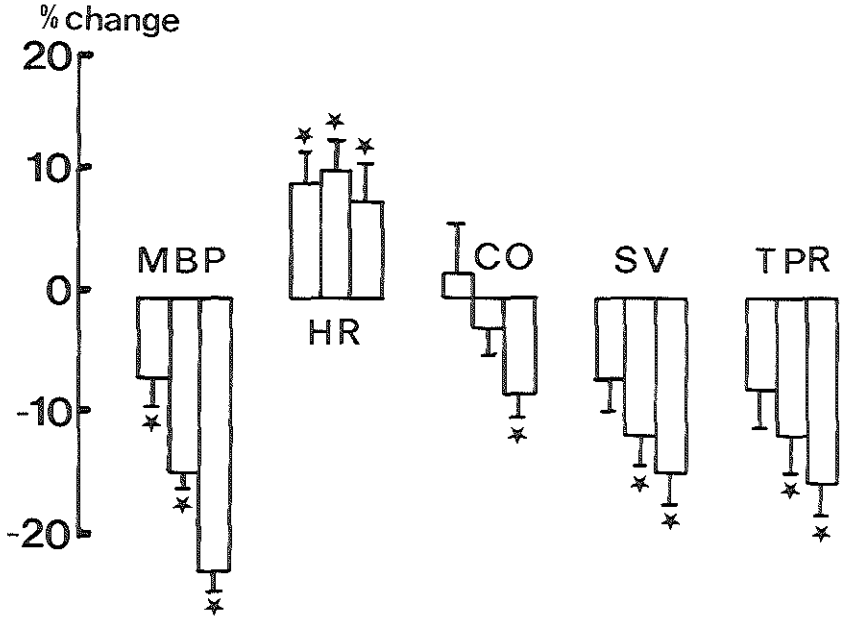


*Figure 2. Percentage change in systemic haemodynamic variables measured 15 minutes after the i.v. administration of prazosin, 0.01, 0.03 and 0.10 mg/kg, respectively, in conscious hypertensive rabbits (n=10). MBP, mean blood pressure (132 ± 4 mmHg); HR, heart rate (270 ± 11 beats/min); CO, cardiac output (556 ± 31 ml/min); SV, stroke volume (2.1 ± 0.2 ml); TPR, total peripheral resistance (249 ± 25 mmHg/l.min^{-1}); between brackets: baseline values; *, significant change from baseline values (P < 0.05).*

Regional haemodynamic variables

Figure 3 shows the changes in regional blood flows to the different organs and tissues measured 15 minutes after the successive prazosin administrations (0.01, 0.03 and 0.10 mg/kg) in the conscious hypertensive rabbits. Despite the decrease in cardiac output, the blood supply to the intestines, kidneys and bones was not reduced. As the lungs receive 15 μm microspheres via peripheral arteriovenous anastomoses as well as bronchial arteries, the obtained blood flow values represent the combination of arteriovenous anastomotic and bronchial flow. No alteration was noticed in this variable. After the second and third dose of prazosin a significant decrease in blood flow to the skeletal muscles (-16 ± 5% and -21 ± 8%, respectively) and the skin (-12 ± 4% and -17 ± 7%, respectively) was measured. At the highest dose blood flow was also significantly reduced in the

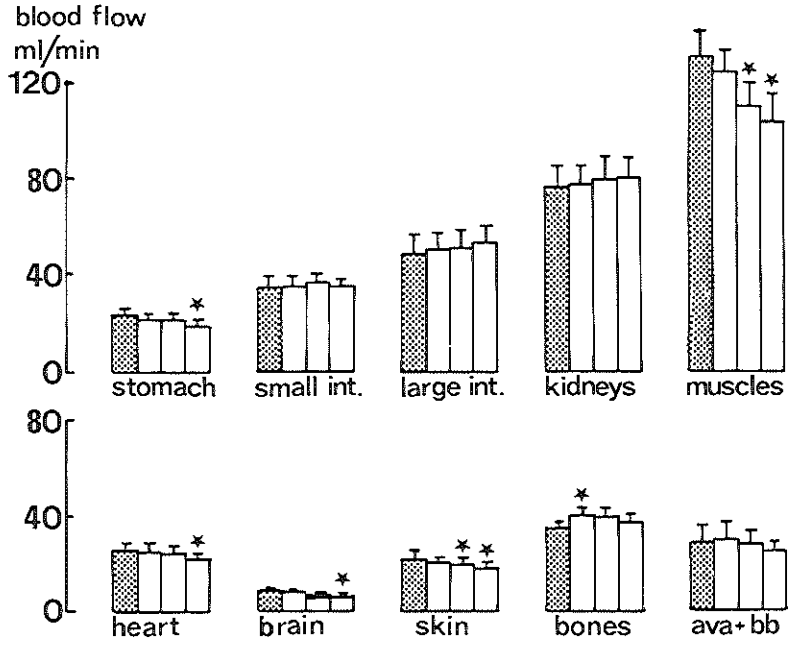


*Figure 3. Effects of prazosin on regional blood flows (ml/min) in conscious hypertensive rabbits (n=10). Dotted bars, baseline values; open bars, values 15 minutes after prazosin, 0.01, 0.03 and 0.10 mg/kg, respectively; AVA + BB, arteriovenous anastomoses + bronchial vascular bed; *, significant change from baseline values (P < 0.05).*

stomach (-22 ± 5%), heart (-11 ± 5%) and brain (-15 ± 6%). Remarkably, in the brain only the blood flow to the cerebral hemispheres was significantly lowered by prazosin, 0.10 mg/kg, (-18 ± 5%) whereas that to the cerebellum and brain stem was not significantly changed (-5 ± 9% and +1 ± 10%, respectively). Similarly, in the heart only the blood flows to the left ventricular wall (-12 ± 5%) and intra-ventricular septum (-12 ± 4%) were significantly reduced at the highest prazosin dose whereas blood flows to atria (-1 ± 16%) and right ventricle (-6 ± 5%) were not significantly affected.

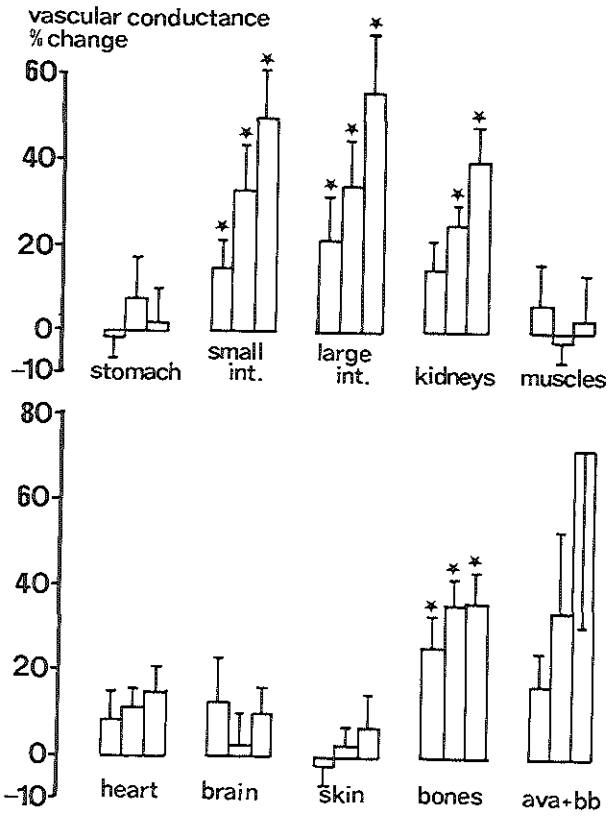


*Figure 4. Effects of prazosin on regional vascular conductances in percentage change from baseline values 15 minutes after the i.v. administration of prazosin, 0.01, 0.03 and 0.10 mg/kg, respectively, in conscious hypertensive rabbits (n=10). AVA + BB, arteriovenous anastomoses + bronchial vascular bed; *, significant change from baseline values (P < 0.05).*

Figure 4 shows that prazosin produced a significant vasodilatation (increase in vascular conductance) in the intestines, kidneys and bones. The mean vascular conductance of arteriovenous anastomoses + bronchial vascular bed also increased, however, the changes were not significant. Prazosin did not significantly affect the vascular conductance in the different regions of the brain. In the heart a tendency for the vascular conductance to increase was noticed. A significant vasodilatation was measured after the second and third prazosin dose in the right ventricle where the vascular conductance increased by 19 ± 6% and 22 ± 6%, respectively. Prazosin did not significantly alter the vascular conductance in the atria, the left ventricle or the intra-ventricular septum.

DISCUSSION

Using the radioactive microsphere technique we studied the acute haemodynamic profile of prazosin in conscious hypertensive rabbits. A dose-dependent decrease in arterial blood pressure accompanied by an increase in heart rate was measured after the acute administration of the α_1-adrenoceptor antagonist. The latter effect is probably due to a reflex-mediated increase in sympathetic activity and a withdrawal of the vagal tone as a result of a decrease in firing frequency of baroreceptors since in sinoaortic denervated rabbits the tachycardia produced by prazosin was greatly diminished and no longer significant (Hamilton et al., 1982). The cardiac stimulation observed in the present study attained a peak value within a few minutes after each prazosin administration. Subsequently, a decline was observed during the following 15 minutes despite the persistance of pronounced hypotension. A similar response was observed by Cavero (1982) after a constant infusion of prazosin in conscious normotensive rabbits. As reported previously for normotensive rabbits (Hamilton et al., 1982), the maximal increase in heart rate produced by prazosin was still less than that observed after the acute administration of more selective arterial vasodilators, such as hydralazine (Bolt and Saxena, 1984b) and felodipine (Bolt and Saxena, 1984c), in rabbits with cellophane perinephritic

hypertension. McCall and Humphrey (1981) have reported a central action of prazosin in anaesthetized cats where the drug caused a reduction in sympathetic nervous discharge. Thus it is possible that this central action partly opposes the baroreceptor-induced changes in autonomic nervous system activity. In addition, the rather moderate reflex tachycardia observed with prazosin may be related to dilatation of venous capacitance vessels (Patel, 1981; Schultz and Westfall, 1982) which results in a decrease in venous return. In contrast, an increase in venous return is usually measured with more selective arterial vasodilators which leads to an activation of atrial stretch receptors (Bainbridge reflex) and thereby contributes to the positive chronotropic effects observed with these agents (Cavero and Roach, 1980; Spokas and Wang, 1980). The decrease in cardiac output and stroke volume observed in the present study can also be attributed to the prazosin-induced dilatation of the venous capacitance vessels and the subsequent reduction in venous return. Prazosin produced a dose-dependent decrease in total peripheral resistance which illustrates the vasodilatory action of the α_1-adrenoceptor antagonist on arterial resistance vessels.

The fall in cardiac output observed at the higher prazosin doses did not result in a generalized reduction in blood flow to the different organs and tissues. The decrease in the amount of blood received by the skeletal muscles accounted for the major part of the deficit. Prazosin also reduced the blood supply to the skin and, at the highest dose used, to the heart, brain and stomach. The vascular conductance was not significantly affected by prazosin in the organs and tissues mentioned. Thus, one might conclude that the resting α_1-adrenergic tone in these vascular beds is low. On the other hand, it is possible that the vasodilatory effects of prazosin are obscured by changes in vascular tone due to tissue autoregulation and the hypotension-induced activation of cardiovascular reflex mechanisms (Reid, 1980). Although vascular α_1-adrenoceptors are blocked, the reflex-mediated increase in sympathetic activity after prazosin administration (Reid and Hamilton, 1980) can still induce peripheral vasoconstriction via the activation of the renin-angiotensin system (Graham and Pettinger, 1979) and the stimulation of postsynaptic vascular α_2-adrenoceptors (Drew and

Whiting, 1979). In this respect, Horn et al. (1982) noticed a relative preponderance of postsynaptic α_2-adrenoceptors in the femoral vascular bed which is in accordance with the absence of a vasodilator response of prazosin in the skeletal muscle in the present study.

Especially in the heart the changes in blood supply are determined for a major part by changes in metabolic demands (Weber and Janicki, 1979). The latter can be predicted, at least to some extent, from changes in the product of heart rate and systolic pressure. Despite the decrease in the rate/pressure product (-16 ± 2 %) at the highest dose of prazosin a tendency for the vascular conductance to increase was observed in the present study reaching a significant level in the right ventricle. This indicates that a resting α_1-adrenergic tone is probably present in the coronary vascular bed.

The remarkable reduction in the blood supply to the brain may be related to symptoms such as dizziness and even loss of consciousness observed after the acute administration of a relatively large first dose of prazosin in man (Cavero and Roach, 1980). The preferential decrease in blood flow to the higher regions of the brain observed in the present study is possibly due to differences in basal α_1-adrenergic tone in the different regions of the brain. On the other hand, tissue autoregulatory responses, reacting upon a possible increase in metabolic demands in certain areas of the brain, may contribute to the regional differences observed in the present study.

Despite the decrease in cardiac output, the blood flow to the kidneys and intestines remained unchanged due to the prazosin-induced vasodilatation in these vascular beds. In the bones pronounced vasodilatation was already noticed at low doses of prazosin which resulted in an increase in blood flow after the first dose of the α_1-adrenenoceptor antagonist in the conscious hypertensive rabbits. Apparently a significant α_1-adrenergic tone is present in the vascular beds of the kidneys, intestines and bones. In addition, *in-vitro* studies show that in comparison with the peripheral vascular beds, the visceral vascular beds are relatively more sensitive to the sympatholytic action of prazosin (Moulds and Jauernig, 1977).

In conclusion, our results indicate that dilatation of both arterial resistance vessels and venous capacitance vessels -leading to a decrease in total peripheral resistance and cardiac ouput, respectively- contribute to the acute hypotensive effect of prazosin in the conscious hypertensive rabbit. α_1-Adrenoceptor blockade leads to selective vasodilatation in kidneys, intestines and bones, thereby maintaining the blood supply in these vascular beds within normal limits despite the decrease in cardiac output. This, however, is at the expense of those vascular beds where the basal α_1-adrenergic tone appears to be low such as the brain. This latter finding may provide an explanation for the acute side effects of prazosin in man, often referred to as the first dose phenomenon.

REFERENCES

Bolt, G.R., Saxena, P.R. (1983) Systemic and regional hemodynamic characteristics of bilateral cellophane perinephritis hypertension in conscious rabbits. Clin. Exp. Hypert. A5: 885-901.

Bolt, G.R., Saxena, P.R. (1984a) Acute systemic and regional haemodynamic profile of prazosin in conscious hypertensive rabbits. Eur. J. Pharmacol. 104: 151-157.

Bolt, G.R., Saxena, P.R. (1984b) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205-213.

Bolt, G.R., Saxena, P.R. (1984c) Acute systemic and regional hemodynamic effects of felodipine, a new calcium antagonist, in conscious renal hypertensive rabbits. J. Cardiovascular. Pharmacol. 6: 707-712.

Cambridge D., Davey, M.J., Massingham, R. (1977) The pharmacology of antihypertensive drugs with special reference to vasodilators, alpha-adrenergic blocking agents and prazosin. Med. J. Aust. Specl. suppl. 2: 2-6.

Cavero, I., Roach, A.G. (1980) The pharmacology of prazosin, a novel antihypertensive agent. Life Sci. 27: 1525-1540.

Cavero, I. (1982) Effects of prazosin on reflex changes in heart rate evoked by vasopressor and vasodepressor stimuli in conscious rabbits. J. Cardiovasc. Pharmacol. 4: s108-s113.

Drew, G.M., Whiting, S.B. (1979) Evidence for two distinct types of postsynaptic α-adrenoceptors in vascular smooth muscle *in-vivo*. Brit. J. Pharmacol. 67: 207-215.

Graham, R.M., Pettinger, W.A. (1979) Effects of prazosin and phentolamine on arterial pressure, heart rate, and renin activity: Evidence in the conscious rat for the functional significance of the presynaptic alpha-receptor. J. Cardiovasc. Pharmacol. 1: 497-502.

Hales, J.R.S. (1974) Radioactive microsphere technique for studies of the circulation. Clin. Exp. Pharmacol. Physiol. suppl 1: 31-46.

