PHARMACOLOGICAL INVESTIGATIONS IN A SWINE MODEL OF CHRONIC LEFT VENTRICULAR DYSFUNCTION

PHARMACOLOGISCHE STUDIES BIJ EEN MODEL VOOR CHRONISCHE LINKER VENTRIKEL DYSFUNCTIE IN VARKENS

PROEFSCHRIFT

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ter nagedachtenis aan mijn vader
aan mijn moeder

CONTENTS

Chapter 1

Introduction

Introduction

In most textbooks heart failure is defined as a state in which the pumping action of the heart is insufficient to meet the metabolic demands of the various organs and tissues. Harris (1987) has however pointed out that total body oxygen consumption in heart failure is not necessarily abnormal and he defines heart failure as a condition in which the heart and the vascular system (load of the heart) need compensatory mechanisms to main central aortic pressure. The underlying cause of heart failure can thus have a myocardial as well as a vascular cause.

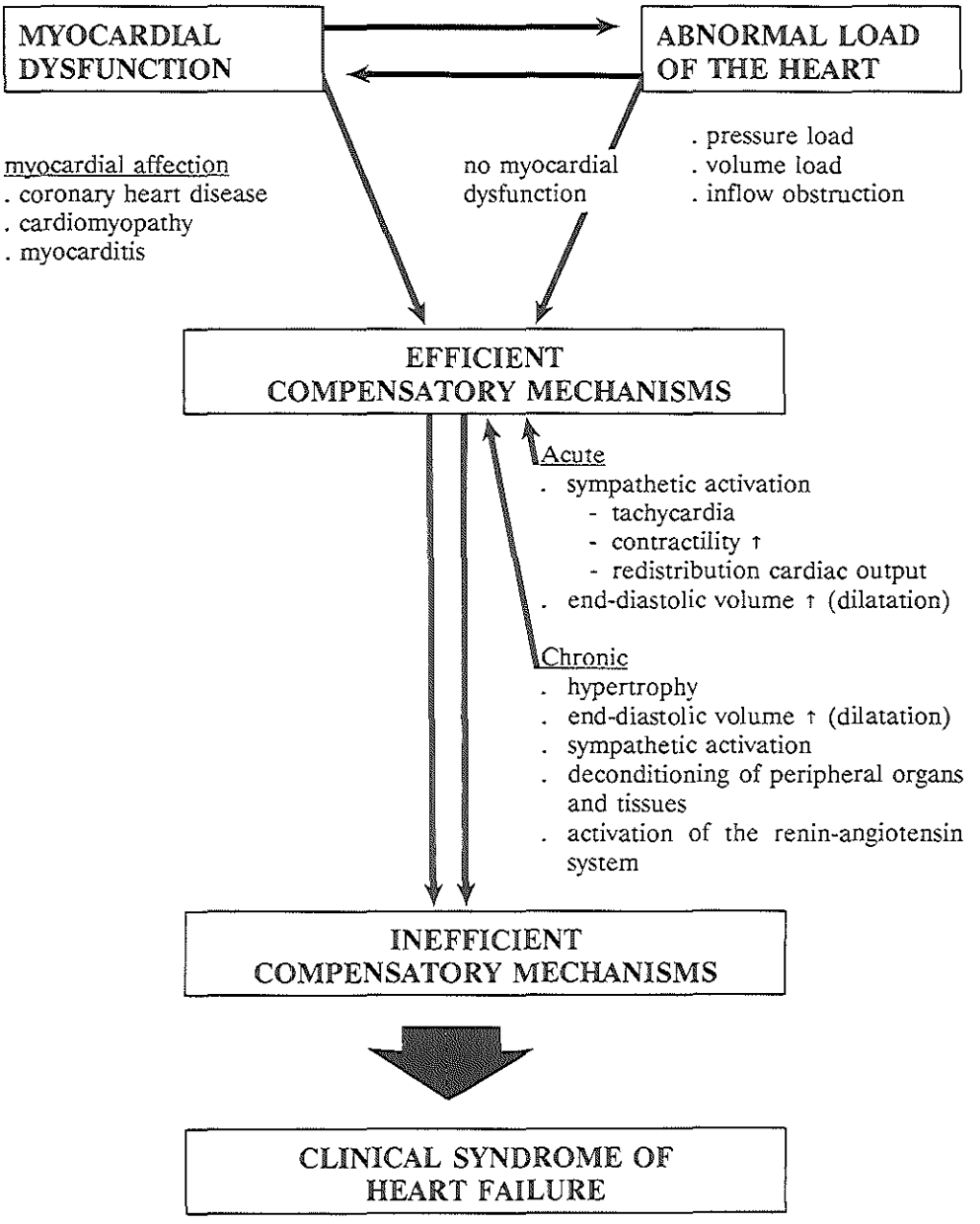
Figure 1 depicts a number of compensatory mechanisms which are activated to maintain central aortic blood pressure and perfusion of a number of vital organs such as the heart and the brain, both of which are primarily under autoregulatory control. As the disease progresses these early adaptations may prove to be insufficient and in spite of further adaptations, clinical signs of heart failure become evident (fig. 1)

In over 80% of all patients, the main causes of heart failure are coronary artery disease and hypertension (Sutton et al., 1990). There is a male predominance because of the higher rate of coronary artery disease, corresponding with a fourfold increased risk of heart failure. Therefore, the factors predisposing for heart failure are similar to those for coronary artery disease and include: elevated blood pressure, diabetes, elevated total cholesterol and obesity (McKee et al., 1971 and Kannel and Cupples, 1988). The prevalence of heart failure in persons in their 50s is about 1% and rises progressively with age up to 10% in persons over 80 years old. The annual incidence also increases with age from 0.2% (between 45 and 54 years) to 4.0% in men between 85 and 94 years of age (Kannel and Belager, 1991). Once diagnosed, heart failure is highly lethal showing a two year mortality rate of 37% for men and 33% for women and increases to 82% and 67% respectively after 6 years (Kannel and Belanger, 1991).

Animal models for chronic heart failure

Investigating separate aspects of the pathophysiology, or new interventions in patients with heart failure is limited by existing medication. It will therefore in the future almost be impossible to evaluate the effects of a new pharmacological agent in patients who are not already receiving treatment with digitalis, diuretics, etc. In addition, not all parameters contributing to the disease such as regional organ blood flows can reliably be studied. Therefore several animal models have been developed. According to the

Fig. 1 *Pathophysiology of cardiac decompensation*



methods used for producing heart failure, these experimental models can be divided into the categories: pressure overloading, volume overloading, coronary occlusion, atrial and ventricular pacing and the administration of agents toxic to the myocyte (Smith and Nutall, 1985).

Models based on abnormal load of the heart

Pressure overloading

Pressure overloading is produced by outflow-obstruction of the left or the right ventricle: i.e. constriction of the pulmonary artery, the aorta, the pulmonary valves or by inducing a valvular or supravalvular stenosis. Alternatively occlusion of a renal artery can be used. The models are summarised in table 1. Models using outflow obstruction are generally characterized by a variable gradient across this stenosis and left ventricular hypertrophy. In contrast, models with renal artery occlusion all show high systemic blood pressure and secondary left ventricular hypertrophy, while proliferative vascular changes occur. Consequently models for heart failure induced by pressure overload are most commonly used for the investigation of the pathogenesis of hypertrophy and vascular changes on the subcellular level. These models are less suitable for the assessment of the effects of pharmacological interventions, because outflow obstruction or hypertension is an infrequent isolated cause of clinical heart failure.

Volume overloading

The creation of an arteriovenous shunt is an approach to produce a model for high output failure (table 2). Alternatively, heart failure induced by aortic valve insufficiency combined with abdominal aorta banding is applied in rabbits (Gilson et al., 1990). Natarajan (1979) has described an experimental model employing volume loading induced by an atrial septal defect in cats. Models exploiting arteriovenous shunts are characterized by a low systemic vascular resistance and a high cardiac output. In general models of volume overloading are most frequently used to study electrolyte- and hormonal changes in heart failure.

Table 1. *Animal models for congestive heart failure induced by pressure overloading.*

method	animal	reference
pulmonary artery banding	cats	Spann et al 1967.
		Bassett and Gelband 1973.
	lambs	Burrington 1978.
	pigs	Welham et al 1978.
	ponies	Manohar et al. 1981.
(supra)valvular aortic stenosis	dogs	Rogers et al. 1971.
		Copeland et al. 1974.
		Allard et al. 1979.
	rabbits	Leclerq et al. 1978.
mitral stenosis	dogs	Haddy et al. 1953
aortic constriction	rats	Mercadier et al. 1981.
		Desjardins et al. 1988.
	rabbits	Alexander et al. 1953.
		Leclercq et al. 1978.
		Gilson et al. 1990.
	dogs	Kleinman et al. 1978.
		Fujii et al. 1988.
	lambs	Burrington 1978.
	guinea pigs	Siri et al. 1989
renal hypertension	rats	Mercadier et al. 1981.
		Noma et al. 1988.
	dogs	Marcus et al. 1981.

Table 2. *Animal models for congestive heart failure induced by volume overloading.*

method	animal	reference
artrial septal defect	cats	Natarajan et al. 1979
aortic regurgitation	rabbit	Gilson et al. 1990
mitral regurgitation	dogs	Morias et al. 1957. Spratt et al. 1983.
arteriovenous shunts	rats	Flaim et al. 1979 Garcia and Diebold 1990a Garcia et al. 1990b
	dogs	Ferguson et al. 1954 Villarreal et al. 1989

Models based on myocardial dysfunction

Coronary occlusion

The most frequent used method of left ventricular failure, is induced by occlusion of a coronary artery (table 3). Coronary artery occlusion can be performed extravascular (ligation) or intravascular (diffuse embolisation or placing an occlusive device in a coronary artery). Coronary ligation is easy to perform in rats (Selye et al. 1960 and Tikkanen et al. 1987). Early mortality, however, ranges between 10% (Selye et al. 1960) and 50% (Pfeffer et al, 1979) within 24 hours after ligation. A further limitation of this model is that not all animals show histological evidence of a myocardial infarction (Pfeffer et al. , 1979). In dogs and pigs, coronary artery occlusion causes less early mortality after 1 hour. In addition, in larger animals, left ventricular and systemic hemodynamic parameters can be studied in more detail.

Diffuse left ventricular damage can be studied in models of coronary artery embolization (table 3).

Heart failure induced by coronary occlusion mimics reliably the hemodynamic derangements in the majority of patients with ischemic cardiomyopathy: decreased stroke volume, increased systemic vascular resistance and increased left ventricular end-diastolic

pressure, while mean arterial blood pressure often will not be affected. Furthermore, secondary neurohormonal changes are very similar to the clinical situation (Anand et al, 1989). In animal models of coronary artery occlusion, however preexisting coronary lesions are absent, and left ventricular failure induced acutely, while in man myocardial lesions develop over a longer period. Consequently, coronary occlusion in animals causes in most causes either cardiogenic shock or changes fully compensating for systemic effects occur. An advantage is that in this model peripheral reflexes remain intact. Because of the latter this model is frequently used for the evaluation of new therapeutic compounds.

Table 3. Animal models for congestive heart failure induced by coronary occlusion.

method	animal	reference
coronary ligation	rats	Selye et al. 1960.
		Pfeffer et al. 1979.
		Tikkanen et al. 1987.
		Tikkanen et al. 1990.
	dogs	Stramba-Badiale et al.1991.
	pigs	Brooks et al. 1977.
		van der Giessen et al. 1989.
coronary embolism		
thrombogenic device	dogs	Kordenat et al. 1972.
sephadex beads	mini-pigs	Nutall et al. 1985.
microspheres	rats	Gorodetskaya et al. 1990.
	dogs	Reikerås et al. 1986.
		Sabbah et al. 1991.
liquid mercury	dogs	Chandraratna et al. 1973.
		Leddy et al. 1983.
thrombus formation	dog	Salazar 1961.

Atrial and ventricular pacing

A popular model for heart failure uses ventricular pacing in dogs (table 4). Ventricular pacing at a frequency between 220 and 260 beats/min for a period up to 8 weeks produces symptoms of heart failure within 7 days (Riegger et al., 1984), which consist of enlargement of both ventricles and increased pulmonary capillary and pulmonary artery pressure. After 10-20 days of pacing, these changes become irreversible. As in the coronary artery ligation model, peripheral responses are maintained. Sudden deaths do occur although less than in the coronary artery ligation models. A disadvantage is that if pacing is stopped, hemodynamic parameters remain unstable for several days (Riegger et al., 1982 and Fitzpatrick et al., 1989). Therefore, studying the hemodynamic responses to drugs does not yield reproducible results in the ventricular pacing model. It is, however well suited to study neurohumoral adaptations in the early or chronic phase of heart failure.

Table 4. Animal models for congestive heart failure induced by pacing.

method	animal	reference
atrial pacing	pigs	Spinale et al. 1990.
ventricular pacing	dogs	Coleman et al. 1971.
		Riegger and Liebau, 1982.
		Armstrong et al. 1986.
		Moe et al. 1990.
		Riegger et al. 1984.
		Sasayama et al. 1991.
		Wilson et al. 1987.
	pigs	Chow et al. 1990.
	sheeps	Fitzpatrick et al. 1989.

Non-ischemic cardiomyophathy

Several models for cardiomyopathy are available, either hereditary or induced by infection, irradiation or drugs (table 5). The Syrian hamster is well known for an autosomal recessive gene causing cardiomyopathy in some strains, which is maintained

Table 5. *Animal models for congestive heart failure induced by non-ischemic myocardial disease.*

method	animal	reference
heriditary cardiomyopathy	hamsters	Opie, 1966. Capasso et al. 1989. Sen et al. 1990.
round heart disease	turkeys	Einzig et al. 1980.
Trypanosoma cruzi	mouses	Morris et al. 1989.
Brisket disease	cows	Will et al. 1962. Hecht et al. 1962.
adriamycin	rats rabbits	Mettler et al. 1977. Arnolda et al. 1985. Langton et al. 1989. Wanless et all. 1987.
doxorubicin	rabbits	Doherty and Cobbe, 1990. Shenasa et al. 1990.
epirubicin	rabbits	Elisson and Björkman,1988.
monocrotaline	rats	Kay et al. 1967. Ceconi et al. 1989. Pelá et al. 1990.
taurine deficiency	cats	Pion et al. 1987.
vitamin E deficiency	rats	Fedelesova et al. 1971.
irradiation	dogs	Stone et al. 1964. Gillette et al. 1985. McChesney et al. 1988.
repetitive transmyocardial DC shock	dog	Mehta et al. 1978. Carlyle and Cohn 1983. Carlyle and Cohn 1990.

in the colony by selective breeding (Bajusz et al., 1969).
Especially anticancer agents may induce chronic low output cardiac failure. In rabbits, adriamycin (Arnolda et al., 1985, Langton et al., 1989 and Wanless et al., 1987), doxorubicin (Doherty and Cobbe, 1990 and Shenasa et al., 1990) and epirubicin (Elisson and Björkman, 1988) have been used to induce heart failure. For instance, after 8 weeks of twice weekly treatment with adriamicin i.v. (1 mg/kg) Wanless (1987) and Arnolda (1985) reported That cardiac output had decreased and systemic vascular resistance had increased.
Thoracic irradiation of adult beagle dogs produces an increase in left ventricular wall thickness and heart rate and a decrease in left ventricular ejection fraction (McChesney et al., 1988).
Heart failure can also be accomplished by specific nutritional deficiencies. Pion (1987) described a reversible model for heart failure caused by taurine deficiency in cats, while in rats a model has been described using vitamin E deficiency (Fedelesova et al., 1971).
Destruction of myocardial tissue by repeated DC-countershock induces low output failure in dogs. Methods using irradiation or DC-countershock are however limited by the damage induced to other organs (lungs, pericardium) and their tendency to exhibit conduction-defects (AV-block). Methods for producing heart failure by non-ischemic myocyte damage are mainly used in the evaluation of subcellular pathological processes and are less suitable for the study of pharmacological interventions.

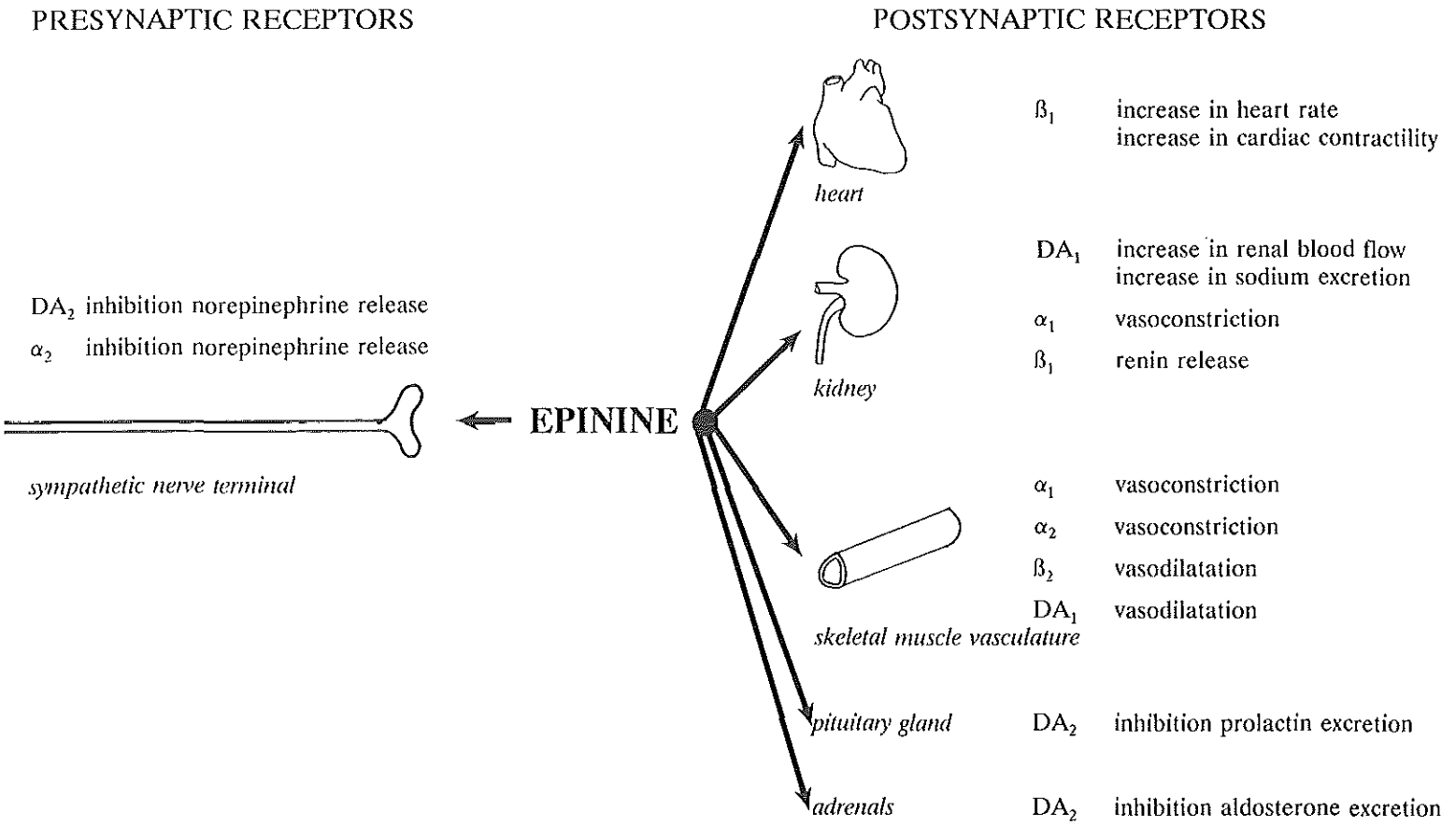
Even a larger number of models for heart failure are available, than shown above. Each model may have some use in heart failure research. None of these models is, however capable to reproduce all the clinical characteristics of heart failure in man.

Aim of the study

In this thesis we first investigated the cardiovascular actions of several classes of drugs in conscious pigs in which heart failure was induced by occluding a coronary artery 3-4 weeks before the studies were performed and compared the results to those observed in normal conscious animals. Positive inotropic agents and vasodilators are frequently used to treat heart failure as they may improve the depressed myocardial contractility and lower the elevated systemic vascular resistance. In chapter 2 and 3 the effects of the 1,4-dihydropyridine derivative calcium antagonists nisoldipine and elgodipine are studied because of their capability to unload the heart by reducing systemic vascular resistance in normal anesthetized pigs (Duncker et al., 1986 and Sassen et al., 1990) and by

increasing cardiac output in normal conscious pigs (Duncker et al., 1987).
To study the potential advantage of vasodilators with positive inotropic properties to vasodilators alone we also investigated the actions of the phosphodiesterase inhibitor pimobendan with nisoldipine in the porcine model for heart failure (Chapter 4).
In chapter 5 we describe the effects of a novel class of drugs which causes systemic vasodilation by potassium channel activation. The results of bimakalim are compared to the nicotinamide derivative nicorandil, which in addition to their nitrate-like properties, also possess potassium channel activator properties (Taira, 1989).
One of the most prominent drugs in the treatment of ischaemic heart disease is nitroglycerin, despite the frequent occurrence of tolerance. In chapter 6 we first evaluated 5 novel nitrate-esters in normal conscious pigs. In the second part nitroglycerin and one of the novel compounds, CEDO 8956, were selected for further study of distribution of cardiac output in conscious pigs with chronic coronary artery occlusion.
Tachycardia is an unwanted situation, especially in patients with coronary artery disease. Selective bradycardic agents, which exert an anti-ischemic effect because of their ability to reduce oxygen demand and improve oxygen supply of the myocardium and especially of the subendocardial layers (Schamhardt et al., 1981, Krumpl et al., 1988 and Indolfi et al., 1989), may thus have some benefit in patients with left ventricular dysfunction, provided that the negative chronotropic properties are not accompanied by negative inotropic properties. We therefore studied the effects of UL-FS 49, a specific bradycardic agent and compared the results to those of the non-selective beta-adrenoceptor antagonist propranolol.
Dopaminergic drugs have also shown some promise in the treatment of heart failure in particular because of their ability to improve renal blood flow. Until recently treatment with this class of drugs was limited by their poor absorption. Recently a novel orally active dopamine-like drug, ibopamine, which is itself pharmacologically inactive, but is rapidly hydrolysed to epinine (N-methyldopamine). Epinine is, however not only a non-selective and non-specific dopamine agonist, but also stimulates alpha- and beta-adrenoceptors (Randolph et al., 1983 and Pochiari et al., 1986). The effects of epinine are summarized in figure 2. Epinine may thus exert a positive inotropic effect by activating $ß_1$ and α_1 adrenoceptors and possibly DA_1 receptors (Zhao et al., 1990). Vasodilation may occur because of activation of DA_1, DA_2 and $ß_2$ receptors, whereas both α_1 and α_2 adrenoceptors could cause an unwanted vasoconstriction. Finally the DA_1 and DA_2 receptors may cause natriuresis. The presence of dopamine receptors in pigs has been well established in a number of vascular beds, but its role, if any, in the coronary circulation and myocardium is unknown. We therefore first studied the role of these receptors in the heart, using intravenous infusions of dopamine and intracoronairy

Figure 2. *Effects of epinine.*



infusions of the specific DA_1 agonist fenoldopam. These experiments were performed in anaesthetized animals (chapter 8). In chapter 9 and 10 we describe the effects of epinine on systemic hemodynamics and regional blood flows in conscious pigs with an intact coronary circulation. In chapter 11 the acute systemic hemodynamic effects of dopamine and dobutamine were studied in conscious pigs with and without left ventricular dysfunction.

Finally in chapter 12 we describe the effect of premedication, which is most frequently used in pigs, on the regional distribution of cardiac output. Both the quantitative and the qualitative effects of these agents were a major reason to perform most studies in conscious animals.

Refenrences.

Alexander N, Edmondson HA and Druny DR. The production of experimental congestive heart failure in rabbits. *Circ Res* 1; 491-498, 1953.

Allard JR, O'Niell MJ and Hoffman JIE. Valvular subcoronairy aortic stenosis in dogs. *Am J Physiol* 236 (*Heart Circ Physiol* 5); H780-784, 1979.

Anand IS, Ferrari R, Kalra GS, Wahi PL, Poole-Wilson PA and Harris PC. Edema of cardiac origin; Studies of body water and sodium, renal function, hemodyanmic indexes, and plasma hormones in untreated congestive cardiac failure. *Circulation* 89; 299-305, 1989.

Armstrong PW, Stopps TP, Ford SE, de Bold AJ. Rapid ventricular pacing in the dog: pathophysiologic studies of heart failure. *Circulation* 74; 1075-1084, 1986.

Arnolda L, McGrath B, Cocks M, Sumithran E and Johnston C. Adriamycin cardiomy opathy in the rabbit: an animal model of low output cardiac failure with activation of vasoconstrictot mechanisms. *Cardiovasc Res* 19; 378-382, 1985.

Bajusz B, Baker JR, Nixon CW and Homburger F. Spontaneous hereditary myocardial degeneration and congestive failure in a strain of Syrian hamster. *Ann NY Acad Sci* 156; 105-129, 1969.

Bassett AL and Gelband H. Chronic partial occlusion of the pulmonary artery in cats. *Circ Res* 32; 15-26, 1973.

Brooks H, Holland R and Al-Sadir J. Right ventricular performance during ischemia: an anatomic and hemodynamic analysis. *Am J Physiol* 233 (*Heart Circ Physiol* 2); H500-513, 1977.

Burrington JD. Response to experimental coarctation of aorta and pulmonic stenosis in fetal lambs. *J Thorac Cardiovasc Surg* 75; 819-826, 1978.

Capasso J, Olivetti G and Anversa P. Mechanical en electrical properties of

cardiomyopathic heart of Syrian hamsters. *Am J Physiol* 257 (*Heart Circ Physiol* 26); H 1836-1842, 1989.

Carlyle PF and Cohn JA. A nonsurgical canine model of chronic left ventricular myocardial dysfunction. *Am J Physiol* 244 (*Heart Circ Physiol* 13); H769-774, 1983.

Carlyle PF and Cohn JA. Systemic and regional hemodynamic effects of alpha-adrenoceptor blockade in chronic left ventricular dysfunction in conscious dog. *Am Heart J* 120; 619-624, 1990.

Ceconi C, Condorelli E, Quinzanini M, Rodella A, Ferrari R and Harris P. Noradrenaline, atrial natriuretic peptide, bombesin and neurotensin in myocardium and blood of rats in congestive cardiac failure. *Cardiovasc Res* 23; 674-682, 1989.

Chandraratna PAN, Oleksyn TW, Schenk EA and Cohen J. Coronary embolization with metallic mercury. *Cardiovasc Res* 7; 614-622, 1973.

Chow E, Woodard JC and Farrar DJ. Rapid ventricular pacing in pigs : an experimental model of congestive heart failure. *Am J Physiol* 258 (*Heart Circ Physiol* 27); H1603-1605, 1990.

Coleman HN, Taylor RR, Pool PE, Whipple GH, Covell JW, Ross J and Braunwald E. Congestive heart failure following chronic tachycardia. *Am Heart J* 81; 790-798, 1971.

Copeland JG, Maron BJ, Luka NL, Ferrans VJ and Michaelis LL. Experimental production of aortic valvular stenosis: short-term and long-term studies in dogs. *J Thorac Cardiovasc Surg* 67; 371-379, 1974.

Desjardins S, Mueller RW and Cauchy MJ. A pressure overlaod model of congestive heart failure in rats. *Cardiovasc Res* 22; 696-702, 1988.

Doherty JD and Cobbe SM. Electrophysiological changes in animal model of chronic cardiac failure. *Cardiovasc Res* 24; 309-316, 1990.

Duncker DJ, Hartog JM, Hugenholtz PG, Saxena PR and Verdouw PD. The effects of nisoldipine (Bay K 5552) on cardiovascular performance and regionalblood flow in pentobarbital-anaesthetized pigs with or without ß-adrenoceptor blockade. *Br J Pharmacol* 88; 9-18, 1986.

Duncker DJ, Saxena PR and Verdouw PD. The effects of nisoldipine alone and in combination with beta-adrenoceptor blockade on systemic haemodynamics and myocardial performance in conscious pigs. *Eur Heart J* 8; 1332-1339, 1987.

Einzig S, Staley NA, Mettler E, Nicoloff DM and Noren GR. Regional myocardial blood flow and cardiac function in a naturally occurring congestive cardiomyopathy of turkeys. *Cardiovasc Res* 14; 396-407, 1980.

Elisson LO and Björkman S. Congestive heart failure in rabbits after a single intrapleural administration of a low dose of doxorubicin or epirubicin. *Pharmacol & Toxicol* 62; 84-89, 1988.

Fedelesova M, Sulakhe PV, Yates JC and Dhalla NS. Biochemical basis of heart function. IV. Energy metabolism and calcium transport in hearts of vitamin E deficient rats. *Can J Physiol Pharmacol* 49; 909-918, 1971.

Ferguson TB, Gregg DE and Shadle OW. Effect of blood and saline infusion on cardiac performance in normal dogs and dogs with arteriovenous fistulas. *Circ Res* 2; 565-572, 1954.

Fitzpatrick MA, Nicholls MG, Espiner EA, Ikram H, Bagshaw P and Yandle TG. Neurohumoral changes during onset and ofset of ovine heart failure: role of ANP. *Am J Physiol* 256 (*Heart Circ Physiol* 25); H 1052-1059, 1989.

Flaim SF, Minteer WJ, Nellis SH and Clark DP. Chronic arteriovenous shunts: evaluation of a model for heart failure in rat. *Am J Physiol* 258 (*Heart Circ Physiol* 27); H 634-641, 1990.

Fujii AM, Gelpi RJ, Mirsky I and Vatner SF. Systolic and diastolic dysfunction during atrial pacing in conscious dogs with left ventricular hypertrophy. *Circ Res* 62; 462-470, 1988.

Garcia R and Diebold S. Simple, rapid and effective mothod of producing aortocaval shunts in the rat. *Cardiovasc Res* 24; 430-432, 1990.

Garcia R, Lachance D and Thibault G. Atrial natriuretic factor release and natriuresis in rats with high-output heart failure. *Am J Physiol* 259 (*Heart Circ Physiol* 28); H1374-1379, 1990.

van der Giessen WJ, van Woerkens LJ, Duncker DJ, Roelandt JRTC and Verdouw PD. Acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J Cardiovasc Pharmacol* 14; 653-658, 1989.

Gillette EL, McChesney SL and Hoopes PJ. Isoeffect curves for radiation induced cardiomyopathy in dogs. *Int J Radiat Oncol Biol Phys* 11; 2091-2097, 1985.

Gilson N, el Houda Bouanani N, Corin A and Crozatier B. Left ventricular function and ß-adrenoceptors in rabbit failing heart. *Am J Physiol* 258 (*Heart Circ Physiol* 27); H634-641, 1990.

