CARDIOVASCULAR PHARMACOLOGY OF VASODILATING DRUGS IN THE PIG

A STUDY ON DIHYDROPYRIDINE CALCIUM-CHANNEL BLOCKERS, PYRIDAZINONE-DERIVATIVES AND NICORANDIL

CARDIOVASCULAIRE PHARMACOLOGIE VAN VAATVERWIJDERS IN HET VARKEN

EEN STUDIE BETREFFENDE DIHYDROPYRIDINE CALCIUM-KANAAL BLOKKEERDERS, PYRIDAZINONE-DERIVATEN EN NICORANDIL

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Chapter 1.
General introduction and aim of the thesis

1.1 Classification of vasodilating drugs

Vasodilating drugs can be classified on basis of the mechanism of action but also on the ultimate vasodilator profile, i.e. venodilation, arteriodilation or a combination of both. In the following sections a brief overview has been presented of the classification of vasodilating drugs according to these two criteria.

1.1.1 Mechanism of action

Vasodilation can be induced basically by three different mechanisms of action: Interference with the neural or humoral (the renin-angiotensin-aldosterone system) control of vasomotor tone and an action on the vascular wall.

Neural mechanism

The cardiovascular control centers of the medulla oblongata receive their input from higher brain centers and from the afferent nerve endings of mechanoreceptors (Fig. 1). From these medullary centers signals are mediated to the heart via both sympathetic and parasympathetic nerve fibers and to the vasculature via predominantly sympathetic nerve fibers. Drugs may interact with the neural regulation of vasomotor tone at different levels. For example, veratrum alkaloids decrease the sympathetic activity by an increase in sensitivity of the baroreceptors. Examples of drugs affecting the higher brain centers are the sedatives, hypnotics, tranquilizers, rauwolfia alkaloids and α-methyldopa. The latter, as well as the α_2-adrenoceptor agonist clonidine, also directly affects the medullary centers. On the level of the ganglia, ganglion-blockers like hexamethonium impair neural transmission. At the adrenergic nerve endings interference with noradrenaline synthesis (α-methyldopa), storage (rauwolfia alkaloids), or release into the synaptic cleft (guanethidine, bretylium) can reduce the noradrenaline-mediated vasomotor tone. Finally, interference with neural control can be accomplished by blockade of post-junctional α_1-(present on veins and arterioles) and

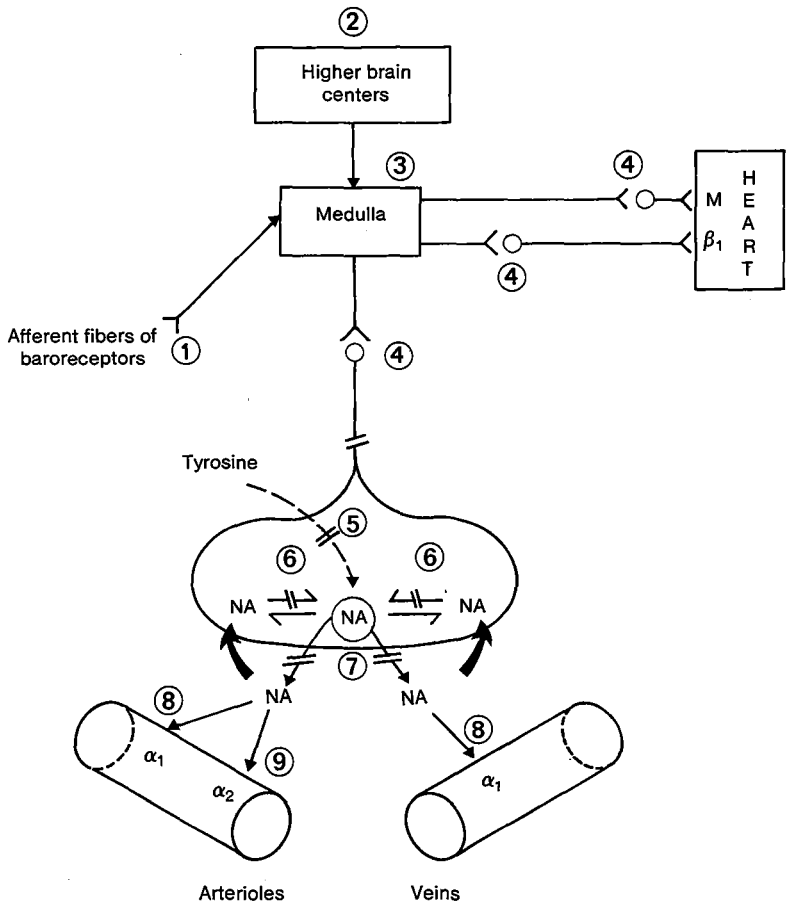


Fig. 1
Interference with the neural regulation of vasomotor tone by drugs at different levels of the nervous system. 1 = Veratrum alkaloids; 2 = sedatives, hypnotics, tranquillizers and α-methyldopa; 3 = α-methyldopa and clonidine; 4 = ganglion-blocking drugs; 5 = α-methyldopa; 6 = rauwolfia alkaloids; 7 = adrenergic blocking drugs; 8 = α_1-adrenoceptor blockers; 9 = α_2-adrenoceptor blocker. Presynaptic receptors have been omitted from this figure. For a description see text.

α_2-(present principally on arterioles) adrenoceptors with prazosin (α_1), yohimbine (α_2) and phentolamine (α_1 and α_2). The role of pre-junctional receptors will not be discussed here; for a review on this subject see Langer and Armstrong (1986).

Renin-angiotensin-aldosterone system

In addition to the neural control, vasomotor tone can be influenced humorally via the renin-angiotensin-aldosterone system (for an extensive review see Dzau and Pratt, 1986). Several factors including an increase in sympathetic nerve activity, a reduction in renal perfusion pressure and the anti-diuretic hormone concentration, a decreased sodium and/or chloride load and other changes in plasma electrolyte concentrations (K^+ , Mg^{2+}) enhance the release of the proteolytic enzyme renin (for a review see Derkx, 1987) from the juxtaglomerular cells in the afferent arterioles of the kidneys (Fig. 2). By cleaving the leucyl-leucine bond, renin converts angiotensinogen into angiotensin-I which in turn is converted into angiotensin-II via the angiotensin converting enzyme. Angiotensin-II exerts a number of pharmacological actions, such as an aldosterone-mediated sodium retention, arterio- and venoconstriction and cardiostimulation. Drugs may interfere with the actions of the renin-angiotensin-aldosterone system by β-adrenoceptor blockade at the level of the juxtaglomerular cells or by inhibition of renin or the angiotensin converting enzyme. Furthermore, the action of angiotensin-II can be blocked by the angiotensin-II antagonists and the angiotensin-II-induced increase in the aldosterone activity can be offset by aldosterone inhibitors like spironolactone (Fig. 2).

Vascular cellular mechanism

Vasodilating drugs which induce vasodilation at the level of the vascular wall are often considered to be "true vasodilators". Some of the events involved in the vascular contraction and possible sites of interference with contractile processes by vasodilating drugs have been schematically depicted in Fig. 3; for details, one may refer to review reports (Bolton, 1979; Cauvin et al., 1983; Van Zwieten, 1984). The free calcium concentration in the cytosol can be increased by a calcium release from intracellular stores and transmembrane influx through receptor- and potential operated channels. The increase in the free calcium concentration in the cytosol leads via

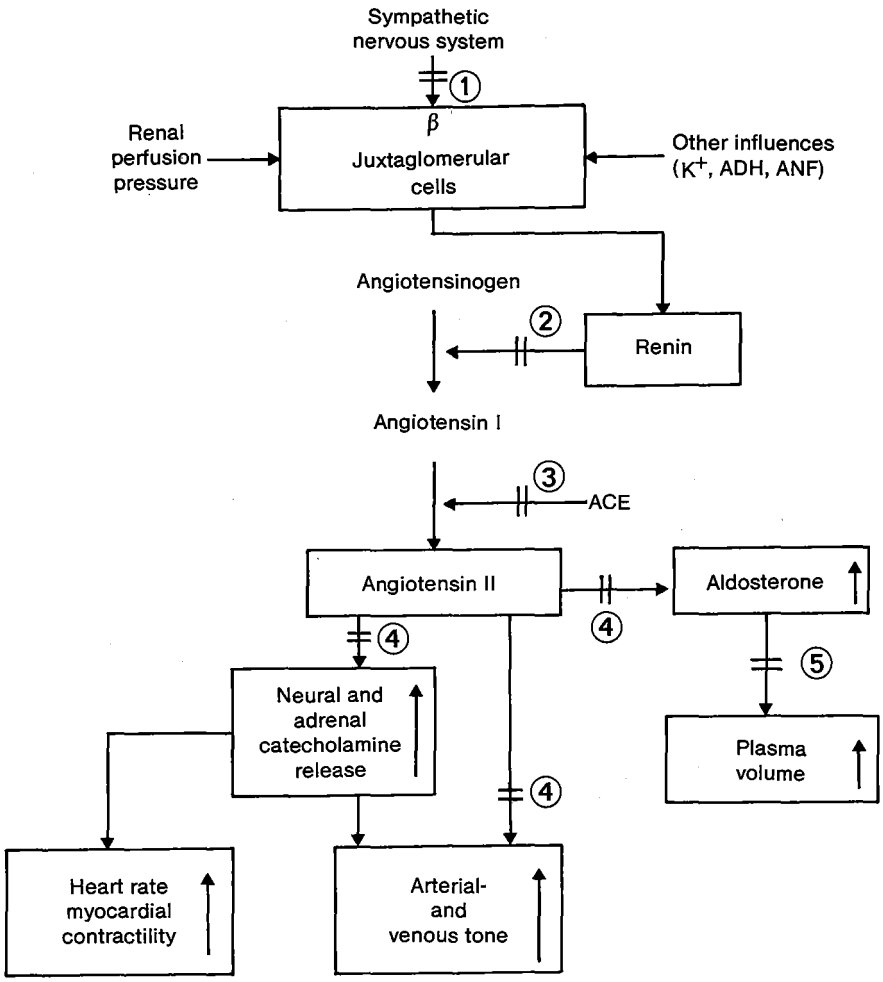


Fig. 2
Interference with the humoral regulation of vasomotor tone by drugs at different levels of the renin-angiotension-aldosterone system. 1 = β-adrenoceptor blockers; 2 = renin inhibitors; 3 = angiotensin converting enzyme inhibitors; 4 = angiotensin-II antagonists; 5 = aldosterone antagonists. For a description see text.

calcium-calmodulin binding to kinase activation and, finally, to actin-myosin activation resulting in smooth muscle contraction. Interference with the influx of extracellular calcium can be induced by blockade of the receptors regulating the receptor operated channels (α_2-adrenoceptor and probably the angiotensin-II receptor and 5-hydroxytryptamine$_2$ receptor), by hyperpolarization which inhibits influx through the potential operated channels or by blocking the potential- and probably also receptor operated channels with calcium-channel blockers. Calcium-channel blockers do not seem to antagonize the α_1-adrenoceptor-mediated vasoconstriction since these receptors induce a release of calcium from intracellular stores rather than mediate an influx of calcium through calcium-channels (Van Zwieten and Timmermans, 1983). Drugs that increase the intracellular cAMP- or cGMP content cause relaxation probably due to inhibition of kinase activity (cAMP and cGMP), a reduction in the free calcium concentration (cAMP) or inhibition of calcium influx (cGMP). An increase in these two second messengers can be accomplished by β_2-adrenoceptor stimulation (cAMP), an inhibition of cAMP breakdown by phosphodiesterase-inhibitors, an enhancement of the cyclase-activity either "directly", for example by nitrates, or via the release of endothelium derived compounds such as the endothelium derived relaxing factor(s) or prostacyclin. Vasodilation can thus also be induced via the endothelium by the "endothelium-dependent" vasodilators of which acetylcholine, ATP, ADP, substance P, bradykinin, arachidonic acid and the phospholipase A_2 inhibitor quinacrine are representatives. These substances need, in contrast to the "endothelium-independent" vasodilators, like the calcium-channel blockers and nitrates, an intact endothelium for their vasodilator actions and a lack thereof may lead to vasoconstriction rather than vasodilation (see Furchgott, 1983). The (or one of the) endothelium derived relaxing factor(s) has recently been claimed to be nitric oxide (Moncada et al, 1987).

1.1.2 Vasodilator profile: arterial, venous and mixed vasodilation.

Vasodilator drugs often act preferentially on the arterial or venous vasculature (Table 1), but the mechanism underlying this preference is not always fully understood. Phentolamine, which blocks both the α_1-and α_2-adrenoceptors, dilates the arterioles more so than the veins (Miller et al., 1976), whereas the α_1-blocker prazosin dilates the venous and arterial

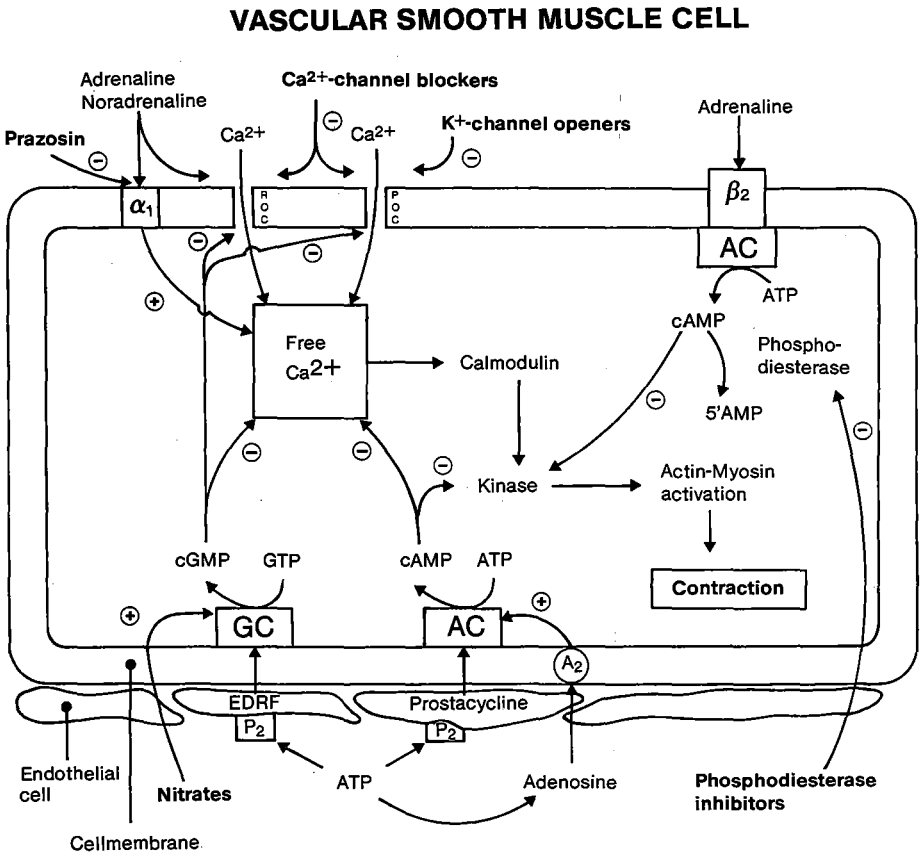


Fig. 3
Schematic representation of some of the pathways involved in the contraction of a vascular smooth muscle cell and possible sites of interefence with vasoconstriction by vasodilating substances. ROC = receptor operated channels; POC = potential operated channel; AC = adenylate cyclase; GC = guanylate cyclase; EDRF = endothelium derived relaxing factor; α_1 = α_1-adrenoceptor; β_2 = β_2-adrenoceptor; P_2 = purinergic$_2$-receptor. A_2 = adenosine$_2$-receptor. For a description see text.

vasculature to a similar extent (see Scriabine and Taylor, 1986). It can be speculated that these differences are due to the distribution of α-adrenergic receptors. While both post-synaptic α_1 and α_2-adrenoceptors have been clearly shown in 'in-vivo' studies to be present in arterioles, the α_1-adrenoceptor seems to be the dominant receptor in the venous capacitance vessels in several species (see Langer and Hicks, 1984). The calcium-channel blockers act predominantly on the arterial side which might be due to the distribution of α_2-adrenoceptors as suggested above. The α_2-adrenoceptor-mediated vasoconstriction has namely been associated with an influx of extracellular calcium and been demonstrated to be susceptible to calcium-channel blockade (Van Zwieten and Timmermans, 1983). However, a great variety exists between different tissues and species with respect to α-adrenoceptor distribution and the susceptibility of the α-adrenoceptors to calcium-channel blockers (Timmermans and van Meel, 1983; Cauvin et al., 1983; Vanhoutte, 1985). This indicates that no firm conclusions can be drawn when trying to explain the profile of the above mentioned drugs. Hydralazine, minoxidil and to a lesser extent, angiotensin converting enzyme inhibitors also have a preference for arterial vessels. Nitrates, like nitroglycerine and isosorbide-dinitrate, act predominantly on the venous vasculature whereas nitroprusside and, as mentioned above, prazosin act equally on both the arterial and venous vessels.

Table 1. Classification of vasodilating drugs based on their preference for arterial, venous or both types of blood vessels.

Arterial	**Venous**	**Arterial + Venous**
Hydralazine	Nitroglycerin	Prazosin
Minoxidil	Isosorbide-dinitrate	Nitroprusside
Calcium-channel blockers		
Angiotensin converting enzyme inhibitors		
Phentolamine		

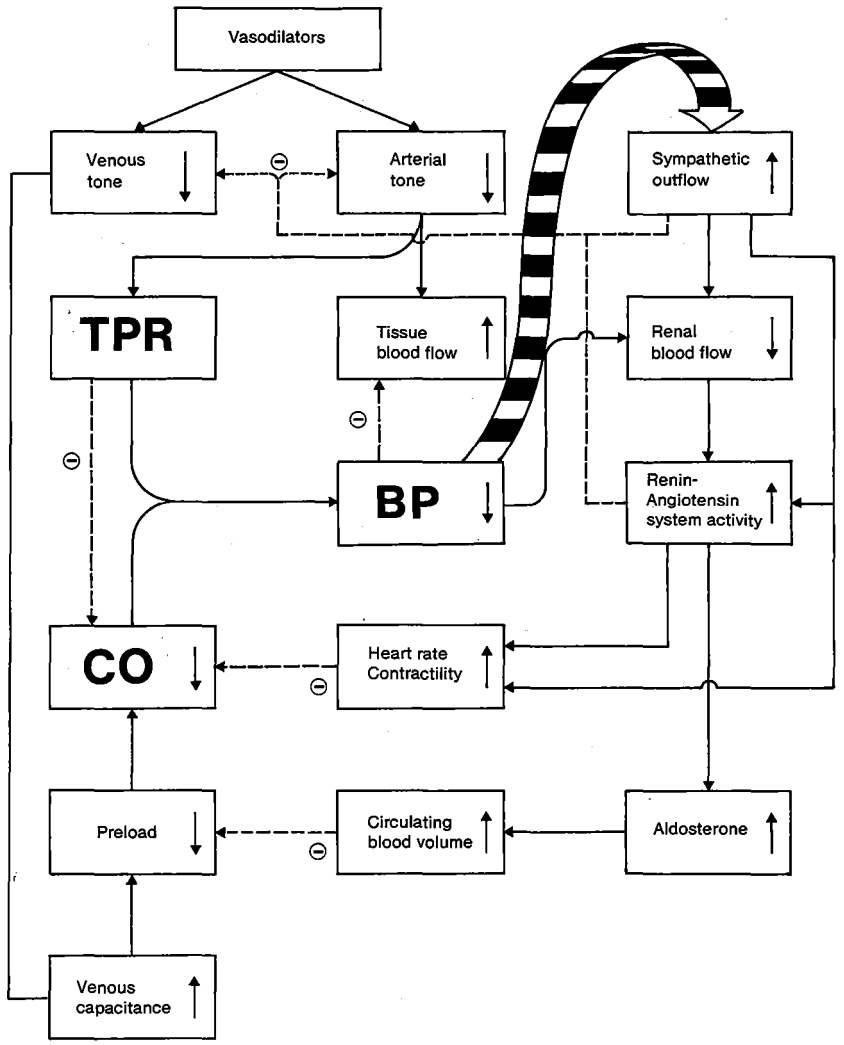


Fig. 4
Schematic representation of the changes in systemic hemodynamic variables and in tissue blood flow and some of the consequences of counterregulatory mechanisms invoked during treatment with vasodilating drugs. TPR = total peripheral resistance; CO = cardiac output; BP = blood pressure. The interrupted (with a minus sign) and uninterrupted lines indicate, respectively, the reduction and facilitation of the variable involved.

1.2 Hemodynamic consequences of arterial and venous vasodilation

The systemic and regional hemodynamic profiles of vasodilators have been recently reviewed (Saxena and Bolt, 1986). Briefly, arterial vasodilation results in a decrease in total peripheral vascular resistance and consequently in a decrease in arterial blood pressure, whereas venous vasodilation leads to an increase in venous capacitance and, therefore, a reduction in ventricular filling pressure, stroke volume and cardiac output (Fig. 4). Thus vasodilation on either side results in a drop in arterial blood pressure. In an attempt to maintain arterial blood pressure, several counterregulatory mechanisms (neural as well as humoral) become operative; these counterregulatory responses may modify the direct vasodilator actions of vasodilating drugs on organs and tissues (Fig. 4).

The effects of counterregulation are unique for the left ventricle since this part of the heart compromises its own blood supply during part of the cardiac cycle. Only 15% of the coronary blood flow, nourishing almost exclusively the subepicardium, occurs during systole because of the extravascular compression and wall tension developed during this part of the cardiac cycle (see Berne and Rubio, 1979). The remaining 85% of coronary flow occurs during diastole and supplies the subendocardial and, to a lesser extent, subepicardial layers. In the subendocardial layers, which depend thus entirely on diastole for their perfusion, vasodilator reserve is exhausted at a higher perfusion pressure (70 mmHg in dogs) than in the subepicardial layers (40 mmHg; Winbury and Howe, 1979). In the physiological state, like during exercise, vasodilator reserve in the subendocardial layers is sufficient to meet the increased oxygen-demand of the myocardium. The increase in heart rate, which causes a reduction in duration of diastole, does not exhaust vasodilator reserve because even during heavy exercise arterial blood pressure is maintained or elevated. On the other hand, the reduction in blood pressure (by peripheral vasodilation) and the baroreceptor reflex-mediated tachycardia (especially during acute administration of vasodilators) decrease both the perfusion pressure of the subendocardial layers and the perfusion time (Fig. 5). As a result the increase in subendocardial blood flow is less than the increase in subepicardial flow. This holds true especially for arterial vasodilation since with venodilation preload, and thus intramyocardial pressure in the subendocardial layers, is reduced which favorably influences the perfusion pressure of the subendocardial layers. For myocardium supplied by

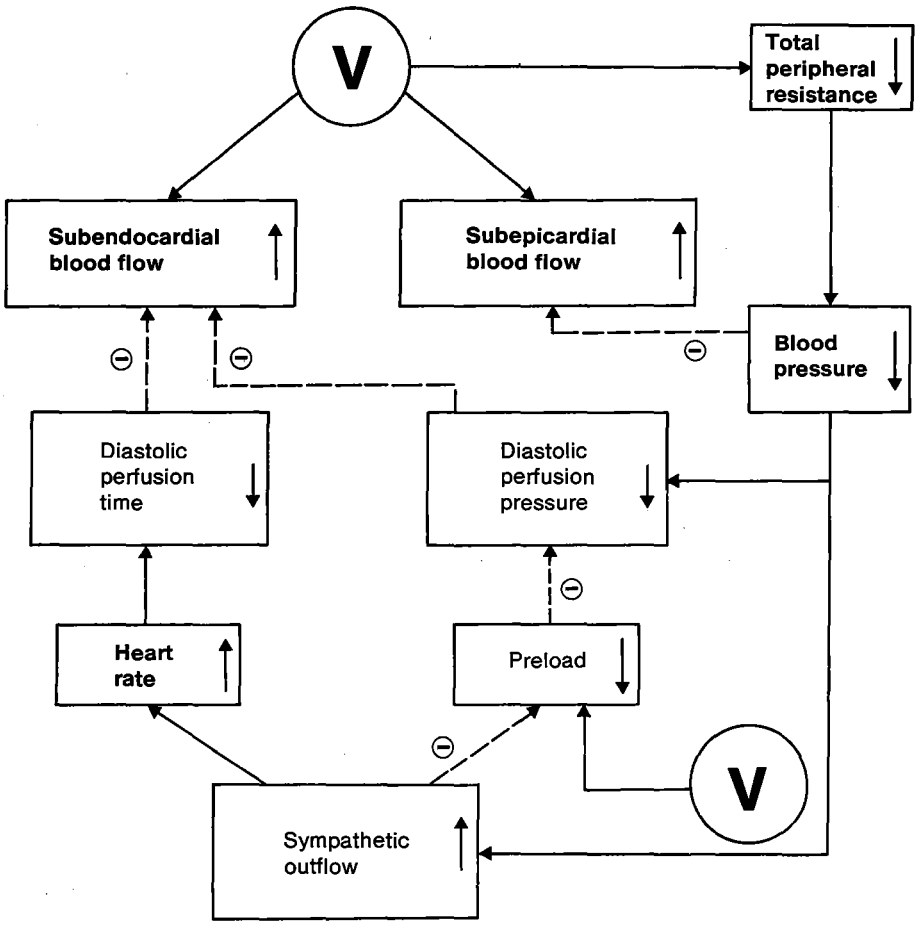


Fig. 5
Vasodilation and its consequences for the perfusion of the different myocardial layers. V = vasodilator; The interrupted lines (with a minus sign) and interrupted lines indicate, respectively, the reduction and facilitation of the variable involved. For a description see text.

non-diseased coronary arteries the reduction in the subendocardial-subepicardial blood flow ratio induced by the vasodilating drugs has no clinical implications as subendocardial blood flow is still commensurate with the needs of the subendocardial layers. However, when a coronary artery is obstructed and vasodilator reserve is already reduced, the combination of a decrease in arterial blood pressure and a reflex-tachycardia may have deleterious effects on the perfusion of especially the subendocardial layers (see section 1.3 coronary artery disease).

1.3 Therapeutic use of vasodilating drugs

Hypertension

The use of vasodilators in hypertension is a rational approach since these drugs antagonize the major hemodynamic disturbance in most forms of hypertension namely the increase in total peripheral resistance. To prevent counterregulatory mechanisms from abolishing the beneficial actions of vasodilators, a combination therapy with β-adrenoceptor antagonists and/or diuretic agents is often needed in the clinical situation. Furthermore, during chronic treatment β-adrenoceptor antagonists have an additive hypotensive action which may allow reduction of the doses of the drugs used.

Coronary artery disease

Nitrates, which have a more marked action on the venous side, have been used for decades in the treatment of angina pectoris due to different causes. The beneficial actions of nitrates is primarily ascribed to the reduction of cardiac preload and, to some extent, afterload (Williams et al., 1965; Burggraff and Parker, 1974) which decrease ventricular dimension and wall tension and therefore lower myocardial oxygen demand. The decrease in wall tension also increases the effective diastolic perfusion pressure of the subendocardial layers. Another more recently appreciated factor contributing to the anti-ischemic actions of nitrates is that these drugs dilate the large epicardial vessels and thereby reduce the severity of the coronary artery stenosis (Brown et al., 1981).

In the last decade calcium-channel blockers have been under investigation in the treatment of coronary artery disease. Although their efficacy has been proven in coronary artery spasm, the usefulness of calcium-channel blockers in stable angina pectoris remains a matter of debate as in the clinical setting

arterial vasodilators not always favorably influence the myocardial oxygen balance. By reducing the afterload the arterial vasodilators often cause an increase in heart rate, a major determinant of myocardial oxygen-demand. The increase in heart rate also reduces the duration of the diastolic perfusion time. This, together with the reduction in perfusion pressure by the hypotensive action of these drugs may be harmful for, in particular, flow to the subendocardial layers. An increase in blood flow can still occur via dilation at the site of the coronary artery stenosis (which is not always possible), via dilation of coronary collaterals (which may not always be present) or via dilation in the terminal arteriolar bed (which is generally believed to be maximal during myocardial ischemia). But vasodilators have also been reported to induce "coronary steal" either from subendocardium to subepicardium distal to a fixed coronary artery stenosis (Gross and Warltier, 1981; Weintraub et al., 1981; Gewirtz et al., 1984) or from ischemic myocardium distal to a completely occluded coronary artery to normally perfused myocardium (Gross and Warltier, 1981). These observations have led to the concept that in ischemic myocardium arterial vasodilators reduce the distal coronary perfusion pressure by vasodilation in non-ischemic myocardium, thereby "stealing" blood from perfusion pressure dependent myocardium. Recently, however, several studies have suggested that vasodilation is not maximal in ischemic myocardium in which case vasodilator therapy may still be useful (Gorman et al., 1984; Heusch and Deussen, 1984; Aversano and Becker, 1985; Canty and Klocke, 1985; Pantely et al., 1985). Furthermore, combination therapy with β-adrenoceptor antagonists has proven superior to monotherapy with either class of drugs. This has revived interest in vasodilators as a therapeutic in (stable) angina pectoris.

Congestive Heart Failure

A role for vasodilators in heart failure (a situation in which the heart is unable to pump blood at a rate commensurate with the metabolic needs of body tissues), seems at first less obvious than in the case of hypertension since the primary cause for heart failure is often the myocardium. However, the peripheral vasculature responds to cardiac pump failure with constriction of the arterioles and veins causing a further deteriorating of myocardial performance. The aim of vasodilator therapy is therefore to reduce pre- and afterload of the heart, thereby normalizing the ventricular dimension and

pump function (Cohn and Franciosa, 1977; Chatterjee and Parmley, 1977). Furthermore, in the failing circulation the arteriolar constriction is particularly pronounced in the renal, splanchnic, dermal and muscular regions (see Drexler et al., 1985, 1986a) and vasodilators should therefore preferably dilate these vascular beds in order to normalize perfusion thereof.

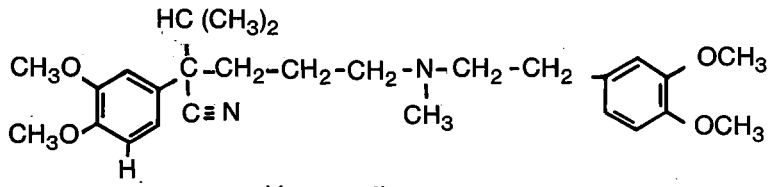
Migraine

The pathogenesis of migraine and related headaches remains controversial. Several hypotheses, like the neuronal, vascular, or ischemia hypothesis (Blau, 1987), have not been able to completely explain the clinical signs of migrainous headaches. The reason for these different theories regarding the pathophysiology of migraine may be that migraine, like hypertension, is a syndrome where the underlying causative factors may vary in different patients.

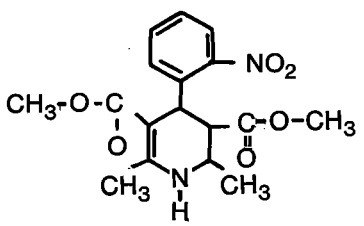
The introduction of vasodilators (calcium-channel blockers) in the treatment of migraine is based on two separate reasonings. The first involves the hypothesis by which advocates initial, mainly intracranial, vasoconstriction during the prodromal phase, followed by extracranial vasodilation causing the head pain (Wolff, 1963). Although this concept of a pure vascular basis of migraine headache may be an oversimplification (Olesen et al., 1981; Bruyn, 1984), a number of studies have found a reduced cerebral blood flow during the initial phase of migraine (O'Brien, 1971; Skinhoj, 1973; Simard and Paulson, 1973; Norris et al., 1975; Henry et al., 1978; Sakai and Meyer, 1978). Yamamoto and Meyer (1980) considered that calcium-channel blockers would prevent the initial cerebral vasospasm and, therefore, also mitigate the subsequent painful vasodilation during the headache phase. Indeed, some studies indicate that nimodipine (Gelmers, 1983; Meyer and Hardenberg, 1983) and nifedipine (Kahan et al., 1983), may be of value in migraine prophylaxis. The second reasoning, based on which another calcium-channel blocker flunarizine was introduced assumes that migraine is due to a focal cerebral ischaemia (Amery, 1982). The resulting accumulation of calcium and the consequent cell damage and migraine headache would then be suppressed by calcium-channel blockers.

Another observation which appears to be of interest is the high potency of nimodipine in antagonizing rabbit basilar artery contractions induced by 5-hydroxytryptamine (Towart, 1981); the latter is released (and then depleted)

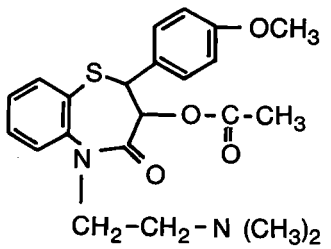
from blood platelets during migraine headaches (Lance, 1982). 5-Hydroxytryptamine and several antimigraine drugs (Johnson and Saxena, 1978; Saxena and Verdouw, 1982; Saxena et al.,1983; Saxena, 1987) are extremely effective in constricting cranial extracerebral arteriovenous anastomoses, which may open up during the headache phase of migraine (Heyck, 1969; Saxena, 1978). The relationship between 5-hydroxytryptamine, arteriovenous shunting and calcium-channel blockers is, however, not known.



Verapamil



Nifedipine



Diltiazem

Fig. 6
Chemical structure of the three 'first generation' calcium-channel blockers verapamil, nifedipine and diltiazem. Note the great differences in structure.

1.4 Drugs used in this thesis

Calcium-channel blockers

In the early sixties German scientists (Lindner, 1960; Haas and Hartfelder, 1962) observed that prenylamine and verapamil exerted, besides coronary vasodilator actions, negative inotropic effects on isolated cat and rabbit

myocardium. At first these drugs were believed to be β-adrenoceptor blocking agents as they opposed the catecholamine-induced effects on the heart (Melville and Benfey, 1965; Haas and Busch, 1967). However, Fleckenstein et al. (1967) reported that the effects of prenylamine and

TABLE 2 RELATIVE EFFECTS OF CALCIUM ANTAGONISTS IN EXPERIMENTAL PREPARATIONS COMPARED WITH THERAPEUTIC LEVELS IN MAN*

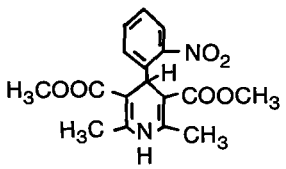
	Verapamil	Nifedipine	Diltiazem
Therapeutic level in man			
ng/ml	80-400	25-100	50-300
molecular weight	455	346	415
molar weight	$2\text{-}8 \times 10^{-7}$ M	$0.5\text{-}2 \times 10^{-7}$ M	$1\text{-}7 \times 10^{-7}$ M
protein binding	about 90 %	about 95 %	about 85 %
molar value, corrected for protein binding	$2\text{-}8 \times 10^{-8}$ M	$0.3\text{-}1 \times 10^{-8}$ M	$1\text{-}5 \times 10^{-8}$ M
Isolated coronary artery contraction 50 % inhibition	10^{-7} M	10^{-8} M	10^{-7} M
Myocardial depression 40 % depression of contractile force	5×10^{-6} M	5×10^{-7} M	10^{-4} M
Fast sodium current depression	10^{-4} M	no effect	10^{-4} M
Slowing of heart rate by 20 %	10^{-6} M	10^{-5} M	10^{-8} M
Relative effect on AV node vs contractile force	6.5:1	1:1	20:1

* From Singh and Opie (1984)

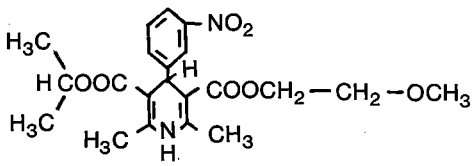
verapamil were the result of inhibition of the influx of calcium into the myocardial cells. Consequently, these agents were called calcium-antagonists and they were ascribed two main properties: 1) suppression of the slow calcium current in ventricular muscle and 2) the reversal of such suppression by an increase in external calcium concentration (Fleckenstein, 1968, 1977). Later, other properties were discovered such as the protection against isoprenaline-induced myocardial calcium overload and inhibition of

calcium-induced tension development in vascular smooth muscle. With the gradual elucidation of their main mechanism of action these agents are now more generally called calcium-entry blockers or calcium-channel blockers.

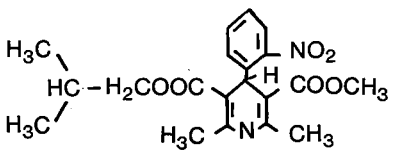
During the last decade the "first generation" calcium-channel blockers verapamil, nifedipine and diltiazem, which are structurally unrelated to each other (Fig. 6), have become widely used in cardiovascular disorders. With the differences in chemical structure also the affinity for the myocardium and the vasculature varies widely (Table 1). In recent years the so called "second generation" agents, analogues of the first generation drugs, have been developed in an attempt to obtain substances with greater specificity for either cardiac (sinus-node, AV-node, myocytes) or vascular (specific vascular bed) tissues. In this respect especially the dihydropyridine-derivatives



Nifedipine



Nimodipine



Nisoldipine

Fig. 7
Chemical structure of the dihydropyridine calcium-channel blockers nifedipine, nisoldipine and nimodipine.

(nifedipine, nisoldipine, nimodipine; Fig. 7) have grown in number. Compared to the parent compound nifedipine, some of these agents have been claimed to have a greater selectivity for vascular tissue over cardiac tissue (Kazda et al., 1980) or for cerebral or coronary arterial beds over other regional beds (Takenaka et al., 1976; Kazda et al., 1980, 1982).

Current indications for the clinical use of calcium-channel blockers vary from stable angina pectoris (Scheidt, 1982), angina at rest and coronary vasospasm (Schroeder, 1982), hypertension, hypertrophic cardiomyopathy and myocardial infarction (Toggart and Zelis, 1982) and cardiac arrhythmias (Singh et al., 1982) to prophylaxis of migraine (Gelmers, 1983) and cerebral ischemia due to post-hemorrhagic cerebral vasospasm (Allen et al., 1983).

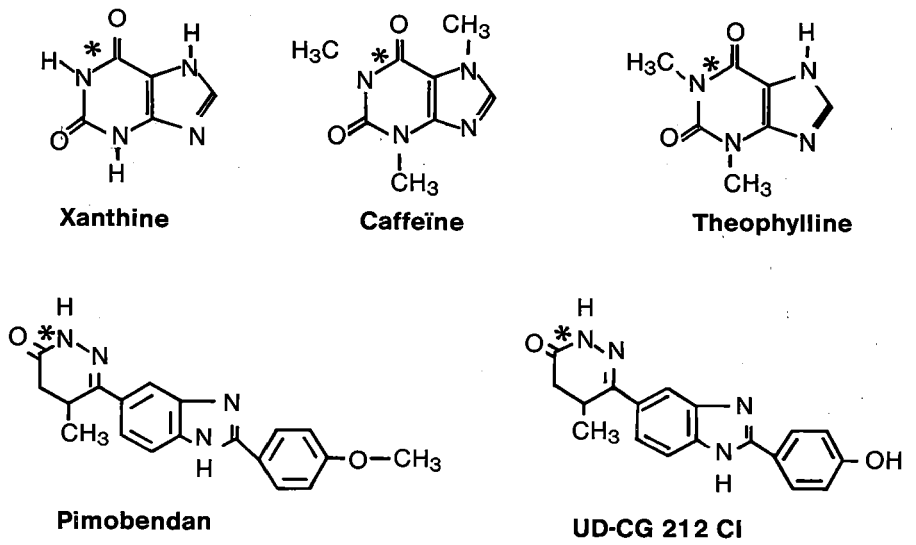


Fig. 8
Chemical structure of xanthine, the methylxanthines caffeine and theophylline and the pyridazinone-derivatives pimobendan and its O-demethylmetabolite UD-CG 212 Cl. *All substances contain the "active" part of the molecule which is thought to be responsible for the phosphodiesterase-inhibiting actions of these drugs.

Pyridazinone-derivatives

This class of drugs, to which pimobendan and its O-demethylmetabolite UD-CG 212 Cl (2-(4-hydroxy-phenyl)-5-(5-methyl-3-oxo-4,5-dihydro-2H-6-pyridazinyl)benzimidazole HCl) belong (Fig. 8), exert vasodilator actions on the arterial as well as on the venous side (Diederen et al., 1982). Besides vasodilator effects the pyridazinone-derivatives possess cardiostimulatory actions (Diederen et al., 1982; Van Meel, 1985). The mechanism through which the pyridazinone-derivatives exert their actions was at first thought to be via phosphodiesterase-inhibition (see Honerjäger et al., 1984; Berger et al., 1985); they share with the phosphodiesterase inhibiting (methyl) xanthines the so called "active site" of the molecule which is thought to be responsible for phosphodiesterase-inhibition (Fig. 8). However, in addition, the cardiotonic actions may also be due to an increased sensitivity of myocardial contractile proteins to calcium (Rüegg et al., 1984; Van Meel et al., 1986) or a prolongation of the action potential, allowing more calcium to enter the myocyte (Honerjäger et al., 1984).

The cardiovascular profile, i.e. vasodilation of venous and arterial beds and positive inotropic actions, appears to be particularly favorable for the therapy of congestive heart failure (Cohn and Franciosa, 1978). Indeed, pimobendan has recently been reported to improve cardiac pump function and normalize preload in patients with congestive heart failure at rest (Brand and Hagemeyer, 1987) and during exercise (Hagemeyer and Brand, 1987).

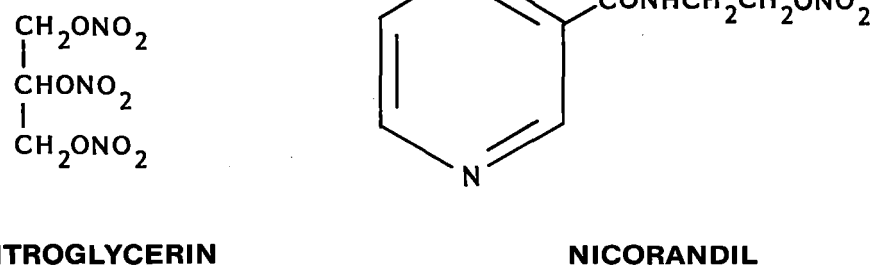


Fig. 9
Chemical structure of the classical nitrate nitroglycerin and the nitrate-like substance nicorandil.

Nicorandil

Nicorandil (SG-75, N-(2-hydroxyethyl) nicotinamide nitrate; Fig. 9) is a new anti-anginal drug with coronary vasodilator properties (Uchida et al., 1978). In vitro studies have shown that besides the nitrate-like action, an increase in intracellular cGMP (Fig. 3), nicorandil also enhances the conductance of potassium-channels thereby hyperpolarizing the cell membrane (Taira, 1987). As a result nicorandil, in addition to the classical nitrate venodilatory action, has a potent effect on the arterial smooth muscle vasculature and dilates epicardial conductance as well as intramyocardial resistance vessels (Taira, 1987; Suryapranata et al., 1988). At clinical dose-range the drug possesses negligible negative inotropic actions (Belz et al., 1984).

The profile of venous and arterial dilation, together with the reported anti-ischemic actions (Uchida, 1978; Thorman et al., 1982, 1983) suggest that nicorandil might be useful in the treatment of heart failure, coronary artery disease and hypertension.

1.5 Aim of the thesis

In this thesis the cardiovascular pharmacological actions of a number of vasodilating drugs have been studied and compared in pigs. Firstly, we compared the vasodilator profile of the dihydropyridine-derivatives nisoldipine, nimodipine and nifedipine and also studied the claimed preference of nisoldipine for the coronary circulation (Kazda et al., 1980; Serruys et al., 1985; Drexler et al., 1986b) and of nimodipine for the cerebral circulation (Kazda et al., 1982) (chapters 2-5). Secondly, since the perfusion of organs and tissues may be compromised in several pathological disorders for which vasodilating drugs might be employed (ischemic heart disease, heart failure, hypertension), we compared the vasodilator profiles of the dihydropyridine-derivatives nisoldipine and nimodipine with those of pimobendan, UD-CG 212 CL and nicorandil after intravenous administration in anesthetized pigs (chapters 6-9). Thirdly, in view of the potentially useful combination of vasodilators and β-adrenoceptor antagonists to reduce hypotension-induced baroreceptor reflex-mediated tachycardia, we compared the systemic hemodynamic actions of the different vasodilating drugs in conscious pigs in the absence or presence of β-adrenoceptor blockade (chapters 9-11). Finally, the long-held view that vasodilation in ischemic myocardium is maximal (see

Berne and Rubio, 1979) and, therefore, vasodilators may induce coronary "steal" (Weintraub et al., 1981; Gross and Warltier, 1981; Gewirtz et al., 1984), may not hold true. Recently, vasodilator therapy has been shown to induce an improvement of perfusion and function of ischemic myocardial areas by recruiting vasodilator reserve (Gorman et al., 1984; Heusch and Deussen, 1984; Aversano and Becker, 1985; Canty and Klocke, 1985; Pantely et al., 1985). In view of these new findings, lending support to the possible usefulness of vasodilator drugs in myocardial ischemia, we investigated the effects of nisoldipine on perfusion of myocardium distal to a fixed concentric coronary artery stenosis in conscious pigs (chapter 12). In addition, the possible anti-ischemic action of this drug was studied during myocardial ischemia-induced in animals subjected to treadmill-exercise (chapter 13).

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VASODILATORY PROFILE OF DIHYDROPYRIDINE-DERIVATIVES

CHAPTER 2

THE EFFECTS OF NISOLDIPINE (BAY K 5552) ON CARDIOVASCULAR PERFORMANCE AND REGIONAL BLOOD FLOW IN PENTOBARBITAL-ANAESTHETIZED PIGS WITH OR WITHOUT β-ADRENOCEPTOR BLOCKADE

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The effects of nisoldipine (Bay K 5552) on cardiovascular performance and regional blood flow in pentobarbital – anaesthetized pigs with or without β-adrenoceptor blockade

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1 The effects of the 1,4-dihydropyridine derivative nisoldipine, infused intravenously (i.v.) at 3 different rates (0.25, 0.5 and 1.0 $\mu g\,kg^{-1}\,min^{-1}$), were studied in anaesthetized pigs on cardiovascular performance with or without β-adrenoceptor blockade produced by propranolol.

2 Nisoldipine caused dose-dependent decreases in arterial blood pressure (30%), systemic vascular resistance (30%) and left ventricular filling pressure (15%), but raised heart rate (25%) and LV *dP/dt max* (20%). Cardiac output was not significantly affected.

3 Transmural myocardial blood flow and vascular conductances increased dose-dependently after nisoldipine. The elevation in blood flow to the left ventricle favoured epicardial layers. Endocardial blood flow showed small increases as the changes in conductance of the endocardial layer more than compensated for the loss in perfusion pressure. The endo-epi blood flow ratio decreased from 1.16 ± 0.05 to 0.70 ± 0.01. Myocardial O_2-consumption was unaltered as the decrease in arterial-coronary venous O_2-content difference (30%) was balanced by the increase in transmural blood flow.

4 Nisoldipine increased blood flow to skeletal muscle (500%), stomach (50%) and adrenals (25%), but decreased that to the liver (50%), spleen (25%) and kidneys (25%). No changes were noticed in the small intestine, skin and brain. In spite of differential effects on blood flow, vascular conductance in all organs and tissues, with the exception of the liver, increased.

5 After β-adrenoceptor blockade the responses of mean arterial blood pressure, cardiac output and systemic vascular resistance to nisoldipine remained virtually unchanged, but the elevations in heart rate and LV *dP/dt max* were abolished, as was the decrease in left ventricular filling pressure.

6 A higher dose of nisoldipine was required after β-adrenoceptor blockade to elicit significant vasodilatation in the epi- and endocardial layers. However, the reduction in endo-epi blood flow ratio by nisoldipine was not affected by propranolol. Myocardial O_2-consumption tended to decrease as the diminution in the arterial-coronary venous O_2-content difference (30%) slightly exceeded the increase of left ventricular blood flow (30%).

7 Except for the brain and liver, effects of nisoldipine on regional vascular conductances were attenuated after β-adrenoceptor blockade.

Introduction

β-Adrenoceptor antagonists and calcium channel blockers are widely used in the treatment of hypertension and ischaemic heart disease. Since these drugs act through different mechanisms, their combined use might be attractive. Some of the 1,4 dihydropyridines (nisoldipine, felodipine), a subgroup of the calcium channel blocking agents, exert a strong vasodilator effect at concentrations that only slightly affect myocardial contractile behaviour. β-Adrenoceptor antagonists usually lower cardiac output and thereby decrease perfusion of most organs and tissues (van Boom & Saxena, 1983). Since significant lowering of blood pressure can be expected with nisoldipine, combined use of these drugs could be detrimental for some of these organs particularly when their perfusion depends on perfusion pressure. The effects of nisoldipine on the distribution of cardiac output have been studied during rest and exercise by Drexler *et al.* (1985), but regional blood flow data on nisoldipine after β-adrenoceptor blockade have not been docu-

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mented. Furthermore, only limited experimental data are available on the effects of the combination of β-adrenoceptor antagonists and calcium channel blockers (Wolffenbuttel & Verdouw, 1983; Warltier *et al.*, 1984a). We therefore evaluated the cardiovascular effects, in particular the distribution of cardiac output, of varying doses of nisoldipine with and without β-adrenoceptor blockade in the domestic swine.

Methods

General

After an overnight fast Yorkshire pigs (20–30 kg) were anaesthetized with 120 mg azaperone i.m. and 150 mg metomidate i.v. (both compounds: Janssen Pharmaceutica, Beerse, Belgium), intubated and ventilated with a mixture of O_2 and N_2O (1:2). Respiratory rate and tidal volume were adjusted in order to keep arterial blood gases within normal limits. A double lumen 8 French (F) catheter was placed in the superior caval vein for administration of sodium pentobarbitone (20 mg kg^{-1} h^{-1}), and pancuronium bromide (4 mg), while two 7F catheters were positioned in the inferior caval vein, for infusions of Haemaccel (to replace blood loss), propranolol and nisoldipine. Left ventricular and aortic pressures were obtained with 8F Millar micro-tipped catheters. Ascending aortic blood flow was measured by placing an electromagnetic flow probe around the vessel after thoracotomy. Cardiac output was derived by adding myocardial blood flow (measured with radioactive microspheres; see below) to ascending aorta blood flow. Oxygen (O_2) saturation and haemoglobin were determined in blood samples withdrawn from the abdominal aorta and the great cardiac vein. Myocardial O_2-consumption was calculated by multiplying the difference between the aortic O_2 content and that of the great cardiac vein, by myocardial blood flow. A stabilization period of at least 30 min was allowed before baseline data were collected.

Regional blood flow

Distribution of cardiac output was determined by the radioactive microsphere method (for details, see Saxena & Verdouw, 1985). Microspheres of 15 ± 1 μm (mean ± s.d.) diameter, labelled with 5 different isotopes (^{103}Ru; ^{113}Sn; ^{46}Sc; ^{95}Nb and ^{141}Ce), were injected in random order via a cannula inserted into the left atrial appendage. To calibrate flow measurements, an arterial reference blood sample was withdrawn (10 ml min^{-1}) starting 10 s before and continuing until 1 min after completion of each microsphere injection. At the end of each experiment the animal was killed and various organs and tissues (see later) were dissected out, weighed, and placed in plastic vials for counting radioactivity. Data were processed by use of a set of computer progammes described elsewhere (Saxena *et al.*, 1980).

Experimental protocol

Fifteen animals received three continuous 10 min infusions of nisoldipine (0.25, 0.5 and 1.0 μg kg^{-1} min^{-1}), seven without and eight after β-adrenoceptor blockade with propranolol (0.5 mg kg^{-1} ± 0.5 mg kg^{-1} h^{-1}). Microspheres were injected and haemodynamic data obtained at baseline and at the end of each infusion rate. An additional batch of microspheres was injected 15 min after administration of propranolol in the animals that received the β-adrenoceptor antagonist. The adequacy of the dose of propranolol to provide β-adrenoceptor blockade and the stability of the preparation have been described in an earlier communication (Wolffenbuttel & Verdouw, 1983).

Statistical analysis

Statistical analysis was performed by use of a two-way analysis of variance followed by the Duncan new multiple range test (Steel & Torrie, 1980). *P* values less than 0.05 were considered to be statistically significant.

Drugs

Apart from the anaesthetics, the only drugs used were propranolol hydrochloride (ICI-Farma, Rotterdam, The Netherlands) and nisoldipine (Bay K 5552, Bayer AG, Wuppertal, West-Germany), dissolved in a mixture of polyethylene glycol 400, glycerol and water. The nisoldipine solution (0.1 mg ml^{-1}) was diluted with 0.9% w/v NaCl immediately before use. The effects of the solvent on haemodynamics were negligible (unpublished data from this laboratory).

Results

Baseline values of the two groups of animals and the effects of propranolol are presented in Tables 1 and 2.

Systemic haemodynamics

Nisoldipine caused dose-dependent increases in heart rate (up to 25%), while mean arterial blood pressure decreased dose-dependently up to 30% (Figure 1). The decline in blood pressure was mainly due to vasodilatation in peripheral vascular beds since cardiac output was virtually unchanged. Myocardial contractility (assessed as LV $dP/dt\ max$), was not compromised by this calcium channel blocker. Left

Table 1 Baseline values of cardiovascular parameters for the animals that received nisoldipine without (group 1, $n = 7$) and after β-adrenoceptor blockade (group 2, $n = 8$)

	Group 1	*Group 2*	
		Before propranolol	*After propranolol*
Systemic circulation			
Heart rate (beats min^{-1})	92 ± 4	96 ± 3	84 ± 3*
Mean arterial blood pressure (mm Hg)	84 ± 3	81 ± 2	77 ± 2
LV *dp/dt max* (mm Hg s^{-1})	2450 ± 250	2330 ± 270	1660 ± 170*
Cardiac output (l min^{-1})	2.8 ± 0.2	3.1 ± 0.3	2.6 ± 0.2*
LV end-diastolic pressure (mm Hg)	10.1 ± 0.9	12.1 ± 0.8	11.4 ± 1.0
Systemic vascular resistance (mm Hg l^{-1} min)	31 ± 4	27 ± 2	32 ± 3*
Coronary circulation			
LV transmural blood flow (ml min^{-1} g^{-1})	1.41 ± 0.18	1.45 ± 0.10	1.15 ± 0.12*
Endo-epi blood flow ratio	1.16 ± 0.05	1.10 ± 0.03	1.09 ± 0.04
Arterial-coronary venous oxygen content difference (mmol l^{-1})	3.4 ± 0.2	3.7 ± 0.3	3.4 ± 0.2
Myocardial O_2 consumption (μmol min^{-1} g^{-1})	4.6 ± 0.3	5.4 ± 0.5	3.8 ± 0.4*

LV *dP/dt max* = maximal rate of rise of left ventricular pressure; LV = left ventricular. Endo-epi blood flow ratio = ratio of the endocardial and epicardial blood flows. Data are presented as mean ± s.e.mean; $*P < 0.05$ vs before propranolol.

Table 2 Baseline values of organ blood flows and vascular conductances for the animals which received nisoldipine without (group 1, $n = 7$) and after β-adrenoceptor blockade (group 2, $n = 8$)

	Flow (ml min^{-1} 100 g^{-1})			*Conductance* (10^{-2} ml min^{-1} mm Hg^{-1} 100 g^{-1})		
	Group 1	*Group 2*		*Group 1*	*Group 2*	
		Before propranolol	*After propranolol*		*Before propranolol*	*After propranolol*
LA	115 ± 31	123 ± 9	108 ± 4	140 ± 42	153 ± 12	139 ± 19
LVT	141 ± 18	145 ± 10	115 ± 12*	172 ± 28	178 ± 11	147 ± 13*
LV-endo	149 ± 15	150 ± 11	117 ± 12*	180 ± 23	185 ± 11	150 ± 13*
LV-epi	132 ± 20	137 ± 10	110 ± 12*	161 ± 29	168 ± 11	141 ± 14
RA	133 ± 32	155 ± 22	146 ± 26	163 ± 44	191 ± 26	185 ± 31
RV	110 ± 21	120 ± 11	106 ± 14	134 ± 29	148 ± 13	135 ± 16
Liver	46 ± 7	54 ± 10	43 ± 10*	56 ± 9	65 ± 12	55 ± 12
Spleen	131 ± 17	96 ± 12	73 ± 9*	131 ± 17	118 ± 14	94 ± 10*
Stomach	15.2 ± 1.6	21 ± 3	17.4 ± 2.5	18 ± 2	26 ± 4	23 ± 3
Small intest.	30 ± 4	32 ± 6	27 ± 4*	36 ± 5	40 ± 8	35 ± 5
Kidneys	277 ± 47	304 ± 28	265 ± 24	339 ± 66	375 ± 34	341 ± 29
Adrenals	214 ± 39	237 ± 49	188 ± 39*	260 ± 54	293 ± 60	238 ± 47
Skel. muscle	4.6 ± 0.9	4.0 ± 0.4	3.0 ± 0.5	5.6 ± 1.1	4.9 ± 0.5	3.8 ± 0.6
Skin	0.65 ± 0.13	1.10 ± 0.39	0.68 ± 0.13	0.82 ± 0.20	1.41 ± 0.52	0.88 ± 0.18
Brain	29 ± 5	26 ± 2	24 ± 2	35 ± 7	32 ± 2	30 ± 2

LA = left atrium; LVT = left ventricular transmural; LV-endo = left ventricular endocardium; LV-epi = left ventricular epicardium; RA = right atrium; RV = right ventricle; small intest. = small intestine; Skel. muscle = skeletal muscle. Data are presented as mean ± s.e.mean; $*P < 0.05$ vs before propranolol.

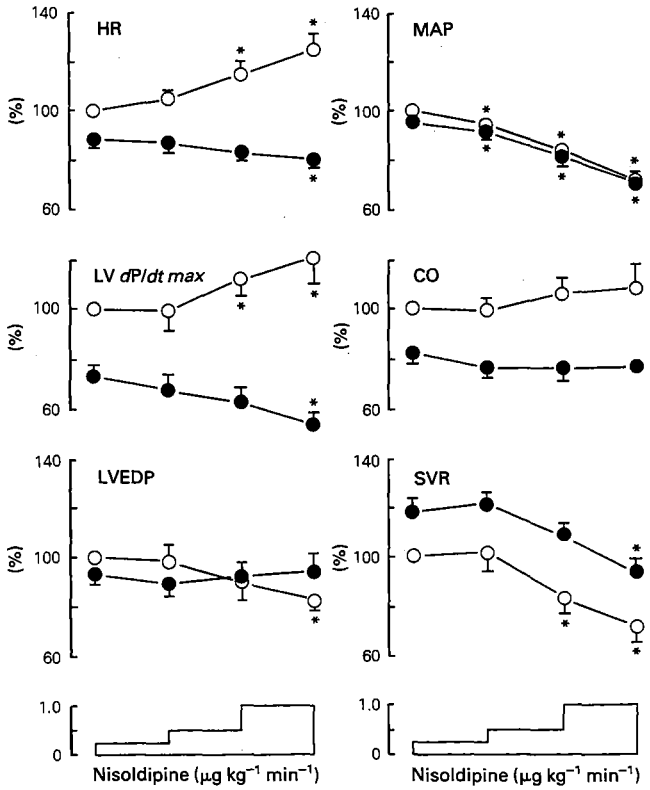

Figure 1 Effects of continuous 10 min infusions of nisoldipine without (○) or after (●) β-adrenoceptor blockade with propranolol on heart rate (HR), mean arterial blood pressure (MAP), myocardial contractility (LV *dP/dt max*), cardiac output (CO), left ventricular end-diastolic pressure (LVEDP) and systemic vascular resistance (SVR). Data are expressed as percentage of baseline values (pre-propranolol values in the β-blocked animals). $*P<0.05$ vs pre-nisoldipine values.

ventricular end-diastolic pressure (LVEDP) declined by 15% after the highest dose.