Hamilton, C.A., Reid, J.L. Zamboulis, C. (1982) The role of presynaptic α-adrenoceptors in the regulation of blood pressure in the conscious rabbit. Br. J. Pharmacol. 75: 417424.

Heymann, M.A., Payne, B.D. Hoffman, J.I.E. Rudolph, A.M. (1977) Blood flow measurements with radionuclide-labelled particles. Progr. Cardiovasc. Dis. 20: 55-79.

Horn, P.T., Kohli, J.D. Listinski, J.L. Goldberg, L. (1982) Regional variation in the α-adrenergic receptors in the canine resistance vessels. Naunyn-Schmiedeberg's Arch. Pharmacol. 318: 166-172.

Jauernig, R.A., Moulds, R.F.W. Shaw, J. (1978) The action of prazosin in human vascular preparations. Arch. Int. Pharmacodyn. 231: 81-89.

Massingham, R., Hayden, M.L. (1975) A comparison of the effects of prazosin and hydrallazine on blood pressure, heart rate and plasma renin activity in conscious renal hypertensive dogs. Eur. J. Pharmacol. 30: 121-124.

McCall, R.B., Humphrey, S.J. (1981) Evidence for a central depressor action of postsynaptic α_1-adrenergic antagonists. J. Autonom. Nerv. Syst. 3: 9-23.

Moulds, R.F.W., Jauernig, R.A. (1977) Mechanism of prazosin collapse. Lancet 1: 200-201.

Page, I.H. (1939) Production of persistent hypertension by cellophane perinephritis. J. Amer. Med. Ass. 113: 2046-2048.

Patel, P., Bose, D. Greenway, C. (1981) Effects of prazosin and phenoxybenzamine on alpha- and beta-receptor-mediated responses in intestinal resistance and capacitance vessels. J. Cardiovasc. Pharmacol. 3: 1050-1059.

Reid, J.L. (1980) Vasodilators in the management of hypertension. Progr. Pharmacol. 3/4: 77-82.

Reid, J.L., Hamilton, C. (1980) Catecholamines and blood pressure regulation: The role of alpha-adrenoceptors, J. Cardiovascular. Pharmacol. 2(suppl. 3): s325-s335.

Saxena, P.R., Schamhardt, H.C. Forsyth, R.P. Loeve, J. (1980) Computer programs for the radioactive microsphere technique. Computer Programs Biomed. 12: 63-84.

Schultz, J.C., Westfall, D.P. (1982) A pharmacological analysis of the α-adrenoceptor antagonism by prazosin in arteries and veins. Blood Vessels 19: 79-87.

Siegel, S. (1956) Non-Parametric Statistics for Behavioural Sciences (McGraw-Hill Kogakusha, Tokyo).

Spokas, E.G., Wang, H.H. (1980) Regional blood flow and cardiac response to hydralazine. J. Pharmacol. Exp. Ther. 212: 294-303.

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

Warren, D.J., Ledingham, J.G.G. (1972) Chronic left atrial catheterization in the rabbit. Pflüg. Arch. 335: 167-172.

Weber, K.T., Janicki, J.S. (1979) The metabolic demand and oxygen supply of the heart: Physiological and clinical considerations. Amer. J. Cardiol. 44: 772-729.

CHAPTER 8:
HAEMODYNAMIC PROFILE AND HYPOTENSIVE MECHANISM OF KETANSERIN IN CONSCIOUS HYPERTENSIVE RABBITS

The radioactive microsphere technique was used to study the systemic and regional haemodynamic effects of ketanserin in conscious renal hypertensive rabbits. In order to characterize the hypotensive mechanism of the compound, we evaluated its antagonism towards 5-hydroxytryptamine$_2$ ($5HT_2$) and α_1-adrenergic receptors at hypotensive doses and compared the cardiovascular profile of ketanserin with that of the α_1-selective adrenoceptor antagonist prazosin.

Ketanserin (0.1, 0.3 and 1.0 mg/kg, *i.v.*) produced a biphasic effect on the arterial blood pressure. A pronounced fall in blood pressure of short duration accompanied by tachycardia preceded a more moderate and longer lasting dose-related hypotensive effect. The presence of an adequate autonomic nervous system activity seems to be required for the prolonged hypotensive action of ketanserin since in animals pretreated with hexamethonium (30 mg/kg) the blood pressure, after an initial decrease, returned to baseline values within a few minutes after each ketanserin dose.

Ketanserin inhibited the pressor responses produced by 5-HT (10, 30 and 100 µg/kg, *i.v.*) and phenylephrine (3, 10 and 30 µg/kg, *i.v.*) indicating that, at hypotensive doses, the compound antagonized both $5HT_2$ receptors and α_1-adrenoceptors. At doses which caused a comparable degree of α_1-adrenoceptor blockade, ketanserin (0.1, 0.3 and 1.0 mg/kg, *i.v.*) as well as prazosin (0.01, 0.03 and 0.10 mg/kg, *i.v.*) decreased the blood pressure as a result of a reduction in total peripheral resistance. However, whereas especially at the lower doses of ketanserin the cardiac output increased, a moderate decrease in this variable contributed to the hypotensive effect of the highest dose of prazosin. Both compounds increased the vascular conductance in the kidneys, gastro-intestinal tract and bones, whereas that in the skin and skeletal muscles was not significantly altered. In contrast to prazosin, ketanserin also caused vasodilatation in the coronary and cerebral vascular beds.

The results suggest that, in addition to a direct vasodilator effect of short duration, ketanserin has a prolonged hypotensive action in conscious hypertensive rabbits which is predominantly due to α_1-adrenoceptor blockade.

Ketanserin, a selective 5-HT_2 receptor antagonist with α_1-adrenoceptor blocking properties (Leysen et al., 1981; Van Nueten et al., 1981; Kalkman et al., 1983), has been shown to reduce the blood pressure in animals (Fozard, 1982; Kalkman et al., 1982) and humans (De Cree et al., 1981; Wenting et al., 1984), by a mechanism which is still a matter of debate. In hypertensive patients ketanserin can lower the arterial blood pressure without causing any attenuation of the pressor response to phenylephrine (Wenting et al., 1984; Zabludowski et al., 1984). This pharmacological evidence has been used to support the concept that a blockade of 5-HT_2 receptors is responsible for the antihypertensive effect of the drug (Janssen, 1983). In contrast, studies in spontaneously hypertensive rats provide arguments that ketanserin lowers the blood pressure merely by a competitive blockade of postsynaptic α_1-adrenoceptors (Fozard, 1982; Kalkman et al., 1982; Cohen et al., 1983a). In addition, a central action has been suggested to contribute to the hypotensive action of the compound (McCall and Schuette, 1984; Mylecharane et al., 1984). In order to provide further information on the hypotensive mechanism of ketanserin we studied its blood pressure lowering effect in conscious hypertensive rabbits and have tried to relate this effect to the blockade of $5HT_2$ and α_1-adrenergic receptors in these animals. In addition, the changes in regional blood flows and vascular resistances after ketanserin administration were determined to characterize the complete haemodynamic profile of this new antihypertensive drug. Since we have recently studied the effects of the α_1-selective adrenoceptor anatagonist prazosin using the same methodology (Bolt and Saxena, 1984a), it enabled us to compare the complete haemodynamic profiles of the two compounds in conscious hypertensive rabbits. The data presented in this chapter have been published elsewhere (Bolt and Saxena, 1984b; Bolt and Saxena, 1985).

METHODS

Animals and surgical procedures

All experiments were performed on conscious hypertensive New

Zealand White rabbits (2.4 - 4.0 kg). Hypertension was induced by bilateral cellophane wrapping of the kidneys, six to ten weeks before the experiment, according to the method of Page (1939). In the animals used for the microsphere experiments a left-sided thoracotomy was performed nine to fourteen days before the experiment in order to cannulate the left atrial appendage with a nylon catheter for the administration of microspheres (Warren and Ledingham, 1972). Subsequently, four to eight days before the experiment the left carotid artery was cannulated in these animals for the measurement of blood pressure and heart rate during the experiments and the withdrawal of arterial blood samples. The different surgical procedures together with the systemic and regional haemodynamic characteristics of bilateral cellophane perinephritis hypertension in rabbits have been described in detail elsewhere (Bolt and Saxena, 1983; chapter 2 and 3).

Measurement of haemodynamic variables

On the day of the experiment the animals were placed in a rabbit restrainer box. The marginal ear vein was cannulated under local anaesthesia (lidocaine 2%) for the administration of the drugs. Subsequently, a catheter was inserted into the central ear artery in the animals used for the 5-HT and phenylephrine experiments. A Statham P23Dc pressure transducer was connected to either the central ear artery catheter or the carotid catheter (microsphere experiments) for the recording of the arterial blood pressure and the heart rate using a Grass model 7 polygraph. Mean blood pressure was obtained by electronically damping of the blood pressure signals. Cardiac output and regional blood flows were measured with the radioactive microsphere technique, using the reference blood sample method (Hales, 1974; Heymann et al., 1977). Nen-Trac microspheres with a nominal diameter of 15 ± 1 (SD) μm and labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc were used. Cardiac output (ml/min) and regional blood flows (ml/min) were calculated using a set of computer programs especially designed for the radioactive microsphere technique (Saxena et al., 1980; relevant information is given in chapter 2). Regional vascular conductances were obtained by dividing respective tissue blood flows (ml/min) by mean arterial blood pressure (mmHg).

Experimental protocol

Systemic and regional haemodynamics

The effects of ketanserin on systemic and regional haemodynamic variables were studied in ten conscious hypertensive rabbits using the radioactive microsphere technique. After a stabilization period of at least 30 minutes the first batch of microspheres was injected to determine baseline values of cardiac output and regional haemodynamic variables. Heart rate and arterial blood pressure values were recorded continuously. After measuring baseline values, each animal received three cumulative doses (0.1, 0.3 and 1.0 mg/kg, *i.v.*) of ketanserin. Ten minutes after each dose when heart rate and blood pressure response had stabilized, a batch of microspheres was injected. An arterial blood sample (0.3 ml) was withdrawn immediatly after each microsphere injection to measure pH-, P_{CO_2} - and P_{O_2} -values, using an ABL-2 (Radiometer, Copenhagen). Values of arterial blood gases and pH were not affected by ketanserin. Before and 10 minutes after the successive doses of ketanserin (0.1, 0.3 and 1.0 mg/kg) the respective values were: pH, 7.41 ± 0.01, 7.41 ± 0.01, 7.39 ± 0.02 and 7.40 ± 0.01; P_{CO_2}, 33 ± 1, 32 ± 1, 31 ± 1 and 30 ± 1 mmHg; and P_{O_2}, 94 ± 3, 93 ± 3, 95 ± 3 and 95 ± 4 mmHg. In a previous study (Bolt and Saxena, 1984a; chapter 7) we have measured the effects of prazosin (0.01, 0.03 and 0.10 mg/kg, *i.v.*) in conscious hypertensive rabbits using the same experimental protocol, except that the microspheres were injected 15 minutes after the subsequent doses of prazosin when stable heart rate and blood pressure responses were measured.

5-HT-antagonistic activity

The effects of 5-HT on the heart rate and arterial blood pressure were studied in seven conscious hypertensive rabbits. After an appropriate equilibration period increasing doses of 5-HT (10, 30 and 100 µg/kg, *i.v.*) were administered at 5 minutes intervals. Hexamethonium (30 mg/kg, *i.v.*) was administered 30 minutes after the measurement of the control responses. When steady baseline values of heart rate and blood pressure were reached the three 5-HT doses were again administered. This was repeated three times at 30 minutes intervals in the presence of ketanserin, 0.1, 0.3 and 1.0 mg/kg, *i.v.*, respectively.

α_1-adrenoceptor blocking properties

The effects of ketanserin and prazosin on the pressor response induced by phenylephrine, 3, 10 and 30 μg/kg, *i.v.*, administered at 5 minutes intervals were studied in two groups of eight rabbits. In the first group the subsequent doses of phenylephrine were administered four times at 30 minutes intervals in the absence and presence of ketanserin, 0.1, 0.3 and 1.0 mg/kg, *i.v.*, respectively. In the second group the effects of prazosin, 0.01, 0.03 and 0.10 mg/kg, *i.v.*, on the phenylephrine pressor response were measured using the same experimental protocol.

Statistical evaluation

All data, expressed as mean (±SEM) in the text, have been statistically evaluated with non-parametric tests (Siegel, 1956). Initially, the Friedman's two way analysis of variance was used to establish whether the samples represented different populations. The Wilcoxon matched-pairs signed ranks test was applied to test the "significance" ($p < 0.05$, two-tailed) of the changes in haemodynamic variables from baseline values.

Drugs

The following drugs were used: 5-hydroxytryptamine creatinine sulphate (Merck, Darmstadt, W.Germany), hexamethonium bromide (Fluka, Buchs, Switzerland), phenylephrine hydrochloride (Sigma Chemical Company, St.Louis, Missouri). Ketanserin tartrate and prazosin hydrochloride were generously supplied by Dr. J.M. Van Nueten of Janssen Pharmaceutica (Beerse, Belgium) and by Pfizer B.V. (Brussels, Belgium), respectively. 5-HT, phenylephrine and hexamethonium were dissolved in physiological saline. Ketanserin and prazosin were dissolved in distilled water. Concentrations were such that a volume less than 0.5 ml was injected at a time.

RESULTS

Systemic haemodynamic variables

The cumulative doses of ketanserin (0.1, 0.3 and 1.0 mg/kg)

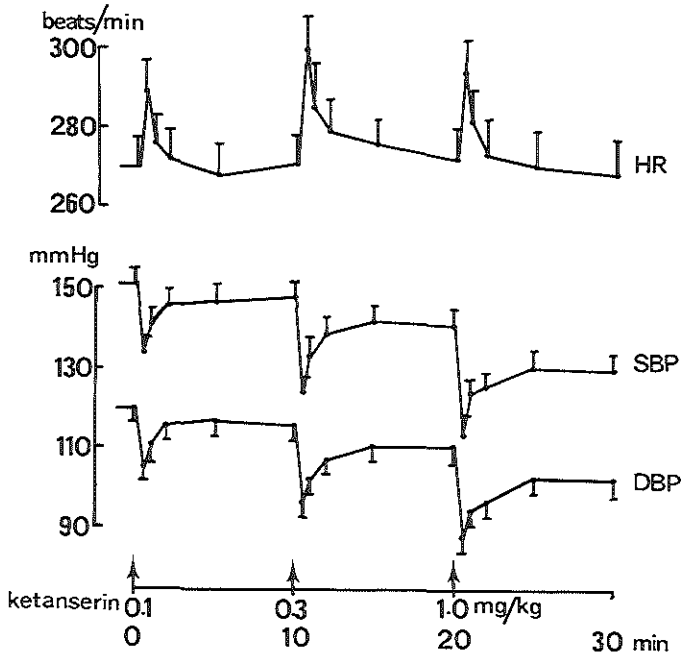


Figure 1. Time course of heart rate (HR), systolic (SBP) and diastolic (DBP) blood pressure responses after the i.v. administration of ketanserin, 0.1, 0.3 and 1.0 mg/kg, in conscious hypertensive rabbits (n=10).

administered at 10 minutes intervals in conscious hypertensive rabbits produced transient falls in systolic and diastolic blood pressure of about 2 minutes duration which were followed by a more moderate and longer lasting dose-related hypotensive effect (figure 1). A transient tachycardia accompanied the initial fall in blood pressure reaching a maximum within 30 seconds after each ketanserin administration. By 2 minutes the changes in heart rate were no longer significant.

In animals pretreated with hexamethonium, 30 mg/kg, only a transient fall in blood pressure was observed after ketanserin, 0.1, 0.3 and 1.0 mg/kg; baseline values were reached within 4 minutes after each ketanserin dose (figure 2).