Gorodetskaya EA, Dugin SF, Medvedev OS and Allabergenova AE. Simple method to produce acute heart failure by coronary vessel embolization in closed chest rats with microspheres. *J Pharmacol Methods* 24; 43-51, 1990.

Haddy FJ, Ferrin AL, Hannon DW, Alden JF, Adams WL and Baronofsky ID. Cardiac function in experimental mitral stenosis. *Circ Res* 1; 219-225, 1953.

Harris P. Congestive cardiac failure: central role of arterial blood pressure. *Br Heart J* 58; 190-203, 1987.

Hecht HH, Kuida H, Lange RL, Thorne JL and Brown AM. Brisket disease; clinical features and hemodynamic observations in altitude-dependent right heart failure of

cattle. *Am J Med* 32; 171-183, 1962.

Indolfi C, Guth BD, Miura T, Miyazaki S, Schultz R and Ross J. Mechanisms of improved ischemic regional dysfunction by bradycardia. *Circulation* 80; 983-993, 1989.

Kannel WB and Belanger AJ. Epidemiology of heart failure. *Am Heart J* 121; 951-957, 1991.

Kannel WB and Cupples A. Epidemiology and risk profile of cardiac failure. *Cardiovasc Drugs Ther* 2; 387-395, 1988.

Kay JM, Harris P and Heath D. Pulmonary hypertension in rats produced by the ingestion of Crolalaria spectabilis seeds. *Thorax* 22; 176-9, 1967.

Kleinman LH, Wichsler AS, Rembert JC, Fedor JM and Greenfield JC. A reproducible model of moderate to severe concentric left ventricular hypertrophy. *Am J Physiol* 234 (*Heart Circ Physiol* 3); H515-519, 1978.

Kordenat RK, Kezdi P and Stanley EL. A new catheter technique for producing experimental coronary thrombosis and selective coronary visualization. *Am Heart J* 83; 360-364, 1972.

Krumpl G, Winkler M, Schneider W and Raberger G. Comparison of the haemodynamic effects of the selective bradycardic agents UL-FS 49, with those of propranolol during treadmill excercise. *Br J Pharmacol* 94; 55-64, 1988.

Langton D, Jover BF, Trigg L, Fullerton M, Blake DW and McGrath BP. Regional distribution of the cardiac output and renal responses to atrial natriuretic peptide infusion in rabbits with congestive heart failure. *Clin Exp Pharmacol Physiol* 16; 939-951, 1989.

Leclercq JF, Sebag C and Swynghedauw B. Experimental cardiac hypertrophy in rabbits after aortic stenosis or incompetence or both. *Biomedicine* 28; 180-184, 1978.

Leddy CL, Wilen M and Franciosa JA. Effects of a new angiotensin converting enzyme inhibitor, enalapril, in acute and chronic left ventricular failure in dogs. *J Clin Pharmacol* 23; 189-198, 1983.

Manohar M, Bisgard GE, Bullard V and Rankin JHG. Blood flow in the hypertrophied right ventricular myocardium of unaneasthetized ponies. *Am J Physiol* 240 (*Heart Circ Physiol* 9); H881-888, 1981.

Marcus ML, Mueller TM and Eastham CL. Effects of short- and long term left ventricular hypertrophy on coronary circulation. *Am J Physiol* 241 (*Heart Circ Physiol* 10); H358-362, 1981.

McChesney SL, Gillette EL and Orton EC. Canine cardiomyopathy after whole heart and partial lung irradiation. *J Radiation Oncology Biol Phys* 14; 1169-1174, 1988.

McKee PA, Castelli WP, McNamara PM and Kannel WB. The natural history of congestive heart failure: The Framingham study. *N Eng J Med* 285; 1441-1446, 1971.

Mehth J, Runge W, Cohn JN and Carlyle P. Myocardial damage after repetitive current shock in the dog: correlation between left ventricular end-diastolic perssure and extent of myocardial necrosis. *J lab Clin Med* 91; 272-279, 1978.

Mercadier JJ, Lompe AM, Wisnewski C, Samuel JL, Bercovici J, Swynghedauw B and Schwartz K. Myosin isoenzyme changes in serveral models of rat cardiac hypertrophy. *Circ Res* 49; 525-532, 1981.

Mettler FP, Young DM and Ward JM. Adriamycin-induced cardiotoxicity (cardiomyopathy and congestive heart failure) in rats. *Cancer Res* 37; 2705-2713, 1977.

Moe GW, Angus C, Howard RJ, de Bold AJ. and Armstrong P.W. Pathophysiological role of changing atrial size and pressure in modulation of atrial natriuretic factor during evolving experimental heart failure. *Cardiovasc Res* 24; 570-577, 1990.

Morais DJ, Richart TS, Fritz AJ, Acree PW, Davila JC and Glover RP. The production of chronic experimental mitral insufficiency. *Ann Surg* 145; 500-508, 1957.

Morris SA, Weiss LM, Factor S, Bilezikian JP, Tanowitz H and Wittner M. Verapamil ameliorates clinical, pathologic and biochemical manifestations of experimental chagasic cardiomyopathy in mice. *JACC* 14; 782-789, 1989.

Natarajan G, Carey RA, Coulson RL, Bove AA and Spann JF. A new technique for production of chronic atrial septal defect in cat without thoracotomy. *Biol and Med* 616; 515-518, 1979.

Noma K, Brändle M and Jacob R. Evaluation of left ventricular function in an experimental model of congestive heart failure due to combined pressure and volume overload. *Basic Res Cardiol* 83; 58-64, 1988

Nutall A, Smith HJ and Loveday BE. A clinically relevant model of heart failure: effects of ticlopidine. *Cardiovasc Res* 19; 187-192, 1985.

Opie LH. The heart muscle in muscular dystrophy with special reference to involvement of the cardiovascular system in the hereditary myopathy of the hamster. *Ann NY Acad Sci* 138; 213-229, 1966.

Pelá G, Missale C, Raddino R, Condorelli E, Spano PF and Visioli O. β_1- and β_2-receptors are diffentially desensitized in an experimental model of heart failure. *J Cardiovasc Pharmacol* 16; 839-846, 1990.

Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA and Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ Res* 44; 503-512, 1979.

Pion PD, Kittleson MD, Rogers QR and Morris JG. Myocardial failure in cats associated with low plasma taurine: A reversible cardiomyopathy. *Science* 237; 764-768, 1987.

Pocchiari F, Pataccini R, Castelnovo P, Longo A, Paro M and Casagrande C. Ibopamine,

an orally active dopamine-like drug: Metabolism and pharmacokinetics in dogs. *Arzneim Forsch* 36; 341-344, 1986.

Randolph WC, Swagzdiz JE, Joseph GL and Gifford R. Circulating levels of epinine and epinine conjugates in rat, dog and man following oral administration of ibopamine. *Pharmacologist* 25; 117, 1983.

Reikerås O, Gunnes P, Sørlie D, Ekroth R and Mjøs OD. Hemodynamic and metabolic effects of dopamine and insulin during acute left ventricular failure in dogs. *J Cardiovasc Pharmacol* 8; 303-308, 1986.

Riegger AJG and Liebau G. The renin-angiotensin-aldosteron system, antidiuretic hormone and sympathetic nerve activity in an experimental model of congestive heart failure in the dog. *Clin Sci* 62; 465-469, 1982.

Riegger AJG, Liebau G, Holzschuh M, Witkowski D, Steilner H and Kochsiek K. Role of the renin-angiotensin system in the development of congestive heart failure in the dog as assessed by chronic converting-enzyme blockade. *Am J Cardiol* 53; 614-618, 1984.

Rogers WA, Bishop SP and Hamlim RL. Experimental production at supravalvular aorta stenosis in dogs. *J Appl Physiol* 30; 917-920, 1971.

Sabbah HN, Stein PD, Kono T, Gheorghiade M, Levine TB, Jafri S, Hawkins ET and Goldstein S. A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *Am J Physiol* 260 (*Heart Circ Physiol* 29); H1379-1384, 1991.

Salazar AE Experimental myocardial infarction: Induction of coronary thrombosis in the intact closed-chest dog. *Circ Res* 9; 1351-1356, 1961.

Sasayama S, Asanoi H and Ishizaka S. Mechanics of contraction and relaxation of the ventricle in experimental heart failure produced by rapid ventricular pacing in the conscious dog. *Eur Heart J* 12 (suppl.C); 35-41, 1991.

Sassen LMA, Soei LK, Koning MMG and Verdouw PD. The central and regional cardiovascular responses to intravenous and intracoronary administration of the phenyldihydropyridine elgodipine in anaesthetized pigs. *Br J Pharmacol* 99; 355 363, 1990.

Schamhardt HC, Verdouw PD and Saxena PR. Improvement of perfusion and function of ischaemic porcine myocardium after reduction of heart rate by alinidine. *J Cardiovasc Pharmacol* 3; 728-738, 1981.

Selye H, Bajusz E, Grasso S and Mendell P. Simple techniques for surgical occlusion of coronary vessels in the rat. *Angiology* 11; 398-407, 1960.

Sen L, O'Niell M, Marsh JD and Smith TW. Myocyte structure, function, and calcium kinetics in the cardiomyopathic hamster heart. *Am J Physiol* 259 (*Heart Circ Physiol*

28); H 1533-1543, 1990.

Shenassa H, Calderone A, Vermeulen M, Paradis P, Stephens H, Cardinal R, de Champlain J and Rouleau JL. Chronic doxorubicin induced cardiomyopathy in rabbits: mechanical, intracellular action potential, and ß adrenergic characteristics of the failing myocardium. *Cardiovasc Res* 24; 591-604, 1990.

Siri FM, Nordin C, Factor SM, Sonnenblick E and Aronson R. Compensatory hypertrophy and failure in gradual pressure-overloaded guinea pig heart. *Am J Physiol* 257 (*Heart Circ Physiol* 26); H1016-1024, 1989.

Smith HJ and Nutall A. Experimental models of heart failure. *Cardiovasc Res* 19; 181-186, 1985.

Spann JF, Bucceno RA, Sonnenblick EH and Braunwald E. Contractile state of cardiac mucle obtained from cats with experimental produced ventricular hypertrophy and heart failure. *Circ Res* 21; 341-354, 1967.

Spinale FG, Hendrick DA, Crawford FA and Carabello BA. Relationship between bioimpedance, thermodilution, and ventriculographic measurements in experimental congestive heart failure. *Cardiovasc Res* 24; 423-429, 1990.

Spratt JA, Olsen CO, Tyson GS, Glower DD, Davis JW and Rankin JS. Experimental regurgitation; Physiological effects of correction on left ventricular dynamics. *J Thorac Cardiovasc Surg* 86; 479-489, 1983.

Stone HL, Bishop VS and Guyton AC. Progressive changes in cardiovascular function after unilateral heart irradition. *Am J Physiol* 206; 289-293, 1964.

Stramba-Badiale M, Vanoli E, de Ferrari GM, Cerati D, Foreman RD and Schwartz PJ. Sympathetic-parasympathetic interaction and accentuated antagonism in conscious dogs. *Am J Physiol* 260 (*Heart Circ Physiol* 29); H335-H340, 1991.

Sutton GC Epidemiologic aspects of heart failure. *Am Heart J* 120; 1538-1540. 1990.

Taira N. Nicorandil as a hybrid bewteen nitrates and potassium channel activators. *Am J Cardiol* 63; 18J-24J, 1989.

Tikkanen T, Helin K, Tikkanen T, Sybertz EJ, Vemulapalli S, Sariola H, Näveri H and Fyrquist F. Elevation of plasma atrial natriuretic peptide in rats with chronic heart failure by SCH 39370, a neutral metalloendopeptidase inhibitor. *J Pharmacol Exp Ther* 254; 641-645, 1990.

Tikkanen T, Svartström-Fraser M, Tikkanen I, Sariola H and Fyhrquist F. Haemodynamic effects of atrial natriuretic peptide in rats with heart failure. *Eur J Pharmacol* 140; 187-193, 1987.

Villarreal D, Freeman RH and Brands MW. DOCA administration and artial natriuretic factor in dogs with chronic heart failure. *Am J Physiol* 257 (*Heart Circ Physiol* 26); H739-745, 1989.

Wanless RB, Anand IS, Poole-Wilson PA and Harris P. An experimental model of chronic cardiac failure using adriamycin in the rabbit: central haemodynamics and regional blood flow. *Cardiovasc Res* 21; 7-13, 1987.

Welham KC, Silove ED and Wyse RKH. Experimental right ventricular hypertrophy and failure in swine. *Cardiovasc Res* 12; 61-65, 1978.

Will DH, Alexander AF, Reeves JT and Grover RF. High altitude-induced pulmonary hypertension in normal cattle. *Circ Res* 10; 172-177, 1962.

Wilson JR, Douglas P, Hickey WF, Lanoce V, Ferraro N, Muhammad A and Reichek N. Experimental congestive heart failure produced by rapid ventricular pacing in dog: cardiac effects. *Circulation* 75; 857-867, 1987.

Zhao R, Fennell WH and Abel FL. Effects of dopamine D_1 and dopamine D_2 receptor agonists on coronary and peripheral hemodynamics. *Eur J Pharmacol* 190; 193-202, 1990.

Chapter 2

Nisoldipine improves blood flow to skeletal muscles in conscious pigs with chronic heart failure.

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Nisoldipine improves blood flow to skeletal muscles in conscious pigs with chronic heart failure

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KEY WORDS: Heart failure, myocardial infarction, systemic haemodynamics, regional blood flows, coronary collateral flow, nisoldipine, pigs.

We studied the acute effects of the calcium antagonist nisoldipine in 10 conscious pigs with chronic heart failure. Left ventricular dysfunction was induced by permanent ligation of the left circumflex coronary artery. Two to three weeks after myocardial infarction the effects of four consecutive 10 min intravenous infusions of nisoldipine (0·05; 0·1; 0·25 and 0·5 $\mu g\ kg^{-1}\ min^{-1}$) or its solvent on systemic haemodynamics were evaluated. In addition, we used the radioactive microsphere technique to study the distribution of cardiac output after each dose of nisoldipine. Nisoldipine significantly ($P<0·05$) increased heart rate (from 144 ± 9 to 161 ± 8 beats min^{-1}), cardiac output (from $2·1\pm0·1$ to $2·9\pm0·2$ l min^{-1}), stroke volume (from 14 ± 1 to 18 ± 1 ml) and left ventricular dP/dt_{max} (from 2600 ± 100 to 3500 ± 250 mmHg s^{-1}), but had no effect on arterial blood pressure. Left ventricular end-diastolic pressure (from 19 ± 2 to 16 ± 1 mmHg) and systemic vascular resistance (from 52 ± 3 to 37 ± 3 mmHg min l^{-1}) decreased after nisoldipine. The nisoldipine-induced increase in cardiac output did not affect blood flow to the kidneys, brain, liver or skin, but perfusion of the stomach (84%), adrenals (84%) and normal myocardium (from 200 ± 25 to 321 ± 38 ml min^{-1} 100 g^{-1}) as well as infarcted myocardium (from 41 ± 8 to 61 ± 19 ml min^{-1} 100 g^{-1}) increased significantly. The largest fraction of the increase in cardiac output was, however, used for the improvement of skeletal muscle blood flow (from 13 ± 5 to 62 ± 13 ml min^{-1} 100 g^{-1}). It is concluded that nisoldipine increases cardiac output in conscious pigs with heart failure, primarily by decreasing peripheral vascular resistance. The increment in cardiac output is distributed preferentially to the skeletal muscles.

Introduction

In congestive heart failure, although blood flow to skeletal muscle may be normal at rest[1–3], increases are less than in normal subjects during exercise[2,4]. For this last observation both the reduction in cardiac output and the inability of peripheral conductance vessels to dilate to appropriate stimuli have been held responsible[4,5]. An improvement of cardiac output, however, does not necessarily imply that regional blood flows to all tissues improve to the same extent. For instance, in patients who undergo a heart transplantation the improvement in systemic haemodynamics is not accompanied by parallel changes in limb blood flow[6]. The same phenomenon appears to play a role during muscle deconditioning by immobilization[7].

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Some vasodilating drugs, such as nitrates, improve systemic haemodynamics in heart failure, but there may not be an increase in exercise tolerance because the skin benefits more from the improved cardiac output than the skeletal muscles do[8]. Recent studies with ACE-inhibitors suggest that improvement in skeletal muscle blood flow, which is only achieved with chronic treatment, is accompanied by an increase in exercise tolerance[9]. The first generation calcium antagonists are also potent vasodilators, but considerable reservation exists about their use when left ventricular function is depressed, because of suspected negative inotropic properties. Nisoldipine, however, exerts in vitro a 4–10 times stronger vasodilatory and 4–10 times weaker cardiodepressant action compared with equimolar doses of nifedipine[10]. Indeed, in a number of in vivo experiments, the negative inotropic actions of nisoldipine have been shown to be negligible[10,11]. In anaesthetized pigs with a normal

left ventricular function, nisoldipine exerts a potent vasodilatory action on several organs and tissues including skeletal muscle[12]. Regional blood flows in anaesthetized animals may, however, differ due to a redistribution of cardiac output in favour of the arteriovenous anastomoses opened by the anaesthesia[13] and which are abundantly present in the skin[14,15]. In order to circumvent possible interactions of anaesthetic agents on regional blood flows, we evaluated the acute effects of nisoldipine on systemic haemodynamics and distribution of cardiac output in conscious pigs with chronic heart failure induced by coronary artery ligation.

Materials and methods

SURGICAL PROCEDURE

After an overnight fast, 10 Yorkshire pigs (18–22 kg), pretreated with 600 mg of a mixture of procaine penicillin-G and benzathine penicillin-G intramuscularly (Duplocillin, Gist-Brocades NV, Delft, The Netherlands), were sedated with an intramuscular injection of 30 mg kg^{-1} ketamine HCl (Aeskoket, Aesculaap BV, Boxtel, The Netherlands). After endotracheal intubation the animals were connected to a respirator for mechanical ventilation with a mixture of oxygen and nitrous oxide (1:2) to which 1–4% (v/v) enflurane was added. Under sterile conditions a jugular vein and a common carotid artery were cannulated for infusion of drugs or solvent and measurement of arterial blood pressure, respectively. The chest was opened via the third left intercostal space and an electromagnetic flow probe (13–15 mm; Skalar, Delft, The Netherlands) was positioned around the ascending aorta for the measurement of aortic blood flow. The heart was exposed via the fifth intercostal space. A tip manometer pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) 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. A second left atrial cannula was used for the injection of radioactively labelled microspheres (15 ± 1 μm; ^{141}Ce, ^{113}Sn, ^{103}Ru, ^{95}Nb, ^{46}Sc; NEN Chemicals GmbH, Dreieich, F.R.G.).

Thereafter, the proximal segment of the left circumflex coronary artery (LCX) was ligated permanently for the induction of a myocardial infarction. After 30 min, during which serious ventricular arrhythmias (sustained ventricular tachycardia or ventricular fibrillation) were treated with immediate DC countershock, the chest was closed. Catheters and wires were tunnelled subcutaneously to the back, and the animals were allowed to recover from surgery.

The animals received 500 mg amoxicilline (Flemoxin, Gist-Brocades NV, Delft, The Netherlands) daily, while the catheters were flushed with heparinized saline solution. During the first 14 postoperative days the animals were adapted to the laboratory facilities, while systemic haemodynamic parameters were monitored. The experimental protocol was executed when systemic haemodynamics remained stable for at least 90 min, usually 2–3 weeks after induction of the myocardial infarction. All measurements were done while the animals were quietly resting in a constraining jacket.

EXPERIMENTAL PROTOCOL

Four consecutive 10 min intravenous infusions with incremental dosages of nisoldipine (0·05, 0·1, 0·25 and 0·5 μg kg^{-1} min^{-1}) were administered. At the end of each infusion period, recordings were made of heart rate, arterial blood pressure, aortic blood flow, left ventricular pressure and its first derivative (LVdP/dt; obtained by electronic differentiation). For a representative tracing, see Fig. 1. Thereafter, approximately 10^6 microspheres were injected into the left atrium, while a blood reference sample (8·8 ml min^{-1}) was withdrawn from the cannula in the aorta. The stability of the systemic haemodynamic condition of the animals was evaluated by infusion of the solvent of nisoldipine using identical infusion rates as with nisoldipine. The stability of the regional blood flows could not be evaluated during the solvent infusions, because of the limited number of different isotopes available. However, we have observed in conscious animals with normal left ventricular function that the solvent neither affected systemic haemodynamics nor regional blood flows (unpublished data from our laboratory). Furthermore, in our laboratory repeated blood flow measurements by the microsphere technique have been shown to have a variability of less than 6%[16].

After completion of the experiments, the animals were killed with an overdose of sodium pentobarbitone and several organs and tissues were dissected out. Both iliopsoas muscles were dissected as skeletal muscle. The heart was dissected after fixation in a 10% formaldehyde solution. The infarcted (fibrotic) area was separated from the non-infarcted

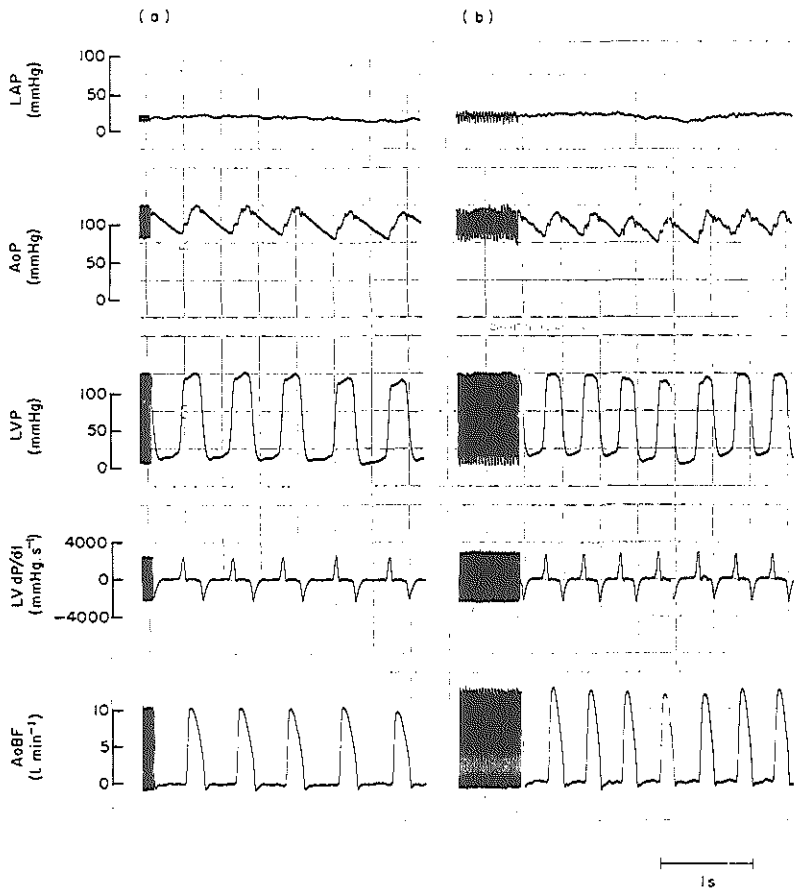

Figure 1 Representative tracing of simultaneously recorded haemodynamic parameters during baseline (a) and after the highest dose (0·5 μg kg^{-1} min^{-1}) of nisoldipine (b). From top to bottom are shown, left atrial pressure (LAP), arterial blood pressure (AoP), left ventricular pressure (LVP) and its first derivative (LVdP/dt) and aortic blood flow (AoBF).

area of the left ventricle, while the latter was divided in epi, meso- and endocardial layers of equal thickness for the measurement of regional myocardial blood flows and the ratio between the normalized endocardial and epicardial blood flow. Because the fibrotic segment could not be reliably divided into layers of equal thickness, we determined only transmural flow in the centre of this segment. All tissues were placed in plastic vials and the radioactivity in these vials was counted for 10 min in a gamma-scintillation counter (Packard, Minaxi autogamma 5000) using suitable windows for discriminating isotopes. Regional blood flows were analysed using specially developed computer programmes[17].

DRUGS

Nisoldipine (Bay K 5552; Bayer AG, Wuppertal, F.R.G.) was prepared from a stock solution (0·5 mg ml^{-1}, dissolved in polyethylene glycol 400, glycerol and water). The nisoldipine solution and the solvent

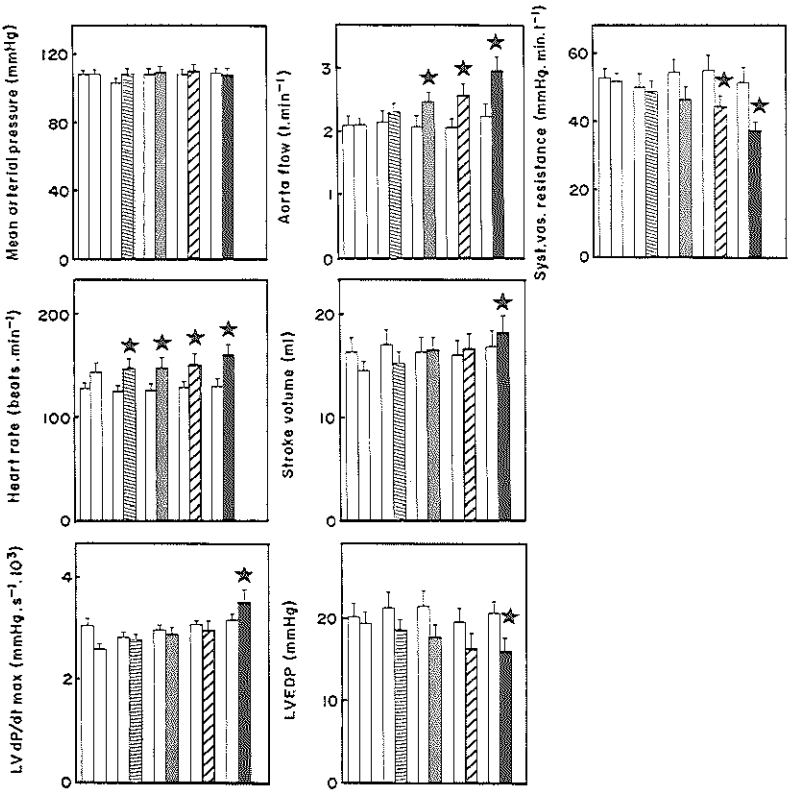

Figure 2 Systemic haemodynamic effects of four consecutive doses of nisoldipine (hatched bars) or its solvent (open bars) in conscious pigs with chronic heart failure. The set of open bars at the left side of each graph represent the pre-drug baseline values of the animals receiving the solvent (left) or nisoldipine (right). Nisoldipine □ 0, ▨ 50, ▩ 100, ▨ 250, ■ 500 ng kg^{-1} min^{-1}. Syst. vas. resistance = systemic vascular resistance; LVEDP = left ventricular end-diastolic pressure. *$P<0.05$, drug-induced changes statistically significant vs solvent.

were diluted with 0·9% w/v NaCl solution immediately before use and administered while protected from light.

STATISTICAL ANALYSIS

All data are presented as mean ± s.e. mean. The significance of the effects of nisoldipine or the solvent was evaluated by Duncan's new multiple-range test once an analysis of variance for one series of repeated measurements (or two series, where appropriate) had revealed that the samples represented different populations (random block design). A P value less than 0·05 was considered statistically significant.

Results

SYSTEMIC HAEMODYNAMICS

The solvent exerted no significant effect on any of the systemic haemodynamic parameters (Fig. 2) Nisoldipine raised cardiac output dose-dependently from 2·1 ± 0·1 to 2·9 to 0·21 min^{-1} ($P<0.05$) by increasing both heart rate (from 144 ± 9 to 161 ± 8 beats min^{-1}, 23%) and stroke volume (from 14 ± 1 to 18 ± 1 ml, 25%). Left ventricular dP/dt_{max} increased by 37% (from 2600 ± 100 to 3500 ± 250 mmHg s^{-1}). Mean arterial blood pressure did not change, which implies that systemic vascular resistance, calculated as the ratio of mean arterial blood pressure and

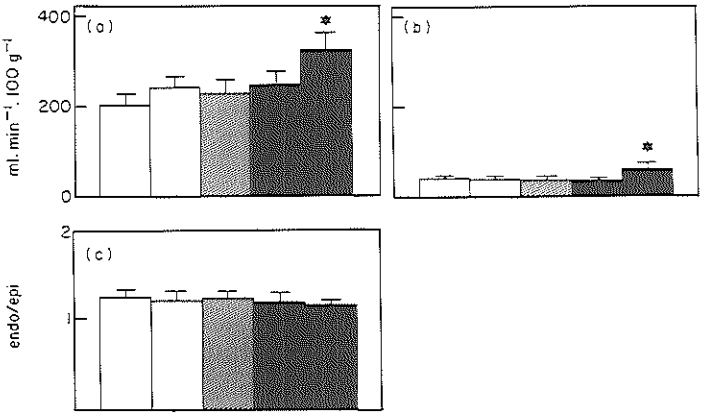

Figure 3 The effect of four consecutive doses of nisoldipine on myocardial blood flow in non-infarcted (a) and infarcted area (b) of left ventricle in animals with chronic heart failure. Nisoldipine □ 0, ▨ 50, ▨ 100, ▩ 250, ■ 500 ng kg^{-1} min^{-1}. Endo/epi = ratio of normalized blood flow in subendo- and subepicardium of non-infarcted left ventricle. *P0·05, drug-induced changes statistically significant vs baseline (open bars).

cardiac output, had decreased (from 53 ± 3 to 37 ± 3 mmHg min^{-1}). Left ventricular end-diastolic pressure decreased from 19 ± 2 to 16 ± 1 mmHg ($P < 0·05$).

MYOCARDIAL BLOOD SUPPLY

Perfusion of the myocardial segment not supplied by the left circumflex coronary artery increased gradually from 200 ± 25 ml min^{-1} 100 g^{-1} to 321 ± 38 ml min^{-1} 100 g^{-1} ($P < 0·05$) at the highest dose (Fig. 3) The increase was homogeneously distributed over the different layers as the normalized subendo-epi blood flow ratio (1·25 ± 0·03) did not change. The increase in transmural blood flow (23 ± 9% and 70 ± 20% after infusion rates of 0·25 μg kg^{-1} min^{-1} and 0·5 μg kg^{-1} min^{-1}, respectively) exceeded the increase in myocardial oxygen demand, calculated as the product of heart rate and left ventricular systolic blood pressure (5 ± 3% and 13 ± 4% after the two highest infusion rates). Based on this observation, it is reasonable to assume that the decrease in the coronary vascular resistance (up to 35 ± 5% after the highest dose) was a true vasodilatory response to nisoldipine rather than a consequence of an increased metabolic demand. Perfusion of the central zone of the myocardium supplied by the occluded left cirumflex coronary artery was 21 ± 4% of that of the non-affected myocardium just prior to drug administration. Nisoldipine also increased flow to the core of the fibrotic myocardium (Fig. 3), but the increments were considerably less (20 ± 5 ml min^{-1} 100 g^{-1} increase from baseline with the highest dose) than for the normal myocardium.