After propranolol the effects of nisoldipine on systemic haemodynamics were only slightly modified. Instead of an increase, we now observed either no changes (first 2 doses) or slight decreases (highest dose) in heart rate and LV *dP/dt max* during increasing nisoldipine infusion rates. These decreases, however, were not statistically different from those observed at the same time period in animals that received propranolol only; compare data reported earlier by Wolffenbuttel & Verdouw (1983). Mean arterial blood pressure and cardiac output responses to nisoldipine were similar to those without β-adrenoceptor blockade, while LVEDP did not change.

Coronary haemodynamics and myocardial O_2-consumption

Nisoldipine caused a considerable elevation of left ventricular blood flow (up to 55% at the end of the highest infusion rate, Figure 2). The microsphere data revealed that the epicardial layers especially benefited from the increase in flow and, as a result, the endo-epi blood flow ratio decreased dose-dependently by up to 40%. The combined effects of the changes in the determinants of myocardial O_2-demand resulted in unaltered O_2-consumption as the decrease in arterial-coronary venous O_2-content difference was balanced by the increase in blood flow.

The nisoldipine-induced increases in blood flow

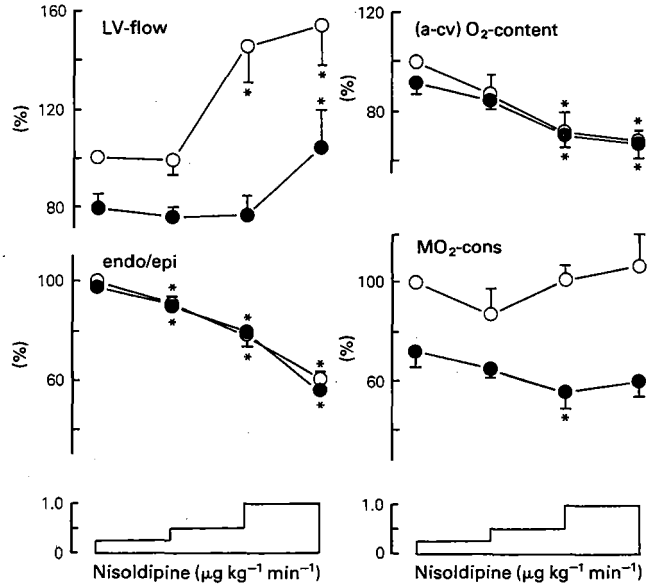

Figure 2 Effects of continuous 10 min infusions of nisoldipine without (○) or after (●) β-adrenoceptor on left ventricular (LV) blood flow, myocardial O_2-consumption (MO_2-cons), the ratio of blood flows in the endo- and epicardium (endo/epi) and the arterial-coronary venous O_2-content difference ((a-cv) O_2-content). Data are expressed as percentage of baseline values (pre-propranolol values in the β-blocked animals). *$P < 0.05$ vs pre-nisoldipine values.

were considerably less after β-adrenoceptor blockade (up to 30% after the highest dose). Inspection of Figure 2 reveals that, after propranolol, a higher infusion rate of nisoldipine was required to enhance transmural myocardial blood flow. Although transmural flow was reduced by propranolol, the latter had no effect on the nisoldipine-induced decrements in endo-epi blood flow ratio. The arterial-coronary venous O_2-difference again decreased, causing slight accentuation on nisoldipine-induced decreases in myocardial O_2-consumption. After β-adrenoceptor blockade, nisoldipine caused lesser increments in transmural conductance (flow/pressure), more so in the right ventricle than in the left ventricle (Figure 3). Right and left atria showed responses similar to those of the ventricles. Although the endo-epi blood flow ratio decreased, endocardial blood flow was maintained under both experimental conditions and was even augmented after the second dose of nisoldipine in untreated animals (Figure 4). Vascular conductances in the endo- and epicardial layers of the left ventricle increased during nisoldipine infusions, but the responses weakened in the β-blocked animals.

Cardiac output distribution

Nisoldipine infusions did not exert a uniform effect on the various regional vascular beds (Figure 5). Perfusion of some organs and tissues increased (skeletal muscles, stomach and adrenals), decreased (liver, spleen and kidneys), or was maintained (small intestine, brain and skin). Decreases in flow were, with the exception to the liver, always less than the drop in mean arterial blood pressure. Therefore, vascular conductance in all organs and tissues, except the liver, increased (Figure 6). The greatest vasodilator response was elicited in the skeletal muscles (up to 700% increase), followed by the skin (140% with the highest dose), stomach (120%), adrenals (60%) and brain (50%). The increases in vascular conductance in the spleen and kidneys were significant only at the second dose.

After β-adrenoceptor blockade the changes in conductances were less pronounced at the higher doses of nisoldipine, except for the brain and liver. The vasodilator response remained most marked in the skeletal muscle as conductance still increased by

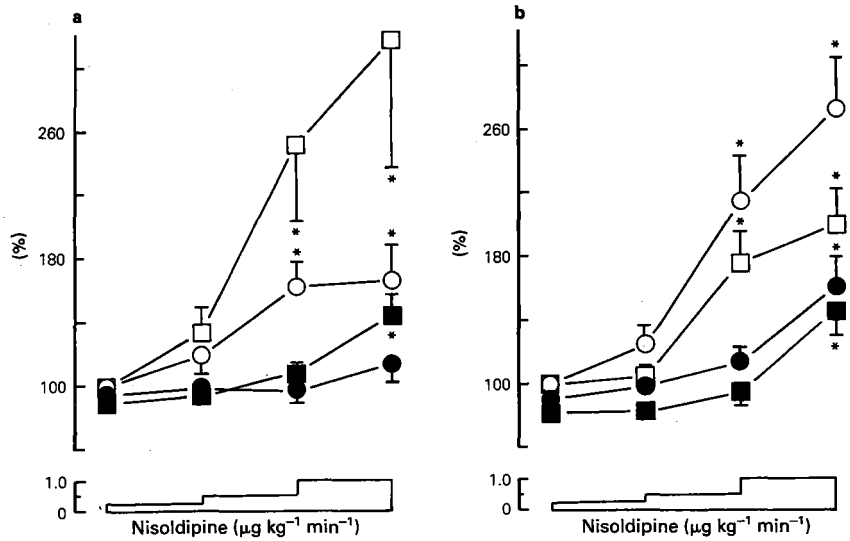

Figure 3 Effects of continuous 10 min infusions of nisoldipine without (open symbols) or after (closed symbols) β-adrenoceptor blockade on vascular conductance in the myocardium. In (a) are shown the conductances in the left (□,■) and right (○,●) atrium. In (b) are shown the conductances in the left (□,■) and right (○,●) ventricle. Data are expressed as percentage of baseline values (pre-propranolol values in the β-blocked animals). $^*P < 0.05$ vs pre-nisoldipine values.

600%, followed by the skin (70%), brain (60%) and stomach, small intestine and adrenals (40%). Conductance in the liver again decreased.

Discussion

Effects of nisoldipine without β-adrenoceptor blockade

As reported by many investigators (Kazda *et al.*, 1980; Vogt *et al.*, 1980; Warltier *et al.*, 1981; Vogt & Kreuzer, 1983; Verdouw *et al.*, 1984; Warltier *et al.*, 1984a,b; Drexler *et al.*, 1985) the major haemodynamic effect of nisoldipine was a reduction of the systemic vascular resistance leading to a decline in mean arterial blood pressure. Presumably due to the baroceptor reflex, heart rate increased, which is consistent with the findings of some investigators (Kazda *et al.*, 1980; Vogt *et al.*, 1980; Warltier *et al.*, 1984a), but at variance with those of others (Vogt & Kreuzer, 1983; Verdouw *et al.*, 1984). The absence of an increase in heart rate after oral administration of nisoldipine reported by Vogt & Kreuzer (1983) might be the result of the moderate decrease in mean arterial blood pressure reported in that study. Also the already enhanced sympathetic drive might have played a role, as the patients in their study suffered from chronic congestive heart failure. An explanation for the discrepancy in heart rate responses with an earlier study performed in our laboratory (Verdouw *et al.*, 1984), might be the higher infusion regimen (2 and 4 $\mu g\,kg^{-1}\,min^{-1}$) used in those experiments. Warltier *et al.* (1981) also reported in anaesthetized dogs dissimilar effects on heart rate as a 15% increase was observed after 1 $\mu g\,kg^{-1}\,min^{-1}$ whereas there was virtually no change after 3 $\mu g\,kg^{-1}\,min^{-1}$. Higher doses might lead to a greater direct negative chronotropic effect (Kazda *et al.*, 1980, Hof & Scholtysik, 1983) and a stronger suppression of the baroceptor reflex (Warltier *et al.*, 1984b). That the experimental conditions are important is illustrated by Warltier *et al.* (1984a) who found an increase in heart rate in conscious dogs after intravenous nisoldipine in doses up to 25 $\mu g\,kg^{-1}\,min^{-1}$, while we observed similar changes after oral administration up to 500 $\mu g\,kg^{-1}$ in the conscious pig (unpublished data).

Nisoldipine did not affect myocardial O_2-consumption, as the elevation of heart rate was balanced by decreases in arterial blood pressure and preload. Rousseau *et al.* (1984) also described no effect on myocardial O_2-consumption in angina pectoris patients in spite of a decline in pressure-rate product.

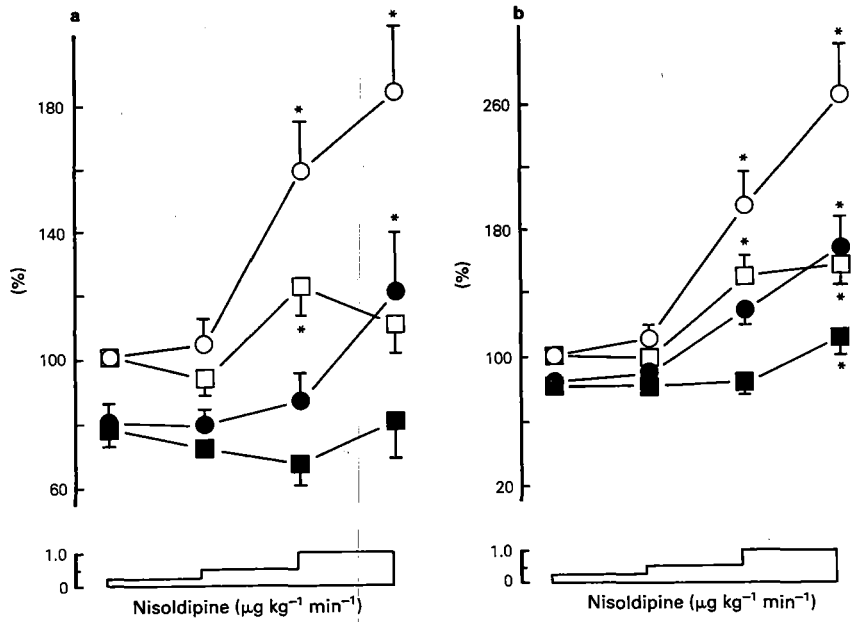

Figure 4 Effects of continuous 10 min infusions of nisoldipine without (open symbols) or after (closed symbols) β-adrenoceptor blockade on blood flows to (a) and conductances in (b) the left ventricular epicardial (○,●) and endocardial (□,■) layers. Data are expressed as percentage of baseline values (pre-propranolol values in the β-blocked animals. $*P < 0.05$ vs pre-nisoldipine values.

Kazda *et al.* (1980), however, found that nisoldipine lowered myocardial O_2-consumption in the anaesthetized dog, which might have been due to the decrease in heart rate in their experiments.

Augmented transmural myocardial blood flow, also demonstrated by other investigators (Kazda *et al.*, 1980; Warltier *et al.*, 1981; 1984a; Rousseau *et al.*, 1984) was completely accounted for by the increase in epicardial blood flow. Although the endo-epi blood flow ratio declined during infusions of higher concentrations of nisoldipine, no deleterious effect was exerted on the endocardium as endocardial blood flow was maintained or even enhanced. Warltier *et al.* (1981) also documented similar changes in endo-epi blood flow ratio, after 1 $\mu g\,kg^{-1}\,min^{-1}$, while subendocardial perfusion was augmented in spite of tachycardia. Serruys *et al.* (1985) found in man that a 30% decrease in total systemic vascular resistance was accompanied by a 50% decrease in coronary vascular resistance. From these observations they prematurely concluded that nisoldipine is primarily a coronary vasodilator. In our study a dose of 1 $\mu g\,kg^{-1}\,min^{-1}$ produced a 30% and 50% decrease in systemic and coronary vascular resistances, respectively. However, the various regions contributed very differently, as vascular conductance in skeletal muscle increased 7 fold, while that in the kidneys, spleen and liver was hardly affected, or even diminished.

The data on organ and tissue perfusion demonstrate that the vasodilator action of nisoldipine is most marked in skeletal muscle, as reported with other dihydropyridines (Hof, 1983; Bolt & Saxena, 1984a). Only in the liver was a vasoconstrictor response observed. Drexler *et al.* (1985) reported in rats a general vasodilatation, although at the dose used (1.6 $\mu g\,kg^{-1}\,min^{-1}$) this was not always statistically significant. Higher organ and tissue conductances were in most cases sufficient to compensate for the loss of perfusion pressure. Blood flow was therefore maintained in most regions. The kidneys are known to possess an autoregulatory mechanism for maintaining stable blood flow, which is primarily myogenic in nature (Thurau & Kramer, 1959; Hashimoto *et al.*, 1980). Hashimoto *et al.* (1980) also reported the capacity of calcium channel blockers to interfere with this autoregulation. Our data show that only with the

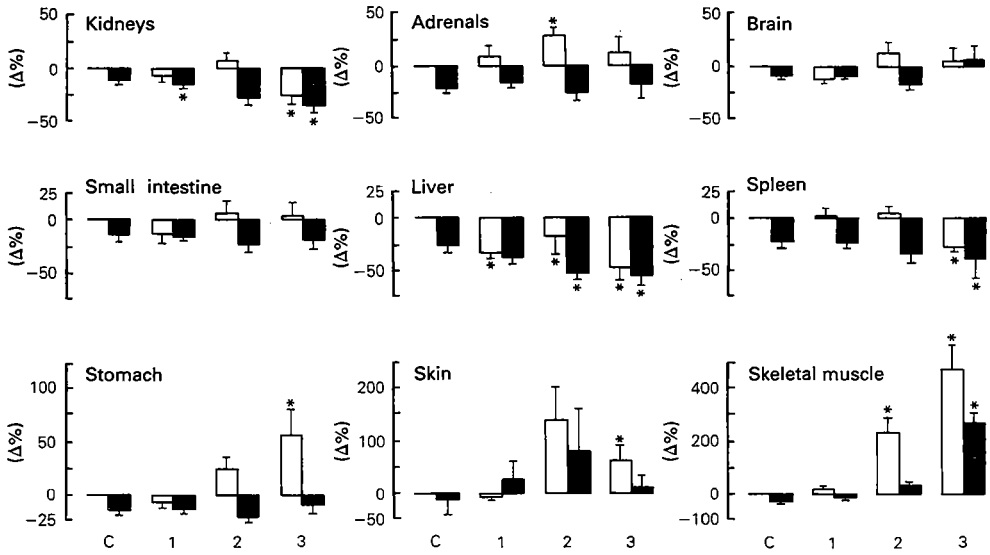

Figure 5 Effects of continuous 10 min infusions of nisoldipine (0.25; 0.5 and 1.0 $\mu g\,kg^{-1}\,min^{-1}$, depicted as 1, 2 and 3 respectively) without (□) or after (■) β-adrenoceptor blockade on tissue blood flows. C denotes control state or, in the β-blocked group, the effect of propranolol. Data are expressed as percentage of baseline values (pre-propranolol values in the β-blocked animals). $^*P < 0.05$ vs pre-nisoldipine values.

second dose was there an increase in conductance, while after the highest dose renal blood flow decreased significantly as conductance remained constant.

Effects of nisoldipine after β-adrenoceptor blockade

Consistent with the findings of Kazda *et al.* (1980), we observed that pretreatment of the animals with the β-adrenoceptor antagonist propranolol did not attenuate nisoldipine-induced decreases in arterial blood pressure and systemic vascular resistance, but abolished the increments in heart rate and LV *dP/dt max*. It therefore appears that the latter effects are mediated primarily via a reflex augmentation of the sympathetic nervous system (Gross *et al.*, 1979; Spedding, 1982; Bolt & Saxena, 1984a). However, in certain circumstances, an additional mechanism resistant to β-adrenoceptor blockade, namely a withdrawal of parasympathetic tone, may also be involved (Nakaya *et al.*, 1983; Warltier *et al.*, 1984a).

After propranolol, nisoldipine caused a slight decrease in myocardial O_2-consumption and a less marked increase in coronary blood flow. This can partly be explained by a decreased metabolic demand, as shown by Vatner & Hintze (1982). In contrast, Warltier *et al.* (1984a) found no difference in coronary blood flow responses to nisoldipine under the two experimental conditions but, in their experiments, the tachycardia following nisoldipine was not completely eliminated by β-adrenoceptor blockade. The endo-epi blood flow ratio showed similar responses to nisoldipine irrespective of the presence or absence of propranolol. However, individual vasodilator responses of epi- and endocardium were attenuated by propranolol. Except for the liver and brain, the same was the case in other organs and tissues. It is interesting to recall that β-adrenoceptor blockade interferes with vasodilator responses to another arteriolar vasodilator, hydralazine (Bolt & Saxena, 1984b).

Finally, we would like to compare the effects of nisoldipine in the present series of experiments with those of another dihydropyridine analogue, nifedipine, which was infused directly into the left anterior descending coronary artery (Wolffenbuttel & Verdouw, 1983). As can be expected, intracoronary infusions of nifedipine (up to 0.5 $\mu g\,kg^{-1}\,min^{-1}$) seem to produce a greater cardiodepressant effect (arterial pressure, cardiac output and LV *dP/dt max* decreased up to 23%, 18% and 35%, respectively), which was either unchanged or attenuated in propranolol-treated animals. In spite of a slight additional cardiodepres-

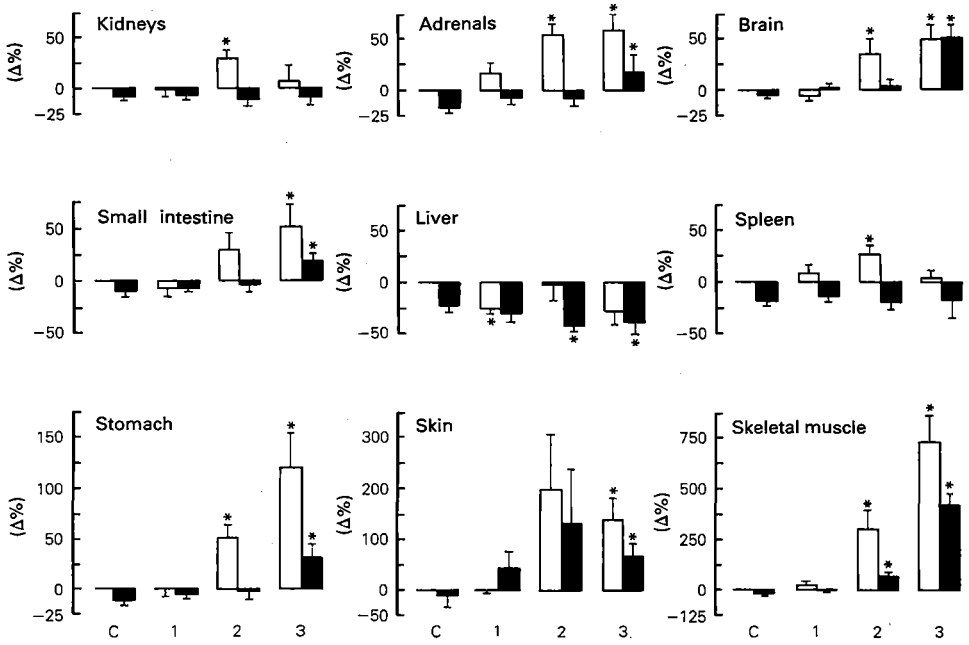

Figure 6 Effects of continuous 10 min infusions of nisoldipine (0.25; 0.5 and 1.0 $\mu g\,kg^{-1}\,min^{-1}$, depicted as 1, 2 and 3 respectively) without (□) or after (■) β-adrenoceptor blockade on regional vascular conductances. C denotes control state or, in the β-blocked group, the effect of propranolol. Data are expressed as percentage of baseline values (pre-propranolol values in the β-blocked animals). $*P < 0.05$ vs pre-nisoldipine values.

sant effect of nifedipine in the presence of propranolol, coronary blood flow increases were similar to those when nifedipine was given alone.

Conclusions

Nisoldipine has been shown to be a potent vasodilator which lowers blood pressure but increases heart rate and coronary blood flow without changing myocardial O_2 consumption. After propranolol, the reflex mediated cardiostimulatory responses to nisoldipine are eliminated but the cardiac function is not compromised. Therefore, a combination of nisoldipine and β-adrenoceptor antagonists is an attractive possibility when the therapeutic aim is to reduce the work of the heart and maintain cardiac perfusion. However, complications have been reported when calcium channel blockers and β-adrenoceptor antagonists are administered concurrently to patients with impaired cardiovascular performance (Opie & White, 1980; Robson & Vishwanath, 1982; Packer *et al.*, 1982; Oesterle & Schroeder, 1982). Indeed, our data, although obtained in acute experiments in anaesthetized animals with normal cardiovascular performance, also show that perfusion of some organs (in particular adrenals, kidneys, liver, spleen and stomach) decrease after combined use of the two drugs. Therefore, when cardiovascular performance is already impaired, nisoldipine might better be employed without β-blockade as it improves myocardial O_2-balance, while myocardial function is maintained. Also, the reduction of afterload facilitates left ventricular emptying, while organ and tissue perfusion is better preserved than when β-blockade is present.

The supply of nisoldipine by Bayer AG, Wuppertal, West Germany and of propranolol by ICI-Farma, Rotterdam, The Netherlands, is gratefully acknowledged. Mr R.J. Rensen and Mr R.H. van Bremen are thanked for their technical assistance and Miss P.H. Vegter for preparing the manuscript.

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CHAPTER 3

THE EFFECTS OF NISOLDIPINE ALONE AND IN COMBINATION WITH BETA-ADRENOCEPTOR BLOCKADE ON SYSTEMIC HAEMODYNAMICS AND MYOCARDIAL PERFORMANCE IN CONSCIOUS PIGS.

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The effects of nisoldipine alone and in combination with beta-adrenoceptor blockade on systemic haemodynamics and myocardial performance in conscious pigs

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KEY WORDS: nisoldipine, propranolol, regional blood flow, beta-adrenoceptor blockade, calcium-antagonist, vasodilatation.

The peak effects of 10 mg nisoldipine p.o. with or without 80 mg propranolol p.o. on systemic and regional haemodynamics in conscious pigs were investigated. Nisoldipine increased heart rate (70%), cardiac output (67%) and maxLVdP/dt (75%), but decreased mean arterial pressure (21%) as systemic vascular conductance increased by 120%. Left ventricular systolic and end-diastolic pressures were not affected. Vasodilatation occurred in most organs. The increase in left ventricular blood flow (150%) favoured the epicardial (195%) over the endocardial (110%) layers. As a result the endo–epi blood flow ratio decreased by 30%.

When nisoldipine was administered simultaneously with propranolol, heart rate (29%), cardiac output (35%) and systemic vascular conductance (65%) increased, but maxLVdP/dt did not change. Mean arterial (18%) and left ventricular systolic (10%) pressure decreased; left ventricular end-diastolic pressure was again unaffected. In most organs vasodilatation was attenuated, but still present, compared to the changes after nisoldipine alone. The increase in epicardial blood flow (70%) again exceeded that in endocardial blood flow (35%), however, the endo–epi ratio decreased by only 15%. In the presence of propranolol, nisoldipine did not exert a negative inotropic action while the reflex-tachycardia was attenuated. In addition, no detrimental effects on perfusion of regional vascular beds were observed.

Introduction

Calcium-channel blockers and beta-adrenoceptor antagonists are widely used in the treatment of coronary artery disease and hypertension. As they act through different mechanisms, the combined use of these drugs might be a useful therapeutic tool[1–4]. In this respect a combination of beta-adrenoceptor antagonists and the 1,4-dihydropyridine derivatives seems especially attractive. The potent vasodilatory effect of this subgroup of calcium-channel blockers often induces a reflex-mediated cardio-stimulatory response that may completely override their direct cardioinhibitory action, which is less marked than that of other calcium-channel blockers such as verapamil and diltiazem. Although beneficial effects of a combination of nifedipine and beta-adrenoceptor antagonists have been reported[1,2,5], adverse interactions like severe cardiodepression, which are more frequently observed with verapamil[4,5] or diltiazem[6], have also been documented[4–8].

Recently nisoldipine, another dihydropyridine derivative, has been shown *in vitro* to exert a 4–10 times stronger vasodilatory effect and an equal or weaker cardiodepressant action compared to nifedipine at equimolar doses[9]. *In vivo*, nisoldipine causes pronounced vasodilatation of the systemic and coronary bed and elicits marked reflex-mediated increases in heart rate and myocardial contractility parameters[9–11]. Although some reports on systemic haemodynamic effects following a combination of nisoldipine with beta-blockers are available[11–13], regional haemodynamic investigation is restricted to one report in anaesthetized pigs The following study was performed to define whether oral administration of nisoldipine,

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when combined with that of propranolol, might have detrimental effects on myocardial performance and on perfusion of regional vascular beds in conscious pigs.

Materials and methods

GENERAL

After an overnight fast, Yorkshire pigs (18–20 kg, $N=7$) pretreated with a mixture of procaine penicillin-G and benzathine-penicillin-G (Duplocillin, Gist-Brocades N.V., Delft, The Netherlands), both 300 000 units i.m., were sedated with 30 mg kg^{-1} ketamine HCl i.m. (Aescoket, Aesculaap B.V., Boxtel, The Netherlands). The animals were intubated and connected to a respirator for artificial ventilation with a mixture of O_2 and N_2O (1 : 2) to which 1% halothane was added. A jugular vein and common carotid artery were cannulated for administration of drugs and measurement of mean arterial blood pressure, respectively. The chest was opened via the left fifth intercostal space to expose the heart. A transducer ($P_{4\cdot5}$, Konigsberg Instruments Inc. Pasedena, California, U.S.A.) was implanted into the apex of the left ventricle of the heart for recording left ventricular pressure. The left atrium was cannulated for recording left atrial pressure which, together with the aortic blood pressure, was used for calibration of the Konigsberg transducer signals. The aorta was approached through the third intercostal space and an electromagnetic flowprobe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta. Cardiac output was calculated by adding myocardial blood flow (see later) to ascending aorta blood flow. Catheters and wires were tunnelled subcutaneously to the back, the chest was closed and the animals allowed to recover. During the next 14 days the animals received daily intravenous bolus injections of 500 mg amoxicilline (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and in addition, during the first week, 500 mg kanamycin (Kamynex, Gist-Brocades N.V., Delft, The Netherlands) to prevent infection. The catheters were flushed daily with an isotonic saline solution containing heparin (500 I.U. ml^{-1}) to avoid clotting of blood in the lumen. After a 1 week postoperative recovery period, at least four sessions were held to adapt the animals to the experimental and laboratory facilities. The experimental protocol was executed 2–3 weeks after the operation. All tracings were written on a Graphtec Linearcorder (F WR 3701, Ankersmit, Breda, The Netherlands). Arterial acid–base balance and oxygenation during the experiments were similar to those reported for young Yorkshire pigs by Lagerwey[14]: $pH=7{\cdot}45\pm0{\cdot}02$, $PCO_2=37\pm3$ mmHg, $PO_2=81\pm5$ mmHg, HbO_2-saturation $=92\pm3\%$.

MEASUREMENT OF REGIONAL BLOOD FLOWS

Regional blood flows were measured by use of the radioactive microsphere technique. Although the microsphere technique offers the advantage of simultaneously measuring blood flow to a large number of tissues in conscious animals with relatively little surgical trauma, an inherent drawback of this method is that only a limited number of isotopes can be employed. Therefore only one dose of nisoldipine with and without propranolol could be used in each individual animal; the dose selected was one that elicited a reasonably marked pharmacological response (see later).

Microspheres (15 ± 1 (SD) μm diameter), labelled with either ^{141}Ce, ^{113}Sn, ^{103}Ru or ^{95}Nb (NEN Chemicals GmbH, Dreieich, West Germany) and suspended in saline containing a drop of Tween 80, were injected in random order into the left atrium over a period of 30–45 s[15].

At the end of each experiment the animal was killed with an overdose of pentobarbitone sodium. The various tissues, as specified later, were dissected out, weighed and placed in plastic vials. The radioactivity in the vials containing the tissues was counted for 5–10 min in a γ-scintillation counter (Packard, model 5986) equipped with a multichannel pulse height analyser (Conrac) using suitable windows for discriminating the different isotopes used[16]. The microsphere and other data were processed by a PDP-11/70 computer using a set of programmes especially developed for the microsphere technique[16]. The amount of blood flow distributed to the various tissues (Q_{tis}) was calculated as follows:

$$\dot{Q}_{tis}(ml\ min^{-1}) = (I_{tis}/I_{tot}) \times CO$$

where I_{tis}, I_{tot} and CO are, respectively, radioactivities (cpm) in the individual tissues and the total number of microspheres injected (calculated as the difference in the radioactivity present in the glass vial before and after injection), and the cardiac output (in ml min^{-1}). Tissue vascular conductance (flow/pressure) was calculated by dividing respective tissue blood flow values by mean arterial blood pressure.

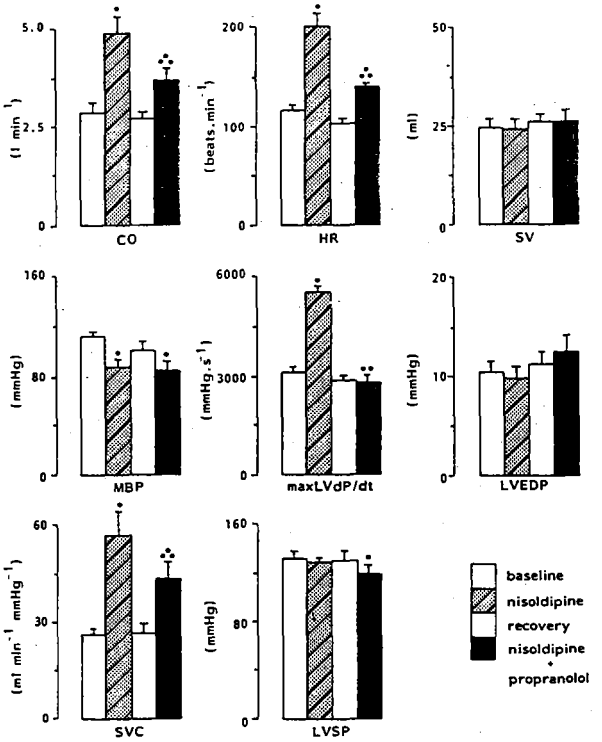

Figure 1 Effects of nisoldipine (10 mg p.o.) without and with propranolol (80 mg p.o.) on systemic haemodynamics in seven conscious pigs. CO, cardiac output; HR, heart rate; SV, stroke volume; MBP, mean arterial blood pressure; maxLVdP/dt, maximal rate of rise of left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; SVC, systemic vascular conductance; LVSP, left ventricular systolic blood pressure. Data are expressed as mean ± SEM; $*P \leq 0{\cdot}05$ *vs.* baseline or recovery. **Change induced by nisoldipine with propranolol significantly different ($P \leq 0{\cdot}05$) from that by nisoldipine without propranolol.

EXPERIMENTAL PROTOCOLS

In pigs fasted for 24 h, 10 mg nisoldipine (equivalent to $0{\cdot}40 \pm 0{\cdot}02$ mg kg^{-1}) was administered orally (p.o.) after baseline data had been recorded and the first batch of radioactive microspheres had been injected for the determination of regional blood flows. All measurements were repeated when nisoldipine-induced changes in heart rate had reached a stable peak level (50 ± 6 min after administration). As a pilot-study in eight pigs had revealed that, 24 h after ingestion of 10 mg of the drug, plasma-levels of nisoldipine could no longer be detected and that haemodynamic parameters had returned to baseline, the next day the same protocol was repeated, but now 80 mg p.o. propranolol (equivalent to $2{\cdot}88 \pm 0{\cdot}44$ mg kg^{-1}) was given along with the 10 mg nisoldipine tablet. This second part of the protocol was performed in the same animals to avoid inter-animal differences. Data were again obtained during baseline and at the peak heart rate effect (48 ± 4 min after administration). In the pilot study ($N = 8$) we had observed that peak changes in heart rate (from 108 ± 5 to 160 ± 12 beats min^{-1}) corresponded with peak plasma concentrations

($3{\cdot}14 \pm 0{\cdot}64$ ng mg^{-1}) of nisoldipine reached about 1 h after the drug administration. When in three of these animals nisoldipine and propranolol were administered simultaneously, the peak concentration of nisoldipine was $2{\cdot}81 \pm 0{\cdot}70$ ng ml^{-1} which was not statistically significantly different from that obtained after nisoldipine alone.

STATISTICAL ANALYSIS

Unless otherwise stated, all data are presented as mean ± standard error of mean. Statistical analysis was performed by use of Duncan's new multiple-range test once a parametric two-way analysis of variance (randomized block design) had revealed that the samples represented different populations[17]. A P value of 0·05 or less (two-tailed) was considered to be statistically significant.

DRUGS

The only drugs used in this study were tablets of nisoldipine (Bay k 5552, Bayer AG, Wuppertal, F.R.G.) and propranolol hydrochloride (ICI-Farma, Rotterdam, The Netherlands).

Results

SYSTEMIC HAEMODYNAMICS

Peak effects of nisoldipine (10 mg p.o.) on systemic haemodynamics are presented in Fig. 1. Nisoldipine caused marked increases in cardiac output (67%), heart rate (70%), and maxLVdP/dt (75%). Despite the increase in cardiac output, mean arterial blood pressure dropped by 21%. Consequently, the calculated systemic vascular conductance increased considerably (120%). No changes were observed in left ventricular systolic or end-diastolic pressure and stroke volume. After simultaneous administration of nisoldipine (10 mg p.o.) and propranolol (80 mg p.o.), the increases in cardiac output (35%), heart rate (29%) and systemic vascular conductance (65%) were much less, and that in maxLVdP/dt even abolished, when compared to those after nisoldipine alone. Mean arterial blood pressure, however, decreased by a similar magnitude (18%), while left ventricular systolic pressure was now slightly reduced (10%). Left ventricular end-diastolic pressure and stroke volume were again unaffected.

CORONARY HAEMODYNAMICS

Administration of nisoldipine alone increased both the transmural left ventricular blood flow (150%) and vascular conductance (225%) but, as

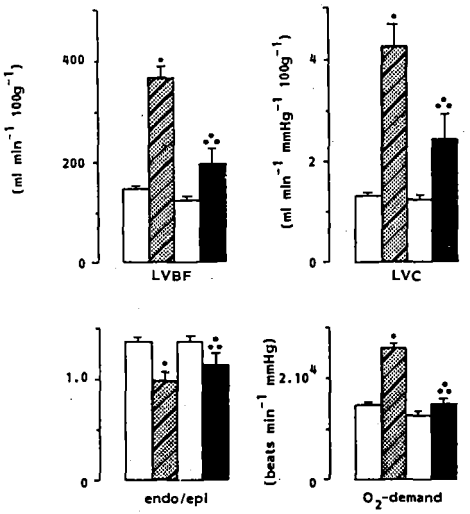

Figure 2 Effects of nisoldipine (10 mg p.o.) without and with propranolol (80 mg p.o.) on coronary haemodynamics in seven conscious pigs. LVBF, left ventricular blood flow; LVC, left ventricular conductance; endo/epi, ratio between endocardial and epicardial normalized blood flows; O_2-demand, heart rate × left ventricular systolic pressure. The four different columns represent, respectively, baseline, nisoldipine, recovery and nisoldipine with propranolol. Data are expressed as mean ± SEM. *$P \leq 0{\cdot}05$ *vs.* baseline or recovery. **Changes induced by nisoldipine with propranolol significantly different ($P \leq 0{\cdot}05$) from that by nisoldipine without propranolol.

the elevations in blood flow and conductance favoured the epicardial (195% and 280%, respectively) over the endocardial (110% and 170%, respectively) layers, the endocardial–epicardial (endo–epi) blood flow ratio decreased by 30% (Fig. 2). As the double product (left ventricular systolic pressure × heart rate), an index of myocardial oxygen demand, increased only by 60%, it would appear that the enhancement in coronary blood flow, i.e. oxygen supply, exceeded the increased oxygen demand. When nisoldipine was combined with propranolol, the elevations in transmural, epicardial and endocardial blood flows (50, 70 and 34%, respectively) and conductances (95, 120 and 70%, respectively) were only moderate, but still statistically significant ($P \leq 0{\cdot}05$). Furthermore, the reduction in endo–epi blood flow ratio (15%) did not reach the level induced by treatment with the

dihydrophyridine derivative alone, while the double product was only slightly (< 10%) enhanced.

DISTRIBUTION OF CARDIAC OUTPUT

The effects of nisoldipine on regional haemodynamics have been presented in Figs 3 and 4. Nisoldipine caused the largest increase in blood flow to the right atrium (700%), followed by the right ventricle (280%), stomach (190%), skeletal muscle (150%) left ventricle (150%, Fig. 2), small intestine (120%), adrenals (110%) and brain (30%). Renal and hepatic artery blood flow as well as flow to the skin and spleen were not significantly affected. When nisoldipine was administered simultaneously with propranolol, increases in blood flow were again observed, though generally (except skeletal muscles) of a lesser magnitude, in the right atrium (260%), skeletal muscles (250%), stomach (110%), right ventricle (105%), small intestine (70%) and left ventricle (50%). Blood flow to the adrenals, brain, liver and kidneys was not significantly affected while that to the skin (50%) and spleen (35%) decreased. As nisoldipine induced a moderate decrease in blood pressure, vascular conductances increased in most organs, except the liver, skin and spleen (Fig. 4). The most pronounced increase was observed in the right atrium (900%), followed by the right ventricle (400%), stomach (280%), skeletal muscles (230%), left ventricle (225%), small intestine (180%), adrenals (170%), brain (60%) and kidneys (50%). With propranolol, nisoldipine induced the greatest vasodilatory response in the right atrium (360%) followed by skeletal muscle (300%), stomach (160%), right ventricle (160%), small intestine (110%), left ventricle (95%) and brain (50%). No statistically significant changes were noted in the liver, skin, spleen, adrenals and kidneys.

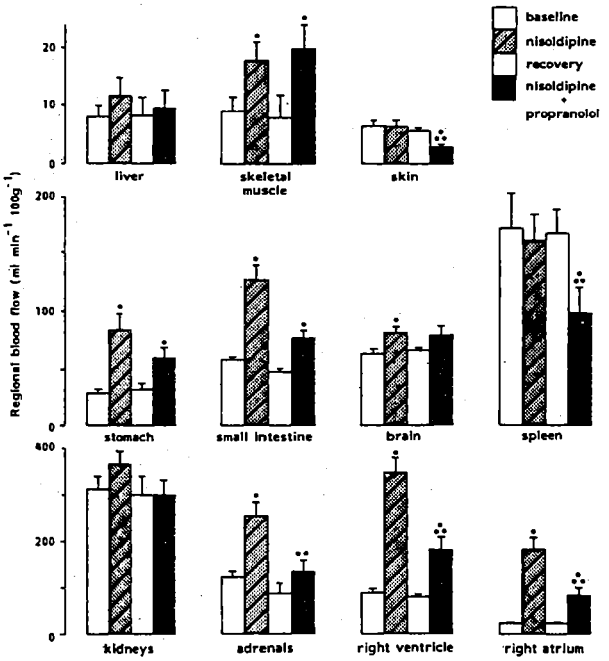

Figure 3 Regional blood flow in seven conscious pigs after nisoldipine (10 mg p.o.) without and with propranolol (80 mg p.o.). Data are expressed as mean ± SEM. *$P \leq 0.05$ *vs.* baseline or recovery. **Change induced by nisoldipine with propranolol significantly different ($P < 0.05$) from that by nisoldipine without propranolol.

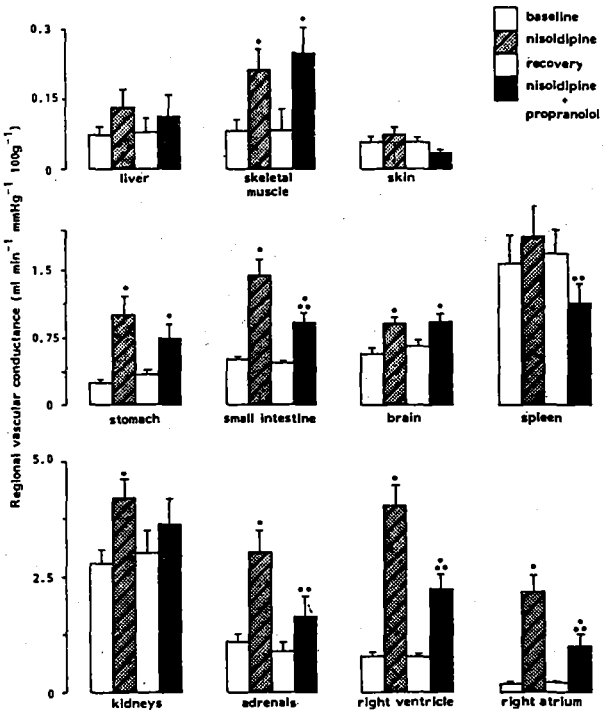

Figure 4 Regional vascular conductance in seven conscious pigs after nisoldipine (10 mg p.o.) without and with propranolol (80 mg p.o.). Data are expressed as mean ± SEM. *$P \leq 0.05$ *vs.* baseline or recovery. **Change induced by nisoldipine with propranolol significantly different ($P \leq 0.05$) from that by nisoldipine without propranolol.

Discussion

EFFECTS OF NISOLDIPINE ALONE

As has been reported by many investigators[9-13,18-22], we also observed that nisoldipine induced a pronounced systemic vasodilatation. This resulted in only a moderate fall in mean arterial blood pressure since cardiac output was markedly elevated. Due to the baroceptor-reflex, contractility and heart rate were augmented but stroke volume remained unchanged. These observations are in general agreement with data obtained after i.v. infusions of nisoldipine in anaesthetized pigs[12]. The major difference is that cardiac output in the anaesthetized animals was unchanged as the reflex-tachycardia was accompanied by a concomitant reduction in stroke volume. It appears that this reduction resulted from the use of pentobarbitone anaesthesia, since equal infusion rates in conscious pigs caused a significant increase in cardiac output solely due to a tachycardia as stroke volume was maintained (unpublished data from this laboratory). In most studies the elevation of cardiac output results primarily from an increase in heart rate, with stroke volume being either unchanged[10,18] or increased[10,19]. In rats with high baseline heart rates (up to 400 beats min^{-1}), the elevation in cardiac output is primarily caused by an augmentation of stroke volume[20,21].

Due to the reflex-tachycardia, the oxygen demand (rate-pressure product) was moderately enhanced. The increase in coronary blood flow, however, exceeded this enhancement, thereby

increasing the oxygen supply — oxygen demand ratio. Serruys *et al.*[22] reported after nisoldipine in humans an unchanged double product and oxygen consumption, while coronary blood flow was increased by 30%. Similar improvements of myocardial oxygen balance have also been demonstrated by other investigators[9,12,19]. The decrease in endocardial–epicardial blood flow ratio is probably due to the combination of an increased heart rate and hypotension[23]. Nevertheless, an increase in endocardial blood flow was present, which far exceeded the increase in oxygen demand.

EFFECTS OF NISOLDIPINE AND BETA-ADRENOCEPTOR BLOCKADE

Although the elevation of systemic vascular conductance was reduced after combined nisoldipine and propranolol, the drop in blood pressure was not different from that observed after nisoldipine alone. This was due to the attenuated increase in cardiac output, which again depended entirely on the increase in heart rate. An increase in heart rate by nisoldipine in the presence of beta-adrenoceptor blockade has also been reported by others[11,13] and this probably results from withdrawal of parasympathetic tone[24], as the atria and the conductance tissue of the left ventricle are richly innervated by the parasympathetic nervous system[25]. The left ventricular myocardium is only scarcely innervated by the parasympathetic system and this might explain why maxLVdP/dt was unaffected by the combination. In contrast, in anaesthetized pigs[12] both heart rate and maxLVdP/dt were significantly decreased by the combination suggesting attenuation of cardiovascular reflexes during pentobartital anaesthesia.

Due to the beta-adrenoceptor blockade the oxygen demand remained essentially unchanged, as the attenuated increase in heart rate was accompanied by a decrease in left ventricular systolic pressure. Furthermore, as heart rate was only moderately increased, the duration of diastole, i.e. the perfusion time of the endocardium, was not reduced as much as after nisoldipine alone. As a result, the endo–epi blood flow ratio decreased less. As in anaesthetized pigs[12], vasodilatation was inhibited in almost all organs studied (except skeletal muscles), when nisoldipine was administered together with propranolol. It is known that beta-adrenoceptor antagonists may generally reduce responses to vasodilator drugs because beta-adrenoceptor blockade leads to a reflex increase in sympathetic tone, which interferes with vasodilatation, and activation of vasoconstrictor alpha-adrenoceptors.

We conclude that nisoldipine is a potent systemic and coronary vasodilator in the conscious pig. The combination of nisoldipine and beta-adrenoceptor blockade exerted no cardiodepressant action, while the reflex-tachycardia was attenuated. Our data, obtained in conscious pigs with a normal coronary circulation, support the recent findings of Silke *et al.*[13] in patients with stable angina pectoris. In addition, we observed no detrimental effects of the combination on perfusion of regional vascular beds.

Nisoldipine was generously supplied by Bayer A.G. (Wuppertal), West Germany), while propranolol was provided by ICI Farma (Rotterdam, The Netherlands). We would like to thank the Laboratory for Experimental Surgery for their skilful assistance during surgery and the post-surgical period and Mr J. P. C. Heiligers for technical assistance during the experiments. Our special thanks go to Ms P. H. Vegter for her assistance in the preparation of this manuscript.

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CHAPTER 4

NIMODIPINE-INDUCED CHANGES IN THE DISTRIBUTION OF CAROTID BLOOD FLOW AND CARDIAC OUTPUT IN PENTOBARBITONE-ANAESTHETIZED PIGS.

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Nimodipine-induced changes in the distribution of carotid blood flow and cardiac output in pentobarbitone-anaesthetized pigs

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1 In view of the claimed effectiveness of nimodipine in migraine and its possible selectivity for cerebral vessels, we investigated the effects of nimodipine in anaesthetized pigs on the fractionation of carotid arterial blood flow into non-nutrient (arteriovenous anastomoses; AVAs) and nutrient (capillary) parts, and on regional tissue blood flows and vascular conductances.

2 Intracarotid infusions of nimodipine (0.05–1.25 $\mu g\,kg^{-1}\,min^{-1}$) redistributed carotid blood flow in favour of its nutrient compartment, particularly to the skeletal muscles and tongue. Vascular conductance in the non-nutrient (AVAs) compartment decreased (40%), most likely, as a result of 'steal' following profound (5.5 fold) arteriolar dilatation.

3 Intravenous infusions of nimodipine (0.05–6.25 $\mu g\,kg^{-1}\,min^{-1}$) caused hypotension, bradycardia, a decrease in conduction in the non-nutrient fraction, and an increase in conduction in the nutrient fraction (mostly in the skeletal muscles, but also in the gastrointestinal tract, cerebral hemispheres, heart and adrenals).

4 Probably due to the hypotensive effect, only skeletal muscle blood flow increased. The nimodipine-induced increase in vascular conductance in the skeletal muscles showed regional variation; the effect was most pronounced in the cheek muscles, followed by the muscles of the chest, abdominal, trunk and gluteal regions.

5 We conclude that: (i) AVA flow seems to represent a 'reserve' perfusion which can be readily diverted to tissues in the case of increased metabolism and/or vasodilatation, (ii) though the overall response to nimodipine of carotid blood flow distribution qualitatively resembles that to some anti-migraine drugs, the relevance of such acute effects in the prophylactic usefulness of nimodipine in migraine remains to be ascertained, and (iii) nimodipine lacks a selective cerebral vasodilator action in the anaesthetized pig.

Introduction

One of the most significant recent advances in drug development has been the discovery of agents interfering with calcium (Ca^{2+}) channels. These drugs are now being used for the treatment of cardiovascular disorders such as angina pectoris, cardiac arrhythmias and hypertension (see Fleckenstein, 1983). The Ca^{2+} channel antagonists exhibit considerable heterogeneity with respect to their effects on the heart and different vascular smooth muscle preparations (Cauvin *et al.*, 1983; Fleckenstein, 1983; Nayler, 1983; Peroutka, 1983). The dihydropyridine derivative nimodipine appears to have a preferential action on cerebral vessels; it antagonizes vasoconstrictor effects of 5-hydroxytryptamine (5-HT), blood and carboxylic thromboxane A_2 on the rabbit basilar artery more effectively than on the rabbit saphenous artery (Towart, 1981; Towart & Perzborn, 1981; Towart *et al.*, 1982). Clinically, nimodipine has shown potential in the therapy of cerebral vascular spasm following subarachnoid haemorrhage (Auer *et al.*, 1982; Grotenhuis *et al.*, 1984; Kostron *et al.*, 1984) and in the treatment of migrainous headaches (Gelmers, 1983; Meyer & Hardenberg, 1983).

In the present investigation we have addressed

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ourselves to two questions. Firstly, in view of the possible involvement of cephalic arteriovenous anastomoses (AVAs) in the pathophysiology of migraine (Heyck, 1969; Saxena, 1978; 1984), we have studied the effects of local infusions of nimodipine on the fractionation of carotid blood flow into nutrient (capillary) and non-nutrient (AVA) parts in the anaesthetized pig. The second part of this study deals with regional haemodynamics. Though several studies have shown that basal cerebral blood flow may increase in some species (Harper *et al.*, 1981; Kazda *et al.*, 1982; McCalden *et al.*, 1984; Mohamed *et al.*, 1984), only Haws *et al.* (1983), using rabbits, have directly compared the cerebrovascular effects of nimodipine with those on some other tissues. Therefore, the effects of intravenous (i.v.) infusions of nimodipine have been studied on regional tissue blood flows and vascular conductances to determine whether the drug causes a selective vasodilatation in the cerebral vascular bed of another species (pig).

Methods

Three series of experiments were performed. In the first series, intracarotid administration of the drug solvent was used as a control for evaluation of the stability of the preparation. In a second group of animals, the effects of local infusions of nimodipine on the total common carotid artery blood flow and its distribution were determined. Finally, i.v. infusions were used to evaluate the effects of nimodipine on cardiac output and its distribution. A wide range of doses of nimodipine was selected; the difference between the first and last infusion rate was 25 and 125 fold in the intracarotid and i.v. experiments, respectively.

Experimental preparation

After an overnight fast, 25 Yorkshire pigs (mean body weight ± s.e.mean, 25.7 ± 0.8 kg; age 12–16 weeks) were initially sedated with 120 mg (i.m.) azaperone (Stresnil) and 150 mg (i.v.) metomidate (Hypnodil). After the animals had been intubated, they were connected to a respirator for intermittent positive pressure ventilation with oxygen and nitrous oxide (1:2). Respiratory rate and tidal volume were adjusted to keep arterial blood gases, measured with an ABL-3 (Radiometer, Copenhagen), within normal limits (pH, 7.35–7.45; Po_2, 90–150 mmHg, Pco_2, 35–45 mmHg); sodium bicarbonate (8.4%, w/v) was infused, if necessary, until base excess was near zero. An electric blanket was used to maintain the animal's temperature around 37°C. A continuous i.v. infusion of pentobarbitone sodium (15–25 mg kg^{-1} h^{-1}) and an i.v. bolus of 4 mg pancuronium bromide (Pavulon) were used to maintain anaesthesia. Arterial blood pressure (obtained via a 7F catheter placed in the left femoral artery and connected to a Statham pressure transducer) and heart rate (counted from ECG signals) were monitored on a Gould Brush recorder. Catheters in both femoral veins and the other femoral artery were used for i.v. administration of drugs and fluids, and for monitoring arterial blood gases.

In the animals which received intracarotid infusions of nimodipine, both common carotid arteries were dissected free in the neck and bilateral cervical vagosympathectomy was performed to avoid reflex influences on the carotid circulation. Two 0.5 mm (external diameter) needles, connected to suitable polyethylene tubings, were inserted directly into one of the common carotid arteries for the infusion of nimodipine and the injection of microspheres, respectively. Blood flow in this artery was measured with a 2.5 or 3 mm (i.d.) calibrated flow probe connected to a sine wave electromagnetic blood flowmeter (Skalar, Delft). In the animals used to study the effects of i.v. administration of nimodipine on the distribution of cardiac output, a mid-sternal thoracotomy was performed. A cannula was inserted into the left atrial appendage for injection of the microspheres and a catheter in the femoral artery was employed to withdraw a reference blood sample during microsphere injection (Saxena *et al.*, 1980). Ascending aorta blood flow was measured with a suitable electromagnetic flow probe placed around the vessel. Cardiac output was derived by adding myocardial blood flow (measured with radioactive microspheres; see below) to the ascending aorta blood flow.

Distribution of common carotid blood flow and cardiac output

Injection of radioactive microspheres Radioactive microspheres (15 ± 1 [s.d.] μm diameter), labelled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb or ^{46}Sc (NEN Chemicals GmbH, Dreieich, West Germany) and suspended in saline containing a drop of Tween 80, were used (for details, see Saxena & Verdouw, 1982, 1984). Prior to use, the spheres were deaggregated by mechanical agitation. The distribution of common carotid arterial blood flow was determined by injecting $1–2 \times 10^5$ microspheres into the artery against the direction of blood flow over a period of 15–20 s. The distribution of cardiac output was determined similarly except that $1–2 \times 10^6$ microspheres were injected into the left atrium. Starting about 5 s before the injection of microspheres, blood was withdrawn (rate: 12 ml min^{-1}) from a femoral artery for a total period of 60–65 s.

Counting of radioactivity At the end of each experiment the animal was killed with an overdose of

pentobarbitone sodium and the various tissues, as specified later, were dissected out, weighed and placed in vials. The radioactivity in the vials containing the tissues and blood samples was counted for 5–10 min in a γ-scintillation counter (Packard, model 5986) equipped with a multichannel pulse height analyser (Conrac) using suitable windows for discriminating the different isotopes used (Saxena *et al.*, 1980).

Calculations The microsphere and other data were processed by a PDP-11/70 computer using a set of programmes especially developed for the microsphere technique (Saxena *et al.*, 1980). The amount of carotid blood distributed to the individual tissues ($\dot{Q}_{tis[car]}$) was calculated by: $\dot{Q}_{tis[car]}$ (ml min^{-1}) = (I_{tis}/I_{tot}) × $\dot{Q}_{car}$ and $\dot{Q}_{tis[car]}$ (%) = (I_{tis}/I_{tot}) × 100, where I_{tis} and I_{tot} are, respectively, the radioactivity (c.p.m.) in a particular tissue and that detected in all tissues (i.e. tissues of the head, including the complete brain, and the neck and lungs) collectively, and $\dot{Q}_{car}$ is carotid blood flow (ml min^{-1}) (see Saxena & Verdouw, 1982). The amount of cardiac output distributed to the various tissues ($\dot{Q}_{tis}$) was calculated as: $\dot{Q}_{tis}$ (ml min^{-1}) = (I_{tis}/I_{art}) × $\dot{Q}_{art}$ and $\dot{Q}_{tis}$ (%) = ($\dot{Q}_{tis}/CO$) × 100, where I_{tis} and I_{art} are, respectively, the radioactivity (c.p.m.) in a particular tissue and that of the arterial blood sample, while $\dot{Q}_{art}$ is the rate of withdrawal of blood samples and CO is cardiac output in ml min^{-1}. The various tissues selected were: heart, kidneys, adrenal glands, skeletal muscles from several regions, skin, spleen, liver, small and large intestine, including caecum and rectum (gastrointestinal tract), eyes and a large part of the brain (cerebral hemispheres). Tissue vascular conductance was calculated by dividing respective blood flow values by mean arterial blood pressure.

The values determined for lungs, when microspheres were injected into the carotid artery, represent the AVA part of the carotid circulation (see Johnston & Saxena, 1978; Saxena & Verdouw, 1982). In the case of left atrial injection, the lungs receive microspheres via both peripheral AVAs and bronchial arteries; however, the contribution via the latter route appears to be only about 1% (Baile *et al.*, 1982). Thus, even in this case, the values for 'lung blood flow' can be used as an index of peripheral AVA flow (i.e. the non-nutrient part of cardiac output). The nutrient part of cardiac output was calculated by subtracting 'lung blood flow' from cardiac output.

Experimental protocol

In all experiments, the baseline values were obtained following a stabilization period of 60 min after completion of the surgical procedures. The measurements consisted of recordings of the heart rate, mean arterial blood pressure and common carotid artery blood flow (or cardiac output), while a batch of microspheres was injected into the carotid artery (or into the left atrium). In the common carotid blood flow distribution experiments, three successively increasing intracarotid infusions of nimodipine (0.05, 0.25 and 1.25 μg kg^{-1} min^{-1}) were then administered for 10 min each. Microspheres were injected at the end (10 min) of the 0.05 and 0.25 μg kg^{-1} min^{-1} infusions, and 2 and 10 min after the last infusion step (1.25 μg kg^{-1} min^{-1}). In the cardiac output distribution experiments, successively increasing i.v. doses of 0.05, 0.25, 1.25 and 6.25 μg kg^{-1} min^{-1} of nimodipine were infused for a period of 10 min each; microspheres were injected at the end of each i.v. infusion period. Control experiments were performed with the drug solvent which was injected into the common carotid artery in amounts (0.5, 2.5 and 12.5 μl kg^{-1} min^{-1}) which corresponded to the three intracarotid doses of nimodipine; 10 min after each infusion, microspheres were injected into the carotid artery.

Data presentation and statistical analysis

Except as mentioned otherwise, all data in the text and

Table 1 Effects of intracarotid administration of nimodipine ($n = 9$) and equivalent amounts of its solvent ($n = 6$) on mean arterial blood pressure and heart rate in pigs

		% change by nimodipine (μg kg^{-1} min^{-1}) or solvent[a]			
	Baseline values	*0.05*	*0.25*	*1.25 (2 min)*	*1.25 (10 min)*
		Mean arterial pressure (mmHg)			
Solvent	104 ± 7	−2 ± 1	−3 ± 1		−4 ± 1*
Nimodipine	105 ± 4	−14 ± 3***	−24 ± 4***	−34 ± 4*	−47 ± 5***
		Heart rate (beats min^{-1})			
Solvent	100 ± 4	−8 ± 1*	−11 ± 1*		−12 ± 2*
Nimodipine	114 ± 8	−7 ± 2	−17 ± 5*	−18 ± 4*	−23 ± 4***

[a]The corresponding doses of solvent were 0.5, 2.5 and 12.5 μl kg^{-1} min^{-1}; *Significantly different from the respective baseline value; **Change significantly more than the respective change caused by the drug solvent.

illustrations are presented as means ± s.e.mean. In general absolute values have been given but in order to facilitate comparison between the effects of nimodipine and its solvent (carotid blood flow distribution experiments), we have presented data in Tables 1 and 2 as % changes from the respective baseline values. These values are means of the % changes in each animal, as are the other % changes included in the text. The significance of the effects of the solvent or nimodipine on the different variables was evaluated by Duncan's new multiple-range test once an analysis of variance (randomized block design) had revealed that the samples represented different populations. The baseline values and the effects of the solvent were compared similarly but, in this case, one-way analysis of variance was used (Saxena, 1985). A P value of 0.05 or less (two-tailed) was considered statistically significant.

Drugs

No drugs other than the anaesthetics and nimodipine (Bay e 9736; Bayer, Wuppertal) were used in this study. The nimodipine solvent used was a mixture of polyethylene glycol 400, glycerol and water. The nimodipine solution (0.1 mg ml^{-1}) and the solvent were diluted with 0.9% w/v NaCl solution immediately before use.