The effects of ketanserin, 0.1, 0.3 and 1.0 mg/kg, on mean arterial blood pressure, cardiac output and total peripheral resistance 10 minutes after the administration in conscious hypertensive rabbits are shown in figure 3. The hypotensive

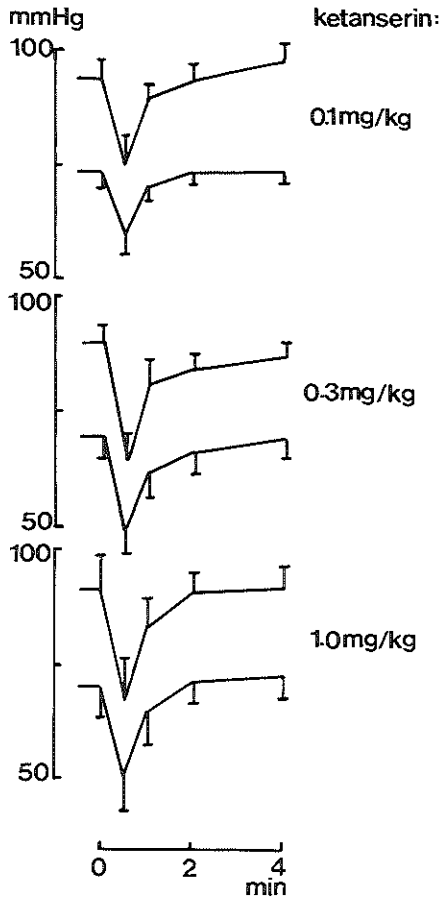


Figure 2. Effects of ketanserin, 0.1 (n=7), 0.3 (n=7) and 1.0 (n=5) mg/kg, i.v. on systolic and diastolic blood pressure in conscious hypertensive rabbits pretreated with hexamethonium, 30 mg/kg.

effect of ketanserin resulted from a reduction in total peripheral resistance. Especially at the lower doses of ketanserin an increase in cardiac output opposed the ketanserin-induced reduction in total peripheral resistance. In figure 3 are also shown the effects of prazosin (0.01, 0.03 and 0.10 mg/kg, i.v.) on mean blood pressure, cardiac output and total peripheral resistance measured 15 minutes after the successive administrations in conscious hypertensive rabbits. In contrast to ketanserin, a moderate fall in cardiac output contributed to the hypotensive effects of the highest dose of prazosin.

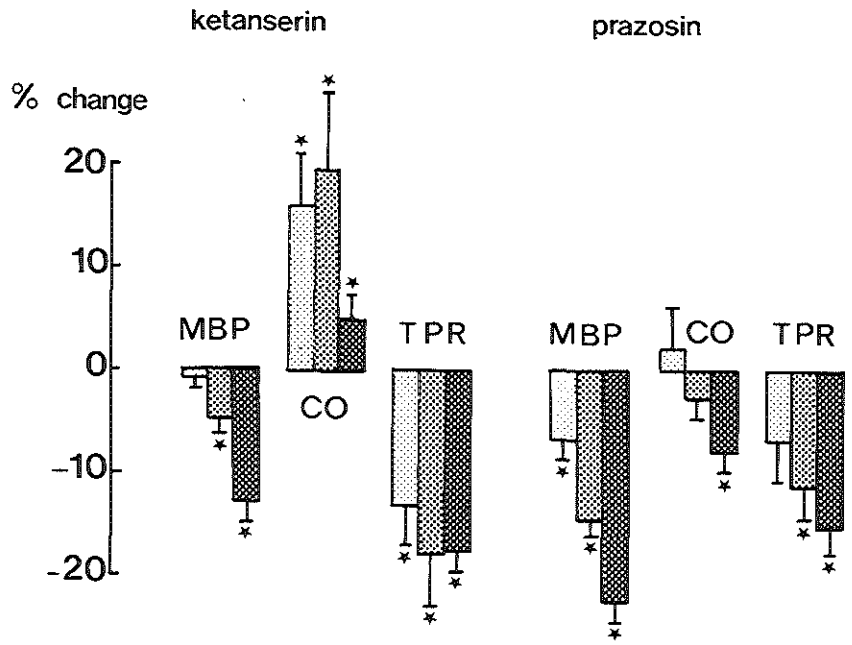


*Figure 3. Effects of ketanserin and prazosin on mean blood pressure (MBP), cardiac output (CO) and total peripheral resistance (TPR) expressed as percentage change from baseline values 10 minutes after ketanserin, 0.1 (▒), 0.3 (▒) and 1.0 (▒) mg/kg, respectively, (n=10) and 15 minutes after prazosin, 0.01 (▒), 0.03 (▒) and 0.10 (▒) mg/kg, respectively, (n=10) in conscious hypertensive rabbits. *, significant change from baseline values ($p < 0.05$). Baseline values for ketanserin: MBP, 127 ± 6 mmHg; CO, 422 ± 77 ml/min; TPR, 309 ± 20 mmHg/l.min^{-1}. Data for prazosin have been derived from Bolt and Saxena (1984a, chapter 7).*

Regional haemodynamic variables

Figure 4 shows the regional blood flow values before and after the successive i.v. administration of ketanserin, 0.1, 0.3 and 1.0 mg/kg in conscious renal hypertensive rabbits. The three doses of ketanserin increased the blood supply to the kidneys and gastro-intestinal tract. After the first and second dose the blood flow to the heart, brain and bones was also enhanced, whereas that to the skin was not affected. In the skeletal muscles the blood flow decreased after ketanserin, 1.0 mg/kg.

Figure 5 shows the changes in regional vascular conductances 10 minutes after ketanserin, 0.1, 0.3 and 1.0 mg/kg, respectively. Ketanserin produced a significant vasodilatation (increase in

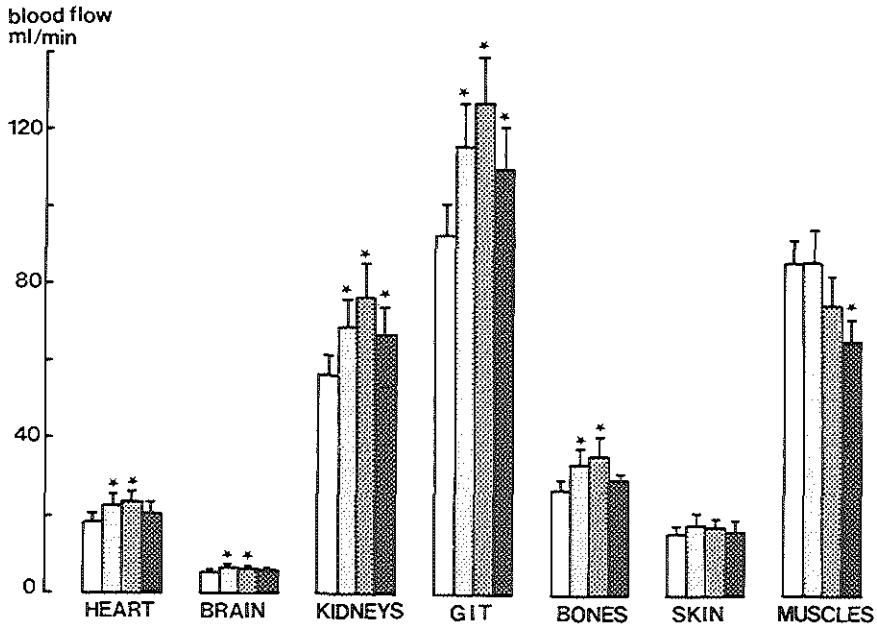


*Figure 4. Effects of ketanserin, 0.1, 0.3 and 1.0 mg/kg, respectively, on regional blood flows (ml/min) 10 minutes after i.v. administration in conscious hypertensive rabbits (n=10). □, baseline values; ▒, ▒ and ▒, values after ketanserin, 0.1, 0.3 and 1.0 mg/kg, respectively; GIT, gastro-intestinal tract; *, significant change from baseline values ($p < 0.05$).*

conductance) in the heart, brain, kidneys, gastro-intestinal tract and bones. The increase in vascular conductance was most pronounced in the latter three vascular beds. In the skin and skeletal muscles the changes were not significant. A more selective vasodilatation was observed with prazosin, 0.01, 0.03 and 0.10 mg/kg, 15 minutes after i.v. administration in conscious hypertensive rabbits. A significant increase in the vascular conductance was measured in the kidneys, gastro-intestinal tract and bones whereas the changes in the heart, brain, skin and skeletal muscles were not significant.

5-HT- and α_1-antagonistic properties

A transient bradycardia accompanied by a fall in blood pressure was observed after the i.v. administration of 5-HT (10, 30 and 100 µg/kg) in conscious hypertensive rabbits (figure 6).

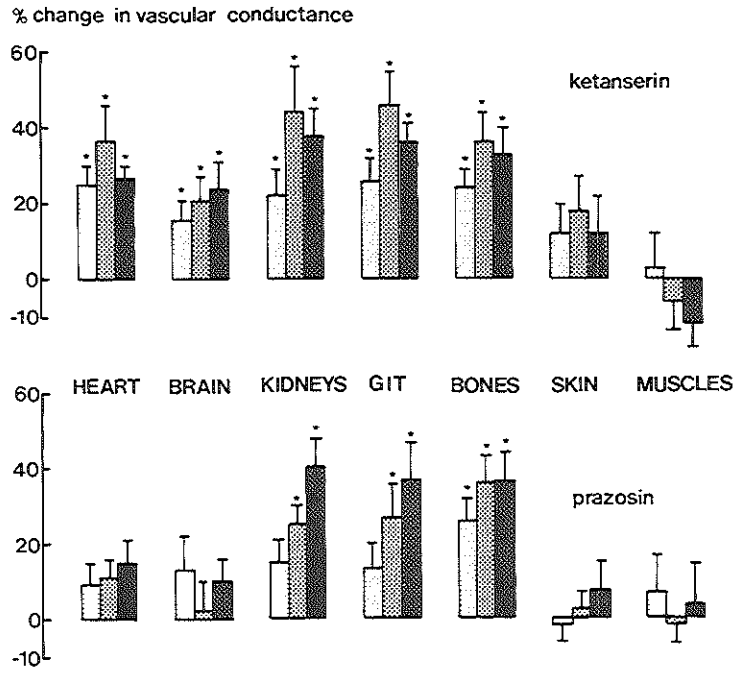


*Figure 5. Effects of ketanserin and prazosin on regional vascular conductances in conscious hypertensive rabbits, expressed as percentage change from baseline values 10 minutes after ketanserin, 0.1 ( ), 0.3 ( ) and 1.0 ( ) mg/kg, respectively, (n=10) and 15 minutes after prazosin, 0.01 ( ), 0.03 ( ) and 0.10 ( ) mg/kg, respectively (n=10). GIT, gastro-intestinal tract; *, significant change from baseline values ($p < 0.05$). Data for prazosin have been adapted from Bolt and Saxena (1984a, chapter 7).*

This effect was followed by a more prolonged hypotensive response accompanied by tachycardia. Pretreatment with a ganglionic blocker (hexamethonium, 30 mg/kg), which led to an increase in heart rate and a decrease in blood pressure, antagonized the first phase of the 5-HT responses. The amine now caused a moderate increase in blood pressure, again followed by a prolonged hypotensive effect (figure 6). Pretreatment with hexamethonium prevented the alterations in heart rate produced by ketanserin and 5-HT indicating an effective ganglionic blockade after hexamethonium, 30 mg/kg (figure 6). The pressor response produced by 5-HT in the ganglion blocked animals was rather small probably partly due to the opposing vasodilator response of 5-HT (22). Nevertheless, ketanserin, 0.1 and 0.3 mg/kg, effectively

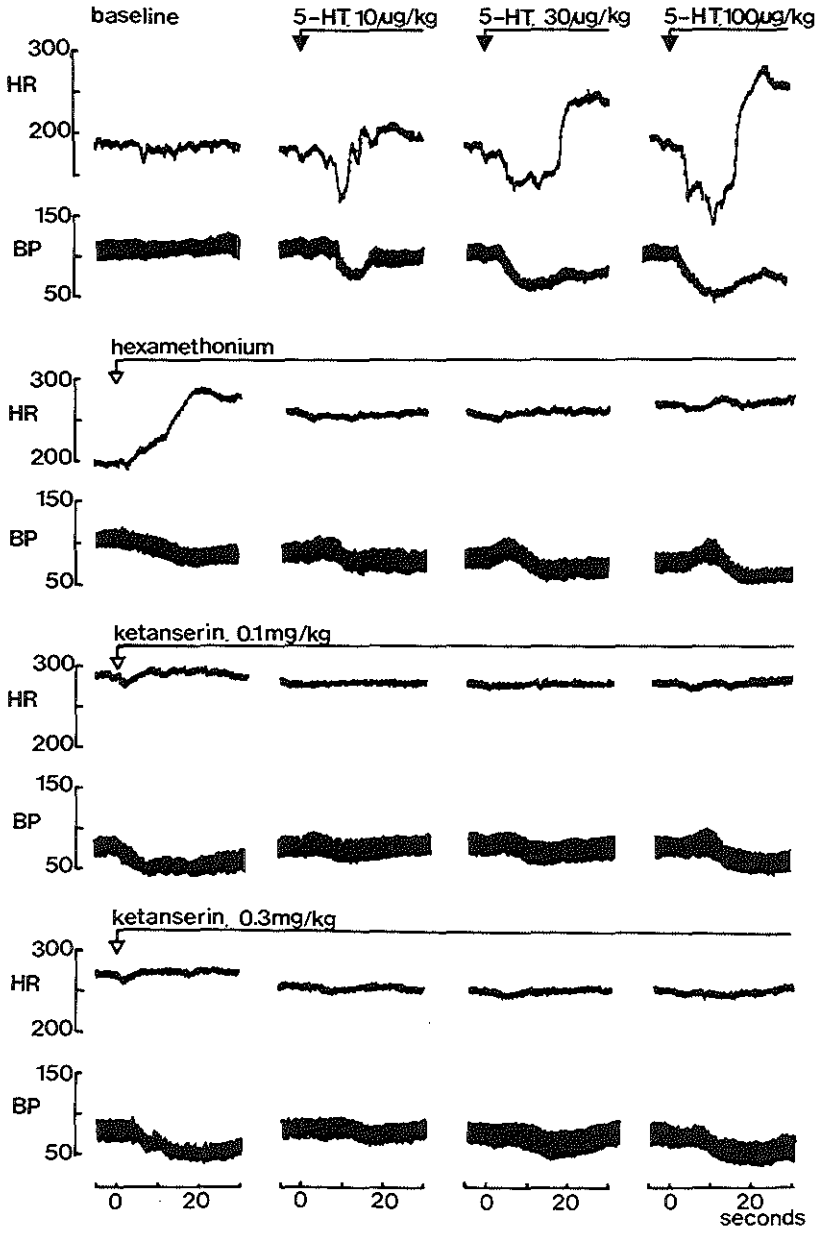


Figure 6. Heart rate (beats/min) and arterial blood pressure (mmHg) responses following injections of 5-HT (10, 30 and 100 µg/kg, i.v.) in a conscious hypertensive rabbit before and after pretreatment with hexamethonium (30 mg/kg, i.v.), in the absence and presence of ketanserin, 0.1 and 0.3 mg/kg, i.v. Pretreatment with hexamethonium blocks bradycardia by 5-HT and unmaskes a moderate pressor response which can be succesfully antagonized by ketanserin.

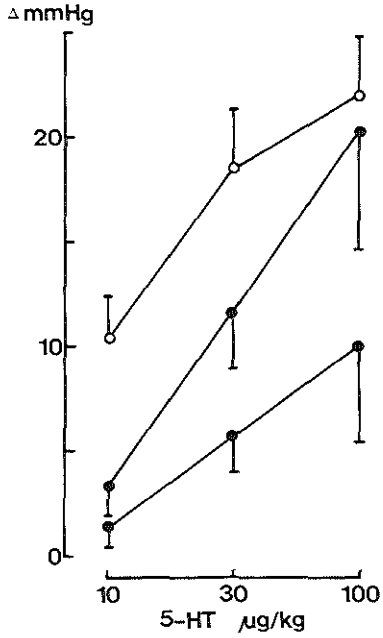


Figure 7. Changes in mean blood pressure produced by 5-HT, 10, 30 and 100 µg/kg, i.v. in conscious hypertensive rabbits (n=7) pretreated with hexamethonium (30 mg/kg) in the absence (open circles) and presence (closed circles) of ketanserin 0.1 and 0.3 mg/kg (from top to bottom, respectively).

antagonized the increase in blood pressure as is shown by the shift in the pressor response curve produced by 5-HT (figure 7).