REGIONAL BLOOD FLOWS

Nisoldipine caused a five-fold increase in skeletal muscle flow and almost doubled blood flow to the adrenal glands and stomach (Fig. 4) Perfusions of a number of other organs such as kidneys, brain, skin and small intestine were, however, not affected by the drug (Fig. 4). From the arterial blood pressure and regional blood flow data it can be easily concluded that vasodilation only occurred in those organs in which flow had increased.

Discussion

SYSTEMIC HAEMODYNAMIC CHANGES INDUCED BY INFARCTION AND NISOLDIPINE

In pigs occlusion of the proximal left circumflex coronary artery lasting 60 min or longer produces a distinct myocardial infarction and left ventricular dilatation without a considerable loss of animals[18]. Left ventricular dysfunction is manifested most clearly by the higher left ventricular filling pressure and systemic vascular resistance and the lower

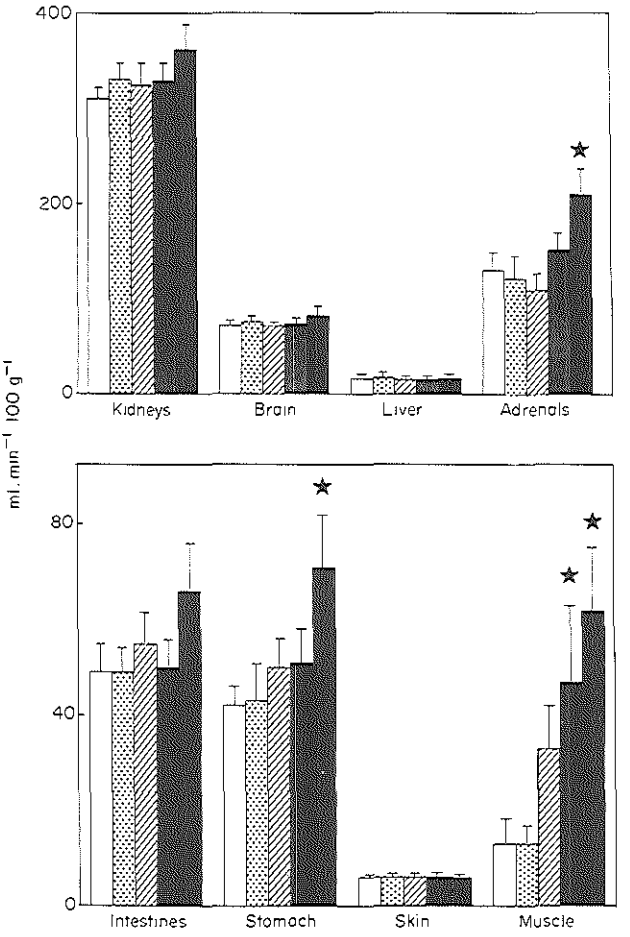

Figure 4 The effects of four consecutive doses of nisoldipine on organ blood flows in conscious pigs with chronic heart failure. Nisoldipine □ 0, ▨ 50, ▨ 100, ▩ 250, ■ 500 ng kg^{-1} min^{-1}. *$P<0.05$, drug-induced changes statistically significant vs baseline.

cardiac output and $LVdP/dt_{max}$ than in identically instrumented conscious pigs, but without the coronary artery ligation[11]. The changes in the haemodynamic parameters recorded 2–3 weeks after the infarction are comparable with those induced by chronic rapid ventricular pacing[19,20]. The fact that after an adequate number (usually 6–8) of adaptation sessions the preparation remained stable during infusion of the solvent for at least 50 min demonstrates that the model is suitable for the evaluation of acute pharmacological interventions.

The highest dose of nisoldipine in the present study, which exerts only very minor effects in pigs without coronary ligation[11], lowered left ventricular filling pressure (17%) and systemic vascular resistance (28%) and increased cardiac output (41%), stroke volume (26%) and $LVdP/dt_{max}$ (37%), while arterial blood pressure remained unchanged. Although significant systemic vasodilation occurred, reflex-mediated tachycardia was only mild (13%), probably due to progressive adrenergic-receptor downregulation[21].

MYOCARDIAL BLOOD FLOWS

Transmural blood flow to the myocardium, adjacent to the segment perfused by the left circumflex coronary artery was 200 ± 25 ml min^{-1} $100\ g^{-1}$ before drug administration. This value is about 15–20% higher than what we have reported before for normal myocardium of conscious pigs[22]. The higher baseline heart rate (15%) of the animals in the present study is probably the most important factor contributing to this finding. The nisoldipine-induced increase in transmural left ventricular blood flow is in agreement with the results of studies in anaesthetized as well as conscious animals, including pigs[12,22,23]. In these studies, the subepicardium benefitted more from the increase in transmural flow than the subendocardium. In the animals with heart failure, we did not observe such a preference for the subepicardial layers, most likely due to the relatively small increase in heart rate and negligible effect on arterial blood pressure[24]. Perfusion of the fibrotic segment was surprisingly high (21% of the adjacent normal myocardium) in view of the lack of coronary collaterals in pigs without coronary artery obstruction. We and others have repeatedly shown that after an acute coronary artery occlusion in pigs, the ischaemic myocardium receives less than 5% of its pre-occlusion blood flow[25–27]. Coronary collaterals can be induced in pigs by applying a fixed critical stenosis[26] or an ameroid constrictor[25] around a native coronary artery. In these models collaterals are formed which can provide the myocardium with up to 80% of the blood flow at rest, which depends on the site of the stenosed artery, the time allowed for the formation of the collaterals and the pharmacological intervention[28]. However, little is known about the formation of collaterals after complete ligation of a coronary artery. As these vessels do not develop within 48 h[29], it is doubtful that they will be able to salvage any myocardium. The increase in collateral flow by nisoldipine may therefore be of limited clinical significance.

REGIONAL BLOOD FLOWS

During chronic heart failure in swine incremental dosages of nisoldipine increased blood flow to adrenals and stomach, but that to the kidneys, brain, liver and skin remained unaffected. In contrast, studies with the calcium antagonist diltiazem in experimental heart failure in conscious rats showed that this compound increased blood flow to normal myocardium, adrenals, kidneys, liver, skin and brain, while blood flow to skeletal muscle remained unaffected at rest, and only slightly increased compared with saline during exercise[30]. The most significant observation in the present study was the five-fold increase in skeletal muscle blood flow. As more than 10% of the total body weight consists of skeletal muscle in this species, it is unlikely that all muscle groups will benefit equally. This is supported by the observation with nimodipine in anaesthetized pigs, in which the flow to a number of skeletal muscle groups increased, but the response varied considerably with the location[31]. Studies with an experimental heart failure model in rats showed that significant drug-induced blood flow redistribution within skeletal muscle may be determined by the fibre-type of the muscle groups[3]. Studies in patients with congestive heart failure have shown that an improvement of the impaired vasodilator response or an improvement of the reduced maximal oxygen consumption may be a prerequisite for improved exercise tolerance[9,32]. The results of the present study indicate that the enhancement of skeletal muscle perfusion by nisoldipine, if maintained during chronic administration, may improve the impaired vasodilator response observed in patients with moderate to severe heart failure.

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References

[1] Massie B, Conway M, Yonge R *et al.* Skeletal muscle metabolism in patients with congestive heart failure: relation to clinical severity and blood flow. Circulation 1987; 76: 1009–19.

[2] Wilson JR, Martin JL, Schwartz D, Farraro N. Exercise intolerance in patients with chronic heart failure: role of impaired nutritive flow to skeletal muscle. Circulation 1984; 69: 1079–87.

[3] Drexler H, Faude F, Höing S, Just H. Blood flow distribution within skeletal muscle during exercise in the presence of chronic heart failure: effect of milrinone. Circulation 1987; 76: 1344–52.

[4] Zelis R, Flaim SF. Alterations in vasomotor tone in congestive heart failure. Prog Cardiovasc Dis 1982; 24: 437–59.

[5] Zelis R, Mason DT, Braunwald E. A comparison of the effects of vasodilator stimuli on peripheral resistance vessels in normal subjects and in patients with congestive heart failure. J Clin Invest 1968; 47: 960–70.

[6] Sinoway LI, Minotti JR, Davis D *et al.* Delayed reversal of impaired vasodilation in congestive heart failure after heart transplantation. Am J Cardiol 1988; 61: 1076–9.

[7] Sinoway LI, Minotti J, Sirio C, Musk TI, Mathews W, Zelis R. The effects of prolonged unilateral forearm

immobilization on maximal peripheral blood flow. J Am Coll Cardiol 1986; 7: 224A.
[8] Leier CV, Huss P, Magorien RD, Unverferth DV. Improved exercise capacity and differing arterial and venous tolerance during chronic isosorbide dinitrate therapy for congestive heart failure. Circulation 1983; 67: 817–22.
[9] Drexler H, Banhardt U, Meinertz T, Wollschläger H, Lehmann M, Just H. Contrasting peripheral short-term and long-term effects of converting enzyme inhibition in patients with congestive heart failure. Circulation 1989; 79: 491–502.
[10] Kazda S, Garthoff B, Meyer H *et al.* Pharmacology of a new calcium antagonistic compound, isobutylmethyl-1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridinedicarboxylate (Nisoldipine, Bay k5552). Arzneim Forsch/Drug Res 1980; 30: 2144–62.
[11] Duncker DJ, Saxena PR, Verdouw PD. Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. Eur J Pharmacol 1988; 156: 401–9.
[12] Duncker DJ, Hartog JM, Hugenholtz PG, Saxena PR, Verdouw PD. The effects of nisoldipine (Bay K5552) on cardiovascular performance and regional blood flow in pentobarbital-anaesthetized pigs with or without beta-adrenoceptor blockade. Br J Pharmacol 1986; 88: 9–18.
[13] Kaihara S, Van Heerden PD, Migita T, Wagner HN Jr. Measurement of distribution of cardiac output. J Appl Physiol 1968; 25: 696–700.
[14] Saxena PR, Verdouw PD. Tissue blood flow and localization of arteriovenous anastomoses in pigs with microspheres of four different sizes. Pflügers Arch 1985; 403: 128–35.
[15] Hales JRS, Fawcett AA, Benett JW. Radioactive microsphere partitioning of blood flow between capillaries and arteriovenous anastomoses in skin of conscious sheep. Pflügers Arch 1978; 376: 87–91.
[16] Schamhardt HC. Regional myocardial perfusion and performance. PhD Thesis. Erasmus University Rotterdam, 1980.
[17] Saxena PR, Schamhardt HC, Forsyth RP, Loeve J. Computer programs for the radioactive microsphere technique. Determination of regional blood flows and other haemodynamic variables in different experimental circumstances. Comp Program Biomed 1980; 12: 63–84.
[18] Van der Giessen WJ, Verdouw PD, Ten Cate FJ, Essed CE, Rijsterborgh H, Lamers JMJ. In vitro cyclic AMP induced phosphorylation of phospholamban: an early marker of long-term recovery of function following reperfusion of ischaemic myocardium? Cardiovasc Res 1988; 22: 714–8.
[19] Riegger GA, Liebau G, Holzschuh M, Witkowski D, Steilner H, Kochsiek K. Role of the renin-angiotensin system in the development of congestive heart failure in the dog as assessed by chronic converting-enzyme blockade. Am J Cardiol 1984; 53: 614–8.
[20] Wilson JR, Douglas P, Hickey WF *et al.* Experimental congestive heart failure produced by rapid ventricular pacing in the dog: cardiac effects. Circulation 1987; 75: 857–67.
[21] Bristow MR, Ginsburg R, Minobe W *et al.* Decreased catecholamine sensitivity and beta-adrenergic-receptor density in failing human hearts. N Engl J Med 1982; 307: 205–11.
[22] Duncker DJ, Saxena PR, Verdouw PD. The effects of nisoldipine alone and in combination with beta-adrenoceptor blockade on systemic haemodynamics and myocardial performance in conscious pigs. Eur Heart J 1987; 8: 1332–9.
[23] Warltier DC, Meils CM, Gross GJ *et al.* Blood flow in normal and acutely ischemic myocardium after verapamil, diltiazem and nisoldipine (Bay k5552), a new dihydropyridine calcium antagonist. J Pharmacol Exp Ther 1981; 218: 296–302.
[24] Domenech RJ, Goich J. Effect of heart rate on regional coronary blood flow. Cardiovasc Res 1976; 10: 224–31.
[25] Schaper W. The collateral circulation of the heart. Amsterdam: Elsevier/North Holland, 1971.
[26] Millard RW. Induction of functional coronary collaterals in the swine heart. Basic Res Cardiol 1981; 76: 468–73.
[27] Verdouw PD, Wolffenbuttel BHR, Van der Giessen WJ. Domestic pigs in the study of myocardial ischaemia. Eur Heart J 1983; 4: 61–7.
[28] Bloor CM, White FC, Lammers RJ. Cardiac ischemia and coronary blood flow in swine. In: Swine in cardiovascular research. Stanton HC, Mersmann HJ, eds. Boca Raton, Florida: CRC Press, Inc., 1986, vol II, 87–104.
[29] Bloor CM, White FC. Functional development of the coronary collateral circulation during coronary artery occlusion in the conscious dog. Am J Pathol 1972; 67: 483–500.
[30] Drexler H, Depenbusch JW, Truog AG, Zelis R, Flaim SF. Effects of diltiazem on cardiac function and regional blood flow at rest and during exercise in a conscious rat preparation of chronic heart failure (myocardial infarction). Circulation 1985; 71: 1262–70.
[31] Duncker DJ, Heiligers J, Mylecharane EJ, Saxena PR, Verdouw PD. Nimodipine-induced changes in the distribution of carotid blood flow and cardiac output in pentobarbitone-anaesthetized pigs. Br J Pharmacol 1986; 89: 35–46.
[32] Creager MA, Massie BM, Faxon DP *et al.* Acute and long-term effects of enalapril on the cardiovascular response to exercise and exercise tolerance in patients with congestive heart failure. J Am Coll Cardiol 1985; 6: 163–70.

Chapter 3

Cardiovascular effects of elgodipine in conscious pigs with a normal coronary circulation and in conscious pigs with a healed myocardial infaction.

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Cardiovascular Effects of Elgodipine in Conscious Pigs with a Normal Coronary Circulation and in Conscious Pigs with a Healed Myocardial Infarction

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Summary: The cardiovascular effects of the phenyldihydropyridine derivative elgodipine (0.3, 1, 3, 10, and 30 μg/kg/min) were studied in normal conscious pigs and in pigs with chronic left ventricular dysfunction (LVD, caused by coronary artery occlusion) without and after β-adrenoceptor blockade with propranolol (0.5 mg/kg + 0.5 mg/kg/h). In normal pigs, elgodipine increased cardiac output from 2.57 ± 0.09 to 5.21 ± 0.24 L/min ($p < 0.05$) as a result of a doubling of the heart rate. Mean arterial blood pressure decreased from 94 ± 2 to 76 ± 3 mm Hg ($p < 0.05$) as a result of a decrease in systemic vascular resistance. Left ventricular (LV) dP/dt_{max} increased (by up to 78 ± 9%), but left ventricular end-diastolic pressure (LVEDP) remained unchanged. After propranolol administration elgodipine did not increase LV dP/dt_{max}, and the increase in heart rate was attenuated, resulting in a smaller increase in cardiac output (from 2.11 ± 0.13 to 3.09 ± 0.23 L/min, $p < 0.05$), but an unchanged vasodilator response. In pigs with LVD, elgodipine increased cardiac output and LV dP/dt_{max} less than in normal animals, but the vasodilator response was not affected. LVEDP decreased from 14.6 ± 1.6 to 11.7 ± 2.5 mm Hg ($p < 0.05$). In animals with LVD, propranolol caused a more severe depression of systemic hemodynamics, but did not modify the cardiovascular responses to elgodipine. Its cardiovascular profile suggests that elgodipine may not only be useful in the treatment of cardiovascular disorders for which other dihydropyridines are already in use, but also in mild chronic heart failure. **Key Words:** Myocardial infarction—Systemic hemodynamics—Calcium antagonist—Elgodipine—β-Adrenoceptor-blockade—Conscious pigs.

Clinical evidence has been presented showing that the second generation of dihydropyridine calcium antagonists, because of their higher vascular selectivity, may be more useful in the treatment of mild heart failure (1–4) than the first generation. The negative reports on the first-generation calcium-channel blockers have stressed their negative inotropic actions as the major factor in the failure to improve left ventricular dysfunction (LVD) (5,6). Elgodipine (isopropyl (2-(N-methyl-N-(4-fluorbenzyl)-amine)-ethyl,2,6-dimethyl-4-(2',3'-methylenedioxyphenyl)-1.5-dihydropyridine-3,5-dicarboxylate, monohydrochloride), IQB 875 D) is a novel compound, which has been shown to relax smooth muscle by inhibition of the slow inward calcium current (7) in vitro. Subsequent studies in anesthetized pigs have indeed shown that elgodipine caused vasodilation of the systemic and coronary vascular beds (8), while pulmonary artery pressure was lowered in anesthetized dogs (9). In these studies (8,9) heart rate did not increase in spite of a profound lowering of arterial blood pressure, but the anesthetic regimen may have blunted a reflex-mediated tachycardia. It is, however, noteworthy that nisoldipine raised heart rate in anesthetized pigs (10).

In order to establish the cardiovascular profile of elgodipine without interference of anesthetic agents, we now describe the actions of elgodipine in conscious instrumented pigs. Measurements were

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repeated after β-adrenoceptor blockade to eliminate the contribution of the sympathetic nervous system. Because some of the cardiovascular effects of calcium-channel blockers might be modified under pathological conditions (11), we also present the acute cardiovascular effects of elgodipine in conscious pigs with a 3-week-old myocardial infarction.

METHODS

Surgical procedures

After an overnight fast, 21 crossbred Landrace–Yorkshire pigs of either sex (20–25 kg), pretreated with 600 mg of a mixture of procaine penicillin-G and benzathine penicillin-G i.m. (Duplocillin, Gist-Brocades NV, Delft, The Netherlands), were sedated with an injection of 30 mg/kg ketamine HCl i.m. (Aeskoket, Aesculaap BV, Boxtel, The Netherlands). Subsequently the animals were intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1:2) to which 1–4% (vol/vol) enflurane was added. Under sterile conditions, a jugular vein and a common carotid artery were cannulated for infusion of drugs or solvent and measurement of arterial blood pressure, respectively. The chest was opened via the third left intercostal space, and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for the measurement of aortic blood flow. The heart was exposed via the fifth intercostal space, and a Konigsberg pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) 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 signal. Throughout the duration of the experiments, the readings of the Konigsberg system were verified using the fluid-filled systems. In 13 animals the proximal segment of the left circumflex coronary artery (LCXCA) was permanently ligated in order to induce a myocardial infarction (11). After 30 min, during which serious ventricular arrhythmias (sustained ventricular tachycardia or ventricular fibrillation) were treated with immediate DC countershock, the chest was closed and the wires were tunneled subcutaneously to the back, and the animals were allowed to recover from surgery. The animals received daily doses of 500 mg amoxicillin (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and, during the first week only, kanamycin 500 mg (Kanymex, Gist Brocades N.V., Delft, The Netherlands), to prevent infection. Catheters were flushed daily with an isotonic saline solution containing 500 IU/ml heparin. During the first 3 weeks of the postoperative recovery period the animals were adapted to the laboratory facilities (8–10 sessions) while hemodynamic parameters were monitored. The experimental protocols were executed when systemic hemodynamics had remained stable for at least 1 h, usually 3 weeks after instrumentation. All measurements were done while the animals were quietly resting in a constraining jacket.

Mortality

All eight animals with intact coronary circulation were used for the study. Of the 13 pigs in which the LCXCA was occluded, four died suddenly during the postoperative period, most likely as a result of ventricular arrhythmia. One animal was excluded because of an infection.

Experimental protocols

Each animal received five consecutive 10-min infusions of elgodipine at increasing infusion rates of 0.3, 1.0, 3.0, 10, and 30 μg/kg/min or five consecutive infusions of the solvent of elgodipine without and after β-adrenoceptor blockade with propranolol (0.5 mg/kg followed by an infusion of 0.5 mg/kg/h). Volumes of the solvent corresponded with those administered during the elgodipine infusions.

The dose of propranolol provides a stable cardiovascular condition and adequate β-adrenoceptor blockade, i.e., dose ratios >20 for heart rate, left ventricular (LV) dP/dt_{max}, and diastolic blood pressure (12). Elgodipine infusions were started 10 min after administration of the propranolol bolus. Data were obtained at baseline and at the end of each infusion step, and, in the propranolol-treated animals, also 10 min after administration of the β-adrenoceptor antagonist when the cardiovascular variables had reached a new stable level. Execution of consecutive experiments in the same animal was separated by an interval of at least 24 h. After an experiment in which propranolol was used, the interval was at least 48 h. The study was performed according to the guidelines approved by the American Physiological Society and approved by the Ethical Committee for Animal Experimentation of the Erasmus University Rotterdam.

Drugs

Apart from the anesthetics used during surgery and the antibiotics and heparin used during the postsurgical period, the only drugs used in this study were elgodipine (courtesy of Dr. A. Galiano, Instituto de Investigacion y Desarrollo, Quimico Biologico SA, Madrid, Spain) and propranolol hydrochloride (ICI Farma, Rotterdam, The Netherlands). Stock solutions (15 μg/kg/ml) of elgodipine were made in a mixture consisting of 3% (vol/vol) ethanol in 0.9% wt/vol NaCl. Further dilutions were made in 0.9% wt/vol NaCl. Preparation of the solution and infusion of the drug took place while the drug was protected from light.

Statistical analysis

All data are expressed as mean ± SEM. Statistical significance of the elgodipine-induced changes was calculated by comparing the drug-induced changes from baseline with the solvent-induced changes after an analysis of variance had revealed that the samples represented different populations. Statistical significance was accepted at $p < 0.05$. A Bonferroni procedure was used because of multiple comparisons.

RESULTS

Baseline values and hemodynamic stability

Effects of solvent infusions. At baseline the most striking difference between the normal pigs and the pigs with myocardial infarction was noticed in the left ventricular end-diastolic pressure (LVEDP), which was twice as high in the animals in which the LCXCA was permanently occluded (Table 1). LV

dP/dt_{max} was 30% lower in the animals with the infarctions than in the normal animals (Table 1).

Intravenous administration of incremental infusion rates of the solvent did not affect any of the systemic hemodynamic parameters in either group of animals during the 50-min duration of the study period (Table 1).

Normal conscious animals

Systemic hemodynamic effects of elgodipine. Figure 1 shows that for infusion rates >1 μg/kg/min, elgodipine caused a dose-dependent increase in cardiac output (up to 103 ± 9% with the highest dose; $p < 0.05$), which was almost exclusively due to the increase in heart rate (up to 82 ± 18%, $p < 0.05$), as stroke volume (not shown) increased only with the highest dose (12 ± 4%, $p < 0.05$). Mean arterial pressure (MAP) started to decrease when the infusion rate was increased to 3 μg/kg/min and had fallen by 19 ± 3% ($p < 0.05$) by the end of the highest infusion rate (30 μg/kg/min). Since the decrease in MAP was accompanied by an increase in cardiac output, it can be calculated that systemic vascular resistance (SVR) had decreased. Systemic vasodilation already occurred during the infusion of 1 μg/kg/min and had resulted in a 59 ± 3% decrease in SVR at the highest dose ($p < 0.05$) (Fig. 1). Arterial pulse pressure, taken as the difference between systolic and diastolic arterial pressure, was not affected (not shown). LVEDP did not change, but LV dP/dt_{max} increased up to 78 ± 9% ($p < 0.05$) above its baseline value of 3,720 ± 370 mm Hg/s.

Effects of β-adrenoceptor blockade. Propranolol decreased cardiac output (15%), heart rate (18%), and LV dP/dt_{max} (25%), while systemic vascular resistance (22%) and LVEDP (24%) increased (Table 2) (all values $p < 0.05$). MAP and stroke volume were not affected. These changes were very similar to those described previously in conscious pigs (12).

Systemic hemodynamic effects of elgodipine after β-adrenoceptor blockade. β-adrenoceptor blockade did not modify the vasodilator response to elgodipine, but abolished the increase in LV dP/dt_{max} (Fig. 1). The elgodipine-induced increases in heart rate were considerably attenuated, but not abolished. Cardiac output increased, but the increments were considerably less than before β-adrenoceptor blockade. LVEDP was not affected.

Conscious animals with a 3-week-old infarction

Systemic hemodynamic effects of elgodipine. The three lowest doses of elgodipine had only a very minor effect on cardiac output (Fig. 2). At the two highest doses cardiac output increased, but the increments were less than in the normal animals. This was primarily due to a smaller increase in heart rate (maximal increases of 71 ± 13 and 104 ± 7 beats/min for the animals with chronic LVD and normal animals, respectively, $p < 0.05$). The vasodilator capacity of elgodipine was not affected as compared to that of the normal animals, but there were marked differences in the responses of left ventricular filling pressure and LV dP/dt_{max}. LVEDP was reduced by 3.0 ± 1.1 mm Hg at the

TABLE 1. *Systemic hemodynamics during infusion of solvent in conscious pigs with an intact coronary circulation and conscious pigs with a 3-week-old infarction*

			Time after start of solvent infusion (min)				
		Baseline	10	20	30	40	50
HR	N	117 ± 4	115 ± 4	115 ± 5	115 ± 5	118 ± 6	117 ± 7
	I	128 ± 6	128 ± 7	127 ± 7	127 ± 6	128 ± 7	131 ± 7
CO	N	2.54 ± 0.15	2.50 ± 0.16	2.50 ± 0.16	2.48 ± 0.16	2.48 ± 0.16	2.56 ± 0.20
	I	2.35 ± 0.13	2.36 ± 0.12	2.34 ± 0.13	2.36 ± 0.13	2.35 ± 0.13	2.38 ± 0.13
SV	N	21 ± 1	21 ± 1	21 ± 1	21 ± 1	20 ± 2	21 ± 2
	I	19 ± 2	19 ± 1	19 ± 2	20 ± 1	19 ± 2	19 ± 2
SAP	N	113 ± 6	116 ± 5	114 ± 5	115 ± 5	114 ± 5	111 ± 4
	I	108 ± 6	105 ± 6	106 ± 6	106 ± 6	107 ± 6	108 ± 6
MAP	N	97 ± 5	101 ± 4	100 ± 4	100 ± 4	99 ± 4	98 ± 4
	I	86 ± 5	85 ± 5	85 ± 5	85 ± 5	87 ± 5	88 ± 5
DAP	N	78 ± 5	84 ± 4	81 ± 4	83 ± 4	81 ± 4	81 ± 3
	I	69 ± 3	68 ± 3	69 ± 3	69 ± 3	70 ± 3	71 ± 4
SVR	N	38 ± 3	40 ± 3	40 ± 3	40 ± 3	40 ± 3	38 ± 3
	I	37 ± 1	37 ± 1	37 ± 1	37 ± 1	38 ± 1	38 ± 1
LV dP/dt_{max}	N	3,340 ± 280	3,310 ± 290	3,300 ± 330	3,480 ± 270	3,410 ± 330	3,450 ± 310
	I	2,750 ± 210[a]	2,760 ± 180	2,780 ± 200	2,770 ± 190	2,770 ± 210	2,770 ± 220
LVEDP	N	5.0 ± 0.4	5.3 ± 0.4	5.1 ± 0.5	4.7 ± 0.4	4.6 ± 0.4	5.3 ± 0.2
	I	15.6 ± 2.0[a]	15.8 ± 2.0	15.6 ± 2.0	15.5 ± 2.1	15.6 ± 2.0	15.6 ± 2.1

HR, heart rate (beats/min); N, normal coronary circulation (n = 8); I, occluded left circumflex coronary artery (n = 8); CO, cardiac output (L/min); SV, stroke volume (ml); SAP, systolic arterial blood pressure (mm Hg); MAP, mean arterial blood pressure (mm Hg); DAP, diastolic arterial blood pressure (mm Hg); SVR, systemic vascular resistance (mm Hg · min/L); LV dP/dt_{max}, maximum rate of rise of left ventricular pressure (mm Hg/s); LVEDP, left ventricular end-diastolic pressure (mm Hg).

Data are mean ± SEM. [a]$p < 0.05$ versus N.

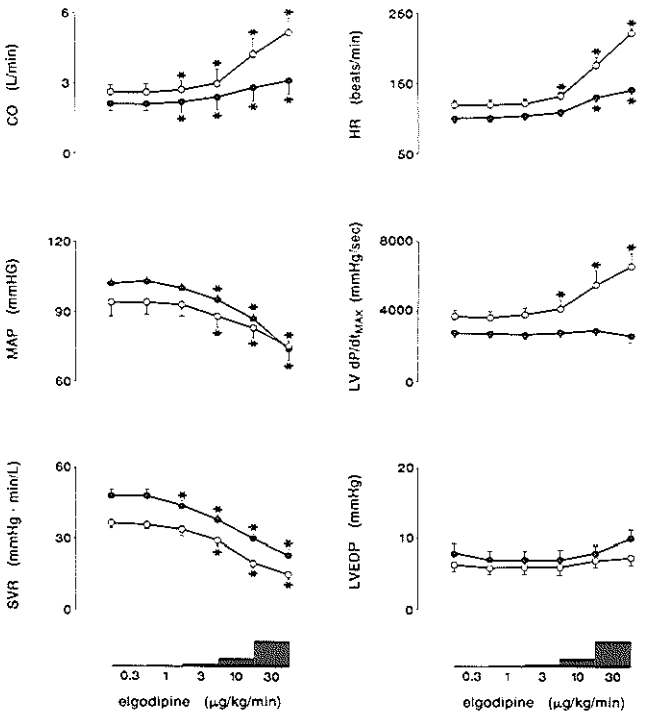

FIG. 1. Cardiovascular effects of elgodipine in conscious pigs with an intact coronary circulation without (open circles, n = 8) and after β-adrenoceptor blockade (solid circles, n = 8) with propranolol. CO, cardiac output; HR, heart rate; MAP, mean arterial blood pressure; LV dP/dt_{max}, maximal rate of rise of left ventricular pressure; SVR, systemic vascular resistance; LVEDP, left ventricular end-diastolic pressure; *, elgodipine-induced changes are significantly different ($p < 0.05$) from solvent-induced changes (see Table 1). Data have been presented as mean ± SEM.

highest dose ($p < 0.05$) in the animals with the infarctions, whereas it remained unchanged in the normal animals. The largest difference was, however, seen in the response of LV dP/dt_{max} at the two highest doses of elgodipine. The sharp increase in LV dP/dt_{max} observed in the normal animals was absent in the animals with the 3-week-old infarction.