Results

Effects of intracarotid infusions of solvent and nimodipine

Arterial blood pressure and heart rate Baseline mean arterial blood pressure and heart rate did not differ

Table 2 Effects of intracarotid administration of nimodipine ($n = 9$) and equivalent amount of its solvent ($n = 6$) on total carotid blood flow and its distribution in pigs

		% change by nimodipine (μg kg^{-1} min^{-1}) or solvent[a]			
	Baseline values	*0.05*	*0.25*	*1.25 (2 min)*	*1.25 (10 min)*
		Blood flow (% carotid flow)			
AVA-fraction					
Solvent	82 ± 1	3 ± 2	2 ± 3		1 ± 3
Nimodipine	82 ± 2	−31 ± 6***	−48 ± 5***	−63 ± 5*	−65 ± 5***
Nutrient-fraction[b]					
Solvent	18 ± 1	−13 ± 9	−11 ± 13		−1 ± 16
Nimodipine	18 ± 2	122 ± 18***	232 ± 23***	323 ± 32*	268 ± 29***
		Blood flow (ml min^{-1})			
Total carotid					
Solvent	166 ± 17	2 ± 1	3 ± 2		2 ± 2
Nimodipine	232 ± 20	4 ± 8	10 ± 7	9 ± 7	−14 ± 7*
AVA-fraction					
Solvent	137 ± 15	6 ± 2	5 ± 3		3 ± 3
Nimodipine	190 ± 18	−31 ± 4***	−44 ± 5***	−60 ± 6*	−70 ± 4***
Nutrient-fraction					
Solvent	29 ± 3	−11 ± 10	−8 ± 13		1 ± 18
Nimodipine	40 ± 5	135 ± 34***	264 ± 29***	323 ± 32*	208 ± 14***
		Conductance (ml min^{-1} mmHg^{-1})			
Total carotid					
Solvent	1.6 ± 0.2	5 ± 1	7 ± 3*		7 ± 3*
Nimodipine	2.2 ± 0.2	21 ± 10	47 ± 10***	67 ± 8*	68 ± 16***
AVA-fraction					
Solvent	1.3 ± 0.2	8 ± 2*	8 ± 4*		7 ± 3*
Nimodipine	1.8 ± 0.2	−20 ± 4***	−27 ± 4***	−40 ± 7*	−44 ± 5***
Nutrient-fraction					
Solvent	0.28 ± 0.02	−9 ± 10	−5 ± 13		6 ± 20
Nimodipine	0.38 ± 0.04	175 ± 42***	385 ± 48***	551 ± 53*	512 ± 68***

[a]The corresponding doses of solvent were 0.5, 2.5 and 12.5 μl kg^{-1} min^{-1}; [b]Includes both extracerebral and cerebral (2%) components. *Significantly different from the respective baseline value; **Change significantly more than the respective change caused by the drug solvent.

significantly in the animals subsequently treated with the solvent or nimodipine (Table 1). Intracarotid administration of the solvent had little or no effect on mean arterial blood pressure but caused slight decreases in heart rate. Nimodipine produced substantial decreases in arterial blood pressure at all three infusion rates, but heart rate decreased significantly more than that with the equivalent amount of solvent only at the highest rate ($1.25\,\mu g\,kg^{-1}\,min^{-1}$) of infusion (Table 1).

Fractionation of carotid blood flow into non-nutrient and nutrient parts The effects of nimodipine and its solvent on the distribution of carotid blood flow are shown in Table 2. Though the baseline values of total carotid blood flows in the two series differed significantly, none of the other baseline values was significantly different. Infusions of the solvent caused little or no change in either the blood flow to or the conductance in the carotid vascular bed (both in non-nutrient and nutrient parts). On the other hand, there was a marked redistribution of blood flow with nimodipine. AVA blood flow and conductance decreased dose-dependently by up to 70 and 44%, respectively, but these decreases were associated with marked increases in nutrient blood flow and conductance (Table 2). Therefore, total carotid artery blood flow was not affected until 10 min after the infusion of the highest dose when flow had significantly decreased by 14%. Since mean arterial blood pressure had decreased with nimodipine, there were increases in the calculated total carotid conductance, but these were much smaller than the increases observed in the nutrient part.

Tissue distribution of the nutrient part of carotid blood flow Figures 1 and 2 show that the various tissues in the head were not equally affected by nimodipine. The

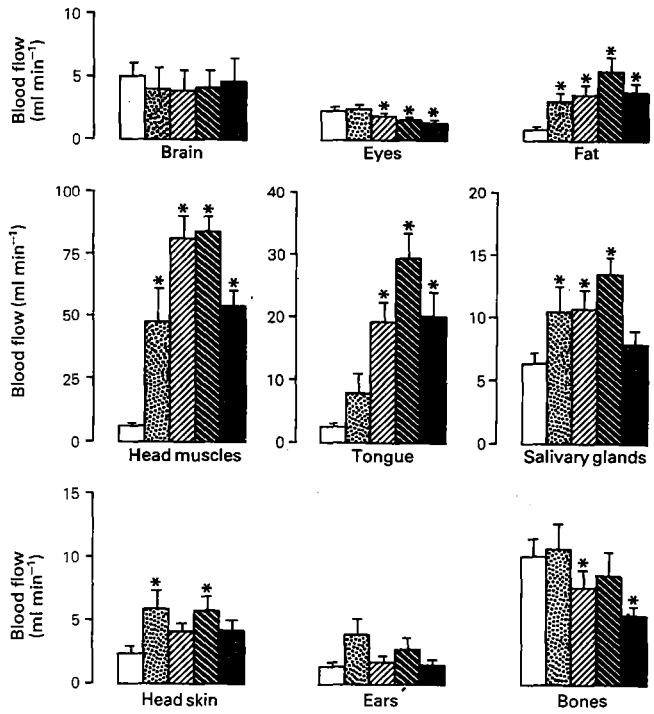

Figure 1 Effects of intracarotid administration of nimodipine on tissue distribution of carotid nutrient blood flow in pigs ($n = 9$). The five columns represent values at baseline (open columns), and after nimodipine ($\mu g\,kg^{-1}\,min^{-1}$) infusions of 0.05 (stippled columns), 0.25 (hatched, dark lines on white background, columns) and 1.25 at 2 min (hatched, white lines on dark background columns) and at 10 min (solid columns). *Significantly different from the respective baseline value.

contribution via the carotid artery to nutrient blood supply of the eyes and bones decreased, but that to the fat, skeletal muscles, tongue, salivary glands and skin increased. The increases in the blood flow to the skeletal muscles (6–14 fold) and tongue (2–12 fold) were particularly marked. No significant change was noticed in the brain and ears (Figure 1).

Vascular conductance increased in all tissues except the eyes and bones (Figure 2). The magnitude of the vasodilator response varied greatly. The largest increase in conductance (20 fold) was observed in skeletal muscles and tongue, followed by fat (9 fold), skin (4 fold) and salivary glands (3 fold). Conductance of the cerebral vascular bed showed the least increase (1.6 fold) and that, too, only after 10 min of the highest infusion rate.

Effects of i.v. infusions of nimodipine

Systemic haemodynamics The effects of nimodipine on systemic haemodynamic variables are shown in Table 3. The drug decreased mean arterial blood pressure and heart rate dose-dependently (by up to 60 ± 4 and $36 \pm 5\%$, respectively). During baseline condition, $55 \pm 2\%$ of cardiac output ($2.9\,l\,min^{-1}$) was used for the nutrition of tissues, while $45 \pm 2\%$ bypassed tissues via AVAs. Nimodipine decreased cardiac output but, except for the highest dose ($38 \pm 7\%$), these effects were relatively minor (<15%). The decrease in cardiac output was entirely in the AVA-part which was reduced by 12 ± 3, 41 ± 5, 70 ± 4 and $87 \pm 3\%$, respectively, by the four infusion rates (0.05, 0.25, 1.25 and $6.25\,\mu g\,kg^{-1}\,min^{-1}$) used. The nutrient part of cardiac output remained unchanged, or even increased (after $1.25\,\mu g\,kg^{-1}\,min^{-1}$ of nimodipine). Systemic vascular conductance increased (up to $65 \pm 13\%$) after the two highest doses of the drug. This increase in the systemic conductance (Table 3) was due to the increase in conductance of the nutrient part of the vascular beds of the various tissues

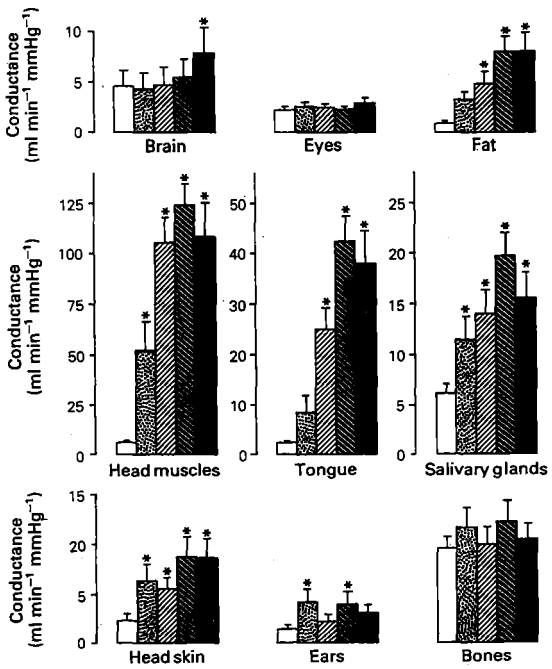

Figure 2 Effects of intracarotid administration of nimodipine on tissue conductance in the nutrient part of carotid vascular bed of pigs ($n = 9$). The five columns represent values at baseline (open columns), and after nimodipine ($\mu g\,kg^{-1}\,min^{-1}$) infusions of 0.05 (stippled columns), 0.25 (hatched, dark on white background, columns) and 1.25 at 2 min (hatched, white lines on dark background, columns) and at 10 min (solid columns). *Significantly different from the respective baseline value.

Table 3 Systemic haemodynamic effects of i.v. administration of nimodipine in pigs (n = 10)

		Nimodipine ($\mu g\,kg^{-1}\,min^{-1}$)			
	Baseline values	0.05	0.25	1.25	6.25
MAP (mmHg)	84 ± 4	78 ± 4	63 ± 4*	45 ± 2*	34 ± 3*
HR (beats min^{-1})	98 ± 3	95 ± 3	92 ± 4	87 ± 5*	63 ± 5*
CO (l min^{-1})	2.9 ± 0.2	2.5 ± 0.1	2.4 ± 0.1*	2.4 ± 0.1*	1.7 ± 0.2*
AVA flow (l min^{-1})	1.3 ± 0.1	1.1 ± 0.1*	0.7 ± 0.1*	0.3 ± 0.04*	0.2 ± 0.04*
NCO (l min^{-1})	1.6 ± 0.1	1.4 ± 0.1	1.7 ± 0.1	2.1 ± 0.1*	1.5 ± 0.2
SVC (ml min^{-1} $mmHg^{-1}$)	35 ± 3	34 ± 3	41 ± 3	54 ± 3*	51 ± 5*
AVAC (ml min^{-1} $mmHg^{-1}$)	16 ± 1	15 ± 2	12 ± 1*	8 ± 1*	5 ± 1*
NVC (ml min^{-1} $mmHg^{-1}$)	19 ± 2	19 ± 2	29 ± 3*	46 ± 3*	46 ± 5*

MAP, mean arterial blood pressure; HR, heart rate; CO, cardiac output; AVA flow, peripheral arteriovenous anastomoses blood flow; NCO, nutrient part of cardiac output. SVC, AVAC and NVC are conductances of, respectively, total systemic, peripheral AVA and nutrient channels.
*Significantly different from the respective baseline value.

(2.4 fold with the highest dose), as the conductance of AVAs decreased dose-dependently to 30% of baseline values.

Tissue blood flow and conductance The changes in nutrient cardiac output were not equally distributed (Figure 3). The perfusion of the skeletal muscles

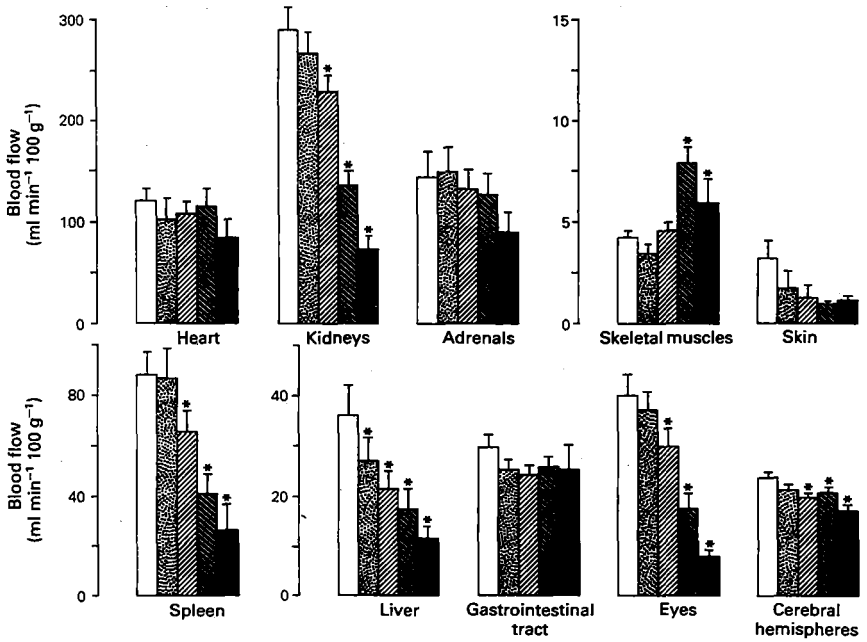

Figure 3 Effects of i.v. administration of nimodipine on tissue distribution of nutrient cardiac output in pigs ($n = 10$). The five columns represent values at baseline (open columns), and after infusions of 0.05 (stippled columns), 0.25 (hatched, dark lines on white background, columns), 1.25 (hatched, white lines on dark background, columns) and 6.25 (solid columns) $\mu g\,kg^{-1}\,min^{-1}$ of nimodipine. *Significantly different from the respective baseline value.

increased, while that of other organs either did not change (heart, adrenal glands, skin and gastrointestinal tract) or even decreased (kidneys, spleen, liver, eyes and cerebral hemispheres). Consequently, nutrient vascular conductance (Figure 4) of some organs increased (heart, adrenal glands, skeletal muscles, gastrointestinal tract and cerebral hemispheres) and of others either remained unchanged (skin and liver) or decreased (kidneys, spleen and eyes). The most pronounced increase was in the conductance of skeletal muscles (271 ± 57%) which was followed by gastrointestinal tract (124 ± 19%) and cerebral hemispheres (98 ± 16%). On the other hand, conductance of the vascular bed of the eye was reduced by 49 ± 7%, which was followed by the renal vascular bed (41 ± 6%).

Skeletal muscles of different regions As shown in Table 4, the effects of nimodipine varied in the skeletal muscles obtained from different regions. With the highest dose the average increase in vascular conductance was 9 fold in the cheek muscles, 4 fold in the chest muscles, 3 fold in the abdominal muscles and only 2 fold in the trunk and gluteal muscles.

Discussion

Systemic haemodynamics

Both intracarotid and i.v. administration of nimodipine caused a fall in arterial blood pressure. Since even the local intracarotid infusions of the drug substantially lowered blood pressure, it would appear that the drug quickly appeared in the systemic circulation. Whether the cervical vagosympathetic nerves were sectioned (intracarotid infusions) or not (i.v. infusions), the hypotensive effect of nimodipine was

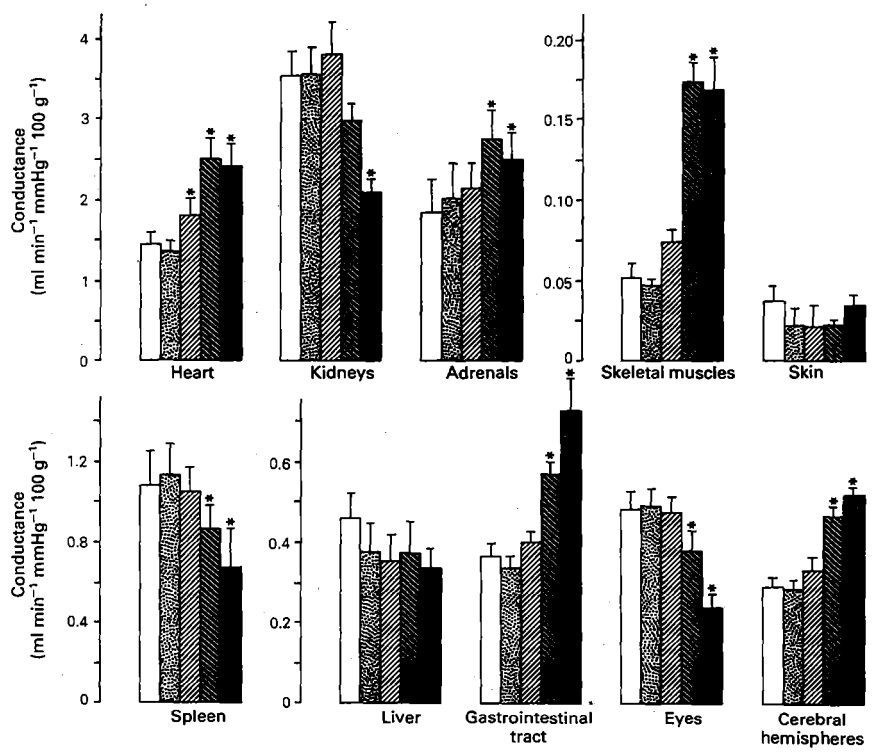

Figure 4 Effects of i.v. administration of nimodipine on nutrient regional vascular conductance in pigs ($n = 10$). The five columns represent values at baseline (open columns), and after infusions of 0.05 (stippled columns), 0.25 (hatched, dark lines on white background, columns), 1.25 (hatched, white lines on dark background, columns) and 6.25 (solid columns) $\mu g\,kg^{-1}\,min^{-1}$ of nimodipine. *Significantly different from the respective baseline value.

Table 4 Effect of i.v. administration of nimodipine on skeletal muscles of different regions of pigs ($n = 10$)

		Nimodipine ($\mu g kg^{-1} min^{-1}$)			
	Baseline values	0.05	0.25	1.25	6.25
		Blood flow ($ml\ min^{-1}\ 100\ g^{-1}$)			
Cheek	3.5 ± 0.6	3.1 ± 0.4	4.5 ± 1.2	14 ± 2*	11 ± 2*
Chest	2.6 ± 0.4	2.1 ± 0.4	2.9 ± 0.5	4.8 ± 0.7*	4.3 ± 1.1*
Abdomen	2.0 ± 0.2	1.7 ± 0.2	1.7 ± 0.3	3.2 ± 0.4*	2.7 ± 0.8
Right trunk	5.5 ± 0.5	4.6 ± 0.9	5.3 ± 1.0	9.0 ± 1.3*	6.6 ± 1.8
Left trunk	5.9 ± 0.8	4.4 ± 0.8	5.4 ± 1.0	8.9 ± 1.9*	6.1 ± 1.8
Right gluteus	4.4 ± 0.5	3.7 ± 0.5	5.0 ± 0.7	6.9 ± 1.2*	5.4 ± 1.6
Left gluteus	5.3 ± 0.4	4.5 ± 0.6	6.7 ± 0.8	9.6 ± 1.3*	6.6 ± 1.4
		Conductance ($ml\ min^{-1}\ mmHg^{-1}\ 100\ g^{-1}$)			
Cheek	0.04 ± 0.01	0.04 ± 0.01	0.07 ± 0.02	0.31 ± 0.05*	0.32 ± 0.04*
Chest	0.03 ± 0.01	0.03 ± 0.01	0.05 ± 0.01	0.11 ± 0.01*	0.12 ± 0.02*
Abdomen	0.02 ± 0.004	0.02 ± 0.004	0.03 ± 0.005	0.07 ± 0.1*	0.07 ± 0.02*
Right trunk	0.07 ± 0.01	0.06 ± 0.01	0.09 ± 0.01	0.19 ± 0.02*	0.19 ± 0.03*
Left trunk	0.07 ± 0.01	0.06 ± 0.01	0.08 ± 0.01	0.19 ± 0.03*	0.17 ± 0.03*
Right gluteus	0.05 ± 0.01	0.05 ± 0.01	0.08 ± 0.01	0.15 ± 0.03*	0.15 ± 0.04*
Left gluteus	0.06 ± 0.01	0.06 ± 0.01	0.11 ± 0.01*	0.21 ± 0.02*	0.19 ± 0.03*

*Significantly different from the respective baseline value.

not accompanied by any evidence of reflex activation of the sympathetic nervous system via the baroreceptors; both heart rate and cardiac output decreased in the present experiments (Tables 1 and 3). The decrease in cardiac output (largely, if not entirely, confined to the non-nutrient part) was moderate and, therefore, the hypotensive effect of nimodipine was mainly due to an increase in systemic vascular conductance.

The lack of reflex effects during nimodipine-induced hypotension in anaesthetized pigs is in contrast to the effects of the structurally related Ca^{2+} channel antagonists nifedipine (Gross *et al.*, 1979) and felodipine (Bolt & Saxena, 1984) in conscious animals, where both heart rate and cardiac output increase prominently. This discrepancy appears to be, at least partly, due to the anaesthetic agents used in the present experiments; in conscious or anaesthetized (fentanyl plus etomidate) patients undergoing cardiac bypass surgery, nimodipine increases cardiac output (Boldt *et al.*, 1985), and therefore causes less hypotension. However, heart rate is not or only slightly elevated by nimodipine, even in conscious rabbits (Haws *et al.*, 1983) or man (Boldt *et al.*, 1985), but is increased by nisoldipine, another related Ca^{2+} channel antagonist, in anaesthetized pigs (Duncker *et al.*, 1986).

Carotid haemodynamics

The results obtained in the present experiments confirm our previous observations that a large (about 80%) fraction of common carotid blood flow in the pig is shunted via AVAs (Saxena & Verdouw, 1982; 1984; Verdouw *et al.*, 1984a,b), which are mainly located in the skin and ears (Saxena & Verdouw, 1985). Local infusions of nimodipine, but not of its solvent, redistributed carotid arterial blood flow in favour of the nutrient (tissue, arteriolar) component at the expense of the non-nutrient (AVA) component. Unlike 5-HT where the increased nutrient flow is mainly distributed to the skin and ears (Saxena and Verdouw, 1982; 1984), nimodipine enhanced primarily that to the skeletal muscles and tongue.

The dilatation of arterioles by nimodipine is apparently due to Ca^{2+} channel blockade, but the mechanism responsible for the decrease in AVA flow and conductance needs further examination. A baroreceptor reflex-mediated stimulation of the sympathetic nervous system seems unlikely for the reasons discussed above and, moreover, in young pigs as used in the present experiments, AVAs are only poorly constricted via noradrenergic mechanisms (Verdouw *et al.*, 1984b). Another possibility may be that nimodipine in some way interferes with the release of an endogenous substance responsible for opening up AVAs in the pig. Though the formation of endothelium-derived relaxing factor (EDRF) in some arterial preparations *in vitro* is Ca^{2+}-dependent (Singer & Peach, 1982; Rubanyi *et al.*, 1985), and can be inhibited by Ca^{2+} channel antagonists (Singer & Peach, 1982), it is not yet known if such an effect of Ca^{2+} channel antagonists is observed *in vivo* or if EDRF can indeed relax AVAs. Thus, it follows that the reduction in AVA flow and conductance most probably results from 'steal' as a consequence of

profound dilatation in the nutrient vascular channels caused by nimodipine. This suggestion is indirectly supported by comparing similar data found with 5-HT, which elicits an 'active' constriction of AVAs. In the face of a 3 fold increase in nutrient vascular conductance, 5-HT reduces AVA conductance by over 80% (Saxena & Verdouw, 1982), whereas in association with an even greater increase (5.5 fold) in nutrient conductance, nimodipine decreased AVA conductance by only about 40% (Table 2). This haemodynamic redistribution as observed with nimodipine may be of physiological significance; the data in this study reinforce the idea that, in situations of increased metabolism and/or vasodilatation, nutrient blood supply to tissues can be readily made available from arteriovenous shunting which apparently represents 'reserve' perfusion.

Arteriovenous shunting has been implicated in the pathophysiology of migraine where profound vasodilatation in these large shunt vessels may lead to a reverse 'steal' (Heyck, 1969; Saxena, 1978; 1984). Indeed anti-migraine drugs, particularly the ergot alkaloids effective in the treatment of individual attacks (Johnston & Saxena, 1978; Schamhardt *et al.*, 1979; Spierings & Saxena, 1980), as well as 5-HT (Saxena & Verdouw, 1982) which may also alleviate migraine attacks (Kimball *et al.*, 1960; Lance, 1982), cause an 'active' constriction of AVAs. Though the overall effect of nimodipine on the distribution of carotid artery blood flow into nutrient and non-nutrient fractions qualitatively resembles that of the above drugs, nimodipine is less efficacious and, as discussed above, has a 'passive' effect on AVAs. Probably because of this passive and moderate effect of AVAs, in contrast to the ergot alkaloids, nimodipine has recently been found to be ineffective in the treatment of individual attacks of classic migraine (Jensen *et al.*, 1985). Whether the acute effects of nimodipine on carotid haemodynamics, as observed in this study, are related to the beneficial properties of the drug reported after long term prophylactic use in migraine (Gelmers, 1983; Meyer & Hardenberg, 1983) remains to be ascertained.

Regional haemodynamics

The regional haemodynamic effects of nimodipine on the nutrient fraction were not uniform. Only skeletal muscle blood flow increased. Since arterial blood pressure decreased, conductance increases (i.e. vasodilatation) were observed (in decreasing order of magnitude) in the skeletal muscles, the gastrointestinal tract, the cerebral hemispheres, the heart and the adrenals. Cerebral vasodilatation has been observed in all studies, but nimodipine, used in doses similar to those in the present experiments, causes variable effects on basal cerebral blood flow. In anaesthetized animals, the drug elicits no (Haws & Heistad, 1984) or a moderate (18–50%) increase in cerebral blood flow (Harper *et al.*, 1981; Kazda *et al.*, 1982; McCalden *et al.*, 1984; Mohamed *et al.*, 1984), probably due to a fall in systemic perfusion pressure. In unanaesthetized rabbits, however, the drug produced a 2 fold increase in cerebral blood flow despite the usual fall in blood pressure (Haws *et al.*, 1983). The other differences with respect to tissue blood flow changes were that Haws *et al.* (1983) observed an increase in the flow to the heart, no change in the flow to the kidneys and only a moderate increase in the flow to the skeletal muscles (masseter). In our experiments, renal blood flow decreased substantially and the increase in blood flow to the skeletal muscles, which showed a regional variation, was very pronounced in the cheek muscles. Skeletal muscle blood flow is also greatly increased by other dihydropyridine Ca^{2+} channel antagonists such as nifedipine, darodipine, felodipine and nisoldipine (Hof, 1983; 1984; 1985; Bolt & Saxena, 1984; Duncker *et al.*, 1986), but not by verapamil or diltiazem (Hof, 1983).

Lastly, though this study did not demonstrate a selective vasodilatation in the brain with nimodipine, it may be that when cerebral (and other) vessels are in spasm or have a higher basal tone (e.g. in animals without anaesthesia, or in clinical situations) nimodipine may show a different spectrum of activity. Hof (1984, 1985) has convincingly shown that the pattern of anti-vasoconstrictor effects of Ca^{2+} channel antagonists (darodipine and verapamil) differs considerably from their pattern of vasodilatation, indicating that the selectivity of action of these drugs, and the influx of extracellular Ca^{2+} (Deth & Van Bremen, 1977; Bolton, 1979), depend not only on the vascular bed but also on the presence of vasoconstrictor influences.

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CHAPTER 5

ENHANCEMENT OF VASOCONSTRICTOR AND ATTENUATION OF VASODILATOR EFFECTS OF 5-HYDROXYTRYPTAMINE BY THE CALCIUM CHANNEL BLOCKERS NIMODIPINE AND NIFEDIPINE IN THE PIG.

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Enhancement of vasoconstrictor and attenuation of vasodilator effects of 5-hydroxytryptamine by the calcium channel blockers nimodipine and nifedipine in the pig

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As calcium (Ca^{2+}) channel blockers are effective against the vasoconstrictor responses to 5-hydroxytryptamine (5-HT) in vitro, and a favourable response is claimed for these drugs in migraine prophylaxis, we studied the interaction between nimodipine or nifedipine, and 5-HT for effects on carotid haemodynamics in the anaesthetized pig. Intracarotid infusions of nimodipine (0.25 $\mu g \cdot kg^{-1} \cdot min^{-1}$), nifedipine (0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or 5-HT (2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) caused a redistribution of carotid blood flow in favour of the nutrient (capillary) fraction at the expense of the non-nutrient (arteriovenous anastomoses; AVA) fraction. Compared to those of 5-HT, the effects of the Ca^{2+} channel blockers on cranial AVAs were much weaker and the increase in the capillary fraction was observed mainly in the skeletal muscles, rather than in the skin and ears as with 5-HT. When 5-HT was infused in the presence of nimodipine or nifedipine, the amine-induced vasoconstrictor responses in the total carotid vascular bed and its AVA fraction were either not attenuated or were increased while the vasodilator responses were reduced. We conclude that: (i) in contrast to what was found in vitro, the 5-HT-induced vasoconstriction in vivo, involving either '5-HT_1-like' (AVAs) or 5-HT_2 (arterioles) receptors, was not antagonized by nimodipine or nifedipine; (ii) the attenuation of the 5-HT-induced dermal vasodilatation by the two Ca^{2+} channel blockers is most likely to be the result of a 'steal' due to the profound vasodilatation in the skeletal muscle region; and (iii) the comparatively mild reduction in AVA conductance caused by the Ca^{2+} channel blockers may be one of the reasons for their inability to abort acute attacks of migraine. The increase in nutrient blood flow is of potential benefit, but whether this property of the Ca^{2+} channel blockers is linked to their usefulness in migraine prophylaxis remains to be ascertained.

Arteriovenous anastomosis; Calcium channel blockers; Carotid artery; 5-Hydroxytryptamine; Migraine; Nifedipine; Nimodipine; Radioactive microspheres; Blood flow (regional)

1. Introduction

Though the magnitude of the effect varies with individual drugs, calcium (Ca^{2+}) channel blockers inhibit the influx of extracellular Ca^{2+} into cardiac and vascular smooth muscle cells. These drugs are primarily effective in the treatment of cardiovascular disorders such as hypertension, cardiac arrhythmias and angina pectoris.

Recently, some Ca^{2+} channel blockers were reported to show therapeutic promise in cerebral vascular spasm following subarachnoid haemorrhage and in migraine prophylaxis (Louis, 1981; Auer et al., 1982; Gelmers, 1983; Meyer and Hardenberg, 1983). These beneficial effects claimed for Ca^{2+} channel blockers in migraine, and

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perhaps also in subarachnoid haemorrhage, may be related to 5-hydroxytryptamine (5-HT). It is known that, after an initial increase in the pre-headache period, the concentration of 5-HT in blood decreases (together with increased urinary excretion of 5-hydroxyindole acetic acid) during migraine headaches (see Lance, 1978; Fozard, 1982). Therefore, while headache itself seems to be associated with vasodilatation in the non-nutrient (arteriovenous anastomoses; AVAs) part of the cranial non-cerebral circulation, possibly linked to a low blood 5-HT concentration (Heyck, 1969; Saxena, 1978), an enhanced release of 5-HT could be involved in the initial cerebral vasoconstriction. Indeed, in vitro studies have shown convincingly that the drugs which block Ca^{2+} channels can also attenuate the vascular smooth muscle contractions elicited by 5-HT (Towart, 1981; Müller-Schweinitzer and Neuman, 1983; Van Nueten, 1984). The present series of experiments were designed to study the interaction between 5-HT and two dihydropyridine Ca^{2+} channel blockers, nimodipine and nifedipine, on the distribution of common carotid artery blood flow in the anaesthetized pig.

2. Materials and methods

2.1. General

Three series of experiments were performed in young Yorkshire pigs (22-28 kg). In the first series (n = 6), infusions into the common carotid artery were used to evaluate the effects of 5-HT on the distribution of total common carotid artery blood flow, before and during local infusions of nimodipine. A similar protocol was followed in the second series (n = 5) but now we used nifedipine instead of nimodipine. A third group of animals (n = 6), served to study the carotid hemodynamic effects of intracarotid infusion of nifedipine alone.

2.2. Experimental set-up

After an overnight fast the pigs were initially sedated with 120 mg azaperone (Stresnil) i.m., and 120-150 mg metomidate (Hypnodil) i.v., intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture (1 : 2) of oxygen and nitrous oxide. A continuous i.v. infusion of pentobarbitone sodium (20 $mg \cdot kg^{-1} \cdot h^{-1}$) was administered throughout the experiment to maintain anesthesia. Respiratory rate and tidal volume were adjusted or sodium bicarbonate (8.4% w/v) was infused (until base excess was near zero) to keep arterial blood gases within normal limits (pH, 7.35-7.45; P_{O_2}, 90-150 mmHg; P_{CO_2}, 35-45 mmHg). Body temperature was kept around 37 °C with an electric blanket. Aortic blood pressure was monitored with a microtipped Millar catheter inserted into the left femoral artery. Both carotid arteries were dissected free in all animals and bilateral vagosympathectomy was performed to avoid reflex effects on the carotid circulation. Blood flow in one of the common carotid arteries was measured with a precalibrated flow probe connected to a sine wave electromagnetic blood flowmeter (Skalar, Delft, The Netherlands). Three 0.5 mm (external diameter) hubless needles, connected to suitable polyethylene tubing, were inserted directly into the main artery for intracarotid infusions of 5-HT, nimodipine or nifedipine and injection of radioactive microspheres.

2.3. Distribution of common carotid artery blood flow

2.3.1. Injection of radioactive microspheres

The distribution of carotid blood flow into nutrient (tissue; capillary) and non-nutrient (AVA) fractions was determined by injecting into the common carotid artery a batch of $1\text{-}2 \times 10^5$ (15 ± 1 (s.d.) μm) microspheres, labeled with ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}NB, or ^{46}Sc (NEN Chemicals GmbH, Dreieich, West Germany) over a 15-20 s period, against the direction of blood flow (Saxena and Verdouw, 1982).

2.3.2. Counting of the radioactivity

The animals were killed at the end of each experiment with an overdose of pentobarbitone sodium. The various tissues of the head, and the lungs, heart and kidneys were dissected out, weighed and placed in vials. The radioactivity in these vials was counted for 5-10 min in a γ-scintil-

lation counter (Packard, model 5986) equipped with a multichannel analyser (Contrac) using suitable windows for discriminating the different isotopes (Saxena et al., 1980).

2.3.3. Calculations

The microsphere and other data were processed by a PDP-11/70 computer with a set of specially developed programmes (Saxena et al., 1980). The fraction of common carotid blood flow distributed to the various organs ($\dot{Q}_{tis[car]}$) was calculated as:

$$\dot{Q}_{tis[car]}(ml \cdot min^{-1}) = (I_{tis}/I_{tot}) \times \dot{Q}_{car} \text{ and}$$

$$\dot{Q}_{tis[car]}(\%) = (I_{tis}/I_{tot}) \times 100$$

where I_{tis} and I_{tot} are, respectively, the radioactivity (c.p.m.) in a particular tissue and that detected in all tissues collectively, and $\dot{Q}_{car}$ is total carotid blood flow ($ml \cdot min^{-1}$). Vascular conductance was calculated by dividing the respective blood flow values by the mean arterial blood pressure. The values obtained for lungs when microspheres were injected into the carotid artery represent the AVA part of the carotid circulation (see Saxena and Verdouw, 1982).

2.4. Experimental protocols

Baseline values were obtained for all experiments after the preparation had been in a stable hemodynamic condition for at least 30 min after completion of the surgical procedures. The measurements consisted of the recording of heart rate, mean arterial blood pressure and common carotid artery blood flow, while a batch of microspheres was injected for the determination of nutrient (tissue; capillary) and non-nutrient (AVA) blood flow. In the first and second series, the measurements were repeated following a 10 min intracarotid infusion of 5-HT ($2.0\ \mu g \cdot kg^{-1} \cdot min^{-1}$) and again, in the first series, after a recovery period of 20 min. An intracarotid infusion of $0.25\ \mu g \cdot kg^{-1} \cdot min^{-1}$ of nimodipine (first series) or $0.75\ \mu g \cdot kg^{-1} \cdot min^{-1}$ of nifedipine (second series) was then started and was continued for 20 min; the doses of the Ca^{2+} channel blockers were chosen after preliminary experiments and approximated those used in patients (for example, see Gelmers, 1983; Hugenholtz et al., 1984; Jensen et al., 1985). All measurements were repeated 10 min after the start of infusion of either Ca^{2+} channel blocker, both before and 10 min after another infusion of 5-HT ($2.0\ \mu g \cdot kg^{-1} \cdot min^{-1}$). In the third series data were recorded before and 10 and 20 min after an infusion of nifedipine ($0.75\ \mu g \cdot kg^{-1} \cdot min^{-1}$) had been started and once again 30 min after the infusion had been stopped.

2.5. Data presentation and statistical evaluation

Unless stated otherwise, all data in the text and illustrations are presented as means ± S.E.M. The significance of the differences between the variables was evaluated by Duncan's new multiple-range test after an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). The paired t-test was used to compare the 5-HT-induced changes during the infusion of nimodipine or nifedipine to the 5-HT-induced changes observed before the Ca^{2+} channel blockers. A P value of less than 0.05 (two-tailed) was considered to be statistically significant.

2.6. Drugs

The drugs, other than anaesthetics, used in this study were 5-hydroxytryptamine creatinine sulphate (Janssen Chimica, Beerse, Belgium), nimodipine and nifedipine (Bayer A.G., Wuppertal, F.R.G.). The doses refer to the base of the substances. The Ca^{2+} channel blockers were dissolved in a mixture of glycerol (60 g), H_2O (100 g) and polyethylene glycol (ad 1129 g), and the stock solution (nimodipine: $0.2\ mg \cdot ml^{-1}$; nifedipine: $0.1\ mg \cdot ml^{-1}$) was diluted with 0.9% NaCl to the concentrations used (nimodipine: $0.25\ \mu g \cdot kg^{-1} \cdot min^{-1}$; nifedipine: $0.75\ \mu g \cdot kg^{-1} \cdot min^{-1}$). The above solvent mixture has no effect on systemic or carotid haemodynamics (Duncker et al., 1986b).

3. Results

3.1. Systemic hemodynamics

Neither heart rate nor mean arterial blood pressure was affected by intracarotid infusions of 2.0

$\mu g \cdot kg^{-1} \cdot min^{-1}$ of 5-HT in the control period (fig. 1). Intracarotid administration of nimodipine (0.25 $\mu g \cdot kg^{-1} \cdot min^{-1}$) or nifedipine (0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$) did not change the heart rate but diminished blood pressure by 21 ± 3 and $20 \pm 3\%$, respectively. In the presence of either Ca^{2+} channel blocker, 5-HT was again unable to induce major changes in these hemodynamics parameters; only an $18 \pm 5\%$ increase in heart rate was noticed with 5-HT during treatment with nimodipine. The heart rate was unchanged during a 20 min infusion of 0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of nifedipine (series 3), while the mean arterial blood pressure decreased by 22 ± 1 and $32 \pm 3\%$ after 10 and 20 min, respectively. The values at baseline, at 10 and 20 min infusion of nifedipine and at 30 min after the end of the infuson were: 93 ± 4, 72 ± 3, 63 ± 2 and 75 ± 2 mmHg, respectively.

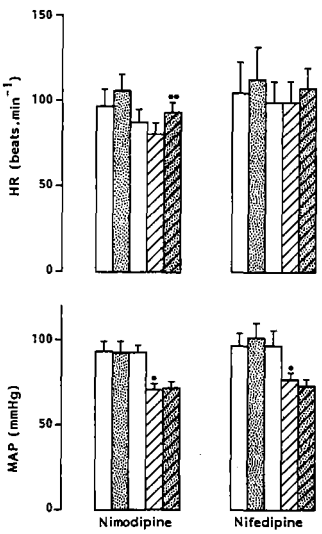

Fig. 1. Heart rate (HR) and mean arterial blood pressure (MAP) values in pigs before and after infusions of 5-HT (2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) given during the control period and during intracarotid infusions of nimodipine (0.25 $\mu g \cdot kg^{-1} \cdot min^{-1}$; left panels) or nifedipine (0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$, right panels). Note that while 5-HT did not cause any change in MAP and increased HR only slightly during nimodipine infusion, both Ca^{2+} channel blockers decreased MAP without affecting HR. * $P < 0.05$ vs. baseline or recovery; ** $P < 0.05$ vs. nimodipine or nifedipine. □ Baseline; ▨ 5-HT; □ recovery; □ nimodipine (left) or nifedipine (right); ▨ 5-HT + nimodipine (left) or nifedipine (right).

3.2. *Carotid hemodynamics*

3.2.1. *Distribution of common carotid artery blood flow during baseline*

As found earlier (Saxena and Verdouw, 1982; Verdouw et al., 1984b), under control (baseline) conditions, a major fraction of the total common carotid artery blood flow (244 ± 21 ml · min^{-1}; $n = 17$) was shunted via AVAs because $86 \pm 2\%$ of

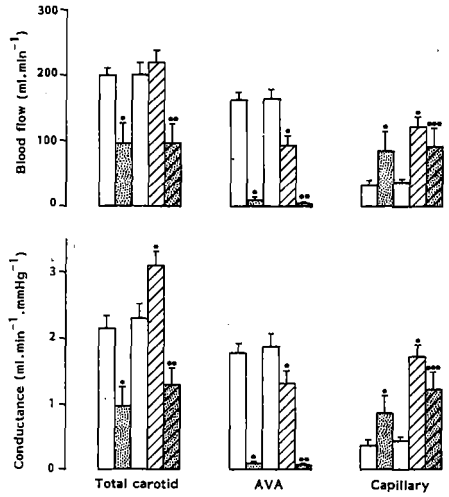

Fig. 2. Effect of intracarotid infusion of nimodipine (0.25 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on the changes induced by intracarotid infusions of 5-HT (2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in the total carotid blood flow and its distribution into non-nutrient (AVA) and nutrient (tissue; capillary) parts in pigs. Nimodipine, like 5-HT, reduced blood flow, and vascular conductance in the AVA fraction but increased the capillary fraction. Total carotid conductance was increased by nimodipine but was decreased by 5-HT. The 5-HT-induced vasoconstriction in the total carotid bed and its AVA fraction was not affected by nimodipine, but the tissue vasodilatation was reduced. * $P < 0.05$ vs. baseline or recovery; ** $P < 0.05$ vs. nimodipine; *** the 5-HT-induced change during nimodipine infusion was significantly different ($P < 0.05$) from the 5-HT-induced change before nimodipine. □ Baseline; □ 5-HT; □ recovery; ▨ nimodipine; ▨ 5-HT + nimodipine.

the injected microspheres was detected in the lungs whereas only a very minor fraction ($1.6 \pm 0.4\%$) was used to supply the brain. The blood flow to the extracerebral tissues was: skin, 1.5 ± 0.2 ml · min^{-1} ($0.6 \pm 0.1\%$); skeletal muscles, 6.1 ± 0.6 ml · min^{-1} ($2.6 \pm 0.2\%$) ears, 0.9 ± 0.2 ml · min^{-1} ($0.4 \pm 0.1\%$); tongue, 2.0 ± 0.5 ml · min^{-1} ($0.9 \pm 0.2\%$); salivary glands, 5.8 ± 1.1 ml · min^{-1} ($2.6 \pm 0.5\%$); eyes, 2.0 ± 0.3 ml · min^{-1} ($0.9 \pm 0.1\%$) and bone, 9.2 ± 1.3 ml · min^{-1} ($4.2 \pm 0.6\%$). Finally, less than 2% of the carotid blood flow was distributed to the contralateral half of the head and less than 0.1% of the microspheres was detected in the heart and kidneys, signifying an efficient entrapment of the spheres in the lung vasculature after their escape via the cranial AVAs.

3.2.2. Effects of nimodipine on the 5-HT-induced changes in the distribution of common carotid artery blood flow and vascular conductance

The effects of intracarotid infusions of 5-HT, before and during nimodipine infusion, on the distribution of carotid blood flow into nutrient (tissue; capillary) and non-nutrient (AVA) fractions are shown in fig. 2. 5-HT caused a decrease in total common carotid artery blood flow ($54 \pm 14\%$), which was entirely due to a reduction ($75 \pm 14\%$) in AVA blood flow; nutrient flow more than doubled. Similar changes were observed in vascular conductances, as the blood pressure was not much affected by 5-HT. In spite of a reduction in blood pressure by nimodipine, the total carotid blood flow was maintained as the total carotid

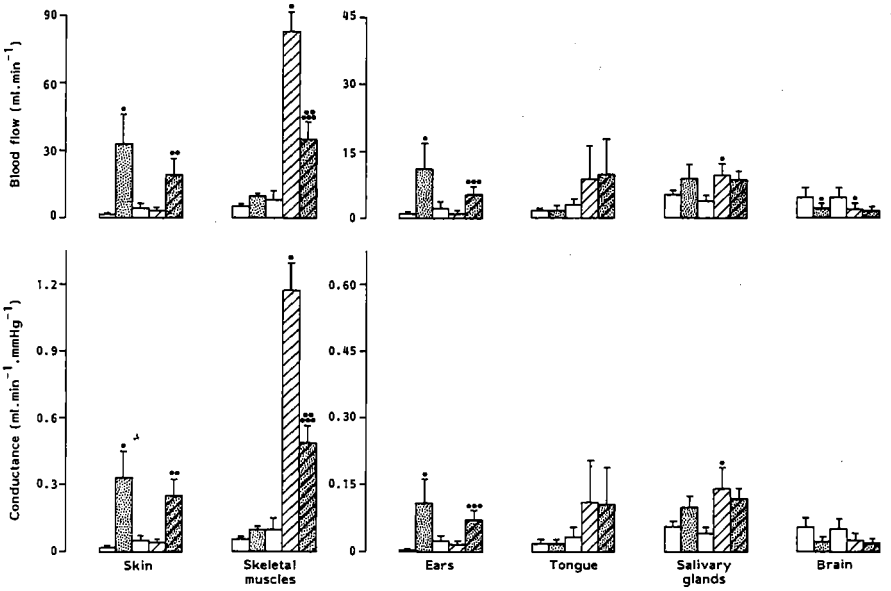

Fig. 3. Effect of intracarotid infusion of nimodipine (0.25 μg·kg^{-1}·min^{-1}) on the vasodilatation induced in various tissues by intracarotid infusions of 5-HT (2.0 μg·kg^{-1}·min^{-1}). While 5-HT produced its most prominent vasodilator effect in the skin and ears, nimodipine selectively and potently increased blood flow to and vascular conductance in the skeletal muscles. During nimodipine infusion, 5-HT caused vasoconstriction in the skeletal muscles, and its vasodilator response in the tissues was reduced. * $P < 0.05$ vs. baseline or recovery; ** $P < 0.05$ vs. nimodipine; *** the 5-HT-induced change during nimodipine infusion was significantly different ($P < 0.05$) from the 5-HT-induced change before nimodipine. □ Baseline; □ 5-HT; □ recovery; ▨ nimodipine; ▨ 5-HT + nimodipine.

conductance increased (41 ± 9%). This was accompanied by a large elevation (300 ± 25%) in vascular conductance in the nutrient part, which outweighed the 19 ± 7% reduction in vascular conductance in AVAs. Although nimodipine markedly affected vascular conductance in both the total carotid vascular bed and its AVA fraction, it had no influence on 5-HT-induced vasoconstrictor responses. In contrast, nimodipine converted the amine-evoked vasodilatation into vasoconstriction in the nutrient part.

Of all tissues and organs, the skin and ears showed the largest vasodilator response to 5-HT (18- and 12-fold increase in vascular conductance, respectively). Skeletal muscles exhibited a marked vasodilatation (15-fold increase in vascular conductance) during nimodipine infusion. Vascular conductance was also increased, but only moderately so in the salivary glands (fig. 3). In the presence of nimodipine, the 5-HT-induced vasodilatation in the skin and ears tended to be less marked. However, in the skeletal muscles 5-HT now elicited a marked vasoconstriction which reduced the nimodipine-induced vasodilatation by more than 50%. Although the carotid artery contribution to the blood supply of the brain decreased slightly in response to both 5-HT and nimodipine, vascular conductance in the brain did not change significantly. Tongue vessels were not affected by either drug.

3.2.3. Effects of nifedipine on the 5-HT-induced changes in the distribution of common carotid artery blood flow and vascular conductance

5-HT induced vasodilatation in the nutrient fraction of the carotid vascular bed (conductance increased by 350 ± 85%). This vasodilatation was unable to compensate for the decline (by 80 ± 5%) in AVA conductance, resulting in a 24 ± 3% reduction in vascular conductance of the total carotid bed (fig. 4). The decrease in mean arterial blood pressure during intracarotid nifedipine infusion was compensated for by a 4-fold elevation in vascular conductance of the capillary fraction which, by compensating for the slight decrease in AVA conductance, enhanced total carotid conductance. As a result, total carotid blood flow was maintained during nifedipine infusion. The 5-HT-induced vasoconstriction in the total carotid vascular bed was enhanced in the presence of nifedipine but that in the AVA part was unchanged. On the contrary, the vasodilator response to 5-HT in the nutrient (tissue; capillary) fraction was completely abolished (fig. 4).

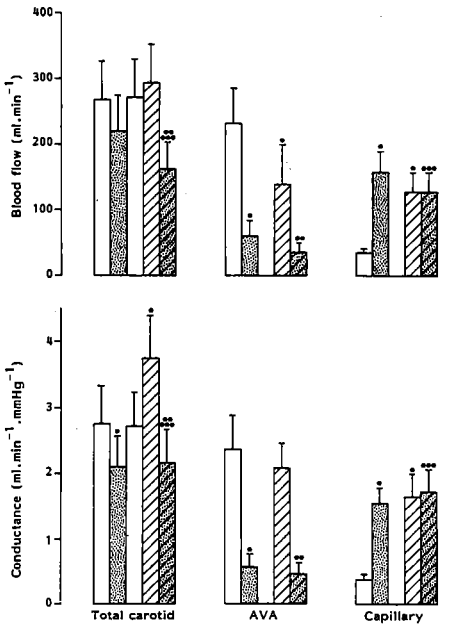

Fig. 4. Effect of intracarotid infusion of nifedipine (0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on the changes induced by intracarotid infusions of 5-HT (2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$) in the total carotid blood flow and its distribution into non-nutrient (AVA) and nutrient (tissue; capillary) parts in pigs. Nifedipine, like 5-HT, reduced blood flow to and vascular conductance in the AVA fraction but increased the capillary fraction. Total carotid conductance was increased by nifedipine but was decreased by 5-HT. The 5-HT-induced vasoconstriction was either not affected (AVA fraction) or was even enhanced (total carotid vascular bed) by nifedipine, but the 5-HT-induced tissue vasodilatation was reduced. * $P < 0.05$ vs. baseline; ** $P < 0.05$ vs. nifedipine; *** the 5-HT-induced change during nifedipine infusion was significantly different ($P < 0.05$) from the 5-HT-induced change before nifedipine. □ Baseline; ▨ 5-HT; □ recovery; ▨ nifedipine; ▨ 5-HT + nifedipine.

5-HT caused a 30-fold increase in vascular con-

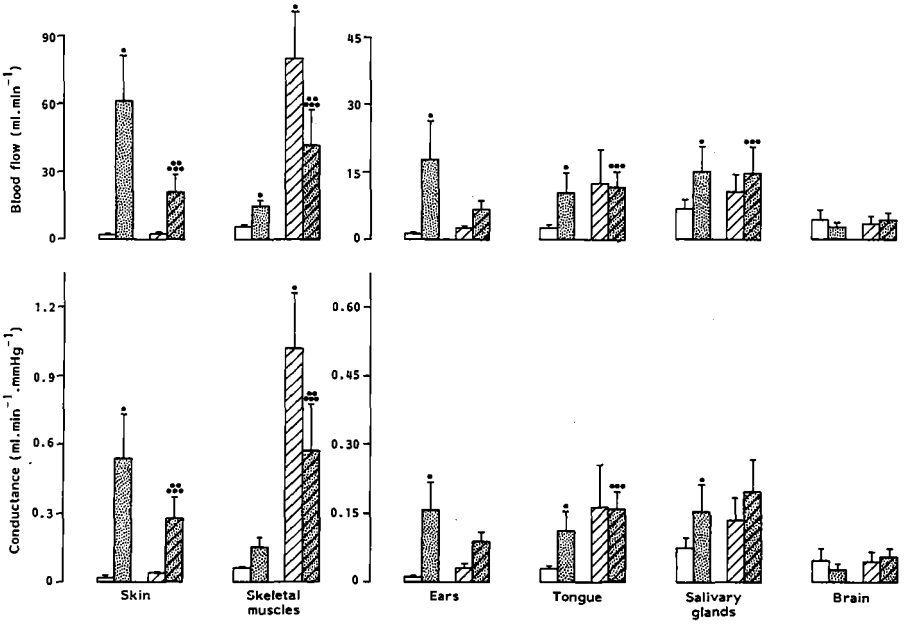

Fig. 5. Effect of intracarotid infusion of nifedipine (0.75 $\mu g \cdot kg^{-1} \cdot min^{-1}$) on the vasodilatation induced in various tissues by intracarotid infusions of 5-HT (2.0 $\mu g \cdot kg^{-1} \cdot min^{-1}$). While 5-HT produced its most prominent vasodilator effect in the skin and ears, nifedipine selectively increased blood flow to, and vascular conductance in the skeletal muscles. During nifedipine infusion, 5-HT caused vasoconstriction in the skeletal muscles and the vasodilator response in the tissues was reduced. * $P < 0.05$ vs. baseline; ** $P < 0.05$ vs. nifedipine; *** the 5-HT-induced change during nifedipine infusion was significantly different ($P < 0.05$) from the 5-HT-induced change before nifedipine. □ Baseline; ▨ 5-HT; ▨ nifedipine; ▩ 5-HT + nifedipine.

TABLE 1

Effects of 20 min intracarotid infusions of 0.75 $\mu g \cdot min^{-1} \cdot kg^{-1}$ nifedipine on carotid haemodynamics.

	Baseline value	Nifedipine		30 min of recovery
		10 min	20 min	
Blood flow ($ml \cdot min^{-1}$)				
Total carotid	272 ±31	270 ±37	227 ±31 [a,b]	224 ±30 [a]
AVA fraction [c]	244 ±28	154 ±26 [a]	134 ±25 [a]	193 ±29 [a]
Tissue fraction	28 ± 5	115 ±26 [a]	92 ±26 [a]	31 ± 6
Skeletal muscles	7.4 ± 1.4	84 ±24 [a]	60 ±21 [a]	10 ± 2
Vascular conductance ($ml \cdot min^{-1} \cdot mmHg^{-1}$)				
Total carotid	3.00± 0.43	3.80± 0.56 [a]	3.71± 0.58 [a]	3.03± 0.47
AVA fraction	2.69± 0.40	2.19± 0.41 [a]	2.21± 0.49 [a]	2.61± 0.46
Tissue fraction	0.30± 0.05	1.59± 0.35 [a]	1.50± 0.44 [a]	0.41± 0.08
Skeletal muscles	0.08± 0.02	1.18± 0.33 [a]	0.97± 0.35 [a]	0.13± 0.03

[a] $P < 0.05$ vs. baseline; [b] $P < 0.05$ min vs. 10 min observations. [c] AVA, arteriovenous anastomotic.

ductance in the skin (fig. 5), which was significantly attenuated when the animals were treated with nifedipine. Vasodilator responses to 5-HT (12- and 4-fold increase in vascular conductance, respectively) in the ears and tongue were also attenuated. 5-HT reduced the nifedipine-induced elevation (almost 16-fold) in vascular conductance in the skeletal muscles by approximately 50%.

3.2.4. Effects of nifedipine infusions alone

Table 1 shows the changes in the distribution of common carotid artery blood flow observed during a 20 min intracarotid infusion of nifedipine ($0.75\ \mu g \cdot kg^{-1} \cdot min^{-1}$) without concomitant administration of 5-HT. As described above, nifedipine decreased AVA flow and vascular conductance but increased capillary blood flow and vascular conductance, particularly to the skeletal muscles. There were no differences between the effects of nifedipine at the 10 and the 20 min period, except that the total carotid blood flow was decreased significantly after 20 min. The carotid hemodynamic variables returned towards baseline values (except total carotid blood flow) 30 min after the nifedipine infusion was stopped.

4. Discussion

4.1. Carotid vascular responses to 5-HT

As reported previously (Saxena and Verdouw, 1982; Verdouw et al., 1984a,b), a large fraction (about 80%) of the total common carotid blood flow in the anesthetized pig was shunted via AVAs located in the skin and ears (Saxena and Verdouw, 1985a). Infusion of 5-HT drastically changed the distribution of carotid blood flow so that, with or without a concomitant decrease in total carotid blood flow, the non-nutrient (AVA) fraction decreased and the nutrient (tissue; capillary) fraction, particularly that to the skin and ears, increased; recovery from the effects of 5-HT was observed within 20 min (Saxena and Verdouw, 1982; Verdouw et al., 1984b). These hemodynamic effects are due to activation of vascular $5\text{-}HT_2$ receptors causing constriction, mainly of the 'large' conductance vessels (Heistad et al., 1976; Saxena and Verdouw, 1982), and vascular '$5\text{-}HT_1$-like' receptors mediating constriction of AVAs and relaxation of arterioles (Saxena and Verdouw, 1985b; Saxena et al., 1986). Besides confirming the above findings with 5-HT, we now report the carotid vascular effects of two Ca^{2+} channel blockers of the dihydropyridine type (nimodipine and nifedipine) and their influence on the responses to 5-HT.

4.2. Carotid vascular effects of nimodipine and nifedipine

Local intracarotid infusions of both nimodipine and nifedipine resulted in a redistribution of carotid blood flow in favour of the nutrient fraction and at the expense of the non-nutrient fraction. While in the case of 5-HT the increased nutrient flow was distributed mainly to the skin and ears (Saxena and Verdouw, 1982; Verdouw et al., 1984b; present results), nimodipine and nifedipine selectively affected the skeletal muscles. It is of interest to recall that vascular conductance in, and blood flow to the skeletal muscles is also increased by other dihydropyridine-type Ca^{2+} channel blockers (darodipine, felodipine and nisoldipine), but not by verapamil or diltiazem (Hof, 1983; Bolt and Saxena, 1984; Duncker et al., 1986a,b). Low affinity but high capacity dihydropyridine binding sites, as yet unassociated with any specific functional response, have recently been described in the skeletal muscles (Miller and Freedman, 1984). It is tempting to suggest that the vascular effects in the skeletal muscles may be related in some way to the above binding sites.

The relaxation of arterioles is apparently due to Ca^{2+} channel blockade but the mechanism responsible for the decrease in AVA flow and conductance is less obvious. It is unlikely to be a baroreceptor reflex-mediated stimulation of the sympathetic nervous system since the animals were bilaterally vagosympathectomized. Moreover, in young pigs AVAs are only poorly constricted via noradrenergic mechanisms (Verdouw et al., 1984a). Thus, the reduction in AVA flow most likely results from 'steal' due to the profound vasodilatation in the nutrient vascular channels. This impression is enhanced when the data for nimodipine and nifedipine are compared to those for

5-HT. While 5-HT reduced the vascular conductance of AVAs by approximately 85% in the face of a 3-fold increase in conductance of the nutrient fraction, nimodipine and nifedipine reduced AVA conductance by, respectively, only 25 and 18%. These latter effects were accompanied by even greater increases in nutrient conductance (4.6- and 5.3-fold, respectively). It therefore seems that, in contrast to 5-HT which actively constricts AVAs, the Ca^{2+} channel blockers cause a 'passive' reduction in AVA flow and conductance.