The effects of ketanserin and prazosin on the pressor response produced by phenylephrine (10, 30 and 100 µg/kg) in the conscious untreated hypertensive rabbits are shown in figure 8. Ketanserin (0.1, 0.3 and 1.0 mg/kg) shifted the three points curve dose-dependently. A comparable α_1-adrenoceptor blocking action was observed with prazosin, 0.01, 0.03 and 0.10 mg/kg.

DISCUSSION

Cardiovascular profile of ketanserin

The fall in blood pressure observed after ketanserin administration in conscious hypertensive rabbits was biphasic; an initial pronounced but transient decrease in blood pressure preceded a more moderate and longer lasting dose-related hypotensive effect. The transient tachycardia, which accompanied the initial fall in blood pressure, probably resulted from the hypotension-induced activation of the baroreceptor reflex and the subsequent increase in sympathetic activity and withdrawal of the

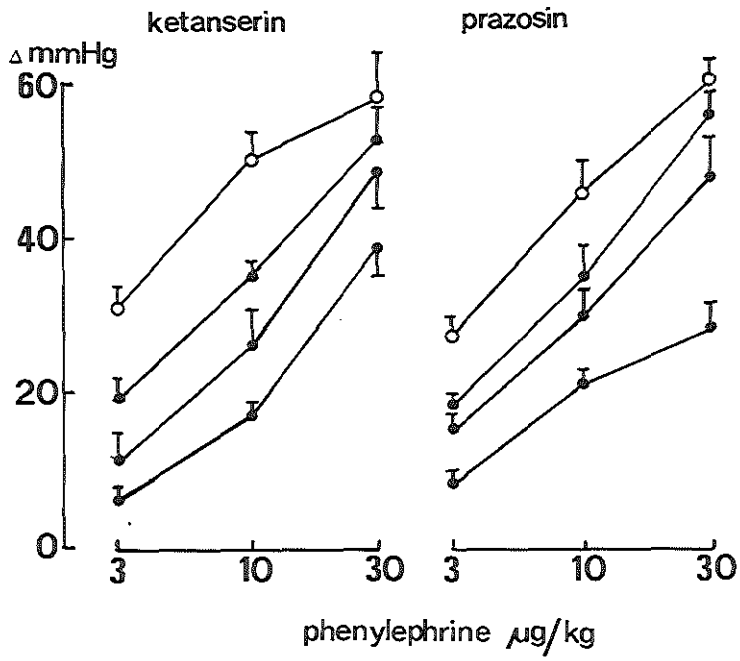


Figure 8. Changes in mean blood pressure produced by phenylephrine, 3, 10 and 30 µg/kg, i.v. in conscious hypertensive rabbits in the absence (open circles) and presence (closed circles) of ketanserin, 0.1, 0.3 and 1.0 mg/kg (from top to bottom, respectively, n=8) and prazosin, 0.01, 0.03 and 0.10 mg/kg (from top to bottom respectively, n=8).

vagal tone (Wenting et al., 1984). Indeed, the ketanserin-induced changes in heart rate were not observed after ganglionic blockade. The absence of reflex tachycardia during the prolonged hypotensive action of ketanserin may be related to the reported interference of the drug with the autonomic nervous system activity (Fozard, 1982; Mylecharane et al., 1984), where it causes a decrease in sympathetic outflow (McCall and Schuette, 1984). Remarkably, a similar heart rate response is observed at hypotensive doses of the α_1-selective adrenoceptor antagonist prazosin in conscious normotensive (Cavero, 1982) and hypertensive (Bolt and Saxena, 1984a) rabbits. Pronounced tachycardia is only observed during the first few minutes after the acute administration of prazosin despite sustained hypotension.

The prolonged fall in blood pressure observed after

ketanserin administration in the conscious hypertensive rabbits was due to a decrease in total peripheral resistance which illustrated the arterial vasodilator properties of ketanserin. An increase in cardiac output, possibly as a result of the reduction in afterload secondary to the fall in total peripheral resistance, was observed after ketanserin, 0.1 and 0.3 mg/kg. The less pronounced increase in cardiac output after ketanserin, 1.0 mg/kg, may be due to a reduction in venous return as a result of dilatation of venous capacitance vessels. In comparison, a transient increase in cardiac output has been observed after ketanserin administration in hypertensive patients despite the fact that a reduction in cardiac filling pressure indicated a reduced venous return (Wenting et al., 1984).

Due to the ketanserin-induced vasodilatation in the heart, brain, kidneys, gastro-intestinal tract and bones, the increase in cardiac output resulted in an enhanced blood supply to these vascular beds. An increase in renal blood flow has also been observed after ketanserin administration in hypertensive patients (Wenting et al., 1984). In the cutaneous vascular bed the vascular conductance and blood flow remained unchanged, whereas in the skeletal muscles the vascular conductance tended to decrease resulting in a reduced blood flow to the muscles at the higher ketanserin doses. Apparently, ketanserin has minor effects in the two latter vascular beds. However, it is possible that reflex-mediated vasoconstriction and tissue autoregulation obscure a direct vasodilator activity of ketanserin in certain vascular beds in the conscious animals (Bolt and Saxena, 1984c).

Hypotensive mechanism of ketanserin

Direct vasodilator action

The biphasic blood pressure response after ketanserin administration in conscious hypertensive rabbits has also been observed in anaesthetized rats (Kalkman et al., 1982) where the initial fall in blood pressure was ascribed to a possible direct vasodilator action of ketanserin. Also in the hindleg of the dog a vasodilator effect of ketanserin has been demonstrated which could not be blocked by methysergide or sympathetic denervation

(Rion et al., 1983) indicating that this effect is not mediated by either 5-HT_2 nor α_1-adrenergic receptors. In the present study the transient decrease in blood pressure was still observed after ganglionic blockade which shows that the initial fall in blood pressure does not depend upon an autonomic nervous tone and may be due to a direct vasodilator action of ketanserin which has also been shown for other structurally related quinazolinedione derivatives (Rion et al, 1983). Although in the present study this initial vasodilatation does not play a role in the prolonged hypotensive action of ketanserin, it is possible that when higher doses are given or when a different route of administration is used the direct vasodilator action may contribute in the antihypertensive properties of the compound. In this respect, the observed hypotensive effect of ketanserin in patients with autonomic insufficiency (Wenting et al., 1984) may possibly be attributed to a direct vasodilator effect of the drug.

5-HT receptor blockade

The pressor response of 5-HT, though only moderate, could be visualized after ganglionic blockade which prevents the fall in heart rate and blood pressure that result from the changes in autonomic nervous activity secondary to activation of the Bezold-Jarisch reflex (Paintal, 1973). The pressor response of 5-HT has been attributed to a direct vasoconstriction mediated via 5-HT_2 receptors aided indirectly by an augmentation of noradrenaline and angiotensin II responses (Van Nueten et al., 1981). These effects can be antagonized by ketanserin due to the 5-HT_2 receptor blocking properties of the compound which may play an important role in the hypotensive mechanism (Van Nueten et al., 1981; Janssen, 1983; Wenting et al., 1984). Indeed, at hypotensive doses ketanserin effectively antagonized the 5-HT-induced pressor responses in the conscious hypertensive rabbits pretreated with hexamethonium. Although this antagonism may facilitate the vasodilator effect of 5-HT mediated via "atypical" 5-HT receptors (Saxena and Verdouw, 1982; Saxena and Verdouw, 1984; Verdouw et al., 1984), until now, one has not been able to demonstrate an important physiological role for 5-HT in the maintenance of the arterial blood pressure. In addition, hypotensive properties have not been shown for other selective

5-HT_2 receptor antagonists (Fozard, 1982; Cohen et al., 1983b; Kalkman et al., 1983). Finally, Amery et al. (1984) have shown that, although longterm treatment with ketanserin reduced the blood pressure in hypertensive patients, 5-HT_2 receptor blockade could not be demonstrated when assessed by platelet aggregation. Thus, despite the 5-HT-antagonistic properties at hypotensive doses of ketanserin, a contribution of 5-HT receptor blockade in the hypotensive action of ketanserin remains to be established.

α_1-adrenoceptor blockade

Ketanserin also possessed α_1-adrenoceptor blocking properties at hypotensive doses as was shown by the shift in the 3-points phenylephrine pressor response curve after ketanserin, 0.1, 0.3 and 1.0 mg/kg, in the conscious hypertensive rabbits. Similar results were obtained in studies in pithed rats where the inhibitory effects of ketanserin on the pressor responses of the α_1-selective adrenoceptor stimulants methoxamine (Kalkman et al., 1982) and phenylephrine (Fozard, 1982) were measured. The decrease in blood pressure produced by the α_1-selective adrenoceptor antagonist prazosin at doses that caused a comparable shift in the phenylephrine pressor response curves indicates that in conscious hypertensive rabbits, as in rats (Fozard, 1982; Kalkman et al., 1982; Cohen et al., 1983a), the blockade of α_1-adrenoceptors by itself can be held responsible for the prolonged hypotensive action of ketanserin. In addition, in the animals pretreated with hexamethonium the blood pressure returned to baseline values within a few minutes which indicates the requirement of an autonomic nervous tone for the prolonged hypotensive action of ketanserin. In contrast, in hypertensive patients hypotensive doses of ketanserin (10 mg/kg, i.v.) did not alter the pressor effects of phenylephrine, suggesting that ketanserin may lower blood pressure independently of α_1-adrenoceptor blockade (Wenting et al., 1984; Zabludowski et al., 1984). Nevertheless, a certain degree of α_1-adrenergic tone seems to be required for the compound to exert its full antihypertensive action in man because pretreatment with prazosin blunted the antihypertensive effect of ketanserin in hypertensive patients (Wenting et al., 1984). In addition, after chronic treatment with ketanserin, Fagard et al., (1984) observed a

reduction of the pressor response to methoxamine in patients with essential hypertension. However, it may be that after chronic oral treatment with ketanserin (120mg/day) plasma levels were higher, causing a more pronounced α_1-adrenoceptor blockade, than after a single *i.v.* administration of 10 mg ketanserin (Wenting et al., 1984; Zabludowski et al., 1984).

In contrast to prazosin, ketanserin moderately increased the cardiac output in the present study. It may be that the direct vasodilator action of ketanserin -which is not observed with prazosin (Bolt and Saxena, 1984a)- contributed to the relatively more pronounced cardiac stimulation during the prolonged hypotensive phase of ketanserin. On the other hand, both, the less pronounced increase in cardiac output after ketanserin, 1.0 mg/kg and the decrease in this variable after prazosin, 0.1 mg/kg, can be explained by a reduction in venous return secundary to dilatation of venous capacitance vessels (Patel et al., 1981; Bolt and Saxena, 1984; Wenting et al., 1984), which becomes more prominent when higher doses of the drugs are used.

The remarkable similarities in the regional haemodynamic profile of ketanserin and prazosin also indicate an important role for the α_1-adrenoceptor blocking properties in the hypotensive mechanism of ketanserin. It is tempting to attribute the additional vasodilatation produced by ketanserin in the coronary and cerebral vascular beds to the 5-HT_2 receptor blocking properties of ketanserin that prazosin does not have (Kalkman et al, 1983). However, other explanations are possible. Myocardial autoregulation plays an important role in the blood flow to the heart (Weber and Janicki, 1979). If we consider the decrease in the cardiac output and the more pronounced reduction in blood pressure observed with prazosin it is possible that the myocardial metabolic demands are less than after ketanserin administration. This may explain the difference between the effects of the two drugs on the coronary vascular bed. In addition, possible differences in the pharmacokinetic properties of ketanserin and prazosin as well as the direct vasodilator action of ketanserin which prazosin does not have, may contribute to the differences in the cardiovascular profiles of the two compounds.

by α_1-adrenoceptors or 5-HT receptors, an autonomic nervous tone seems to be required for the prolonged hypotensive action of ketanserin. In addition, considering the α_1-adrenoceptor blocking activity of ketanserin at hypotensive doses and the similarities in the cardiovascular profile of ketanserin and prazosin, blockade of α_1-adrenoceptors probably plays a predominant role in the hypotensive mechanism of ketanserin in conscious hypertensive rabbits.

REFERENCES

Amery, A., Fagard, R., Fiocchi, R., Lijnen, P., Staessen, J., Vermylen, J. (1984) Antihypertensive action and serotonin-induced platelet aggregation during long-term ketanserin treatment in hypertensive patients. J. Cardiovasc. Pharmacol. 6: 182-185.

Bolt, G.R., Saxen, P.R. (1983) Systemic and regional hemodynamic characteristics of bilateral cellophane perinephritis hypertension in conscious rabbits. Clin. Exp. Hypertension A5: 885-901.

Bolt, G.R., Saxena, P.R. (1984a) Acute systemic and regional haemodynamic effects of prazosin in conscious hypertensive rabbits. Eur. J. Pharmacol. 104: 151-157.

Bolt, G.R., Saxena, P.R. (1984b) Characterization of the hypotensive mechanism of ketanserin in conscious renal hypertensive rabbits. Brit. J. Pharmacol. 82: 227p.

Bolt, G.R., Saxena, P.R. (1984c) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205-213.

Bolt, G.R., Saxena, P.R. (1985) Cardiovascular profile and hypotensive mechanism of ketanserin in rabbits. Hypertension, in press.

Cavero, I. (1982) Effects of prazosin on reflex changes in heart rate evoked by vasopressor and vasodepressor stimuli in conscious rabbits. J. Cardiovasc. Pharmacol. 4: s108-s112.

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

Cohen, M.L., Fuller, R.W., Kurz, K.D. (1983b) LY 53857, a selective new potent seretonergic (5-HT_2) receptor antagonist, does not lower blood pressure in the spontaneously hypertensive rat. J. Pharmacol. Exp. Ther. 227: 327-332.

De Cree, J., Leempoels, J., De Cock, W., Geukens, H., Verhaegen, H. (1981) The antihypertensive effects of a pure and selective serotonin-receptor blocking agent (R41468) in elderly patients. Angiology 32: 137-144.

Fagard, R., Fiocchi, R., Lijnen, P., Staessen, J., Moerman, E., De Schaepdryver, A., Amery, A. (1984) Haemodynamic and humoral responses to chronic ketanserin treatment in essential hypertension. Brit. Heart J. 51: 159-156.

Fozard, J.R. (1982) Mechanism of the hypotensive effect of ketanserin. J. Cardiovasc. Pharmacol. 4: 829-838.

Hales, J.R.S. (1974) Radioactive microsphere technique for studies of the circulation. Clin. Exp. Pharmacol. Physiol. suppl 1: 31-46.

Heymann, M.A., Payne, B.D., Hoffman, J.I.E., Rudolph, A.M. (1977) Blood flow measurements with radionuclide-labelled particles. Progr. Cardiovasc. Dis. 20: 55-79.

Janssen, P.A.J. (1983) 5-HT_2 receptor blockade to study serotonin-induced pathology. Trends Pharmacol. Sci. 4: 198-206.

Kalkman, H.O., Timmermans, P.B.M.W.M., Van Zwieten, P.A. (1982) Characterization of the antihypertensive properties of ketanserin (R41468) in rats. J. Pharmacol. Exp. Ther. 222: 227-231.

Kalkman, H.O., Harms, Y.M., Van Gelderen, E.M., Batink, H.D., Timmermans, P.B.M.W.M., Van Zwieten, P.A. (1983) Hypotensive activity of serotonin antagonists; correlation with alpha$_1$-adrenoceptor and serotonin receptor blockade. Life. Sci. 32: 1499-1505.

Leysen, J.E., Awouters, F., Kennis, L., Laduron, P.M., Vandenberk, J., Janssen, P.A.J. (1981) Receptor binding profile of R41468, a novel antagonist at 5-HT_2 receptors. Life Sci. 28: 1015-1022.

McCall, R.B., Schuette, M.R. (1984) Evidence for an alpha$_1$ receptor-mediated central sympathoinhibitory action of ketanserin. J. Pharmacol. Exp. Ther. 228: 704-710.