TABLE 2. *Hemodynamic effects of β-adrenoceptor blockade with propranolol in eight normal conscious pigs and eight conscious pigs with a 3-week-old infarction*

	Normal pigs		Pigs with infarction	
	Baseline	Propranolol	Baseline	Propranolol
HR	119 ± 7	101 ± 5[a]	125 ± 6	103 ± 4[a]
MAP	99 ± 4	102 ± 4	91 ± 3	93 ± 3
CO	2.46 ± 0.10	2.11 ± 0.13[a]	2.36 ± 0.07	1.79 ± 0.06[a,b]
SVR	38.8 ± 1.3	47.5 ± 3.0[a]	38.6 ± 1.9	53.0 ± 1.7[a,b]
LVEDP	6.6 ± 0.9	8.1 ± 1.2[a]	11.9 ± 1.1	15.3 ± 1.4[a,b]
LV dP/dt_{max}	3,670 ± 260	2,760 ± 210[a]	2,760 ± 160	1,950 ± 130[a]
SV	20.3 ± 1.1	20.4 ± 1.1	19.3 ± 1.1	17.6 ± 1.0[a]

HR, heart rate (beats/min); MAP, mean arterial blood pressure (mm Hg); CO, cardiac output (L/min); SVR, systemic vascular resistance (mm Hg · min/L); LVEDP, left ventricular end-diastolic pressure (mm Hg); LV dP/dt_{max}, maximum rate of rise of left ventricular pressure (mm Hg/s); SV, stroke volume (ml).

Data are mean ± SEM.

[a] $p < 0.05$ versus baseline.

[b] Propranolol-induced change is significantly different ($p < 0.05$) from propranolol-induced change in normal pigs.

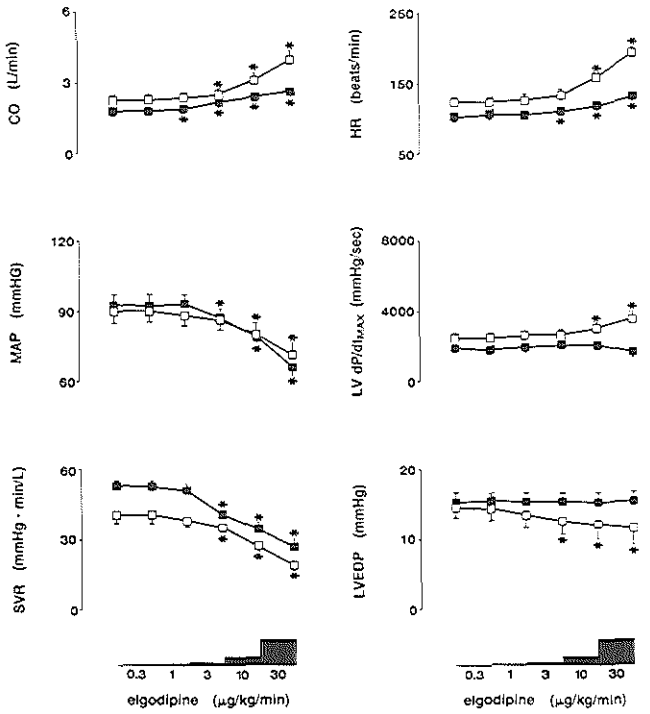

FIG. 2. Cardiovascular effects of elgodipine in conscious pigs with a 3-week-old myocardial infarction without (open squares, n = 8) and after β-adrenoceptor blockade (solid squares, n = 8) with propranolol. For further details see Fig. 1 legend.

Effects of β-adrenoceptor blockade. The same dose of propranolol as used in the normal conscious pigs now caused a slight decrease (8%) in stroke volume, whereas stroke volume in the normal animals remained unchanged (Table 2). The decrease in cardiac output, both in absolute values and as a percentage of baseline, was larger in the animals with the infarction than in the normal animals (0.58 ± 0.05 vs. 0.35 ± 0.06 L/min, respectively, $p < 0.05$). Furthermore, the increases in SVR, and LVEDP were also significantly larger as compared to the normal animals (Table 2).

Systemic hemodynamic effects of elgodipine after β-adrenoceptor blockade. β-adrenoceptor blockade again did not modify the vasodilator response to elgodipine (SVR 53% without and 50% after β-blockade). However, β-blockade with propranolol attenuated the elgodipine-induced increase in cardiac output because of a smaller increase in heart rate (57% without, compared to 30% after β-blockade, respectively, $p < 0.05$). LVEDP, in contrast to the experiments without β-adrenoceptor blockade, was not lowered (Fig. 2).

DISCUSSION

The present study confirms the vasodilator response of the systemic vascular bed to elgodipine as observed in anesthetized pigs (8), for conscious normal animals as well as for animals with chronic LVD. Compared to the other dihydropyridines evaluated in the same model, the vasodilator potency of elgodipine is of the same order as that of nifedipine and nimodipine, but less than that of nisoldipine (12). In contrast to our observations in the anesthetized animals, we now observed a marked increase in cardiac output, which was due to an increase in heart rate. It is therefore most likely that the presence of anesthesia in the earlier study (8) blunted a reflex-mediated tachycardia. The increase in cardiac output was also responsible for the smaller decrease in arterial blood pressure in these conscious animals than in the anesthetized animals. Parallel to the increase in heart rate there was an increase in LV dP/dt_{max}, which was abolished when elgodipine was administered after β-adrenoceptor blockade. Elgodipine, however,

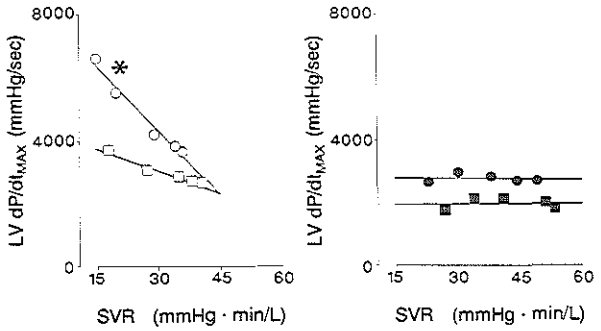

FIG. 3. Relationship between elgodipine-induced systemic vascular resistance (SVR) and LV dP/dt_{max} in conscious pigs with intact coronary circulation before (open circles, n = 8) and after (solid circles, n = 8), β-adrenoceptor blockade, and in conscious pigs with a 3-week-old infarction before (open squares, n = 8) and after (solid squares, n = 8) β-blockade. Each data point is the mean of eight observations. *Indicates that the slope of the linear regression line of the normal animals was significantly different ($p < 0.05$) from the mean slope of the linear regression line of the animals with the infarction, as calculated by comparing the slopes of the individual animals using an unpaired Student's *t* test (two-tailed).

still caused a moderate increase in heart rate after propranolol administration. This increase in heart rate after β-adrenoceptor blockade is not unique to elgodipine, since it has also been reported for other dihydropyridine derivatives in pigs (12), dogs (13), and humans (14), and may be due to parasympathetic withdrawal (15).

In the animals with LVD due to myocardial infarction, the vasodilator response to elgodipine was similar to that observed in the normal animals, but the increase in the already depressed LV dP/dt_{max} was considerably less. This can be most clearly illustrated by plotting the relationship between SVR and LV dP/dt_{max} (Fig. 3). The smaller increase in LV dP/dt_{max} may be one of the factors that contributed to the smaller increase in cardiac output by elgodipine in the animals with the 3-week-old infarction. A similar observation has also been reported for nisoldipine (11). Figure 4 demonstrates that the smaller increase in heart rate is not the only factor responsible for the smaller increase in LV dP/dt_{max} in the animals with the 3-week-old infarction, and may be an indication of exhaustion of the inotropic reserve of the noninfarcted (i.e., left anterior descending coronary artery–perfused) myocardium (11).

Similar to earlier observations with other dihydropyridines (12), elgodipine did not affect left ventricular filling pressure in normal animals. A modest but significant decrease was detected in the animals with LVD. This observation is not unique. In an earlier study we showed a significant, albeit relatively small, effect for nisoldipine (11). Not all second-generation dihydropyridines exert this property; clinical studies in patients with congestive heart failure have shown no effect of nicardipine (4) on pulmonary capillary wedge pressure, whereas data on felodipine (1,2) and isradipine (3,16) are inconclusive. The reason for this effect of elgodipine is not clear. At first glance it appears that the effect may be related to the increase in heart rate, since no change in LVEDP was observed after the animals were pretreated with propranolol (Fig. 2). However, in the normal animals the changes in heart rate were even more striking, but LVEDP did not change. Furthermore, if heart rate changes are important, an effect should also have been observed with the aforementioned dihydropyridines (3,4). Further studies are therefore necessary to confirm the lowering of elevated left ventricular filling pressures by elgodipine.

The absence of negative inotropy and the lowering of the loading conditions of the heart is of interest for the treatment of mild congestive heart failure. Now evidence is accumulating that the value of positive inotropy in the treatment of this disorder may be of limited value (17). Several other second-generation dihydropyridines, such as nisoldipine (18), nicardipine (4,19), felodipine (1,2,20), and isradipine (3,21), which have a cardiovascular profile similar to that of elgodipine, have shown promise in clinical trials of congestive heart failure. We therefore conclude that further studies with elgodipine to investigate its usefulness in the management of heart failure in humans may be indicated. Special

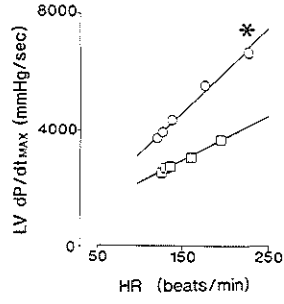

FIG. 4. Relationship between heart rate and LV dP/dt_{max} in conscious pigs with an intact coronary circulation (circle) and in conscious pigs with a 3-week-old infarction (square). For further details see Fig. 3 legend.

attention should be given to the effects of chronic treatment, because the acute effects do not necessarily reflect the long-term effects. A further point of attention should be the effect of elgodipine on neurohumoral activation, because vasodilators such as the dihydropyridines may also increase plasma norepinephrine and plasma renin activity activation in response to the hypotensive action of these drugs (22,23). However, this effect may be blunted by the defective baroreceptor activity in patients with advanced heart failure. Furthermore, there is evidence that calcium-channel blockers inhibit angiotensin II–mediated aldosterone release (24). It has been suggested that vasodilators, like the more vascular-selective second-generation dihydropyridines, while markedly improving vascular compliance, may unload the ventricle to a far greater extent and thereby more than offset any negative inotropic effect or tendency to activate neuroendocrine systems (25).

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REFERENCES

1. Emanuelsson H, Hjalmarson A, Holmberg S, Waagstein F. Acute hemodynamic effects of felodipine in congestive heart failure. *Eur J Clin Pharmacol* 1985;28:489–93.
2. Dunselman PHJM, Kuntze CEE, van Bruggen A, et al. Efficacy of felodipine in congestive heart failure. *Eur Heart J* 1989;10:354–64.
3. Greenberg B, Siemienczuk D, Broudy D. Hemodynamic effects of PN 200-110 (isradipine) in congestive heart failure. *Am J Cardiol* 1987;59:70B–4.
4. Greenbaum RA, Wan S, Evans TR. The acute haemodynamic effects of nicardipine in patients with chronic left ventricular failure. *Eur J Clin Pharmacol* 1986;30:383–6.
5. Elkayam U, Weber L, McKay CR, Rahimtoola SH. Differences in hemodynamic response to vasodilation due to calcium channel antagonism with nifedipine and direct acting agonism with hydralazine in chronic refractory congestive heart failure. *Am J Cardiol* 1984;54:126–31.
6. Murakami T, Hess OM, Krayenbuehl HP. Left ventricular function before and after diltiazem in patients with coronary artery disease. *Circulation* 1985;5:723–30.
7. Roman C, De Miguel B, Giráldez A, Galiano A. Study of agonism and antagonism of elgodipine on different types of receptors. *Eur J Pharmacol* 1990;183:2411–2.
8. Sassen LMA, Soei LK, Koning MMG, Verdouw PD. The central and regional cardiovascular responses to intravenous and intracoronary administration of the phenyldihydropyridine elgodipine in anaesthetized pigs. *Br J Pharmacol* 1990; 99:355–63.
9. De Miguel B, Galiano A, Landin L, et al. Elgodipine vs. nicardipine: haemodynamic changes in anaesthetized dogs. *Eur J Pharmacol* 1990;183:1341–2.
10. Duncker DJ, Hartog JM, Hugenholtz PG, Saxena PR, Verdouw PD. The effects of nisoldipine (Bay K 5552) on cardiovascular performance and regional blood flow in pentobarbital-anaesthetized pigs with or without beta-adrenoceptor blockade. *Br J Pharmacol* 1986;88:9–18.
11. Van der Giessen WJ, Van Woerkens LJ, Duncker DJ, Roelandt JRTC, Verdouw PD. The acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J Cardiovasc Pharmacol* 1989;14: 653–8.
12. Duncker DJ, Saxena PR, Verdouw PD. Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *Eur J Pharmacol* 1988;156:401–9.
13. Warltier DC, Zyvoloski MG, Gross GJ, Brooks HL. Comparative actions of dihydropyridine slow channel calcium blocking agents in conscious dogs: systemic and coronary hemodynamics with and without combined beta-adrenergic blockade. *J Pharmacol Exp Ther* 1984;230:367–75.
14. Silke B, Verma SP, Midtbo KA, et al. A haemodynamic study of the effects of combined slow-channel blockade (nisoldipine) and beta-blockade (metoprolol) in coronary heart disease. *Int J Cardiol* 1986;13:231–41.
15. Nakaya H, Schwartz A, Millard RW. Reflex chronotropic and inotropic effects of calcium channel-blocking agents in conscious dogs. Diltiazem, verapamil and nifedipine compared. *Circ Res* 1983;52:302–11.
16. Van den Toren EW, Van Bruggen A, Ruegg PC, Lie KI. Hemodynamic effects of an intravenous infusion of isradipine in patient with congestive heart failure. *Am J Med* 1988:84[suppl 3B]:97–101.
17. Erdman D. The value of positive inotropy in acute and chronic heart failure. *J Cardiovasc Pharmacol* 1989;14:S36–41.
18. Vogt A, Kreuzer H. Hemodynamic effects of nisoldipine in chronic congestive heart failure. *Arzneimittelforsch* 1983; 33:877–9.
19. Rymann KS, Kubo SH, Lystash J, Stone G, Cody RJ. Effect of nicardipine on rest and exercise hemodynamics in chronic congestive heart failure. *Am J Cardiol* 1986;58:583–8.
20. Timmis AD, Jewitt DE. Studies with felodipine in congestive heart failure. *Drugs* 1985;29:66–75.
21. McGrath BP, Newman R, Older P. Hemodynamic study of short- and long-term isradipine treatment in patients with chronic ischemic congestive heart failure. *Am J Med* 1989; 86:75–80.
22. Barjon JN, Rouleau JL, Bichet D, Juneau C, DeChamplain J. Chronic renal and neurohormonal effects of the calcium entry blocker nisoldipine in patients with congestive heart failure. *J Am Coll Cardiol* 1987;9:622–30.
23. Packer M. Calcium channel blockers in chronic heart failure: the risk of physiologically rational therapy. *Circulation* 1990;82:2254–7.
24. Facunding JL, Catt KJ. Dependence of aldosterone stimulation in adrenal glomerulosa cells on calcium uptake: effects of lanthanum and verapamil. *Endocrinology* 1980;107:1345–53.
25. Francis GS. Calcium channel blockers and congestive heart failure. *Circulation* 1991;83:336–8.

Chapter 4

Acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure.

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Acute Hemodynamic Effects of Nisoldipine and Pimobendan in Conscious Pigs with Chronic Heart Failure

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Summary: The acute systemic hemodynamic effects of the calcium antagonist nisoldipine and the pyridazinone-derivative pimobendan, a phosphodiesterase inhibitor with vasodilating as well as positive inotropic properties, were studied in conscious pigs with chronic heart failure. Left ventricular (LV) dysfunction, manifested by a 25% decrease in cardiac output (CO), a 35% increase in systemic vascular resistance (SVR), and a doubling of the left ventricular filling pressure, was induced by a proximal ligation of the left circumflex coronary artery. Two to three weeks after myocardial infarction, cumulative 10-min infusions of either nisoldipine (0.05, 0.1, 0.25, and 0.5 μg/kg/min), pimobendan (2.5, 5, 12.5, and 25 μg/kg/min) or the solvents were administered. Infusion of the solvents did not affect any of the hemodynamic variables. Both nisoldipine and pimobendan normalized CO and exhibited a similar cardiac profile [systemic vasodilatation, reduction in left ventricular filling pressure, and an increase in heart rate (HR)] except for the significantly ($p < 0.05$) larger increase in $LVdP/dt_{max}$ with pimobendan (85%) than with nisoldipine (45%). In animals with heart failure, lower doses of both nisoldipine (twice) and pimobendan (four times) were needed to elicit a 30% reduction in SVR than in animals with normal pump function. For both drugs, the slope of the line describing the vasodilatory and positive inotropic properties shifted more in favor of the vasodilatory actions during heart failure ($p < 0.05$). We conclude that in swine with chronic LV dysfunction nisoldipine, despite its lack of inotropic properties, appeared to improve ventricular function to the same extent as the primary positive inotropic agent pimobendan. **Key Words:** Heart failure—Myocardial infarction—Systemic hemodynamics—Nisoldipine—Pimobendan—Conscious pigs.

Vasodilator drugs may be of benefit in chronic heart failure because of their capability of unloading the heart by reducing systemic vascular resistance (SVR) and thereby improving cardiac output (CO). This is usually associated with a reduction in wall stress and left ventricular (LV) filling pressure. Because of their vasodilatory properties, calcium channel blockers have been studied extensively in heart failure etiologies: secondary to impaired coronary blood flow and myocardial infarction, increased SVR, pulmonary hypertension, and LV diastolic dysfunction due to hypertrophic cardiomyopathy. Clinical studies describing the effects of calcium antagonists in patients with LV dysfunction are not unequivocal. Some investigators report beneficial effects on acute as well as long-term hemodynamics and exercise tolerance (1–4), and others describe no effect or even aggravation of LV failure (5–8). A potential drawback to the use of calcium channel blockers may be their negative inotropic action. Although this action may be counterbalanced by a reflex-mediated sympathetic response in some patients, compensation may be insufficient because of elevated levels of catecholamines and limited cardiac reserve in others. Nisoldipine is a calcium channel blocker which belongs to the class of the 1,4-dihydropyridine derivatives. The compound exerts in vitro a 4–10 times stronger vasodilatory and an equal or weaker cardiodepressant action compared to equimolar doses of nifedipine (9). The potent coronary and systemic vasodilatory responses have been confirmed in the intact animal

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(9–13) and in humans (14–16). We describe the systemic hemodynamic actions of nisoldipine in domestic pigs with induced chronic heart failure. To determine if vasodilators with positive inotropic properties are superior in this model, we also evaluated the actions of the benzimidazole-pyridazinone derivative pimobendan, a phosphodiesterase inhibitor which also has calcium-sensitizing properties (17).

MATERIALS AND METHODS

Surgical procedures

After an overnight fast, Yorkshire pigs (17–24 kg), pretreated with 600 mg of a mixture of procaine penicillin-G and benzathine penicillin-G intramuscularly (i.m.) (Duplocillin, Gist-Brocades NV, Delft, The Netherlands), were sedated with an i.m. injection of 30 mg/kg ketamine HCl (Aeskoket, Aesculaap BV, Boxtel, The Netherlands). After endotracheal intubation, the animals were connected to a respirator for mechanical ventilation with a mixture of oxygen and nitrous oxide (1:2) to which 1–4% (vol/vol) enflurane was added. Under sterile conditions, a jugular vein and a common carotid artery were cannulated for infusion of drugs or solvent and measurement of arterial blood pressure (BP), respectively. The chest was opened through the third left intercostal space, and an electromagnetic flow probe (13–15 mm; Skalar, Delft, The Netherlands) was positioned around the ascending aorta for measurement of aortic blood flow. The heart was exposed through the fifth intercostal space. A tip manometer pressure transducer (P_4, Konigsberg Instruments, Pasadena, CA, U.S.A.) was implanted into the left ventricle of the heart through its apex for recording of LV pressure. The left atrium was cannulated for recording left atrial pressure which, together with arterial BP, was used to calibrate the Konigsberg transducer signals. Subsequently, the proximal segment of the left circumflex coronary artery was ligated permanently for induction of a myocardial infarction. After 30 min, the chest was closed. In this period, serious ventricular arrhythmias [substained ventricular tachycardia (VT) or ventricular fibrillation (VF)] were treated with immediate DC countershock. Catheters and wires were tunnelled subcutaneously to the back, and the animals were allowed to recover from surgery. The animals received 500 mg amoxicilline daily (Flemoxin, Gist Brocades NV, Delft, The Netherlands), while the catheters were flushed with heparinized saline solution. During the first 14 postoperative days, the animals were adapted to the laboratory facilities. The experimental protocol was executed 2–3 weeks after instrumentation and induction of the myocardial infarction.

Experimental animals

Eleven animals underwent the surgical procedures. During the first 30 min after coronary artery occlusion, VF occurred in four of these animals. Defibrillation was successful in all. One animal died suddenly the day after surgery. During the course of the study, no other animal died.

To establish whether ligation of the left circumflex coronary artery affected cardiac pump function in the chronic phase, we compared the baseline data of the systemic hemodynamic parameters with those of 20 other animals (mean weight 22 kg) that had undergone the identical surgical procedures except for coronary artery ligation. The baseline data of seven of these animals were described previously (18).

Experimental protocols

Three series of experiments were performed. In two series, four consecutive 10-min intravenous (i.v.) infusions with incremental dosages of either drug were administered. The infusion rates were 0.05, 0.1, 0.25, and 0.5 μg/kg/min for nisoldipine (n = 6) and 2.5, 5, 12.5, and 25 μg/kg/min for pimobendan (n = 6). In the third series of experiments (n = 8) either the solvent of pimobendan (n = 4) or that of nisoldipine (n = 4) was administered with infused volumes identical to those of the active drugs. At the end of each 10-min infusion period, when parameters had reached a stable level, heart rate (HR), aortic blood flow, LV pressure and its first derivative ($LVdP/dt$), and arterial BP were recorded. Some animals were used for more than one protocol. In such animals, infusion of the drugs or solvents was randomized and separated by at least 2 days.

After completion of the experiments, the animals were killed with an overdose of pentobarbital sodium. The hearts were removed, and the infarcted (fibrotic) area and the noninfarcted area of the left ventricle were excised after fixation for 7 days in a 10% formaldehyde solution. Infarct size was expressed as percentage (wt/wt) of the left ventricle.

Drugs

Nisoldipine infusions (Bay K 5552; Bayer AG, Wuppertal, F.R.G.) were prepared from a stock solution (0.5 mg/ml) dissolved in equal volumes of polyethylene glycol (PEG) 400 and glycerol. The nisoldipine solution and the solvent were diluted with a 0.9% wt/vol NaCl solution immediately before use and administered while protected from light. Pimobendan (UD-CG 115 BS; Dr. Karl Thomae GmbH, Biberach an der Riss, F.R.G.) was dissolved in a mixture of PEG 200 and saline (1:1).

Statistical analysis

All data are mean ± SEM. Significance of the effects of the drugs or the solvents was evaluated by Duncan's new multiple-range test once an analysis of variance (ANOVA) had revealed that the samples represented different populations; $p < 0.05$ was considered statistically significant.

RESULTS

Arterial acid–base balance and blood gases

The arterial blood gases during the experiments were pH = 7.41 ± 0.04, Pco_2 = 44 ± 4 mm Hg, Po_2 = 87 ± 6 mm Hg, and HbO_2-saturation = 91 ± 2%.

Evaluation of the heart failure model

Occlusion of the left circumflex coronary artery resulted in a fibrotic area weighing 14 ± 2% of the total LV mass. Baseline values of systemic hemodynamic variables differed significantly from those of animals in our laboratory that underwent identical surgical procedures, except for the coronary artery occlusions (Table 1). CO in the animals with

TABLE 1. *Systemic hemodynamics of conscious pigs with and without chronic occlusion of the LCXCA*

Hemodynamics	Control (n = 20)	LCXCA occlusion (n = 10)
CO (L/min)	2.73 ± 0.13	2.06 ± 0.18[a]
HR (beats/min)	124 ± 3	133 ± 7
SV (ml)	22.1 ± 1.0	15.4 ± 1.5[a]
LV$dP/dt_{max}$ (mm Hg/s)	3,180 ± 180	2,800 ± 170
LVEDP (mm Hg)	10.3 ± 1.0	19.8 ± 1.4[a]
MAP (mm Hg)	105 ± 4	106 ± 3
SVR (mm Hg/L · min)	38 ± 2	51 ± 5[a]

LCXCA, left circumflex coronary artery; CO, cardiac output; HR, heart rate; SV, stroke volume; LV$dP/dt_{max}$, maximum rate of rise of left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; MAP, mean arterial blood pressure; SVR, systemic vascular resistance.
[a] $p < 0.05$ versus control.

heart failure (2.06 ± 0.18 L/min) was 25% lower than in the nonligated animals (2.73 ± 0.13 L/min) because the increase in HR in the infarcted animals infarcted animals was insufficient to compensate for the decrease in stroke volume (SV). Mean arterial BP (MAP) was maintained because of a 35% increase in SVR. From the other determinants of cardiac performance, LV end-diastolic BP (LVEDP) was doubled, whereas the contractility index LV$dP/dt_{max}$ was 10% lower in the animals with heart failure despite the slightly higher HR.

Stability of the model during the 50-min observation period

After the adaptation sessions were completed, the systemic hemodynamic variables remained stable during administration of solvents for at least 50 min (Fig. 1).

Effects of nisoldipine in heart failure

Infusion at a rate of 0.05 μg/kg/min had no effect on any of the hemodynamic variables, but when the infusion rate was increased most parameters changed gradually (Fig. 1). CO was restored to normal when nisoldipine was infused at a rate of 0.5 μg/kg/min. This increase in CO was caused by in-

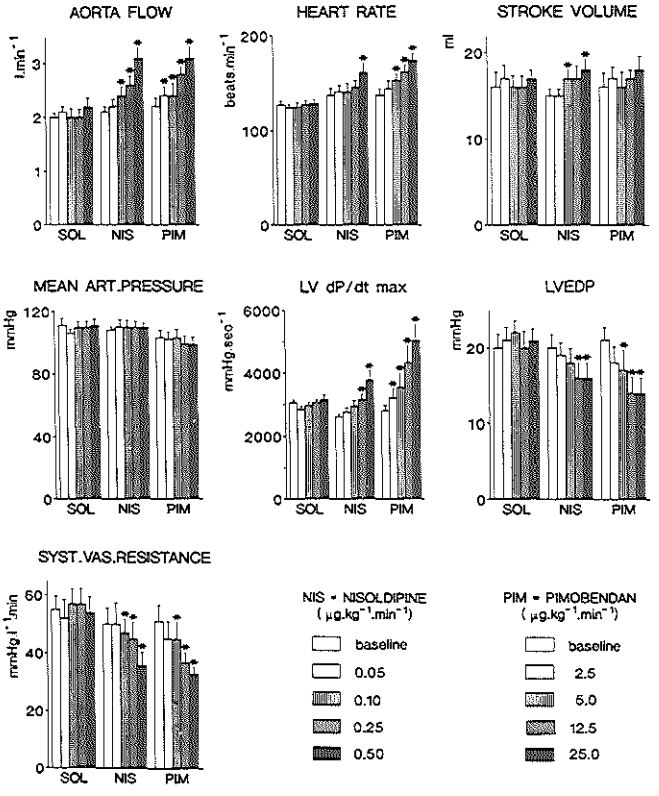

FIG. 1. Systemic hemodynamic effects of nisoldipine (NIS) and pimobendan (PIM) in conscious pigs with heart failure. Stability of the model was investigated by infusion of solvents (SOL). LVEDP, left ventricular end-diastolic pressure. Data are mean ± SEM; *p < 0.05, drug-induced changes statistically significant versus solvent-induced changes.

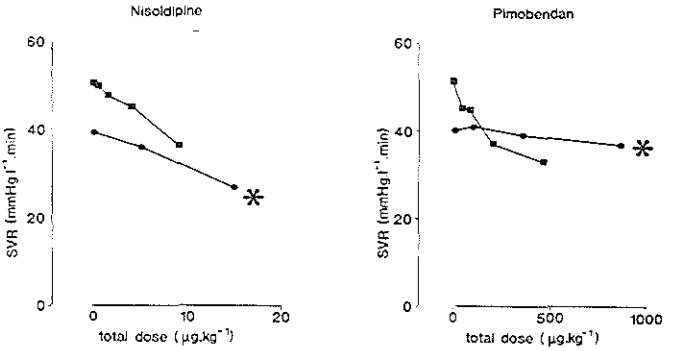

FIG. 2. Vasodilatory actions of nisoldipine and pimobendan in conscious pigs without (solid circles) and with (solid squares) heart failure. Doses are the total cumulative dose. SVR, systemic vascular resistance. Responses in the animals without heart failure are from earlier studies by our laboratory (18,23). *Mean slope of the line for normal animals significantly different ($p < 0.05$) from the mean slope for animals with heart failure. Statistical significance was determined by calculating slopes of the regression lines of individual animals and comparing the mean slope of each group of animals by unpaired Student's *t* test.

creases in both HR (27%) and SV (20%). MAP did not change as SVR was normalized. LVEDP decreased from 20 ± 2 to 16 ± 2 mm Hg ($p < 0.05$), but was still elevated as compared with values of the normal control animals, while LV$dP/dt_{max}$ increased to levels above those usually measured in conscious animals without coronary artery obstructions.

Effects of pimobendan in heart failure

The lowest infusion rate (2.5 μg/kg/min) had no effect on any of the cardiovascular parameters, but increasing the infusion rate to 25 μg/kg/min normalized CO, primarily owing to an increase in HR (Fig. 1). Except for a more pronounced increase ($p < 0.05$) in LV$dP/dt_{max}$ with pimobendan (85%) than with nisoldipine (45%), the cardiovascular profile of both drugs was very similar after CO was normalized by both drugs.