4.3. Modification of carotid vascular responses to 5-HT by nimodipine and nifedipine

When nifedipine was infused alone for 20 min (series 3 experiments), the effects of the drug were not much different whether measured at 10 or 20 min. This suggests that the changes (from values after 10 min nifedipine infusion) obtained after simultaneous infusions of nifedipine and 5-HT (series 2 experiments) were in fact due to 5-HT and not to a major change in the effects of nifedipine with a longer period of infusion. The same may also hold true for nimodipine, though this drug was not infused alone for the full 20 min period.

A comparison of the responses to 5-HT before and after the infusions of the two Ca^{2+} channel blockers revealed three interesting facts: (i) the vasoconstrictor response in the total carotid vascular bed not only remained unattenuated but seemed to be enhanced; (ii) the increase in the tissue fraction due to 5-HT was completely eliminated (total capillary flow) or reduced (skin and ears); and (iii) blood vessels in the skeletal muscles – strongly dilated under the influence of the Ca^{2+} channel blockers – responded to 5-HT with a marked vasoconstriction.

The lack of attenuation by the Ca^{2+} channel blockers, nimodipine and nifedipine, of the 5-HT-induced constrictions in vivo of AVAs (mediated by '5-HT_1-like' receptors) or of 'small' resistance vessel (arterioles; mediated by 5-HT_2 receptors) suggests that, in contrast to the 5-HT-induced constrictions of 'large' conducting arteries in vitro (Towart, 1981; Müller-Schweinitzer and Neuman, 1983; Van Nueten, 1984), these responses are not dependent on influx of extracellular Ca^{2+}. A similar conclusion has recently been reached by others. Except for verapamil which has some affinity for 5-HT_2 binding sites (see Kalkman et al., 1984), a number of Ca^{2+} channel blockers (diltiazem, flunarizine, nimodipine or darodipine) failed to antagonize the 5-HT-induced elevations of perfusion and arterial blood pressures in the rat (Cavero and Lefèvre-Borg, 1981; Kalkman et al., 1984) and the decrease in total peripheral AVA flow in the cat (Hof et al., 1985). Hof et al. (1985) also observed that 5-HT, which did not significantly alter coronary haemodynamics, caused an unmistakable coronary vasoconstriction when given after darodipine.

Unlike the vasoconstrictor responses, the 5-HT-induced vasodilatation, particularly that in the skin vasculature, was reduced by the two Ca^{2+} channel blockers. It appears to us that the attenuated increase in flow to, and the vascular conductance in the skin is secondary to the strong vasodilatation elicited by the two Ca^{2+} channel blockers in other parts of the nutrient bed mainly of the skeletal muscles. When the blood vessels of skeletal muscles were strongly relaxed under the influence of the Ca^{2+} channel blockers, it is logical to expect that 5-HT elicited no further vasodilatation but caused arterial vasoconstriction which is mediated by 5-HT_2 receptors (Verdouw et al., 1984b; Saxena and Lawang, 1985; Meschig et al., 1985). Unlike the vasoconstriction in 'large' vessels in vitro, this vasoconstriction was not inhibited by nifedipine and nimodipine in vivo (see above).

4.4. Possible implications in migraine

Cerebral blood flow has been reported to decrease, probably as a result of 'spreading depression of Leão', in 'classical' but not in 'non-classical' migraine patients (Lauritzen and Olesen, 1984). If the release of 5-HT is involved, Ca^{2+} channel blockers probably do not act via this mechanism as shown by their ineffectiveness against the vasoconstrictor responses to 5-HT in vivo.

As mentioned at the outset, sudden opening of cranial AVAs in the headache phase (Heyck, 1969;

Saxena, 1978), probably associated with the decrease in blood 5-HT level (Lance, 1978; Fozard, 1982), has also been pathophysiologically implicated in migraine. Compatible with this suggestion, antimigraine drugs – particularly the ergot alkaloids – effective in the treatment of individual attacks (Johnson and Saxena, 1978; Spierings and Saxena, 1980) as well as 5-HT (Saxena and Verdouw, 1982; Verdouw et al., 1984b) which has been reported to alleviate acute attacks (Kimball et al., 1960) decrease AVA shunting by eliciting an 'active' constriction of cranial AVAs. The 'passive' reduction of AVA flow induced by the two Ca^{2+} channel blockers may be one of the reasons for their inability to abort acute attacks of migraine (Jensen et al., 1985). The redistribution of carotid blood flow towards the nutrient compartment, as observed in the present study, should obviously be considered as potentially beneficial. Whether this property of Ca^{2+} channel blockers is linked to the usefulness of these drugs in the prophylactic therapy of migraine (Gelmers, 1983; Meyer and Hardenberg, 1983) remains to be ascertained.

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VASODILATORY PROFILE OF PYRIDAZINONE-DERIVATIVES

CHAPTER 6

CARDIOVASCULAR PROFILE OF PIMOBENDAN, A BENZIMIDAZOLE-PYRIDAZINONE DERIVATIVE WITH VASODILATING AND INOTROPIC PROPERTIES

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CARDIOVASCULAR PROFILE OF PIMOBENDAN, A BENZIMIDAZOLE-PYRIDAZINONE DERIVATIVE WITH VASODILATING AND INOTROPIC PROPERTIES

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P.D. VERDOUW, J.M. HARTOG, D.J. DUNCKER, W. ROTH and P.R. SAXENA, *Cardiovascular profile of pimobendan, a benzimidazole-pyridazinone derivative with vasodilating and inotropic properties,* European J. Pharmacol. 126 (1986) 21–30.

Intravenous infusions of 0.01-0.1 mg · kg^{-1} · min^{-1} of pimobendan, a benzimidazole-pyridazinone derivative in pigs with normal coronary circulation caused dose-dependent changes in heart rate (10-35%), left ventricular systolic pressure (−5 to −45%), left ventricular filling pressure (−20 to −40%) but had only a minor effect on the maximum rate of rise of left ventricular pressure (max LVdP/dt; 10-20%). The decrease in mean arterial blood pressure was primarily due to systemic vasodilation; peripheral resistance and cardiac output decreased by up to 40 and 14%, respectively. Vasodilation occurred in several vascular beds, but was particularly pronounced in the adrenals, stomach, small intestine and myocardium. Although the increase in myocardial blood flow favoured the epicardium, vascular conductance in both the endo- and epicardial layers was significantly increased. Myocardial O_2 consumption ($M\dot{V}O_2$) was not affected despite the increase in heart rate. Bolus injections of 0.1-0.5 mg · kg^{-1} pimobendan produced similar changes in all haemodynamic variables, except max LVdP/dt which now increased by 30-70%. As in the infusion experiments, cardiac output tended to decrease due to a pronounced reduction in ventricular preload probably as a result of venodilation and the consequent reduction in cardiac filling. However, in animals where max LVdP/dt and cardiac output were reduced and pre- and/or after-load were increased by partial occlusion of the left anterior descending coronary artery, pimobendan clearly increased both max LVdP/dt and cardiac output. Pretreatment with propranolol did not modify any of the cardiovascular responses to pimobendan, thereby excluding the involvement of a β-adrenoceptor mechanism. Pimobendan is thus a compound with vasodilator and positive inotropic properties that improves cardiac output in animals with severe myocardial ischaemia. The finding that the mild tachycardia caused by pimobendan was not accompanied by an increase in $M\dot{V}O_2$ warrants investigation to evaluate its usefulness in the treatment of heart failure.

Distribution of cardiac output	Myocardial O_2 consumption	Benzimidazole pyridazinone	Pimobendan
β-Adrenoceptor blockade	Phosphodiesterase inhibitor	Inotropic agent	Tachycardia
Myocardial ischaemia	UD-CG 115 BS	Vasodilation	Pig

1. Introduction

Heart failure is a pathophysiological state in which the heart is unable to pump blood at a rate commensurate with the metabolic needs of body tissues. Although myocardial dysfunction is often the cause of heart failure, other conditions (for example, a sudden overload of the heart or a reduction of cardiac filling) can lead to heart failure even in the presence of a normal myocardium (Braunwald, 1984). Thus, in dealing with heart failure one should consider not only the myocardium but also the peripheral circulation as,

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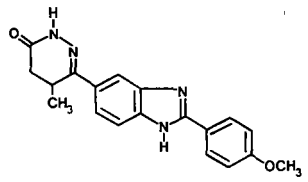


Fig. 1. Chemical structure of pimobendan (UD-CG 115 BS).

in addition to a depressed myocardial contractility, elevated left ventricular filling pressure and systemic vascular resistance are among prominent characteristics of heart failure. It is therefore obvious that positive inotropic agents which also cause peripheral vasodilation may be useful in the treatment of heart failure.

In an attempt to provide efficient substitutes for cardiac glycosides, which have a narrow therapeutic margin, many positive inotropic agents acting via different mechanisms including inhibition of cardiac phosphodiesterases, have been described during the past several years (see Farah et al., 1984). One such compound, pimobendan (UD-CG 115 BS; fig. 1) has been shown to increase myocardial contractility and cause peripheral vasodilation (Diederen et al., 1982; Van Meel, 1985) possibly due to elevation of cyclic AMP levels following inhibition of myocardial phosphodiesterase (Honerjäger et al., 1984). In this report we give a detailed description of the cardiovascular profile of the drug, including its effects on regional vascular beds and myocardial performance.

2. Materials and methods

2.1. Experimental set-up

After an overnight fast Yorkshire pigs (22-28 kg) were sedated with 120 mg azaperone i.m. and anaesthetized with 150 mg i.v. metomidate. The levels of arterial blood gases (ABL-3, Acid-Base Laboratory, Radiometer, Copenhagen) were controlled by artificial ventilation with a mixture of $O_2 : N_2O$ (1 : 2). Catheters were placed in the superior vena cava for administration of pentobarbital (20 $mg \cdot kg^{-1} \cdot h^{-1}$), sodium bicarbonate (8.4%, when needed), pimobendan and haemaccel; the latter was given to replace blood loss. Micro-tipped Millar catheters were used for measurements of the left ventricular and central aortic blood pressures. An 8 F catheter was positioned in the descending aorta for the withdrawal of arterial blood samples. The muscle relaxant pancuronium (4 mg) was administered before the heart was exposed via a midsternal split. Electromagnetic flow probes (Skalar, Delft, The Netherlands) were placed around the ascending aorta and the left anterior descending coronary artery (LAD). The great cardiac vein was cannulated and the left atrial appendage was catheterized for the injection of microspheres.

Myocardial wall thickness was monitored with a 5 MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, PA, USA). The wall thickness at end-diastole (EDT) and end-systole (EST) was used to calculate systolic wall thickening (swt) as:

$$\text{swt}\,(\%) = 100 \times (\text{EST-EDT})/\text{EDT}$$

and the mean velocity of swt ($\bar{V}_{swt}$) as:

$$\bar{V}_{swt} = (\text{EST-EDT})/\text{DS}$$

where DS is the duration of the isovolumic contraction phase and the ejection time.

The distribution of cardiac output was measured using the microsphere technique (see Saxena and Verdouw, 1985; Verdouw et al., 1985). About 2×10^6 microspheres (15 ± 1 (S.D.)μm diameter, NEN Company, Dreieich, West Germany), labelled with either ^{46}Sc, ^{103}Ru, ^{141}Ce, ^{95}Nb or ^{113}Sn, were injected via a cannula placed in the left atrium of the heart. A reference arterial blood sample was withdrawn (flow rate, 10 $ml \cdot min^{-1}$) starting just before and continuing for a period of about 1 min after the injection of microspheres. At the end of the experiment several organs (see later) were excised, weighed and placed in vials. The heart was removed and fixed in 4% formalin for at least 24 h. The details of the counting of radioactivity and processing of data have been described previously (Saxena et al., 1980).

Myocardial oxygen consumption ($M\dot{V}O_2$) was estimated by multiplying the difference in the O_2

content of the arterial and coronary venous blood by coronary blood flow.

2.2. Experimental protocols

Four series of experiments were performed to characterize the cardiovascular actions of pimobendan. In the first series (n = 8), four consecutive 10 min infusions (0.01, 0.025, 0.05 and 0.10 mg · $kg^{-1} \cdot min^{-1}$) were used. At the end of each infusion all cardiovascular variables were recorded and arterial blood was drawn for the determination of plasma concentrations of the parent compound and its major metabolite (UD-CG 212 BS). In the second series (n = 9), each animal received three consecutive bolus injections of pimobendan at 10 min intervals. The doses (0.1, 0.25 and 0.5 mg · kg^{-1}) were chosen such that the animals received the same amount of drug as administered during each comparable infusion period in the first series.

To evaluate cardiac pump function during pathological conditions, blood flow in the left anterior descending coronary artery was reduced in 4 animals (third series) by tightening a J-shaped clamp placed around the vessel until the systolic wall thickening was reduced to 20-30% of the baseline value. After the haemodynamic values had been stable for at least 15 min these animals were given pimobendan (0.25 mg · kg^{-1} + 0.05 mg · $kg^{-1} \cdot min^{-1}$). Lastly, in order to establish whether or not some of the actions of pimobendan were caused via β-adrenoceptor stimulation, the cardiovascular effects of the drug were studied in 5 pigs (fourth series) before and after pretreatment with propranolol (0.5 mg · kg^{-1} followed by 0.5 mg · $kg^{-1} \cdot h^{-1}$). This dose regimen of propranolol provides adequate β-adrenoceptor blockade in pigs (Wolffenbuttel and Verdouw, 1983).

2.3. Determination of plasma concentrations

The plasma concentrations of pimobendan and its O-demethylmetabolite, UD-CG 212 BS were determined using an HPLC assay with fully automated drug preconcentration on solid supports (Roth, 1983). Briefly, the drugs were extracted on a reverse phase column and simultaneously preconcentrated after injection of whole plasma. The compounds were measured by means of fluorescence detection (332 nm/405 nm) after HPLC separation on reversed phase ODS-hypersil (particle size: 5 μm). The eluent composition was methanol/water 590/460 (v/v) + 2.5 g ammonium acetate per liter eluent (total amount 2.625 g). Post column, a mixture of methanol/orthophosphoric acid 85%/water (300/100/100, v/v/v) was added with a flow rate of 0.2 ml · min^{-1} via a T-fitting in order to optimize the fluorescence (increase in fluorescence by a factor of 2). The lower limit of detection for both compounds was about 1 ng/ml. Pimobendan and UD-CG 212 BS themselves were used as external standards.

2.4. Data presentation and statistical analysis

All data are presented as means ± S.E.M. Absolute values are given in the tables and in fig. 3, but % changes from baseline values are shown in the other figures to facilitate comparison between different variables. However, all statistical analyses were performed on the actual data values. The significance of the difference between the means of any two groups was compared by applying Duncan's new multiple range test once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Saxena, 1985). Statistical significance was accepted at $P < 0.05$ (two-tailed).

2.5. Drugs

The only drugs used in this study were the anaesthetics, haemaccel, propranolol hydrochloride and pimobendan (4,5-dihydro-6-[2-(4-methoxyphenyl)-1H-benzimidazol-5-yl]-5-methyl-3(2H)-pyridazinone; UD-CG 115 BS). Pimobendan was dissolved in a mixture of polyethylene glycol 200 and saline (1 : 1).

3. Results

3.1. Intravenous infusion of pimobendan in animals with normal coronary circulation

3.1.1. Plasma concentrations of pimobendan and UD-CG 212 BS

The plasma concentrations of the parent drug increased rapidly during intravenous infusions of pimobendan. There was a more than 14-fold difference between the concentrations noted at the end of the first and the last infusion period. The concentration of the major metabolite, UD-CG 212 BS, increased only 6-fold during the same period (table 1) suggesting saturation of the enzymes metabolizing pimobendan during higher infusion rates.

3.1.2. Systemic haemodynamics

The most pronounced effect of pimobendan was the vasodilation in systemic vascular beds as the dose-dependent decreases in arterial blood pressure (up to 45%) agreed very closely with those in systemic vascular resistance (table 1). Diastolic arterial pressure declined slightly more than systolic arterial blood pressure (up to 52 and 44%, respectively). Pimobendan also decreased the left ventricular filling pressure, indicating reduced venous return and, therefore, venodilation. This probably accounts for the slight decrease in cardiac output at the two highest infusion rates. Max LVdP/dt was moderately enhanced (up to 20%) with the two lowest doses, but was not different from baseline during the two highest infusion rates.

3.1.3. Regional haemodynamics

Table 2 shows that, despite the reduction in cardiac output, pimobendan increased blood flow to the adrenals, stomach, small intestine and brain, but decreased that to the kidneys and spleen. Hepatic arterial (liver) and skeletal muscle blood flow did not change significantly after pimobendan. Since arterial blood pressure was decreased by the drug, vascular conductance increased dose dependently in most organs (fig. 2). The most marked effect was on the stomach (up to 325%) followed by adrenals (200%), small intestine (190%), myocardium (160%; see following section), skeletal muscles (140%) and brain (130%). No differences existed between the effects of pimobendan on different brain areas (cerebral hemispheres, diencephalon, cerebellum and brainstem; data not shown in the figure). Vascular conduc-

TABLE 1

Cardiovascular actions of continuous 10 min pimobendan infusions in 8 anaesthetized open-chest pigs. Abbreviations: CO, cardiac output; HR, heart rate; SV, stroke volume; LVEDP, left ventricular end-diastolic pressure; LVdP/dt, maximal rate of rise of left ventricular pressure; SVR, systemic vascular resistance; SAP, systolic arterial pressure (mm Hg); DAP, diastolic arterial pressure (mm Hg). All data were obtained after 10 min of infusions. All data are presented as means ± S.E.M. * $P < 0.05$ vs. baseline.

	Baseline	Pimobendan ($mg \cdot kg^{-1} \cdot min^{-1}$)			
		0.01	0.025	0.05	0.1
Total dose injected ($mg \cdot kg^{-1}$)	–	0.1	0.35	0.85	1.85
Plasma concentration ($\mu g \cdot l^{-1}$)					
Pimobendan	–	100 ± 10	274 ± 19	689 ± 35	1441 ± 61
UD-CG 212 BS	–	3.1 ± 0.3	9.1 ± 0.6	14.4 ± 1.2	20.2 ± 1.8
CO ($l \cdot min^{-1}$)	2.16 ± 0.11	2.15 ± 0.11	2.01 ± 0.12	1.90 ± 0.11 *	1.85 ± 0.14 *
HR ($beats \cdot min^{-1}$)	84 ± 4	92 ± 4 *	104 ± 5 *	112 ± 6 *	114 ± 7 *
SV (ml)	26 ± 2	24 ± 1	20 ± 2 *	17 ± 2 *	17 ± 2 *
LVEDP (mm Hg)	10 ± 1	8 ± 1 *	6 ± 1 *	6 ± 1 *	6 ± 1 *
LVdP/dt ($mm\ Hg \cdot s^{-1}$)	1460 ± 160	1680 ± 170 *	1770 ± 210 *	1620 ± 210	1570 ± 230
SVR ($mm\ Hg \cdot l^{-1} \cdot min$)	39 ± 3	37 ± 3	30 ± 2 *	25 ± 1 *	22 ± 1 *
SAP (mm Hg)	102 ± 4	95 ± 5 *	79 ± 3 *	64 ± 3 *	57 ± 2 *
DAP (mm Hg)	63 ± 4	59 ± 4	49 ± 4 *	37 ± 3 *	30 ± 2 *

TABLE 2

Effect of continuous 10 min pimobendan infusions on organ blood flow (ml·min^{-1}·100 g^{-1}) in 8 anaesthetized open-chest pigs. All data are presented as means ± S.E.M.; * P = < 0.05 vs. baseline.

	Baseline	Pimobendan (mg·kg^{-1}·min^{-1})			
		0.01	0.025	0.05	0.1
Kidneys	322 ± 27	351 ± 22	318 ± 32	217 ± 28 *	156 ± 23 *
Liver	30 ± 9	27 ± 8	24 ± 6	22 ± 4	21 ± 5
Spleen	80 ± 13	101 ± 20	84 ± 17	51 ± 11 *	33 ± 8 *
Adrenals	136 ± 22	166 ± 24 *	189 ± 29 *	194 ± 19 *	181 ± 20 *
Stomach	14 ± 1	17 ± 2	19 ± 2 *	24 ± 3 *	27 ± 3 *
Small intestine	21 ± 1	22 ± 2	22 ± 1	26 ± 2 *	28 ± 2 *
Skeletal muscles	7 ± 1	7 ± 1	6 ± 1	7 ± 2	8 ± 1
Brain	22 ± 2	22 ± 1	22 ± 2	23 ± 1	25 ± 2 *

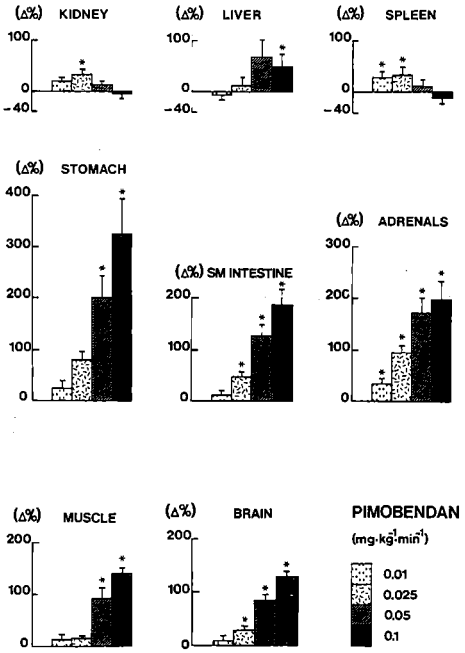


Fig. 2. Effect of intravenous infusion of pimobendan on regional vascular conductances. Pre-infusion values of vascular conductances (in ml·min^{-1}·mm Hg^{-1}·100 g^{-1}) were: kidneys: 3.98 ± 0.39; liver: 0.37 ± 0.10; spleen: 0.94 ± 0.13; stomach 0.18 ± 0.02; small intestine: 0.25 ± 0.03; adrenals: 1.68 ± 0.29; skeletal muscles 0.08 ± 0.02 and brain: 0.27 ± 0.02. Data are presented as percentage changes from baseline, but the statistical analysis was performed on the actual data. * P < 0.05 vs. baseline.

tance in the kidneys, liver and spleen was much less affected. All three showed increases at lower doses (< 50%) but the effect tended to decrease as perfusion pressure started to drop severely.

3.1.4. Myocardial blood flow and performance

Coronary blood flow increased up to 30% in spite of the decline in arterial blood pressure (fig. 3). This increase in flow was not evenly distributed transmurally as the epicardium benefited more (up to 43%) than the endocardial layers (up to 15%) during the first three infusion rates. There was even a slight decrease in the endocardial blood flow at the highest infusion rate (0.1 mg·kg^{-1}·min^{-1}). Consequently, the endo-epi flow ratio decreased gradually from 0.99 ± 0.03 to 0.70 ± 0.02 (P < 0.05) at the end of the highest infusion rate. Coronary venous O_2 saturation increased dose dependently from 37 ± 5 to 58 ± 3% (P < 0.05). Hence $M\dot{V}O_2$ was not significantly affected (fig. 3). Regional myocardial function showed no changes for systolic wall thickening but a gradual increase in the velocity of thickening was noticed. Figure 4 shows that, although the vasodilator response to pimobendan in the epicardial layers exceeded that in the endocardial layers, vascular conductance in the latter was also doubled at the end of the highest infusion rate. Though not shown in the figure, the increases in vascular conductance in the right ventricle (up to 180 ± 12%) and left atrium (up to 110 ± 20%) were very similar to those in the left ventricle.

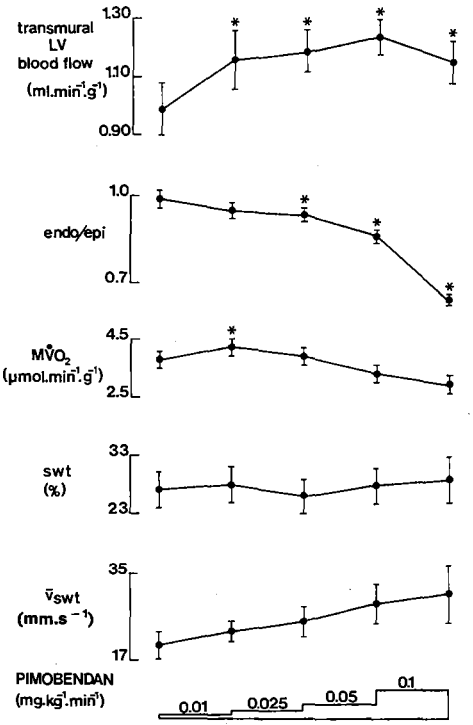


Fig. 3. Left ventricular performance after intravenous infusions of pimobendan at increasing rates. From top to bottom: transmural left ventricular blood flow, the endo-epi blood flow ratio, left ventricular O_2 consumption ($M\dot{V}O_2$), regional systolic wall thickening (swt) and the mean velocity of wall thickening ($\bar{V}_{swt}$). * $P < 0.05$ vs. baseline.

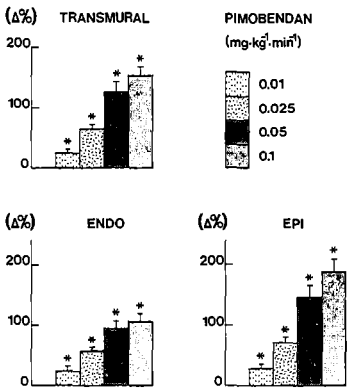


Fig. 4. Effect of intravenous infusion of pimobendan on left ventricular vascular conductance. Vasodilation of the epicardial vessels exceeded that of the endocardial vessels, which explains the decrease in the endo-epi blood flow ratio. Pre-infusion transmural conductance was 1.26 ± 0.22 ml·min^{-1}·mm Hg^{-1}·100 g^{-1}. * $P < 0.05$ vs. baseline. Data are presented as percentage changes from baseline but the statistical analysis was performed on the actual data.

3.2. Bolus injections in animals with normal coronary circulation

3.2.1. Plasma concentrations of pimobendan and UD-CG 212 BS

Following each bolus injection of pimobendan, the plasma levels of the parent drug increased sharply but, at 10 min, they were considerably lower than those after infusions of the corresponding amounts. As was the case in the infusion experiments, the plasma concentration of the metabolite UD-CG 212 BS increased but less steeply than that of the parent drug (table 3).

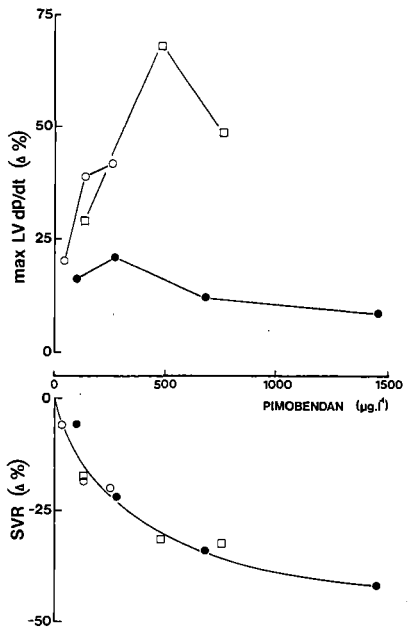
3.2.2. Systemic haemodynamics

As in the infusion experiments, a pronounced vasodilation accompanied by tachycardia occurred after bolus injections of pimobendan (table 3). Cardiac output decreased slightly (19%) after the highest dose as the increase in heart rate (up to 35%) was not sufficient to balance the decrease in stroke volume (up to 40%). The latter appeared to be caused by the fall in left ventricular filling pressure (up to 62%) as systemic vascular resistance decreased (up to 40%) and max LVdP/dt increased by almost 70% in spite of the fall in diastolic arterial blood pressure (table 3). The substantial increase in max LVdP/dt after the bolus injections contrasts sharply with the minor changes observed when the drug was infused (series 1). This is best illustrated in fig. 5 which shows that the larger increases occurred at comparable reductions in systemic vascular resistance. Another striking feature was that the increase in max LVdP/dt was lower with 0.5 mg · kg^{-1} than with 0.25 mg · kg^{-1} despite similar after- and preload reductions.

TABLE 3

Cardiovascular actions of cumulative bolus injections of pimobendan in 9 anaesthetized open-chest pigs. Abbreviations: CO, cardiac output; HR, heart rate; SV, stroke volume; LVEDP, left ventricular end-diastolic pressure; LVdP/dt, maximal rate of rise of left ventricular pressure; SVR, systemic vascular resistance; SAP, systolic arterial pressure; DAP, diastolic arterial pressure; [a] peak refers to the time at which the effect on max LVdP/dt was maximum. All data are presented as means ± S.E.M.; * $P < 0.05$ vs. baseline.

	Baseline	Pimobendan ($mg \cdot kg^{-1}$)					
		0.1 (n = 9)		0.25 (n = 9)		0.5 (n = 7)	
		Peak [a]	10 min	Peak	10 min	Peak	10 min
Total dose injected ($mg \cdot kg^{-1}$)	–	0.1		0.35		0.85	
Plasma concentration ($\mu g \cdot l^{-1}$)							
Pimobendan	–	124 ± 22	34 ± 3	484 ± 59	129 ± 12	757 ± 68	273 ± 26
UD-CG 212 BS	–	1.7 ± 0.4	5.4 ± 1.6	7.8 ± 1.6	11.2 ± 1.4	12.4 ± 1.9	17.4 ± 1.8
CO ($l \cdot min^{-1}$)	2.46 ± 0.18	2.46 ± 0.16	2.37 ± 0.15	2.47 ± 0.12	2.21 ± 0.17	2.40 ± 0.20	2.06 ± 0.16 *
HR ($beats \cdot min^{-1}$)	102 ± 5	110 ± 5 *	114 ± 6 *	121 ± 7 *	128 ± 5 *	127 ± 5 *	135 ± 6 *
SV (ml)	24 ± 1	24 ± 1	21 ± 1 *	21 ± 1	17 ± 1 *	19 ± 1 *	15 ± 1 *
LVEDP (mm Hg)	9 ± 1	6 ± 1 *	6 ± 1 *	5 ± 1 *	5 ± 1 *	4 ± 1 *	4 ± 1 *
LVdP/dt ($mm\ Hg \cdot s^{-1}$)	1750 ± 20	2230 ± 20 *	2080 ± 20 *	3100 ± 30 *	2540 ± 20 *	2690 ± 30 *	2570 ± 20 *
SVR ($mm\ Hg \cdot l^{-1} \cdot min$)	38 ± 5	32 ± 4 *	36 ± 4	26 ± 4 *	31 ± 3 *	25 ± 3 *	29 ± 3 *
SAP (mm Hg)	107 ± 5	98 ± 5 *	96 ± 5 *	80 ± 5 *	81 ± 5 *	77 ± 4 *	76 ± 3 *
DAP (mm Hg)	71 ± 4	63 ± 5 *	66 ± 5	47 ± 4 *	53 ± 4 *	45 ± 4 *	48 ± 4 *



3.3. *Bolus injections in animals with ischaemic hearts*

Reduction of the lumen of the left anterior descending coronary artery did not affect heart rate but led to an increase in the left ventricular end-diastolic pressure (from 8.4 ± 1.4 to 19.6 ± 1.7 mm Hg) and diminutions in max LVdP/dt (25%), cardiac output (by 26%), stroke volume (23%) and regional systolic wall thickening (from 24 ± 2 to 9 ± 2%). A large fall in mean arterial blood pressure (9%) was prevented by the vasoconstriction (23%) in systemic vascular beds. After the preparation had been stable for 15 min, pimobendan (0.25 $mg \cdot kg^{-1}$ over 2 min + 0.05 $mg \cdot kg^{-1}$ ·

Fig. 5. Changes in max LVdP/dt (top) and total systemic vascular resistance (SVR, bottom) as a function of arterial plasma concentrations of pimobendan after infusions or bolus injections (peak and 10 min later). Notice that the changes in SVR but not those in max LVdP/dt depend on the plasma concentrations. The latter is true even when the effects on heart rate and pre- and afterload are taken into account (compare tables 1 and 3). * $P < 0.05$ vs. baseline. □ Pimobendan bolus (peak); ○ pimobendan bolus (10 min); ● pimobendan infusion.

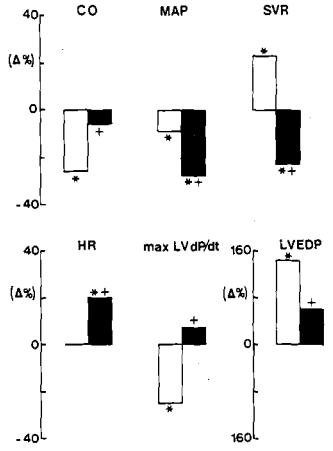


Fig. 6. Effect of pimobendan (0.25 mg·kg^{-1} + 0.05 mg·kg^{-1}·min^{-1}) in 4 pigs with a partial stanosis in the left anterior descending coronary artery. The ischemia (□) and pimobendan (■) induced changes are given as percentage change from baseline. Baseline values were: cardiac output (CO, 2.44 ± 0.25 l·min^{-1}); mean arterial blood pressure (MAP, 74 ± 5 mm Hg); systemic vascular resistance (SVR, 31 ± 5 mm Hg·l^{-1}·min); heart rate (HR, 81 ± 1 beats·min^{-1}); max LVdP/dt (1630 ± 220 mm Hg·s^{-1}) and left ventricular end-diastolic blood pressure (LVEDP, 8.4 ± 1.4 mm Hg). * $P < 0.05$ vs. baseline; $^{+}$ $P < 0.05$ vs. ischaemia. Data are presented as percent changes from baseline but the statistical analysis was performed on the actual data.

min^{-1} for 5 min) was administered. The drug lowered left ventricular filling pressure to 12.6 ± 1.8 mm Hg and systemic vascular resistance (45%), but increased heart rate (20%) and max LVdP/dt (30%). Stroke volume did not change significantly, perhaps due to a reduction in left ventricular filling following the increase in heart rate. These observations are akin to those in the animals with a normal coronary circulation. However, the increase in cardiac output in the ischaemic preparation (20%, fig. 6) was at variance with the results of these experiments.

3.4. Cardiovascular actions of pimobendan after β-adrenoceptor blockade

A bolus of 0.5 mg·kg^{-1} propranolol followed by an infusion of 0.5 mg·kg^{-1}·h^{-1} lowered heart rate (from 89 ± 14 to 75 ± 7 beats·min^{-1}), max LVdP/dt (from 1930 ± 290 to 1280 ± 110 mm Hg·s^{-1}) and mean arterial blood pressure (from 83 ± 2 to 74 ± 4 mm Hg). Cardiac output was reduced to the same extent as arterial blood pressure so that peripheral resistance did not change. Pimobendan-induced tachycardia, increase in cardiac contractility and systemic vasodilation were not modified in the presence of β-blockade. The changes caused by 0.5 mg·kg^{-1} pimobendan before and after propranolol, respectively, were 27 ± 6 and 27 ± 5% (heart rate), 49 ± 13 and 47 ± 14% (max LVdP/dt) and −32 ± 6 and −38 ± 5% (systemic vascular resistance). Coronary venous O_2 saturation, which had decreased from 18 ± 3 to 13 ± 3% after β-blockade, increased dose dependently to 39 ± 7% ($P < 0.05$) during pimobendan administration, a response again not different from that observed with pimobendan alone.

4. Discussion

The investigation showed that pimobendan caused a vasodilation and cardiac stimulation which were not mediated by β-adrenoceptors. The vasodilator activity of pimobendan was demonstrated in animals that received the drug either as infusions or as bolus injections. The magnitude of the effect was closely related to the arterial plasma concentrations and appeared to be independent of the rate of drug administration. In contrast, the inotropic effects of pimobendan, evaluated by changes in max LVdP/dt, were less obvious. Bolus injections caused marked increases, but when the plasma levels were raised gradually by infusion, the changes in max LVdP/dt were only minimal. One must, however, be careful when using max LVdP/dt as an index of contractility as this parameter also depends on heart rate and pre- and afterload, all of which changed considerably with the drug. In pigs, an increase in heart rate from 60 to 100 beats·min^{-1} causes a 15% increase in max LVdP/dt, but further increases have no additional effect (Verdouw et al., 1980a,b; Scheffer and Verdouw, 1983). Since the heart rate ranged from 80 to 140 beats·min^{-1} in the present study, it is unlikely that tachycardia alone could account for

the increase in max LVdP/dt. Moreover, because of the fall in left ventricular end-diastolic (probably due to venodilation) and arterial blood pressures, the increases in inotropic state were underestimated by the changes in max LVdP/dt. At variance with the changes in total systemic vascular resistance, the effects of pimobendan on max LVdP/dt were not related to the plasma concentrations of the drug (fig. 5). During the infusion experiments higher concentrations were accompanied by lower max LVdP/dt increments, even at comparable changes in heart rate, pre- and afterload. It has been suggested that the metabolite UD-CG 212 BS possesses positive inotropic properties (Meyer, unpublished data). However, in our study the concentrations of UD-CG 212 BS increased at a similar rate after infusion or bolus injections of pimobendan (see tables 1 and 3), yet the pattern of max LVdP/dt changes differed widely. Therefore, our data do not suggest a positive inotropic action of UD-CG 212 BS in the concentration range attained in this investigation.

A combination of vasodilation and positive inotropy increases cardiac output by facilitation of ventricular emptying and augmentation of the velocity of wall thickening (Cohn and Franciosa, 1978; Miller et al., 1977; 1981; Verdouw et al., 1981). The results obtained with pimobendan during coronary artery occlusion-induced myocardial ischemia show that, in a setting where cardiac contractility and output are depressed in the presence of a high pre- and/or afterload, the pimobendan-induced increase in cardiac inotropy is translated into a salutary effect on the pump function of the heart. It would appear, therefore, that the drug may be useful in clinical heart failure where a similar pathophysiological state is encountered. The finding that cardiac output did not increase in the animals with normal hearts emphasizes the role of left ventricular filling and systemic vascular resistance.

The effects of pimobendan on regional vascular beds are relevant to the therapy of congestive heart failure. Vasodilation occurred in all beds but, for the kidneys and spleen, this happened only with the lowest doses. When perfusion pressure started to fall severely, the vascular beds of these organs tended to constrict. For some other organs (stomach, adrenals, heart, brain) the vasodilator response increased dose dependently. In the heart, vasodilation was more marked in the epicardium than in the endocardium (endo-epi ratio was reduced) and, as a result, perfusion of the epicardium increased and that of the endocardium was maintained despite a marked reduction in perfusion pressure. The smaller effect on the endocardial blood flow may have been at least partly due to an increase in heart rate and the consequent reduction of the duration of diastole caused by pimobendan. Moreover, the vasodilator capacity of the endocardial vessels appears to be less than that of the epicardial vessels (Winbury and Howe, 1979). The reduction in endo-epi ratio does not appear to be deleterious because myocardial function was improved by the drug, even in the animals with an ischaemic heart.

A potential disadvantage of cardiotonic agents is that they may increase $M\dot{V}O_2$, not least by the accompanying tachycardia. In the case of pimobendan, these untoward effects were apparently balanced by reductions in pre- and afterload since $M\dot{V}O_2$ did not change following drug administration. The data on $M\dot{V}O_2$ also confirm that vasodilation in the coronary vascular bed was due to a direct action of pimobendan and was not the consequence of an increased myocardial metabolic demand.

In summary, pimobendan presents itself as a compound with vasodilator and positive inotropic properties that improves cardiac output in a model of severe ischaemia. The finding that the mild tachycardia elicited by the drug was not accompanied by an increase in $M\dot{V}O_2$ warrants investigation to evaluate its usefulness in the treatment of heart failure. This is especially true since, in the clinical setting, the heart rate may even be reduced because of a reduction in reflex sympathetic activity as a consequence of an improvement in left ventricular function.

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CHAPTER 7

USEFULNESS OF PIMOBENDAN IN THE TREATMENT OF HEART FAILURE.

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Usefulness of Pimobendan in the Treatment of Heart Failure

By D. J. Duncker[1], F. J. van Dalen[1], J. M. Hartog[1], J. M. J. Lamers[2], R. J. Rensen[1], P. R. Saxena[3], and P. D. Verdouw[1]

Summary: *The effects of the benzimidazole-pyridazinone pimobendan (UD-CG 115 BS) on systemic haemodynamics, myocardial performance and the distribution of cardiac output were studied in open-chest anaesthetized pigs. After intravenous bolus injections (0.1–0.5 mg · kg^{-1}, n = 7) increases in heart rate (up to 37%), LVdP/dt$_{max}$ (up to 54%) and decreases in systemic vascular resistance (up to 33%) and left ventricular filling pressure (up to 50%) were observed, while cardiac output was unchanged. Vasodilation occurred in nearly all regional vascular beds, but was most pronounced in the adrenals (200%), followed by stomach (150%), small intestines (130%), heart (125%) and brain (110%). O_2-consumption was not affected in spite of the increases in heart rate and myocardial inotropy. To evaluate the direct effects on the myocardium, pimobendan was also infused (1–5 μg · kg^{-1} · min^{-1}, n = 7) directly into the left anterior descending coronary artery. In addition to a marked vasodilation of the coronary bed (140%), also a lowering of the left ventricular filling pressure (up to 20%) and cardiac output (15%) was observed, but no changes in regional myocardial function, LVdP/dt$_{max}$ and systemic vascular resistance occurred. Immediately after intracoronary bolus injections (1 mg · kg^{-1}, n = 4), vasodilation of the coronary vessels was apparent, but myocardial contractility was not affected. This may explain that cyclic AMP content, determined in biopsies excised 30 s after injection, was unaltered. It may be concluded that pimobendan exerts actions on the cardiovascular system which may be useful in the treatment of heart failure.*

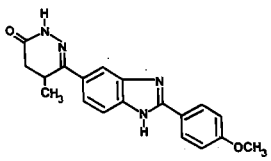
Zusammenfassung: *Nützlichkeit von Pimobendan bei der Behandlung der Herzinsuffizienz*

Die Wirkungen des Benzimidazol-pyridazinons Pimobendan (UD-CG 115 BS) auf systemische Hämodynamik, myokardiale Leistung und Verteilung des Herzminutenvolumens wurden an narkotisierten Schweinen mit eröffnetem Brustkorb untersucht. Nach intravenösen Bolusinjektionen (0,1–0,5 mg · kg^{-1}, n = 7) wurde eine Zunahme der Herzfrequenz (bis 37%) und der LVdP/dt$_{max}$ (bis 54%) sowie eine Abnahme des systemischen Gefäßwiderstandes (bis 33%) und des linksventrikulären Füllungsdruckes (bis 50%) beobachtet, während das Herzminutenvolumen unverändert blieb. Eine Vasodilatation war in fast allen regionalen Gefäßbetten zu verzeichnen, jedoch war sie besonders augenfällig in den Nebennieren (200%), gefolgt vom Magen (150%), dem Dünndarm (130%), dem Herzen (125%) und Gehirn (110%). Der O_2-Verbrauch blieb trotz der Steigerung der Herzfrequenz und des myokardialen Inotropismus unbeeinflußt. Um die unmittelbaren Effekte auf das Myokard zu bewerten, wurde Pimobendan direkt in die linke vordere absteigende Koronararterie infundiert (1–5 μg · kg^{-1} · min^{-1}, n = 7). Außer einer deutlichen Vasodilatation in den Kranzgefäßbetten (140%) wurde auch eine Abnahme des linksventrikulären Füllungsdruckes (bis 20%) und des Herzminutenvolumens (15%) beobachtet, doch es traten keine Veränderungen bei der regionalen myokardialen Funktion, der LVdP/dt$_{max}$ und dem systemischen Gefäßwiderstand ein. Unmittelbar nach intrakoronaren Bolusinjektionen (1 mg · kg^{-1}, n = 4) war die Erweiterung) der Herzkranzgefäße augenfällig, die Kontraktilität des Myokards wurde jedoch nicht beeinflußt. Dies könnte eine Erklärung dafür sein, daß der Gehalt an zyklischem AMP, der in 30 s nach Injektion exzidiertem Biopsiematerial bestimmt wurde, unverändert war. Daraus kann geschlossen werden, daß die Effekte, die Pimobendan auf das kardiovaskuläre System ausübt, bei der Behandlung der Herzinsuffizienz nützlich sein könnten.

Key words: *Cardiotonic drugs · Pimobendan, pharmacology · UD-CG 115 BS*

1. Introduction

The ideal drug for the treatment of heart failure should augment cardiac pump function by improving myocardial contractility, and (or) reducing left ventricular pre- and afterload, without increasing myocardial O_2-demand. In recent years a large series of new compounds (i.e. sulmazole, amrinone, milrinone) has been shown to meet the above mentioned requirements in the experimental animal. In clinical practice, however, the high incidence of side effects has prevented these drugs from totally replacing cardiac glycosides, despite the unwanted properties of the latter. In this study we describe some investigations with the benzimidazole-pyridazinone pimobendan (UD-CG 115 BS*), see formula diagram), which is structurally related to sulmazole [1]. Pimobendan increases myocardial contractility in the canine preparation [1, 2], possibly due to phosphodiesterase inhibition [3]. Diederen et al. also provided evidence that the drug is active on the venous vasculature [2]. We have shown that intravenous infusion of the drug also causes a marked systemic vasodilation in the pig [3a]. However, in these experiments the positive inotropic potency of pimo-



*) Manufacturer: Dr. Karl Thomae GmbH, Biberach an der Riss (Federal Republic of Germany).

bendan was of relatively minor importance. This discrepancy in inotropy between our study and those reported by others [1, 2] could not only be due to differences in the species used and experimental model but also be caused by differences in the administration: intravenous bolus injections versus infusions. In an attempt to reconcile these differences we studied the effect of pimobendan after intravenous bolus injections in anaesthetized pigs. Radioactively labelled microspheres were used to determine the effects on organ blood flow. In a second series of experiments we also infused the drug directly into a coronary artery to separate the direct actions on the myocardium from those on the vascular beds. Finally, the effects on myocardial cAMP levels were evaluated after selective coronary artery bolus injections.

2. Materials and methods

2.1. Experimental set-up

After an overnight fast Yorkshire pigs (22–28 kg) were sedated with 120 mg azaperone i.m., and anaesthetized with 150 mg intravenous metomidate [4]. Arterial blood gases (ABL-3, Acid-Base Laboratory, Radiometer, Copenhagen, Denmark) were controlled by artificial ventilation with a mixture of $O_2 : N_2O$ (1 : 2). Catheters were placed in the superior vena cava for administration of pentobarbital (20 mg · kg^{-1} · h^{-1}), sodium bicarbonate (8.4%, when needed), pimobendan and polygeline (Haemaccel®, Behringwerke AG, Mahrburg/Lahn, FR Germany) to replace blood loss. Microtipped Millar catheters (Millar, Instr., Houston, TX, USA) were used for measurement of the blood pressures in the left ventricular cavity and central aorta. An 8 F catheter was positioned in the aorta descendens for the withdrawal of blood samples. Before the heart was exposed via a midsternal split, 4 mg of the muscle relaxant pancuronium bromide was administered. A precalibrated electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the ascending aorta. The great cardiac vein was cannulated and the left atrial appendage was catheterized for the injection of microspheres. In some animals the left anterior descending coronary artery (LADCA) was cannulated for intracoronary administration of pimobendan.

Myocardial wall thickness tracings, monitored with a 5 MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, PA, USA), were used to evaluate regional myocardial performance. The wall thickness at end-diastolic (EDT) and end-systole (EST) were used to calculate systolic wall thickening (swt) as

$$swt\ (\%) = 100 \times (EST\text{-}EDT)/EDT$$

and the mean velocity of swt (∇_{swt}) as

$$\nabla_{swt} = (EST\text{-}EDT)/DS$$

where DS is the duration of the isovolumic contraction phase and the ejection time [4]. In some experiments (n = 3) regional function was estimated from segment length changes obtained from a pair of ultrasonic dimension gauges (5 MHz), placed subendocardially [5] and connected to a 4-channel ultrasonic dimension system (Model 401, Schuessler and associates, Cardiff by the Sea, CA, USA). The segment length at end-diastole (EDSL) and at end-systole (ESSL) were used to calculate systolic segment length shortening (sls) as

$$sls\ (\%) = 100 \times (EDSL\text{-}ESSL)/EDSL$$

and the mean velocity of sls (∇_{sls}) as

$$\nabla_{sls} = (EDSL\text{-}ESSL)/DS.$$

Distribution of cardiac output was measured using the radioactive microsphere technique [4]. Just before the injection of about 2×10^6 microspheres (15 ± 1 μm diameter, NEN Company, Dreieich, FR Germany) labelled with either ^{46}Sc, ^{103}Ru, ^{141}Ce, ^{95}Nb or ^{113}Sn, into the left atrium, the withdrawal of an arterial reference sample was started (flow rate 10 ml · min^{-1}) and continued for a period of about 1 min after injection of the microspheres was completed. After the experiment organs and tissues were excised, weighed and placed in vials. Details of the counting of the radioactivity and processing of the data have been described in detail [6, 7].

2.2. Experimental protocols

Three series of experiments were performed. In the first series (n = 7) 3 consecutive i.v. bolus injections (0.1, 0.25 and 0.5 mg · kg^{-1}) were used at 10-min intervals. Haemodynamic data were obtained during peak response and 10 min after administration of the bolus. At this time radioactive microspheres were injected to determine regional blood flows. In a second series of experiments (n = 7) pimobendan was directly infused into the left anterior descending coronary artery at increasing infusion rates (1, 2.5 and 5 μg · kg^{-1} · min^{-1}), each rate again lasting 10 min. In the last series (n = 4) the role of cAMP was studied. To minimize the effect of the systemic circulation on myocardial performance pimobendan was administered directly into the left anterior descending coronary artery. Cyclic AMP and cyclic GMP levels were determined in segments perfused by the LADCA and remote from this artery. To this end 0.5 g biopsies were rapidly taken from the ventricle areas, and directly frozen in isopentane cooled in liquid N_2. The frozen tissue was pulverized in a mortar while liquid N_2 was continuously added. The powder was mixed with 2.5 ml 0.9% perchloric acid (PCA), thawed and centrifuged thereafter. The PCA pellet was washed once more with 2.5 ml 0.9% PCA and further used for protein estimation. Cyclic AMP and cyclic GMP were determined, using the Amersham's cyclic AMP and cyclic GMP assay kits (Amersham, UK), in the combined supernatants after neutralization with 0.7 mol/l K_3PO_4 and 10 min centrifugation at 12,000 g [8].

2.3. Statistical analysis

Statistical analysis was performed using parametric tests [9]. After each dose of pimobendan, changes from baseline were calculated separately in each experiment and the significance of these changes was determined by using the Duncan new multiple-range test, once an analysis of variance had established that the data represented different populations. Statistical significance was accepted at $p < 0.05$ (two-tailed). All data have been expressed as mean ± standard error of mean (mean ± SEM).

2.4. Drugs

The only drugs used in this study were the anaesthetics, Haemaccel and pimobendan (supplied by Dr. Karl Thomae GmbH, Biberach an der Riss, FR Germany) which was dissolved in a mixture of polyethylene glycol 400 and saline (1 : 1).

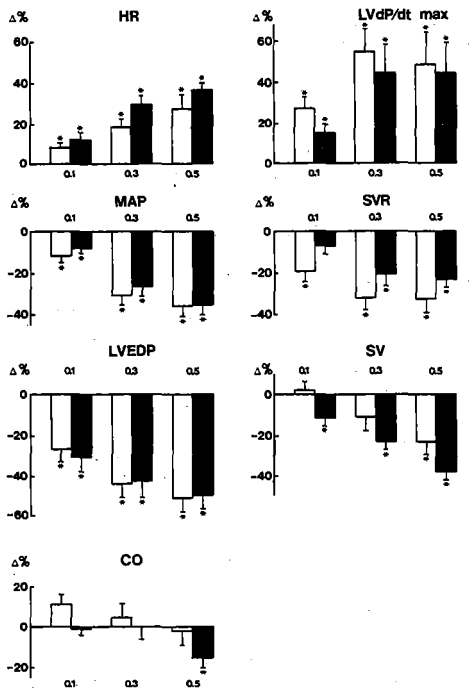


Fig. 1: Haemodynamic effects of cumulative bolus injections (mg · kg^{-1}) of pimobendan (blank columns = i.v. bolus peak; dark columns = i.v. bolus 10 min). The following parameters are shown: heart rate (HR: baseline 99 ± 6 beats · min^{-1}); maximum rate of rise of left ventricular pressure ($LVdP/dt_{max}$: 1680 ± 140 mmHg · s^{-1}); mean arterial blood pressure (MAP: 92 ± 5 mmHg); systemic vascular resistance (SVR: 41 ± 5 mmHg · l^{-1} · min); left ventricular end-diastolic pressure (LVEDP: 9.7 ± 1.0 mmHg); stroke volume (SV: 24.2 ± 1.7 ml) and cardiac output (CO: 2.39 ± 0.23 l · min^{-1}). All data are presented as percentage change from baseline (mean ± SEM). * $p < 0.05$ vs baseline.

3. Results

3.1. Intravenous bolus injections in pigs

3.1.1. Haemodynamics

Within minutes after each bolus injection mean arterial blood pressure decreased up to 36% in a dose-dependent manner. This was caused by arterial vasodilation as cardiac output was not significantly affected (Fig. 1). From the determinants of cardiac output, heart rate increased up to 37% but stroke volume decreased to 62% of baseline. This occurred in spite of the reduction in afterload and the increase in $LVdP/dt_{max}$ (44%, Fig. 1). Therefore the 50% decrease in left ventricular filling pressure must have been responsible for the decline in stroke volume.

3.1.2. Distribution of cardiac output

10 min after administration of the lowest dose (0.1 $mg \cdot kg^{-1}$), none of the conductances of the regional vascular beds differed from their respective baseline values (Table 1), but after 0.25 and 0.50 $mg \cdot kg^{-1}$, a profound vasodilation of the regional beds not only prevented a significant fall in perfusion of most organs and tissues but even caused slight increases in that of the myocardium (40%, Table 2). The increases in conductance were in declining order: adrenals (200%), stomach (150%), small intestine (130%), heart (125%) and brain (110%). In Table 1 the results for total brain are presented because only minor differences existed between the effects on the hemispheres (100%), diencephalon (110%), cerebellum (130%) and brainstem (150%). The vasodilatory response of the vascular beds of the kidneys, liver and spleen did not reach levels of significance.

Table 1: Conductance of regional vascular beds after cumulative intravenous bolus injections of pimobendan in 7 anaesthetized pigs.

	Baseline	Pimobendan ($mg \cdot kg^{-1}$)		
		0.1	0.25	0.5
SVC	27.7 ±3.0	30.0 ±3.3	35.3 ±4.6*	37.5 ±3.7*
Kidneys	4.05 ±0.86	4.34 ±1.02	4.28 ±0.52	5.66 ±0.75
Liver	0.50 ±0.19	0.50 ±0.19	0.42 ±0.18	0.64 ±0.18
Spleen	1.52 ±0.17	1.70 ±0.18	2.01 ±0.43	1.87 ±0.21
Adrenals	2.01 ±0.41	2.47 ±0.45	2.92 ±0.46*	4.31 ±0.31*
Stomach	0.22 ±0.06	0.22 ±0.05	0.30 ±0.05*	0.38 ±0.05*
Small intestine	0.23 ±0.03	0.24 ±0.03	0.31 ±0.04*	0.40 ±0.04*
Muscle	0.050±0.015	0.045±0.009	0.051±0.014	0.067±0.014*
Skin	0.015±0.005	0.049±0.011*	0.054±0.022*	0.044±0.011*
Left atrium	1.05 ±0.24	1.30 ±0.27	1.69 ±0.28*	2.15 ±0.33*
Left ventricle	1.26 ±0.22	1.44 ±0.27	1.85 ±0.37*	2.59 ±0.50*
Right ventricle	1.27 ±0.30	1.53 ±0.35	2.26 ±0.50*	3.07 ±0.63*
Total brain	0.28 ±0.05	0.32 ±0.05	0.39 ±0.05*	0.50 ±0.05*

All conductances are in $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$, except those of the systemic vascular tree (SVC, $ml \cdot min^{-1} \cdot mmHg^{-1}$). All data are presented as mean ± SEM. * $p < 0.05$ vs baseline.

Table 2: Perfusion of regional vascular beds after cumulative intravenous bolus injections of pimobendan in 7 anaesthetized pigs.

	Baseline	Pimobendan ($mg \cdot kg^{-1}$)		
		0.1	0.25	0.5
CO	2.49± 0.24	2.44± 0.20	2.37± 0.23	2.26± 0.20
Kidneys	359±72	343±63	310±56	351±59
Liver	45.2 ±16.7	40.7 ±13.8	29.8 ±11.7	38.0 ±10.2
Spleen	139±18	141±17	141±31	113±12
Adrenals	182±39	203±35	206±32	259±11
Stomach	19.6 ± 5.3	17.9 ± 3.7	20.5 ± 3.8	23.0 ± 3.6
Small intestine	19.9 ± 2.7	19.7 ± 1.8	21.1 ± 2.4	23.7 ± 1.7
Muscle	4.45± 1.34	3.67± 0.73	3.48± 0.94	3.94± 0.72
Skin	1.32± 0.37	4.19± 1.12	3.95± 1.95	2.68± 0.67
Left atrium	93±20	104±17	115±18	130±19
Left ventricle	113±20	118±19	128±23	157±29
Right ventricle	112±24	123±22	155±32*	183±34*
Total brain	25.3 ± 3.8	26.1 ± 3.1	26.0 ± 2.6	29.5 ± 2.2

All flow data are in $ml \cdot min^{-1} \cdot 100\ g^{-1}$, except cardiac output (CO) which is presented in $l \cdot min^{-1}$. All data are presented as mean ± SEM. * $p < 0.05$ vs baseline.

Table 3: Haemodynamic effects of cumulative 10-min intracoronary infusions of pimobendan in 7 anaesthetized pigs.

	Baseline	Pimobendan ($\mu g \cdot kg^{-1} \cdot min^{-1}$)		
		1	2.5	5
CO	2.10±0.25	1.99±0.22*	1.88±0.22*	1.82±0.22*
HR	82±4	85±4	87±4	92±5*
SV	26±3	23±3*	22±3*	20±3*
$LVdP/dt_{max}$	1690±70	1730±100	1780±120	1990±200
LVEDP	10.0 ±0.4	9.3 ±0.7	8.6 ±0.9	8.0 ±0.9*
MAP	84±5	79±5*	76±5*	69±5*
SVR	43±4	43±5	43±5	40±4

Abbreviations: CO = cardiac output ($l \cdot min^{-1}$); HR = heart rate ($beats \cdot min^{-1}$); SV = stroke volume (ml); $LVdP/dt_{max}$ = maximum rate of rise of left ventricular pressure ($mmHg \cdot s^{-1}$); LVEDP = left ventricular end-diastolic pressure (mmHg); MAP = mean arterial blood pressure (mmHg); SVR = systemic vascular resistance ($mmHg \cdot l^{-1} \cdot min$). All data are presented as mean ± SEM. * $p < 0.05$ vs baseline.

3.1.3. Myocardial performance

Coronary blood flow increased dose-dependently up to 140% of the pre-treatment value (113 ± 20 $ml \cdot min^{-1} \cdot 100\ g^{-1}$) because of a doubling of the coronary vascular conductance. The increase in flow was most beneficial for the epicardial layers as perfusion of the endocardial layers remained unchanged (not shown). Consequently the endo-epi blood flow ratio decreased from 1.06 ± 0.04 to 0.83 ± 0.03 ($p < 0.05$). The oxygen content in the coronary venous blood rose as the oxygen saturation in the great cardiac vein increased from 26 ± 4 to 44 ± 3% ($p < 0.05$). Myocardial O_2-consumption was not significantly affected, which is not surprising because the double product (heart rate × systolic arterial blood pressure) was also not affected as the elevations in heart rate were balanced by the decreases in arterial blood pressure.

3.2. Intracoronary infusions

3.2.1. Haemodynamics

In spite of the low doses (1–5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) the intracoronary infusions caused dose-dependent decreases in arterial blood pressure (up to 18%, $p < 0.05$) because of a fall in cardiac output (up to 15%, $p < 0.05$) as systemic vascular resistance was not affected (Table 3). This decrease in cardiac output occurred despite a moderate dose-dependent increase in heart rate. Consequently stroke volume was decreased. Of its determinants only left ventricular filling was reduced, reflected by a 20% decrease in LVEDP, while systemic vascular resistance was not affected and $LVdP/dt_{max}$ tended to increase. Although arterial blood pressure decreased, no redistribution of cardiac output occurred as the conductances of the regional vascular beds were not significantly affected (Table 4).