Mylecharane, E.J., Phillips, C.A., Markus, J.K., Shaw, J. (1984) Evidence for a central component to the hypotensive action of ketanserin. J. Cardiovasc. Pharmacol., in press.

Page, I.H. (1939) The production of persistent hypertension by cellophane perinephritis. J. Amer. Med. Ass. 113: 2046-2049.

Paintal, A.S. (1973) Vagal sensory receptors and their reflex effects. Physiol. Rev. 53: 159-227.

Patel, P., Bose, D., Greenway, D. (1981) Effects of prazosin and phenoxybenzamine on alpha- and beta-receptor-mediated responses in intestinal resistance and capacitance vessels. J. Cardiovasc. Pharmacol. 3: 1050-1059.

Rion, R., Mena, M.A., Hong, E. (1983) On the mechanism of action of ketanserin, a selective antagonist of the S_2 receptors with antihypertensive activity. Proc. West. Pharmacol. Soc. 26: 373-375.

Saxena, P.R., Schamhardt, H.C., Forsyth, R.P., Loeve, J. (1980) Computer programs for the radioactive microsphere technique. Computer Programs Biomed. 12: 63-84.

Saxena, P.R., Verdouw, P.D. (1982) Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow. J. Physiol. (Lond) 332: 501-520.

Saxena, P.R., Verdouw, P.D. (1984) Effects of methysergide and 5-hydroxytryptamine (5-HT) on carotid blood flow distribution in pigs: further evidence for the presence of atypical 5-HT receptors. Brit. J. Pharmacol. 82: 817-826.

Siegel, S. (1956) Non-Parametric Statistics for Behavioural Sciences. (McGraw-Hill Kogakusha, Tokyo).

Van Nueten,J.M., Janssen, P.A.J., Van Beek, J., Xhonneux, R., Verbeuren, T.J., Vanhoutte, P.M. (1981) Vascular effects of ketanserin (R41468), a novel antagonist of 5-HT_2 serotonergic receptors. J. Pharmacol. Exp. Ther. 218: 217-230.

Verdouw, P.D., Jennewein, H.M., Heiligers, J., Duncker, D.J., Saxena, P.R. (1984) Redistribution of carotid artery blood flow by 5-HT: Effects of the 5-HT_2 receptor antagonists ketanserin and WAL 1307. Eur. J. Pharmacol. 102: 499-509.

Warren, D.J., Ledingham, J.G.G. (1972) Chronic left atrial catheterization in the rabbit. Pflüg. Arch. 335: 167-172.

Weber, K.T., Janicki, J.S. (1979) The metabolic demand and oxygen supply of the heart: Physiologic and clinical considerations. Amer. J. Cardiol. 44: 722–729.

Wenting, G.J., Woittiez, A.J.J., Man in 't Veld, A.J., Schalekamp, M.A.D.H. (1984) 5-HT, alpha-adrenoceptors, and blood pressure; effects of ketanserin in essential hypertension and autonomic insufficiency. Hypertension 6: 100–109

Zabludowski, J.R., Zoccali, C., Isles, C.G., Murray, G.D., Robertson, J.L.S., Inglis, C.G., Fraser, R., Ball, S.G. (1984) Effect of 5-hydroxytryptamine type 2 receptor antagonist, ketanserin, on blood pressure, the renin-angiotensin system and sympatho-adrenal function in patients with essential hypertension. Brit. J. Clin. Pharmacol. 17: 309–316.

CHAPTER 9:
VASODILATORS AND REGIONAL BLOOD FLOW

At present a wide variety of vasodilator drugs has become available for antihypertensive therapy. In accordance with the differences in mechanism of action, each drug produces a heterogeneous pattern of vasodilator responses leading to a characteristic redistribution of the regional blood flows. A more profound knowledge of the regional haemodynamic profiles of different types of vasodilators may contribute to a better and rational application of these drugs in antihypertensive therapy tailored to particular needs of a patient.

The dominant haemodynamic disturbance in the established phase of most hypertensive forms is an increase in total peripheral resistance. Consequently vasodilators appear to be a rational approach to treatment. Despite this rationale, the usefulness of vasodilators in clinical practise is rather limited because of pronounced activation of cardiovacular reflex mechanisms that follows the decrease in blood pressure produced by vasodilator drugs (Reid, 1979), leading to undesirable changes in haemodynamic variables (figure 1). However, at present vasodilator drugs have gained renewed interest now that the most common problems experienced with these drugs, reflex-mediated cardiac stimulation and salt and water retention, can be counteracted by concurrent administration of β-adrenoceptor antagonists and diuretic agents. In addition, new compounds, such as converting enzyme inhibitors and α_1-selective adrenoceptor antagonists, have been developed which indirectly relax vascular smooth muscle through interference with pressor systems (figure 1) and whose blood pressure lowering effects appear to cause a less pronounced activation of cardiovascular reflex mechanisms. Considering the revival of interest in vasodilator drugs, a reconsideration of the haemodynamic profiles of these drugs seems to be justified.

The changes in systemic haemodynamic variables that accompany

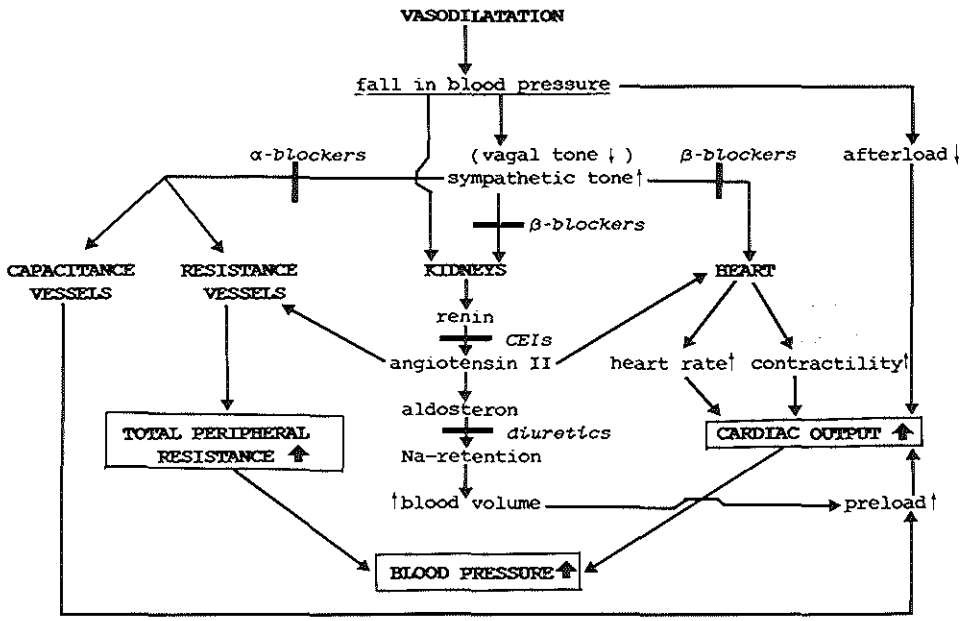


Figure 1. Schematic representation of the compensatory responses to vasodilator treatment and the interference by β-adrenoceptor antagonists, α-adrenoceptor antagonists, converting enzyme inhibitors (CEIs) and diuretics.

the fall in blood pressure during antihypertensive treatment with vasodilator drugs are rather well studied. However, less attention has been paid to the regional haemodynamic effects of these agents. The available information comes mainly from in-vitro studies on isolated vascular preparations and from in-vivo studies on separate vascular beds. Thus, a comparative review of the regional haemodynamic effects of vasodilator drugs consists of a tedious assembly of data obtained from different studies in various species. In addition, from a therapeutic point of view, more relevant information is obtained when studies are performed on conscious hypertensive animals. This guarantees a normal functioning of the different cardiovascular reflex-mechanisms which play an important role in the haemodynamic profile of antihypertensive drugs. Taking these considerations into account, we have used the radioactive microsphere technique to study the acute systemic as well as regional haemodynamic effects of a series of antihypertensive drugs of different types

in conscious rabbits with bilateral cellophane perinephritis hypertension. This experimental form of hypertension is characterized by a rather generalized increase in peripheral vascular resistances with the most pronounced change in the renal vascular bed, the latter resulting in a decrease in blood flow to the kidneys. The cardiac output is moderately decreased due to a reduction in stroke volume (Bolt and Saxena, 1983). Plasma renin activity is normal (Campbell et al., 1973). Similar haemodynamic characteristics have been described for late and severe essential hypertension in man (Lund-Johansen, 1983) as well as for many other experimental models of hypertension (Zandberg, 1984). The results obtained with the different drugs have been published elsewhere, separately for each drug (Bolt and Saxena, 1984a,b,c, 1985a,b). This paper, which has been accepted for publication (Bolt and Saxena, 1985c), serves to compare the haemodynamic profiles of five "direct" and "indirect" vasodilators and to discuss the pharmacological and therapeutic consequences. For this purpose we have selected the arterial vasodilator hydralazine, the calcium antagonist felodipine, the converting-enzyme inhibitor captopril, the $5HT_2$ receptor antagonist ketanserin and the α_1-adrenoceptor antagonist prazosin.

SYSTEMIC HAEMODYNAMIC VARIABLES

The hypotensive action of vasodilator drugs results from dilatation of arterial resistance vessels and the consequent reduction in total peripheral resistance. However, the degree by which the total peripheral resistance is reduced in order to produce a certain fall in blood pressure varies considerably amongst different vasodilators depending upon the concomittant changes in cardiac output which either oppose (when cardiac output increases) or contribute to (when cardiac output decreases) the hypotensive action of the drug. This is illustrated in figure 2 by the acute effects of hydralazine, 0.3, 1.0 and 3.0 mg/kg, felodipine, 0.003, 0.01 and 0.03 mg/kg, captopril, 0.1, 0.3 and 1.0 mg/kg, ketanserin, 0.1, 0.3 and 1.0 mg/kg, and prazosin, 0.01, 0.03 and 0.10 mg/kg, on systemic haemodynamic variables in

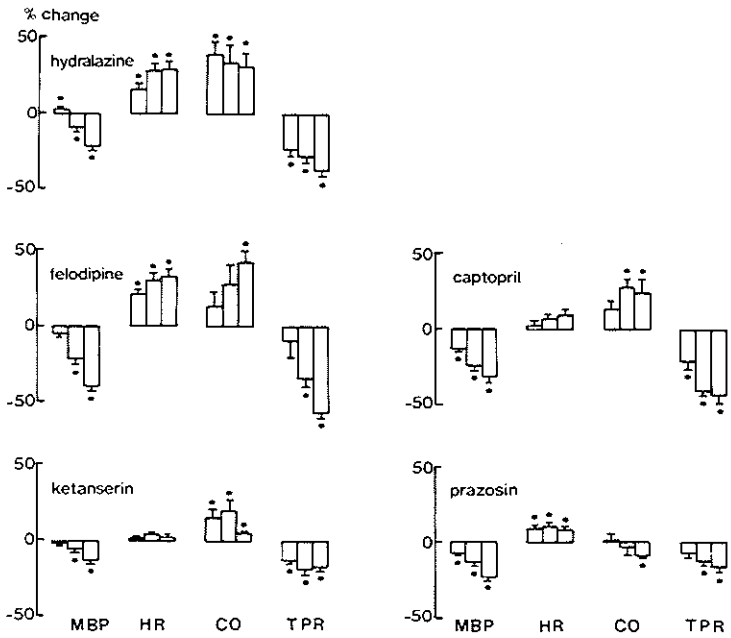


*Figure 2. Effects of hydralazine (0.3, 1.0 and 3.0 mg/kg, n=10), felodipine (0.003, 0.01 and 0.03 mg/kg, n=8), captopril (0.1, 0.3 and 1.0 mg/kg, n=8), ketanserin (0.1, 0.3 and 1.0 mg/kg, n=10) and prazosin (0.01, 0.03 and 0.10 mg/kg, n=10) on mean blood pressure (MBP), heart rate (HR), cardiac output (CO) and total peripheral resistance (TPR), expressed as percentage change from baseline values in conscious hypertensive rabbits. The changes were measured 10 minutes after the i.v. administration of the cumulative doses of felodipine, captopril and ketanserin, and 15 minutes after the i.v. administration of the cumulative doses of hydralazine and prazosin. *, significant change from baseline values ($p < 0.05$).*

conscious rabbits with bilateral cellophane perinephritis hypertension. To decrease the blood pressure, a relatively large fall in total peripheral resistance is necessary to overcome the increase in cardiac output produced by hydralazine, felodipine, captopril and ketanserin, whereas a reduction in cardiac output partly contributes to the acute hypotensive action of prazosin.

The cardiac function depends on four major determinants: preload, afterload, contractile state of the heart and heart rate (Ribner et al., 1982). The fall in afterload, secondary to the reduction in total peripheral resistance, is a common mechanism by which vasodilator drugs tend to increase cardiac output, especially when myocardial function is depressed. The contractile

state of the heart and the heart rate are, amongst other factors, influenced by changes in antonomic nervous activity. Secondary to the fall in blood pressure, the baroreceptor reflex becomes activated which results in cardiac stimulation via an increase in sympathetic activity and a reduction in vagal tone. In the present study a pronounced increase in heart rate (> 30 %) was observed with hydralazine and felodipine. A marked but transient tachycardia was also noticed immediatly after each ketanserin and prazosin administration but the heart rate response returned to baseline values within a few minutes after each dose of ketanserin, and stabilized at about 10 % above baseline values within 15 minutes after each prazosin administration. Captopril did not significantly alter the heart rate. The absence of a pronounced reflex-tachycardia during the prolonged hypotensive action of captopril, ketanserin and prazosin may be due to the reported inhibitory effects of these compounds on the autonomic nervous system activity and the consequent interference with the baroreceptor reflex (Cavero and Roach, 1980; Unger et al., 1983; McCall and Schuette, 1984).

The cardiac stimulation together with the fall in afterload is, however, not sufficient for the cardiac output to increase. An enhanced venous return of blood to the heart is necessary in order to keep up with an increased pumping capacity. Because the venous return is determined by the sum of local blood flows through peripheral tissues (Guyton, 1981), the arterial dilatation produced by vasodilator drugs and the consequent enhanced tissue perfusion can lead to an increase in venous return, particularly when venous capacitance vessels are not or only slightly dilated by the drug. In addition, the reflex-mediated increase in sympathetic tone due to the activation of the baroreceptor reflex may even lead to α-receptor mediated constriction of venous capacitance vessels thereby further enhancing venous return (Ribner et al., 1982). On the other hand, a dilatation of venous capacitance vessels will reduce the venous return and, consequently, decrease the cardiac output in the normal functioning heart (Ribner et al., 1982). Apart from dilatation of arterial resistance vessels, prazosin has been shown to affect venous capacitance vessels as well (Cavero and Roach, 1980), which explains the moderate fall in cardiac output observed after the

administration of prazosin, 0.1 mg/kg, in conscious hypertensive rabbits (figure 2).

An increase in venous return has also been suggested to participate in the cardiac stimulation observed with arterial vasodilators via the stimulation of atrial "stretch" receptors, thereby activating the Bainbridge reflex (Spokas and Wang, 1980). This may explain the positive chronotropic effects and the pronounced increase in cardiac output observed in the present study after hydralazine administration at doses which do not decrease the blood pressure despite the fall in total peripheral resistance (Spokas and Wang, 1980).

In conclusion, the changes in systemic haemodynamic variables that accompany the fall in blood pressure produced by vasodilators of different types vary considerably depending upon the respective site of action (arterial and/or venous vessels) and the more or less pronounced activation of cardiovascular reflex mechanisms.