DISCUSSION

Evaluation of the model

In pigs, occlusion of the left circumflex coronary artery (LCXCA) causes a less severe impairment of cardiac pump function than a proximal occlusion of the left anterior descending coronary artery (LADCA). This correlates well with the observation that the area affected by occlusion of the LCXCA is much smaller (15%) than the area affected by occlusion of the LADCA (40%). The smaller mass of infarcted myocardium probably also accounts for the smaller loss of animals due to sudden death (19). Nevertheless, the loss of pump function (25%) and the increases in LV filling pressure (100%) and SVR (35%) in the present study compare well with what has been observed in other models of heart failure such as rapid ventricular pacing (20,21). The contractility index LV$dP/dt_{max}$ was only mildly affected (−10%). This probably results not only from the size of the infarcted area, but also from a compensatory mechanism in the adjacent myocardium. An important feature is the stability of the model after adequate adaptation sessions which, together with maintenance of peripheral reflexes, is paramount for study of acute interventions. Because of the chronic nature of coronary artery occlusion, the cardiac effects in these pigs, which do not possess coronary collaterals, are confined to the myocardium perfused by the nonoccluded vessels (22).

Effects of nisoldipine and pimobendan

Both compounds decreased SVR as well as LV filling pressure. Because nisoldipine increased SV and to a lesser extent HR and pimobendan primarily increased HR, both compounds were able to normalize CO. The vasodilatory properties of nisoldipine have been well documented (9–16). In the present study, this occurred at doses slightly lower than has been described for conscious pigs with a normal coronary circulation and pump function

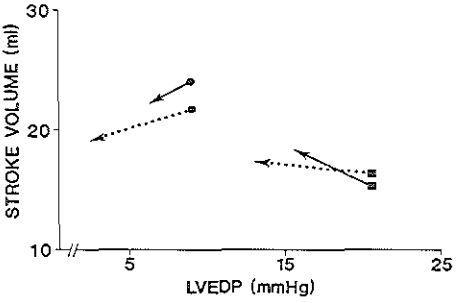

FIG. 3. Effects of nisoldipine (solid line) and pimobendan (dotted line) on relation between left ventricular end-diastolic pressure (LVEDP) and stroke volume (SV) in conscious pigs with (solid squares) and without (solid circles) heart failure. In animals with filling pressures in the normal range, a reduction in filling pressure was accompanied by a decrease in SV. Responses of the animals without heart failure are from earlier studies by our laboratory (18,23).

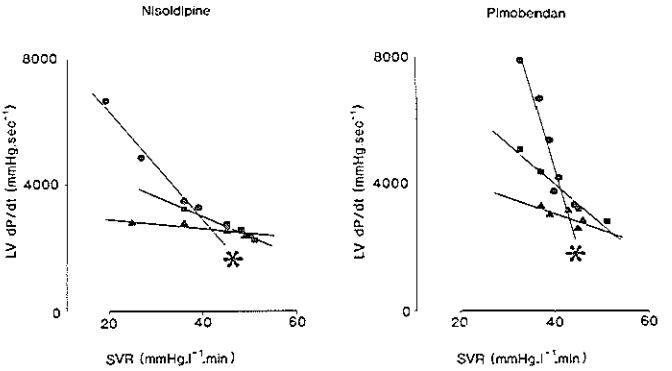

FIG. 4. Relationship between vasodilatory and positive inotropic actions of nisoldipine and pimobendan in conscious pigs with heart failure (solid squares) and in pigs without heart failure before (solid circles) and after (solid triangles) β-adrenoceptor blockade with propranolol. SVR, systemic vascular resistance; LV*dP/dt*, maximum rate of rise in left ventricular pressure. Responses in the animals without heart failure are from earlier studies by our laboratory (18,23). *Mean slope for the normal animals (solid circles) was significantly different ($p < 0.05$) from both the slopes of normal animals after β-adrenoceptor blockade and that of animals with heart failure. Statistical analysis is described in the legend to Fig. 2.

(Fig. 2) (23). The systemic vasoconstriction may have potentiated the vasodilatory action of nisoldipine. However, the vasodilatory actions of nisoldipine in the study of Duncker and colleagues (23) was only marginally affected when SVR was increased by pretreatment with propranolol. Therefore, other factors such as humoral changes which occur in this chronic heart failure model (W. J. van der Giessen, F. H. M. Derkx, F. Boomsma, P. D. Verdouw, unpublished observations, 1988) may also contribute. More striking was the observation that pimobendan, which exhibited only minimal vasodilatory actions in conscious pigs with normal pump function (18), was a potent vasodilator under the conditions described in the present study (Fig. 2).

The larger increase in LV$dP/dt_{max}$ in the pimobendan-treated animals was not translated into a larger increase in SV as compared with nisoldipine. The slightly larger decrease in LV filling pressure in the pimobendan-treated animals may be the factor responsible for this observation. The importance of not lowering LV filling pressure too greatly was also demonstrated in normal conscious pigs in which SV was either unchanged or decreased after administration of the compounds (Fig. 3).

The slope of the linear regression lines describing the relationship between vasodilatory and positive cardiostimulatory properties was shifted in favor of vasodilatory actions during heart failure, approaching (LV$dP/dt_{max}$) or parallelling (HR) the slope of normal conscious pigs pretreated with propranolol (Figs. 4 and 5). In chronic heart failure, reflex-mediated responses can be blunted (24), which may be due to depletion of norepinephrine (NE) from cardiac sympathetic nerve endings (25) and down-regulation of β-adrenoceptors (26). Furthermore, in heart failure sympathetic activity is often already enhanced (27) which results, as in exercise, in attenuated baroreceptor-mediated reflexes. Although these mechanisms may be operative in the present model, their contributions appear to be limited, as basal HR was not significantly increased, and HR and LV$dP/dt_{max}$ increased considerably (although less than in normal animals) after nisoldipine and pimobendan-induced vasodilatation. However, we used an acute administration of the drugs. With chronic treatment, the baroreceptors reset, which

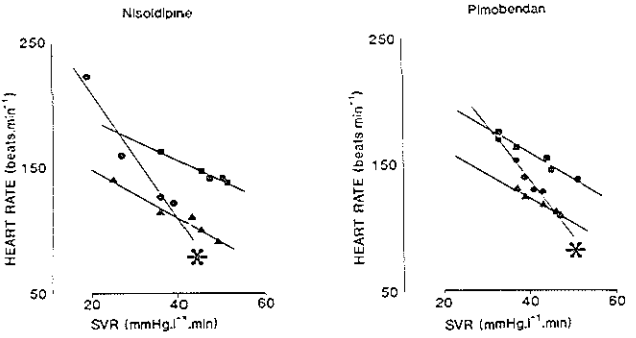

FIG. 5. Relationship between vasodilatory and positive chronotropic actions of nisoldipine and pimobendan in conscious pigs with heart failure (solid squares) and in pigs without heart failure before (solid circles) and after (solid triangles) β-adrenoceptor blockade with propranolol. SVR, systemic vascular resistance. Responses in the animals without heart failure are from earlier studies by our laboratory (18,23). *Mean slope for normal animals (solid circles) was significantly different ($p < 0.05$) from both the slopes of normal animals after β-adrenoceptor blockade and that of animals with heart failure. Statistical analysis is described in the legend to Fig. 2.

could shift the response even further in the direction of vasodilation.

We conclude that in swine with chronic LV dysfunction pimobendan, despite its positive inotropic properties, had no clear advantage over nisoldipine. Further studies are needed to evaluate which organs and tissues benefit most from the increase in CO. In earlier studies in anesthetized animals with normal LV function, we showed that pimobendan increased blood flow to the splanchnic circulation rather than the skeletal muscles (28).

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REFERENCES

1. Belloci F, Ansalone G, Santarelli P, et al. Oral nifedipine in the long-term management of severe congestive heart failure. *J Cardiovasc Pharmacol* 1982;46:476–80.
2. Magorien RD, Leier CV, Kolibash AJ, Barbush TJ, Unverfeth DV. Beneficial effects of nifedipine on rest and exercise myocardial energetics in patients with congestive heart failure. *Circulation* 1984;70:884–90.
3. Walsh RW, Porter CB, Starling MR, O'Rourke RA. Beneficial hemodynamic effects of intravenous and oral diltiazem in severe congestive heart failure. *J Am Coll Cardiol* 1984; 3:1044–50.
4. Miller AB, Conetta DA, Bass TA. Sublingual nifedipine: acute effects in severe chronic congestive heart failure secondary to idiopathic dilated cardiomyopathy. *Am J Cardiol* 1985;55:1359–62.
5. Chew CYC, Hecht HS, Collett JT, McAllister RG, Singh BN. Influence of the severity of left ventricular dysfunction on hemodynamic responses to intravenously administered verapamil in ischemic heart disease. *Am J Cardiol* 1981; 47:917–22.
6. Klein HO, Ninio R, Oren V, et al. The acute hemodynamic effects of intravenous verapamil in coronary artery disease: assessment by equilibrium gated radionuclide ventriculography. *Circulation* 1983;67:101–10.
7. Josephson MA, Singh BN. Use of calcium antagonists in ventricular dysfunction. *Am J Cardiol* 1985;55:81–8.
8. Murakami T, Hess OM, Krayenbuehl HP. Left ventricular function before and after diltiazem in patients with coronary artery disease. *J Am Coll Cardiol* 1985;5:723–30.
9. Kazda S, Garthoff B, Meyer H, et al. Pharmacology of a new calcium antagonistic compound, isobutyl methyl 1,4-dihydro-2,6-dimethyl-4-(2-nitrophenyl)-3,5-pyridine-carboxylate (nisoldipine, Bay k 5552a). *Arzneim Forsch (Drug Res)* 1980;30:2144–62.
10. Warltier DC, Zyvoloski MG, Gross GJ, Brooks HL. Comparative effects of dihydropyridine slow calcium channel blocking agents in conscious dogs: systemic and coronary hemodynamics with and without combined beta-adrenergic blockade. *J Pharmacol Exp Ther* 1984;230:367–75.
11. Drexler H, Flaim SF, Fields RH, Zelis R. Effects of nisoldipine on cardiocirculatory dynamics and cardiac output distribution in conscious rats at rest and during treadmill exercise. *J Pharmacol Exp Ther* 1985;232:376–81.
12. Duncker DJ, Hartog JM, Hugenholtz PG, Saxena PR, Verdouw PD. The effects of nisoldipine (Bay k 5552) on cardiovascular performance and regional blood flow in pentobarbital-anaesthetized pigs with or without beta-adrenoceptor blockade. *Br J Pharmacol* 1986;88:9–18.
13. Duncker DJ, Saxena PR, Verdouw PD. The effects of nisoldipine alone and in combination with beta-adrenoceptor blockade on systemic haemodynamics and myocardial performance in conscious pigs. *Eur Heart J* 1987;8:1332–9.
14. Vogt A, Neuhaus KL, Kreuzer H. Hemodynamic effects of the new vasodilator drug Bay k 5552 in man. *Arzneim Forsch (Drug Res)* 1980;30:2162–4.
15. Silke B, Frais MA, Muller P, Verma SP, Reynolds G, Taylor SH. Haemodynamic dose-response effects of intravenous nisoldipine in coronary artery disease. *Br J Clin Pharmacol* 1985;20:675–80.
16. Serruys PW, Suryapranata H, Planellas J, et al. Acute effects of intravenous nisoldipine on left ventricular function and coronary hemodynamics. *Am J Cardiol* 1985;56:140–6.
17. Fujino K, Sperelakis N, Solaro RJ. Differential effects of D- and L-pimobendan on cardiac myofilament calcium sensitivity. *J Pharmacol Exp Ther* 1988;247:519–23.
18. Duncker DJ, Hartog JM, Levinsky L, Verdouw PD. Systemic haemodynamic actions of pimobendan (UD-CG 115 BS) and its *O*-demethylmetabolite UD-CG 212 CL in the conscious pig. *Br J Pharmacol* 1987;91:609–15.
19. Verdouw PD, JM Hartog. Provocation and suppression of ventricular arrhythmias in domestic swine. In: *Swine in cardiovascular research*, vol II. Stanton HC, Mersmann HJ, eds. Boca Raton, FL: CRC Press, 1986:121–56.
20. Riegger GAJ, Liebau G, Holzschuh M, Witkowski D, Steilner H, Kochsiek K. Role of the renin-angiotensin system in the development of congestive heart failure in the dog as assessed by chronic converting-enzyme blockade. *Am J Cardiol* 1984;53:614–8.
21. Wilson JR, Douglas P, Hickey WF, et al. Experimental congestive heart failure produced by rapid ventricular pacing in the dog: cardiac effects. *Circulation* 1987;75:857–67.
22. Van der Giessen WJ, ten Cate FJ, Lamers JMJ, Essed CE, Rijsterborgh H, Verdouw PD. In vitro cyclic AMP-induced phosphorylation of phospholamban: an early marker of long term recovery of function following reperfusion of ischaemic myocardium? *Cardiovasc Res* 1988;22:714–8.
23. Duncker DJ, Saxena PR, Verdouw PD. Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *Eur J Pharmacol* 1988;156:401–9.
24. Goldstein RE, Beiser GD, Stampfer M, Epstein SE. Impairment of autonomically mediated heart rate control in patients with cardiac dysfunction. *Circ Res* 1978;36:571–8.
25. Chidsey CA, Braunwald E, Morrow AG, Mason DT. Myocardial norepinephrine concentration in man. Effects of reserpine and of congestive heart failure. *N Engl J Med* 1963;269:653–9.
26. Bristow MR, Ginsburg R, Minobe W, et al. Decreased catecholamine sensitivity and beta adrenergic receptor density in failing human hearts. *N Engl J Med* 1982;307:205–11.
27. Levine TB, Francis GS, Goldsmith SR. Activity of the sympathetic nervous system and renin-angiotension system assessed by plasma hormone levels and their relation to hemodynamic abnormalities in congestive heart failure. *Am J Cardiol* 1982;49:1659–66.
28. Verdouw PD, Hartog JM, Duncker DJ, Roth W, Saxena PR. Cardiovascular profile of pimobendan, a benzimidazole-pyridazinone derivative with vasodilating and inotropic properties. *Eur J Pharmacol* 1986;126:21–30.

Chapter 5

Cardiovascular effects of the novel potassium channel opener bimakalim in conscious pigs with and without myocardial infarction. A comparitive study with nicorandil.

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Cardiovascular Effects of the Novel Potassium Channel Opener Bimakalim in Conscious Pigs after Myocardial Infarction. A Comparative Study with Nicorandil.

Summary

In conscious pigs potassium channel opener bimakalim (37.5 - 300 $ng.kg^{-1}.min^{-1}$) and nicorandil (10-80 $\mu g.kg^{-1}.min^{-1}$) cardiac output increased from 2.7 ± 0.1 to 3.8 ± 0.2 $l.min^{-1}$ and from 2.7 ± 0.1 to 3.9 ± 0.3 $l.min^{-1}$ (both $p<0.05$), respectively due to increases in heart rate. Mean arterial blood pressure (104 ± 4 mmHg) decreased gradually to 91 ± 6 with bimakalim and to 84 ± 5 mmhg with nicorandil (both $p<0.05$), due to similar decreases in systemic vascular resistance. $LVdP/dt_{max}$ also increased with both drugs (up to 48 ± 11% and 69 ± 7%) but left ventricular end-diastolic pressure remained unchanged with bimakalim, while it gradually decreased from 9 ± 1 to 5 ± 1 mmhg ($p<0.05$) with nicorandil.

In the pigs with a 3 weeks old myocardial infarction, the vasodilator responses to bimakalim and nicorandil were not affected, but the increases in heart rate and $LVdP/dt_{max}$ were attenuated compared to the effects in the normal animals. Non-selective beta-adrenoceptor blockade did not affect the vasodilatory capacity, but attenuated the increases in heart rate and $LVdP/dt_{max}$ of bimakalim. From the present study we may conclude that in conscious pigs bimakalim was approximately 100 times more potent as an arterial vasodilator than nicorandil. The potential negative inotropic actions of bimakalim are negligible and the compound may therefore be of interest in clinical situations which require a lowering of systemic vascular resistance.

Introduction

Potassium channel activators open ATP-sensitive K^+ channels which leads to hyperpolarisation of the smooth muscle cell membrane and thereby limits calcium availability via voltage-dependent Ca^{2+} channels (Cook, 1988, Buckingham et al. 1986 and Hamilton et al., 1986). The benzopyran-derivative bimakalim (EMD 52 692, SR 44866) is a novel potassium channel activator, which is 5 times more potent than cromakalim (BRL 34915) and 100 times more potent than nicorandil (Findlay et al. 1989, De Peyer et al., 1989, Richer et al., 1990 and Gericke et al., 1989).

In anaesthetized pigs bimakalim decreased arterial blood pressure due to systemic

vasodilatation, increased heart rate and had no effect on left ventricular filling pressure and the inotropic state of the myocardium (Sassen et al., 1990). It has well been established, however, that anesthetic agents may interfere with the cardiovascular actions of pharmacological agents. In the present study we therefore describe the cardiovascular effects of bimakalim in conscious animals. In view of the clinical application of the drug we used two groups of animals: one group with a normal myocardial function and another with a healed myocardial infarction because of a coronary artery ligation 3 weeks before the measurements were made.
The cardiovascular profile of bimakalim was also compared to that of nicorandil, a compound which is structurally related to nitrates, but possesses also potassium channel activator properties (Taira, et al., 1979, Sakai et al., 1980, Taira, 1981, Taira, 1989, Verdouw et al., 1987 and Sassen et al., 1989).

Methods

Surgical procedures

After an overnight fast, cross-bred Landrace x Yorkshire pigs (18-22 kg) were instrumented under sterile conditions for the measurement of left ventricular pressure (Konigsberg pressure transducer), left atrial- and aortic pressures and ascending aortic blood flow (electromagnetic flow probe) as described earlier (Van der Giessen et al., 1989, Van Woerkens et al. 1991 and Van Woerkens et al, in press).
In 12 animals the proximal segment of the left circumflex coronary artery (LCXCA) was permanently ligated for the induction of a myocardial infarction. After 30 min, during which period serious ventricular arrhythmias (sustained ventricular tachycardia or ventricular fibrillation) were treated with immediate DC countershock, the chest was closed and the wires were tunnelled subcutaneously to the back, and the animals were allowed to recover from surgery. During the first weeks of the post operative recovery period the animals were adapted to the laboratory facilities (8 to 10 sessions of 1 hour), while hemodynamic parameters were monitored. The experimental protocols were executed when systemic hemodynamics remained stable for at least one hour. All experiments were carried out while the animals were quietly resting in a constraining jacket.
Three of the 12 pigs with the coronary artery ligation died suddenly during the early post-operative period, most likely secondary to ventricular arrhythmias.

Experimental protocols

Each animal received 4 consecutive ten min infusions of bimakalim (37.5, 75, 150 and 300 $ng.kg^{-1}.min^{-1}$) or nicorandil (10, 20, 40 and 80 $\mu g.kg^{-1}.min^{-1}$) or 4 consecutive infusions of the solvent. Volumes of the solvent corresponded with those administered during the active drug infusions.
In both animal models the hemodynamic effects of bimakalim were also studied after non-selective beta-adrenoceptor blockade with 0.5 $mg.kg^{-1}$ + 0.5 $\mu g.kg^{-1}.h^{-1}$ propranolol (Duncker et al., 1987).
Data were obtained at baseline and at the end of each infusion step. Experiments in the same animal were separated by at least 24 hours.

Ethical considerations

The study was performed according to the guidelines approved by the American Physiological Society and approved by the Ethical Committee for Animal Experimentation of the Erasmus University Rotterdam.

Statistical analysis

All data are expressed as mean ± standard error of the mean (SEM). Statistical significance of the drug-induced changes was calculated by comparing the drug-induced changes from baseline with the solvent-induced changes after an analysis of variance had revealed that the samples represented different populations. Statistical significance was accepted at $p<0.05$. A Bonferroni-procedure was used because of multiple comparisons.

Drugs

The only drugs used during the experiments were bimakalim (EMD 52 692) (4-(1,2-dihydro-2-oxo-1-pyridyl)2,2-dimethyl-2H-1-benzopyran-6-carbonitrite, courtesy of Dr. P. Schelling, E. Merck, Darmstadt, Germany), nicorandil (N-(2-hydroxyethyl)nicotinamide nitrate, Rhone-Poulenc, Amstelveen, The Netherlands) and propranolol hydrochloride (ICI Farma, Rotterdam, The Netherlands). Bimakalim was dissolved in saline containing 4% (v/v) polyethylene glycol to obtain an infusion rate of 2 $ml.min^{-1}$ for administration of the highest dose.. Nicorandil was dissolved in 1.5 ml ethyl alcohol and further diluted with saline to infuse identical volumes during drug administration.

Table 1 *Systemic hemodynamics during infusion of solvent in conscious pigs with an intact coronary circulation (N: n = 6)and in conscious pigs with a 3 weeks old infarction (I: n = 9)*

		Solvent (ml/min)				
		Baseline	0.25	0.5	1	2
HR	N	116 ± 8	118 ± 10	118 ± 10	115 ± 10	116 ± 10
	I	117 ± 8	117 ± 8	117 ± 7	118 ± 8	122 ± 8
CO	N	2.63 ± 0.04	2.63 ± 0.04	2.62 ± 0.04	2.59 ± 0.05	2.63 ± 0.05
	I	2.43 ± 0.08*	2.44 ± 0.08	2.43 ± 0.07	2.45 ± 0.06	2.47 ± 0.06
SV	N	23.2 ± 1.8	22.9 ± 2.1	22.8 ± 2.0	23.2 ± 2.0	23.2 ± 2.1
	I	21.5 ± 1.7	21.8 ± 1.8	21.6 ± 1.8	21.7 ± 2.0	21.0 ± 1.8
SAP	N	126 ± 5	125 ± 4	125 ± 3	126 ± 3	127 ± 4
	I	116 ± 4*	115 ± 4	117 ± 4	115 ± 4	114 ± 4
MAP	N	99 ± 3	98 ± 4	99 ± 3	99 ± 3	100 ± 3
	I	96 ± 4	96 ± 4	99 ± 5	98 ± 5	96 ± 5
DAP	N	73 ± 4	75 ± 5	74 ± 4	74 ± 3	74 ± 4
	I	80 ± 3*	80 ± 4	83 ± 5	81 ± 4	81 ± 4
SVR	N	37.6 ± 1.6	37.5 ± 1.6	37.7 ± 1.6	38.2 ± 1.4	38.1 ± 1.4
	I	39.5 ± 1.2	39.3 ± 1.4	40.6 ± 1.7	39.8 ± 1.5	39.0 ± 1.8
$LVdP/dt_{max}$	N	3540 ± 230	3500 ± 260	3400 ± 220	3450 ± 280	3670 ± 240
	I	2960 ± 410*	2950 ± 380	2960 ± 350	2910 ± 360	2970 ± 410
LVEDP	N	8 ± 1	8 ± 1	8 ± 1	8 ± 1	8 ± 1
	I	13 ± 2*	13 ± 2	14 ± 2	14 ± 2	14 ± 2

*HR = heart rate (beats/min); CO = cardiac output (l/min); SV = stroke volume (ml); SAP = systolic arterial blood pressure (mmHg); MAP = mean arterial blood pressure (mmHg); DAP = diastolic arterial blood pressure (mmHg); SVR = systemic vascular resistance (mmHg/min/l); $LVdP/dt_{max}$ = maximum rate of rise of left ventricular pressure (mmHg/s); LVEDP = left ventricular end-diastolic pressure (mmHg); * $P<0.05$ I vs N.*

Results

Baseline values and hemodynamic stability during solvent infusion (Table 1)

Baseline hemodynamic values showed that occlusion of the left circumflex coronary artery 3 weeks earlier resulted in a 10% decrease in cardiac output, a 30% decrease in $LVdP/dt_{max}$ and a 60-100% increase in left ventricular end-diastolic pressure (Table 1). These results are similar to those reported earlier (Van der Giessen et al., 1989, Van Woerkens et al., 1991 and Van Woerkens et al., in press).
Intravenous administration of incremental infusion rates of both vehicle solutions did not affect any of the systemic hemodynamic parameters in either group of animals during the 50 min duration of the study period (Table 1).

Systemic hemodynamic effects in normal conscious animals (Figure 1)

Bimakalim at dose higher than 75 $ng.kg^{-1}.min^{-1}$ increased cardiac output dose-dependently up to 42 ± 6% ($p<0.05$), due to an increase in heart rate (up to 62 ± 14%, $p<0.05$). Mean arterial blood pressure decreased (from 104 ± 4 to 91 ± 6 mmHg, $p< 0.05$), which was the consequence of a pronounced systemic vasodilation (systemic vascular resistance decreased dose dependently from 39 ± 3 $mmHg.l^{-1}.min$ to 24 ± 3 $mmHg.l^{-1}.min$). dP/dt_{max} increased (up to 48 ± 11%, $p< 0.05$), while left ventricular end-diastolic pressure remained unchanged.
Nicorandil showed a cardiovascular profile comparable to that of bimakalim, the only difference being that nicorandil decreased left ventricular end-diastolic pressure al all doses.

Hemodynamic effects in conscious animals with an infarct (Figure 2)

Bimakalim exerted in these animals similar hemodynamic actions as in pigs with the intact coronary circulation, except for a smaller increase in heart rate (up to 37 ± 4% versus 62 ± 14% in the normal animals) and a small but significant decrease of left ventricular end-diastolic pressure at the highest dose (18 ± 6%, $p<0.05$).
Nicorandil again exhibited a similar cardiovascular profile as bimakalim. The decrease in left ventricular end-diastolic pressure, however, was more pronounced after nicorandil (up to 24 ± 5%).

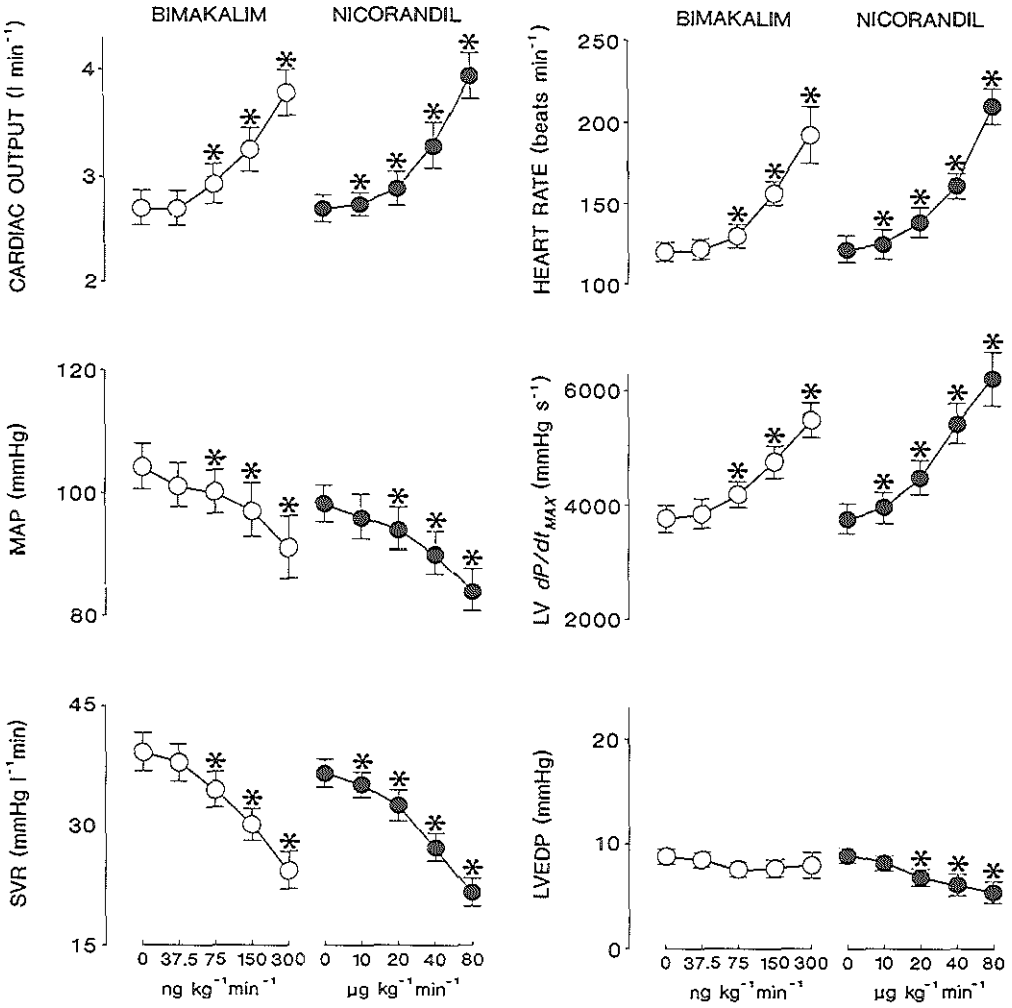

Figure 1. *Systemic hemodynamic effects of bimakalim (n=6) and nicorandil (n=8) in normal conscious pigs. MAP = mean arterial blood pressure; LV dP/dt_{max} = maximal rate of rise of left ventricular pressure; SVR = systemic vascular resistance; LVEDP = left ventricular end-diastolic pressure; Data are means ± SEM; * Drug induced change from baseline significantly different ($p<0.05$) compared to the comparable solvent induced change from baseline (see Table 1).*

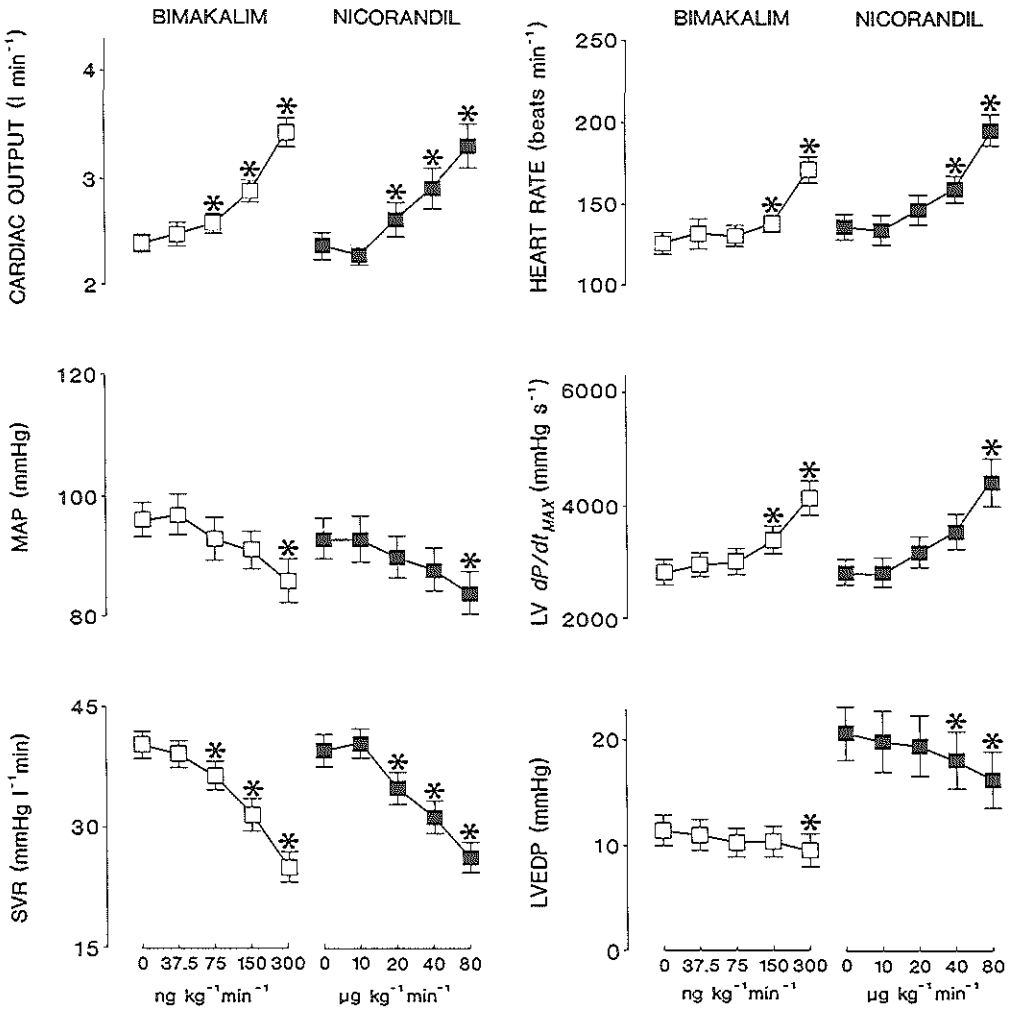

Figure 2. *Systemic hemodynamic effects of bimakalim (n=8) and nicorandil (n=9) in conscious pigs with a three week old infarction. MAP = mean arterial blood pressure; LV dP/dt_{max} = maximal rate of rise of left ventricular pressure; SVR = systemic vascular resistance; LVEDP = left ventricular end-diastolic pressure; Data are means ± SEM; * Drug induced change from baseline significantly different ($p<0.05$) compared to the comparable solvent induced change from baseline (see Table 1).*