Table 4: Conductance of regional vascular beds after cumulative 10-min intracoronary infusions of pimobendan in 7 anaesthetized pigs.

	Baseline	Pimobendan ($\mu g \cdot kg^{-1} \cdot min^{-1}$)		
		1	2.5	5
SVC	25.0 ±2.6	25.1 ±2.6	24.8 ±2.6	26.3 ±2.4
Kidneys	4.23 ±0.56	3.77 ±0.30	4.52 ±0.85	4.65 ±1.10
Liver	0.43 ±0.13	0.44 ±0.17	0.49 ±0.15	0.47 ±0.17
Spleen	1.74 ±0.38	1.78 ±0.39	2.00 ±0.54	1.46 ±0.36
Adrenals	2.51 ±0.26	2.70 ±0.37	3.03 ±0.47	3.32 ±0.67
Stomach (n = 3)	0.18 ±0.05	0.19 ±0.06	0.18 ±0.06	0.19 ±0.06
Muscle	0.038±0.006	0.036±0.007	0.041±0.008	0.039±0.009
Left atrium	1.14±0.13	1.16 ±0.15	1.33 ±0.19	1.47 ±0.18

All conductances are in $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$, except of the systemic vascular tree (SVC, $ml \cdot min^{-1} \cdot mmHg^{-1}$). All data are presented as mean ± SEM.

Table 5: Myocardial performance after cumulative 10-min intracoronary (LADCA) infusions of pimobendan in 7 anaesthetized pigs.

	Baseline	Pimobendan ($\mu g \cdot kg^{-1} \cdot min^{-1}$)		
		1	2.5	5
Control segment				
Flow				
epi	125±11	118±10	129±18	122±14
endo	138±14	128±12	127±13	124±11
endo/epi	1.07±0.06	1.09±0.06	1.02±0.08	1.04±0.06
Conductance				
epi	1.48±0.07	1.49±0.07	1.66±0.17	1.77±0.15
endo	1.62±0.13	1.62±0.11	1.67±0.13	1.81±0.10
LADCA segment				
Flow				
epi	132±17	143±16	163±19	172±27
endo	143±17	154±19	179±18	191±25
endo/epi	1.18±0.09	1.16±0.12	1.18±0.09	1.21±0.09
Conductance				
epi	1.57±0.16	1.81±0.16*	2.13±0.16*	2.53±0.34*
endo	1.68±0.16	1.93±0.16	2.32±0.12*	2.77±0.26*
Cor ven O_2-sat	24±4	44±5*	51±5*	63±6*
O_2-consumption	0.53±0.07	0.40±0.05	0.39±0.07	0.30±0.03*

Abbreviations: LADCA = left anterior descending coronary artery; flow in $ml \cdot min^{-1} \cdot 100\ g^{-1}$; conductance in $ml \cdot min^{-1} \cdot mmHg^{-1} \cdot 100\ g^{-1}$; cor ven O_2-sat in %; O_2-consumption in $mmol \cdot min^{-1} \cdot 100\ g^{-1}$. All data are presented as mean ± SEM. * $p < 0.05$ vs baseline.

3.2.2. Myocardial performance

Because of the fall in perfusion pressure, a slight decrease in perfusion of the myocardium adjacent to the LADCA perfused segment was observed (Table 5). A minor increase in conductance prevented that this decrease in perfusion reached levels of significance. The increase in the conductance of both the endo- and epicardial layers from the area perfused by the LADCA prevented that the fall in perfusion pressure was detrimental to their perfusion. Systolic wall thickening (swt), mean velocity of swt, systolic segment length shortening (sls) and mean velocity of sls did not change significantly from their respective baseline values of 25 ± 4%, 19.8 ± 3.4 mm · s^{-1}, 24 ± 5% and 5.6 ± 1.1 mm · s^{-1}. The most marked changes were again in the coronary venous O_2-content, as the saturation in the great cardiac vein increased from 24 ± 4 to 63 ± 6% ($p < 0.05$). These increases in coronary venous O_2-saturation were caused by a lowering in regional myocardial O_2-consumption (Table 5), which occurred although the double product was hardly affected.

3.3. Myocardial cyclic AMP and cyclic GMP levels after intracoronary pimobendan administration

Within seconds after intracoronary bolus injections (1 mg · kg^{-1}) a very pronounced vasodilation of the coronary vascular bed occurred (visible by a bright red colour of the myocardium and confirmed by the extreme high O_2-saturations in the great cardiac vein (up to 75%)), but $LVdP/dt_{max}$ and regional myocardial wall thickening of the area perfused by the LADCA were not significantly affected. At that moment the heart was excised rapidly and treated for cyclic AMP and cyclic GMP determinations. Cyclic AMP in the LADCA-perfused myocardium tended to be slightly lower (6.13 ± 0.42 vs 7.27 ± 0.50 pmol/mg protein, $p > 0.05$), while cyclic GMP was slightly higher (0.343 ± 0.115 vs 0.143 ± 0.24 pmol/mg protein) compared to respective data obtained from a segment remote from the myocardium perfused by the LADCA.

4. Discussion

Intracoronary infusions were used to minimize the extracardial effects of pimobendan to enable us to evaluate more directly the drug's inotropic potency. In spite of the low doses (1–5 $\mu g \cdot kg^{-1} \cdot min^{-1}$) the intracoronary infusions caused a decrease in cardiac output, as the decrease in stroke volume was not compensated by the acceleration of heart rate. This diminution in stroke volume was the result of a reduction in preload as the responses of afterload and myocardial contractility did not favor such a reduction. Because no dilation of the systemic arterial bed was noted, our data suggest that at these low doses a larger sensitivity exists for the venous than for the arterial vessels, confirming the earlier observations reported by Diederen et al. [2]. Higher doses (0.1–0.5 mg · kg^{-1} i.v.) caused a marked systemic arterial vasodilation in addition to the venodilation. It is noteworthy that the effects on the arterial vessels appear to be shorter lasting than on the veins, as after 10 min the effects on the arterial vessels were considerably lower than the peak effects obtained after 2 min, while the effects on the venous vasculature persisted. The arterial vasodilation is one of the factors that prevented a fall in cardiac output during the intravenous bolus injections. Another was the significant rise in myocardial contractility, reflected by the increase in $LVdP/dt_{max}$. Although the latter is not a true index for myocardial contractility because of its dependence on heart rate and pre- and afterload, the experimental evidence in this study warrants such a conclusion as the pre- and afterload induced-changes in $LVdP/dt_{max}$ tend to underestimate the inotropic changes [10, 11]. Moreover, we have repeatedly shown that in the anaesthetized pig $LVdP/dt_{max}$ is only slightly modified when heart rate varies between 100 and 140 beats · min^{-1} [12, 13]. While intravenous bolus injections (0.1–0.5 mg · kg^{-1}) revealed increases in myocardial inotropy, such an action was not noticeable during the intracoronary infusions. That the intracoronary infusion rates were too low is ruled out when one takes into account that after intravenous administration only about 2% of the pimobendan passes through the coronary circulation. Moreover, intracoronary bolus injections (up to 1 mg · kg^{-1}) were also unable to elicit a positive inotropic action, while arterial vasodilation was remarkably present. In these experiments, we excised the hearts approximately 30 s after administration, to avoid extracardial influences of pimobendan on myocardial performance. The absence of significant changes in myocardial cAMP content therefore does not exclude the possibility of phosphodiesterase inhibition as a mechanism for the drug's inotropic potency [3], but rather indicates that the vessels respond much more rapidly (within seconds) than the tissue.

After intravenous bolus injection vasodilation occurred in all regional vascular beds. The increase in conductance was enough to maintain or even augment regional blood flow in spite of the fall in perfusion pressure. In the myocardium, the epicardial layers benefited more than the endocardial layers yielding a 20% decrease in the endo-epi blood flow ratio. Domenech and Goich [14] reported that tachycardia does not affect the endo-epi blood flow ratio in normotensive animals because of the endocardial vasodilatory reserve capacity, but in dilated coronary vascular beds raising the heart rate resulted in a decrease of the endo-epi blood flow ratio [14]. Therefore, the pimobendan-induced tachycardia and decrease in perfusion pressure are very likely the responsible factors for the uneven distribution of the flow after administration of the drug. The intracoronary infusions of pimobendan were accompanied by smaller changes in heart rate and arterial blood pressure, which explains why the different myocardial layers were now equally affected. Since the myocardial O_2-consumption was not increased after the intravenous and even decreased during the intracoronary experiments we conclude that the increase in flow was not the result of an autoregulatory response mediated by an increased O_2-demand. The decrease in O_2-consumption after the intracoronary infusions probably underestimates the true vasodilatory response to the pimobendan infusions because of a metabolic-induced vasoconstriction. Since the double product of heart rate and left ventricular systolic pressure was unchanged in the intracoronary experiments, other factors may have played a role in the lowering of the myocardial O_2-consumption. A decrease in left ventricular wall tension due to the decrease in LVEDP seems to be such a factor. In the intravenous experiments the double product was also unchanged, but now the decrease in LVEDP was balanced by an increase in $LVdP/dt_{max}$ resulting in an unchanged myocardial O_2-consumption.

In conclusion, our data suggest that pimobendan at low doses acts primarily on the venous system but at higher doses the effects on the arterial vessels are also prominent. Depending on the rate of administration, a significant positive inotropic response can be elicited. In view of our observations with pimobendan, it is of interest that Wilmshurst et al. [15] reported amrinone to be devoid of any beneficial effect in patients with cardiac failure when administered directly into the coronary artery. Intravenous administration, on the other hand, improved cardiovascular performance due to vasodilation of the venous and arterial beds. Only in a small number of patients myocardial contractility increased probably due to catecholamine release. These observations suggest that the beneficial actions of some of the so-called positive inotropic agents might be due to normalization of left ventricular dimensions as a result of a reduction in both preload and afterload rather than to a significant increase in myocardial contractility. However, the above described properties of pimobendan are useful in the treatment of heart failure and side effects permitting, clinical studies appear to be warranted.

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CHAPTER 8

CARDIOVASCULAR EFFECTS OF UD-CG 212 CL, A METABOLITE OF PIMOBENDAN, IN ANAESTHETIZED PIGS.

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Cardiovascular effects of UD-CG 212 CL, a metabolite of pimobendan, in anaesthetized pigs

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Systemic and regional haemodynamic effects of UD-CG 212 CL (0.5-16 $\mu g \cdot kg^{-1} \cdot min^{-1}$), the major metabolite of the vasodilator and cardiotonic drug pimobendan, were studied in anaesthetized pigs. The drug caused a dose-dependent decrease in left ventricular (LV) end-diastolic and arterial blood pressures while it increased systemic vascular conductance, heart rate and maxLVdP/dt. The decrease in LV end-diastolic pressure was observed at lower plasma concentrations than the increase in systemic vascular conductance. Cardiac output tended to decrease but statistical significance was achieved only with the highest concentration. These effects of UD-CG 212 CL were not altered by the blockade of β-adrenoceptors with propranolol. The vasodilator action of UD-CG 212 CL was noticed in several organs but the effects were relatively more marked (in decreasing order of magnitude) in the adrenals, kidneys, gastrointestinal tract, brain and LV epicardium. Since both arterial pressure and cardiac output decreased, the blood flow increased significantly only in the adrenals and decreased moderately in the spleen, LV endocardium and skeletal muscles. The effects of UD-CG 212 CL on the renal and skeletal muscle haemodynamics were different from those of pimobendan, which causes vasodilatation in the skeletal muscles but not in the kidneys. The results of this study show that, like the parent compound pimobendan, UD-CG 212 CL has independent cardiotonic and vasodilator actions; the latter being more pronounced on the venous side. However, the contribution of this metabolite to the overall pharmacological activity of pimobendan appears to be limited.

UD-CG 212 CL; Pimobendan; Phosphodiesterase inhibitors; Vasodilatation; Regional blood flow; β-Adrenoceptor blockade; Myocardial O_2 consumption; Tachycardia; Inotropic agents (positive); (Pig)

1. Introduction

The last decade has seen considerable attention focused on a new type of cardioactive drugs which could be useful in the treatment of congestive heart failure. These agents are thought to dilate peripheral vascular beds and increase myocardial contractility by elevating cyclic AMP levels following inhibition of phosphodiesterases (Honerjäger et al., 1984; Scholz and Meyer, 1986). One such drug is pimobendan (UD-CG 115 BS) which in vivo experiments have shown to act more potently on the venous than on the arterial side; the positive inotropic action is only moderate (Diederen et al., 1982; Van Meel, 1985; Verdouw et al., 1986; Duncker et al., 1986c). Recently, the major metabolite of pimobendan, UD-CG 212 CL (fig. 1), was found in in vitro studies to increase cardiac contractile force (Meyer et al., 1985; Scholz

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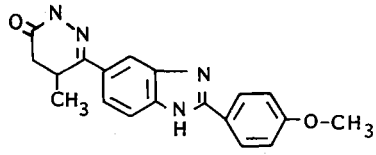
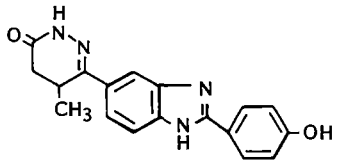


Pimobendan (UD-CG 115 BS)



UD-CG 212 CL

Fig. 1. Chemical structure of pimobendan and its major metabolite UD-CG 212 CL.

and Meyer, 1986). The major goal of the present study was to investigate the complete systemic and regional haemodynamic profile of UD-CG 212 CL. Additionally, in view of the possible contribution of UD-CG 212 CL to the systemic haemodynamic effects of pimobendan (Meyer et al., 1985; Scholz and Meyer, 1986), we have measured the plasma concentrations of UD-CG 212 CL and compared them with concentrations achieved following infusions of the parent drug pimobendan (Verdouw et al., 1986) to assess the extent of such a contribution.

2. Materials and methods

2.1. Experimental set-up

After a 24 h fast, Yorkshire pigs (23-28 kg) were sedated with 120 mg azaperone (Stresnil®) i.m., anaesthetized with 150 mg metomidate (Hypnodil®), intubated and ventilated with a mixture of O_2 and N_2O (1 : 2). Respiratory rate and tidal volume were set to keep arterial blood gases, measured with an ABL-3 (Radiometer, Copenhagen, Denmark), within normal limits ($7.35 < pH < 7.45$; $35\ mmHg < PCO_2 < 45\ mmHg$; $90\ mmHg < PO_2 < 150\ mmHg$). Cathethers placed in the superior vena cava were used for administration of α-chloralose ($100\ mg \cdot kg^{-1}$) and pentobarbital sodium ($5\ mg \cdot kg^{-1} \cdot h^{-1}$) for anaesthesia, Haemaccel® (Behringwerke AG, Marburg, FRG) to replace blood loss, and UD-CG 212 CL. An 8F catheter was positioned in the descending aorta for withdrawal of blood samples. Left ventricular and aortic blood pressures were obtained with 8F Millar microtipped catheters (Millar Instruments Houston, Texas, USA). Prior to exposing the heart via a midsternal split, 4 mg of the muscle relaxant pancuronium bromide (Pavulon®) was administered. Ascending aortic blood flow was measured with an electromagnetic flow probe (Skalar, Delft, The Netherlands). Blood samples were collected from the great cardiac vein for the determination of haemoglobin and O_2 saturation. Myocardial O_2 consumption was calculated by multiplying left ventricular blood flow by the arterial-coronary venous O_2 content difference.

The distribution of cardiac output was determined using the radioactive microsphere technique. Microspheres of $15 \pm 1\ \mu m$ (mean ± S.D.) diameter labeled with either ^{103}Ru, ^{113}Sn, ^{46}Sc, ^{95}Nb or ^{141}Ce (NEN Chemicals GmbH, Dreieich, FRG), were injected in random order via a cannula inserted into the left atrial appendage. Flow measurements were calibrated by withdrawal of an arterial reference blood sample at a rate of $10\ ml \cdot min^{-1}$ starting just before and continuing for 1 min after each injection of microspheres. The animal was killed at the end of the experiment and several organs and tissues were excised and treated as described elsewhere (Saxena and Verdouw, 1985). The data were processed using computer programmes developed for the purpose (Saxena et al., 1980).

2.2. Experimental protocols

Two series of experiments were performed. In both series pre-drug systemic haemodynamic data were collected after a stabilisation period of 30-45 min. In the first group (n = 9) this was followed by six consecutive 15 min infusions of 0.5, 1, 2, 4, 8 and 16 $\mu g \cdot kg^{-1} \cdot min^{-1}$ of UD-CG 212 CL.

Measurements were repeated at the end of each infusion at a given rate. Radioactive microspheres were injected at baseline and at the end of the four highest 'infusion rates' (2, 4, 8 and 16 $\mu g \cdot kg^{-1} \cdot min^{-1}$), because of the limited number of isotopes available. Since increases in heart rate and maxLVdP/dt in these experiments were accompanied by a fall in mean arterial blood pressure, the same infusion rates were repeated in a second group of 5 animals after pretreatment with propranolol (0.5 $mg \cdot kg^{-1}$ followed by an infusion of 0.5 $mg \cdot kg^{-1} \cdot h^{-1}$) to exclude effects of a direct or indirect β-adrenoceptor mechanism. Regional blood flows and plasma concentrations were not determined in this series of experiments. An earlier study from our laboratory (Wolffenbuttel and Verdouw, 1983) had shown that the above-mentioned dose regimen for propranolol provides adequate β-adrenoceptor blockade and that systemic haemodynamic parameters change less than 5% over a period of 90 min.

2.3. Determination of plasma drug concentrations

The concentration of UD-CG 212 CL in the plasma was measured using high-performance liquid chromatography (HPLC). The details of the HPLC assay have been described earlier (Roth, 1983; Verdouw et al., 1986). The lower limit for detection of the compound is 1 $ng \cdot ml^{-1}$.

2.4. Statistical analysis

Data are presented as means ± S.E.M. Statistical analysis was performed by use of a parametric two-way analysis of variance (randomized block design), followed by Duncan's new multiple range test (Steel and Torrie, 1980). Statistical significance was accepted at $P < 0.05$ (two-tailed).

2.5. Drugs

The substances used were the anaesthetics, Haemaccel, propranolol hydrochloride (ICI-Pharma, Rotterdam, The netherlands) and UD-CG 212 CL (Dr. Karl Thomas GmBH, Biberach a/d Riss, FRG). The latter was dissolved in a mixture of polyethylene glycol and saline, such that the infusion rates of polyethylene glycol ranged between 0.5 and 1.0 $ml \cdot min^{-1}$. The infusion of the solvent of these rates has no cardiovascular effects (Verdouw et al., 1983).

3. Results

3.1. Cardiovascular actions of UD-CG 212 CL without β-adrenoceptor blockade

3.1.1. Plasma concentrations of UD-CG 212 CL

As the infusion rates (0.5, 1, 2, 4, 8 and 16 $\mu g \cdot kg^{-1} \cdot min^{-1}$) of UD-CG 212 CL were increased, the plasma concentrations reached levels of 9 ± 1 $ng \cdot ml^{-1}$; 21 ± 2 $ng \cdot ml^{-1}$; 44 ± 5 $ng \cdot ml^{-1}$; 87 ± 8 $ng \cdot ml^{-1}$; 170 ± 16 $ng \cdot ml^{-1}$ and 361 ± 32 $ng \cdot ml^{-1}$, respectively. The latter was approximately 20 times the highest UD-CG 212 CL concentration obtained in pimobendan infusion experiments (Verdouw et al., 1986).

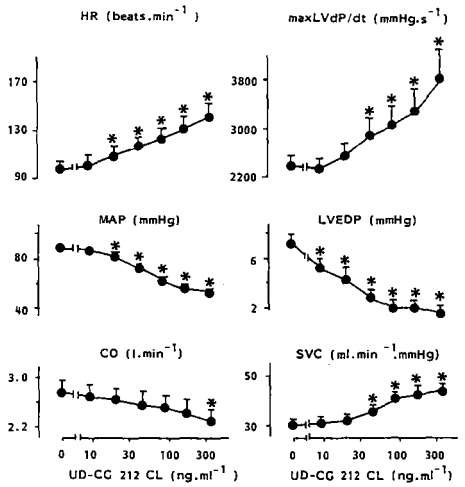


Fig. 2. Systemic haemodynamics at increasing UD-CG 212 CL plasma concentrations. HR, heart rate; maxLVdP/dt, maximal rate of rise of left ventricular pressure; MAP, mean arterial pressure; LVEDP, left ventricular end-diastolic pressure; CO, cardiac output; SVC, systemic vascular conductance. Data are presented as means ± S.E.M. * $P < 0.05$ vs. pre-drug values.

3.1.2. Systemic haemodynamics

UD-CG 212 CL caused dose-related decreases in mean arterial blood pressure (fig. 2), without affecting pulse pressure (not shown). Although the hypotensive action of UD-CG 212 CL was accompanied by a positive chronotropic action (heart rate increased up to 40%) it was not sufficient to prevent a fall in cardiac output, as stroke volume decreased dose dependently from a pre-drug value of 29 ± 2 to 17 ± 2 ml (not shown). For concentrations less than 30 ng · ml^{-1} this decrease in stroke volume was primarily due to a reduced left ventricular filling (left ventricular end-diastolic pressure decreased up to 50%), as the increases in systemic vascular conductance (flow/pressure) by 10% and maxLVdP/dt (15%) would facilitate left ventricular emptying. No additional effects on left ventricular end-diastolic pressure were seen for concentrations higher than 50 ng · ml^{-1}, while maxLVdP/dt increased gradually up to 60% and systemic vascular conductance increased by 45% (fig. 2).

3.1.3. Regional blood flows and vascular conductances

Because of the limited number of microspheres available with different radioactive labels, regional blood flows were determined before the start of the infusion of UD-CG 212 CL and at the end of the four highest infusion rates. Figure 3 shows that the decrease in cardiac output was not equally distributed over all organs. Blood flow to the adrenals was increased at each plasma concentration (up to 100%). Renal blood flow initially tended to increase, but started to decrease at concentrations higher than 40 ng · ml^{-1} and had fallen to below pre-drug values at 360 ng · ml^{-1}. At concentrations higher than 40 ng · ml^{-1}, splenic (up to 45%), skeletal muscle (up to 40%) and left ventricular (up to 20%, for further details see below) blood flow decreased but no significant changes were observed at any concentration in

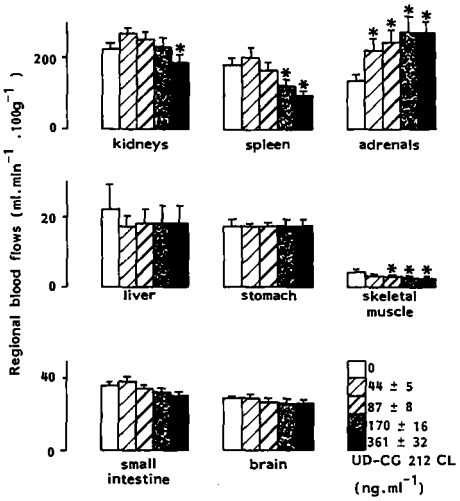


Fig. 3. Effects of i.v. infusions of UD-CG 212 CL on regional blood flows. Data are presented as means ± S.E.M. * $P < 0.05$ vs. pre-drug values.

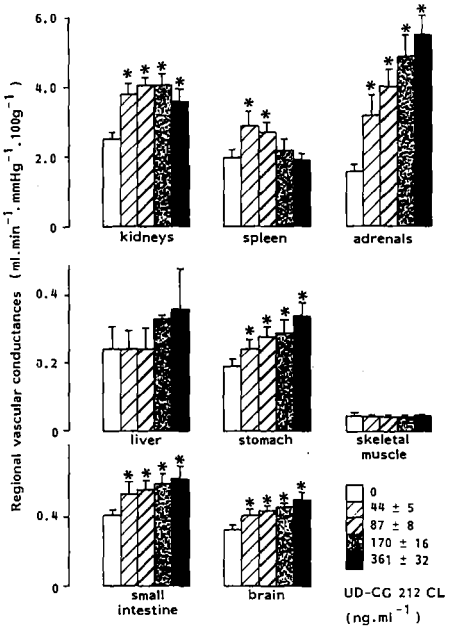


Fig. 4. Effects of i.v. infusions of UD-CG 212 CL on regional vascular conductances. Data are presented as means ± S.E.M. * $P < 0.05$ vs. pre-drug values.

any of the other organs studied (stomach, small intestine, brain, liver and skin (not shown)). Dose-related increases in vascular conductance were observed in the adrenals (up to 300%), stomach (up to 100%), brain (up to 60%) and small intestine (up to 60%; fig. 4). The increase in conductance in the renal bed was independent of the dose, whereas vasodilatation in the spleen only occurred at the lowest two doses. The changes in vascular conductance in skeletal muscle, liver and skin (not shown) did not reach significance.

3.1.4. Coronary circulation

As shown in fig. 5 the blood flow to the two atria and the right ventricle did not change after the administration of UD-CG 212 CL. Since a decrease in arterial blood pressure was observed, vascular conductance in these organs increased dose dependently. Transmural left ventricular blood flow decreased by 12% when UD-CG 212 CL reached arterial plasma levels of 40 ng · ml^{-1}. A further decline (28%) was observed at 170 ng · ml^{-1} (fig. 5). The decreases were confined to the subendocardial layers, yielding a moderately decreased endo-epi blood flow ratio (from 1.23 ± 0.04 to 0.95 ± 0.03, $P < 0.05$, not shown). Vascular conductances of the subendocardial and subepicardial layers were, respectively, virtually unchanged and increased dose dependently (up to 55%).

The O_2 saturation in the great cardiac vein increased from 15 ± 2 to 21 ± 1% ($P < 0.05$) at 40 ng · ml^{-1} but was not further affected at higher

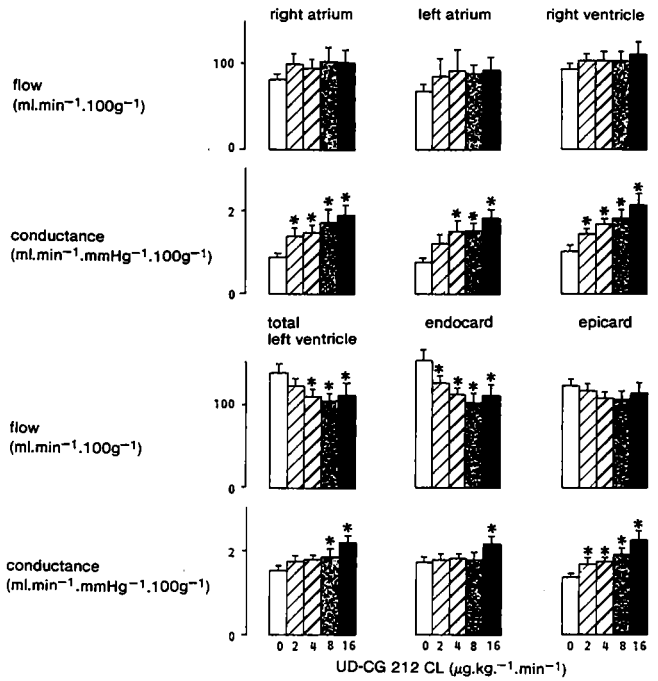


Fig. 5. Myocardial blood flows and vascular conductances with increasing rate of UD-CG 212 CL i.v. infusion. The plasma concentrations at baseline and at the end of the infusions at each rate were: 0 ng·ml^{-1}, 44±5 ng·ml^{-1}, 87±8 ng·ml^{-1}, 170±16 ng·ml^{-1} and 361±32 ng·ml^{-1}, respectively. Data are presented as means±S.E.M. * $P < 0.05$ vs. pre-drug values.

concentrations (not shown). Myocardial O_2 consumption therefore decreased gradually from 5.1 ± 0.4 $\mu mol \cdot min^{-1} \cdot g^{-1}$ to 3.6 $\pm$ 0.5 $\mu mol \cdot min^{-1} \cdot g^{-1}$ ($P < 0.05$; not shown).

3.2. Cardiovascular actions of UD-CG 212 CL after β-adrenoceptor blockade

When UD-CG 212 CL was infused at rates up to 8 $\mu g \cdot kg^{-1} \cdot min^{-1}$ neither the responses of heart rate nor those of maxLVdP/dt, mean arterial blood pressure, cardiac output and left ventricular end-diastolic pressure were significantly modified by the presence of propranolol (not shown).

4. Discussion

In the study just described UD-CG 212 CL dilated both arterial and venous vascular beds and increased myocardial contractility. The data in fig. 2 reveal that at concentrations lower than 30 $ng \cdot ml^{-1}$ UD-CG 212 CL is primarily a venodilator (decrease in left ventricular end-diastolic pressure) and that the arterial vasodilator and maxLVdP/dt increasing properties only become apparent at higher concentrations. Because maxLVdP/dt depends on heart rate and pre- and afterload, its use as an index of inotropy demands caution. In this study the heart rate increased, which could be a factor contributing to the augmentation of maxLVdP/dt (Higgins et al., 1973). However, in the anaesthetized pig, maxLVdP/dt is not significantly affected by a heart rate in the range of 100-150 beats $\cdot min^{-1}$ (Scheffer and Verdouw, 1983). Furthermore, the decreases in diastolic arterial blood pressure and left ventricular end-diastolic pressure tend to reduce maxLVdP/dt (Mason, 1969). It thus seems that the enhancement of maxLVdP/dt by UD-CG 212 CL represents its positive inotropic effect. The increases in heart rate and maxLVdP/dt induced by UD-CG 212 CL were not modified by propranolol. We therefore conclude that these effects are not mediated by β-adrenoceptors, either directly or via enhancement of sympathetic nerve activity (due to baroreceptor reflex) but the possibility of withdrawal of vagal tone cannot be excluded. With respect to the positive inotropic effects of UD-CG 212 CL as well as of pimobendan, evidence from in vitro studies suggests that inhibition of phosphodiesterase is involved, but the extent of this involvement is less than in the case of bipyridine derivatives such as amrinone and milrinone (Scholz and Meyer, 1986).

UD-CG 212 CL caused a pronounced vasodilatation of the systemic arterial bed. Although vasodilatation occurred in most regional beds it was conspicuously absent in skeletal muscle and was of only limited magnitude in the left ventricle. In this respect the effects of UD-CG 212 CL are similar to those of amrinone (Hartog et al., 1986), but quite different from those of other vasodilators such as the calcium channel blockers nimodipine and nisoldipine (Duncker et al., 1986a,b; Verdouw et al., in press), the nitrate-like drug nicorandil (unpublished data from our laboratory) or even pimobendan (Verdouw et al., 1986; Duncker et al., 1986c), which were all evaluated in the same experimental model and elicited moderate to pronounced vasodilatation in these regions. The vasodilatation that occurred in the left ventricle was not sufficient to prevent a reduction in transmural blood flow. As the coronary venous O_2 content increased slightly it is likely that the reduction in flow reflects the diminished metabolic needs (myocardial O_2 consumption decreased up to 30%). The transmural vasodilatation was almost solely confined to the subepicardial layers; endocardial blood flow and the endo/epi ratio decreased. It is known that endocardial blood flow is more susceptible to a decrease in diastolic perfusion time (due to tachycardia in this case) and systemic perfusion pressure (see Feigl, 1983). Alternatively, though less likely, there is the possibility that UD-CG 212 CL has a more potent vasodilator effect on the epicardial layers whereby a 'steal' of endocardial blood flow may take place. Interestingly, a reduction in endo-epi ratio has also been observed for the phosphodiesterase inhibitor amrinone in the same animal model (Hartog et al., 1986), but in animals with ischaemic hearts amrinone significantly increased endocardial blood flow and the endo-epi ratio (Hartog et al., 1987). It therefore appears that the decrease in endocardial blood

flow and endo-epi ratio by UD-CG 212 CL found in this study in animals with a normal heart may not be of much consequence for the clinical situation. Furthermore, in congestive heart failure, the heart rate is usually already high and vasodilator drugs are likely to reduce heart rate, while blood pressure is maintained (due to an increase in cardiac output).

Of particular interest in the treatment of congestive heart failure are the renal and skeletal muscle blood flows. With UD-CG 212 CL vasodilatation occurred in the renal vascular bed at all concentrations. There was even an increase in blood flow at the lower doses despite the fall in arterial blood pressure and cardiac output. However, no vasodilatation occurred in skeletal muscle. In this respect the actions of UD-CG 212 CL were quite different from those of pimobendan which did not affect renal vascular conductance but increased skeletal muscle conductance by 40% at comparable changes in mean arterial blood pressure and cardiac output (Verdouw et al., 1986).

The maximum concentration of UD-CG 212 CL after administration of pharmacologically active doses (10, 25, 50 and 100 $\mu g \cdot kg^{-1} \cdot min^{-1}$) of pimobendan did not exceed 20 $ng \cdot ml^{-1}$ (Verdouw et al., 1986). Around this concentration, UD-CG 212 CL caused only small but significant reductions in left ventricular end-diastolic and mean arterial pressures and increases in heart rate. These effects may partly contribute to the systemic effects of pimobendan. However, in view of the much higher plasma concentrations of UD-CG 212 CL required to cause major haemodynamic effects, it would appear that most cardiovascular changes observed after the administration of pimobendan in the original study (Verdouw et al., 1986) were induced by the parent drug itself.

In summary, the present experiments show that UD-CG 212 CL is a vasodilator agent with positive inotropic actions. The vasodilator effect of the drug is more marked on the venous than on the arterial vascular bed. In addition, the positive inotropic action of UD-CG 212 CL seems to be more potent than that of its parent compound pimobendan.

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VASODILATORY PROFILE OF NICORANDIL

CHAPTER 9

NICORANDIL-INDUCED CHANGES IN THE DISTRIBUTION OF CARDIAC OUTPUT AND CORONARY BLOOD FLOW IN PIGS.

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Nicorandil-induced changes in the distribution of cardiac output and coronary blood flow in pigs

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Summary. The present investigation was conducted to study systemic and regional haemodynamic effects of nicorandil, a potent coronary vasodilator, after intravenous or local intracoronary administration in anaesthetized or conscious pigs. Intravenous infusions of nicorandil for 10 min in both anaesthetized (15, 30, 75 and 150 $\mu g \cdot kg^{-1} \cdot min^{-1}$) and conscious (20, 40 and 80 $\mu g \cdot kg^{-1} \cdot min^{-1}$) pigs reduced arterial blood pressure, stroke volume, left ventricular end-diastolic pressure (LVEDP) and systemic vascular resistance, but increased heart rate and maxLVdP/dt. Since nicorandil decreased LVEDP at doses which did not affect arterial blood pressure, the drug may be considered as a more potent venodilator than arterial dilator. Nicorandil increased cardiac output only in conscious animals due to a more marked tachycardia (85% after 80 $\mu g \cdot kg^{-1} \cdot min^{-1}$) than in anaesthetized animals (30% after 75 $\mu g \cdot kg^{-1} \cdot min^{-1}$). The nicorandil-induced increase in heart rate and maxLVdP/dt, being substantially attenuated in conscious pigs after treatment with propranolol, can be ascribed to a reflex activation of the sympathetic nervous system following the fall in arterial pressure. Although cardiac output did not change in anaesthetized animals, intravenous infusions of nicorandil did cause a redistribution of blood flow in favour of organs such as the heart, adrenals, spleen, small intestine and brain at the expense of that to the stomach and kidneys; hepatic artery and skeletal muscle blood flow did not change. The increase in myocardial blood flow, primarily to the subepicardial layers, was associated with an enhancement in coronary venous oxygen content and was also noticed after intracoronary infusions of nicorandil (0.6, 1.5, 3 and 6 $\mu g \cdot kg^{-1} \cdot min^{-1}$). The above cardiovascular profile suggests a possible usefulness of nicorandil in angina pectoris as well as congestive heart failure. However, caution is needed because the strong hypotensive action and reflex-mediated tachycardia may under certain conditions aggravate myocardial ischaemia, particularly in the subendocardial layers.

Key words: Nicorandil – Systemic haemodynamics – Vasodilatation – Coronary circulation – Regional blood flows – Beta-adrenoceptor blockade – Propranolol – Pigs

Introduction

Nicorandil (N-(2-hydroxyethyl) nicotinamide nitrate; SG-75) is a potent directly-acting coronary vasodilator (Uchida et al. 1978). Upon intra-arterial injection the drug also increases blood flow in the femoral, mesenteric and, to a lesser extent, renal vessels (Sakai et al. 1981). The results obtained in several models of myocardial ischaemia (Aono et al. 1981; Lamping and Gross 1984a, b; Lamping et al. 1984a, b) and in angina pectoris (Uchida 1978; Thormann et al. 1982, 1983) have focused special attention on the potential usefulness of the drug as an anti-anginal agent (Sakai et al. 1983). In addition, since nicorandil decreases both left ventricular end-diastolic and end-systolic volumes and possesses only a negligible negative inotropic action in the therapeutic dose-range, the drug might also be useful in the treatment of heart failure (Belz et al. 1984). In this condition the decrease in cardiac output leads to cardiovascular adjustments often at the expense of renal and skeletal muscle blood flow (Drexler et al. 1986). Therefore, normalization of perfusion of these organs is particularly beneficial. However, few data on the effects of nicorandil on regional blood flows, other than that of the coronary circulation, have so far been reported.

With respect to regional myocardial blood flow, using the radioactive microsphere technique, Gross et al. (Lamping and Gross 1984a; Lamping et al. 1984a, b; Preuss et al. 1985) have reported that, both in anaesthetized and conscious dogs, nicorandil dose-dependently increases transmural left ventricular blood flow with the greatest increases occurring in the subepicardium and mid-myocardium. As a result the subendocardial-subepicardial blood flow ratio (endo/epi) decreases but even then subendocardial blood flow is elevated by more than two-fold (Preuss et al. 1985). Moreover, if the fall in aortic pressure after nicorandil is prevented by use of a cuff around the descending thoracic aorta, collateral blood flow to the subendocardial layers of an ischaemic area increases to a similar extent as (Lamping and Gross 1984b) or in excess of (Lamping and Gross 1984a) that to the subepicardial layers.

The object of the present investigation, performed in young Yorkshire pigs, is three-fold. Firstly, we have studied the regional haemodynamic effects of nicorandil on various tissues. Secondly, an attempt has been made to delineate direct and indirect (secondary to systemic haemodynamic changes) effects of nicorandil on blood flow to different layers of the myocardium. For this purpose the drug was infused directly into a coronary artery. Lastly, since a combination of nicorandil and beta-adrenoceptor antagonists is clinically important, we also report on the systemic haemodynamic effects of nicorandil with or without propranolol in conscious pigs.

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Materials and methods

Anaesthetized pigs. After an overnight fast Yorkshire pigs of either sex (24–26 kg, $n = 17$), were sedated with 120 mg azaperone (Stresnil) i.m., anaesthetized with 150 mg metomidate (Hypnodil) i.v., intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture of oxygen and nitrous oxide (1:2). Respiratory rate and tidal volume were set to keep arterial blood gases within the normal range: $7.35 < pH < 7.45$; $35 \text{ mm Hg} < PCO_2 < 45 \text{ mm Hg}$ and $90 \text{ mm Hg} < PO_2 < 150 \text{ mm Hg}$. 8F catheters were placed in the superior caval vein for administration of 100 mg · kg^{-1} alpha-chloralose followed by an infusion of low dose pentobarbital (5 mg · kg^{-1} · h^{-1}), administration of the muscle relaxant pancuronium bromide (4 mg) prior to thoracotomy, and Haemaceel (Behringwerke A.G., Marburg, FRG) to replace blood loss. Catheters were also positioned in the inferior caval vein for infusion of nicorandil and in the femoral artery for withdrawal of blood samples. Microtipped catheters (8F Millar) were used to measure left ventricular and central aortic blood pressures. After thoracotomy, an electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the ascending aorta, while the great cardiac vein was cannulated for collection of blood in which haemoglobin concentration and oxygen saturation were determined (OSM2, Radiometer, Copenhagen, Denmark). In some animals the left anterior descending coronary artery was also cannulated with a 4F catheter for intracoronary infusions of nicorandil. Myocardial oxygen consumption ($M\dot{V}O_2$), was calculated as the product of coronary blood flow and the difference in the oxygen contents of the arterial and coronary venous blood.

To determine regional blood flows, the left atrial appendage was cannulated for injection of a batch of 1–2.10^6 carbonized plastic microspheres [15 ± 1 µm (SD) in diameter] labeled with either ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce. Full details of the procedures and the calculation of flow data have been reported earlier (Saxena et al. 1980; Saxena and Verdouw 1985).

Conscious pigs. After an overnight fast Yorkshire pigs (18–20 kg, $n = 6$), pretreated with a mixture of procaine penicilline-G and benzathinepenicilline-G (Duplocilline) both 300.000 U i.m., were sedated with 30 mg · kg^{-1} ketamine · HCl i.m., intubated and connected to a respirator for artificial ventilation with a mixture of oxygen and nitrous oxide (1:2) to which 1% halothane was added. After the jugular vein and common carotid artery had been cannulated for infusion of drugs and measurement of mean arterial blood pressure, the chest was opened via the left fifth intercostal space, the heart exposed and a Konigsberg transducer (Konigsberg Instrument Inc., Pasadena, CA, USA) implanted near the apex for recording of left ventricular pressure. The left atrium was cannulated for recording of left atrial pressure, used for calibration of the Konigsberg transducer signals. The aorta was approached through the third intercostal space and an electromagnetic flow probe was positioned around the ascending aorta. Catheters and wires were tunneled subcutaneously to the back, the chest was closed and the animals allowed to recover. During the next days the animals received i.v. 500 mg amoxicilline (Clamoxil) and 500 mg kanamycine (Kamynex) to prevent infection. Daily flushment of the catheters was performed to prevent clotting of blood in the lumen. After recovery of surgery at least 4 sessions were held to adapt the animals to the experimental and laboratory facilities. The experimental protocol was executed 2–3 weeks after surgery.

Experimental protocols. In the anaesthetized animals four consecutive 10 min intravenous (15, 30, 75 and 150 µg · kg^{-1} · min^{-1}; $n = 12$) or coronary (0.6, 1.5, 3.0 and 6.0 µg · kg^{-1} · min^{-1}; $n = 5$) infusions were administered. Systemic haemodynamics were measured and the distribution of coronary blood flow was determined in both series of experiments, but the distribution of cardiac output was only determined during the intravenous infusion experiments. In the conscious pigs the systemic haemodynamic effects of three successive 10-min infusions (20, 40 and 80 µg · kg^{-1} · min^{-1}) without ($n = 6$) and after ($n = 4$) beta-adrenoceptor blockade with propranolol (0.5 mg · kg^{-1} + 0.5 mg · kg^{-1} · h^{-1}) were employed. Regional blood flows were not determined in these animals.

Drugs. The substances used in this study were the anaesthetics, propranolol (ICI-Pharma, Rotterdam, The Netherlands) and nicorandil (Rhône-Poulenc, Amstelveen, The Netherlands). Nicorandil was dissolved in 1.5 ml ethylalcohol and 0.5 ml polyethylene glycol and subsequently final volume was reached by adding isotonic saline. The solvent has no effect on cardiovascular performance in the pig (Duncker et al. 1986b).

Statistical analysis. Analysis was performed by using a parametric two-way analysis of variance (randomized block design) followed by Duncan's new multiple range test (Steel and Torrie 1980). *P*-Values less than 0.05 were considered to be statistically significant.

Results

Intravenous infusions of nicorandil in anaesthetized animals

Systemic circulation. The haemodynamic effects of nicorandil are summarized in Table 1. Mean arterial blood pressure decreased dose-dependently to 55% of the pre-drug value without affecting pulse pressure. This was due to a reduction in systemic vascular resistance, as cardiac output remained unchanged. The maintenance of cardiac output resulted from a reflex-induced tachycardia (heart rate increased up to 30%), as stroke volume decreased from 24 ± 2 ml to 20 ± 1 ml. This decline in stroke volume must have been the result of the reduction in left ventricular filling pressure from 7 ± 1 mm Hg to 4 ± 1 mm Hg as the reduction in arterial blood pressure and the slight increase in maxLVdP/dt would facilitate left ventricular ejection.

Coronary circulation. Although left ventricular transmural blood flow was not affected, its distribution over the myocardium changed in favour of the subepicardial layers, as flow to the subendocardium decreased by 30% of the pre-drug value of 144 ± 9 ml · min^{-1} · 100 g^{-1}, whereas that to the subepicardium increased by 65% of its pre-drug value of 120 ± 8 ml · min^{-1} · 100 g^{-1} (Fig. 1). Transmural blood flow was maintained despite the nicorandil-induced hypotension and, therefore, coronary vasodilation must have taken place; the calculated transmural resistance decreased by up to 45%. The vasodilatation was only limited to the subepicardial layers (60% decrease in vascular resistance) as the resistance of the subendocardial layers was not significantly affected (Fig. 1).

Table 1. Systemic haemodynamics after continuous 10 min intravenous infusions of nicorandil in 12 open-chest anaesthetized pigs

	Baseline	Nicorandil ($\mu g \cdot kg^{-1} \cdot min^{-1}$)			
		15	30	75	150
Cumulative total dose ($\mu g \cdot kg^{-1}$)	–	150	450	1200	2700
CO	2.5 ± 0.2	2.4 ± 0.3	2.7 ± 0.2	2.6 ± 0.2	2.6 ± 0.2
HR	104 ± 5	114 ± 6*	120 ± 5*	126 ± 6*	135 ± 7*
SV	24 ± 2	21 ± 2	21 ± 2*	21 ± 2*	20 ± 1*
LVSP	101 ± 5	94 ± 5*	86 ± 4*	78 ± 3*	69 ± 2*
LVEDP	7.1 ± 0.7	5.6 ± 0.6*	5.0 ± 0.6*	3.6 ± 0.5*	4.2 ± 0.5*
maxLVdP/dt	2630 ± 190	3030 ± 300*	3040 ± 260*	3120 ± 380*	3000 ± 350
DAP	69 ± 4	64 ± 4*	56 ± 4*	47 ± 3*	37 ± 3*
MAP	87 ± 4	81 ± 5*	70 ± 5*	70 ± 4*	48 ± 3*
SVR	37 ± 3	37 ± 5	29 ± 2*	24 ± 2*	19 ± 1*

CO = cardiac output ($l \cdot min^{-1}$); HR = heart ate (beats $\cdot min^{-1}$); SV = stroke volume (ml); LVSP and LVEDP are the left ventricular systolic and end-diastolic pressure, respectively (mm Hg); maxLVdP/dt = maximum rate of rise of left ventricular pressure ($mmHg \cdot s^{-1}$); DAP and MAP are the diastolic and mean arterial blood pressure (mm Hg), respectively; SVR = systemic vascular resistance ($mm\ Hg \cdot l^{-1} \cdot min$); all data are mean ± SEM; * $P < 0.05$ vs. baseline

Table 2. Myocardial blood flows and resistances after continuous 10 min intravenous infusions of nicorandil in 11 open-chest anaesthetized pigs

	Baseline	Nicorandil ($\mu g \cdot kg^{-1} \cdot min^{-1}$)			
		15	30	75	150
Cumulative total dose ($\mu g \cdot kg^{-1}$)	–	150	450	1200	2700
Blood flows					
left ventricle	133 ± 8	142 ± 15	149 ± 17	177 ± 19*	148 ± 23
right ventricle	99 ± 15	122 ± 10	134 ± 15	169 ± 20*	164 ± 26*
left atrium	99 ± 17	119 ± 11	119 ± 16	133 ± 14	113 ± 14
right atrium	104 ± 22	113 ± 10	123 ± 16	113 ± 11	88 ± 11
Resistances					
left ventricle	0.66 ± 0.05	0.57 ± 0.06	0.51 ± 0.07	0.36 ± 0.05*	0.36 ± 0.05*
right ventricle	1.03 ± 0.16	0.66 ± 0.07*	0.57 ± 0.09*	0.41 ± 0.07*	0.37 ± 0.08*
left atrium	1.00 ± 0.16	0.69 ± 0.09*	0.72 ± 0.17*	0.51 ± 0.09*	0.45 ± 0.06*
right atrium	1.01 ± 0.17	0.74 ± 0.09	0.62 ± 0.07	0.56 ± 0.06*	0.59 ± 0.09*

Blood flows are in $ml \cdot min^{-1} \cdot 100\ g^{-1}$ and resistances in $mm\,Hg \cdot ml^{-1}\ min \cdot 100\ g^{-1}$; all data are mean ± SEM; * $P < 0.05$ vs. baseline

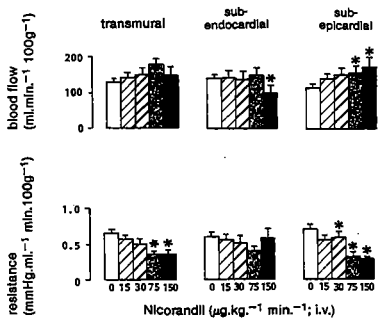

Fig. 1. Left ventricular blood flow and coronary vascular resistance after consecutive 10 min intravenous infusions of nicorandil in 11 anaesthetized pigs. Although transmural blood flow did not change, there was a redistribution in favour of the epicardium. All data have been presented as means ± SEM. * $P < 0.05$ vs. pre-nicorandil

Although left ventricular transmural blood flow did not change, myocardial oxygen consumption decreased from 5.5 ± 0.3 to 4.1 ± 0.7 $\mu mol \cdot min^{-1} \cdot g^{-1}$. This was reflected by the decrease in myocardial oxygen extraction as coronary venous oxygen saturation increased from 21% ± 3% to 36% ± 4% ($P < 0.05$).

Right ventricular blood flow increased dose-dependently up to 55%, but perfusion of the left and right atrium were not significantly affected (Table 2). Consequently, the decrease in vascular resistance was more prominent in the right ventricle (up to 65%) than in the right (up to 40%) or left atrium (up to 55%).

Regional blood flows. Although cardiac output did not change during the nicorandil infusions, blood flow to some organs (adrenals, spleen, small intestine and brain) increased whereas that to others was minimally affected (liver and skeletal muscle) or decreased (kidneys and stomach) (Fig. 2). Blood flow to the stomach decreased at the lowest two concentrations (up to 22%), but returned towards the pre-drug

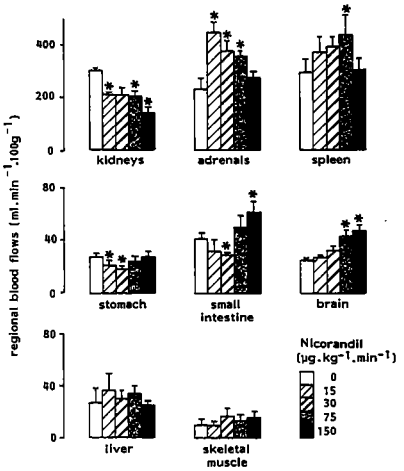

Fig. 2. Regional blood flows after consecutive 10 min intravenous infusions of nicorandil in 12 anaesthetized pigs. All data have been presented as means ± SEM. * $P < 0.05$ vs. pre-nicorandil

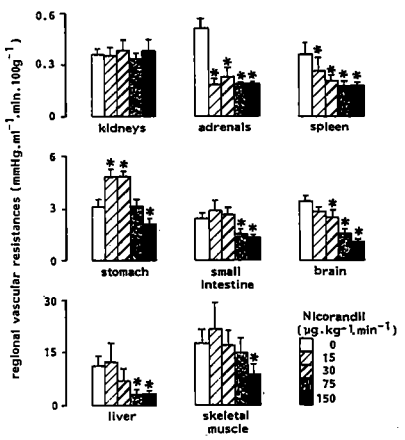

Fig. 3. Regional vascular resistances after consecutive 10 min intravenous infusions of nicorandil in 12 anaesthetized pigs. All data have been presented as means ± SEM. * $P < 0.05$ vs. pre-nicorandil

Table 3. Systemic haemodynamics after consecutive 10 min intracoronary infusions of nicorandil in 5 open-chest anaesthetized pigs

	Baseline	Nicorandil (µg · kg^{-1} · min^{-1})			
		0.6	1.5	3.0	6.0
CO	2.9 ± 0.3	2.9 ± 0.3	2.8 ± 0.2	2.8 ± 0.2	2.9 ± 0.2
HR	104 ± 7	102 ± 7	101 ± 8	106 ± 7	114 ± 9*
SV	28 ± 3	29 ± 3	28 ± 3	27 ± 4	26 ± 4*
LVSP	114 ± 9	110 ± 8	106 ± 6*	104 ± 6*	101 ± 6*
LVEDP	8.3 ± 0.8	7.7 ± 1.1	6.9 ± 1.1*	5.7 ± 1.0*	5.5 ± 0.8*
maxLVdP/dt	2400 ± 220	2310 ± 200	2360 ± 200	2400 ± 190	2550 ± 220
MAP	96 ± 9	93 ± 8	90 ± 6*	89 ± 7*	86 ± 7*
SVR	35 ± 5	34 ± 5	34 ± 4	33 ± 3	31 ± 3

CO = cardiac output (l · min^{-1}); HR = heart rate (beats · min^{-1}); SV = stroke volume (ml); LVSP and LVEDP are the left ventricular systolic and end-diastolic pressure (mm Hg), respectively; maxLVdP/dt = maximum rate of rise of left ventricular pressure (mmHg · s^{-1}); SVR = systemic vascular resistance (mm Hg · l^{-1} · min); all data are mean ± SEM; * $P < 0.05$ vs. baseline

value at higher concentrations. In the small intestine, blood flow was significantly elevated at the highest concentration.

In view of the hypotensive action of nicorandil, vasodilatation must have occurred in all organs in which flow was increased or remained unchanged (Fig. 3). The resistance of the renal vascular bed was unchanged because the decrease in renal blood flow paralleled the fall in perfusion pressure. The biphasic pattern in flow to the stomach was also reflected by a vasoconstriction at low and a vasodilatation at high concentrations. For the small intestine the increase in resistance at low concentrations was not statistically significant.

Intracoronary infusions of nicorandil in anaesthetized animals

Systemic circulation. Infusion rates up to 3 µg · kg^{-1} · min^{-1} did not affect cardiac output, heart rate, stroke volume, maxLVdP/dt and systemic vascular resistance, while a slight (<10%) decrease in blood pressure was observed (Table 3). Only left ventricular end-diastolic pressure was markedly affected as there was a drop from 8.3 ± 0.8 mm Hg to 5.7 ± 1.0 mm Hg. At the highest infusion rate (6 µg · kg^{-1} · min^{-1}) cardiac output was maintained because the slight increase in heart rate (10%) compensated for a similar decrease in stroke volume.

Coronary circulation. In the left anterior descending coronary artery (LADCA) perfused area subepicardial blood flow increased with the highest two infusion rates, but subendocardial blood flow remained unchanged (Fig. 4). Consequently, the endo/epi decreased dose-dependently from 1.15 ± 0.11 to 0.91 ± 0.08 ($P < 0.05$). Transmural myocardial resistance decreased slightly which was primarily due to vasodilatation in the subepicardial layers. Because the

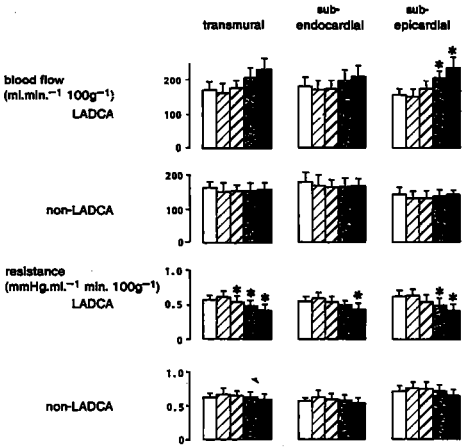

Fig. 4. Myocardial blood flow and coronary vascular resistance after intracoronary infusions of nicorandil into the left anterior descending coronary artery (LADCA) of 5 anaesthetized pigs. No changes were observed in the area adjacent to the LADCA perfused myocardium. The intracoronary infusion rates ($\mu g \cdot kg^{-1} \cdot min^{-1}$) were: □ = 0, ▨ = 0.6; ▩ = 1.5; ▦ = 3.0 and ■ = 6.0. All data have been presented as means ± SEM. * $P < 0.05$ vs. pre-nicorandil

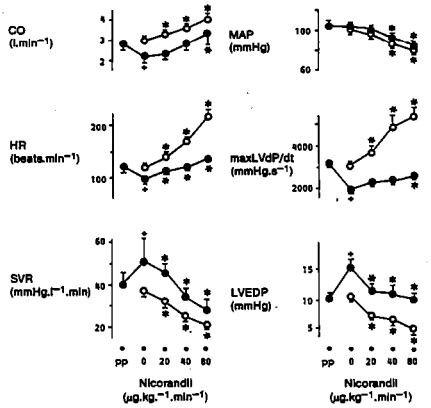

Fig. 5. The effects of continuous 10 min intravenous infusions of nicorandil without (○; $n = 6$) and after (●; $n = 4$) beta-adrenoceptor blockade in conscious pigs. All data have been presented as mean ± SEM. * $P < 0.05$ vs. pre-nicorandil (○). + $P < 0.05$ vs. pre-propranolol (*PP*)

slight increase in transmural blood flow was accompanied by a decreased coronary arterio-venous oxygen content difference, oxygen consumption of the LADCA-perfused area remained unchanged. In the different layers of the myocardium not perfused by the LADCA no significant changes in either blood flow or vascular resistance were observed.

Intravenous infusions of nicorandil in conscious pigs

Systemic circulation. Cardiac output increased dose-dependently from $3.0 \pm 0.2\,l \cdot min^{-1}$ to $4.0 \pm 0.2\,l \cdot min^{-1}$ ($P < 0.05$) due to an increase in heart rate (up to 80%, Fig. 5), which completely negated the dose-related diminution in stroke volume from 24 ± 2 ml to 19 ± 2 ml ($P < 0.05$, not shown). The reason for the decrease in stroke volume was the reduction in left ventricular end-diastolic pressure from 10.6 ± 0.8 mm Hg to 4.6 ± 1.0 mm Hg ($P < 0.05$) as both the reduction in blood pressure (up to 20%) and the increase in maxLVdP/dt (up to 80%) would augment stroke volume. Since the decrease in blood pressure occurred despite an increase in cardiac output, nicorandil caused a vasodilatation of the systemic vascular bed.

The immediate effects of propranolol were similar to those described for pentobarbital-anaesthetized pigs (Wolffenbuttel and Verdouw 1983): decreases in cardiac output (22%), heart rate (19%) and maxLVdP/dt (40%), increases in left ventricular filling pressure (from 10 to 15 mm Hg) and systemic vascular resistance (27%) and no change in mean arterial blood pressure. After beta-adrenoceptor blockade with propranolol, the responses of heart rate and maxLVdP/dt to nicorandil infusions were markedly attenuated, whereas those of the other variables were not significantly affected.

Discussion

Systemic haemodynamics

The present study in conscious and anaesthetized pigs confirms the potent vasodilating properties of nicorandil already reported by others (Uchida et al. 1978; Sakai et al. 1981). At lower concentrations nicorandil exerted a more pronounced effect on preload (left ventricular end-diastolic pressure; LVEDP) than on afterload (systemic arterial pressure) suggesting that the drug is a more potent venodilator than arterial dilator. This is also supported by the results obtained in the intracoronary infusion experiments where doses were increased in small steps and LVEDP already decreased at doses which had a negligible effect on systemic arterial pressure.