REGIONAL HAEMODYNAMIC VARIABLES

Vascular resistance

The fall in total peripheral resistance produced by vasodilator drugs is in general not uniformly distributed over the various organs and tissues. Figure 3 shows the acute changes in regional vascular resistances in 7 peripheral vascular beds after the administration of increasing doses of hydralazine, felodipine, captopril, ketanserin and prazosin in conscious hypertensive rabbits. Hydralazine preferentially decreased the vascular resistance in the cerebral, coronary and renal vascular beds whereas the muscular bed was not significantly affected by the drug and a remarkable vasoconstriction was observed in the gastro-intestinal tract and skin. With the exception of the cutaneous vascular bed, a rather generalized peripheral vasodilatation was produced by felodipine although, quantitatively, marked regional differences were noticed. A uniform fall in peripheral vascular resistances was measured after captopril administration. Both, ketanserin and prazosin, dilated the renal and gastro-intestinal circulation and did not significantly change the vascular resistance in the muscles and skin. In contrast to prazosin, ketanserin also caused a moderate

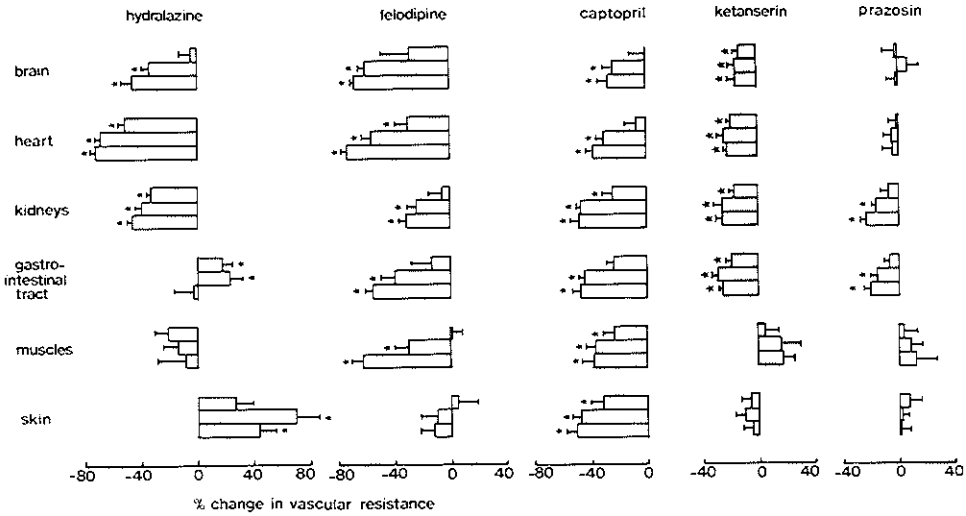


*Figure 3. Acute effects of hydralazine, felodipine, captopril, ketanserin and prazosin on regional vascular resistances (mmHg/ml.min^{-1}) expressed as percentage change from baseline values in conscious hypertensive rabbits. Doses and times of measurement are given in figure 1. *, significant change from baseline values ($p < 0.05$).*

decrease in the vascular resistance in the brain and heart.

Ofcourse, the heterogeneous pattern of vasodilator responses observed with each drug is mainly determined by the differences in potency to dilate the blood vessels in various vascular beds. The effectiveness of hydralazine to decrease the vascular resistance in the cerebral, coronary and renal circulation has been frequently reported in studies performed on isolated vascular beds (Ribner et al., 1982; Sakai et al., 1980). The profile observed with the dihydropiridine, felodipine, is in agreement with the findings obtained with other Ca-antagonists of the dihydropyridine group (Hof, 1984). Even the relatively higher doses of the drug necessary to dilate the muscular vascular bed is compatible with the reported higher potency of Ca-antagonists in the coronary vessels when compared with femoral arteries (Mullett et al., 1983). The cutaneous circulation has been shown to be relatively insensitive for the vasodilator action of Ca-antagonists (Vanhoutte and Rimele, 1982). The uniform vasodilatation produced by the converting-enzyme inhibitor, captopril, is remarkable

considering the low plasma renin levels in this "low renin" model of hypertension (Campbell et al., 1973). This may indicate a more prominant role of the renin angiotensin system, possibly of vascular origin, in the maintenance of the increased blood pressure in cellophane perinephritis hypertension than previously suggested (Campbell et al., 1983).

The similarities in the regional haemodynamic profiles of the $5HT_2$-receptor antagonist, ketanserin, and α_1-adrenoceptor antagonist, prazosin, are compatible with the α_1-adrenolytic properties that have also been reported for ketanserin (Kalkman et al., 1982). Indeed, in addition to $5HT_2$ antagonistic activity, a blockade of α_1-adrenoceptors could be demonstrated at the hypotensive doses of ketanserin in conscious hypertensive rabbits (Bolt and Saxena, 1985b). Although it is tempting to attribute the additional vasodilator properties of ketanserin to the antagonism of $5HT_2$ receptors (Janssen, 1983), other explanations (direct vasodilator action of ketanserin, tissue autoregulation, differences in pharmacokinetic properties etc) are possible (Bolt and Saxena, 1985b).

As outlined in figure 1, cardiovascular reflex mechanisms also play an important role in the regional haemodynamic profiles of vasodilators by opposing the direct vasodilator action via an increase in sympathetic activity and the activation of the renin angiotensin system (Reid, 1979). Because several "indirect" vasodilators act through interference with these pressor systems (α-adrenoceptor antagonists, converting enzyme inhibitors), the contribution of the reflex-mediated vasoconstrictor responses in the haemodynamic profile also varies amongst the different drugs used. The increase in vascular resistance in the gastro-intestinal circulation and the skin observed after hydralazine administration (figure 3) is apparently reflex mediated, because a generalized vasodilator response which included the femoral and mesenteric vascular beds has been reported in studies in blood perfused circulations isolated from the autonomic nervous system (Ribner et al., 1982; Sakai et al., 1980). These data also show that cardiovascular reflex mechanisms can still operate in the presence of hydralazine, which indicates that the mechanism of action of the drug does not involve a basic cellular pathway. In this respect, it has been demonstrated that

the vasodilator action of hydralazine depends for a large part on the presence of an intact endothelium, whereas angiotensin II and catecholamines mediate their pressor responses via receptors on the vascular smooth muscle cell (Spokas et al., 1984).

Reflex mediated vasoconstriction was not observed after the administration of felodipine. This is in agreement with data showing that Ca-antagonists of the dihydropyridine group attenuate the vasoconstrictor effects of α-adrenergic stimulants and angiotensin II (Hof, 1984). The generalized vasodilatation observed after converting-enzyme inhibition without any sign of reflex-mediated vasoconstriction suggests that the renin angiotensin system plays an important role in the acute regulation of the blood pressure.

Due to the α_1-adrenolytic activity of ketanserin and prazosin, vasoconstrictor responses, mediated via α_1 receptors, are blocked. However, it is possible that a vasodilator action of these compounds, for instance in the muscular and cutaneous vascular beds, is counteracted by the stimulation of vascular α_2 receptors and angiotensin II. In this respect, an increased activity of the renin-angiotensin system has been measured after the acute administration of ketanserin and prazosin in animal and clinical studies (Cavero and Roach, 1980; Janssen, 1983). In addition, in a comparitive study on the renal and femoral vascular bed a preponderance of α_2 receptors has been demonstrated in the femoral bed whereas vascular α_1 receptors dominated in the renal circulation (Horn et al., 1982), which is compatible with the dilator response of prazosin in the renal bed and the unchanged resistance in the muscular bed observed in the present study.

Finally, especially in the brain and heart, tissue autoregulation may participate in the observed haemodynamic profiles by maintaining the blood flows to these essential organs in accordance with the metabolic demands. Consequently, the observed changes in vascular resistance must be considered in view of the changes in blood flow to these organs and will therefore be discussed in the following section of this paper.

Regional blood flows

The peripheral dilatation in combination with the changes in cardiac output will greatly alter the distribution of regional

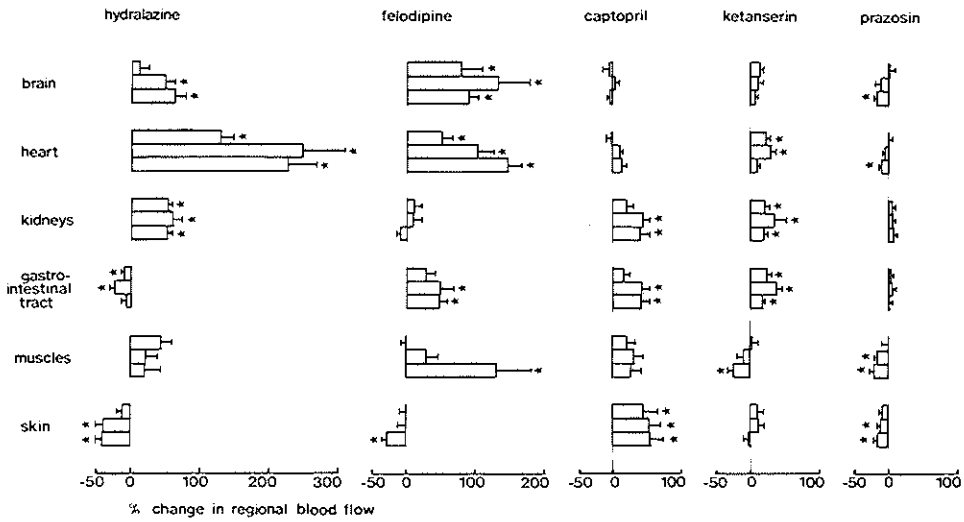


*Figure 4. Acute effects of hydralazine, felodipine, captopril, ketanserin and prazosin on regional blood flows (ml/min), expressed as percentage change from baseline values. Doses and times of measurement are given in figure 1. *, significant change from baseline value ($p < 0.05$).*

blood flows (figure 4). Apart from a qualitative description of the changes in regional blood flows, one has to consider the contribution of each vascular bed in the total circulation. Large amounts of blood are received by the kidneys, gastro-intestinal tract and skeletal muscles. In conscious hypertensive rabbits these circulations receive about 13, 27 and 21 % of the cardiac output, respectively (Bolt and Saxena, 1983). A relatively small but selective dilatation in one of these vascular beds will therefore have important consequences for the total circulation and may lead to underperfusion of other vascular beds which are not dilated by the drug, especially when cardiac output remains unchanged or decreases. This "steal" effect is for instance illustrated by the changes in regional blood flow observed after prazosin administration in conscious hypertensive rabbits (figure 4). The selective dilatation in the kidneys and gastro-intestinal tract produced by the α_1-adrenoceptor antagonist results in unchanged blood flows to these vascular beds despite the fall in cardiac output after prazosin, 0.1 mg/kg. However, this is at the expense of the blood supply to other organs and tissues, amongst

which essential organs such as the heart and brain. The remarkable fall in cerebral perfusion observed in the present study may be related to the serious side effects such as dizziness and even loss of consciousness observed after the acute administration of a relatively large first dose of prazosin in hypertensive patients, often refered to as "the first dose phenomenon of prazosin" (Cavero and Roach, 1982).

Because of the strong autoregulatory mechanism in the brain which under normal circumstances maintains the cerebral blood flow at a constant level over a wide blood pressure range, vasodilator drugs are generally considered as relatively ineffective in changing the blood flow to the brain. However, it has been demonstrated that in hypertension the lower limit to autoregulation is shifted to a higher blood pressure level, which indicates that hypertensive patients are more susceptible to cerebral underperfusion after an abrupt fall in blood pressure (Graham et al., 1983). This may play a role in the effects of prazosin on the blood flow to the brain. On the other hand, a pronounced vasodilatation and an increase in blood flow to the brain was observed after hydralazine and felodipine administration in the conscious hypertensive rabbits (figure 4). Considering the strong cerebral autoregulatory mechanism, it is possible that an increase in metabolic activity, for instance in the cardio-acceleration and/or vasomotor centres located in medulla and pons contribute to the increase in blood flow in the cerebral vascular bed. This is supported by the finding that the changes observed with hydralazine are completely due to an enhanced blood supply to brainstem and cerebellum whereas the blood flow to the cortex is not altered by the drug (Bolt and Saxena, 1984b). Similarly, the changes produced by felodipine were most pronounced in the "lower" regions of the brain.

Autoregulatory responses also play a role in the observed changes in the coronary vascular bed. The product of heart rate and systolic pressure is generally considered as a determinant of the metabolic activity of the heart. An increase in this variable is observed at the lower doses of hydralazine and felodipine, whereas the rate/pressure product decreased with captopril, ketanserin and prazosin (figure 5). Thus an increased metabolic activity probably partly determined the increase in blood flow to

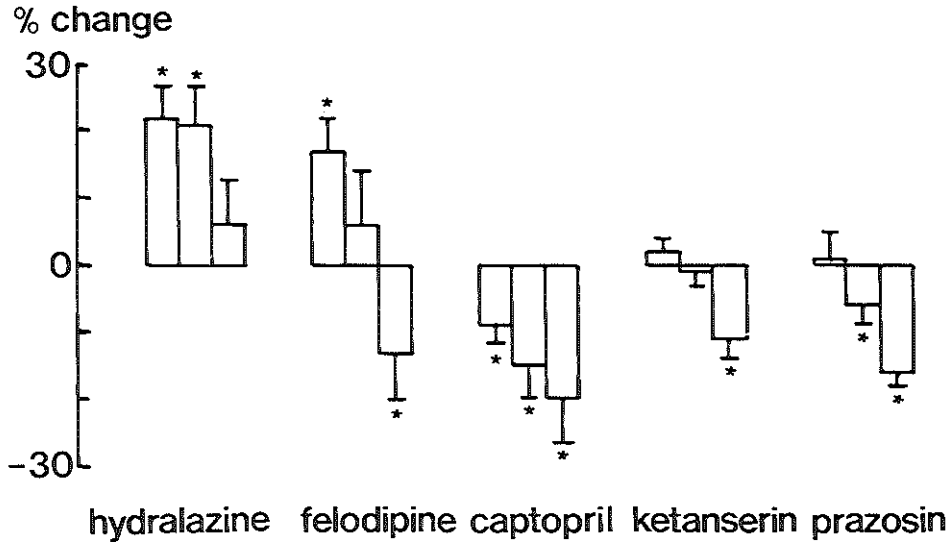


*Figure 5. Acute effects of hydralazine, felodipine, captopril, ketanserin and prazosin on the double product of heart rate (beats/min) and the systolic blood pressure (mmHg) expressed as percentage change from baseline values in conscious hypertensive rabbits. Doses and times of measurement are given in figure 1. *, significant change from baseline values ($p < 0.05$).*

the heart with the first two drugs at the lower doses used. On the other hand, the moderate fall in coronary blood flow after prazosin, 0.1 mg/kg, is not necessarily a negative aspect of the drug in view of the lowered metabolic activity (figure 5).

Despite the observed increase in coronary blood flow, single treatment with arterial vasodilators has been reported to induce angina pectoris in hypertensive patients (Ribner et al., 1982). Apart from the possible involvement of a coronary "steal" effect in patients with coronary stenosis (Ribner et al., 1982), an additional explanation is offered by the finding that in the present study both, hydralazine and felodipine, preferentially dilated and increased the blood flow to the outer layers of the left ventricular wall (Bolt and Saxena, 1984a,b). A decrease in the weight-normalized endocardial/epicardial blood flow ratio was measured with these drugs whereas this variable was not significantly altered by captopril, ketanserin or prazosin (table 1). This is probably due to the decrease in diastolic perfusion time, secondary to the increase in heart rate observed with

hydralazine and felodipine, and the changes in myocardial wall stress (Boudoulais et al., 1979). Indeed, pretreatment with the β_1-adrenoceptor antagonist atenolol not only opposed the hydralazine-induced cardiac stimulation in conscious hypertensive rabbits, but also protected against the undesirable changes in regional blood flow distribution in the left ventricular wall (Bolt and Saxena, 1984b). Thus, it seems posssible that after single treatment with arterial vasodilators the relatively small increase in blood flow to the endocardial layer of the left ventricular wall is insufficient to keep up with the increased metabolic demands especially in patients with limited coronary reserve.

Each drug reduced the vascular resistance in the renal vascular bed (figure 3). However, an increase in blood flow to the kidneys was only observed after hydralazine, captopril and ketanserin (figure 4), which is in agreement with the reported effects of these drugs on the renal circulation in hypertensive patients (Ribner et al., 1982; Mullett et al., 1983; Unger et al., 1983). Due to the moderate fall in cardiac output, prazosin did not significantly increase the blood flow to the kidneys. A relatively less pronounced effect on the renal vascular bed with, consequently, minor changes in renal blood flow, seemes to be a common characteristic of Ca-antagonists of the dihydropyridine group (Hof, 1984). Because the blood flow to the kidneys is known to be reduced in most forms of experimental and clinical hypertension, an improved blood supply to these organs may be considered as a favourable aspect of the haemodynamic profile of vasodilator drugs.