Table 2 *Hemodynamic effects of 10 min i.v. infusion bimakalim after beta-adrenoceptor blockade with propranolol 0.5 mg.kg^{-1} + 0.5 mg.kg^{-1}.h^{-1} in normal conscious pigs (N) and in conscious pigs with a 3 weeks old infarction (IF)*

		Baseline pre-propranolol	Baseline	bimakalim (ng.kg^{-1}.min^{-1})			
				37.5	75	150	300
HR	N	122 ± 4	103 ± 5^{+}	106 ± 3	110 ± 4*	123 ± 3*	141 ± 3*
	IF	136 ± 6	95 ± 6^{+}	100 ± 4	98 ± 6	100 ± 7	112 ± 8*
MAP	N	94 ± 4	95 ± 4	93 ± 4*	91 ± 5*	87 ± 5*	79 ± 5*
	IF	100 ± 5	96 ± 3	96 ± 4	94 ± 3	91 ± 3	84 ± 4*
CO	N	2.73 ± 0.08	2.29 ± 0.06^{+}	2.35 ± 0.06	2.54 ± 0.08*	2.80 ± 0.15*	3.32 ± 0.21*
	IF	2.45 ± 0.09	1.66 ± 0.05$^{+\circ}$	1.69 ± 0.06	1.84 ± 0.07*	1.99 ± 0.10*	2.31 ± 0.20*
SVR	N	35.6 ± 2.1	42.8 ± 3.0^{+}	41.0 ± 3.0*	37.5 ± 3.3*	32.8 ± 3.5*	25.7 ± 3.1*
	IF	40.8 ± 2.6	57.8 ± 1.8$^{+\circ}$	56.9 ± 1.9	51.1 ± 0.8*	45.8 ± 1.4*	37.2 ± 3.1*
LVEDP	N	6.7 ± 0.9	10.1 ± 0.7^{+}	9.7 ± 0.6	9.4 ± 0.9	9.1 ± 1.1	9.6 ± 1.2
	IF	15.2 ± 3.4	21.8 ± 4.8$^{+\circ}$	20.8 ± 4.9	20.8 ± 4.8	20.3 ± 5.1	18.5 ± 5.3
$LVdP/dt_{max}$	N	3660 ± 210	2360 ± 180^{+}	2500 ± 200	2700 ± 280*	2890 ± 250*	3230 ± 270*
	IF	3240 ± 310	2100 ± 500^{+}	2120 ± 510	2250 ± 560	2330 ± 600	2590 ± 560*
SV	N	22.6 ± 1.1	22.3 ± 1.4	22.1 ± 1.2	23.1 ± 1.4	23.0 ± 1.8	24.0 ± 1.7
	IF	18.1 ± 1.1	17.0 ± 1.2	17.0 ± 0.9	19.1 ± 1.5*	20.1 ± 1.6*	20.8 ± 1.6*

*HR = heart rate (beats/min); MAP = mean arterial blood pressure (mmHg); CO = cardiac output (l/min); SVR = systemic vascular resistance (mmHg/min/l); LVEDP = left ventricular end-diastolic pressure (mmHg); $LVdP/dt_{max}$ = maximum rate of rise of left ventricular pressure (mmHg/s); SV = stroke volume (ml). Data are presented as mean ± SEM. * $p<0.05$ versus Baseline; $^{+}$ Baseline significantly different ($p<0.05$) versus pre-propranolol; $^{\circ}$ propranolol induced changes at Baseline significantly different ($p<0.05$) from propranolol induced changes in normal animals.*

Hemodynamic effects of bimakalim after beta-adrenoceptor blockade (Table 2)

Non-selective beta-adrenoceptor blockade did not effects the vasodilator response to bimakalim in either group of animals. Also the rise in cardiac output after bimakalim was similar. However, in the normal conscious pigs, the increases in heart rate (39 ± 8% at the highest dose) and $LVdP/dt_{max}$ (36 ± 5%) were attenuated compared to the increments without beta-adrenoceptor blockade. This blunting effect of beta-adrenoceptor blockade on the responses of heart rate and $LVdP/dt_{max}$ after bimakalim infusion was more pronounced in the animals with impaired left ventricular function. Due to this effect on heart rate the bimakalim-induced increase in cardiac output was also attenuated. In contrast to the animals with a normal cardiac function, but similar to the experiments without propranolol in the same model, infusion of bimakalim after beta-adrenoceptor blockade resulted in the animals after myocardial infarction in a decrease of 16 ± 6% ($p<0.05$) of the left ventricular end-diastolic pressure at the highest dose.

Discussion

The present study, performed in conscious pigs, shows that bimakalim is a potent vasodilator, both in normal pigs and in pigs with a three week old myocardial infarction. The study also indicates that the arterial vasodilator capacity of bimakalim exceeds that of nicorandil by approximately a factor of 100. This confirms the earlier observations with the two compounds in anaesthetized pigs (Sassen et al., 1990 and Verdouw et al., 1987).

There are, however, also some distinct differences compared to the observations in anaesthetized animals. For instance, in the same dose range as in the present study, cardiac output and $LVdP/dt_{max}$ did not change. While left ventricular end-diastolic pressure and stroke volume decreased during infusion of bimakalim in anaesthetized pigs (Sassen et al., 1990). The major reason for the increase in cardiac output in the present study is the maintained stroke volume, as the increases in heart rate were similar in both studies. The finding that in the normal conscious animals left ventricular end-diastolic pressure did not change is certainly an important factor that stroke volume did not change in the present study, and most likely also contributed to the modest increase in $LVdP/dt_{max}$.

In the animals with myocardial infarction, we found a mild effect on left ventricular end-diastolic pressure, but only with the highest dose. In this respect the actions of

bimakalim resemble more closely those of the dihydropyridine calcium antagonists nisoldipine and elgodipine (Van der Giessen et al., 1989, Duncker et al., 1987, Van Woerkens et al., 1991 and Sassen et al., 1990) than nicorandil (Sakai et al., 1980 and present study). The more pronounced effect of nicorandil on left ventricular end-diastolic pressure in the normal animals was only the only difference between the two compounds and most likely reflects the nitrate-like properties of nicorandil.

Nonselective beta-adrenoceptor blockade attenuated, but did not abolish, the bimakalim-induced increases in heart rate and $LVdP/dt_{max}$. Modest increases in heart rate after beta-adrenoceptor blockade have also been reported for dihydropyridine-derivatives in pigs (Duncker et al., 1987), dogs (Warltier et al., 1984) and humans (Silke et al., 1986) and are most likely due to withdrawal of parasympathetic tone (Nakaya et al., 1983). However, in view of the chronotropic actions of bimakalim during intracoronairy administration (Sassen et al., 1990), we cannot exclude a direct effect of the drug on heart rate.

Potassium channel activators have potentially negative inotropic properties (Yanagisawa et al., 1988), but the selectivity for vascular smooth muscle outweighs that for the myocardium (Gotanda et al., 1988). Findlay et al. (1989) have indeed shown that bimakalim is capable to inhibit both the electrical and the mechanical activity of cardiac muscle, but the required dose for cardiac muscle was much higher than for vascular smooth muscle. In the present study we still found a moderate 47% increase in $LVdP/dt_{max}$ in the animals with the infarctions, while the increase in heart rate was only 37%, and arterial blood pressure decreased by 115 (Table 2). Since under this condition systemic vascular resistance decreased by 38%, we may conclude that also in conscious animals with even a impaired left ventricular function, the negative inotropic effects of bimakalim are negligible.

From the present study we may conclude that in conscious pigs bimakalim was considerably more potent as an arterial vasodilator than nicorandil. The potential negative inotropic effects of bimakalim proved to be negligible, even when myocardial function was impaired. The compound may therefore be of interest in a variety of clinical situations which require a lowering of systemic vascular resistance.

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References

Buckingham RE, Clapham JC, Hamilton TC, Loryman SD, Norton J and Poyser RH. BRL 34915, a novel antihypertensive agent: comparison of effects on blood pressure and other haemodynamic parameters with those of nifedipine in animal models. *J Cardiovasc Pharmacol* 1986; 8: 798-804.

Cook NS. The pharmacology of potassium channels and their therapeutic potential. *TIPS* 9; 21-28, 1988.

De Peyer JE, Lues I, Gericke R and Häusler G. Characterization of a novel K^+ channel activator, EMD 52 692, in electrophysiological and pharmacological experiments. *Pflügers Arch* 414; S191, 1989.

Duncker DJ, Saxena PR and Verdouw PD. Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *Eur J Pharmacol* 156; 401-409, 1987.

Findlay I, Deroubaix E, Guiraudou P and Coraboeuf E. Effects of activation of ATP-sensitive K^+ channels in mammalion ventricular myocytes. *Am J Physiol* 257 (Heart Circ Physiol 26); H1551-H1559, 1989.

Gericke R, Lues I, De Peyer JE and Haeusier G. Electrophysiological and pharmacological characterization of EMD 52 692. A new potassium channel activator. *Naunyn-Schmiedeberg's Arch Pharmacol* 1989; 339: R62.

Gotanda K, Satoh K and Taira N. Is the cardiovascular profile of BRL 34915 characteristic of potassium channel activation? *J Cardiovasc Pharmacol* 12; 239-246, 1988.

Hamilton TC, Weir SW and Weston AH. Comparison of the effects of BRL 34915 and verapamil on electrical and mechanical activity in rat portal vein. *Br J Pharmacol* 88; 103-11, 1986.

Nakaya H, Schwartz A and Millard RW. Reflex chronotropic and inotropic effects of calcium channel-blocking agents in conscious dogs. Diltiazem, verapamil and nifedipine compared. *Circ Res* 52; 302-311, 1983.

Richer C, Mulder P, Dousseau MP, Gautier P and Guidicelli JF. Systemic and regional haemodynamic interactions between K^+ channel openers and the sympathetic nervous system in the SHR. *Br J Pharmacol* 100; 557-563, 1990.

Sakai K, Ohba Y, Akima M, Kamiyama H, Hinohara Y and Nakano H. Pharmacodynamic and metabolism studies on a new coronary vasodilator N-(2-hydroxyethyl)nicotinamide nitrate (SG-75). *Jpn J Pharmacol* 30; 881-890, 1980.

Sassen LMA, Duncker DJGM, Gho BCG, Diekmann HW and Verdouw PD. Haemodynamic profile of the potassium channel activator EMD 52 692 in

anaesthetized pigs. *Br J Pharmacol* 101; 605-614, 1990.

Sassen LMA, Soei LK, Heere ThJM, Van Woerkens LJ, Saxena PR and Verdouw PD. Nicorandil and cardiovascular performance in anaesthetized pigs with a concentric coronary artery stenosis. *Naunyn-Schmiedeberg's Arch Pharmacol* 340; 733-739, 1989.

Sassen LMA, Soei LK, Koning MMG, Verdouw PD. The central and regional cardiovascular responses to intravenous and intracoronary administration of the phenyldihydropyridine elgodipine in anaesthetized pigs. *Br J Pharmacol* 99; 355-363, 1990.

Silke B, Verma SP, Midtbo KA, et al. A haemodynamic study of the effects of combined slow channel blockade (nisoldipine) and beta-blockade (metoprolol) in coronary heart disease. *Int J Cardiol* 13; 231-241, 1986.

Taira N. Similarity and dissimilarity in the mode and mechanism of action between nicorandil and classical nitrates: an overview. *J Cardiovasc Pharmacol* 10; S1-S9, 1981.

Taira N. Nicorandil as a hybrid between nitrates and potassium channel activators. *Am J Cardiol* 63; 18J-24J, 1989.

Taira N, Satoh K, Yanagisawa T, Imai Y and Hiwatari M. Pharmacological profile of a new coronary vasodilator drug 2-nicotinamidoethyl nitrate (SG-75). *Clin Exp Pharmacol Physiol* 6; 301-316, 1979.

Van der Giessen WJ, Van Woerkens LJ, Duncker DJ, Roelandt JRTC and Verdouw PD. The acute hemodynamic effect of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J Cardiovasc Pharmacol* 14; 653-658, 1989.

Van Woerkens LJ, Schotman SN, Van der Giessen WJ and Verdouw PD. Cardiovascular effects of elgodipine in conscious pigs with a normal coronary circulation and conscious pigs with a healed myocardial infarction. *J Cardiovasc Pharmacol* 17; 976-982, 1991.

Van Woerkens LJ, Van der Giessen WJ and Verdouw PD. Cardiovascular profile of 5 novel nitrate-esters: A comparative study with nitroglycerin in conscious pigs with and without left ventricular dysfunction. *Br J Pharmacol*, 104; 7-14, 1991.

Van Woerkens LJ, Van der Giessen WJ and Verdouw PD. The selective bradycardic effects of UL-FS 49 do not adversely affect left ventricular function in conscious pigs with chronic coronary artery occlusion. Cardiovasc Drugs Ther (in press).

Verdouw PD, Sassen LMA, Duncker DJ, Schmeets IOL, Rensen RJ and Saxena PR. Nicorandil-induced changes in the distribution of cardiac output and coronary blood flow in pigs. *Naunyn-Schmiedeberg's Arch Pharmacol* 336; 352-358, 1987.

Warltier DC, Zyvoloski MG, Gross GJ and Brooks HJ. Comparative actions of

dihydropyridine slow channel calcium blocking agents in conscious dogs: Systemic and coronary hemodynamics with and without combined beta-adrenergic blockade. *J Pharmacol Exp Ther* 230: 367-375, 1984.

Yanagisawa T, Hashimoto H and Taira N. The negative inotropic effect of nicorandil is independent of cyclic GMP changes: a comparison with pinacidil and cromakalim in canine atrial tissue. *Br J Pharmacol* 95: 393-398, 1988.

Chapter 6

Cardiovascular profile of 5 novel nitrate-esters: A comparitive study with nitroglycerin in conscious pigs with and without left ventricular dysfunction.

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Cardiovascular profile of 5 novel nitrate-esters: a comparative study with nitroglycerin in pigs with and without left ventricular dysfunction

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1 Four cumulative 10 min intravenous infusions of 0.05, 0.2, 0.5 and 2.0 mg min^{-1} were used to compare the cardiovascular profile of 5 novel nitrate-esters dissolved in Intralipid 10% to that of nitroglycerin (GTN) in conscious pigs.

2 Infusion of Intralipid 10% alone had no effect on any of the systemic haemodynamic parameters. GTN infusions decreased mean arterial blood pressure dose-dependently from 94 ± 2 mmHg to 79 ± 3 mmHg ($P < 0.05$) and raised cardiac output from 2.74 ± 0.09 l min^{-1} to 3.40 ± 0.18 l min^{-1} ($P < 0.05$) due to an increase in heart rate (by up to 43 ± 3%), as stroke volume decreased slightly. Systemic vascular resistance decreased (by 32 ± 3%) and left ventricular end-diastolic pressure fell from 5.2 ± 0.4 mmHg to 2.2 ± 0.5 mmHg (both $P < 0.05$).

3 The novel compounds CEDO 8811, CEDO 8834 and CEDO 8901 increased cardiac output only at the highest dose (7%, 8% and 9%, respectively). There was no change in mean arterial blood pressure as the increase in cardiac output was counterbalanced by arterial vasodilatation. All three compounds reduced left ventricular end-diastolic pressure slightly.

4 CEDO 8816 was a more potent arterial and venodilator than the aforementioned CEDO compounds, as the decreases in systemic vascular resistance and left ventricular end-diastolic pressure were already significant at lower doses. The fall in stroke volume was fully compensated by the increase in heart rate and as a result cardiac output increased by 11 ± 3% ($P < 0.05$) at the highest dose.

5 CEDO 8956 was the most potent vasodilator of the novel compounds and exhibited a cardiovascular profile similar to that of GTN. Left ventricular end-diastolic pressure decreased significantly during infusion of 0.2 mg min^{-1}. Mean arterial blood pressure decreased by 11 ± 2% ($P < 0.05$) in spite of an increase in cardiac output by up to 20 ± 2% ($P < 0.05$), due to a decrease (by 27 ± 1%, $P < 0.05$) in systemic vascular resistance. The increases in heart rate (20 ± 5%, $P < 0.05$) and $LVdP/dt_{max}$ (38 ± 4%, $P < 0.05$) were, however, considerably less after CEDO 8956 than after GTN.

6 The potential of CEDO 8956 in the treatment of chronic left ventricular dysfunction was evaluated during administration to conscious pigs (21–23 kg), in which the left circumflex coronary artery was ligated 4 weeks earlier. In these animals, baseline values for cardiac output and $LVdP/dt_{max}$ were lower and those of systemic vascular resistance and left ventricular end-diastolic pressure were higher than in the first group of experiments.

7 Both GTN and CEDO 8956 in doses of 0.05 to 2.0 mg min^{-1} increased cardiac output dose-dependently (by up to 34% and 19%, respectively). The decrease in systemic vascular resistance was larger with GTN (35%) than with CEDO 8956 (17%), which resulted in a 13% decrease in mean arterial pressure during infusion of GTN, whereas there was no change in mean arterial pressure during infusion of CEDO 8956. Both compounds increased $LVdP/dt_{max}$ (by 48% and 30%, respectively) and lowered left ventricular end-diastolic pressure to normal levels.

8 At a dose of 1.0 mg min^{-1}, both GTN and CEDO 8956 increased left ventricular blood flow parallel to the increase in myocardial oxygen demand. At this dose, GTN also caused vasodilatation in the vascular beds of the brain, kidneys and adrenals. With CEDO 8956 no significant changes were achieved.

9 We conclude that the cardiovascular profile of CEDO 8956 in both normal animals and in animals with chronic left ventricular dysfunction warrants further study on its usefulness in the treatment of a number of cardiovascular disorders.

Keywords: Nitrate-esters; nitroglycerin; myocardial infarction; left ventricular dysfunction; systemic haemodynamics; coronary blood flow; regional blood flows; conscious pigs.

Introduction

Nitroglycerin (glyceryltrinitrate, GTN) has remained one of the major drugs used in the acute treatment of ischaemic heart disease, despite an attenuation of the therapeutic effects in a substantial group of patients in the long term. In search of new nitrate-like drugs a number of structurally different compounds have been synthesized. In this study we evaluated the systemic haemodynamic effects of these 5 novel nitrate-esters in conscious instrumented pigs and compared the results to those obtained with GTN. We have earlier shown that the magnitude of the responses obtained under pathophysiological conditions, might be different from those obtained under normal conditions (Van der Giessen *et al.*, 1989). We therefore selected the most potent of the novel compounds for further study in conscious pigs with mild left ventricular dysfunction secondary to a chronic coronary artery occlusion. The major characteristics of this model are a depressed cardiac output and myocardial contractility, while left ventricular filling pressure is elevated. Mean arterial blood pressure is maintained by peripheral vasoconstriction. In this model, in addition to the effects on systemic haemodynamics, we also evaluated the effects on regional blood flows using radioactive labelled microspheres.

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Methods

The experimental procedures were approved by the Committee on animal experiments of the Erasmus University Rotterdam, and complied to the guidelines for the use and care of experimental animals as put forward by the council of the American Physiological Society (DHEW Publication No. (NIH) 80-23, 1980).

Surgical procedures

After an overnight fast, 25 cross-bred Landrace x Yorkshire pigs of either sex (19–21 kg at the time of surgery), pretreated with 600 mg of a mixture of procaine penicillin-G and benzathine penicillin-G intramuscularly (Duplocillin, Gist-Brocades NV, Delft, The Netherlands), were sedated with an intramuscular injection of 30 mg kg^{-1} ketamine HCl (Aeskoket, Aesculaap BV, Boxtel, The Netherlands). Subsequently the animals were intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1:2) to which 1–4% (v/v) enflurane was added. Under sterile conditions, a jugular vein and a common carotid artery were cannulated for infusion of drugs or solvent and measurement of arterial blood pressure, respectively. The chest was opened via the third left intercostal space and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for the measurement of aortic blood flow. The heart was exposed via the fifth intercostal space and a pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) 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.

In 16 of the 25 animals, the proximal segment of the left circumflex coronary artery (LCXCA) was permanently ligated for the induction of a myocardial infarction. In these animals the left atrial cannula was also used for the injection of radioactive microspheres to determine regional blood flows. After instrumentation was completed, a period of 30 min was allowed before closure of the chest. During this period ventricular tachycardia and ventricular fibrillation (occurring exclusively in the group with coronary artery ligation) were treated with d.c.-countershock. The chest was then closed and the wires tunnelled to the back, and the animals were allowed to recover from surgery.

Post-surgical period

The animals received daily intravenous doses of 500 mg amoxicillin (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and, during the first week only, 500 mg kanamycin (Kanymex, Gist Brocades N.V., Delft, The Netherlands) to prevent infection. Catheters were flushed daily with an isotonic saline solution containing 500 iu ml^{-1} heparin. During the first month of the post-operative recovery period the animals were adapted to the laboratory facilities (8 to 10 sessions), while haemodynamic parameters were monitored. The experimental protocols were executed when systemic haemodynamics remained stable for at least 1 h, usually 4 weeks after instrumentation. All measurements were done while the animals were quietly resting in a constraining jacket.

Two animals with an intact coronary circulation were excluded from further study because of failure of the electrical signals. From the 16 pigs in which the left circumflex coronary artery was occluded, 5 animals died suddenly during the early post-operative period, most likely secondary to a ventricular arrhythmia. One animal was killed because of an infection.

Experimental protocols

In the animals with the intact coronary circulation 4 consecutive 10 min intravenous infusions with increasing doses of 0.05, 0.2, 0.5 and 2.0 mg min^{-1} (2.2 ± 0.1, 8.7 ± 0.2, 21.8 ± 0.5 and 87 ± 2 $\mu g\,kg^{-1}\,min^{-1}$, respectively) GTN, CEDO 8956, CEDO 8811, CEDO 8816, CEDO 8834 or CEDO 8901 dissolved in Intralipid 10% or equal volumes of Intralipid 10% were administered during separate runs of the protocol. Heart rate, arterial blood pressure, mean aortic blood flow, left ventricular pressure and its first derivative ($LVdP/dt$) were recorded at the end of each infusion period. Stroke volume was calculated by dividing mean aortic blood flow and heart rate, while systemic vascular resistance was determined by dividing mean arterial blood pressure and mean aortic blood flow. In all experiments infusions of different compounds or solvent in the same animals were separated by at least 24 h.

In the animals with the infarction 5 consecutive 10 min intravenous infusions with increasing doses of 0.05, 0.2, 0.5, 1.0 and 2.0 mg min^{-1} (2.3 ± 0.1, 9.2 ± 0.3, 23.0 ± 0.8, 46.1 ± 1.5 and 92 ± 3 $\mu g\,kg^{-1}\,min^{-1}$, respectively) were used to compare the cardiovascular effects of CEDO 8956 to those of GTN. As control, the Intralipid 10% solvent was infused with equal volumes. In these animals regional organ blood flows were also determined at baseline and after infusion of 1 mg min^{-1} of both compounds, by injection of a batch of 1–2 × 10^6 carbonized plastic microspheres (15 ± 1 μm (s.d.) in diameter) labelled with either ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce (NEN Chemicals GmbH, Dreieich, Germany) into the left atrium. To calculate regional blood flows a reference blood sample was withdrawn from the cannula in the carotid artery at a rate of 10 ml min^{-1}, starting 15 s before the injection of microspheres, until 90 s after completion of the injection of the microspheres. At the end of the experiments animals were killed with an overdose of sodium pentobarbitone. From the animals various organs (adrenals, liver, spleen, stomach, small intestine, brain and kidneys) and representative aliquots of several tissues (abdominal skin, skeletal muscle) were excised, weighed and put into vials. The hearts were fixed in formaldehyde (10% v/v) and 48 h later the atria and right ventricle were cut off the left ventricle. The normal myocardium of the left ventricle was divided into three layers of equal thickness: subepicardium, mid-myocardium and subendocardium. The infarcted area of the left ventricle was separated from the normal myocardium, and counted as a whole.

The radioactivity was counted and the amount of blood flow to the various tissues (Q_{tis}) was calculated as:

$$Q_{tis}\ (\text{ml}\,\text{min}^{-1}) = (I_{tis}/I_{art}) \times Q_{art},$$

where I_{tis} and I_{art} are the radioactivity in a particular tissue and that of the arterial blood sample respectively, and Q_{art} is the rate of withdrawal of the blood sample.

Drugs

The drugs used in this study were CEDO 8811 (3,3-diphenyl-1-propanol nitrate), CEDO 8816 (1,6-hexanediol dinitrate), CEDO 8834 (3-phenyl-3-(4-pyridyl)-1-propanol nitrate, 4-methylbenzenesulphonate), CEDO 8901 (1-(4-nitrophenoxy)-2, 3-propanediol dinitrate), and CEDO 8956 (1,4-(*trans*)-di(hydroxymethyl)cyclohexane dinitrate).

All CEDO compounds were synthesized by Cedona Pharmaceuticals, Haarlem, The Netherlands, by standard methods as described elsewhere (European Patent Application EP 0 35 9335 A2) and dissolved in Intralipid 10% (Kabi Vitrum, Stockholm, Sweden) in a concentration of 1 mg ml^{-1}. The compounds were made available by courtesy of Drs. J. Bron and J.F. van der Werf. GTN (Nitro-POHL, G. Pohl-Boskamp GmbH, Hohenlockstedt, Germany) was used in an aqueous solution of 1 mg ml^{-1}.

Statistical analysis

All data are presented as the mean ± s.e.mean. The significance of the effects of the drugs have been evaluated by comparing the changes from baseline induced by the drugs to the changes from baseline during infusion of the solvent, using analysis of variance. Significance was accepted for $P < 0.05$. A

Table 1 Cardiovascular effects of solvent (Intralipid 10%) in 7 conscious pigs with a normal coronary circulation (control) and in 9 conscious pigs with left ventricular dysfunction (LVD) secondary to a chronic occlusion of the left circumflex coronary artery

			Intralipid (ml min^{-1}) *for 10 min*				
		Baseline	*0.05*	*0.2*	*0.5*	*1.0*	*2.0*
CO	Control	2.71 ± 0.11	2.69 ± 0.10	2.71 ± 0.11	2.71 ± 0.12		2.70 ± 0.14
	LVD	2.27 ± 0.15*	2.26 ± 0.15	2.27 ± 0.15	2.27 ± 0.14	2.25 ± 0.14	2.27 ± 0.15
HR	Control	128 ± 4	129 ± 5	127 ± 3	131 ± 4		129 ± 5
	LVD	115 ± 6	114 ± 7	113 ± 5	113 ± 6	113 ± 6	114 ± 6
SV	Control	21.3 ± 0.9	21.1 ± 1.2	21.2 ± 0.7	20.8 ± 1.2		21.0 ± 1.3
	LVD	20.1 ± 1.2	20.1 ± 1.3	20.2 ± 1.2	20.3 ± 1.1	20.2 ± 1.2	20.0 ± 1.1
SAP	Control	114 ± 4	114 ± 4	114 ± 4	115 ± 4		114 ± 4
	LVD	118 ± 4	117 ± 3	117 ± 4	116 ± 4	117 ± 4	119 ± 4
MAP	Control	98 ± 3	99 ± 4	98 ± 3	99 ± 3		99 ± 3
	LVD	95 ± 3	94 ± 3	96 ± 3	95 ± 4	96 ± 4	98 ± 4
DAP	Control	80 ± 4	81 ± 4	79 ± 3	82 ± 4		83 ± 3
	LVD	77 ± 3	76 ± 2	78 ± 3	77 ± 4	79 ± 3	81 ± 4
SVR	Control	36 ± 2	37 ± 2	37 ± 2	37 ± 3		37 ± 3
	LVD	43 ± 2*	42 ± 2	43 ± 2	43 ± 2	44 ± 2	44 ± 2
$LVdP/dt_{max}$	Control	3310 ± 310	3280 ± 340	3380 ± 330	3470 ± 360		3460 ± 360
	LVD	2800 ± 230*	2690 ± 230	2810 ± 220	2860 ± 260	2840 ± 250	2850 ± 230
LVEDP	Control	4.5 ± 0.3	4.2 ± 0.5	4.3 ± 0.4	4.2 ± 0.5		4.5 ± 0.4
	LVD	14.1 ± 1.4*	14.4 ± 1.5	14.0 ± 1.5	13.9 ± 1.6	13.8 ± 1.6	14.6 ± 1.6

CO = cardiac output (l min^{-1}); HR = heart rate (beats min^{-1}); SV = stroke volume (ml); SAP = systolic arterial blood pressure (mmHg); MAP = mean arterial blood pressure (mmHg); DAP = diastolic arterial blood pressure (mmHg); SVR = systemic vascular resistance (mmHg min l^{-1}); $LVdP/dt_{max}$ = maximum rate of rise of left ventricular pressure (mmHg s^{-1}); LVEDP = left ventricular end-diastolic pressure (mmHg); Data have been presented as mean ± s.e.mean; * $P < 0.05$ vs baseline data of pigs with intact coronary circulation (control).