In general nicorandil produced similar effects in both the anaesthetized and the conscious pigs. Apart from the fact that cardiac output increased in the conscious but not in the anaesthetized animals, in both conditions intravenous administration of nicorandil increased heart rate and maxLVdP/dt and decreased stroke volume, LVEDP, systemic vascular resistance and arterial pressure. The increase in cardiac output was due to the more marked tachycardia in the conscious (85% after 80 $\mu g \cdot kg^{-1} \cdot min^{-1}$) than in the anaesthetized (30% after 75 $\mu g \cdot kg^{-1} \cdot min^{-1}$) animals. The nicorandil-induced tachycardia and increase in maxLVdP/dt can be ascribed to a reflex activation of the sympathetic nervous system following the fall in arterial pressure elicited by nicorandil. It is to be appreciated that reflex-activity can be attenuated by anaesthetic agents and, therefore, the magnitude of tachycardia was less in the anaesthetized animals. Moreover, positive chronotropic and inotropic effects were substantially reduced, though tachycardia was not completely

eliminated, when nicorandil was administered to the conscious animals after beta-adrenoceptor blockade with propranolol. The propranolol-resistant tachycardia, as already described with a number of other vasodilators in different species, including man (Man in't Veld et al. 1978; Reid 1979; Nakaya et al. 1983; Bolt and Saxena 1984a; Warltier et al. 1984), are most likely due to a withdrawal of parasympathetic tone. The fall in stroke volume in both conscious and anaesthetized pigs was apparently caused by the decrease in LVEDP as the nicorandil-induced reduction in arterial pressure and enhancement in maxLVdP/dt would tend to facilitate left ventricular ejection.

Regional haemodynamics

Although cardiac output did not change, at lower concentrations there was a redistribution of blood flow in favour of organs such as the adrenals, spleen and brain at the expense of that to the stomach, small intestine and kidneys. At the highest concentration increases in blood flow were observed in the small intestine and brain, while renal blood flow was diminished. Except the kidneys, vascular resistance decreased to different degrees in all organs studied. From a number of studies it is abundantly clear that the direct vascular effects of vasodilators are modified to different extents by counter-regulatory mechanisms, such as tissue autoregulation and baroreceptor activation and, therefore, each vasodilating agent seems to produce a characteristic haemodynamic profile (see Saxena and Bolt 1987).

The reduction in renal blood flow with nicorandil is similar to our previous observations with the dihydropyridine calcium channel blockers nisoldipine (Dunker et al. 1986a) and nimodipine (Duncker et al. 1986b) as well as the pyridazinone derivative pimobendan (Verdouw et al. 1986; Duncker et al. 1986c) which were studied in the same animal model (anaesthetized pigs). However, in contrast, another dihydropyridine calcium channel blocker felodipine, studied in conscious renal hypertensive rabbits, increased renal blood flow (Bolt and Saxena 1984b).

The effects of nicorandil and the calcium channel blockers on skeletal muscle blood flow also differ. Whereas nicorandil and pimobendan (Verdouw et al. 1986; Duncker et al. 1986c) did not affect muscle blood flow, it was markedly increased (100% to 400%) by the calcium channel blockers, felodipine (Bolt and Saxena 1984b), nisoldipine (Duncker et al. 1986a) and nimodipine (Duncker et al. 1986b).

Myocardial oxygen consumption and haemodynamics

Although myocardial oxygen consumption decreased slightly left ventricular blood flow tended to increase which, in view of the increased coronary venous oxygen content, points towards a vasodilatory action of nicorandil on the coronary arterial bed. Despite the unchanged transmural blood flow there was a redistribution in favour of the subepicardium which is in agreement with the observation by other investigators (Preuss et al. 1985). To investigate whether the decrease in endo/epi was due to the hypotension and tachycardia (Domenech and Goich 1976) or to a preference of nicorandil for the subepicardial layers, we infused the substance directly into a coronary artery in order to minimize systemic haemodynamic responses. Except for the highest intracoronary infusion rate, the systemic haemodynamic changes were minimal and transmural myocardial blood flow, and its distribution, of the control area were not affected. However, nicorandil again selectively increased epicardial blood flow suggesting a preferential susceptibility of the subepicardium to the vasodilatory action of nicorandil.

In conclusion, the cardiovascular profile of nicorandil suggests that the drug may be useful during myocardial ischaemia, but caution is warranted because the strong hypotensive action and the reflex-mediated tachycardia might under certain conditions, especially when the vasodilatory reserve of the subendocardial layers is exhausted, aggravate rather than ameliorate myocardial ischaemia by a coronary steal. Administration of the drug to patients with heart failure might also be considered. Because of the high activity of the sympathetic nervous system in a large number of these patients, reflex tachycardia is less likely to occur. Furthermore, reduction of both pre- and afterload by nicorandil might normalize dimensions of the heart and thereby reduce myocardial oxygen consumption. Data on renal and skeletal muscle blood flows do not show such a favourable action of the drug, but it is possible that when vascular tone of these beds is increased during heart failure, vasodilatation in these beds may become more prominent.

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SYSTEMIC HEMODYNAMIC ACTIONS OF VASODILATING DRUGS IN THE ABSENCE OR PRESENCE OF β-ADRENOCEPTOR BLOCKADE

CHAPTER 10

COMPARISON OF THE SYSTEMIC HEMODYNAMIC ACTIONS OF DIHYDROPYRIDINE CALCIUM-CHANNEL BLOCKERS IN CONSCIOUS PIGS WITH OR WITHOUT β-ADRENOCEPTOR BLOCKADE.

Chapter 10

Comparison of the systemic hemodynamic actions of dihydropyridine calcium-channel blockers in conscious pigs with or without β-adrenoceptor blockade.

Introduction

Though the calcium-channel blockers, nifedipine, nisoldipine and nimodipine, have greater vascular than direct cardiac actions, these drugs may show variations in the extent of their preferences for vascular versus cardiac musculature and for venous versus arterial vasculature. Nisoldipine has been reported to be 4 to 10 times more potent on vascular smooth muscle than nifedipine, while it is equipotent or less potent with respect to inhibition of cardiac muscle contraction (Kazda et al., 1980). Nimodipine has been claimed to cause vasodilation in the cerebral bed with very little effect on arterial blood pressure (Kazda et al., 1982). In this investigation in conscious pigs the effects of these calcium-channel blockers on systemic hemodynamic variables have been compared using cumulative intravenous infusions. Since these drugs often induce reflex-mediated cardiostimulation (Warltier et al., 1984; Duncker et al., 1986, 1987b), combination of calcium antagonists with β-adrenoceptor antagonists may be superior to monotherapy in the treatment of coronary artery disease (Dargie et al., 1981; Fox et al., 1981). Therefore, the effects of these dihydropyridine-derivatives have also been studied in animals after pretreatment with propranolol.

Materials and methods

General

The instrumentation of the animals has been described in detail in an earlier publication (Duncker et al., 1987a). After recovery from surgery at least 4 sessions were held to adapt the animals to the experimental and laboratory facilities, before the experimental protocol was executed.

Experimental protocols

Three consecutive 10 min intravenous infusions were used with each drug: 0.5, 1 and 2 $\mu g.kg^{-1}.min^{-1}$ for nisoldipine (n=7) and 1, 2 and 4 $\mu g.kg^{-1}.min^{-1}$ for nimodipine (n=6) and nifedipine (n=6). At least 24 hours later these experiments were repeated 10 min after β-adrenoceptor blockade with intravenous 0.5 $mg.kg^{-1}$ + 0.5 $mg.kg^{-1}.h^{-1}$ propranolol. This dose-regimen

provides adequate β-adrenoceptor blockade in conscious pigs (Duncker et al., 1987c).

In another series of experiments (n=9) we studied the cardiovascular actions of the dihydropyridine solvent to evaluate the hemodynamic stability of our experimental model. The amount of solvent (intravenous 10 min infusions of 0.1, 0.2 and 0.4 $ml.min^{-1}$) corresponded with the amount of solvent infused during the nimodipine experiments and was twice the volume administered during nifedipine and nisoldipine infusions. Finally, we evaluated the hemodynamic stability of β-adrenoceptor blockade after intravenous administration of 0.5 $mg.kg^{-1}$ + 0.05 $mg.kg^{-1}.h^{-1}$ propranolol (n=6).

In each case data were obtained at baseline and at the end (10 min) of each infusion period. In the propranolol-treated animals data were also recorded 10 min after administration of the β-adrenoceptor antagonist at which time cardiovascular parameters had reached a new stable level.

Data presentation and statistical analysis

Data have been presented as mean ± S.E. of the mean. In general data have been presented as absolute values, except for the control experiments (Tables 1 and 2) which have been presented in percentage changes. Statistical analysis was performed by use of Duncan's new multiple-range test once an analysis of variance (randomized block design) had revealed that the samples represented different populations (Steel and Torrie, 1980). P values of 0.05 or less (two-tailed) were considered to be statistically significant.

Drugs

Apart from the anesthetics during surgery and the antibiotics and heparin during the post-surgical period, the drugs used in this study were nisoldipine, nifedipine and nimodipine (Bayer AG, Wuppertal, FRG) and propranolol hydrochloride (ICI Farma, Rotterdam, the Netherlands).

Results

Hemodynamic stability of solvent and propranolol treated animals

Intravenous infusions of solvent did not affect any of the measured systemic hemodynamic parameters (Table 1). Intravenous treatment with propranolol reduced heart rate by 15 ± 2%, $LVdP/dt_{max}$ by 26 ± 4% and cardiac output by 17 ± 3%, while left ventricular end-diastolic blood pressure

Table 1. Systemic hemodynamics during 10 min solvent infusions in 9 conscious pigs.

		% by solvent (ml.min^{-1})		
	baseline	0.1	0.2	0.4
CO	2.4 ± 0.1	−3 ± 2	−1 ± 3	1 ± 3
HR	118 ± 6	−1 ± 1	−2 ± 2	−1 ± 2
SV	21 ± 1	−2 ± 2	1 ± 2	2 ± 2
LVdP/dt$_{max}$	3060 ± 240	−2 ± 2	2 ± 3	5 ± 4
LVEDP[1]	11.0 ± 1.3	0.9 ± 0.7	1.0 ± 0.4	1.0 ± 0.9
SAP	118 ± 2	−1 ± 1	0 ± 1	0 ± 1
MAP	89 ± 3	−1 ± 1	0 ± 1	0 ± 1
DAP	76 ± 3	−1 ± 1	0 ± 2	2 ± 2
SVR	37 ± 2	3 ± 2	1 ± 3	0 ± 2

[1]changes have been expressed in absolute values. CO = cardiac output (l.min^{-1}); HR = heart rate (beats.min^{-1}); SV = stroke volume (ml); LVdP/dt$_{max}$ = maximal rate of rise of left ventricular pressure (mmHg.s^{-1}); LVEDP = left ventricular end-diastolic blood pressure (mmHg); SAP = systolic arterial blood pressure (mmHg); MAP = mean arterial blood pressure (mmHg); DAP = diastolic arterial blood pressure (mmHg); SVR = systemic vascular resistance (mmHg.l^{-1}.min).

increased by 4.6 ± 0.9 mmHg within 10 min after injection of the bolus. Mean arterial blood pressure did not change due to an increase in systemic vascular resistance (19 ± 6%). The propranolol-induced effects did not further change during the remainder of the 40 min period (Table 2).

Nisoldipine with or without β-adrenoceptor blockade

Nisoldipine (0.5, 1 and 2 μg.kg^{-1}.min^{-1}) caused a dose-dependent increase in cardiac output (up to 71 ± 8% with the highest dose) which was entirely due to the increase in heart rate (up to 86 ± 12%) as stroke volume did not change with the lower doses and even slightly decreased after the highest dose (Fig. 1). In spite of the increase in cardiac output, systolic, mean and diastolic arterial blood pressure decreased up to 12 ± 3%, 19 ± 2% and 23 ± 2%, respectively, due to systemic vasodilation (systemic vascular

Table 2. Systemic hemodynamics during propranolol infusions in 6 conscious pigs

		% by propranolol (0.5 $mg.kg^{-1}$ + 0.5 $mg.kg^{-1}.h^{-1}$)			
	baseline	propranolol	10'	20'	30'
CO	2.6 ± 0.1	2.2 ± 0.1+	0 ± 2	-1 ± 1	-1 ± 1
HR	127 ± 7	108 ± 5+	-2 ± 1	-2 ± 1	-2 ± 2
SV	21 ± 1	20 ± 1	3 ± 1	1 ± 1	0 ± 2
$LVdP/dt_{max}$	3280 ± 430	2400 ± 240+	-2 ± 3	-1 ± 3	-2 ± 3
LVEDP[1]	12.2 ± 0.9	16.8 ± 1.2+	-0.7 ± 0.5	0.5 ± 0.4	-0.3 ± 0.8
SAP	122 ± 12	119 ± 10	0 ± 2	-3 ± 2	-2 ± 2
MAP	96 ± 9	93 ± 7	0 ± 2	-4 ± 3	-2 ± 3
DAP	84 ± 8	82 ± 6	-1 ± 2	-4 ± 4	-3 ± 3
SVR	37 ± 4	44 ± 5+	0 ± 3	-2 ± 2	0 ± 3

[1]changes have been expressed in absolute values. CO = cardiac output ($l.min^{-1}$); HR = heart rate ($beats.min^{-1}$); SV = stroke volume (ml); $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure ($mmHg.s^{-1}$); LVEDP = left ventricular end-diastolic blood pressure (mmHg); SAP = systolic arterial blood pressure (mmHg); MAP = mean arterial blood pressure (mmHg); DAP = diastolic arterial blood pressure (mmHg); SVR = systemic vascular resistance ($mmHg.l^{-1}.min$).

resistance decreased by 52 ± 3%). Since the increase in $LVdP/dt_{max}$ (105 ± 10%) and the decrease in systemic vascular resistance would favour an increase in stroke volume, the slight decrease in left ventricular end-diastolic pressure (-2.7 ± 1.1 mmHg) must have been responsible for the reduction in stroke volume after the highest dose.

β-Adrenoceptor blockade did not much affect the nisoldipine-induced increases in cardiac output, despite an attenuation of the positive inotropic response (increase in $LVdP/dt_{max}$; Fig. 1). At the highest dose the increase in stroke volume partly compensated for the attenuated heart rate response. Arterial blood pressure responses were not affected by pretreatment with propranolol, nor was the response of the calculated systemic vascular

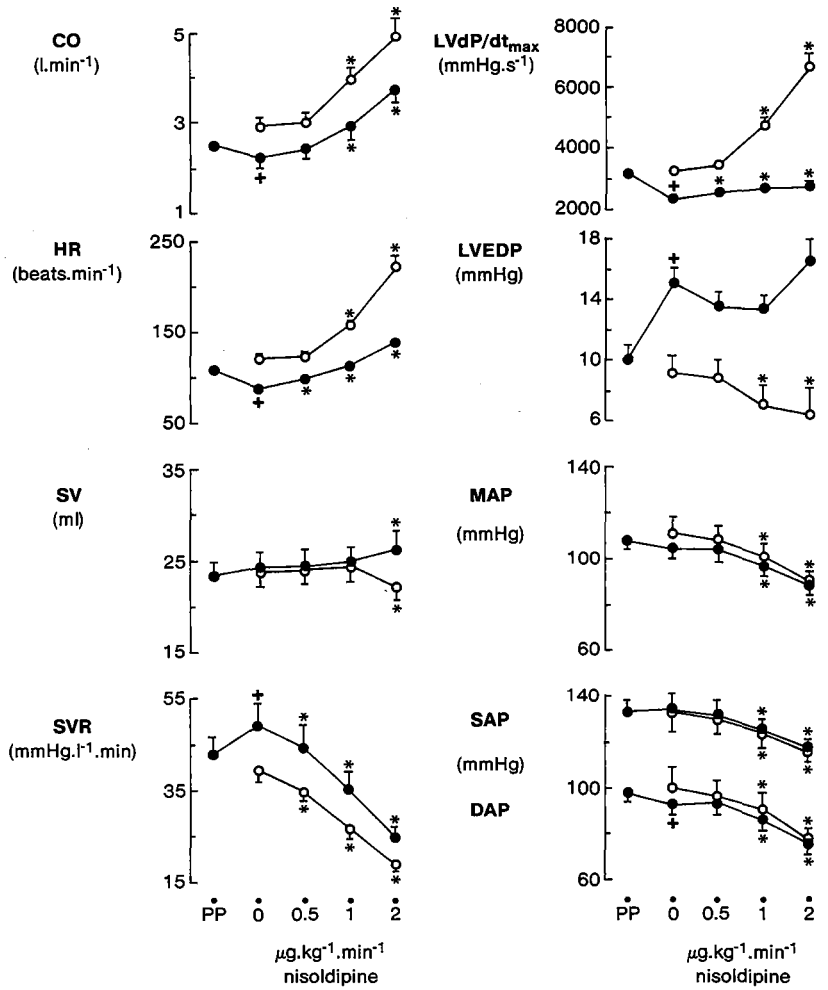

Figure 1.
The effects of consecutive 10 min intravenous infusions of 0.5, 1 and 2 $\mu g.kg^{-1}.min^{-1}$ nisoldipine without (○; n=7) and after (●; n=6) β-adrenoceptor blockade with 0.5 $mg.kg^{-1}$ + 0.5 $mg.kg^{-1}.h^{-1}$ propranolol in conscious pigs. CO = cardiac output; $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure; HR = heart rate; LVEDP = left ventricular end-diastolic blood pressure; SV = stroke volume; MAP = mean arterial blood pressure; SVR = systemic vascular resistance; SAP = systolic arterial blood pressure; DAP = diastolic arterial blood pressure; PP = Pre-propranolol. Data have been presented as mean ± S.E.M.; *nisoldipine-induced changes statistically significant ($P<0.05$); ⁺propranolol-induced changes statistically significant ($P<0.05$).

resistance. β-adrenoceptor blockade abolished the reduction in left ventricular end-diastolic blood pressure caused by nisoldipine.

Nimodipine with or without β-adrenoceptor blockade

Nimodipine (1, 2 and 4 $\mu g.kg^{-1}.min^{-1}$) caused dose-related hemodynamic effects that were similar to those induced by nisoldipine (Fig. 2). Only the responses of stroke volume and left ventricular end-diastolic pressure were slightly different as these parameters did not change with nimodipine.

After β-adrenoceptor blockade responses of cardiac output and heart rate were attenuated and that of $LVdP/dt_{max}$ abolished. Changes in arterial blood pressure were not affected, nor were the actions of nimodipine on stroke volume. Left ventricular end-diastolic pressure tended to increase but this did not reach levels of statistical significance. Finally the systemic vasodilation was slightly attenuated.

Nifedipine with or without β-adrenoceptor blockade

The dose-dependent actions of nifedipine (1, 2 and 4 $\mu g.kg^{-1}.min^{-1}$) were similar to that of the other two dihydropyridines. Increases in cardiac output, heart rate, $LVdP/dt_{max}$ were observed, and decreases in arterial blood pressure and calculated systemic vascular resistance. Like nimodipine, nifedipine did not affect stroke volume and left ventricular end-diastolic pressure. After β-adrenoceptor blockade responses of cardiac output and heart rate were minimally affected but that of $LVdP/dt_{max}$ was blunted. The responses of the other variables were not affected by the presence of propranolol.

Discussion

The most prominent of the hemodynamic responses of the dihydropyridines was systemic vasodilation, with nisoldipine as the most potent of the three drugs. Probably due to the pronounced vasodilation, nisoldipine elicited also the most prominent cardiostimulatory action. The increase in heart rate might have contributed to the increase in $LVdP/dt_{max}$. However, it has been shown that in pigs, although under anesthesia, raising heart rate from 100 to 160 $beats.min^{-1}$ by left atrial pacing has only a minor effect on $LVdP/dt_{max}$ (Scheffer and Verdouw, 1983). Moreover, preliminary observations from our

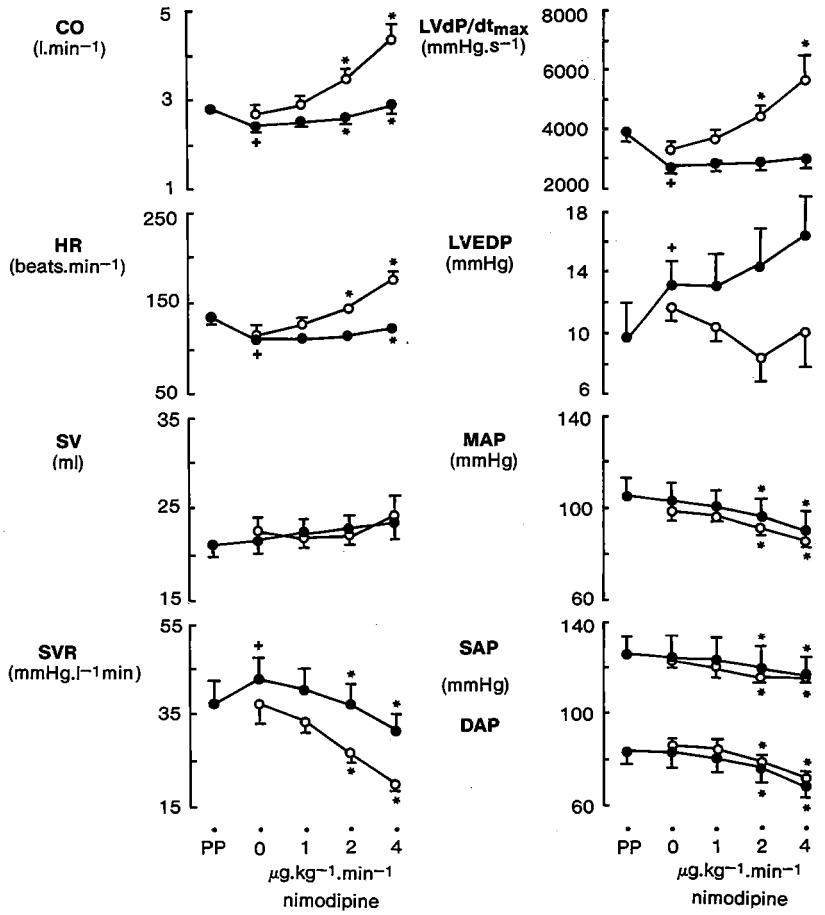

Figure 2.
The effects of consecutive 10 min intravenous infusions of 1, 2 and 4 μg.kg^{-1}.min^{-1} nimodipine without (○; n=6) or after (●; n=6) propranolol in conscious pigs. See legends of Fig. 1 for further details.

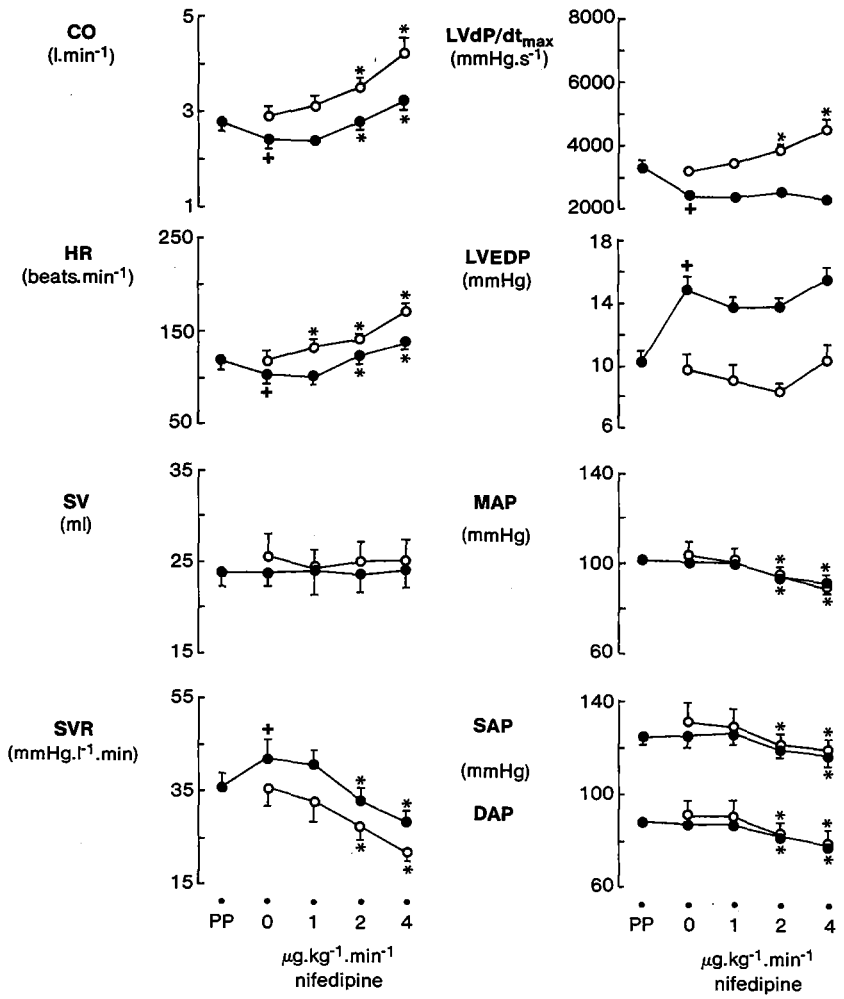

Figure 3.
The effects of consecutive 10 min intravenous infusion of 1, 2 a
4 μg.kg^{-1}.min^{-1} nifedipine without (○; n=6) or after (●; n=6) propranolol
conscious pigs. See legends of Fig. 1 for further details.

laboratory in conscious pigs, showed that the negative chronotropic compound UL-FS 49 (1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-[2-(3,4-dimethoxyphenyl)-ethyl]methylimino]propyl]-2H-3-benzazepin-2-on hydrochloride; Kobinger and Lillie, 1984) reduced heart rate from 140 to 90 beats.min^{-1} without affecting $LVdP/dt_{max}$. It is therefore likely that the increase in $LVdP/dt_{max}$ is predominantly due to direct sympathetic stimulation of the left ventricle rather than mediated by the increase in heart rate. Left ventricular filling pressure was only minimally affected by the three drugs although the nisoldipine-induced decrease was statistically significant. However, this finding has probably no major clinical implication as other substances, like the pyridazinone-derivatives pimobendan and UD-CG 212 Cl and the nitrate-like substance nicorandil which have been studied in the same model, reduced left ventricular filling pressure to a greater extent, while increments in heart rate were less (Duncker et al., 1987a) or equal (Verdouw et al., 1987). Furthermore, when preload was elevated by propranolol none of the three dihydropyridines affected this parameter, in contrast to the above mentioned other substances.

β-Adrenoceptor blockade did not significantly affect the vasodilatory responses. The increases in $LVdP/dt_{max}$ were more potently attenuated than those in heart rate and cardiac output. An increase in heart rate in the presence of β-adrenoceptor blockade has also been reported by other investigators (Warltier et al., 1984; Silke et al., 1986) and may be due to parasympathetic withdrawal (Nakaya et al., 1983). Since the left ventricle is only scarcely innervated by the parasympathetic system (Higgins et al., 1973), myocardial contractility is unable to increase through such a mechanism. Of the three drugs, the effects of nimodipine were affected to a greater extent by the presence of propranolol than were the effects of the two other calcium-channel blockers.

In conclusion, the dihydropyridine calcium channel blockers (nifedipine, nisoldipine and nimodipine) appear potent arterial vasodilators with negligible cardiodepressant actions. The systemic hemodynamical profiles were very similar for all three drugs. In view of the reflex-tachycardia and the negligible negative inotropic actions these substances can be safely combined with β-adrenoceptor blockade without compromising left ventricular pump function.

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CHAPTER 11

SYSTEMIC HAEMODYNAMIC ACTIONS OF PIMOBENDAN (UD-CG 115 BS) AND ITS O-DEMETHYLMETABOLITE UD-CG 212 CL IN THE CONSCIOUS PIG.

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Systemic haemodynamic actions of pimobendan (UD-CG 115 BS) and its *O*-demethylmetabolite UD-CG 212 Cl in the conscious pig

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1 The cardiovascular effects of the pyridazinone-derivatives pimobendan and its *O*-demethylmetabolite UD-CG 212 Cl (2-(4-hydroxy-phenyl)-5-(5-methyl-3-oxo-4,5-dihydro-2H-6-pyridazinyl) benzimidazole HCl) were studied in conscious pigs, employing consecutive intravenous 10 min infusions of 10, 25, 50 and 100 $\mu g\,kg^{-1}\,min^{-1}$ and 2, 4 and 8 $\mu g\,kg^{-1}\,min^{-1}$ respectively.

2 Pimobendan caused dose-dependent increases in $LVdP/dt_{max}$ (up to 115%) and heart rate (up to 30%), while cardiac output was slightly elevated (up to 15%) and stroke volume decreased by 12%. Left ventricular end-diastolic pressure decreased in a dose-related manner from 8.7 ± 1.0 mmHg to 2.7 ± 1.7 mmHg. Mean arterial blood pressure was not significantly affected because systemic vascular resistance decreased dose-dependently up to 15%.

3 After β-adrenoceptor blockade, the pimobendan-induced increases in heart rate and cardiac output were attenuated and the increase in $LVdP/dt_{max}$ almost abolished. The responses of left ventricular end-diastolic and mean arterial blood pressure, systemic vascular resistance and stroke volume were not modified.

4 UD-CG 212 Cl caused dose-related increases in $LVdP/dt_{max}$ (up to 100%) and heart rate (up to 25%). Cardiac output was minimally elevated (up to 8%) as stroke volume decreased dose-dependently up to 15%. As systemic vascular resistance decreased up to 12%, mean arterial blood pressure was slightly reduced (5%). Left ventricular end-diastolic blood pressure decreased dose-dependently from 9.0 ± 0.8 mmHg to 3.8 ± 1.3 mmHg.

5 After β-adrenoceptor blockade, the UD-CG 212 Cl-induced increases in heart rate and $LVdP/dt_{max}$ were attenuated and almost abolished and amounted up to 15% and 20%, respectively. The responses of the other systemic haemodynamic parameters were not significantly modified.

6 We conclude that pimobendan and UD-CG 212 Cl are compounds with marked positive inotropic and venodilator properties in the conscious pig. The attenuation of the inotropic effects by pretreatment with propranolol strongly suggests that, in the conscious pig, the β-adrenergic system is significantly involved in the positive inotropic actions. The lack of effect of β-adrenoceptor blockade on the vasodilator responses to both compounds suggest a mechanism not related to β-adrenergic activity.

Introduction

The pyridazinone-derivative pimobendan (UD-CG 115 BS) has been shown to possess veno- and arteriodilator as well as positive inotropic properties in a number of animal models (Diederen *et al.*, 1982; van Meel, 1985; Verdouw *et al.*, 1986; Duncker *et al.*, 1986). Although the precise mechanism of action of pimobendan is still largely unknown, phosphodiesterase inhibition and an increased sensitivity of contractile proteins to calcium may contribute to its cardiovascular actions (Rüegg *et al.*, 1984; Honerjäger *et al.*, 1984; Scholz & Meyer, 1986). It has also been reported that UD-CG 212 Cl (2-(4-hydroxy-phenyl)-5-(5-methyl-3-oxo-4,5-dihydro-2H-6-pyridazinyl) benzimidazole HCl), the *O*-demethylmetabolite of pimobendan, is more potent at increasing myocardial contractile force than the parent drug itself (Scholz & Meyer, 1986). Confusion therefore exists as to what

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extent the cardiovascular actions ascribed to pimobendan are in fact secondary to the presence of the metabolite. In the present study we describe the cardiovascular actions of pimobendan and UD-CG 212 Cl in the conscious pig and on the possible contribution of UD-CG 212 Cl to the actions of pimobendan. In addition, to obtain information on the relative contribution of factors other than those involving the β-adrenergic system, we also studied the effects of pimobendan and UD-CG 212 Cl after pretreatment with propranolol.

Methods

General

After an overnight fast, Yorkshire pigs (18–20 kg, $n = 6$), pretreated with a mixture of procaine penicillin-G and benzathinepenicillin-G (Duplocillin, Gist-Brocades N.V., Delft, The Netherlands) both 300000 units i.m., were sedated with 30 mg kg^{-1} ketamine HCl i.m. (Aescoket, Aesculaap B.V., Boxtel, The Netherlands). The animals were intubated and connected to a respirator for artificial ventilation with a mixture of O_2 and N_2O (1:2) to which 1% halothane was added. A jugular vein and common carotid artery were cannulated for infusion of drugs and measurement of mean arterial blood pressure, respectively. The chest was opened via the left fifth intercostal space to expose the heart. A transducer ($P_{4.5}$, Konigsberg Instruments Inc. Pasadena, California, USA) was implanted into the left ventricle of the heart through its apex for recording of left ventricular pressure. The left atrium was cannulated for recording of left atrial pressure which, together with the aortic blood pressure, was used for calibration of the Konigsberg transducer signals. The aorta was approached through the third intercostal space and an electromagnetic flowprobe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta. Catheters and wires were tunnelled subcutaneously to the back, the chest was closed and the animals allowed to recover. During the next 14 days the animals received daily intravenous bolus injections of 500 mg amoxicilline (Clamoxil; Beecham Farma B.V., Amstelveen, The Netherlands) and in addition, during the first week, 500 mg kanamycin (Kamynex; Gist-Brocades N.V., Delft, The Netherlands) to prevent infection. Daily flushing of catheters with an isotonic saline solution containing 500 iu heparin per ml (Thromboliquine; Organon Teknika B.V., Boxtel, The Netherlands) was performed to avoid clotting of blood in the lumen. After one week for recovery from surgery, at least 4 sessions were held to adapt the animals to the experimental and laboratory facilities. The experimental protocol was executed 2–3 weeks after the operation. All tracings were on a Graphtec Linearcorder (F WR 3701; Ankersmit, Breda, The Netherlands). Arterial acid-base balance and oxygenation during the experiments were not significantly different from those observed for young conscious Yorkshire pigs by Lagerwey (1973): $pH = 7.41 \pm 0.04$, $P_{CO_2} = 44 \pm 4$ mmHg, $P_{O_2} = 87 \pm 6$ mmHg and HbO_2-saturation of $91 \pm 2\%$.

Experimental protocols

Four series of experiments (6 pigs in each series) were performed. In two series, consecutive 10 min infusions of either drug were administered. For pimobendan the infusion rates were 10, 25, 50 and 100 μg kg^{-1} min^{-1} and for UD-CG 212 Cl, 2, 4 and 8 μg kg^{-1} min^{-1}. Corresponding volumes were 0.2, 0.5, 1.0 and 2.0 ml min^{-1} and 0.5, 1.0 and 2.0 ml min^{-1} for the pimobendan and UD-CG 212 Cl infusions, respectively. At the end of each 10 min infusion period, when parameters had reached a stable level, tracings of left ventricular pressure and its first derivative ($LVdP/dt$; obtained by electronic differentiation), arterial blood pressure, stroke volume and cardiac output were recorded and arterial blood samples were withdrawn for the determination of plasma concentrations of pimobendan and UD-CG 212 Cl. In the other two series of experiments the same protocols were repeated after β-adrenoceptor blockade with propranolol. The latter was dissolved in isotonic saline and administered intravenously as a bolus injection of 0.5 mg kg^{-1} (given over 2 min), immediately followed by a continuous infusion of 0.5 mg kg^{-1} h^{-1} at a rate of 0.2 ml min^{-1}. The infusions of the pyridazinone-derivatives were started 10 min after the bolus administration of propranolol. At this time haemodynamic parameters had reached a stable level. In other experiments we have shown that the isoprenaline dose-ratio for heart rate and $LVdP/dt_{max}$ for this dose of propranolol is more than 20 (unpublished data from this laboratory). Since the volume that was infused during propranolol administration was small (0.2 ml min^{-1}), isotonic saline was not administered to the animals which did not receive propranolol.

Determination of plasma concentrations

The plasma concentrations of pimobendan and UD-CG 212 Cl were determined by use of an h.p.l.c. assay with fully automated drug preconcentration on solid support (Roth, 1983). Briefly, the drugs were extracted on a reverse phase column and simultaneously preconcentrated after injection of whole plasma. The compounds were measured by means of fluorescence detection (332 nm/405 nm) after h.p.l.c separation on reversed phase ODS-hypersil (particle size; 5 μm). The eluent composition was methanol/water (590/460, v/

v) + 2.5 g ammonium acetate per litre eluent (total amount 2.625 g). Post column, a mixture of methanol/orthophosphoric acid 85%/water (300/100/100,v/v/v) was added with a flow rate of 0.2 ml min^{-1} via a T-fitting in order to optimize the fluorescence (increase in fluorescence by a factor of 2). The lower limit of detection for both compounds was about 1 ng ml^{-1}. Pimobendan and UD-CG 212 Cl themselves were used as external standards.

Statistical analysis

Data have been presented as mean of 6 experiments ± s.e.mean. Statistical analysis was performed by use of a parametric two-way analysis of variance (randomized block design), followed by the Duncan new multiple range test (Steel & Torrie, 1980). Statistical significance was accepted at $P < 0.05$ (two-tailed).

Drugs

The only substances used were propranolol hydrochloride (ICI-Pharma, Rotterdam, The Netherlands), pimobendan and its *O*-demethylmetabolite (2-(4-hydroxy-phenyl)-5-(5-methyl -3-oxo-4,5-dihydro-2H-6- pyridazinyl)benzimidazole HCl). Both of the latter compounds were kind gifts from Dr Karl Thomae GmBH, Biberach a/d Riss, FRG and they were dissolved in a mixture of polyethylene glycol 200 and saline (1:1).

Results

Plasma concentrations of pimobendan and UD-CG 212 Cl

Although duration and rate of infusion of pimobendan were the same as for the anaesthetized pigs (Verdouw *et al.*, 1986), the arterial plasma concentrations of pimobendan were considerably less (30–50%) in the conscious animals. On the other hand, the concentrations of UD-CG 212 Cl were very similar in the two preparations. With the lowest two infusion rates of UD-CG 212 Cl, UD-CG 212 Cl plasma concentrations were similar to the UD-CG 212 Cl plasma concentrations measured after the highest two infusion rates of pimobendan (Table 1).

Effects of pimobendan

Pimobendan caused a mild increase in cardiac output (up to 15%) due to a moderate tachycardia (heart rate increased up to 30%), as stroke volume decreased by 12% (Figure 1). $LVdP/dt_{max}$ increased dose-dependently and was more than doubled after the highest infusion rate. Since mean arterial blood pressure was unchanged in the presence of an increased cardiac output, systemic arterial vasodilatation (reflected by a decrease in systemic vascular resistance up to 15%) must have occurred. The effect on the systemic venous

Table 1 Plasma concentrations of pimobendan and UD-CG 212 Cl after continuous intravenous 10 min infusions of pimobendan and UD-CG 212 Cl in conscious pigs

		Pimobendan (μg kg^{-1} min^{-1})			
		10	25	50	100
Total dose administered (μg kg^{-1})		100	350	850	1850
Plasma concentration (ng ml^{-1})					
Pimobendan	−	65 ± 3*	157 ± 7*	364 ± 16*	828 ± 32*
Pimobendan	+	66 ± 3*	172 ± 6*	399 ± 17*	918 ± 35*
UD-CG 212 Cl	−	0 ± 0	9 ± 1*	14 ± 2*	22 ± 4*
UD-CG 212 Cl	+	1 ± 1	7 ± 2*	13 ± 2*	20 ± 3*
		UD-CG 212Cl (μg kg^{-1} min^{-1})			
		2	4	8	
Total dose administered (μg kg^{-1})		20	60	140	
Plasma concentration (ng ml^{-1})					
UD-CG 212 Cl	−	14 ± 1*	29 ± 1*	62 ± 2*	
UD-CG 212 Cl	+	18 ± 2*†	33 ± 1*†	70 ± 2*†	

Values are given as mean of 6 experiments ± s.e.mean; (−) indicates that propranolol was not present and (+) that the animals were pretreated with propranolol (0.5 mg kg^{-1} + 0.5 mg kg^{-1} h^{-1}); *$P < 0.05$ versus each of the lower plasma concentrations in the same series of experiments. †$P < 0.05$ versus plasma level at comparable infusion rate without β-adrenoceptor blockade.

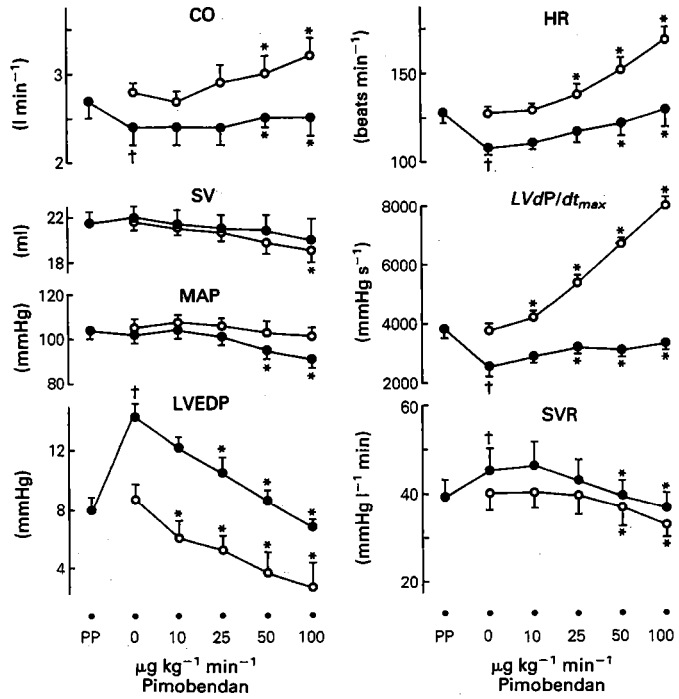

Figure 1 Effects of continuous intravenous 10 min infusions of pimobendan, before (○) or after (●) β-adrenoceptor blockade with propranolol, on systemic haemodynamics in conscious pigs. CO = cardiac output; HR = heart rate; SV = stroke volume; MAP = mean arterial blood pressure; $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure; SVR = systemic vascular resistance. Data have been presented as mean of 6 experiments with s.e.mean shown by vertical lines; †$P < 0.05$ vs pre-propranolol (PP) values; *$P < 0.05$ vs baseline (0).

vasculature was, however, much more pronounced as left ventricular end-diastolic pressure was reduced from 8.7 ± 1.0 mmHg to 2.7 ± 1.7 mmHg.

Pretreatment with propranolol attenuated the increases in heart rate and cardiac output, almost abolished the response of $LVdP/dt_{max}$ but had no effect on the reductions in pre- and afterload (Figure 1).

Effects of UD-CG 212 Cl

UD-CG 212 Cl (0–60 ng ml^{-1}) produced cardiovascular effects similar, both qualitatively and quantitatively, to pimobendan (0–900 ng ml^{-1}), as shown in Figure 2. After β-adrenoceptor blockade the UD-CG 212 Cl-induced increases in heart rate and $LVdP/dt_{max}$ were attenuated and almost abolished, respectively, whereas there was no effect on the changes in systemic vascular resistance, mean arterial blood pressure or left ventricular end-diastolic pressure.

Discussion

In a number of animal models, pimobendan has been shown to dilate the venous and arterial vasculature, as well as exert positive inotropic and chronotropic effects. However, differences in the potency of this drug with respect to the vasodilator and cardiac stimulatory effects have been reported, which may be due to the absence or presence of anaesthesia as well as to differences in species. Diederen *et al.* (1982) reported that in conscious dogs there was a more potent effect on the myocardium than on the vasculature, while in anaesthetized baboons prominent effects on both the vasculature and the myocardium were observed. Van Meel (1985) described in anaesthetized cats a strong venodilator effect besides a potent positive inotropic action. In anaesthetized pigs, pimobendan proved to be a more potent vasodilator, in particular of the venous bed, than a positive

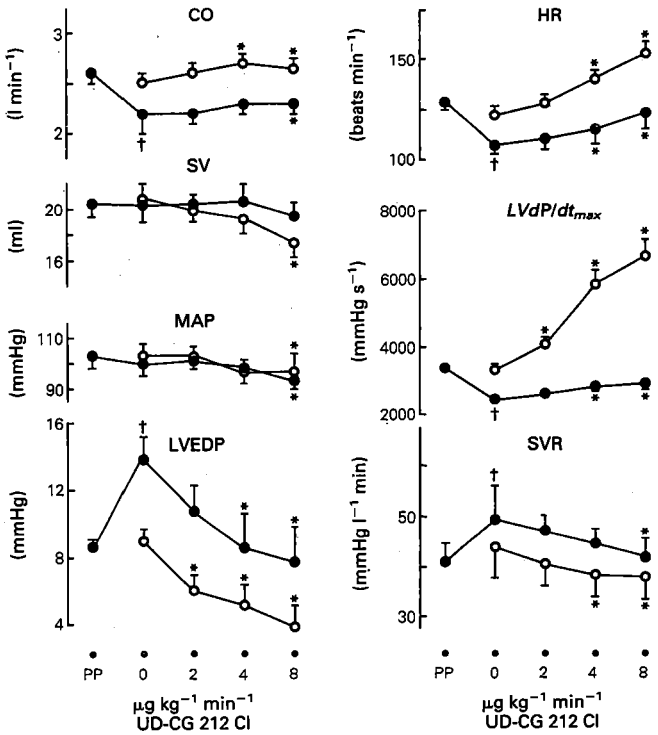

Figure 2 Effects of continuous intravenous 10 min infusions of UD-CG 212 Cl, before (○) or after (●) β-adrenoceptor blockade with propranolol, on systemic haemodynamics in conscious pigs. CO = cardiac output: HR = heart rate; SV = stroke volume; MAP = mean arterial blood pressure; $LVDP/DT_{max}$ = maximal rate of rise of left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; SVR = systemic vascular resistance. Data have been presented as mean of 6 experiments with s.e.mean shown by vertical lines; †$P < 0.05$ vs pre-propranolol (PP) values; *$P < 0.05$ vs baseline (0).

otropic agent (Verdouw *et al.*, 1986; Duncker *et al.*, 86). In the present study pimobendan caused a amatic increase in $LVdP/dt_{max}$, while the vasodilator fect was primarily confined to the venous vas-ılature.

The use of $LVdP/dt_{max}$ as an index of myocardial ntractility is often subject to criticism because of its pendence on heart rate, preload and afterload Mason, 1969). The question therefore remains to hat extent the increase in $LVdP/dt_{max}$ induced by ese pyridazinone derivatives reflects true positive otropy. In anaesthetized pigs we have shown that creasing heart rate, by atrial pacing, from 100 beats in^{-1} to 160 beats min^{-1} has no effect on $LVdP/dt_{max}$ cheffer & Verdouw, 1983). Although we have no ch data in the conscious animal, it appears unlikely that $LVdP/dt_{max}$ would more than double when heart rate increases only by 30%. Furthermore, the reduction in left ventricular filling pressure leads to a underestimation of myocardial contractility changes by using $LVdP/dt_{max}$.

The precise mechanism of action of the pyridazinone derivatives is still largely unknown. However, phosphodiesterase inhibition, an increased sensitivity of contractile proteins to calcium and a prolongation of duration of the action potential, allowing more calcium to enter the cell, have been demonstrated to be involved in their actions in a number of *in vitro* preparations (Rüegg *et al.*, 1984; Honerjäger *et al.*, 1984; Berger *et al.*, 1985; Scholz & Meyer, 1986). The marked attenuation of the pyridazinones-induced increases in $LVdP/dt_{max}$ after pretreatment with

propranolol implies that in the present study the β-adrenergic system, possibly via phosphodiesterase inhibition, contributed significantly to the actions of these drugs. In contrast, in pentobarbitone-anaesthetized pigs, β-adrenoceptor blockade did not modify the responses to pimobendan (Verdouw *et al.*, 1986). One must keep in mind, however, that in the anaesthetized animals, pimobendan caused not only much smaller increases in $LVdP/dt_{max}$ compared to the effects in conscious animals, but also that $LVdP/dt_{max}$ was already severely depressed (baseline 1500 mmHg s^{-1} by the presence of pentobarbitone. Barbiturates have been reported to decrease sympathetic outflow (Roberts, 1980) and this would render phosphodiesterase inhibition less effective than in the conscious state with a higher β-adrenergic activity. Therefore, in the conscious pigs phosphodiesterase inhibition might be involved in the positive inotropic action of pimobendan and UD-CG 212 Cl. However, the increase in $LVdP/dt_{max}$ which was insensitive to β-adrenoceptor blockade in the anaesthetized animals as well as the pyridazinone-induced increase in $LVdP/dt_{max}$ after β-adrenoceptor blockade in the conscious animals, strongly suggest that other mechanisms are also involved in the inotropic actions of these drugs. It is of interest that another pyridazinone-derivative (sulmazole) increased $LVdP/dt_{max}$ by 75% from its baseline value of 2400 mmHg s^{-1} in anaesthetized pigs (Verdouw *et al.*, 1981). Surprisingly the vasodilator actions of pimobendan and UD-CG 212 Cl were not affected by propranolol suggesting that these effects are not mediated by a β-adrenergic mechanism.

UD-CG 212 Cl is more potent at increasing contractile force than its parent compound (Scholz & Meyer, 1986). In the present study UD-CG 212 Cl exerted a cardiovascular action similar to that of pimobendan, but at much lower plasma concentrations. It is therefore feasible that the metabolite contributed to the positive inotropic actions of pimobendan. During the pimobendan infusions, UD-CG 212 Cl plasma levels did not exceed 22 ng ml^{-1}. When this concentration was attained during infusion of the metabolite itself, heart rate and $LVdP/dt_{max}$ were only moderately elevated but left ventricular filling pressure was already markedly reduced. However, at the end of the first infusion period of pimobendan, there was already a pronounced reduction in left ventricular filling pressure, while UD-CG 212 Cl could not yet be detected. It therefore appears that the effects during pimobendan infusion are primarily due to the parent drug itself.

In conclusion pimobendan as well as UD-CG 212 Cl are, in conscious pigs, potent positive inotropic agents and venodilators. The vasodilator effects on the systemic arterial vasculature are much less pronounced than in anaesthetized animals. Since pretreatment with propranolol strongly attenuated, but did not abolish, the increases in myocardial contractility caused by both compounds, phosphodiesterase inhibition could well be involved but this does not account completely for their positive inotropic actions. In contrast, the vasodilator effects are not affected by the presence of β-adrenoceptor blockade and other mechanisms than those operating through the β-adrenergic system must be involved.

We would like to express our gratitude to the Laboratory for Surgery for their skilful assistance during surgery and the post-surgical period. We would like to thank Dr W. Roth for determining the plasma concentrations of pimobendan and UD-CG 212 Cl. Miss P.H. Vegter is gratefully thanked for her assistance in the preparation of the manuscript.

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VASODILATOR THERAPY AND MYOCARDIAL ISCHEMIA

CHAPTER 12

NISOLDIPINE AND PERFUSION OF POST-STENOTIC MYOCARDIUM IN CONSCIOUS PIGS WITH DIFFERENT DEGREES OF CONCENTRIC STENOSIS.

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Chapter 12

Nisoldipine and perfusion of post-stenotic myocardium in conscious pigs with different degrees of concentric stenosis.

Summary

The effects of oral nisoldipine on the perfusion and wall function of a myocardial segment distal to a fixed coronary artery stenosis were studied in 2 groups of conscious pigs with different degrees of stenosis. In group 1 (n=8) systolic wall thickening (SWT) of the post-stenotic segment was more than 15% (27 ± 4%); in group 2 (n=7) SWT was less than 10% (7 ± 1%).

The systemic hemodynamic profiles at baseline and during nisoldipine were similar in both groups. Dose-titrations of nisoldipine (0.24 ± 0.02 $mg.kg^{-1}$ and 0.47 ± 0.04 $mg.kg^{-1}$) were performed to obtain increases in heart rate of 25% and 50%, respectively. These increases were accompanied by increases in cardiac output (up to 50%) and $LVdP/dt_{max}$ (60%), while systemic vascular resistance (35%) and mean arterial blood pressure (10%) were reduced. Left ventricular systolic and end-diastolic blood pressure and stroke volume were not affected.

In both groups, nisoldipine caused increases in blood flow to the non-stenotic area which favoured the subepicardium more than the subendocardium. Blood flow to the post-stenotic area of group 1 was normal at baseline and was only slightly enhanced (preferentially to the subepicardium) by nisoldipine. In the post-stenotic area of group 2 transmural and subendocardial blood flow were lower at baseline compared to the control area. Nisoldipine did not affect subepicardial blood flow but reduced subendocardial blood flow.

In spite of the reflex-mediated positive chronotropic actions of nisoldipine the acute post-stenotic systolic wall thickening was not affected by nisoldipine in either group.

We conclude that, under the experimental conditions employed (concentric stenosis, no coronary collaterals and acute drug administration), nisoldipine does not have a useful effect on post-stenotic myocardial blood flow, particularly in animals with severe stenosis. In view of a possible resetting of the baroreceptors (subsiding of the tachycardia) with chronic treatment and the presence of eccentric stenosis in many patients, additional studies are warranted.

Introduction

Since the myocardial vasculature possesses a great capacity for autoregulation, a coronary artery stenosis reduces coronary perfusion pressure but not necessarily perfusion (Berne and Rubio, 1979; Feigl, 1983). The autoregulatory capacity is most pronounced in the subepicardial layers of the myocardium as vasodilator reserve in these layers is greater than that in the subendocardium. Consequently, progressive narrowing of a coronary artery affects perfusion and causes ischemia in the subendocardium earlier than in the subepicardium.

Until recently it was believed that during myocardial ischemia, coronary vasodilator reserve is completely exhausted as vasodilation in the post-stenotic segment is maximal (Berne and Rubio, 1979). Pharmacological interventions were therefore primarily aimed at reducing myocardial oxygen demand and at increasing myocardial oxygen supply by elevation of perfusion pressure or prolongation of diastolic perfusion time. Unless vasodilation at the site of stenosis is possible (eccentric stenosis or spasm), vasodilation is generally regarded as being potentially harmful since it may induce "coronary steal" from ischemic to non-ischemic transmurium (Warltier et al., 1980) or from post-stenotic subendocardium to subepicardium (Weintraub et al., 1981; Gewirtz et al., 1984). However, evidence is now emerging that coronary vasodilation may not be maximal in ischemic myocardium. In dogs (Aversano & Becker, 1985; Canty and Klocke, 1985) as well as in pigs (Pantely et al., 1985) with myocardial ischemia resulting from a fixed stenosis in a coronary artery, intracoronary administration of adenosine can increase myocardial blood flow. Furthermore, during exercise-induced ischemia in dogs, both α_2-adrenoceptor blockade (Seitelberger et al., 1986) and nifedipine (Heusch et al., 1987) improve blood flow to and function of the myocardium distal to the stenosis.

In the present investigation we studied the effects of the dihydropyridine calcium channel blocker nisoldipine on regional myocardial blood flow distribution and function of the post-stenotic myocardium in conscious pigs with a fixed chronic coronary artery stenosis. To investigate the possible dependency of these effects upon the severity of the stenosis the animals were divided into two groups, one with slight or almost no and the other with marked attenuation of myocardial wall motion. Nisoldipine was administered orally in doses up to 0.5 $mg.kg^{-1}$ which corresponds with doses

used in the clinical setting (Lam et al., 1985; Lopez et al., 1985). Furthermore, Drexler et al. (1986) showed that after oral administration of nisoldipine a more pronounced vasodilation of the coronary bed is obtained than after intravenous application.

Materials and Methods

General

After an overnight fast Yorkshire pigs (18–20 kg), pretreated with a mixture of procaine penicillin–G and benzathine penicillin–G (Duplocillin, Gist–Brocades N.V., Delft, The Netherlands) both 300,000 units i.m., were sedated with 30 $mg.kg^{-1}$ ketamine HCl i.m. (Aescoket, Aesculaap B.V., Boxtel, The Netherlands). The animals were intubated and connected to a respirator for artificial ventilation with a mixture of oxygen and nitrous oxide (1:2) to which 1% halothane was added. A jugular vein and a common carotid artery were cannulated for infusion of drugs and measurement of mean arterial blood pressure, respectively. The chest was opened via the left fifth intercostal space to expose the heart. A transducer ($P_{4.5}$, Konigsberg Instruments Inc. Pasadena, California, USA) was implanted into the left ventricle of the heart through its apex for recording of left ventricular blood pressure. The left atrium was cannulated for the injection of radioactive microspheres (see later) and for recording of left atrial pressure which, together with the aortic blood pressure, was used for calibration of the Konigsberg transducer signals. Regional myocardial function was assessed by sonomicrometry (Triton Technology, San Diego, Ca, USA). One pair of ultrasonic crystals (5 MHz) was implanted in the myocardial area perfused by the left anterior descending coronary artery (LADCA) to measure regional myocardial wall thickness. The wall thicknesses at end–diastole (EDT) and end–systole (EST) were used to calculate systolic wall thickening (SWT) as:

$$SWT\ (\%) = (EST - EDT)/\ EDT \times 100\%$$

and the mean velocity of SWT (V_{swt}) as:

$$V_{swt}\ (mm.s^{-1}) = (EST - EDT)\ /\ DS$$

where DS is the duration of systole (isovolumic contraction phase and ejection time). Systolic wall thickening at the time of surgery (open-chest state) was 32 ± 4%.

The aorta was approached through the third intercostal space and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta. The proximal segment of the LADCA was dissected free from its surrounding tissue and a teflon constrictor, with an internal diameter varying from 1.0 to 2.0 mm, was positioned around the LADCA which resulted in different degrees of loss of systolic wall thickening. Catheters and wires were tunnelled subcutaneously to the back. The chest was closed and the animals allowed to recover. During the next two days the animals received intravenous bolus injections of 500 mg amoxicilline per day (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) to prevent infection. Catheters were flushed daily with an isotonic saline solution containing 500 I.U. heparin per ml (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands) to avoid blood clotting. Prior to surgery the animals had been adapted to the laboratory and experimental facilities. An additional adaptation procedure was performed on the second day after surgery to confirm hemodynamic stability. This was the case in all but two animals showing ventricular arrhythmias (see results). On the next day the arrhythmias had disappeared and the experimental protocol could be executed. The animals were fasted for 18 hours prior to the experiments. All tracings were written on a Graphtec Linearcorder (F WR 3701, Ankersmit, Breda, The Netherlands). Arterial acid-base balance and oxygenation during the experiments were within the following limits: $7.37 < pH < 7.49$; $35\ mmHg < PCO_2 < 45\ mmHg$; $75\ mmHg < PO_2 < 95\ mmHg$. These values are in accordance with earlier reports (see Tumbleson and Schmidt, 1986).

Regional myocardial blood flows

Carbonized plastic microspheres (15 ± 1 (s.d) μm in diameter) labelled with ^{141}Ce, ^{113}Sn, ^{103}Rn or ^{95}Nb (NEN Chemicals GmbH, Dreieich, FRG) and suspended in saline containing a drop of Tween 80, were injected in random order into the left atrium over a period of 30 seconds, while an arterial reference sample was drawn for calibration of the microsphere data.

At the end of each experiment the animal was killed with an overdose of pentobarbitone sodium, the heart excised, the left anterior descending

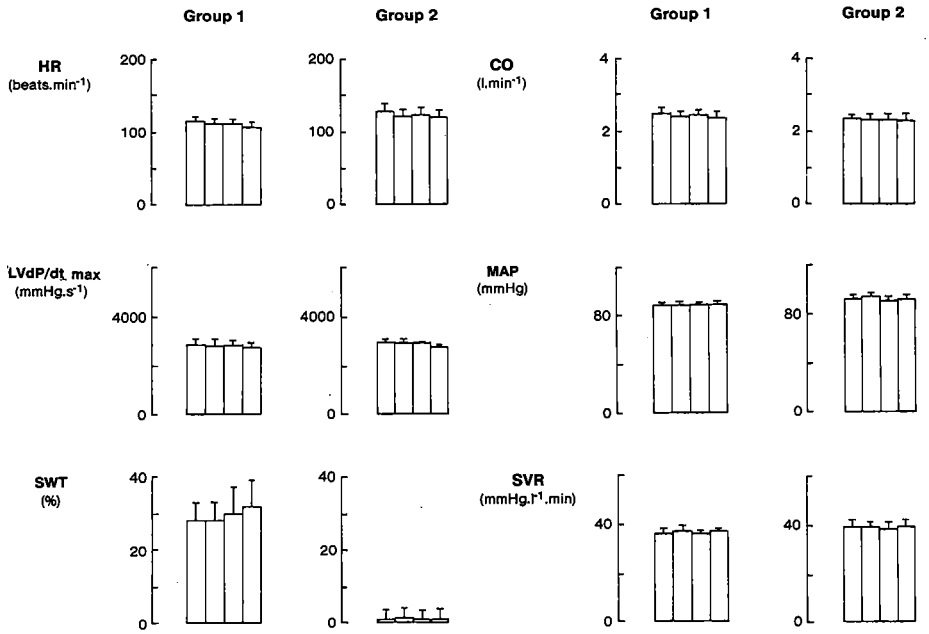

Fig. 1.
Systemic haemodynamics of untreated animals of group 1 (SWT>15%; n=6) and group 2 (SWT<10%; n=6). The four bars represent: baseline, 30 min, 90 min and 150 min after baseline, respectively. HR=heart rate; CO=cardiac output; $LVdP/dt_{max}$=maximal rate of rise of left ventricular blood pressure; MAP=mean arterial blood pressure; SWT=systolic wall thickening; SVR=systemic vascular resistance; Data have been presented as mean ± SEM.

coronary artery (LADCA) cannulated for injection of methylene blue dye to delineate between LADCA and non-LADCA perfused areas, and the heart fixed in 10% formalin for at least 48 hours. The left ventricle was then divided into LADCA and non-LADCA perfused areas. To avoid mixture of both areas, border zone tissue was not sampled. Both areas were separated into three layers of equal thickness from endocardium to epicardium. Details of the radioactive microsphere method and of the calculation of flow data have been reported earlier (Saxena et al., 1980; Verdouw et al., 1985).