The gastro-intestinal, muscular and cutaneous circulations appear to be relatively more sensitive to reflexly initiated vasoconstrictor responses mediated via the autonomic nervous system and the renin-angiotensin system (Ribner et al., 1982). Indeed, with the exception of captopril, all drugs reduced the blood flow to one or more of these three vascular beds (figure 4). Although for clarity reasons these vascular beds are considered in their entirety, regional differences can be observed in each bed. For instance, the fall in blood supply to the gastro-intestinal tract observed with hydralazine, appears to be completely due to a reduced blood flow to the stomach and small intestine; the blood

flow to the large intestine increased after hydralazine, 3.0 mg/kg (Bolt and Saxena, 1984b). Similar differences can be expected in various regions of the skin and skeletal muscles. Although in case of underperfusion, serious side effects may result, these vascular beds are generally not considered as "essential". Nevertheless, they may play an important role in the circulation, because of the large amounts of blood received by these beds. This is for instance illustrated by the effects of the highest dose of felodipine (0.03 mg/kg) which markedly dilated the muscular vascular bed and increased the blood flow to the skeletal muscles thereby "stealing" blood from other areas such as the cerebral bed which exhibits maximal vasodilatation already at lower doses of the drug.

TABLE 1. Acute effects of hydralazine, felodipine, captopril, ketanserin and prazosin on systemic and regional haemodynamic variables and the endocardial/epicardial blood flow ratio in the left ventricular wall.

	hydralazine	felodipine	captopril	ketanserin	prazosin
Dose ($mg.kg^{-1}$)	3.0	0.01	0.3	1.0	0.1
Mean blood pressure (% change)	−21 ± 4	−21 ± 4	−25 ± 3	−13 ± 2	−23 ± 2
Heart rate	+ + +	+ + +	o	o	+
Cardiac Output	+ + +	+ +	+ +	+	−
Total peripheral resistance	− − − −	− − − −	− − − −	− −	− −
Resistance					
brain	− −	− − −	−	−	o
heart	− − − −	− − −	−	−	o
kidneys	− −	−	− −	−	−
Blood flow					
brain	+	+ + +	o	o	−
heart	+ + + +	+ +	o	o	−
kidneys	+	o	+	+	o
Endo/epi ratio	− − −	− − −	o	o	o

−,o,+, indicate relative magnitude of responses with − − − − and + + + + as the largest decrease and increase, respectively; endo/epi ratio, endocardial/epicardial blood flow ratio in the left ventricular wall.

SUMMARY

A summary of the data is given in table 1 which compares the acute changes in systemic haemodynamic variables and regional vascular resistances and blood flows to essential organs in conscious hypertensive rabbits at equal hypotensive doses (with the exception of ketanserin). Although immediatly after the administration of ketanserin, 1.0 mg/kg, a very pronounced fall in blood pressure (> -25 %) was observed, by 10 minutes the blood pressure response had stabilized at only 13 % below baseline level. Because of this initial fall in blood pressure we have avoided the use of ketanserin in doses higher than 1.0 mg/kg. As outlined in table 1, each drug produces a characteristic pattern of vasodilator responses which results in a marked redistribution of the cardiac output. The effects of the vasodilator drugs vary due to differences in dilator potency in the various vascular beds. In addition, cardiovascular reflex mechanisms and tissue autoregulation contribute to a different extent to the observed changes in vascular resistances and regional blood flows.

POSSIBLE CLINICAL RELEVANCE

Clinicians treating hypertensive patients, ought to take into account the haemodynamic characteristics of hypertension and the changes in systemic and regional haemodynamic variables that accompany the fall in blood pressure produced by antihypertensive drugs. A prerequisite for an antihypertensive drug is that it should not reduce the blood flow to essential organs, such as the brain, heart and kidneys. Moreover, when a hypertensive patient suffers from an impaired blood supply to certain vascular beds, an improved blood flow to these areas may be required. The important differences between the haemodynamic profiles of the various vasodilators demonstrated in the present study should help clinicians in making optimal use of the drugs and may therefore contribute to a more adequate application of these compounds in antihypertensive therapy.

It goes without saying that extreme caution is necessary when extrapolating the findings obtained in animal studies to man.

Moreover, it is possible that the haemodynamic profiles of antihypertensive drugs vary depending upon the experimental form of hypertension used. Finally, although the acute effects of drugs are certainly important, these effects may differ from those after chronic administration due to factors such as the possible adaptation of cardiovascular reflex mechanisms, the involvement of volume factors and the tolerance towards drugs. It shall be of considerable importance to investigate the changes in systemic and regional haemodynamic variables during chronic administration of antihypertensive drugs of different types.

REFERENCES

Bolt, G.R., Saxena, P.R. (1983) Systemic and regional hemodynamic characteristics of bilateral cellophane perinephritis hypertension in conscious rabbits. Clin. Exp. Hypertension A5: 885–901.

Bolt, G.R., Saxena, P.R. (1984a) Acute systemic and regional hemodynamic effects of felodipine, a new calcium antagonist, in conscious renal hypertensive rabbits. J. Cardiovasc. Pharmacol. 6: 707–712.

Bolt, G.R., Saxena, P.R. (1984b) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205–213.

Bolt, G.R., Saxena, P.R. (1984c) Acute systemic and regional haemodynamic profile of prazosin in conscious hypertensive rabbits. Eur. J. Pharmacol. 104: 151–157.

Bolt, G.R., Saxena, P.R. (1985a) Acute systemic and regional haemodynamic profile of captopril in conscious rabbits with bilateral cellophane perinephritis hypertension. Accepted for publication in Clin. Exp. Pharmacol. Physiol.

Bolt, G.R., Saxena, P.R. (1985b) Cardiovascular profile and hypotensive mechanism of ketanserin in conscious renal hypertensive rabbits. Accepted for publication in Hypertension.

Bolt, G.R., Saxena, P.R. (1985c) Vasodilators and regional blood flow. Submitted for publication.

Boudoulais, H., Rittgers, S.E., Lewis, R.P., Leier, C.V., Weissler, A.M. (1979) Changes in diastolic time with various pharmacologic agents. Implication for myocardial perfusion. Circulation 1: 164–169.

Campbell, D.J., Skinner, S.H., Day, A.J. (1973) Cellophane perinephritis hypertension and its reversal in rabbits; effect on plasma renin, renin substrate, and renal mass. Circulation Res. 33: 105–113.

Cavero, I., Roach, A.G. (1980) The pharmacology of prazosin, a novel antihypertensive agent. Life Sciences 27: 1525–1540.

Graham, D.I., Lee, W.R., Cumming, A.M.M., Robertson, J.I.S., Jones, J.V. (1983) Hypertension and the intracranial and ocular circulations: effects of antihypertensive treatment. In Handbook of Hypertension Vol. 1 ed. J.I.S. Robertson, pp. 174–201, Amsterdam: Elsevier Science Publishers.

Guyton, A.C. (1981) The relationship of cardiac output and arterial pressure control. Circulation Res. 64: 1079–1088.
Hof, R.P. (1984) Selective effects of different calcium antagonists on the peripheral circulation. Trends Pharmacol. Sci. 5: 100–102.
Horn, P.T., Kohli, J.D., Listinski, J.L., Goldberg, L. (1982) Regional variation in the alpha-adrenergic receptors in the canine resistance vessels. Naunyn-Schmiedeb. Arch. Pharmacol. 318: 166–172.
Janssen, P.A.J. (1983) 5-HT_2 receptor blockade to study serotonin-induced pathology. Trends Pharmacol. Sci. 4: 198–206.
Kalkman, H.O., Timmermans, P.B.M.W.M., Van Zwieten, P.A. (1982) Characterization of the antihypertensive properties of ketanserin (R 41 468) in rats. J. Pharmacol. Exp. Therapeut. 222: 227–231.
Lund-Johansen, P. (1983) The hemodynamics of essential hypertension. In Handbook of Hypertension Vol. 1 ed. J.I.S. Robertson, pp. 151–173, Amsterdam: Elsevier Science Publishers.
McCall, R.B., Schuette, M.R. (1984) Evidence for an alpha-1 receptor-mediated central sympathoinhibitory action of ketanserin. J. Pharmacol. Exp. Therapeut. 228: 704–710.
Mullett, M. Gharaibeh, M, Warltier, D.C., Gross, G.J. (1983) The effect of diltiazem, a calcium channel blocking agent, on vasoconstrictor responses to norepinephrine, serotonin and potassium depolarization in canine coronary and femoral arteries. Gen. Pharmacol. 14: 259–264.
Reid, J.L. (1979) Vasodilators in the management of hypertension. Progr. Pharmacol. 3/4: 77–82.
Ribner, H.S., Bresnahan, D., Hsieh, A.M., Silverman, R., Tommaso, C., Coath, A., Askenazi, J. (1982) Acute hemodynamic responses to vasodilator therapy in congestive heart failure. Progr. Cardiovasc. Dis. 25: 1–43.
Sakai, K., Akima, M., Adachi, J. (1980) Pharmacological features of the coronary, renal, mesenteric, and femoral vascular beds of rats revealed by intra-arterial administration of drugs. J. Cardiovasc. Pharmacol. 2: 453–470.
Spokas, E.G., Wang, H.H. (1980) Regional blood flow and cardiac response to hydralazine. J. Pharmacol. Exp. Ther. 212: 294–303.
Spokas, E.G., Folco, G., Quilley, J., Chander, P., McGiff, J.C. (1983) Endothelium mechanism in the vascular action of hydralazine. Hypertension 5 (suppl 1): 107–111.
Unger, T., Ganten, D., Lang, R.E. (1983) Converting enzyme inhibitors: antihypertensive drugs with unexpected mechanisms. Trends Pharmacol. Sci. 4: 514–519.
Vanhoutte, P.M., Rimele, T.J. (1982) Calcium and alpha-adrenoceptors in activation of vascular smooth muscle. J. Cardiovasc. Pharmacol. 14: 259–264.
Zandberg, P. (1984) Animal models in experimental hypertension: relevance to drug testing and discovery. In Handbook of Hypertension Vol. 3 ed. P.A. Van Zwieten, pp. 6–45, Amsterdam: Elsevier Science Publishers.

SUMMARY

At present many different antihypertensive drugs are available which lower the blood pressure through various mechanisms of action (chapter 1). Accordingly, it is expected that the haemodynamic changes that accompany the fall in blood pressure during antihypertensive treatment with different drugs vary considerably. The aim of the studies described in this thesis is to characterize and compare the haemodynamic profiles of various types of antihypertensive drugs with special emphasis on the drug-induced changes in regional blood flows. For this purpose the radioactive microsphere technique was used to study the acute effects of a series of "direct" and "indirect" vasodilators in conscious hypertensive rabbits.

In order to test the suitability of the radioactive microsphere technique for successive measurements of systemic and regional haemodynamic variables in conscious animals, a control study was performed in which the effects of three subsequent injections of physiological saline (0.1 ml/kg, *i.v.*) on baseline values of cardiac output and regional blood flows were determined in conscious normotensive rabbits using microspheres with a diameter of 15 μm and labeled with different isotopes (chapter 2). No consistent changes were produced by the successive saline administrations in any of haemodynamic variables measured. It was therefore concluded that at least four different measurements can be made reliably in a period of one hour.

The effects of the drugs were studied in conscious rabbits with bilateral cellophane perinephritis hypertension. In chapter 3 a comparison is made between the values of systemic and regional haemodynamic variables in normotensive rabbits and in hypertensive rabbits 6 weeks after encapsulation of the kidneys with cellophane. Bilateral cellophane perinephritis hypertension was characterized by a rather generalized increase in peripheral vascular resistances with the most pronounced changes in the renal vascular bed, the latter resulting in a marked decrease in blood flow to the kidneys. The cardiac output was moderately reduced in the hypertensive rabbits due to a fall in stroke volume. A negative correlation was observed between the weight of the left ventricular wall and the weight-normalized blood flow to this area suggesting inadequate coronary perfusion as myocardial hypertrophy becomes more pronounced.

Chapter 4 describes the acute haemodynamic profile of the converting enzyme inhibitor captopril (0.1, 0.3 and 1.0 mg/kg, *i.v.*) in conscious hypertensive rabbits. The drug effectively reduced the blood pressure in this "low plasma

renin" model of hypertension. A moderate increase in cardiac output was observed at the higher doses of the drug. The rather generalized peripheral vasodilatation (increase in vascular conductance) produced by captopril suggests that an increased activity of the renin-angiotensin system in tissues such as the vascular wall and the brain may be involved in the maintenance of the increased blood pressure in rabbits with cellophane perinephritis.

The effects of the arterial vasodilator hydralazine (0.3, 1.0 and 3.0 mg/kg, *i.v.*), alone and in combination with the selective β_1-adrenoceptor antagonist atenolol (1.0 mg/kg, *i.v.*), are described in chapter 5. The fall in blood pressure produced by hydralazine was accompanied by an increase in heart rate and cardiac output. The drug caused vasodilatation and increased the blood flow in the heart, brain and kidneys, whereas a, probably reflex-mediated, vasoconstriction was measured in the skin, stomach and small intestine resulting in a reduced blood supply to these vascular beds. In addition, hydralazine increased the arteriovenous anastomotic flow. Atenolol inhibited the cardiac stimulation and thereby accentuated the hypotensive action of hydralazine, 0.3 mg/kg. The synergistic effect on the blood pressure response was not obserbed after hydralazine, 3.0 mg/kg, due to the increase in cardiac output at this hydralazine dose despite effective β-adrenoceptor blockade. Moreover, atenolol interfered with the vasodilator response of hydralazine in the heart, skeletal muscles and arteriovenous anastomoses. Probably secundary to the changes in diastolic perfusion time and myocardial wall stress, the β-adrenoceptor antagonist increased the weight-normalized endocardial/epicardial blood flow ratio in the left ventricular wall and thereby abolished the decrease in this variable produced by hydralazine. This finding may be related to the protection offered by β-adrenoceptor antagonists against the vasodilator-induced attacks of angina pectoris in patients with limited coronary reserve.

A characterization of the acute haemodynamic profile of the calcium antagonist felodipine (3, 10 and 30 μg/kg, *i.v.*) is given in chapter 6. Felodipine effectively reduced the blood pressure and increased the heart rate and cardiac output. With the exception of the cutaneous vascular bed, a rather generalized peripheral vasodilatation was produced by the drug although, quantitatively, marked regional differences were noticed. This resulted in an increased blood supply to the heart, brain, intestines and, at the higher doses used, skeletal muscles. As observed with hydralazine, the calcium antagonist reduced the endocardial/epicardial blood flow ratio, which illustrated the negative aspects of single treatment with this arterial vasodilator.

The acute effects of the selective α_1-adrenoceptor antagonist prazosin (0.01, 0.03 and 0.10 mg/kg, *i.v.*) are described in chapter 7. Prazosin

decreased the blood pressure by reducing total peripheral resistance. A moderate fall in cardiac output contributed to the hypotensive effect of the highest dose of prazosin used. Only a slight increase in heart rate was observed 15 minutes after the successive prazosin administrations. The drug produced a rather selective vasodilatation in the kidneys, intestines and bones which resulted in unchanged blood flows to these vascular beds despite the fall in cardiac output after prazosin, 0.10 mg/kg. However, this was at the expense of the blood supply to the heart, brain, skeletal muscles and skin. The remarkable decrease in cerebral perfusion may be related to the "first dose phenomenon" of prazosin in man characterized by side-effects such as dizziness and even loss of consciousnes.