Bonferroni correction was used because of comparison for multiple measurements. Statistical significance of the regression lines was determined by calculating the slopes of the regression lines of the individual animals and comparing the mean slope of each group of animals by unpaired Student's *t* test.

Results

Systemic haemodynamics during infusion of Intralipid (10%) in normal conscious pigs and in conscious pigs with chronic left ventricular dysfunction

Table 1 illustrates that infusion of Intralipid 10% did not lead to significant changes in any of the systemic haemodynamic parameters in the normal conscious pigs and in the animals with chronic left ventricular dysfunction. The table also shows that significant differences existed between the baseline values of both groups of animals. Similar to earlier reported findings (Van der Giessen *et al.* 1989; Van Woerkens *et al.*, 1991), we observed that occlusion of the left circumflex coronary artery had no effect on mean arterial blood pressure, because peripheral vasoconstriction counterbalanced the 20% decrease in cardiac output but that $LVdP/dt_{max}$ was 20% lower and left ventricular end-diastolic pressure had tripled in the animals with the occluded left circumflex coronary artery.

Systemic haemodynamic effects of nitroglycerin and the 5 novel nitrate-esters in normal conscious pigs

Intravenous administration of GTN caused a dose dependent increase in cardiac output (24 ± 5%, $P < 0.05$, Figure 1). Since the 43 ± 3% increase in heart rate exceeded the percentage increase in cardiac output it is obvious that stroke volume decreased ($P < 0.05$). In spite of the increase in cardiac output, mean arterial blood pressure decreased gradually. The changes in stroke volume and mean arterial pressure were only significant during the highest two infusion rates. Systemic vascular resistance had already decreased significantly after

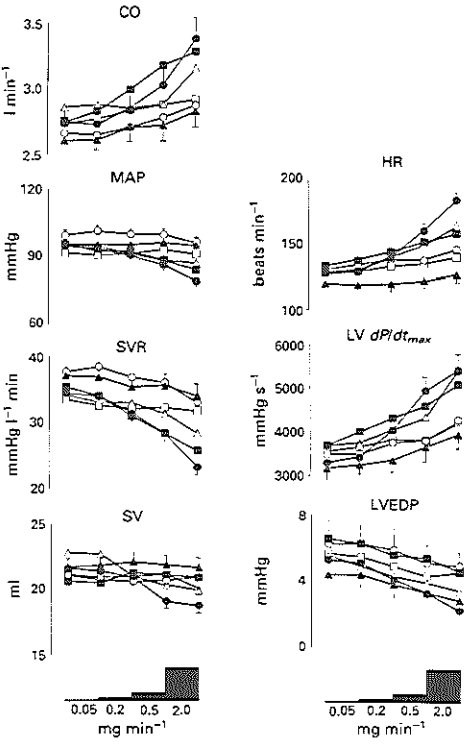

Figure 1 Systemic haemodynamic effects of nitroglycerin (GTN) (●) and 5 novel nitrate-esters CEDO 8811 (□), CEDO 8816 (△), CEDO 8834 (○), CEDO 8901 (▲) and CEDO 8956 (■) in conscious pigs (23–25 kg) with an intact coronary circulation. CO = cardiac output; HR = heart rate; MAP = mean arterial blood pressure; $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure; SVR = systemic vascular resistance; LVEDP = left ventricular end-diastolic pressure; SV = stroke volume. For reasons of clarity most of the bars for the s.e.mean and the levels of significance have been omitted in the figure (for details see the text). Data are the mean of 7 observations. The bars below the graphs depict the dose of the compounds in mg min^{-1}.

infusion of the second dose. $LVdP/dt_{max}$ increased dose-dependently. At each dose the increase in $LVdP/dt_{max}$ ($5 \pm 3\%$, $23 \pm 4\%$, $49 \pm 7\%$ and $63 \pm 4\%$, respectively) was larger than the increase in heart rate ($1 \pm 1\%$, $9 \pm 2\%$, $26 \pm 2\%$ and $43 \pm 3\%$, respectively). Left ventricular end-diastolic pressure decreased after infusion of the second dose and was further lowered during infusion of the highest dose.

No adverse reactions were observed after administration of any of the CEDO compounds. From these novel compounds, CEDO 8811, CEDO 8834 and CEDO 8901 were the least active as cardiac output increased only at the highest dose (by $7 \pm 2\%$, $8 \pm 2\%$ and $9 \pm 2\%$, respectively, all $P < 0.05$). Mean arterial blood pressure did not change during infusion of these compounds. Figure 1 also shows that the other haemodynamic parameters changed only moderately. With all three compounds the increases in $LVdP/dt_{max}$ ($21 \pm 7\%$, $20 \pm 5\%$ and $25 \pm 3\%$ after the highest dose, respectively, all $P < 0.05$) were larger than those in heart rate ($12 \pm 4\%$, $10 \pm 2\%$ and $6 \pm 4\%$ after the highest dose, respectively). Left ventricular end-diastolic pressure decreased slightly during the last two infusion steps.

CEDO 8816 was a slightly more potent arterial and venodilator than the three other CEDO compounds as systemic vascular resistance started to decrease (by $7 \pm 3\%$, $P < 0.05$) during infusion of $0.5\,mg\,min^{-1}$ and had decreased by $16 \pm 4\%$ ($P < 0.05$) after infusion of the highest dose. Left ventricular end-diastolic pressure had already decreased significantly during infusion of $0.2\,mg\,min^{-1}$. Stroke volume decreased ($8 \pm 2\%$, $12 \pm 4\%$ and $12 \pm 2\%$ after 0.2, 0.5 and $2.0\,mg\,min^{-1}$, respectively, $P < 0.05$), despite the reduction in afterload and the moderate positive inotropic effect of CEDO 8816 (reflected by a $46 \pm 8\%$ increase in $LVdP/dt_{max}$ against only a $27 \pm 5\%$ increase in heart rate at the highest dose). The fall in stroke volume was, however, fully compensated by the increase in heart rate ($9 \pm 1\%$, $16 \pm 4\%$ and $27 \pm 5\%$, respectively). Consequently, cardiac output increased slightly ($11 \pm 3\%$, $P < 0.05$) at the highest dose.

CEDO 8956 caused dose-dependent increases in cardiac output of $9 \pm 2\%$, $16 \pm 2\%$ and $20 \pm 2\%$ (all $P < 0.05$) during infusion of 0.2, 0.5 and $2.0\,mg\,min^{-1}$, respectively (Figure 1). The increases in cardiac output were secondary to the increases in heart rate, which were $9 \pm 5\%$, $14 \pm 4\%$ and $20 \pm 5\%$, respectively. At variance with the observations made during infusion of GTN, there were no decreases in stroke volume. Mean arterial blood pressure decreased by $7 \pm 2\%$ and $11 \pm 2\%$ during infusion of 0.5 and $2.0\,mg\,min^{-1}$, respectively. Systemic vascular resistance had already decreased by $12 \pm 2\%$ ($P < 0.05$) during infusion of $0.2\,mg\,min^{-1}$. During the highest infusion rate there was a further decrease ($27 \pm 1\%$, $P < 0.05$). Left ventricular end-diastolic pressure started to decrease during infusion of $0.2\,mg\,min^{-1}$ ($19 \pm 5\%$, $P < 0.05$) and further decreased by $33 \pm 5\%$ ($P < 0.05$) during infusion of $2.0\,mg\,min^{-1}$. $LVdP/dt_{max}$ started to increase significantly during infusion $0.2\,mg\,min^{-1}$ ($17 \pm 1\%$, $P < 0.05$). With the two higher infusion rates, the increments in $LVdP/dt_{max}$ were $25 \pm 2\%$ and $38 \pm 4\%$, respectively ($P < 0.05$).

Effects of nitroglycerin and CEDO 8956 on systemic haemodynamics in conscious pigs with chronic left ventricular dysfunction

In these animals cardiac output increased dose-dependently during infusion of GTN (Table 2). Similar to the animals with normal left ventricular function, the increase in cardiac output was due to an increase in heart rate. The increase in heart rate ($53 \pm 8\%$) exceeded the increase in cardiac output ($36 \pm 9\%$) and stroke volume must therefore have decreased. Mean arterial blood pressure started to decrease during infusion of $0.5\,mg\,min^{-1}$ and had fallen by $12 \pm 2\,mmHg$ ($P < 0.05$) at the end of the highest infusion rate. Because cardiac output increased, the decrease in arterial blood pressure must have been caused by arterial vasodilatation. Systemic vascular resistance was lowered by $8 \pm 2\%$ ($P < 0.05$) during infusion of $0.2\,mg\,min^{-1}$, and had fallen by $35 \pm 3\%$ after the last dose. $LVdP/dt_{max}$ increased dose-dependently by up to $48 \pm 6\%$ ($P < 0.05$), while left ventricular end-diastolic pressure was gradually lowered from $11.2 \pm 1.0\,mmHg$ to $5.6 \pm 1.2\,mmHg$ ($P < 0.05$).

The most striking difference between CEDO 8956 and GTN was that mean arterial blood pressure was unaffected during infusion of the former, whereas it decreased with GTN. Since cardiac output increased dose-dependently by up to 18% after CEDO 8956, we can calculate that the decrease in systemic vascular resistance (16%) was less than that observed during infusion of GTN (35%). As with GTN, an increase in heart rate was responsible for the increase in cardiac output.

Table 2 Cardiovascular effects of nitroglycerin (GTN) and CEDO 8956 (CED) in 10 conscious pigs with left ventricular dysfunction secondary to occlusion of the left circumflex coronary artery

			Infusions of GTN or CEDO 8956 for 10 min ($mg\,min^{-1}$)				
		Baseline	*0.05*	*0.2*	*0.5*	*1.0*	*2.0*
CO	GTN	2.12 ± 0.13	2.12 ± 0.12	2.24 ± 0.12*	2.45 ± 0.12*	2.61 ± 0.12*	2.84 ± 0.19*
	CED	2.32 ± 0.15	2.32 ± 0.15	2.38 ± 0.15	2.50 ± 0.14*	2.61 ± 0.16*	2.77 ± 0.19*
HR	GTN	117 ± 8	120 ± 8	127 ± 7*	139 ± 8*	157 ± 6*	174 ± 6*
	CED	116 ± 7	118 ± 8	124 ± 8	135 ± 8*	142 ± 7*†	150 ± 8*†
SV	GTN	18.7 ± 1.3	18.2 ± 1.3	18.0 ± 1.2	18.2 ± 1.5	16.9 ± 1.1	16.7 ± 1.3
	CED	20.5 ± 1.3	20.1 ± 1.4	19.6 ± 1.3	18.8 ± 1.2	18.5 ± 1.1	19.2 ± 1.1
SAP	GTN	112 ± 4	112 ± 4	109 ± 4	104 ± 3	101 ± 2*	99 ± 3*
	CED	112 ± 4	111 ± 4	106 ± 3*	104 ± 3*	106 ± 4	107 ± 4*†
MAP	GTN	92 ± 2	92 ± 3	90 ± 3	86 ± 2*	82 ± 2*	80 ± 3*
	CED	93 ± 3	91 ± 3	89 ± 3	88 ± 3	90 ± 3†	90 ± 4†
DAP	GTN	72 ± 2	75 ± 3	74 ± 3	71 ± 2	69 ± 2*	68 ± 2
	CED	74 ± 3	75 ± 3	73 ± 3	76 ± 3	76 ± 3†	78 ± 4†
SVR	GTN	44.8 ± 2.4	44.7 ± 2.7	41.1 ± 2.3*	36.0 ± 1.8*	31.9 ± 1.3*	28.7 ± 1.2*
	CED	41.0 ± 1.9	40.4 ± 1.8	38.2 ± 1.6*	35.9 ± 1.5*	35.2 ± 1.7*†	34.0 ± 2.0*†
$LVdP/dt_{max}$	GTN	2530 ± 240	2600 ± 240	2740 ± 240	3080 ± 290*	3300 ± 320*	3700 ± 350*
	CED	2620 ± 260	2620 ± 260	2950 ± 290	2950 ± 290*	3310 ± 380*	3440 ± 410*
LVEDP	GTN	11.2 ± 1.0	10.9 ± 1.0	9.9 ± 0.8	8.6 ± 0.8*	6.3 ± 1.0*	5.6 ± 1.2*
	CED	12.4 ± 1.3	11.7 ± 1.4*	10.9 ± 1.5*	9.7 ± 1.7*	8.5 ± 1.7*	7.6 ± 1.7*

CO = cardiac output ($l\,min^{-1}$); HR = heart rate ($beats\,min^{-1}$); SV = stroke volume (ml); SAP = systolic arterial blood pressure (mmHg); MAP = mean arterial blood pressure (mmHg); DAP = diastolic arterial blood pressure (mmHg); SVR = systemic vascular resistance ($mmHg\,min\,l^{-1}$); $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure ($mmHg\,s^{-1}$); LVEDP = left ventricular end-diastolic pressure (mmHg); Data have been presented as mean ± s.e.mean; * change from baseline statistically different ($P < 0.05$) from solvent-treated animals. †CEDO 8956-induced change from baseline statistically different ($P < 0.05$) from GTN-induced change.

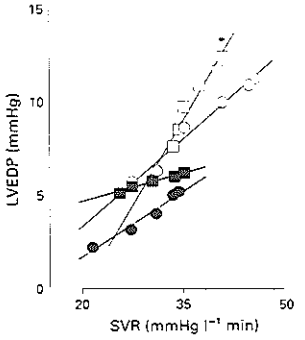

Figure 2 Relation between systemic vascular resistance (SVR) and left ventricular end-diastolic pressure (LVEDP) during incremental infusion rates of nitroglycerin (GTN) (●) and CEDO 8956 (■) in normal pigs and during incremental infusion rates of GTN (○) and CEDO 8956 (□) in conscious pigs with chronic left ventricular dysfunction. The slope of the CEDO 8956 regression line was significantly steeper in the animals with left ventricular dysfunction than in the normal animals (* $P < 0.05$).

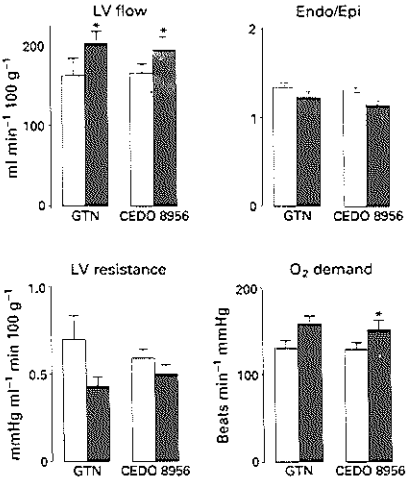

Figure 3 Transmural blood flow (LV flow) and its distribution (Endo/Epi), the coronary vascular resistance (LV resistance) remote from the site of infarction and O_2 demand in conscious pigs with a 4 week occlusion of the left circumflex coronary artery. Data were obtained at Baseline (□) and after a 10 min infusion of nitroglycerin (GTN) and CEDO 8956 at a rate of $46.1 \pm 1.5\,\mu g\,kg^{-1}\,min^{-1}$ (▨). * $P < 0.05$ vs baseline.

The increase in heart rate was significantly less with CEDO 8956 than with GTN. $LVdP/dt_{max}$ also increased (up to 30%), and like GTN, the increments were parallel to those in heart rate. Left ventricular end-diastolic pressure, however, decreased to the same degree as with GTN during the infusions of CEDO 8956.

Figure 2 illustrates the relation between the changes in systemic vascular resistance and in left ventricular end-diastolic pressure for the different experimental conditions. The figure shows that the venodilator capacity of CEDO 8956 was increased in the pigs with chronic left ventricular dysfunction as compared to the normal pigs.

Effects of nitroglycerin and CEDO 8956 on myocardial blood flow in animals with chronic left ventricular dysfunction

Because of the limited number of available isotopes, regional flow data could only be obtained at baseline and after administration of a single dose of each of the two drugs. The infusion rate of $1\,mg\,min^{-1}$ was chosen for both GTN and CEDO 8956. There were no differences in the baseline values of the two drugs (Figure 3). Myocardial O_2 demand, calculated as the product of heart rate and left ventricular systolic pressure, increased similarly during infusion of both GTN and CEDO 8956. Both GTN and CEDO 8956 increased transmural perfusion of the myocardium nourished by the non-occluded left anterior descending coronary artery. There was a modest trend towards a preferential increase of perfusion of the subepicardial layers, but the decreases in the ratio of the normalized endocardial and epicardial blood flows (endo/epi-ratio) did not reach levels of statistical significance with either drug. The decrease in the coronary vascular resistance was more pronounced with GTN than with CEDO 8956 (Figure 3). The perfusion of the central part of the myocardium in the distribution of the occluded left circumflex coronary artery increased significantly during infusion of GTN (from $25 \pm 4\,ml\,min^{-1}\ 100\,g^{-1}$ to $39 \pm 7\,ml\,min^{-1}\ 100\,g^{-1}$, $P < 0.05$), but not during that of CEDO 8956 (from $30 \pm 5\,ml\,min^{-1}\,100\,g^{-1}$ to $36 \pm 4\,ml\,min^{-1}\,100\,g^{-1}$).

Right ventricular blood flow increased more during administration of CEDO 8956 (from $105 \pm 9\,ml\,min^{-1}\ 100\,g^{-1}$ to $153 \pm 17\,ml\,min^{-1}\ 100\,g^{-1}$, $P < 0.05$) than during infusion of GTN (from $106 \pm 21\,ml\,min^{-1}$ to $136 \pm 13\,ml\,min^{-1}\ 100\,g^{-1}$). This difference was caused by the different effects of the drugs on arterial perfusion pressure as the decreases in coronary vascular resistance were very similar with both compounds (from $0.97 \pm 0.11\,mmHg\,min\,ml^{-1}\ 100\,g$ to $0.67 \pm 0.07\,mmHg\,min\,ml^{-1}\ 100\,g$ for GTN and from $0.96 \pm 0.10\,mmHg\,min\,ml^{-1}\ 100\,g$ to $0.67 \pm 0.09\,mmHg\ min\,ml^{-1}\ 100\,g$ for CEDO 8956).

Effects of nitroglycerin and CEDO 8956 on peripheral organ perfusion in conscious pigs with chronic left ventricular dysfunction

Infusion of $1\,mg\,min^{-1}$ of GTN resulted in significant increases in blood flow to the adrenals and the kidneys (Table 3). Calculation of the regional vascular resistances revealed that GTN induced vasodilatation in the vascular beds perfusing the brain, kidneys, adrenals and spleen but that with this dose no effect was observed in skeletal muscle, skin and liver (hepatic artery circulation only) (Table 4). CEDO 8956 was less potent at this dose in lowering systemic vascular resistance than GTN, which explains that with CEDO 8956 only a significant reduction in the resistance of the vascular bed of the adrenals was observed and that the changes in the cerebral and renal vascular resistances did not reach a level of statistical significance.

Discussion

GTN is an effective therapeutic agent in the treatment of a number of cardiovascular disorders including myocardial ischaemia, myocardial infarction and congestive heart failure (Mason *et al.*, 1971; Flaherty *et al.*, 1975; Flaherty, 1983; Packer, 1983). The principal mechanism is a direct relaxation of vascular smooth muscle and a number of investigators believed that the effect on the venous capacitance vessels was more pronounced than on the arterial resistance vessels, while others have reported less marked differences. The present study as far as performed in normal conscious pigs demonstrates a marked reduction in both left ventricular end-

Table 3 Effects of 10 min intravenous infusions of nitroglycerin (GTN) and CEDO 8956 at a rate of 1 mg min^{-1} on regional blood flows in 10 conscious pigs with left ventricular dysfunction secondary to chronic occlusion of the left circumflex coronary artery

	Baseline	*GTN*	*Baseline*	*CEDO 89*
Brain	75 ± 5	82 ± 2	71 ± 2	78 ± 4
Kidneys	421 ± 32	496 ± 40*†	424 ± 28	437 ± 28
Liver	22 ± 5	24 ± 6	22 ± 4	23 ± 5
Spleen	275 ± 22	299 ± 32	273 ± 26	228 ± 23
Adrenals	119 ± 12	219 ± 17*†	144 ± 18	166 ± 13
Stomach	47 ± 6	52 ± 7	50 ± 8	50 ± 10
Small intestine	52 ± 5	52 ± 3	50 ± 5	55 ± 7
Skin (abdomen)	6.7 ± 0.9	6.4 ± 1.1	6.9 ± 0.8	5.5 ± 0.6
Muscle (gluteus maximus)	4.9 ± 1.3	4.4 ± 0.6	3.9 ± 0.3	4.9 ± 0.7
Muscle (erector spinae)	3.8 ± 0.5	3.0 ± 0.4	3.3 ± 0.4	4.1 ± 1.1

Blood flows are expressed in ml min^{-1} 100 g^{-1} and are shown as mean ± s.e.mean. * $P < 0.05$ vs baseline; † $P < 0.05$ for GTN-induced change from baseline vs CEDO 8956-induced change from baseline.

Table 4 Effects of 10 min intravenous infusions of nitroglycerin (GTN) and CEDO 8956 at a rate of 1 mg min^{-1} on regional vascular resistances in 10 conscious pigs with left ventricular dysfunction secondary to a chronic occlusion of the left circumflex coronary artery

	Baseline	*GTN*	*Baseline*	*CEDO 895*
Brain	1.26 ± 0.09	1.01 ± 0.03*†	1.31 ± 0.05	1.17 ± 0.07
Kidneys	0.23 ± 0.02	0.18 ± 0.02*†	0.23 ± 0.02	0.21 ± 0.02
Liver	8.8 ± 3.1	6.9 ± 2.1	6.5 ± 1.7	11.1 ± 6.2
Spleen	0.35 ± 0.03	0.32 ± 0.05†	0.36 ± 0.03	0.43 ± 0.05
Adrenals	0.86 ± 0.10	0.40 ± 0.04*†	0.74 ± 0.09	0.57 ± 0.05*
Stomach	2.2 ± 0.3	1.8 ± 0.2	2.2 ± 0.3	2.1 ± 0.2
Small intestine	1.9 ± 0.2	1.6 ± 0.1	2.1 ± 0.3	1.9 ± 0.3
Skin (abdomen)	17 ± 3	17 ± 3	15 ± 2	18 ± 2
Muscle (erector spinae)	27 ± 3	31 ± 4	32 ± 4	30 ± 4

Vascular resistances are expressed in mmHg min ml^{-1} 100 g and are reported as mean ± s.e.mean. * $P < 0.05$ vs baseline; † $P < 0.05$ for GTN-induced change from baseline vs CEDO 8956-induced change from baseline.

diastolic pressure and systemic vascular resisance during infusion of GTN. When GTN was administered in animals with chronic left ventricular dysfunction the effects on left ventricular end-diastolic pressure and systemic vascular resistance were not different from those in the normal animals (Figure 2).

Of the new compounds, CEDO 8956 demonstrated a cardiovascular profile similar to that of GTN, although in the normal conscious pigs, the effect on left ventricular filling pressure was less pronounced than during GTN infusion. It could be argued that the slightly smaller increase in heart rate during infusion of CEDO 8956 was a contributing factor to this observation, but earlier studies with a number of dihydropyridine derivatives have shown that in the range of the heart rates, reported in this study, the effect of heart rate on left ventricular end-diastolic pressure is minimal (Duncker *et al.*, 1988). In the animals with left ventricular dysfunction, however, CEDO 8956 lowered left ventricular end-diastolic pressure as effectively as GTN (Table 2), while the effect on the systemic vasculature remained comparable to that induced in the normal animals (Figure 2). Both compounds increased cardiac output by an increase in heart rate rather than in stroke volume, which would be more desirable. One must keep in mind, however, that the left ventricular filling pressures in the animals with left ventricular dysfunction, although elevated were not extremely high. It is very likely that reduction of higher left ventricular filling pressure would result in a more favourable effect on stroke volume. Nevertheless, an increase in heart rate, which is a common observation with all vasodilators, increases the myocardial oxygen demand and will, in particular in hypertrophied hearts, decrease coronary flow reserve (Hoffman, 1990). Adjuvant treatment with a specific bradycardic agent may then be attractive to unload the heart without reflex-mediated tachycardia and to increase stroke volume and coronary flow reserve (Verdouw *et al.*, 1981; Canty *et al.*, 1990). The finding that CEDO 8956 did not lower mean arterial blood pressure is not necessarily a disadvantage as Cruickshank *et al.* (1987) have shown that lowering high arterial blood pressure too much may be harmful.

Contrary to earlier reported observations in dogs (Capurro *et al.*, 1977), nitroglycerin has no beneficial effect on total coronary blood flow and its distribution within the myocardium of the pig after an acute coronary artery occlusion (Most *et al.*, 1978). In the present study GTN increased blood flow to the myocardium remote from the distribution of the occluded left circumflex coronary artery. The increase appeared to be related to the increase in myocardial oxygen demand, estimated by the heart rate-left ventricular systolic pressure product. In contrast to dogs, pigs do not usually have a significant coronary collateral circulation, which may explain the differences in the observations made by Capurro *et al.* (1977) and Most *et al.* (1978). After chronic occlusion a substantial collateral flow has been observed in the fibrotic tissue of the porcine myocardium, the magnitude of which can be altered by pharmacological interventions (Van der Giessen *et al.*, 1990a). In the present study GTN also increased the flow to the fibrotic segment. It is questionable whether this increase in flow has any clinical significance as we have not been able to recruit any function by inotropic stimulation (dobutamine infusion) in this infarcted segment (unpublished observation). CEDO 8956, albeit less, also showed an increase in flow to the normal segment. A smaller increase in the heart rate-left ventricular systolic pressure product may be an index for myocardial oxygen demand, the basis for this observation. The present data cannot be used to speculate on the effects of CEDO 8956 on myocardium which is perfused by partially occluded coronary arteries. Several studies (Macho & Vatner, 1981; Hintze & Vatner, 1983) have shown that the large epicardial vessels dilate in response to nitroglycerin. Dilatation of the stenosis, which is independent of an increase in coronary artery blood flow (Adachi *et al.*, 1987) may be a major mechanism in improving an impaired oxygen balance. The design of

the present study does not provide insight into a possible dilator effect on the large epicardial vessels by CEDO 8956.

The effects of GTN on organ blood flows other than the myocardium have only been addressed in a limited number of studies. Most of these studies are in anaesthetized animals in which GTN was administered with the aim of producing severe hypotension. Colley & Sivarajan (1984) observed that during GTN infusion in halothane-anaesthetized dogs, blood flows to the brain, kidneys, liver, gastrointestinal tract and skeletal muscle were maintained, despite a reduction of mean arterial blood pressure from 82 ± 4 mmHg to 45 ± 3 mmHg, due to a 50% decrease in systemic vascular resistance. Norlén (1988) found in methomidate-anaesthetized pigs that doses of GTN which decreased systemic vascular resistance by 28% and mean arterial blood pressure by 33%, blood flows to the heart, brain, kidneys, adrenal glands, stomach and liver (hepatic artery) were not affected, but that perfusion of the spleen had decreased by 25%. The present study differs considerably from these studies, as the dose of GTN at which regional blood flows and vascular resistances were determined, lowered mean arterial blood pressure by only 12 mmHg from its baseline of 92 mmHg. Furthermore the studies were performed in conscious animals with a lowered cardiac output due to impairment of left ventricular function. In the present study cerebral blood flow was maintained and renal blood flow increased, due to local vasodilatation (Table 4). The same dose of CEDO 8956 ($1\,mg\,min^{-1}$) had no effect on mean arterial blood pressure and cardiac output increased only by 12%. The effects on the regional vascular beds were therefore less pronounced than with GTN and only the vascular resistance of the adrenals was significantly decreased.

Both GTN and CEDO 8956 lacked an effect on skeletal muscle blood flow. Our present results are in agreement with an earlier report using isosorbide-dinitrate (Leier *et al.*, 1983). On the other hand we have shown an acute improvement in skeletal muscle perfusion by the calcium channel blocker nisoldipine (Van der Giessen *et al.*, 1990b). This may be important as Creager *et al.* (1985) showed in patients with heart failure that an improvement in exercise tolerance was paralleled by an increase in fore-arm blood flow. Due to the limited number of different microspheres available, regional organ blood flow data were not studied during infusion of the Intralipid 10% solvent. In an earlier paper (Van der Giessen *et al.*, 1990b), however, we showed that peripheral organ blood flows remained unchanged in conscious pigs, when systemic haemodynamic parameters remained stable, as was the case with infusion of the solvent in this report (Table 1).

In conclusion, from the novel compounds only CEDO 8956 had a cardiovascular profile similar to that of GTN and was therefore selected for further study in the conscious animals with chronic left ventricular dysfunction. In this model, CEDO 8956 normalized the depressed cardiac output by lowering left ventricular filling pressure and systemic vascular resistance. The radioactive labelled microsphere data showed that CEDO 8956, similar to GTN, caused dilatation of the coronary vascular bed remote from the infarcted area. The observations that the preload reduction by CEDO 8956 was more pronounced when left ventricular filling pressures were elevated, and that for the same decreases in left ventricular filling pressure the increase in heart rate by CEDO 8956 was less than with GTN may be attractive for cardiac patients. The cardiovascular profile of CEDO 8956, therefore warrants further studies to investigate its potential usefulness in the clinical setting. In further studies attention should be paid to the duration of action and the development of tolerance which could attenuate the therapeutic effects.

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References

ADACHI, H., TOMOIKE, H., NISHIJIMA, H., HAYASHI, Y., YAMADA, A. & NAKAMURA, M. (1987). Effects of verapamil and nitroglycerin on coronary and systemic hemodynamics in conscious dogs. *J. Pharmacol. Exp. Ther.*, **241**, 1072–1078.

CANTY, J.M., GIGLIA, J. & KANDATH, D. (1990). Effect of tachycardia on regional function and transmural myocardial perfusion during graded coronary pressure reduction in conscious dogs. *Circulation*, **82**, 1815–1825.

CAPURRO, N.L., KENT, K.M., SMITH, H.J., AAMODT, R. & EPSTEIN, S.E. (1977). Acute coronary occlusion: prolonged increase in collateral flow following brief administration of nitroglycerin and methoxamine. *Am. J. Cardiol.*, **39**, 679–683.

COLLEY, P.S. & SIVARAJAN, M. (1984). Regional blood flow in dogs during halothane anesthesia and controlled hypotension produced by nitroprusside or nitroglycerin. *Anesth. Analg.*, **63**, 503–510.

CREAGER, M.A., MASSIE, B.M., FAXON, D.P., FRIEDMAN, S.D., KRAMER, B.L., WEINER, D.A., RYAN, T.J., TOPIC, N. & MELIDOSSIAN, C.D. (1985). Acute and long-term effects of enalapril on the cardiovascular response to exercise and exercise tolerance in patients with congestive heart failure. *J. Am. Coll. Cardiol.*, **6**, 163–170.