Experimental protocols

The experiments were performed in 18 instrumented pigs. In 12 of these animals stability of systemic hemodynamic variables and regional myocardial wall function was evaluated over a 150 min period. The effects of orally administered nisoldipine on systemic hemodynamics, myocardial blood flows and regional left ventricular wall function of the LADCA perfused segment were studied in 15 pigs. The two doses of nisoldipine (0.24 ± 0.02 and 0.47 ± 0.04 $mg.kg^{-1}$) were selected in each animal prior to surgery and were such that elicited peak tachycardia (30–60 min after drug administration) of approximately 30 and 60 $beats.min^{-1}$, respectively. Measurements consisting of systemic hemodynamics and regional myocardial wall function were made and a batch of microspheres injected at baseline and at peak heart rate effect of the first dose of nisoldipine. In all but the first three animals systemic hemodynamic measurements were repeated at 30 min, 60 min and 120 min after peak-effect. Twentyfour hours later, when it is known that previously administered nisoldipine is no longer detectable in the plasma (see Duncker et al., 1987) the same protocol was performed, but now employing the higher dose of the drug.

Data presentation and statistical analysis

Animals were categorized into two groups defined by the loss of systolic wall thickening of the post-stenotic segment at the time of the experiment; each group was analyzed separately. Group 1 consisted of animals with a systolic wall thickening of 15% or more; group 2 consisted of animals with a systolic wall thickening of 10% or less. No animals with a systolic wall thickening between 10% and 15% were present.

All data have been presented as mean ± SE of mean. Statistical analysis was performed using Duncan's New Multiple range-test once a parametric two-way analysis of variance (randomized block design) had revealed that the samples represented different populations.

Drugs

Except for the anesthetics during surgery and the antibiotics during the post-surgical period the only drug used in this study was nisoldipine (Bayer A.G., Wuppertal, F.R.G.).

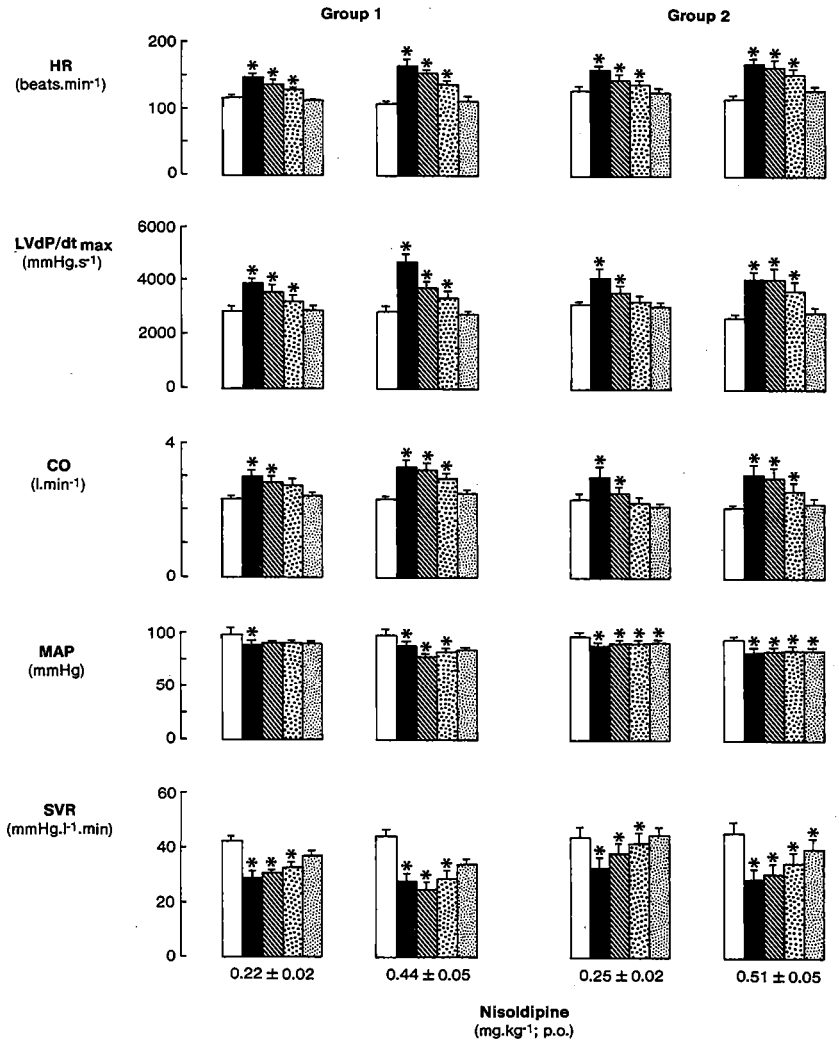

Fig. 2.
Effects of nisoldipine on systemic haemodynamics in group 1 (SWT>15%; n=8) and in group 2 (SWT<10%; n=7). The five bars represent: baseline (□), peak-response to nisoldipine (■) and 30 min (▨), 60 min (▩) and 120 min (▩) after peak-response, respectively. HR = heart rate; $LVdP/dt_{max}$ = maximal rate of rise of left ventricular blood pressure; MAP = mean arterial blood pressure; CO = cardiac output; SVR = systemic vascular resistance. Data have been presented as mean ± SEM; *P<0.05 vs baseline.

Results

Arrhythmias during the post-surgical period

Two animals died the night following surgery. Because post-mortem examination was negative ventricular fibrillation might have been the cause of death. In two other animals ventricular arrhythmias (>5 premature ventricular contractions.min^{-1}) were observed during the first adaptation session after surgery, but not on the following days. Arrhythmias were not observed in any of the other animals, neither during adaptation, nor during the course of the experiments.

Stability of systemic hemodynamics and regional myocardial wall function

Hemodynamical data obtained during 150 min in which the animals received no treatment have been presented in Fig. 1. In both group 1 and group 2 none of the parameters changed significantly from its baseline value during the course of the experiment. Moreover, there were no differences between the systemic hemodynamic parameters of the two groups despite the difference in regional systolic wall function.

Nisoldipine-induced responses

Systemic hemodynamics. The nisoldipine-induced increases in heart rate were accompanied by responses that were very similar in both groups (Fig. 2). Calculated systemic vascular resistance decreased dose-dependently (up to 36 ± 4% and 37 ± 4% after the highest dose in group 1 and 2, respectively), as mean arterial blood pressure was reduced from 98 ± 5 to 88 ± 5 mmHg and from 95 ± 4 to 83 ± 5 mmHg after the highest dose and cardiac output increased considerably (43 ± 5% and 46 ± 7%). The increase in cardiac output resulted primarily from a dose-dependent increase in heart rate (up to 53 ± 6% and 50 ± 7%, in group 1 and 2, respectively) as stroke volume was unchanged from its baseline value (21 ± 1 ml and 19 ± 2 ml). Left ventricular systolic (124 ± 8 mmHg and 122 ± 2 mmHg) and end-diastolic (12.1 ± 1.0 mmHg and 12.8 ± 1.9 mmHg) blood pressure were also unchanged from their respective baseline values. Finally $LVdP/dt_{max}$ increased dose-dependently up to 67 ± 8% and 53 ± 7% in group 1 and 2 respectively. The doses needed to elicit these responses were similar for group 1 (0.22 ± 0.03 mg.kg^{-1} and 0.44 ± 0.05 mg.kg^{-1}, p.o.) and group 2 (0.25 ± 0.02 mg.kg^{-1} and 0.51 ± 0.05 mg.kg^{-1}, p.o.).

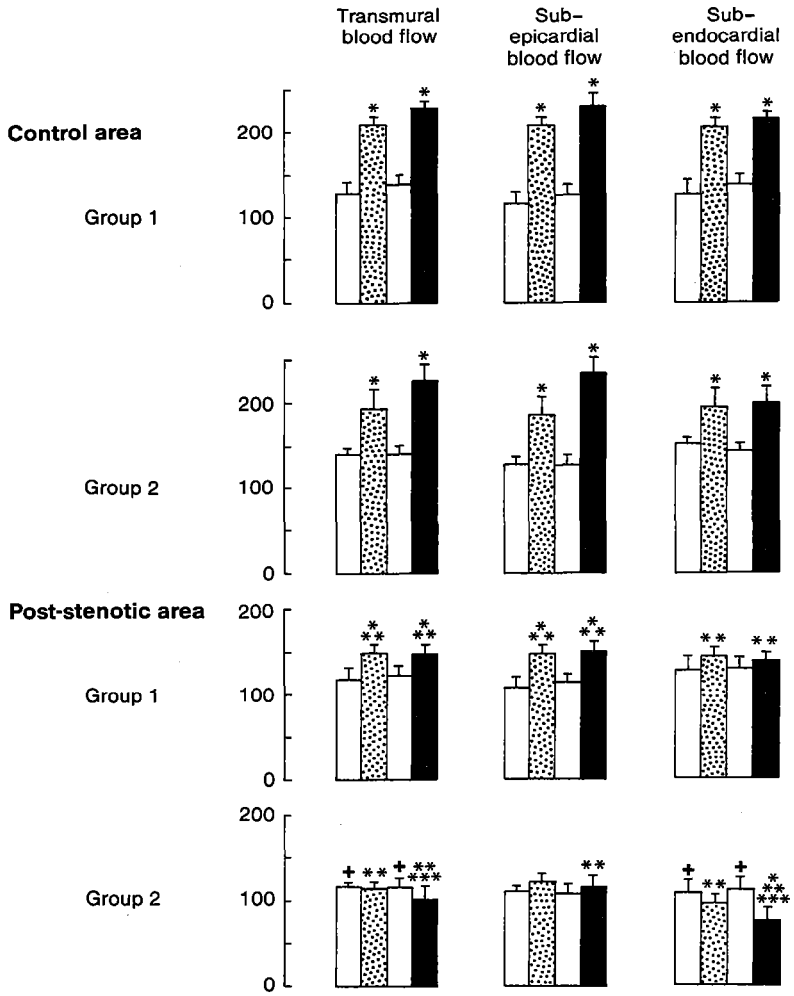

Fig. 3.
Regional myocardial blood flow (ml.min^{-1}.100 g^{-1}) responses to nisoldipine of the control and post-stenotic areas of group 1 (SWT>15%; n=8) and group 2 (SWT<10%; n=7). The four columns represent: control (□), peak-response to nisoldipine (0.22 ± 0.03 mg.kg^{-1} and 0.25 ± 0.02 mg.kg^{-1} p.o. in group 1 and 2, respectively; ▨), control (□) and peak-response to nisoldipine (0.44 ± 0.05 mg.kg^{-1} and 0.51 ± 0.05 mg.kg^{-1} p.o. in group 1 and 2, respectively; ■). Data have been presented as mean ± SEM; *P<0.05 vs baseline; ** nisoldipine-induced response significantly different (P<0.05) from that in the corresponding control area; *** nisoldipine-induced response in group 2 significantly (P<0.05) different from that in group 1. +P<0.05 vs corresponding control area.

Regional myocardial blood flows. Baseline myocardial blood flow values and the nisoldipine-induced responses in the control area of both groups were similar (Fig. 3). Myocardial blood flow to the control areas was dose-dependently enhanced in both groups (up to 76 ± 18% and 64 ± 12% after the highest dose in group 1 and 2, respectively). The increase in transmural blood flow favoured the subepicardial (96 ± 22% and 88 ± 13%, respectively) over the subendocardial (70 ± 21% and 40 ± 13%) layers. In the post-stenotic area of group 1 baseline blood flow values were very similar to those in the control segment, but the nisoldipine-induced responses were markedly different. Nisoldipine caused only slight increases in transmural (25%) and subepicardial (40%) blood flows; however, subendocardial flow remained unchanged (Fig. 3). In the post-stenotic segment of group 2 transmural and subendocardial blood flows at baseline were significantly lower compared with flows of the control area. Nisoldipine did not affect transmural and subepicardial blood flow but reduced subendocardial blood flow by 30% with the highest dose.

Regional myocardial wall function. The effects of nisoldipine on regional wall function are shown in Fig. 4. In neither of the two groups myocardial wall thickness at end-diastole and at end-systole (baseline values: 10.0 ± 0.8 mm and 12.8 ± 0.9 mm in group 1; 10.6 ± 1.1 mm and 11.3 ± 1.2 mm in group 2) was affected. Systolic wall thickening (27 ± 4% for group 1 and 7 ± 1% for group 2 during baseline) was also not affected by the drug in either group. Velocity of wall thickening was enhanced in group 1 but unaffected in group 2.

Discussion

In this investigation nisoldipine was administered orally at two doses which were titrated in each animal based on peak heart rate responses of 30 and 60 beats.min^{-1}, respectively. The tachycardiac effect of the lower dose (25% of baseline value) corresponds well with heart rate changes observed in the clinical setting (Silke et al., 1985; Serruys et al., 1985). Moreover, the peak tachycardiac responses to nisoldipine correlate with peak drug concentrations in the plasma (Duncker et al., 1987).

As reported by many investigators (see Verdouw et al., 1988) we also observed that nisoldipine induced a pronounced systemic vasodilation, which

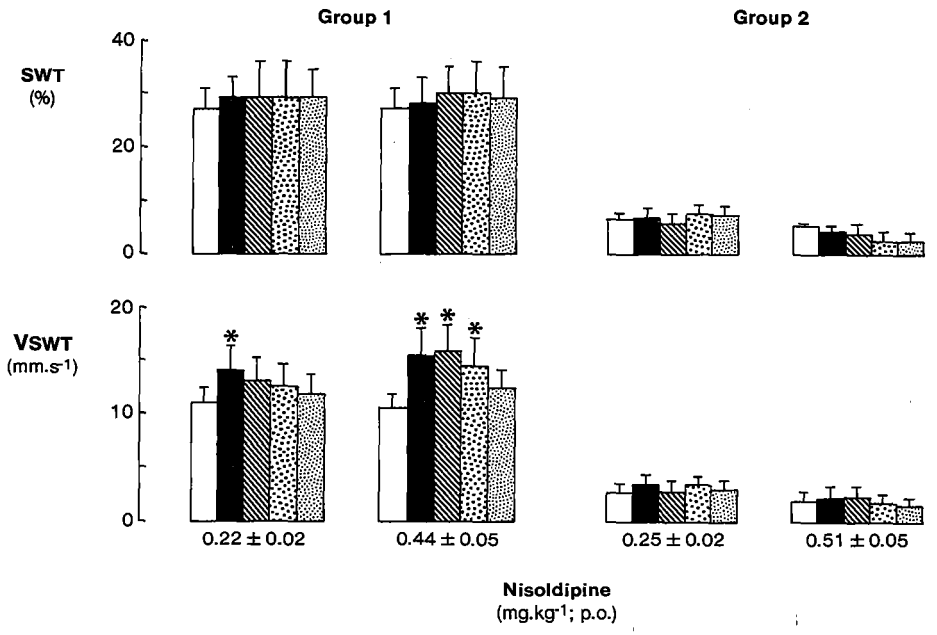

Fig. 4.
Regional left ventricular myocardial wall function responses to nisoldipine of pigs in group 1 (SWT>15%; n=8) and group 2 (SWT<10%; n=7). The five columns represent: baseline (□), peak response to nisoldipine (■) and 30 min (▨), 60 min (▩) and 120 min (▩) after peak-response, respectively. SWT = normalized systolic wall thickening; Vswt = mean velocity of wall thickening. Data have been presented as mean ± SEM; *P<0.05 vs baseline.

resulted in only a moderate reduction in mean arterial blood pressure as cardiac output was markedly elevated. The increase in cardiac output was the result of a reflex-mediated increase in heart rate as stroke volume was maintained. Due to the baroreceptor reflex $LVdP/dt_{max}$ was also enhanced. Left ventricular end-diastolic blood pressure was unchanged, which is in accordance with reported earlier findings (see Verdouw et al., 1988). Nisoldipine, like other dihydropyridine calcium channel-blockers, does not have an effect on preload unless elevated end-diastolic pressures are present (Verdouw et al., 1984; Kimchi et al., 1985). In both groups the cardiovascular profile of nisoldipine was not different from that observed in conscious pigs with a normal coronary circulation (Duncker et al., 1987), which implies that the myocardial ischemia in the present study was not severe enough to produce chronic reduction in global left ventricular pump function.

Nisoldipine potently enhanced myocardial blood flow to the normally perfused areas which is in agreement with data obtained in animals with a normal coronary circulation (for reference see Verdouw et al., 1988) or in the control area of ischemic canine hearts (Warltier et al., 1981). The increase in blood flow favoured the subepicardial layers, a finding observed with many vasodilators (Verdouw et al., 1986, 1987a,b), and this most likely resulted from the moderate hypotension and the reflex-mediated tachycardia (see Feigl, 1983).

In group 1 the stenosis did not affect basal blood flow which correlates well with the almost normal systolic wall thickening of the post-stenotic area. In group 2, however, the stenosis severely reduced systolic wall thickening, while basal transmural and subendocardial but not subepicardial blood flow were decreased. These findings are supported by Gallagher et al. (1985) who reported a severe impairment of systolic wall thickening in the presence of a coronary stenosis despite a normal subepicardial blood flow and subepicardial function. In the post-stenotic segment of group 1 in which the stenosis caused almost no or slight loss of wall function, nisoldipine caused a moderate increase in transmural blood flow which was solely confined to the subepicardial layers. In the post-stenotic myocardial area of the animals in which the stenosis caused marked loss of wall function (group 2), nisoldipine failed to cause an increase in blood flow to the subepicardial layers whereas subendocardial blood flow was decreased. This decrease probably resulted from the hypotension and increase in heart rate. Although subepicardial blood flow was not affected by the drug vasodilation must have occurred as mean aortic blood pressure and hence perfusion pressure was decreased.

Our findings lend further support to the concept that during myocardial ischemia due to a fixed concentric coronary artery stenosis, vasodilators, may not be beneficial (Weintraub et al., 1981; Gross and Warltier, 1981; Gewirtz et al., 1984). However, recent reports have shown that vasodilator reserve may be present in ischemic myocardium distal to a severe coronary artery stenosis and that vasodilators may improve myocardial blood flow and myocardial function (Heusch and Deussen, 1984; Aversano and Becker, 1985, Seitelberger et al., 1986; Heusch et al., 1987). The different observations in those studies and in the present one might be the result of (i) different routes of administration, (ii) absence or presence of collateral circulation and

(iii) different duration of ischemia. In most studies intracoronary administration of drugs was used to minimize systemic effects (Heusch and Deussen, 1984; Aversano and Becker, 1985; Canty and Klocke, 1985; Pantely et al., 1985; Seitelberger et al., 1986). We, on the other hand, used oral administration like in the clinical situation. Systemic administration of a vasodilator results in hypotension which together with a reflex-tachycardia reduces autoregulatory capacity of the myocardial vasculature especially in the subendocardial layers (see Feigl, 1983). Only in the study of Heusch et al. (1987) intravenous administration of nifedipine was employed. However, during exercise-induced ischemia, systemic hemodynamics of the untreated and the nifedipine-treated group did not differ. Furthermore, systemic administration of nifedipine might have enhanced blood flow through collateral vessels, which may be abundantly present in canine hearts. For example, Warltier et al (1981) observed an increase in flow to a totally collateral-dependent area in acutely ischemic dog hearts after intravenous administration of nisoldipine. Since pigs possess very few collaterals and it is unlikely that after induction of ischemia extensive collateral formation has taken place within 5 days (Ramo et al., 1970), such a beneficial effect of nisoldipine was not to be expected in our study. Finally, in all previous studies measurements were made up to maximally 3 hours after induction of ischemia, whereas in our study ischemia was present for more than 2 days when the protocol was executed. To our knowledge, no information is available on the extent of vasodilator reserve during prolonged (more than 3 hours) ischemia. Therefore, it might be that in our animals vasodilator reserve was no longer present at the time of the experiment.

The nisoldipine-induced responses of myocardial blood flow were not accompanied by a worsening of wall function in group 1 and, surprisingly, also not in group 2. An explanation for this observation is not readily found. Nisoldipine might have decreased oxygen demand of the post-stenotic area although the increase in heart rate suggests an increase in oxygen demand rather than a reduction. Another possibility arises from the investigation of Berdeaux et al (1984) who observed an increase in wall function of severely ischemic myocardium after a low dose of prenalterol, a β-adrenoceptor agonist, causing an increase in heart rate of 15 beats.min^{-1}, while after a high dose of prenalterol an increase in heart rate of 40 beats.min^{-1} was accompanied by an unchanged wall function. This suggests

that the reflex-mediated increase in sympathetic activity can cause an increase in wall function even in severely ischemic myocardium, provided that the increase in heart rate is minimal. In our experiments it is possible that these reflex-mediated chronotropic and inotropic actions of nisoldipine balance one another with respect to their effects on wall function.

In conclusion, the findings in the present study, although obtained in normotensive animals with a normal cardiac pump function, suggest that in patients with myocardial ischemia caused by a concentric coronary artery stenosis and with few collaterals, vasodilators may not be beneficial. Therefore, it is important to stratify patients according to the status of their coronary circulation, i.e. presence or absence of collaterals and severity and type of stenosis. Because of its potent systemic vasodilator properties, the drug will most likely also be used in patients with hypertension. In view of this and the probable resetting of the baroreceptors, long-term studies in models with hypertension and myocardial ischemia appear to be worth-while.

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CHAPTER 13

EXERCISE-INDUCED ISCHEMIA IN PIGS: EFFECTS OF NISOLDIPINE WITH OR WITHOUT PROPRANOLOL.

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Chapter 13

Exercise-induced myocardial ischemia in pigs: cardiovascular actions of nisoldipine with or without β-adrenoceptor blockade.

Summary

We evaluated the effects of oral nisoldipine with or without propranolol on exercise-induced myocardial ischemia in conscious pigs with a coronary artery stenosis. Treadmill-running up to 4 $km.h^{-1}$ increased cardiac output (90%), heart rate (90%), $LVdP/dt_{max}$ (80%), left ventricular systolic (15%) and end-diastolic blood pressure (13 ± 3 mmHg), while systolic wall thickening of the post-stenotic left ventricular myocardium was reduced from 29 ± 8% to 19 ± 6%. Nisoldipine neither affected the systemic hemodynamic profile during exercise nor the exercise-induced reductions in myocardial wall function. Propranolol attenuated both the positive chronotropic and inotropic effects and the deterioration of wall function caused by the treadmill-exercise. Combined treatment with the two drugs resulted in a cardiovascular profile similar to propranolol during exercise, but the loss of wall function was now completely prevented. We conclude that, unlike propranolol, nisoldipine was not effective against the exercise-induced ischemia but may have beneficial actions when combined with β-adrenoceptor antagonists.

Introduction

In clinical studies both β-adrenoceptor antagonists and calcium-channel blockers are effective as anti-anginal agents (Prichard et al., 1970; Livesly et al., 1973; Kaltenbach et al., 1979). These two classes of drugs act through different mechanisms and in a number of studies their combination has proven superior to monotherapy with either class (Leon at al., 1980; Dargie et al., 1981; Fox et al., 1981). In this respect the dihydropyridine calcium-channel blockers appear particularly attractive to combine with β-adrenoceptor antagonists since the latter drugs block the baroreceptor reflex-mediated tachycardia. However, the effects of the dihydropyridines are not always predictable as vasodilation per se, depending on the experimental set-up and dose of the vasodilator used, may either induce "coronary steal" (Warltier et al., 1980; Weintraub et al., 1981) or increase blood flow to ischemic regions (Canty and Klocke, 1985; Pantely et al., 1985; Heusch et al., 1987). In the

present study we evaluated the effects of nisoldipine, a dihydropyridine-derivative with potent vasodilator and minimal direct negative inotropic properties (Kazda et al., 1980; Duncker et al., 1987a), on systemic hemodynamics and post-stenotic myocardial wall function during exercise-induced myocardial ischemia in pigs with a subacute coronary artery obstruction. Since nisoldipine may be used in the clinical setting in combination with β-adrenoceptor antagonists, we also investigated the actions of nisoldipine in the presence of propranolol. Oral administration was employed in view of the clinical use of these drugs.

Methods and Materials

Surgical procedure for instrumentation and coronary artery stenosis

After an overnight fast 9 Yorkshire pigs (18–20 kg), pretreated with a mixture of procaine penicillin-G and benzathine-penicillin-G (Duplocillin, Gist-Brocades N.V., Delft, The Netherlands) both 300,000 units i.m., were sedated with 30 $mg.kg^{-1}$ ketamine HCl i.m. (Aescoket, Aesculaap B.V., Boxtel, The Netherlands). The animals were intubated and connected to a respirator for artificial ventilation with a mixture of oxygen and nitrous oxide (1:2) to which 1% halothane was added. A jugular vein and common carotid artery were cannulated for infusion of drugs and measurement of arterial blood pressure, respectively. The chest was opened via the left fifth intercostal space to expose the heart. A transducer ($P_{4.5}$, Konigsberg Instruments Inc. Pasadena, California, USA) was implanted into the left ventricle of the heart through its apex for recording of left ventricular pressure. The left atrium was cannulated for recording of left atrial pressure which, together with the arterial blood pressure, was used for calibration of the Konigsberg transducer signals. Regional myocardial function was assessed by sonomicrometry (Triton Technology, San Diego, Ca, USA). A pair of ultrasonic crystals (5 MHz) was implanted in the left ventricular myocardial segment perfused by the left anterior descending coronary artery (LADCA) to measure regional myocardial wall thickness. The wall thickness at end-diastole (EDT) and end-systole (EST) were used to calculate percentual systolic wall thickening (SWT) as reported earlier (Verdouw et al., 1983). Subsequently, the aorta was approached through the third intercostal space and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta. The proximal segment of the LADCA was dissected free from its

surrounding tissue and a teflon constrictor with an internal diameter varying from 1.0 to 2.0 mm was positioned around the LADCA which resulted in a loss of systolic wall thickening to different degrees. Lastly, catheters and wires were tunnelled subcutaneously to the back and the chest was closed. The animals were allowed to recover from the surgery for 7–8 days during which they were adapted to the laboratory facilities.

During the postoperative period the animals received daily intravenous bolus injections of 500 mg amoxicilline (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) to prevent infection. The catheters were flushed daily with an isotonic saline solution containing 500 I.U. per ml heparin (Thromboliquine, Organon Teknika B.V., Boxtel, The Netherlands) to avoid clotting of blood in the lumen.

Experimental protocol

This study in conscious instrumented pigs with a fixed coronary artery stenosis was conducted in two parts: one part dealt with the effects of nisoldipine on exercise–induced changes in systemic hemodynamic variables and post–stenotic myocardial wall motion in control (untreated) animals and the other part dealt with similar effects of nisoldipine in animals treated with propranolol. For the first part of the study the animals were placed on a treadmill and systemic hemodynamic variables and post–stenotic myocardial wall motion were recorded. The speed of the treadmill was gradually increased first to 3 $km.h^{-1}$ and then to 4 $km.h^{-1}$. These speeds were maintained for a period of 2 min each during which hemodynamic variables became stable and data were collected. Both systemic hemodynamics and wall thickness variables returned towards baseline values within 60 min after cessation of the treadmill–running. Subsequently, the animals were orally administered 10 mg (0.54 ± 0.01 $mg.kg^{-1}$) of nisoldipine and, at the drug–induced peak–response of systemic hemodynamic parameters (30–60 min after administration), measurements were made and the exercise protocol repeated.

For the second part of the study the first exercise–test was performed 60 min after treating the animals with 80 mg (4.36 ± 0.10 $mg.kg^{-1}$) propranolol. In a pilot–study (n=3) we established the extent of β–adrenoceptor blockade at various times after oral administration of 80 mg of propranolol; the tachycardiac responses to isoprenaline (0.1 $\mu g.kg^{-1}$; i.v.) were

37 ± 3 beats.min^{-1} at baseline and 26 ± 6, 14 ± 1, 6 ± 4, 14 ± 7 and 7 ± 4 beats.min^{-1} at 30, 60, 120, 180 and 240 min after propranolol administration. After a 60 min period of recovery from treadmill-exercise nisoldipine (10 mg) was administered and, approximately 30-60 min later (at nisoldipine peak-effect), exercise-tests were again performed. The two parts of the study, as outlined above, were executed on consecutive days, but the order of the two parts was varied. We have shown earlier that nisoldipine (10 mg orally) is completely eliminated from the plasma within 24 hours after administration (Duncker et al., 1987a). Furthermore, the isoprenaline-induced increases in heart rate had returned to baseline levels 24 hours after administration of 80 mg of propranolol orally: 37 ± 3 beats.min^{-1} at baseline versus 33 ± 4 beats.min^{-1} 24 hours after propranolol. We did not perform control experiments to establish the reproducibility of the exercise-induced responses, but studies in dogs (Matsuzaki et al., 1984a) reveal that the responses are identical during exercise performed 3 hours after the first exercise test.

Two of the 9 animals, which did not run on the treadmill satisfactorily, were excluded from the study protocol. Arterial blood pressure measured with a fluid-filled catheter could not be obtained during the exercise period due to disturbances in the signals induced by the running; this parameter was therefore excluded from analysis.

Drugs

Except for the anesthetics during surgery and the antibiotics, the only drugs used in this study were nisoldipine (Bayer A.G., Wuppertal, F.R.G.) and propranolol hydrochloride (ICI-Farma, Rotterdam, The Netherlands).

Data presentation and statistical analysis

All data have been presented as mean ± SE of mean. Statistical analysis was performed using Duncan's New Multiple range test once a parametric two-way analysis of variance (randomized block design) had revealed that the samples represented different populations.

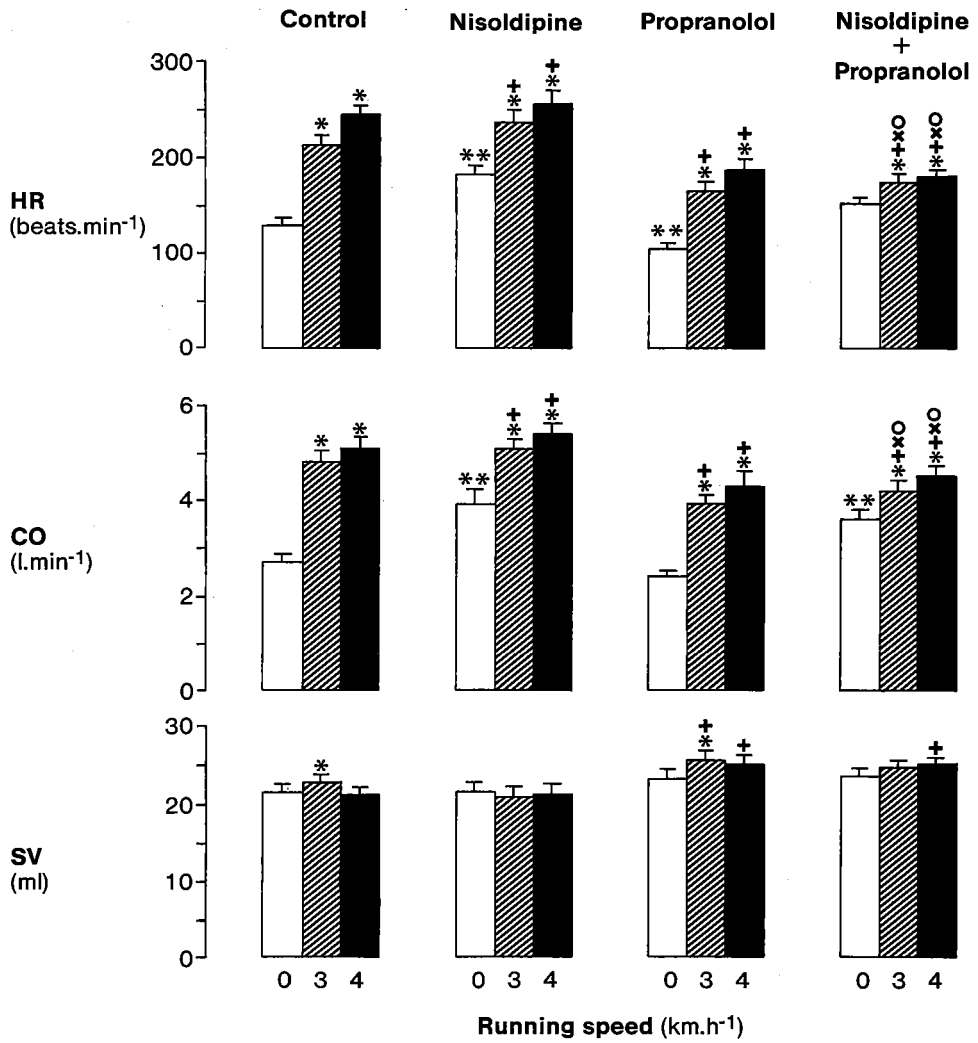

Fig. 1.
Systemic haemodynamic effects of exercise without and after medication with nisoldipine, propranolol or their combination. HR=heart rate; CO=cardiac output; SV=stroke volume. *P<0.05 vs corresponding pre-exercise; **P<0.05 pre-exercise after medication vs pre-exercise without medication; $^{+,x,o}$ exercise-induced response after medication significantly different (P<0.05) from exercise without treatment (+), after nisoldipine (x), or propranolol (O).

Results

Blood chemistry

Arterial acid-base balance and oxygenation at the time of the experiments were within the following limits: 7.37 < pH < 7.50; 35 mmHg < PCO_2 < 45 mmHg; 75 mmHg < PO_2 <95 mmHg, corresponding well with earlier reports (Tumbleson and Schmidt, 1986).

Arrhythmias during the post-surgical period

In two animals ventricular arrhythmias (>5 min^{-1}) were observed during the first adaptation session after surgery but not on the following days. Arrhythmias were not observed in any of the other animals, neither during adaptation, nor during the course of the experiments.

Exercise without treatment

The exercise-induced changes observed in our study are very similar to those reported by other investigators (Sanders et al., 1977). Treadmill exercise up to 4 $km.h^{-1}$ almost doubled heart rate, cardiac output and $LVdP/dt_{max}$ as those parameters had increased at the highest running speed by 91 ± 6%, 92 ± 8% and 83 ± 11%, respectively. Increases were also observed in left ventricular end-diastolic blood pressure (from 7 ± 1 to 20 ± 4 mmHg) and left ventricular systolic pressure (15 ± 3%), while stroke volume was minimally affected (Figs. 1 and 2). Treadmill-running caused speed-dependent reductions in systolic wall thickening (from 29 ± 8% to 19 ± 6% at 4 $km.h^{-1}$) which were primarily due to the decrease in end-systolic wall thickness as end-diastolic wall thickness was not significantly affected (Fig. 3).

Exercise in the presence of nisoldipine

During exercise in the presence of nisoldipine the hemodynamic parameters reached levels very similar to those obtained during the control run, except for left ventricular end-diastolic pressure which was, at both running speeds, consistently lower than in the untreated animals (Figs. 1 and 2). Since nisoldipine increased pre-exercise levels of heart rate, cardiac output and $LVdP/dt_{max}$, the exercise-induced changes in these variables after nisoldipine were less than those observed in the control period. Nisoldipine tended to reduce the exercise-induced decrease in systolic wall thickening (from 26 ± 8%

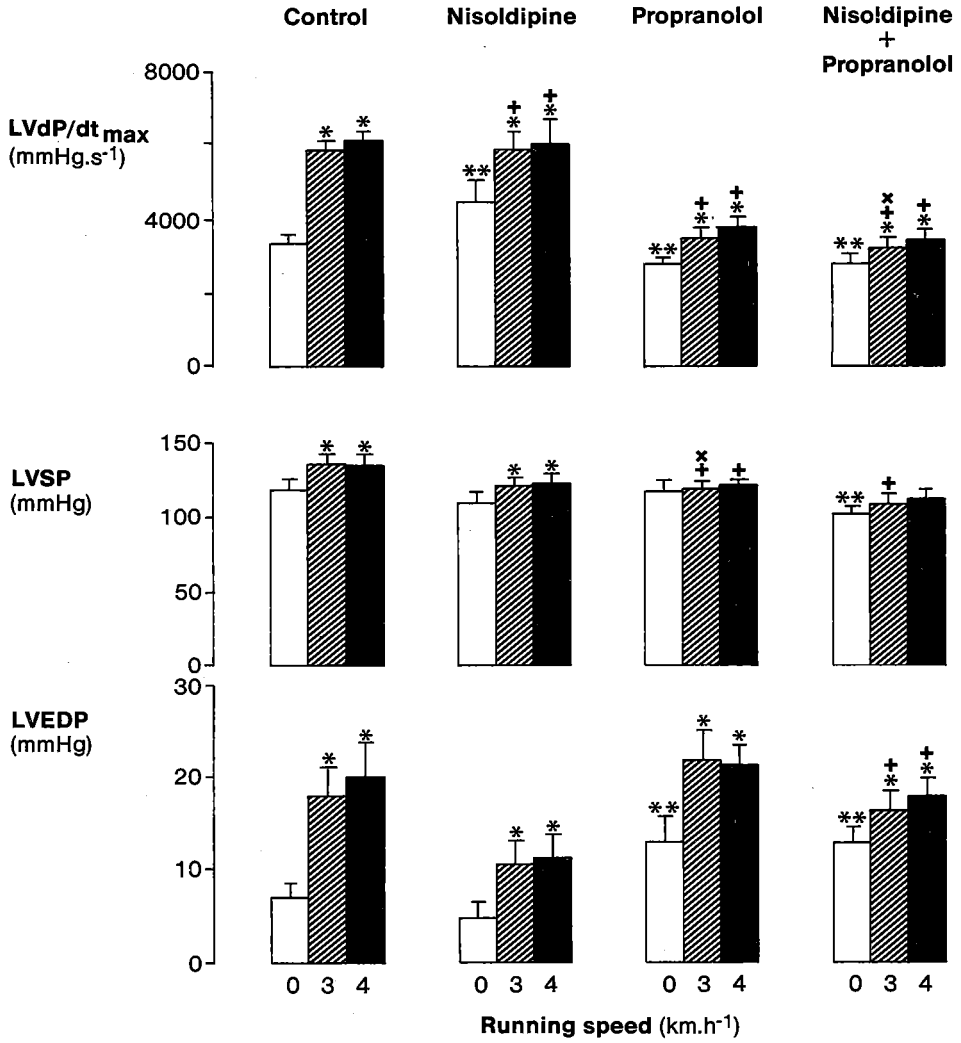

Fig. 2.
Systemic haemodynamic effects of exercise without and after medication with nisoldipine, propranolol, or their combination. LVdP/dt$_{max}$=maximal rate of rise of left ventricular pressure; LVSP=left ventricular systolic blood pressure; LVEDP=left ventricular end-diastolic blood pressure. *P<0.05 vs corresponding pre-exercise; **P<0.05 pre-exercise after medication vs pre-exercise without medication; $^{+,x,o}$ exercise-induced responses after medication significantly (P<0.05) different from exercise-induced responses without treatment (+), after nisoldipine (x), or propranolol (O).

to 19 ± 8%) but this effect did not reach levels of statistical significance (Fig. 3).

Exercise in the presence of propranolol

After β-adrenoceptor blockade with propranolol the exercise-induced increases in heart rate (up to 81 ± 11%), cardiac output (82 ± 13%) and $LVdP/dt_{max}$ (38 ± 5%) were lower than those observed during exercise without medication. Stroke volume increased more ($P<0.05$) than in the untreated animals, while the responses of left ventricular end-diastolic pressures were similar (Figs. 1 and 2). Propranolol also significantly reduced the exercise-induced loss of wall function as systolic wall thickening decreased only from 29 ± 8% to 24 ± 7% (Fig. 3).

Exercise in the presence of propranolol and nisoldipine

Systemic hemodynamic parameters reached levels similar to those obtained during exercise-tests in propranolol-treated animals (Figs. 1 and 2). However, the combination completely prevented the exercise-induced loss of wall function of the post-stenotic segment (Fig. 3).

Discussion

Systemic hemodynamics

The exercise-induced responses of systemic hemodynamics were very similar to those described for exercising pigs with a normal coronary circulation (Sanders et al., 1977; Scheffer and Verdouw, 1983) or with a gradually occluded coronary artery (Bloor et al., 1984; White and Bloor, 1986). The increase in cardiac output was almost exclusively due to the tachycardia as stroke volume remained virtually unchanged. $LVdP/dt_{max}$ was markedly enhanced indicating that global left ventricular function increased. Left ventricular systolic pressure was slightly elevated, while a pronounced increase in left ventricular end-diastolic pressure was observed.

The pre-exercise cardiovascular actions of nisoldipine, propranolol or their combination under resting conditions are in accordance with the findings of other investigators (Warltier et al., 1984; Scriabine and Taylor, 1984; Silke et al., 1985, 1986) as well as those from our laboratory (Duncker et al., 1987a,b). Despite the changes induced by nisoldipine at rest, during exercise the systemic hemodynamic variables reached levels that were similar in

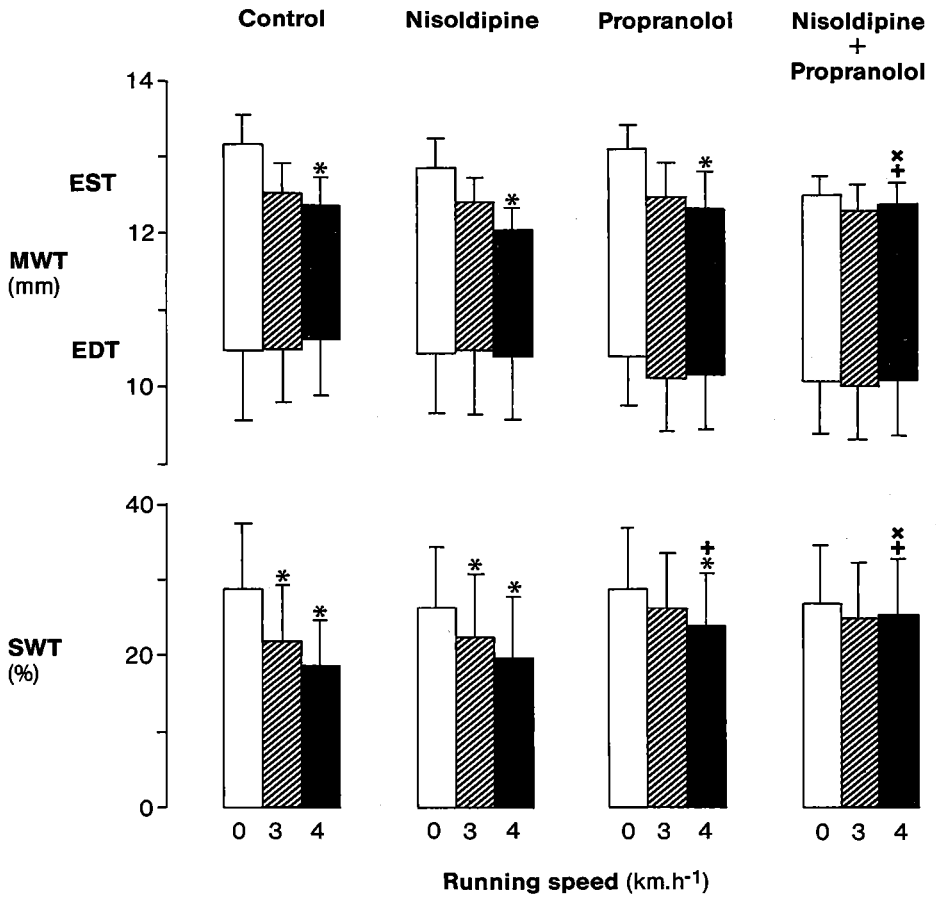

Fig. 3.
Effects of exercise on post-stenotic wall function in the absence or presence of medication with nisoldipine, propranolol, or their combination. MWT=left ventricular myocardial wall thickness; EST = end-systolic thickness; EDT = end-diastolic thickness; SWT= normalized systolic wall thickening. *P<0.05 vs corresponding pre-exercise; **P<0.05 pre-exercise after medication vs pre-exercise without treatment; $^{+,x}$ exercise-induced responses after medication significantly (P<0.05) different from exercise-induced responses without treatment (+), or after nisoldipine (x).

untreated and nisoldipine-treated animals. Only left ventricular systolic and end-diastolic pressures were lower when compared to the group without medication. Another dihydropyridine-derivative, nifedipine, has also been shown not to modify the changes in systemic hemodynamic variables during exercise in dogs with critical coronary stenosis (Heusch et al., 1987). In contrast, Silke et al. (1985, 1986) reported that during exercise nisoldipine-treated patients with coronary artery disease achieved higher heart rate levels but had a lower arterial blood pressure than untreated control patients. These patients were apparently subjected to a mild exercise, as heart rates of only 100 beats.min^{-1} were reached during the control-exercise period. At such a low level of exercise the baroreceptor-reflex is still operative so that nisoldipine could induce further tachycardia.

Pretreatment with propranolol attenuated but not completely eliminated the exercise-induced increases in heart rate, cardiac output, $LVdP/dt_{max}$ and left ventricular systolic blood pressure. These findings, suggesting the involvement, at least in part, of the withdrawal of parasympathetic tone during exercise, are in agreement with data reported earlier in dogs with propranolol (Heyndrickx et al., 1980) and atenolol (Matsuzaki et al., 1984b). Combined treatment with nisoldipine and propranolol resulted during exercise in a hemodynamic state similar to that obtained after propranolol alone. Silke et al. (1986) observed in their patients that addition of nisoldipine to metoprolol caused an increase in cardiac output, heart rate and stroke volume. The reason for this observation might again be the low exercise level to which their patients were subjected and at which the baroreceptor-reflex (parasympathetic withdrawal) is still operative.

Regional myocardial wall function

None of the drug regimen caused a significant increase in wall function at rest, but different patterns emerged during exercise. In our study nisoldipine was ineffective in antagonizing the exercise-induced decrease in systolic wall thickening. In contrast, Heusch et al. (1987) observed an improvement of myocardial performance after nifedipine in exercise-induced myocardial ischemia in dogs. The reason for the difference in observations may be that pigs, unlike dogs, possess few native coronary collaterals. Our experiments were performed 7-9 days after implantation of a constrictor which caused severe impairment of wall function (systolic wall thickening less than 15%) in

only two out of seven animals. Since myocardial ischemia is an important inductor of collateral formation it is not likely that extensive collateral formation took place in most animals in this study. Warltier et al. (1981) have also shown that in anesthetized dogs nisoldipine increased blood flow to a myocardial area totally dependent upon collateral circulation. Therefore, the reason for the difference between our observations and those of Heusch et al. (1987) might be related to the absence of extensive coronary collaterals in our animals.

As we found in this study with pigs, β-adrenoceptor antagonists have been shown to be effective against myocardial ischemia induced by treadmill-exercise in dogs (Kumada et al., 1980; Matsuzaki et al., 1984b; Tomoike et al., 1987). This beneficial effect is most likely due to a reduction in heart rate (Guth et al., 1987) which not only decreases myocardial oxygen demand but also prolongs the diastolic coronary perfusion time to improve myocardial blood flow, especially to the subendocardial layers (Schamhardt et al., 1981; Saxena, 1983; Guth et al., 1987). Though propranolol effectively antagonized (but not eliminated) the exercise-induced loss of systolic wall function, nisoldipine did not. The reason for this difference in the efficacy of the two drugs may lie in their respective pharmacological profile. Propranolol can decrease myocardial oxygen demand (due to negative chronotropic and inotropic effects) as well as enhance blood supply to the jeopardized myocardium (due to increased diastolic perfusion time; Schamhardt et al., 1981). In case of nisoldipine the decrease in myocardial oxygen demand (due to peripheral vasodilation) can be offset by an exercise-induced increase in sympathetic activity. Since the last effect is antagonized by propranolol, the combination of the two drugs may be more effective in averting the deterioration of systolic wall function of post-stenotic myocardium during exercise. Indeed, this seems to be the case in our experiments where after propranolol alone the animals did show some deterioration in systolic wall thickening in the post-stenotic myocardium during treadmill exercise; such changes were completely prevented after the combined treatment with propranolol and nisoldipine.

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DISCUSSION

CHAPTER 14
GENERAL DISCUSSION AND CONCLUSIONS

DISCUSSION

Chapter 14
General discussion and conclusions

14.1 Methodological considerations

The experimental animal

The experimental animal used in the studies presented in this thesis was the farm-bred Yorkshire pig. The use of swine in scientific research has increased rapidly over the last decade. Ample studies demonstrate that the pig is closely related to the human, anatomically and physiologically (Pond and Houpt, 1978; Sack, 1982; Swindle and Bobbie, 1983; Peng et al., 1983; Pluth, 1983; Swindle, 1984). From a cardiovascular point of view the similarities in size and distribution of coronary arteries (Weaver et al., 1986), blood pressure and heart rate (Smith et al., 1964), plasma lipoprotein profile (Mahley and Weisgraber, 1974) and responses to hyperlipidemic diets (Thomas et al., 1983) are of interest. In pigs atherosclerosis occurs spontaneously (Skold and Getty, 1961) and can also be easily induced (Thomas et al., 1983). However, for cardiovascular studies in vivo the dog is perhaps still the most often employed animal, partly because it is easier to handle. However, a disadvantage of the dog for cardiovascular research is that it has an extensive native collateral circulation in the heart and even complete occlusion of coronary arteries may result in no infarction or variable infarct sizes (Schaper, 1971). In contrast pigs, like humans, possess very few native collaterals; the formation of new collaterals can, however, be easily induced (Ramo, 1970; Millard, 1981). Moreover, for exercise studies, swine compare more favorably to human than dogs with respect to heart weight-body weight ratio, VO_2max, cardiac index, regional distribution of cardiac output and acid-base balance (see Mckirnan et al., 1986).

The experiments in this thesis were performed in anesthetized as well as conscious animals. The anesthetized animal model can be used for acute, relatively short-lasting experiments with drugs administered intravenously or intra-arterially (intracoronary or intracarotid). However, the presence of anesthesia may interfere with the autonomic regulation of the cardiovascular system and, therefore, may alter the actions of drugs. For example, pentobarbital anesthesia is known to reduce inotropy of the heart (Sawyer

et al., 1971) which is an undesirable action especially in a model of myocardial ischemia. Also, the baroreceptor reflex appears to be attenuated in the presence of barbiturates (Roberts, 1980; Montgomery et al., 1982; Zimpfer et al., 1982). Although the conscious animal model is more laborious than the anesthetized model, it enables chronic studies, exercise studies, or studies in which oral administration of a drug is employed. Furthermore, in conscious animals there is no interference of anesthesia with circulatory dynamics.

Model for the study of myocardial ischemia

Myocardial ischemia has been defined as the imbalance between the supply of oxygenated blood and the oxygen requirements of the myocardium. The consequence of this imbalance is: 1) a lack of oxygen and metabolic substrates for the production of energy in the form of high energy phosphates such as adenosine triphosphate (ATP) and creatine phosphate and 2) the diminished "wash out" of metabolites of the ensuing anaerobic glycolysis. Anaerobic glycolysis is insufficient to meet the energy requirements of the myocyte. Furthermore, the accumulation of metabolic products, such as lactate, H^+ and purine bases in the heart inhibits anaerobic glycolysis and other metabolic pathways.

Functional features of myocardial ischemia are: 1) loss of contractile function (within seconds after a flow reducing intervention) and 2) electrolyte and electrophysiological changes which may lead to rhythm disturbances (Hillis and Braunwald, 1977). In this thesis we used systolic wall thickening as a measure of regional myocardial function. Systolic wall thickening of the left ventricle is a good measure of local myocardial function (Sasayama et al., 1976) which is closely related to disturbances in oxygen demand/oxygen supply (Kerber et al., 1975; Verdouw et al., 1980; Ross and Franklin, 1976). Another measure of myocardial ischemia, frequently used in the clinical setting, are ST-segments changes. Although ST-segment changes are used to quantitate infarct size (Maroko et al., 1972) poor correlations have been reported between ST-segment changes and infarct size or the severity of myocardial ischemia (Vincent et al., 1977). Metabolic markers (lactate and hypoxanthines) have also been used as a measure of the severity and extent of myocardial ischemia (Parker et al., 1969; Opie et al., 1973; Remme et al., 1977) but they have the disadvantage of being time-dependent as their

production fades during prolonged ischemia (Rovetto et al., 1975; De Jong et al., 1977; Verdouw et al., 1979), probably due to deterioration of cell function.

In chapters 12 and 13 we investigated the effects of nisoldipine on perfusion and/or wall function of a myocardial area distal to a coronary artery stenosis in the proximal part of the left anterior descending coronary artery. The stenosis was produced by use of a teflon ring, implantation of which resulted in a fixed concentric obstruction. In a clinical study by Vlodaver and Edwards (1971) 30% of all (200) atherosclerotic lesions studied at autopsy were of the concentric type, whereas 70% belonged to the eccentric type, either polymorphous or slit-like. In contrast to eccentric stenoses, the vessel in the case of concentric obstructions is circumferentially affected by the atherosclerotic process and responds less well to vasodilatory interventions than eccentric stenoses (see Lichtlen and Ebner, 1986).

Another important factor that determines the effectiveness of vasodilatory treatment is the presence or absence of collateral vessels supplying the post-stenotic myocardium. In the experiments described in chapter 12 the stenosis in the animals was present for a period of 5 days which is not sufficient to allow significant collateral formation (Ramo et al., 1970). In pigs with a longer period of stenosis (7-9 days) as used in chapter 13, 5 out of 7 animals showed no signs of myocardial ischemia under resting conditions. Since myocardial ischemia is a potent inductor of collateral formation (Schaper, 1971), it is not likely that an extensive collateral circulation was present in that group of animals. In a clinical study by Schwartz et al. (1978) collaterals were not observed when the stenosis obstructed less than 90% of the cross-sectional area of the coronary arterial lumen. With a stenosis of 90% to 99% of the luminal area, 22% of the patients demonstrated collaterals; of patients with a complete occlusion, 83% showed collateral supply. In another study (Kober et al., 1978) collaterals were not present when the stenosis was less than 60%; half the patients, with a stenosis of 90% to 99%, showed collaterals and again with complete coronary occlusion collateral vessels were apparent in nearly all patients. These clinical data correspond well with experimental findings (Schaper and Wüsten, 1979; Marcus, 1983) in which collateral formation becomes apparent when the stenosis has reduced the luminal area for more than 80%-90%. At this stage the vasodilator reserve of the post-stenotic bed is exhausted even at rest (see

Klocke et al., 1987) and myocardial ischemia will ensue, reflected by a loss of wall function (Schwartz et al., 1978), thereby promoting collateral-formation. In the above mentioned clinical studies (Schwartz et al., 1978; Kober et al., 1978) no collaterals could be visualized in approximately 30% of all patients. In the study of Kober et al. (1978) 30% of all patients had an obstruction in the left anterior descending coronary artery, without concomitant atherosclerotic processes in other coronary vessels, or collaterals. In the study by Schwartz et al. (1978), 50% of patients with one-vessel disease had no collaterals. In view of the above findings in patients with coronary artery disease, our model seems to correlate well with a subgroup of patients who have one-vessel disease and no or few collaterals.

In chapter 13 acute myocardial ischemia was induced by exercise on a treadmill one week after surgery. The animals were adapted to the laboratory facilities prior to the operation and during the post-surgical period but were not trained on the treadmill to avoid a training-effect per se. We know from earlier experiments in our laboratory (Scheffer and Verdouw, 1983) as well as from other reports (Sanders et al., 1978; Moores et al., 1986) that pigs can be easily trained to perform treadmill exercise. Six out of 9 animals performed the complete exercise protocol on the first day, while in one animal the first day exercise-adaptation was necessary. In two animals no exercise-protocol could be executed because the animals were incapable of running at a constant pace and they were excluded from further study. In only two out of the seven animals employed in the exercise-protocol the fixed stenosis caused a significant loss of systolic wall function at rest and therefore collateral circulation may have been present in these two animals. However, based on the findings by Ramo et al. (1970) the collateral circulation would probably not have been extensive after 1 week of implantation of the constrictor. This also suggests that our animal experimental model mimics patients with one-vessel disease with only poor or no collateral formation.