A characterization of the acute haemodynamic profile and the hypotensive mechanism of ketanserin, a selective 5-HT_2-receptor antagonist with α_1-adrenoceptor blocking properties, is given in chapter 8. An initial pronounced fall in blood pressure, possibly due to a direct vasodilator action of ketanserin, accompanied by tachycardia preceded a more moderate but longer lasting hypotensive effect of the drug (0.1, 0.3 and 1.0 mg/kg, *i.v.*). Especially at the lower doses used, the cardiac output increased. Considering i) the α_1-adrenoceptor blocking properties at hypotensive doses of ketanserin in conscious hypertensive rabbits shown by the inhibition of the pressor response to phenylephrine, ii) the decrease in blood pressure produced by prazosin at doses which caused a comparable degree of α_1-adrenoceptor blockade, iii) the absence of a prolonged hypotensive effect of ketanserin after ganglionic blockade, it was concluded that α_1-adrenoceptor blockade probably plays a predominant role in the hypotensive effects of ketanserin in conscious hypertensive rabbits. In addition, as observed with the α_1-adrenoceptor antagonist prazosin, ketanserin caused vasodilatation in the kidneys, gastro-intestinal tract and bones. In contrast to prazosin, ketanserin also produced a moderate vasodilatation in the heart and brain. Although it is attractive to attribute the differences in the regional haemodynamic profiles to the 5-HT_2-receptor blocking properties of ketanserin, other explanations are possible, e.g. the direct vasodilator action of ketanserin, differences in pharmacokinetic properties and tissue autoregulatory responses.

Chapter 9 summarizes and compares the haemodynamic profiles of hydralazine, felodipine, captopril, prazosin and ketanserin in conscious hypertensive rabbits. The changes in systemic haemodynamic variables that accompany the fall in blood pressure produced by the different drugs vary considerably depending upon the respective site of action (arterial and/or venous vessels) and the more or less pronounced activation of cardiovascular reflex mechanisms. Each drug

produced a characteristis pattern of vasodilator responses which resulted in a redistribution of the cardiac output. The regional haemodynamic profile is determined by the potency of the drug to dilate the various vascular beds. In addition, the reflex-mediated increase in sympathetic vasomotor tone and activation of the renin-angiotensin system, and tissue autoregulatory responses contribute to a different extend to the observed changes in vascular resistances and regional blood flows. The studies presented in this thesis should alert clinicians that important differences exist in the haemodynamic profiles of "direct" and "indirect" vasodilator drugs. This may ultimately lead to a more rational approach to antihypertensive treatment.

SAMENVATTING

Bij de behandeling van hypertensie heeft men de beschikking over een groot aantal antihypertensieve farmaca met diverse werkingsmechanismen. Men mag dan ook verwachten, dat de hemodynamische veranderingen, die gepaard gaan met de daling in bloeddruk tijdens behandeling met antihypertensieve farmaca, sterk uiteenlopen (hoofdstuk 1). Het doel van het, in dit proefschrift beschreven, onderzoek is het hemodynamische profiel van een aantal antihypertensieve farmaca te karakteriseren en te vergelijken, waarbij met name aandacht is besteed aan de veranderingen in de bloeddoorstroming van perifere vaatgebieden. Hiertoe zijn de acute effecten een van vijftal "direkte" en "indirekte" vaatverwijders bestudeerd in niet-genarcotiseerde hypertensieve konijnen.

Om zowel de systemische als de regionale hemodynamische variabelen te kunnen meten, is gebruik gemaakt van de radioactieve microsfeer techniek. Een serie controle experimenten zijn uitgevoerd om de bruikbaarheid van deze methode voor ons onderzoek te toetsen. Hiertoe zijn de acute veranderingen in systemische en regionale variabelen bestudeerd na drie opeenvolgende toedieningen van een fysiologische zoutoplossing (0.1 ml/kg, *i.v.*) in normotensieve niet-genarcotiseerde konijnen, waarbij het hartminuutvolume en de perifere bloeddoorstroming in diverse regionale vaatgebieden zijn gemeten met behulp van microsferen met een diameter van 15 µm en gemerkt met verschillende radioactieve isotopen. Daar er in geen van de hemodynamische variabelen significante veranderingen werden waargenomen, kon worden geconcludeerd, dat het mogelijk is om tenminste vier verschillende metingen te verrichten in een tijdsbestek van een uur.

De effecten van de farmaca zijn bestudeerd in niet-genarcotiseerde konijnen met bilaterale cellofaan perinephritis hypertensie. In hoofdstuk 3 zijn de hemodynamische variabelen in normotensieve en hypertensieve konijnen met elkaar vergeleken. Bilaterale cellofaan perinephritis hypertensie in konijnen werd gekenmerkt door een nagenoeg algemene toename in vaatweerstand in alle organen en weefsels. De toename in vasculaire weerstand was het meest uitgesproken in het renale vaatgebied hetgeen resulteerde in een vermindering van de bloeddoorstroming van de nieren. Het hartminuutvolume was enigzins verlaagd in de hypertensieve konijnen als gevolg van een daling in het slagvolume. Een negatieve correlatie werd waargenomen tussen het gewicht en de bloeddoorstroming per gewichtseenheid van de linkerventrikelwand. Deze waarneming suggereert, dat de coronaire perfusie minder adequaat wordt naarmate de hypertrofie van het myocard toeneemt.

In hoofdstuk 4 staat het acute hemodynamische profiel beschreven van de remmer van het converting enzyme, captopril (0.1, 0.3 en 1.0 mg/kg, *i.v.*) in niet-genarcotiseerde konijnen met cellofaan perinephritis hypertensie. Deze experimentele vorm van hypertensie wordt gekenmerkt door een normale plasma renine activiteit. Desondanks werd een sterke daling in bloeddruk waargenomen na captopril toediening. De hogere doseringen veroorzaakten een geringe toename in het hartminuutvolume. De algemene vasodilatatie in de perifere vaatgebieden, welke werd waargenomen na captopril toediening, suggereert, dat een verhoogde activiteit van het renine-angiotensine systeem in de wand van bloedvaten en/of in hersenweefsel mogelijk een rol speelt bij het instandhouden van de verhoogde bloeddruk in konijnen met cellofaan perinephritis.

De acute effecten van de arteriële vaatverwijder hydralazine (0.3, 1.0 en 3.0 mg/kg, *i.v.*), alsmede de combinatie van atenolol (1 mg/kg, *i.v.*) en hydralazine (0.3 en 3.0 mg/kg, *i.v.*), worden behandeld in hoofdstuk 5. De daling in bloeddruk veroorzaakt door hydralazine ging gepaard met een toename in hartslagfrequentie en hartminuutvolume. Vasodilatatie en een toename in bloeddoorstroming werden waargenomen in het hart, de hersenen en de nieren, terwijl een, waarschijnlijk reflex-gemedieerde, vasoconstrictie werd gemeten in de huid, de maag en de dunne darm. Dit laatste resulteerde in een verminderde doorbloeding van deze vaatgebieden. Daarnaast veroorzaakte hydralazine een toename in de bloeddoorstroming van arterioveneuze anastomoses. De selectieve β_1-adrenoceptor antagonist, atenolol, verminderde de stimulatie van het hart, hetgeen resulteerde in een accentuering van het bloeddrukverlagende effect van hydralazine, 0.3 mg/kg. Het synergistische effect op de bloeddruk werd niet waargenomen na hydralazine, 3.0 mg/kg, als gevolg van een sterke stijging in het hartminuutvolume na deze hydralazine dosis, ondanks een effectieve blokkade van β-adrenerge receptoren. Bovendien verminderde atenolol het vasodilatoire effect van hydralazine in het hart, de skeletspieren en op de arterioveneuze anastomoses. Atenolol veroorzaakte een toename in de ratio van de bloeddoorstroming van endocard en epicard in de linkerventrikelwand, waarschijnlijk als gevolg van een verlenging van de diastolische perfusietijd en een verminderde druk op de ventrikelwand in het hart tijdens behandeling met de β-adrenerge blokker. Hierdoor werd het negatieve effect van hydralazine op deze variabele teniet gedaan. Deze waarneming houdt mogelijk verband met de bescherming, die wordt geboden door β-adrenerge antagonisten, tegen de door vaatverwijders geinduceerde aanvallen van angina pectoris in patienten met een beperkte coronaire reserve.

Een karakterisering van het acute hemodynamische profiel van de calcium antagonist felodipine (3, 10 en 30μg/kg, *i.v.*) wordt gegeven in hoofdstuk 6.

Felodipine veroorzaakte een sterke daling in bloeddruk, welke gepaard ging met een stijging in hartslagfrequentie en hartminuutvolume. Met uitzondering van de huid, werd een nagenoeg algemene vasodilatatie waargenomen in de verschillende organen en weefsels, hoewel kwantitatief duidelijke verschillen werden gemeten. Dit resulteerde in een toename in bloeddoorstroming in het hart, de hersenen, het maagdarmkanaal en, bij de hogere doseringen, in de skeletspieren, terwijl een verminderde doorbloeding van de huid werd geconstateerd na de hoogste felodipine dosis. Net als hydralazine, verlaagde felodipine de ratio van de doorbloeding van endocard en epicard in de linkerventrikelwand. Deze laatste waarneming illustreert het negatieve aspect van behandeling met deze arteriële vaatverwijder.

De acute effecten van de selectieve α_1-adrenoceptor antagonist prazosine (0.01, 0.03 and 0.10 mg/kg, *i.v.*) in niet-genarcotiseerde hypertensieve konijnen staan beschreven in hoofdstuk 7. Naast een verlaging in de totale perifere weerstand, droeg een daling in het hartminuutvolume bij tot het hypotensieve effect van prazosine, 0.10 mg/kg. Slechts een geringe stijging in de hartslagfrequentie werd waargenomen 15 minuten na de opeenvolgende toedieningen van het farmacon. Prazosine veroorzaakte een tamelijk selektieve vasodilatatie in de nieren, de darmen en het botweefsel, hetgeen resulteerde in een onveranderde bloeddoorstroming in deze vaatgebieden, ondanks de daling in het hartminuutvolume na de hoogste prazosine dosis. Echter, dit ging ten koste van de bloedtoevoer naar ondermeer hart, hersenen, skeletspieren en huid. De opmerkelijke daling in de cerebrale bloeddoorstroming verklaart het zogenaamde "first dose phenomenon" van prazosine in mensen, dat wordt gekenmerkt door veschijnselen zoals duizeligheid en bewustzijnsverlies.

Hoofdstuk 8 geeft een karakterisering van het cardiovasculaire profiel en het hypotensieve mechanisme van ketanserine, een selectieve 5-HT_2-receptor antagonist met α_1-adrenoceptor blokkerende eigenschappen. Een initiële daling in bloeddruk van korte duur, mogelijk veroorzaakt door een direkte vasodilatoire werking, welke gepaard ging met tachycardie, werd waargenomen na iedere ketanserine toediening. Dit effect werd gevolgd door een geringer hypotensief effect van langere duur. Met name na toediening van de lagere doseringen werd een toename in het hartminuutvolume geconstateerd. Op grond van i) de α_1-adrenolytische eigenschappen van ketanserine in de hypotensieve doseringen, aangetoond door een remming van de door fenylefrine geinduceerde stijging in bloeddruk, ii) de daling in bloeddruk veroorzaakt door prazosine in doseringen met een vergelijkbare α_1-adrenolytische activiteit, en iii) de afwezigheid van een langdurig hypotensief effect van ketanserine in konijnen na ganglion blokkade met hexamethonium, is de conclusie getrokken, dat de hypotensieve

werking van ketanserine in de hypertensieve konijnen met name berust op blokkade van vasculaire α_1-adrenerge receptoren. Daarnaast veroorzaakte ketanserine, net als prazosine, vasodilatatie in de nieren, het maagdarmkanaal en het botweefsel. Echter in tegenstelling tot prazosine, werd na ketanserine toediening tevens een geringe vasodilatatie waargenomen in het hart en de hersenen. Hoewel het aantrekkelijk is om de verschillen in het regionale hemodynamische profiel van prazosine en ketanserine toe te schrijven aan de 5-HT_2-receptor blokkerende eigenschappen van ketanserine, zijn andere verklaringen mogelijk zoals een direkte vasodilatoire werking van ketanserine, verschillen in farmacokinetische eigenschappen tussen prazosine en ketanserine en de bijdrage van autoregulatie van de bloedtoevoer naar hart en hersenen na prazosine- en ketanserine toediening.

In hoofdstuk 9 zijn de hemodynamische effecten van hydralazine, felodipine, captopril, prazosine en ketanserine samengevat en met elkaar vergeleken. De veranderingen in de systemische hemodynamische variabelen, die gepaard gaan met de daling in bloeddruk na toediening van de diverse "direkte" en "indirekte" vaatverwijders in niet-genarcotiseerde hypertensieve konijnen lopen sterk uiteen afhankelijk van het aangrijpingspunt van het betreffende farmacon (arterieel en/of veneus) en de door het farmacon geinduceerde activatie van cardiovasculaire reflex mechanismen. Tevens produceert elk farmacon een karakteristiek patroon van vasodilatoire effecten, hetgeen resulteert in een herverdeling van het hartminuutvolume over de verschillende weefsels en organen. Het regionale hemodynamische profiel wordt bepaald door de vasodilatoire eigenschappen van het betreffende farmacon in de diverse vaatgebieden. Bovendien spelen de reflectoire verhoging van de sympatische vaattonus, de activatie van het renine-angiotensine systeem en autoregulatie van de bloeddoorstroming in diverse weefsels in verschillende mate een rol bij de acute veranderingen in vasculaire weerstand en bloeddoorstroming van de perifere vaatgebieden. Het onderzoek, omschreven in dit proefschrift, zou clinici attent moeten maken op de belangrijke verschillen in het hemodynamische profiel van verschillende "directe" en "indirecte" vaatverwijders, hetgeen zou kunnen bijdragen tot een meer rationale benadering van de behandeling van hypertensie.

List of publications

G.R. Bolt, P.R. Saxena (1983) Systemic and regional hemodynamic characteristics of bilateral cellophane perinephritis hypertension in conscious rabbits. Clin. Exp. Hypertension A5: 885-901.

G.R. Bolt, P.R. Saxena (1983) Cardiovascular profile of felodipine, a new Ca-antagonist, in conscious renal hypertensive rabbits; a comparison with hydralazine (abstract). Brit. J. Pharmacol. 80: 612p.

G.R. Bolt, P.R. Saxena (1984) Acute systemic and regional hemodynamic effects of felodipine, a new calcium antagonist, in conscious renal hypertensive rabbits. J. Cardiovasc. Pharmacol. 6: 707-712.

G.R. Bolt, P.R. Saxena (1984) Interaction of atenolol with the systemic and regional hemodynamic effects of hydralazine in conscious renal hypertensive rabbits. J. Pharmacol. Exp. Ther. 230: 205-213.

G.R. Bolt, P.R. Saxena (1984) Acute systemic and regional haemodynamic profile of prazosin in conscious hypertensive rabbits. Eur. J. Pharmacol. 104: 151-158.

G.R. Bolt, P.R. Saxena (1984) Characterization of the hypotensive mechanism of ketanserin in conscious renal hypertensive rabbits (abstract). Brit. J. Pharmacol. 82: 120p.

G.R. Bolt, P.R. Saxena (1985) Cardiovascular profile and hypotensive mechanism of ketanserin in rabbits. Accepted for publication in Hypertension.

G.R. Bolt, P.R. Saxena (1985) Acute systemic and regional haemodynamic profile of captopril in conscious rabbits with bilateral cellophane perinephritis hypertension. Accepted for publication in Clin. Exp. Pharmacol. Physiol.

G.R. Bolt, P.R. Saxena (1985) Vasodilators and regional blood flow. Accepted for publication in Ind. J. Pharmacol.

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Curriculum Vitae

De promovendus werd op 30 november 1952 geboren te Opperdoes, een landelijk dorpje gelegen in West-Friesland. Het HBS-b diploma werd in mei 1970 behaald aan de toenmalige RHBS te Enkhuizen. Een Biologie studie aan de Vrije Universiteit in Amsterdam werd in maart 1981 afgerond met het doctoraalexamen, waarna een aanvang werd gemaakt met het in dit proefschrift beschreven promotie onderzoek op het Farmacologisch Instituut van de Erasmus Universiteit in Rotterdam. Sedert januari 1985 is de promovendus als toegevoegd docent verbonden aan de subfaculteit Farmacie van de Rijksuniversiteit in Utrecht.