14.2 Systemic hemodynamic actions of vasodilating drugs in conscious pigs.

In order to facilitate the comparison of the systemic hemodynamic actions of the substances studied in this thesis the relative changes of the different systemic hemodynamics have been depicted along the diagonals of a hexagon (Fig. 1). One must be careful when using percentage changes from baseline

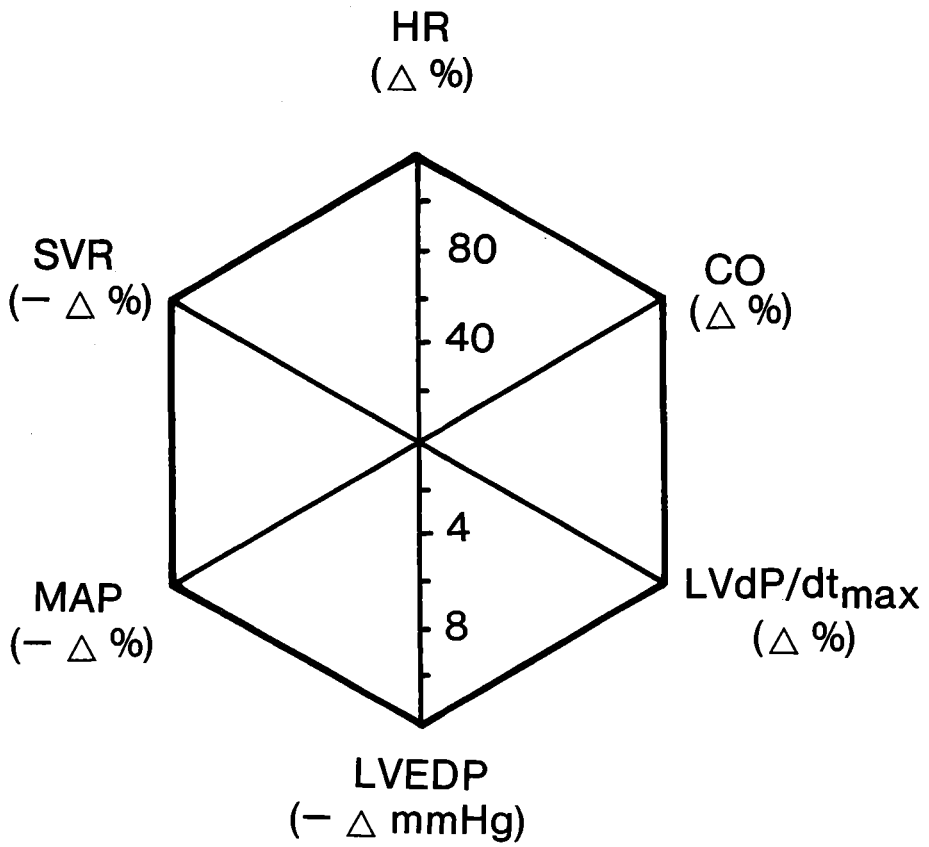

Fig. 1
Schematic representation of the effects on systemic hemodynamic parameters as used in Figs 2 and 3. HR = heart rate; CO = cardiac output; $LVdP/dt_{max}$ = maximal rate of rise in left ventricular pressure; LVEDP= left ventricular end-diastolic pressure; MAP = mean arterial pressure; SVR = systemic vascular resistance. All changes have been expressed as percentage change from baseline (as specified for heart rate), except LVEDP. The changes in the latter have been expressed in absolute values because of the marked effect of β-adrenoceptor blockade on this parameter.

as an indicator of the magnitude of the response of a parameter because such changes depend on the baseline values. Therefore a minimum requirement is that the respective baseline values of a parameter are similar for each drug. No significant differences between any of the baseline values of the parameters existed before β-adrenoceptor blockade. β-adrenoceptor blockade

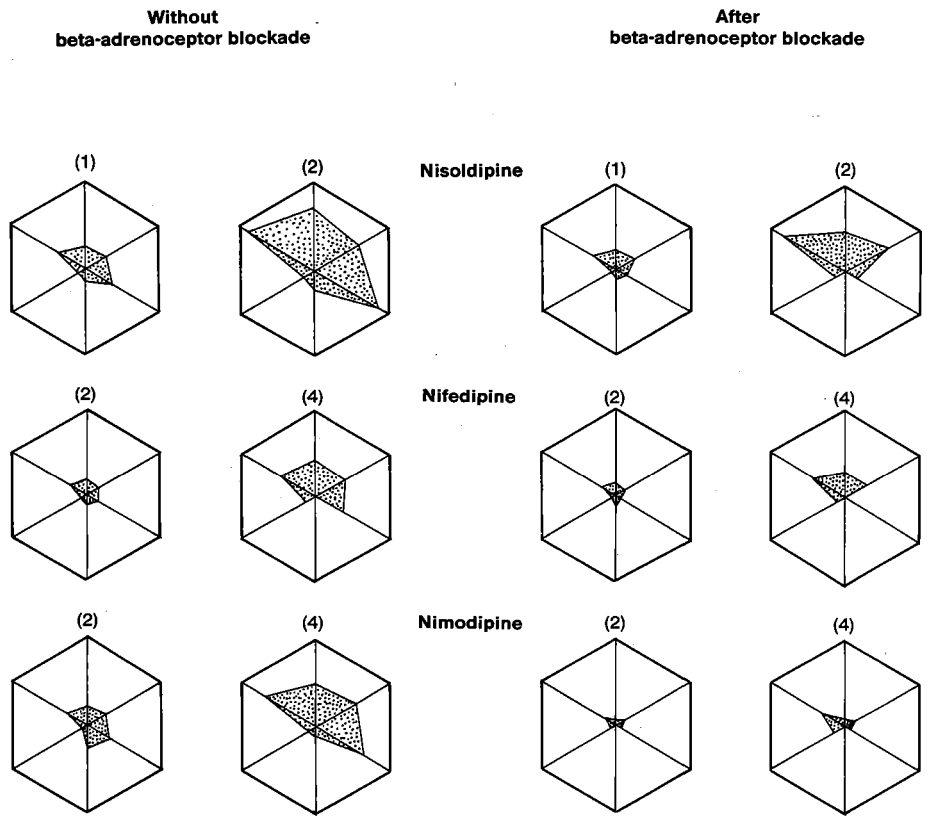

Fig. 2
Systemic hemodynamic responses to three dihydropyridines without and after β-adrenoceptor blockade in conscious pigs. See Fig. 1 for explanation of the diagrams. The numbers in parenthesis indicate the infusion rate of the drugs in $\mu g.kg^{-1}.min^{-1}$.

had a relatively minor effect on all parameters except for LVEDP. The changes in the latter have therefore been expressed in absolute values to allow comparison between the hemodynamic actions of a drug without and after β-adrenoceptor blockade. Inspection of Fig. 2 reveals that in conscious pigs the dihydropyridines nisoldipine, nimodipine and nifedipine have similar

systemic hemodynamic profiles, with arteriodilation (indicated by the decrease in systemic vascular resistance) being the most prominent feature, but that

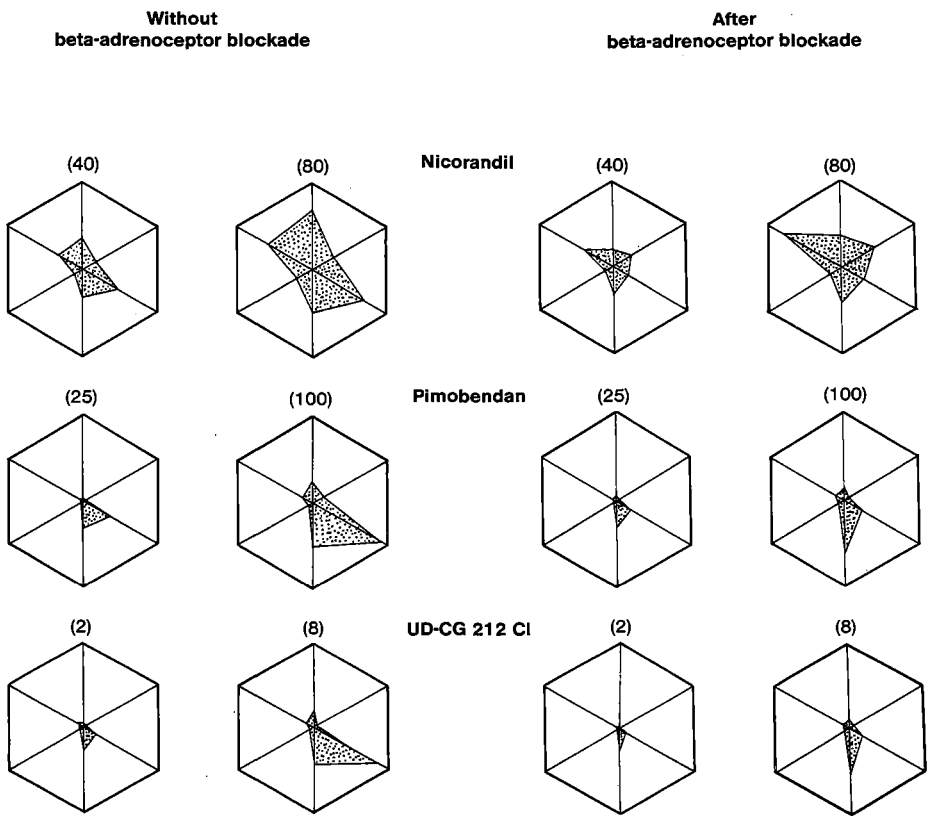

Fig. 3
Systemic hemodynamic responses to nicorandil, pimobendan and UD-CG 212 Cl without and after β-adrenoceptor blockade in conscious pigs. See Fig. 1 for explanation of the diagrams. The number in parenthesis indicate the infusion rate of the drugs in $\mu g.kg^{-1}.min^{-1}$.

they differ somewhat in potency. At variance with the data of the dihydropyridines are those of the three substances presented in Fig. 3. Nicorandil as well as pimobendan and UD-CG 212 Cl markedly decreased left ventricular filling pressure, indicating venodilation. Because of this action

the increase in cardiac output was consistently less than the increase in heart rate with these drugs.

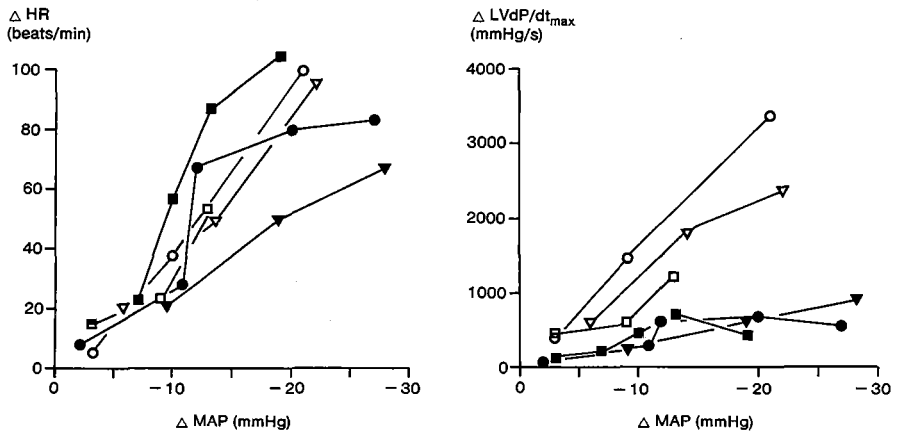

Fig. 4
Changes in heart rate (HR) and the rate of rise in left ventricular pressure (LVdP/dtmax) are plotted as a function of the decrease in mean arterial blood pressure (MAP) for conscious pigs (open symbols) and dogs (closed symbols). The vasodilatory drugs were nifedipine (□,■) nisoldipine (○,●) and nicorandil (▽ , ▼). The data on pigs and dogs were obtained in the laboratories of Dr. Verdouw (Verdouw et al., 1987, chapter 10 this thesis) and Dr. Gross (Warltier et al., 1984; Preuss et al., 1985), respectively.

β-adrenoceptor blockade had no major effects on the arterio- and venodilatory responses of the compounds (Figs. 2 and 3), but attenuated in all cases the positive inotropic and also, to a large extent, the positive chronotropic actions.

If we compare the actions of vasodilating drugs in conscious pigs to those in conscious dogs, it is clear that in both species the drop in blood pressure elicits a similar marked baroreceptor reflex-mediated tachycardia (Fig. 4). In contrast, a reflex-mediated increase in myocardial contractility is much more pronounced in pigs than in dogs. An explanation for this observation is not readily found but one of the possibilities is a denser sympathetic innervation in left ventricular porcine myocardium resulting in a more pronounced reflex-mediated increase in $LVdP/dt_{max}$. It is unlikely that these drugs exert a greater negative inotropic action in dogs than in pigs since after

pretreatment with β-adrenoceptor blockade no significant reductions in $LVdP/dt_{max}$ were observed in conscious dogs (not shown in Fig. 4).

14.3 Influence of anesthesia on the systemic hemodynamic profile of vasodilating drugs.

The influence of anesthesia on systemic hemodynamic responses to vasodilators are indicated in Table 1. In anesthetized animals a certain degree of systemic vasodilation resulted in a much greater fall in arterial blood pressure, apparently due to an attenuation of the baroreceptor reflex which prevented an increase in cardiac output. It is known for some time that pentobarbital anesthesia attenuates the baroreceptor-reflex (Roberts, 1980; Montgomery et al., 1982; Zimpfer et al., 1982). In an earlier study

TABLE 1

Systematic hemodynamic responses induced by intravenously administered vasodilating drugs at comparable reductions in systemic vascular resistance[1] in pigs.

		dose (μg/kg/min)	MAP	SVR	CO	HR	SV	$LVdP/dt_{max}$	LVEDP
Nisoldipine	A	0.25 - 1.0	↓↓	↓↓	→	↑	↓	↑	↓
	C	0.5 - 1.0	↓	↓↓	↑	↑↑	→	↑↑	↓
Nimodipine	A	0.25 - 1.25	↓↓	↓↓	↓	↓	→		
	C	1.0 - 2.0	↓	↓↓	↑	↑	→	↑↑	→
Nicorandil	A	15 - 75	↓↓	↓↓	→	↑	↓	↑	↓↓
	C	20 - 80	↓	↓↓	↑↑	↑↑↑	↓	↑↑↑	↓↓↓
Pimobendan	A	10 - 100	↓↓	↓↓	↓	↑↑	↓	↑	↓↓
	C	10 - 100	→	↓	↑	↑	↓	↑↑↑	↓↓↓
UD-CG 212 CI	A	0.5 - 8	↓↓	↓↓	↓	↑↑	↓↓	↑↑	↓↓↓
	C	2 - 8	→	↓	→	↑	↓	↑↑↑	↓↓↓

Abbreviations: A = anesthetized; C = conscious; MAP = mean arterial pressure; SVR = systemic vascular resistance; CO = cardiac output; HR = heart rate; SV = stroke volume; $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure; LVEDP = left ventricular end-diastolic pressure.

[1] Pimobendan and UD-CG 212 CI administration in conscious pigs induced only a slight reduction in systemic vascular resistance.

Booth et al. (1960) had shown that α-chloralose anesthesia, which we employed in the nicorandil and UD-CG 212 Cl studies, did not affect the baroreceptor-reflex in pigs. The low dose of pentobarbital (5 $mg.kg^{-1}.h^{-1}$) which we added to the α-chloralose anesthesia may therefore be responsible for the attenuated positive chronotropic responses to nicorandil.

At variance with the other three compounds presented in Table 1 the pyridazinone-derivatives showed a somewhat larger percentage increase in heart rate in anesthetized animals. The slightly lower baseline value in anesthetized pigs only partly explains this observation. Another contributing factor might be the minimal systemic vasodilation in conscious animals, which in anesthetized animals was prominent, inducing therefore only a slight increase in heart rate. Furthermore, it cannot be excluded that the positive chronotropic action of these drugs in anesthetized pigs were direct rather than reflex-mediated. The increments in heart rate were, namely, not affected by pretreatment with propranolol.

Finally, in contrast with the similarities between the systemic hemodynamic profiles of nisoldipine and nimodipine in conscious pigs, nimodipine exerted in anesthetized pigs a direct cardiodepressant action while nisoldipine did not. Similar differences in action between nimodipine and nisoldipine have been reported in pentobarbital anesthetized dogs (Maxwell et al., 1982; Satoh et al., 1984).

14.4 Vasodilatory profile of vasodilating drugs.

The responses of regional vascular conductances induced by five vasodilating drugs have been presented along the diagonals of a hexagon (Fig. 5). As can be seen in Fig. 6, the dihydropyridines nisoldipine and nimodipine caused their greatest vasodilatory response in skeletal muscles. Nicorandil, at the middle dose having a profile similar to nimodipine, elicited at the highest dose a more evenly distributed vasodilation in the peripheral beds. This profile at the highest dose was similar to that after the highest dose of pimobendan. Despite the similarity to pimobendan with respect to the systemic hemodynamic actions, UD-CG 212 Cl did not cause significant vasodilation in the left ventricular myocardium and the skeletal muscle vasculature. However, of all drugs studied this compound was the only substance that caused a slight increase in renal vascular conductance.

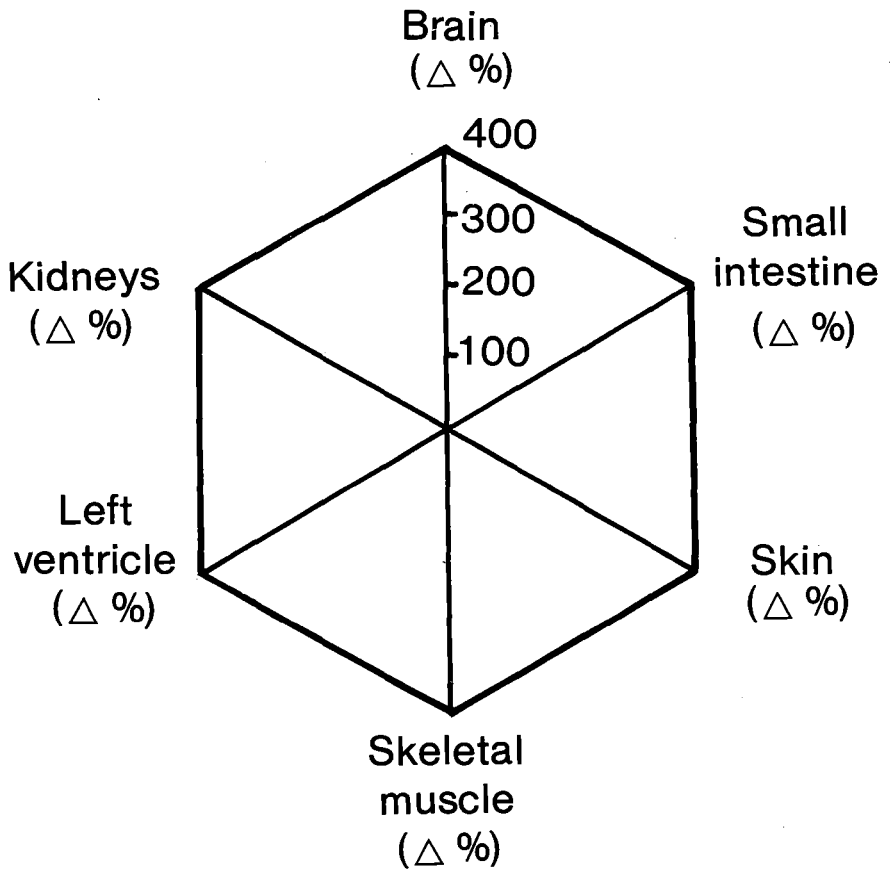

Fig. 5
Schematic representation of the effects on six regional vascular conductances as used in Fig. 5. All changes have been expressed as percentage change from baseline.

In the carotid circulation, intra-arterial infusions of nimodipine and nifedipine produced almost identical vasodilatory profiles (Fig. 7). Here again, the dihydropyridines induced the greatest response in the skeletal muscles.

When the vasodilatory actions of nisoldipine after intravenous administration in anesthetized animals are compared with those of the drug

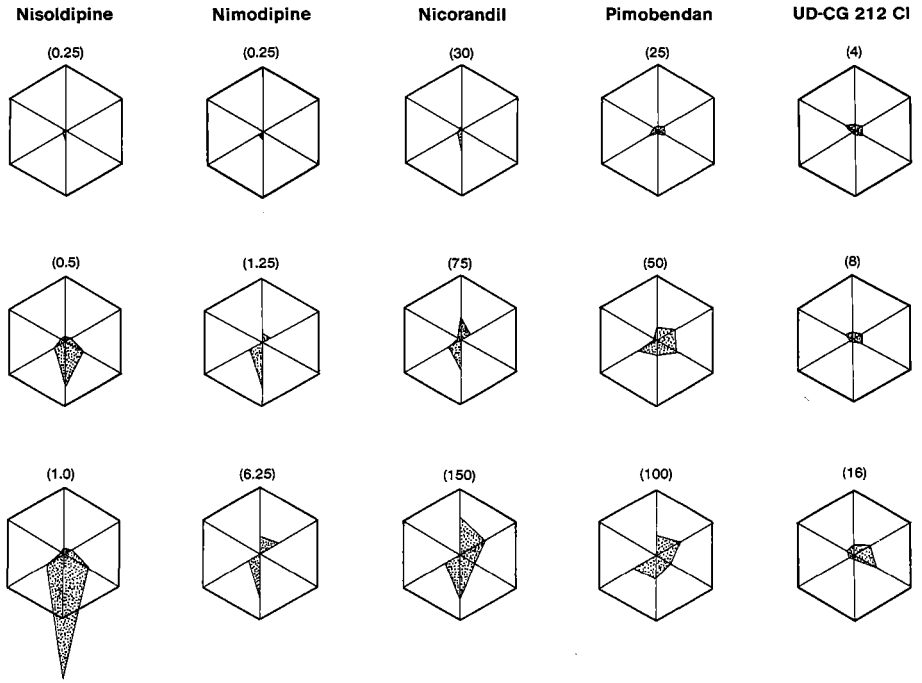

Fig. 6
Regional vasodilatory responses to five vasodilators. See Fig. 5 for explanation of the diagrams. The numbers in parenthesis indicate the infusion rates of the drugs in $\mu g.kg^{-1}.min^{-1}$. Blood pressure reductions at each dose were, respectively, for nisoldipine 5, 15 and 25 mmHg, for nimodipine 21, 38 and 52 mmHg, for nicorandil 18, 28 and 40 mmHg, for pimobendan 21, 35 and 43 mmHg and for UD-CG 212 Cl 28, 34 and 39 mmHg.

after oral administration in conscious pigs (Fig. 8), the most striking feature is the absence of preferential vasodilation in skeletal muscles in the conscious animals. Based on the responses of systemic vascular conductance, the gastrointestinal tract, left ventricle and brain responded dose-dependently. It is not clear whether the differences with regard to vascular conductance responses in the skeletal muscle, and also skin, are due to the anesthesia or the route of administration. A slight response of the skin vasculature to a vasodilator agent in conscious animals is a finding also observed with nisoldipine in rats (Drexler et al., 1985, 1986) and felodipine in rabbits (Bolt and Saxena, 1984) and is possibly due to reflex-mediated sympathetic vasoconstriction. Although the skeletal muscle responses might also be due to the absence of anesthesia, Drexler et al. (1985, 1986) also observed that in

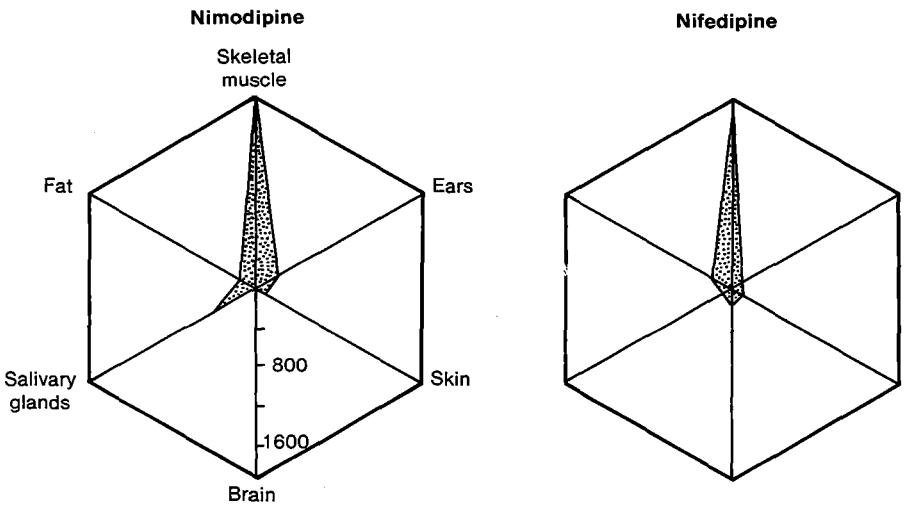

Fig. 7
Effects of intracarotid infusions of nimodipine (0.25 $\mu g.kg^{-1}.min^{-1}$) and nifedipine (0.75 $\mu g.kg^{-1}.min^{-1}$) on regional vascular conductances of ipsilateral tissues and organs in the pig head. Data have been expressed as percentage change from baseline.

conscious rats the largest vasodilatory response in the skeletal muscle occurred after intravenous administration of nisoldipine. In contrast, after an oral dose eliciting similar systemic hemodynamic actions, the greatest vasodilatory response was observed in the gastrointestinal tract. These findings are in agreement with our data and it is therefore possible that the differences that we observed are due to different routes of administration rather than absence or presence of anesthesia.

Finally, in contrast to some reports claiming selectivity of nisoldipine and nimodipine for the coronary and cerebral vascular beds, respectively (Kazda et al., 1980, 1982; Serruys et al., 1985), we observed that all three dihydropyridines exerted their most potent vasodilating action on skeletal muscle vasculature.

14.5 Conclusions and perspectives

The cardiovascular responses induced by the vasodilating drugs studied in this thesis indicate that these drugs might play a role in the treatment of

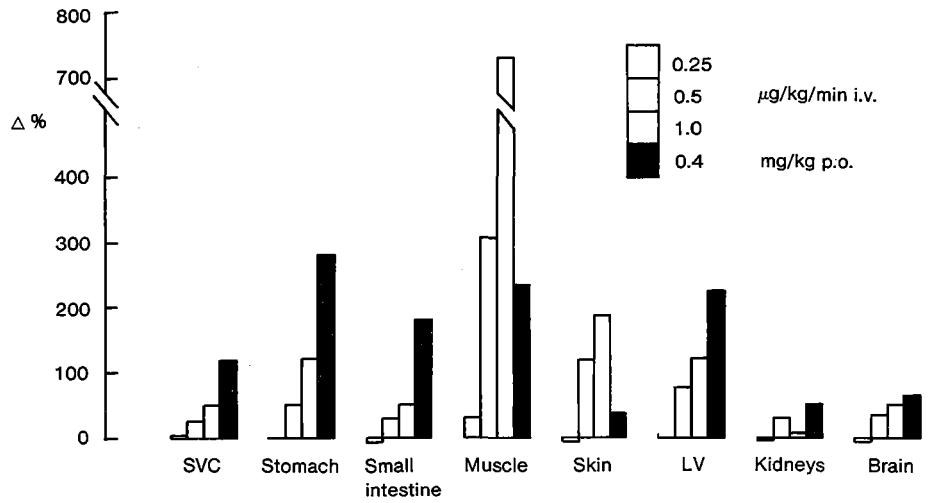

Fig. 8
Comparison of the changes in regional vascular conductances induced by intravenous nisoldipine in anesthetized and oral nisoldipine in conscious pigs. SVC = systemic vascular conductance, LV = left ventricle.

cardiovascular disorders, in particular, coronary artery disease, hypertension and heart failure.

With respect to coronary artery disease and hypertension, the dihydropyridines and nicorandil caused systemic and coronary arteriolar vasodilation. The acute administration of nicorandil and the dihydropyridines induced a baroreceptor reflex-tachycardia which may limit their usefulness, since the combination of hypotension and an increase in heart rate may lead to an underperfusion of the subendocardial layers in the myocardium distal to a coronary artery stenosis. However, during prolonged treatment with vasodilators the baroreceptors usually reset and the reflex-tachycardia fades (Kiowski et al., 1983). But, since pretreatment with the β-adrenoceptor antagonist propranolol attenuated the reflex-tachycardia and in addition

detrimental effects on cardiac pump function and contractility were not observed, a combination of vasodilators and β-adrenoceptor blockade might be more attractive than monotherapy with either drug. Furthermore, during chronic treatment with β-adrenoceptor antagonists, after an initial reflex-mediated compensatory increase in systemic vascular resistance, blood pressure is decreased and this effect may add to the hypotensive actions of vasodilating drugs.

Though most vasodilators, in particular after acute administration, cause a greater vasodilation in the subepicardial than in the subendocardial layers due to their systemic actions and the smaller vasodilatory reserve in the subendocardial layers, they do not seem to have an intrinsic preference for either layer. In this respect, an interesting observation was recently made by Gross and co-workers (Pelc et al., 1987) who reported that so called "endothelium-dependent" vasodilators (acetylcholine and ATP) preferentially caused vasodilation in the subendocardial regions after intracoronary administration whereas "endothelium-independent" vasodilators like nifedipine did not have such a preference. We found that the "endothelium-independent" vasodilator pimobendan did not have a preference, while nicorandil even favoured the subepicardial layers after intracoronary infusions. It might be speculated that, provided the systemic actions are mild, "endothelium-dependent" vasodilators are less likely to induce so called "steal" distal to a coronary artery stenosis due to vasodilation in subepicardial layers and that they may favorably influence myocardial oxygen balance preferentially in the subendocardial layer where ischemia occurs first. Other factors that may determine the possible benefits of vasodilators in coronary artery disease are the nature of the coronary artery stenosis and the absence or presence of a collateral circulation. In this thesis, like in all other experimental models with a coronary artery stenosis, a concentric (fixed) stenosis which is not amenable to vasodilation was used. In the present experiments in pigs, where only few collaterals could have been present, nisoldipine caused an impairment of blood flow to the subendocardial layers during rest, whereas the drug was ineffective against exercise-induced ischemia. It seems therefore important to stratify patients on the basis of the nature of coronary stenosis (concentric or eccentric) and the presence of a collateral circulation in order to obtain a patient population that is most likely to benefit from vasodilating therapy. Furthermore, studies in experimental models with eccentric stenoses and/or

collaterals employing chronic treatment with vasodilators are necessary to obtain more detailed information on the possible usefulness of a certain drug in a particular patient population.

In patients with heart failure a reduction in left ventricular filling pressure is associated with a reduction in mortality (see Packer et al., 1987). Therefore, it appears that nicorandil and the pyridazinone-derivatives are of greater promise in this disorder than are the dihydropyridines of which nisoldipine only after i.v. administration caused a slight decrease in filling pressure. The use of calcium-channel blockers has also not been advised for treatment of congestive heart failure in a recent review article (Packer et al., 1987). Although the "unloading" of the heart by the pyridazinone-derivatives and nicorandil caused a reduction in stroke volume and/or cardiac output in animals with a normal circulation, in animals with failing hearts pimobendan unloaded the left ventricle and increased cardiac pump function, thereby normalizing these parameters (chapter 6). Reflex-tachycardia does not seem to be important in heart failure since the reduction in afterload is often unaccompanied by increases in heart rate due to the already enhanced sympathetic drive (Higgins et al., 1972; Levine et al., 1982). Furthermore, the increase in cardiac pump function prevents a fall in blood pressure.

Another aspect of heart failure is the systemic arteriolar vasoconstriction in the peripheral vascular beds especially in the kidneys, skin, skeletal muscle and gastro-intestinal tract. Nicorandil and pimobendan caused vasodilation in the latter three beds, but failed to increase vascular conductance in the renal bed. On the other hand UD-CG 212 Cl increased renal vascular conductance, but was relatively ineffective on the skeletal muscle vasculature. Interpretation of these regional vasodilatory profiles of drugs that are of potential usefulness in the treatment of congestive heart failure must be done with caution since the vasodilatory profile of a drug in a normal circulation may not be the same as in a circulation with high vascular tone (Hof, 1983; Hof et al., 1985). Further research in experimental models of congestive heart failure seems therefore necessary to elucidate whether the drugs studied in this thesis still exert their potentially beneficial actions.

Finally, marked effects of anesthesia on circulatory control mechanisms suggest that conscious rather than anesthetized animals should be employed in cardiovascular research. This seems particularly true for animal models

dealing with myocardial ischemia and heart failure where counterregulatory or compensatory mechanisms are integral parts of the profile of the disease.

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SUMMARY

SUMMARY

Chapter 1 presents a brief review of literature concerning the mechanism of action, the vasodilator profile and some clinical uses of vasodilating drugs. Thereafter, this thesis describes an investigation in *anesthetized* or *conscious* pigs dealing with the cardiovascular pharmacology of representatives of three groups of vasodilating drugs. The selected drugs were: the dihydropyridine calcium-channel blockers nisoldipine, nimodipine and nifedipine, the pyridazinone-derivatives pimobendan and UD-CG 212 Cl (which may act via phosphodiesterase-inhibition), and the nitrate-like drug nicorandil (which also opens potassium-channels).

The Vasodilator Profile of Dihydropyridine Calcium-channel Blockers

Nisoldipine has been described as having a particularly pronounced and selective vasodilator action on the coronary circulation. In order to evaluate this claim we studied the effects of this substance on distribution of cardiac output. In anesthetized pigs intravenous infusions of nisoldipine caused dose-dependent coronary and systemic vasodilation and increases in heart rate and $LVdP/dt_{max}$ as a result of baroreceptor reflex activation (Chapter 2). Systemic vasodilation was most pronounced in the skeletal muscle vasculature. Coronary vasodilation occurred particularly in the subepicardial layers of the left ventricle. After pretreatment with the β-adrenoceptor antagonist propranolol, vasodilator responses to nisoldipine were relatively unaffected, but the reflex-mediated responses were abolished. In conscious pigs (Chapter 3) oral administration of nisoldipine resulted in marked systemic vasodilation which was most pronounced in the gastrointestinal tract and skeletal muscles. The increase in cardiac output (reflex-mediated) prevented a major drop in arterial blood pressure. Probably due to the attenuated increase in heart rate after combined treatment with propranolol and nisoldipine, the subendocardial-subepicardial blood flow ratio was better maintained than after nisoldipine alone.

We conclude that: (i) nisoldipine is a substance with a potent vasodilator action, (ii) this vasodilation is most pronounced in the skeletal muscles and, (iii) the drug can be safely combined with β-adrenoceptor antagonists without compromising left ventricular pump function.

In view of the claimed selectivity for cerebral vessels and effectiveness in migraine, the effects of nimodipine on the distribution of cardiac output and carotid artery blood flow as well as on the vascular responses to 5-hydroxytryptamine (5-HT) were evaluated (Chapters 4 and 5). Intravenous infusion of nimodipine caused hypotension, bradycardia and a vasodilator response that was most marked in the skeletal muscles. Upon intracarotid administration, both nimodipine and nifedipine redistributed carotid blood flow in favor of the capillary compartment, again particularly to the skeletal muscles. Intracarotid infusion of 5-HT also redistributed carotid artery blood flow, but in favor of the capillary blood flow to the skin. Arteriovenous anastomotic (AVA) blood flow was severely reduced by 5-HT, while the calcium-channel blockers affected it only mildly. The vasoconstrictor response to 5-HT was not affected by the two calcium-channel blocking drugs.

In conclusion: (i) nimodipine lacks selectivity for the cerebral vasculature, (ii) the vasodilator actions of nimodipine and nifedipine are most pronounced in the skeletal muscles, (iii) the comparatively mild reductions in AVA conductance may be one of the reasons for the inability of dihydropyridine-derivatives to abort acute attacks of migraine and (iv) in contrast to 'in-vitro' studies, 5-HT induced vasoconstriction 'in-vivo' is antagonized neither by nimodipine nor by nifedipine.

Vasodilator Profile of Pyridazinone-derivatives

In heart failure peripheral vasoconstriction may play an important role in maintaining or aggravating the failing state of the heart. Vasoconstriction may be particularly pronounced in the renal, dermal, gastrointestinal and skeletal muscle vasculature. We therefore evaluated the effects of the pyridazinone-derivatives pimobendan and UD-CG 212 Cl on cardiovascular dynamics, in particular the distribution of cardiac output. Intravenous infusions of pimobendan in anesthetized pigs caused venous and arterial dilation and an increase in heart rate but had only a minor effect on $LVdP/dt_{max}$ (Chapter 6). Arterial vasodilation was most pronounced in the adrenals, gastrointestinal tract, skeletal muscles and myocardium (subepicardium > subendocardium). Intravenous bolus injection of pimobendan produced similar changes in all systemic and regional hemodynamic variables except that $LVdP/dt_{max}$ now increased markedly (Chapter 7). Cardiac output was slightly reduced in animals with a normal coronary circulation.

However, in animals with an ischemic heart, the drug clearly increased both $LVdP/dt_{max}$ and cardiac output while pre- and afterload were reduced. During intracoronary infusion no preference for the subepicardial layer was observed and the endo-epi blood flow ratio was unaffected.

Intravenous infusion of the O-demethyl metabolite of pimobendan, UD-CG 212 Cl, caused nearly similar systemic hemodynamic actions as the parent drug but at much lower doses (Chapter 8). The plasma concentrations of UD-CG 212 Cl obtained during pimobendan infusions were however too low to ascribe the actions of the parent drug to the formation of the metabolite. The regional arterial vasodilation was most pronounced in the adrenals, kidneys and gastrointestinal tract but was conspicuously absent in the skeletal muscles.

It is concluded that: (i) the pyridazinone-derivatives exert vasodilator and positive inotropic actions which improve cardiac pump function in pigs with severe myocardial ischemia, (ii) although the contribution of the metabolite to the actions of pimobendan seems negligible during acute experiments, it is conceded that it may become important during prolonged treatment; this is particularly interesting in view of the vasodilation by pimobendan in the skeletal muscles and gastrointestinal tract and the vasodilation by UD-CG 212 Cl in the renal vasculature.

Vasodilator Profile of Nicorandil

The vasodilator profile of the nitrate-like substance nicorandil was evaluated in the light of its potential usefulness in coronary artery disease, hypertension and congestive heart failure. Intravenous infusions of nicorandil in anesthetized pigs reduced arterial blood pressure, stroke volume, left ventricular end-diastolic pressure and systemic vascular resistance, but increased heart rate and $LVdP/dt_{max}$ (Chapter 9). Cardiac output was not affected as the increase in heart rate was balanced by the reduction in stroke volume. The nicorandil-induced increases in heart rate and $LVdP/dt_{max}$ were most likely due to a reflex activation of the sympathetic nervous system following the fall in arterial blood pressure. Although cardiac output did not change in animals, intravenous infusions of nicorandil did cause a redistribution of blood flow in favor of organs such as the heart, adrenals, spleen, small intestine and brain at the expense of that to the stomach and kidneys. The increase in myocardial blood flow, primarily to the subepicardial

layers, was associated with an enhancement in coronary venous oxygen content and was also noticed after intracoronary infusions of nicorandil.

We conclude that the cardiovascular profile of nicorandil suggests possible usefulness of the drug in coronary artery disease as well as in congestive heart failure and hypertension. However, the combination of the marked hypotensive effect and reflex-mediated tachycardia may, under certain conditions, aggravate rather than ameliorate myocardial ischemia, particularly in the subendocardial layers.

Systemic hemodynamic actions of vasodilating drugs in the conscious pig in the absence or presence of β-adrenoceptor blockade

Since vasodilating drugs induce reflex-mediated cardiostimulatory responses which may partially offset their beneficial actions, these agents are often combined with β-adrenoceptor blockade. Furthermore, patients with hypertension and/or coronary artery disease which are considered for treatment with vasodilator therapy may already be on β-adrenoceptor antagonist medication. Therefore, we studied the hemodynamic effects of the vasodilators nicorandil, nisoldipine, nimodipine and nifedipine in combination with propranolol.

Intravenous infusions of nicorandil caused a marked systemic vasodilation which led, however, to only a moderate fall in arterial blood pressure due to an increase in cardiac output (Chapter 9). This increase in cardiac output was due to tachycardia as stroke volume and left ventricular filling pressure were reduced. After pretreatment with propranolol the vasodilation was not affected but increases in heart rate and $LVdP/dt_{max}$ were attenuated.

Intravenous infusions of nisoldipine, nimodipine and nifedipine resulted in similar systemic hemodynamic profiles. Of the three dihydropyridines nisoldipine was the most potent substance (Chapter 10). Dose-dependent reduction in systemic vascular resistance induced marked baroreceptor reflex-mediated counterregulation. Systemic vasodilation was not affected by pretreatment with propranolol, but reflex-mediated actions were attenuated (cardiac output and heart rate) or abolished ($LVdP/dt_{max}$). The effects of the dihydropyridines on left ventricular filling pressure were minimal and independent of pretreatment with propranolol.

To evaluate the importance of β-adrenergic tone for the cardiovascular actions of the pyridazinone-derivatives, we studied the effects of pimobendan

and its O-demethyl metabolite UD-CG 212 Cl in combination with propranolol in conscious pigs (Chapter 11). Intravenous infusions of both compounds caused prominent dose-dependent increases in $LVdP/dt_{max}$ and, to a far lesser extent, in heart rate and cardiac output. Stroke volume was slightly reduced due to the decrease in left ventricular end-diastolic pressure. Mean arterial blood pressure was not significantly affected. In contrast to the observations in anesthetized pigs there was a slight decrease in the systemic vascular resistance slightly decreased of the conscious animals. After β-adrenoceptor blockade, increases in heart rate and cardiac output induced by the pyridazinone-derivatives were attenuated and those in $LVdP/dt_{max}$ were almost abolished. The responses of left ventricular end-diastolic and mean arterial blood pressure, systemic vascular resistance and stroke volume were not modified.

In conclusion: (i) combination treatment of nicorandil or the dihydropyridines with β-adrenoceptor blockade seems useful as it attenuates the reflex-mediated cardiostimulatory responses, (ii) these reflex responses are much more pronounced in conscious than in anesthetized animals, and (iii) β-adrenergic tone seems to be necessary for the cardiostimulatory actions of pyridazinone-derivatives.

Vasodilator therapy and myocardial ischemia

Recent studies indicate that vasodilator reserve may still be present despite severe myocardial ischemia. Thus we investigated the effects of nisoldipine on the performance of the myocardium distal to a fixed coronary artery stenosis in conscious pigs at rest (Chapter 12) and during exercise (Chapter 13).

In Chapter 12 the animals studied at rest were divided into two groups depending upon the severity of stenosis as judged by systolic wall thickening (SWT) of the post-stenotic segment: Group 1 (SWT>15%) and Group 2 (SWT<10%). The systemic hemodynamic profiles at baseline and after oral administration of nisoldipine were similar in both groups. Increases in heart rate were accompanied by increases in cardiac output and $LVdP/dt_{max}$, while systemic vascular resistance and mean arterial blood pressure were reduced. Left ventricular systolic and end-diastolic blood pressure and stroke volume were not affected. In both groups, nisoldipine caused increases in blood flow to the control (non-stenotic) myocardial area which favored the subepicardium

over the subendocardium. Blood flow to the post-stenotic area in group 1 animals was normal at baseline but was only slightly enhanced (preferentially to the subepicardium) by nisoldipine. In the post-stenotic area of group 2 animals, transmural and subendocardial blood flows were lower at baseline as compared to the control area. Nisoldipine did not affect subepicardial blood flow but reduced subendocardial blood flow. It is concluded that, under the experimental conditions employed (concentric stenosis, no coronary collaterals and acute drug administration) nisoldipine does not have a beneficial effect on post-stenotic myocardial blood flow, particularly in animals with severe stenosis.

In Chapter 13 the effects of oral nisoldipine with or without propranolol on exercise-induced myocardial ischemia in pigs with a coronary artery stenosis were evaluated. Treadmill-running up to 4 $km.h^{-1}$ increased cardiac output, heart rate, $LVdP/dt_{max}$, left ventricular systolic and end-diastolic blood pressure, while systolic wall thickening of the post-stenotic left ventricular myocardium was reduced. Nisoldipine neither affected the systemic hemodynamic profile during exercise nor the exercise-induced reductions in myocardial wall function. Propranolol attenuated both the positive chronotropic and inotropic effects and the deterioration of wall function caused by the treadmill-exercise. Combined treatment with the two drugs resulted in a cardiovascular profile during exercise similar to that with propranolol alone, but the loss of wall function was now completely prevented. Thus, unlike propranolol, nisoldipine was not effective against the exercise-induced ischemia but may have beneficial actions when combined with β-adrenoceptor antagonists.

Overall conclusions

The pharmacological responses induced by the vasodilating drugs studied in this thesis indicate that these drugs have a hemodynamic profile commensurate with their therapeutic use in cardiovascular disorders like coronary artery disease, hypertension and heart failure. However there are certain aspects which should be further elaborated. Firstly, with respect to the first two disease entities, the baroreceptor reflex mediated actions may potentially offset the benefits of nicorandil and the dihydropyridines. To circumvent these negative effects a combination with β-adrenoceptor blockade appears useful. Secondly, based on the findings with nisoldipine in

our model of myocardial ischemia, nisoldipine may not be effective in patients with concentric stenosis lacking coronary collaterals. It may therefore be important to select patients for therapy with such drug on the basis of the nature of their stenosis and the state of their coronary collateral circulation. Also, chronic studies in experimental models with eccentric stenoses and/or coronary collaterals are necessary to obtain more detailed information on possible benefits from these drugs in a particular patient population. Thirdly, with respect to heart failure the pyridazinone-derivatives present greater promise than other vasodilator drugs studied in this thesis. These drugs reduce preload and enhance myocardial contractility which, in animals with ischemic hearts, normalized cardiac pump function. Finally, the marked effects of anesthesia on circulatory control mechanisms shows that conscious rather than anesthetized animals should be preferred in cardiovascular research especially in experimental models dealing with myocardial ischemia and heart failure.

SAMENVATTING

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In hoofdstuk 1 is een kort overzicht gegeven van het werkingsmechanisme, het vasodilator profiel en een aantal klinische toepassingen van vasodilatantia. Hierna volgt een beschrijving van onderzoek naar de cardiovasculaire pharmacologie van drie groepen van vasodilatantia in genarcotiseerde en niet-genarcotiseerde varkens. De geselecteerde stoffen waren: de dihydropyridine calcium-kanaal blokkeerders nisoldipine, nimodipine and nifedipine, de pyridazinone-derivaten pimobendan en UD-CG 212 Cl (werkingsmechanisme mogelijkerwijs via phosphodiesterase-inhibitie) en de nitraat-achtige stof nicorandil (werkingsmechanisme tevens via kalium-kanalen).

Het vasodilator profiel van dihydropyridine calcium-kanaal blokkeerders

Van nisoldipine is beschreven dat het een selectieve verwijding geeft van het coronaire vaatbed. Teneinde deze eigenschap te toetsen, bestudeerden we de effecten van deze stof op de verdeling van het hartminuutvolume. In genarcotiseerde varkens veroorzaakten intraveneuze infusies van nisoldipine dosis afhankelijke verwijding van zowel het coronaire alsook het systemische vaatbed. Tevens namen tengevolge van activatie van de baroreceptor reflex hartfrequentie en $LVdP/dt_{max}$ toe (Hoofdstuk 2). In het systemische vaatbed trad de dilatatie voornamelijk op in de skeletspieren. In de linker ventrikel was de dilatatie van het coronaire vaatbed groter in de subepicardiale dan in de subendocardiale lagen. Voorbehandeling met de β-adrenoceptor antagonist propranolol, had geen invloed op de vasodilator responsies van nisoldipine maar de reflectoire responsies waren sterk verminderd. Orale toediening van nisoldipine aan varkens (Hoofdstuk 3) leidde in het systemische vaatbed vooral tot dilatatie van de tractus gastro-intestinalis en de skeletspieren. De reflectoire toename in het hartminuutvolume (reflectoir) voorkwam een grote daling van de arteriele bloeddruk. De subendocardiale-subepicardiale perfusie ratio nam minder af na gecombineerde behandeling met propranolol en nisoldipine dan na nisoldipine alleen, ten gevolge van de geringere toename in hartfrequentie.

Concluderend: (i) nisoldipine is een stof met een potent vasodilaterende werking, (ii) deze vasodilatatie is niet-selectief en het meest uitgesproken in de skeletspier en (iii) de stof kan gecombineerd worden met β-adrenoceptor

antagonisten zonder de pompfunctie van de linker ventrikel nadelig te beinvloeden.

In het licht van de gepropageerde selectiviteit voor de cerebrale vaten en effectiviteit in migraine, werden de effecten van nimodipine op de distributie van het hartminuutvolume en de bloedstroom door de arteria carotis communis zonder en tijdens infusies van 5-hydroxytryptamine (5-HT) bestudeerd in genarcotiseerde varkens (Hoofdstukken 4 en 5). Intraveneuze infusie van nimodipine veroorzaakte hypotensie, bradycardie en een vaatverwijding welke het meest uitgesproken was in de skeletspieren. Na toediening in de arteria carotis induceerden nimodipine en nifedipine een redistributie van de carotis bloedstroom ten gunste van het capillaire compartiment, en in het bijzonder van de skeletspieren. Infusie van 5-HT in de carotis gaf een redistributie van de carotis bloedstroom, waarvan nu vrijwel uitsluitend de huid profiteerde. De bloedstroom door de arterioveneuze anastomosen (AVA) werd sterk gereduceerd door 5-HT, terwijl de calcium-kanaal blokkeerders de AVA doorbloeding slechts in geringe mate beinvloedden. De vasoconstrictor responsie na infusie van 5-HT werd niet door de calcium-kanaal blokkeerders geantagoneerd.

We concluderen dat: (i) nimodipine geen selectiviteit ten opzichte van de cerebrale vasculatuur bezit, (ii) de vasodilator effecten van nimodipine en nifedipine het meest uitgesproken zijn in de skeletspieren, (iii) de relatief milde afnamen van de AVA geleiding na toediening van nifedpine en nimodipine wellicht één van de mogelijke verklaringen is voor het onvermogen van dihydropyridine-derivaten om acute aanvallen van migraine te couperen, en (iv) in tegenstellig tot 'in-vitro' studies, de vasoconstrictie door 5-HT 'in-vivo' niet door nimodipine en nifedipine geantagoneerd wordt.

Het vasodilator profiel van pyridazinone-derivaten

Bij hartfalen kan een toename in periphere vaatweerstand leiden tot een verslechtering van de pompfunctie van het hart. Vasoconstrictie is dan vooral sterk in de circulatie van de nier, skeletspier, huid en tractus gastro-intestinalis. We evalueerden daarom de effecten van de pyridazinone-derivaten pimobendan en UD-CG 212 Cl op de cardiovasculaire dynamica en in het bijzonder op de distributie van het hartminuutvolume. Intraveneuze infusies van pimobendan in genarcotiseerde varkens veroorzaakten veneuze en arteriele dilatatie en een toename in hartfrequentie, maar hadden slechts een

gering effect op LVdP/dt_{max} (Hoofdstuk 6). Arteriele vasodilatatie was het meest uitgesproken in de bijnieren, tractus gastro-intestinalis, skeletspieren en myocardium (subepicardium > subendocardium). Intraveneuze bolus injecties van pimobendan produceerden dezelfde veranderingen in alle systemische en regionaal haemodynamische variabelen met uitzondering van LVdP/dt_{max} die nu sterk toenam (Hoofdstuk 7). In dieren met een normale coronair circulatie was er een geringe daling van het hartminuutvolume. Daarentegen leidde toediening van pimobendan aan dieren met een ischaemisch hart tot een stijging van het hartminuutvolume en de LVdP/dt_{max}. Gedurende intracoronaire infusies nam de myocard doorbloeding toe zonder voorkeur voor de subepicardiale lagen.

Intraveneuze infusie van UD-CG 212 Cl, de O-demethyl metaboliet van pimobendan, veroorzaakte systemisch haemodynamische veranderingen die vrijwel gelijk waren aan die van pimobendan. UD-CG 212 Cl veroorzaakte deze veranderingen echter al bij veel lagere doseringen (Hoofdstuk 8). De plasma concentraties van het geformeerde UD-CG 212 Cl tijdens pimobendan infusies zijn echter te laag om de effecten van pimobendan toe te schrijven aan de formatie van de metaboliet. De perifere vasodilatatie was het meest uitgesproken in de bijnieren, nieren en tractus gastro-intestinalis maar opvallend afwezig in de skeletspieren.

Concluderend: (i) de pyridazinone-derivaten bezitten een vasodilator en een positief inotrope werking, (ii) in varkens met een ischaemisch hart leidt dit tot een verbetering van het hartminuutvolume, (iii) hoewel de bijdrage van de metaboliet aan de werking van pimobendan verwaarloosbaar is gedurende acute experimenten, wordt deze wellicht belangrijk bij chronische toediening. Dit is vooral interessant indien men bedenkt dat pimobendan vaatverwijding induceert in de skeletspieren en tractus gastro-intestinalis en UD-CG 212 Cl in de nieren.

Het vasodilator profiel van nicorandil

Het vasodilator profiel van de nitraat-achtige stof nicorandil werd geevalueerd vanwege mogelijke toepassing in coronaire hartziekten, hypertensie en hartfalen. Intraveneuze infusies van nicorandil in genarcotiseerde varkens reduceerde de arteriele bloeddruk, slagvolume, linker ventrikel eind-diastolische bloeddruk en de systemische vasculaire weerstand, maar induceerde een toename in hartfrequentie en LVdP/dt_{max} (Hoofdstuk 9).

Het hartminuutvolume veranderde niet omdat de afname in slagvolume werd gecompenseerd door de toename in hartfrequentie. Er was wel een verandering in de distributie van het hartminuutvolume ten gunste van het hart, dunne darm en hersenen ten koste van de maag en nieren. De toename in de linker ventrikel doorbloeding, voornamelijk naar de subepicardiale lagen, ging gepaard met een toename in de coronair veneuze zuurstof saturatie. Na intracoronaire toediening van nicorandil werden dezelfde resultaten voor het hart gevonden.

Concluderend: het cardiovasculaire profiel van nicorandil suggereert een mogelijke toepassing in coronaire hartziekten, hartfalen en hypertensie. De combinatie van een sterke bloeddruk daling en een reflectoire tachycardie kan onder bepaalde condities, vooral in de subendocardiale lagen, ischaemie induceren.

Systemische haemodynamische werking van vasodilatantia in het niet-genarcotiseerde varken voor en tijdens β-adrenoceptor blokkade.

Aangezien vaatverwijders reflex-gemedieerde cardiostimulerende responsies kunnen induceren die de gunstige effecten geheel of gedeeltelijk opheffen, worden deze stoffen vaak gecombineerd met β-adrenoceptor antagonisten. Tevens worden patienten met een ischaemisch hartziekte en/of hypertensie die voor behandeling met vaatverwijders in aanmerking komen al vaak behandeld met β-adrenoceptor antagonisten. Wij bestudeerden daarom de haemodynamische effecten van de vaatverwijders nicorandil, nisoldipine, nimodipine en nifedipine na voorbehandeling met propranolol. Om mogelijke effecten van anaesthesie uit te sluiten werden de experimenten in niet-genarcotiseerde dieren uitgevoerd.

Intraveneuze infusies van nicorandil induceerden een opmerkelijke systemische vasodilatatie met slechts een geringe daling in de arteriele bloeddruk, omdat het hartminuutvolume toenam (Hoofdstuk 9). Deze toename was het gevolg van een tachycardie omdat het slagvolume afnam. Na voorbehandeling met propranolol was de systemische vasodilatatie ongewijzigd maar de toenamen in hartfrequentie en $LVdP/dt_{max}$ verminderd.

Nisoldipine, nimodipine en nifedipine vertoonden na intraveneuze toediening een overeenkomstig systemisch haemodynamisch profiel met nisoldipine als de meest potente stof (Hoofdstuk 10). Dosis afhankelijke reducties in systemische vasculaire weerstand leidden tot opmerkelijke baroreceptor reflex

gemedieerde effecten. Systemische vasodilatatie werd niet beinvloed door voorbehandeling met propranolol, maar de reflectoir geinduceerde effecten waren verminderd (hartminuutvolume en hartfrequentie) of zelfs volledig geinhibeerd ($LVdP/dt_{max}$). De effecten van de dihydropyridines op de vullingsdruk van de linker ventrikel waren zeer gering en onafhankelijk van voorbehandeling met propranolol.

Om de invloed van de β-adrenerge tonus op de cardiovasculaire werking van de pyridazinone-derivaten te evalueren, bestudeerden we de effecten van pimobendan en UD-CG 212 Cl in niet-genarcotiseerde varkens in combinatie met propranolol (Hoofdstuk 11). Intraveneuze infusies van beide pyridazinone-derivaten veroorzaakten sterke toenamen in $LVdP/dt_{max}$ en in mindere mate in hartfrequentie en hartminuutvolume. Het slagvolume nam een weinig af door de daling van de linker ventrikel vullingsdruk. De gemiddelde arteriele bloeddruk veranderde niet. In tegenstelling tot de bevindingen bij de genarcotiseerde varkens nam de systemische vaatweerstand slechts weinig af in de niet-genarcotiseerde dieren. Na β-adrenoceptor blokkade waren de door de pyridazinone-derivaten geinduceerde stijgingen in hartfrequentie en hartminuutvolume geringer en in $LVdP/dt_{max}$ bijna volledig verdwenen. Onveranderd waren de responsies van de linker ventrikel vullingsdruk, de arteriele bloeddruk, de systemische vasculaire weerstand en het slagvolume.

Samenvattend: (i) combinatie therapie van nicorandil of de dihydropyridine-derivaten met β-adrenoceptor antagonisten lijkt zinvol aangezien dit de reflex-gemedieerde cardiostimulerende responsies verzwakt, (ii) deze reflectoire responsies zijn veel sterker in niet-genarcotiseerde dan in genarcotiseerde dieren, en (iii) de β-adrenerge tonus is van belang voor de cardiostimulerende werking van pyridazinone-derivaten.

Vasodilator therapie en myocard ischaemie

Uit recente studies is gebleken dat vasodilator reserve nog aanwezig kan zijn in ischaemisch myocard. Daarom bestudeerden we de effecten van nisoldipine op het myocard distaal van een gefixeerde coronair stenose in varkens in rust (Hoofdstuk 12) en tijdens inspanning (Hoofdstuk 13). In hoofdstuk 12 waren de dieren die bestudeerd werden verdeeld in twee groepen gebaseerd op de ernst van de stenose. Deze werd beoordeeld met behulp van de procentuele systolische wandverdikking (SW) van het post-stenotische segment: groep 1 (SW>15%) en groep 2 (SW<10%). De systemisch

haemodynamische profielen van groep 1 en 2 waren voor en na orale toediening van nisoldipine hetzelfde. Toenamen in hartfrequentie gingen vergezeld van stijgingen in hartminuutvolume en $LVdP/dt_{max}$, terwijl de systemisch vasculaire weerstand en de gemiddelde arteriele bloeddruk daalden. Linker ventrikel systolische- en eind-diastolische bloeddruk en slagvolume bleven onveranderd. In beide groepen veroorzaakte nisoldipine een toename in doorbloeding van het controle (niet-stenotische) gebied van de linker ventrikel waarvan de subepicardiale lagen het meest profiteerden. De doorbloeding van het post-stenotische gebied in de varkens behorende tot groep 1 was normaal tijdens de controle waarneming maar werd slechts gering verhoogd (preferentieel naar het subepicardium) door nisoldipine. In het post-stenotische gebied van de dieren van groep 2 waren de transmurale en subendocardiale doorbloeding tijdens de controle meting verlaagd ten opzichte van het niet-stenotische gebied. Nisoldipine had geen effect op de subepicardiale bloedstroom maar reduceerde de subendocardiale doorbloeding.

Concluderend: nisoldipine heeft onder de experimentele condities zoals die door zijn ons gebruikt (concentrische stenose, geen coronair collateralen en acute toediening van de stof), geen gunstige effecten op post-stenotische myocardiale bloedstroom, vooral in dieren met een ernstige stenose.

In hoofdstuk 13 werden de effecten van oraal toegediend nisoldipine geevalueerd voor en tijdens β-adrenoceptor blokkade met propranolol op inspannings-gebonden myocard ischaemie in varkens met een coronair stenose. Lopen op een tredmolen tot een snelheid van 4 $km.h^{-1}$ veroorzaakte een toename in hartminuutvolume, hartfrequentie, $LVdP/dt_{max}$, linker ventrikel systolische en eind-diastolische bloeddruk, terwijl de systolische wandverdikking van de post-stenotische linker ventrikel wand werd gereduceerd. Nisoldipine beinvloedde noch het haemodynamisch profiel noch de inspannings-gebonden afname in wandfunctie tijdens inspanning. Propranolol remde zowel de positief chrono- en inotrope effecten als de inspannings-gebonden reductie in wandfunctie. Combinatie therapie resulteerde tijdens inspanning in een cardiovasculair profiel dat gelijk was aan dat verkregen met propranolol. De afname in wandfunctie werd nu echter volledig voorkomen. Dus, nisoldipine had, in tegenstelling tot propranolol, geen effect op het verlies aan functie van het post-stenotische myocard tijdens inspanning, maar heeft eventueel wel een gunstige werking na combinatie met β-adrenoceptor antagonisten.

Eindconclusies

De farmacologische responsies op de vaatverwijders die in dit proefschrift werden bestudeerd geven aan dat deze stoffen een haemodynamisch profiel bezitten dat beantwoord aan de eisen voor een therapeutisch gebruik in cardiovasculaire aandoeningen zoals coronaire hartziekten, hypertensie en hartfalen. Er zijn echter een aantal aspecten die meer aandacht vereisen. Ten eerste , wat betreft hypertensie en coronaire hartziekten, kunnen de baroreceptor reflex gemedieerde effecten mogelijkerwijs de gunstige effecten van nicorandil en de dihydropyridines te niet doen. Om deze negatieve effecten te vermijden lijkt een combinatie met een β-adrenoceptor antagonist nuttig. Ten tweede, gebaseerd op de bevindingen in ons model van myocardiale ischaemie zou het kunnen zijn dat nisoldipine niet effectief is in patienten met een concentrische coronair stenose en een gering ontwikkelde collateraal circulatie. Het lijkt daarom belangrijk om patienten te selecteren voor behandeling met een stof als nisoldipine op basis van de aard van hun stenose en de aanwezigheid van een collateraal circulatie. Daarbij zijn studies in experimentele modellen met eccentrische vernauwingen en/of collateralen noodzakelijk om hieromtrent meer informatie te verkrijgen. Ten derde, wat betreft hartfalen lijken de pyridazinone-derivaten de stoffen met het meest geschikte profiel voor deze entiteit. De pyridazinone-derivaten verlagen de vullingsdruk en verhogen de contractiliteit van de linker ventrikel, wat in dieren met ischaemische harten leidde tot een normalisering van de pompfunctie van het hart. Tenslotte, de sterke effecten van anaesthesie op de cardiovasculaire reflexen laten zien dat genarcotiseerde boven niet-genarcotiseerde dieren te prefereren zijn in het onderzoek van vooral myocard ischaemie en hartfalen.

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CURRICULUM VITAE

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