CRUICKSHANK, J.M., THORP, J.M. & ZACHARIAS, F. (1987). Benefits and potential harm of lowering high blood pressure. *Lancet*, **i**, 581–583.

DUNCKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1988). Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *Eur. J. Pharmacol.*, **156**, 401–409.

FLAHERTY, J.T. (1983). Comparison of intravenous nitroglycerin and sodium nitroprusside in acute myocardial infarction. *Am. J. Med.*, **74**, (suppl. 6B), 53–60.

FLAHERTY, J.T., REID, P.R., KELLY, D.T., TAYLOR, D.R., WEISFELDT, M.L. & PITT, B. (1975). Intravenous nitroglycerin in acute myocardial infarction. *Circulation*, **51**, 132–139.

HINTZE, T.H. & VATNER, S.F. (1983). Comparison of effects of nifedipine and nitroglycerin on large and small coronary arteries and cardiac function in conscious dogs. *Circ. Res.*, (suppl. 1), **52**, 139–146.

HOFFMAN, J.I.E. (1990). Autoregulation and heart rate. *Circulation*, **82**, 1880–1881.

LEIER, C.V., HUSS, P., MAGORIEN, R.D. & UNVERFERTH, D.V. (1983). Improved exercise capacity and differing arterial and venous tolerance during chronic isosorbide dinitrate therapy for congestive heart failure. *Circulation*, **67**, 817–822.

MACHO, P. & VATNER, S.F. (1981). Effects of nitroglycerin and nitroprusside on large and small coronary vessels in conscious dogs. *Circulation*, **64**, 1101–1107.

MASON, D.T., ZELIS, R. & AMSTERDAM, E.A. (1971). Actions of the nitrites on the peripheral circulation and myocardial oxygen consumption: significance in the relief of angina pectoris. *Chest*, **59**, 296–305.

MOST, A.S., WILLIAMS, D.O. & MILLARD, R.W. (1978). Acute coronary occlusion in the pig: effect of nitroglycerin on regional myocardial blood flow. *Am. J. Cardiol.*, **42**, 947–953.

NORLÉN, K. (1988). Central and regional haemodynamics during controlled hypotension produced by adenosine, sodium nitroprusside and nitroglycerin. *Br. J. Anaesth.*, **61**, 186–193.

PACKER, M. (1983). New perspectives on therapeutic application of nitrates as vasodilator agents for severe chronic heart failure. *Am. J. Med.*, **74**, (suppl. 6B), 61–72.

VAN DER GIESSEN, W.J., DUNCKER, D.J., SAXENA, P.R. & VERDOUW, P.D. (1990a). Nimodipine has no effect on the cerebral circulation in conscious pigs, despite an increase in cardiac output. *Br. J. Pharmacol.*, **100**, 277–282.

VAN DER GIESSEN, W.J., VAN WOERKENS, L.J., DUNCKER, D.J., ROELANDT, J.R.T.C. & VERDOUW, P.D. (1989). The acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J. Cardiovasc. Pharmacol.*, **14**, 653–658.

VAN DER GIESSEN, W.J., VAN WOERKENS, L.J., SAXENA, P.R., ROELANDT, J.R.T.C. & VERDOUW, P.D. (1990b). Nisoldipine improves blood flow to skeletal muscles in conscious pigs with chronic heart failure. *Eur. Heart J.*, **11**, 552–559.

VAN WOERKENS, L.J., SCHOTMAN, S.N., VAN DER GIESSEN, W.J. & VERDOUW, P.D. (1991). Cardiovascular effects of elgodipine in conscious pigs with a normal coronary circulation and in conscious pigs with a healed myocardial infarction. *J. Cardiovasc. Pharmacol.*, (in press).

VERDOUW, P.D., HARTOG, J.M. & RUTTEMAN, A.M. (1981). Systemic and regional myocardial responses to AR-L 115 BS, a positive inotropic imidazo-pyridine, in the absence or in the presence of the bradycardiac action of alinidine. *Basic Res. Cardiol.*, **76**, 328–343.

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Chapter 7

The selective bradycardic effects of UL-FS 49 do not adversely effect left ventricular function in conscious pigs with chronic coronary artery occlusion.

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The Selective Bradycardic Effects of UL-FS 49 Do Not Adversely Affect Left Ventricular Function in Conscious Pigs with Chronic Coronary Artery Occlusion

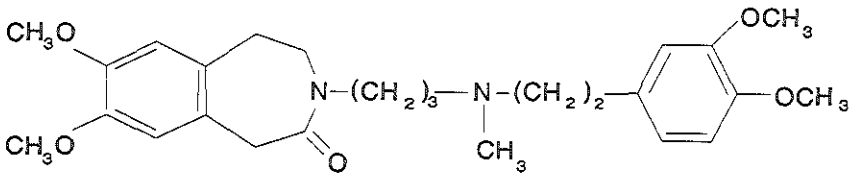
Summary

This study was designed to test whether the selective bradycardic effects of UL-FS 49 (zatebradine) were altered in the setting of chronic mild left ventricular dysfunction secondary to a myocardial infarction. We therefore administered four doses of UL-FS 49 (zatebradine) at 15 min intervals (cumulative doses of 10, 30, 100 and 300 $\mu g\ kg^{-1}$) to 8 normal conscious pigs and to 7 pigs in which the left circumflex coronary artery was occluded 3 weeks previously. Left ventricular dysfunction in this second group of animals was manifested by an increase in left ventricular end-diastolic pressure (LVEDP 11 ± 2 mmHg vs 7 ± 1 mmHg, respectively; $p<0.05$) and a decrease in $LVdP/dt_{max}$ (3020 ± 210 mmHg vs 3720 ± 210 mmHg, respectively; $p<0.05$). The results showed that UL-FS 49 (zatebradine) was equally effective in reducing heart rate in both groups of animals (from 126 ± 4 to 95 ± 2 beats min^{-1} and from 140 ± 5 to 98 ± 6 beats min^{-1} for the normal animals and for the animals with a chronic myocardial infarction (MI), respectively). The duration of left ventricular systole was not affected, but the duration of diastole was prolonged from 290 ± 10 to 420 ± 20 msec in the normal animals and from 250 ± 10 to 430 ± 30 msec in the animals with MI (both $p<0.05$). Up to 100 $\mu g\ kg^{-1}$ UL-FS 49 (zatebradine) did not affect arterial blood pressure, whereas $LVdP/dt_{max}$ and cardiac output decreased by less than 10% in either group. With the highest dose there were decreases in cardiac output (20%) and $LVdP/dt_{max}$ (15%) and a 5-6 mmHg increase in left ventricular end-diastolic pressure, in both groups. The data suggest that UL-FS 49 (zatebradine) in doses up to 100 $\mu g\ kg^{-1}$ may also in the setting of chronic mild left ventricular dysfunction be an attractive agent when heart rate has to be reduced selectively.

Introduction

A reduction in heart rate will improve myocardial oxygen balance by both reducing oxygen demand and improving oxygen supply, in particular of the subendocardial layers, the most vulnerable area of the myocardium. The above are the most important reasons for the anti-ischemic effects of selective bradycardic agents in the treatment of acute

experimental myocardial ischemia (Schamhardt et al, 1981, Guth et al., 1987, Krumpl et al., 1988 and Indolfi et al., 1989). Hoffman (1990) and Canty et al. (1990) recently pointed out that selective bradycardic agents may also be useful to reverse the adverse effect of tachycardia on the lower limit of subendocardial autoregulation. The last group of investigators observed that increasing the heart rate in dogs from 100 beats min^{-1} to 200 beats min^{-1} increased the minimal coronary perfusion pressure needed to ensure normal subendocardial perfusion by 60% (Canty et al., 1990). The mechanisms responsible for this increase in minimal coronary perfusion pressure are believed to be related to the tachycardia-induced myocardial oxygen consumption, which increases autoregulated coronary flow (Drake-Holland et al., 1984) and the tachycardia-induced reduction in diastolic time per minute, which decreases maximal coronary flow (Raff et al., 1972). In normal hearts a tachycardia-induced reduction in coronary flow reserve will not lead to subendocardial ischemia, but if coronary flow reserve is already impaired, for instance due to hypertrophy, myocardial ischemia may ensue when heart rate increases.



UL-FS 49

zatebradine

Figure 1 *The chemical structure of UL-FS 49.*

UL-FS 49 (1,3,4,5-tetra-hydro-7,8-dimethoxy-3[3-[[2-(3,4-dimethoxyphenyl)ethyl]-methylimino]-propyl]-2H-3-benzazepin-2-on-hydro-chloride; Fig. 1) (zatebradine) is a so-called specific bradycardic agent which lowers heart rate by a mechanism other than beta-adrenoceptor or calcium channel blockade (Kobinger and Lillie, 1984 and Lillie and Kobinger, 1986), although there are structural similarities with verapamil. Similar to alinidine, the mode of action of UL-FS 49 may be by an effect on anionic channels (Millar et al., 1981) or on the I_f channel (Bouman et al., 1985 and Snyders et al., 1985). UL-FS 49 has been shown to reduce predominantly heart rate (Kobinger and Lillie, 1984 and Lillie and Kobinger, 1986) and thereby improves perfusion and function of acutely ischemic myocardium (Guth et al,. 1987, Krumpl et al., 1988 and Indolfi et al., 1989). The effects of UL-FS 49 in animals with chronic left ventricular dysfunction have not yet been studied. We have, however, shown that in conscious pigs chronic left ventricular dysfunction may alter the quantitative as well as the qualitative effects of cardiovascular drugs (Van der Giessen et al., 1989 and Van Woerkens et al., 1991. Furthermore, when left ventricular function is chronically impaired, a reduction in heart rate by beta-adrenoceptor blockade often leads to a further deterioration of cardiovascular performance due to unwanted negative inotropy (Van Woerkens et al., 1991). In the present study we therefore evaluated the cardiovascular effects of UL-FS 49 in conscious pigs with a 3 week old myocardial infarction and compared the results to those obtained in a group of pigs with normal hearts, in order to establish whether the cardiovascular effects of UL-FS 49, in particular on diastolic perfusion time, were affected by the chronic left ventricular dysfunction.

Materials and Methods

Surgical procedures

The experimental procedures were approved by the Committee on Animal Experiments of the Erasmus University Rotterdam, and complied to the guidelines for the use and care of experimental animals as put forward by the Council of the American Physiological Society (DHEW Publication No. (NIH) 80-23, 1980).

After an overnight fast, 20 cross-bred Landrace x Yorkshire pigs of either sex (19-21 kg at the time of surgery), pretreated with 600 mg of a mixture of procaine penicillin-G and benzathine penicillin-G intramuscularly (Duplocillin, Gist-Brocades NV, Delft, The Netherlands), were sedated with an intramuscular injection of 30 mg kg^{-1} ketamine HCL (Aeskoket, Aesculaap BV, Boxtel, The Netherlands). Subsequently the animals were intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1:2)

to which 1-4% (v/v) enflurane was added. Under sterile conditions, a jugular vein and a common carotid artery were cannulated for infusion of drugs or solvent and measurement of arterial blood pressure, respectively. The chest was opened via the third left intercostal space and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for the measurement of aortic blood flow. The heart was exposed via the fifth intercostal space and a Konigsberg pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) 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.
In 12 animals the proximal segment of the left circumflex coronary artery (LCXCA) was permanently ligated for the induction of a myocardial infarction. Serious ventricular arrhythmias (sustained ventricular tachycardia or ventricular fibrillation) were treated with immediate DC countershock. After 30 min the chest was closed and the wires were tunnelled subcutaneously to the back, and the animals were allowed to recover from surgery.
The animals received daily intravenous doses of 500 mg amoxicilline (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and, during the first week only, kanamycin 500 mg (Kanymex, Gist Brocades N.V., Delft, The Netherlands) to prevent infection. Catheters were flushed daily with an isotonic saline solution containing 500 IU ml^{-1} heparin. During the first 3 weeks of the post operative recovery period the animals were adapted to the laboratory facilities (8 to 10 sessions), while hemodynamic parameters were monitored. The experimental protocols were executed when systemic hemodynamics and arterial blood gases remained stable for at least one hour, usually 3 weeks after instrumentation. All measurements were done while the animals were quietly resting in a constraining jacket.

Mortality

All eight animals with the intact coronary circulation could be used for the study. From the 12 pigs in which the left circumflex coronary artery was occluded, 4 animals died suddenly during the post-operative period, most likely secondary to a ventricular arrhythmia. One animal was excluded from the study because of failure of the electrical transducers.

Experimental protocols

After baseline hemodynamic measurements were obtained, each animal received 4 bolus infusions of UL-FS 49 of 10, 20, 70, and 200 $\mu g\ kg^{-1}$ (cumulative doses of 10, 30, 100 and 300 $\mu g\ kg^{-1}$) or the solvent of UL-FS 49 at 15 min intervals. At the end of each 15 min period, all hemodynamic measurements were repeated before the next dose was administered. The duration of systole and diastole were determined from the arterial blood pressure recordings. The nadir of diastolic arterial blood pressure was taken as the onset and the occurrence of the incisura as the end of left ventricular ejection. The experiments with the drug and the solvent were separated by at least 24 hours. Volumes of the solvent corresponded with those administered during the UL-FS 49 infusions.

Drugs

UL-FS 49 was supplied by Dr. Karl Thomae GmbH (Biberach an der Riss, Germany). The drug was dissolved in saline (0.9% NaCl w/v) to a concentration of 10 $\mu g\ kg^{-1}\ ml^{-1}$.

Statistical analysis

All data have been presented as mean ± SEM. Differences between the baseline values of the two groups of animals were evaluated using the unpaired student t test (two-tailed). Statistical significance of drug-induced changes was determined by comparison to the solvent-induced changed from the same group. A Bonferroni correction was used because of multiple measurements. Statistical significance was accepted for a probability less than 5%.

Results

Blood gases

Arterial blood gases of the normal animals and the animals with chronic left ventricular dysfunction did not differ and were within the limits of those reported before for conscious pigs (Van Woerkens et al., 1991), measured with an ABL-3 (Radiometer, Copenhagen, Denmark): pH 7.42 ± 0.02, pO_2 75 ± 3 mmHg and pCO_2 40 ± 2 mmHg.

Table 1. *Stability of systemic hemodynamic parameters during solvent infusion in 8 normal conscious pigs (N) and in 7 conscious pigs with a 3 week old myocardial infarction (MI)*

		Baseline	saline (ml)			
			1	2	7	20
HR	N	121 ± 9	123 ± 11	121 ± 11	120 ± 12	122 ± 11
	MI	131 ± 4	130 ± 4	130 ± 3	129 ± 4	131 ± 4
CO	N	2.64 ± 0.05	2.63 ± 0.05	2.63 ± 0.05	2.59 ± 0.07	2.61 ± 0.04
	MI	2.52 ± 0.10	2.52 ± 0.11	2.51 ± 0.10	2.50 ± 0.10	2.50 ± 0.10
SAP	N	129 ± 4	126 ± 4	126 ± 3	127 ± 3	130 ± 5
	MI	119 ± 4	119 ± 4	121 ± 4	120 ± 4	119 ± 5
MAP	N	99 ± 4	97 ± 4	98 ± 4	99 ± 4	100 ± 4
	MI	97 ± 4	97 ± 4	99 ± 4	97 ± 4	98 ± 5
DAP	N	73 ± 5	71 ± 6	72 ± 5	73 ± 4	74 ± 5
	MI	76 ± 2	77 ± 2	78 ± 3	77 ± 2	78 ± 3
$LVdP/dt_{max}$	N	3640 ± 270	3520 ± 280	3420 ± 250	3510 ± 350	3680 ± 310
	MI	2720 ± 200[+]	2680 ± 170	2820 ± 190	2720 ± 200	2670 ± 220
LVEDP	N	9 ± 1	9 ± 1	9 ± 1	9 ± 1	9 ± 1
	MI	14 ± 2[+]	14 ± 2	14 ± 2	15 ± 2	14 ± 2
SV	N	22.2 ± 1.9	22.0 ± 2.4	22.4 ± 2.3	22.1 ± 2.1	22.1 ± 2.1
	MI	19.2 ± 0.8	19.5 ± 0.8	19.3 ± 0.9	19.5 ± 0.9	19.1 ± 0.9

*HR = heart rate (beats min^{-1}); CO = cardiac output (l min^{-1}); SAP, MAP and DAP are systolic, mean and diastolic arterial blood pressure (mmHg), respectively. $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure (mmHg s^{-1}); LVEDP = left ventricular end-diastolic pressure (mmHg); SV = stroke volume (ml). [+]$P<0.05$ vs normal pigs (for baseline measurements only); *$P<0.05$ vs Baseline; Data have been presented as mean ± SEM.*

Table 2. *Systemic hemodynamic effects of UL-FS 49 in 8 normal conscious pigs (N) and in 7 conscious pigs with a 3 week old myocardial infarction (MI)*

		Baseline	UL-FS 49 ($\mu g\ kg^{-1}$)			
			10	20	70	200
Total dose		0	10	30	100	300
HR	N	126 ± 4	125 ± 4	120 ± 4*	114 ± 4*	95 ± 2*
	MI	140 ± 4	138 ± 5	133 ± 6*	117 ± 5*	98 ± 6*
CO	N	2.65 ± 0.11	2.55 ± 0.12*	2.49 ± 0.12*	2.45 ± 0.18*	2.19 ± 0.12*
	MI	2.58 ± 0.11	2.54 ± 0.10	2.46 ± 0.10*	2.36 ± 0.10*	2.02 ± 0.20*
SAP	N	122 ± 5	122 ± 5	121 ± 4	122 ± 4	124 ± 6
	MI	114 ± 5	113 ± 5	114 ± 5	117 ± 5	115 ± 4
MAP	N	99 ± 4	100 ± 4	98 ± 3	97 ± 3	95 ± 4
	MI	93 ± 4	93 ± 4	94 ± 4	94 ± 4	90 ± 3
DAP	N	74 ± 6	77 ± 5	73 ± 5	70 ± 5	66 ± 7
	MI	73 ± 4	71 ± 4	73 ± 4	72 ± 3	66 ± 3
$LVdP/dt_{max}$	N	3720 ± 210	3680 ± 230	3510 ± 230*	3420 ± 280*	3140 ± 280*
	MI	3020 ± 210+	2740 ± 250*	2790 ± 240*	2720 ± 220*	2570 ± 290*
LVEDP	N	7 ± 1	8 ± 1	8 ± 1	9 ± 1*	12 ± 1*
	MI	11 ± 2+	12 ± 2	11 ± 2	13 ± 2	16 ± 3*
SV	N	21.7 ± 1.2	21.2 ± 1.6	21.4 ± 1.5	22.5 ± 1.8	23.6 ± 1.3
	MI	18.6 ± 1.4	18.5 ± 1.2	18.3 ± 1.2	20.0 ± 1.0*	20.7 ± 1.4

*HR = heart rate (beats min^{-1}); CO = cardiac output (l min^{-1}); SAP, MAP and DAP are systolic, mean and diastolic arterial blood pressure (mmHg), respectively. $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure (mmHg s^{-1}); LVEDP = left ventricular end-diastolic pressure (mmHg); SV = stroke volume (ml). +P<0.05 vs normal pigs (for baseline measurements only); *P<0.05 vs Baseline; Data have been presented as mean ± SEM.*

Baseline values of systemic hemodynamic of the normal pigs and the pigs with the infarctions

The baseline values of the animals with the chronically occluded left circumflex coronary artery showed some significant differences compared to the normal conscious animals (Tables 1 and 2). In the former, heart rate was 10% higher and $LVdP/dt_{max}$ was 20% lower than in the normal animals. The differences in mean arterial blood pressure and stroke volume were not significantly different. Left ventricular filling pressure, however, was significantly higher in the animals with chronic left circumflex coronary artery occlusion (14 ± 2 versus 9 ± 1 mmHg, $p<0.05$). Table 1 also shows that the systemic hemodynamic parameters remained stable in both groups of animals during the infusion of the solvent.

Cardiovascular effects of UL-FS 49

Table 2 shows that UL-FS 49 was at least as effective in reducing heart rate in the animals with the infarction as in the normal animals. As a matter of fact, after administration of 100 $\mu g\ kg^{-1}$ (total dose), the reduction in the animals with the infarction was slightly larger (23 ± 3 beats min^{-1}) than in the normal animals (12 ± 2 beats min^{-1}, $p<0.05$). At the other doses the reduction in heart rate was, however, very similar. Cardiac output decreased by less than 10% in both groups until infusion of a cumulative dose of 300 $\mu g\ kg^{-1}$, which caused cardiac output to decrease by approximately 20% in both groups. At any dose, the decrease in heart rate was larger than the decrease in cardiac output, and it therefore follows that stroke volume increased (10-15% at the highest dose). Mean arterial blood pressure was not affected in either group over the entire dose range, which indicates that peripheral vasoconstriction counterbalanced the effect of cardiac output on mean arterial blood pressure at the highest dose. $LVdP/dt_{max}$ decreased in both groups by less than 10% until the last dose was administered.
During baseline conditions, the duration of systole was the same for both groups of animals, but the duration of diastole was significantly reduced in the animals with the infarction (Figure 2). The bradycardic action of UL-FS 49 had no effect on the duration of systole in either group. The duration of diastole was, however, significantly prolonged in a dose dependent manner in both groups of animals (up to 130 ± 20 msec in the normal animals and up to 180 ± 30 msec in the animals with the infarctions).

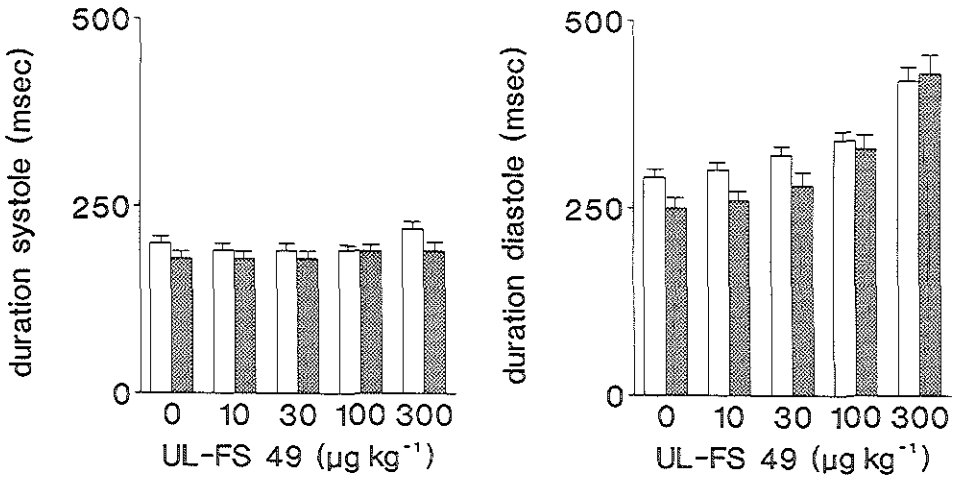


Figure 2 *Effect of UL-FS 49 on the duration of left ventricular systole and on the duration of diastole in 8 normal conscious pigs (□) and in 7 pigs with a 3 week occlusion of the left circumflex coronary artery (▩). The figure illustrates that UL-FS 49 had no effect on the duration of systole but prolonged the duration of diastole to the same extent in both groups of animals. + $p<0.05$ vs normal conscious pigs (only for pre-drug data (0)). *$p<0.05$ vs pre-drug data. The bars indicate the SEM.*

Discussion

In patients with stable angina pectoris and normal left ventricular function, selective bradycardic agents offer only a limited advantage above beta-adrenoceptor antagonists e.g. where effects on other organs (lungs, liver, thyroid) have to be avoided. However, in patients with left ventricular dysfunction the direct effects on the heart may become more important. After a myocardial infarction, the unaffected part of the left ventricle has to compensate for the loss in contractile function of the infarcted segment. This may lead to compensatory hypertrophy, which results in a decreased coronary flow reserve. Both beta-adrenoceptor antagonists and selective bradycardic agents, by increasing diastolic perfusion period may improve coronary perfusion. On the other hand, the negative inotropic effects of beta-adrenoceptor antagonists may interfere with systolic function, leading to the need for further adaptive mechanisms, such as ventricular dilatation, which in itself decreases subendocardial perfusion (Bache, 1988).

Occlusion of the left circumflex coronary artery in pigs leads to an infarct size of at least 15% of the left ventricle (Van der Giessen et al., 1989) and to hypertrophy of the left ventricular anterior wall and septum. Therefore, it may serve as an appropriate model to compare the effects of beta-adrenoceptor antagonists and selective bradycardic agents on global left ventricular function.

The most important finding of the present study is that UL-FS 49 reduced heart rate in conscious pigs with chronic mild left ventricular dysfunction to the same extent as in normal pigs, without adversely affecting cardiac performance. This is important as we have earlier shown that the effects of several drugs were modified when tested in the same model. We have, for example, shown that the positive inotropic actions of the phosphodiesterase inhibitor pimobendan were attenuated, and that the depressant effects of propranolol become more apparent in animals with chronic left ventricular dysfunction when compared to the effects in normal animals (Van der Giessen et al., 1989 and Van Woerkens et al., 1991). On the other hand, the calcium channel blockers nisoldipine and elgodipine, which had no effect on left ventricular end-diastolic pressure in the normal conscious animals, caused a lowering of the elevated left ventricular pressures in the animals with chronic left ventricular dysfunction (Van der Giessen et al., 1989 and Van Woerkens et al., 1991).

UL-FS 49 had no effect on the duration of left ventricular systole, in either group of animals. The prolongation of the RR interval was therefore caused by a prolongation of the duration of diastole. The finding that UL-FS 49, amongst others, had no effect on the duration of systole, implies that the drug does not reduce the myocardial oxygen demand per heart beat, although it will lower the myocardial oxygen demand per min by reducing the number of systoles per min. The prolongation of the duration of diastole per heart beat (47%) or per min (11%) is therefore probably the most important mechanism for the anti-ischemic actions of UL-FS 49 (Guth et al., 1987, Krumpl et al., 1988 and Indolfi et al., 1989).

The negative chronotropic properties of UL-FS 49 have also been studied in conscious dogs (Guth et al., 1987, Krumpl et al., 1988 and Indolfi et al., 1989). It is noteworthy that in this last species three times higher doses than used in the pigs were needed to accomplish similar reductions in heart rate. Furthermore, in these studies in dogs the reductions in heart rate were accompanied by increases (significant or non-significant) in $LVdP/dt_{max}$ (Guth et al., 1987, Krumpl et al., 1988 and Indolfi et al., 1989), whereas in the pigs we found a small but significant decrease in $LVdP/dt_{max}$. The reason for this descrepancy between the two species can not be easily assessed. Nevertheless, in both anesthetized and conscious pigs the relation between the reduction in heart rate and in $LVdP/dt_{max}$ is more favorable for UL-FS 49 than for its congener falipamil (Verdouw

Table 3. *Systemic hemodynamic effects of UL-FS 49 in 3 normal anesthetized pigs during atrial pacing at 110 beats min^{-1}*

	Baseline pre-pacing	UL-FS 49 (μg kg^{-1})					pacing off
Total dose	0	0	10	30	100	300	
HR	101 ± 2	110 ± 1	110 ± 1	110 ± 1	110 ± 1	110 ± 1	74 ± 3*
CO	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.0 ± 0.2	2.0 ± 0.3	2.1 ± 0.3	1.8 ± 0.2
MAP	97 ± 2	99 ± 2	102 ± 5	100 ± 5	100 ± 7	104 ± 9	89 ± 11
$LVdP/dt_{max}$	2420 ± 280	2220 ± 330	2250 ± 370	2200 ± 340	2160 ± 360	2210 ± 420	2300 ± 370
SV	20 ± 2	19 ± 2	19 ± 2	18 ± 2	18 ± 2	19 ± 2	24 ± 2

*HR = heart rate (beats min^{-1}); CO = cardiac output (l min^{-1}); MAP = mean arterial blood pressure (mmHg), respectively. $LVdP/dt_{max}$ = maximal rate of rise of left ventricular pressure (mmHg s^{-1}); SV = stroke volume (ml). Data have been presented as mean ± SEM. * $p<0.05$ vs Baseline pre-pacing.*

et al., 1983) or alinidine (Verdouw et al., 1987 and Verdouw et al., 1980) , two other so-called specific bradycardic agents. We also noticed that falipamil and alinidine both decreased $LVdP/dt_{max}$ dose dependently when heart rate was fixed by atrial pacing and that the magnitude of these changes were very similar during pacing and normal sinus rhythm (Verdouw et al., 1983 and Verdouw et al., 1987). This last observation is most likely explained by the observation that, at least in anesthetized pigs, $LVdP/dt_{max}$ is not very sensitive to changes in heart rate in the range from 100 to 150 beats.min^{-1} (Scheffer and Verdouw, 1983). In three preliminary experiments in anesthetized pigs we have observed that UL-FS 49 did not significantly decrease $LVdP/dt_{max}$, when heart rate was kept constant by atrial pacing at 110 bpm. (Table 3). It thus appears that UL-FS 49 exerts considerable less negative inotropic effects than for instance alinidine or falipamil, when heart rate associated changes in myocardial contractility are excluded.

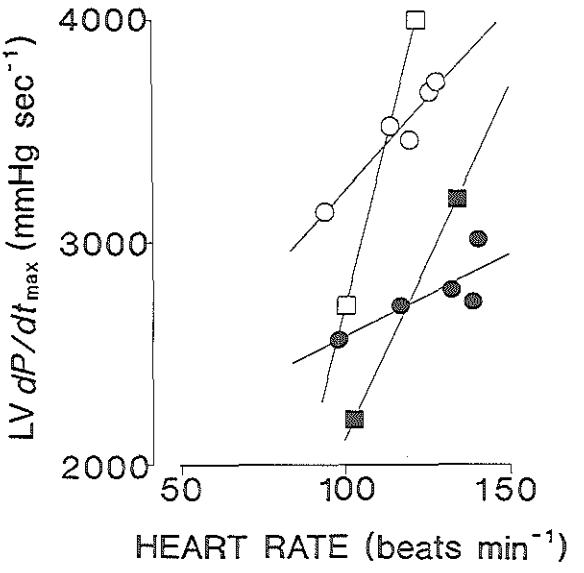


Figure 3. *Relation between changes in heart rate and stroke volume in normal conscious pigs and in conscious pigs with chronic left ventricular dysfunction during administration of UL-FS 49 (○ and ● , respectively) and propranolol (□ and ■ , respectively). Notice that propranolol and UL-FS 49 had opposite effects on stroke volume. The data on propranolol have been reported before (Van Woerkens et al., 1991).*

As was discussed above, beta-adrenoceptor antagonists may also ameliorate myocardial ischemia by a reduction in heart rate. A disadvantage of this class of drugs is that they can substantially decrease myocardial contractility. We have earlier shown that in animals with a 4 week old myocardial infarction, propranolol at a dose (0.5 mg kg^{-1}) which lowered heart rate by 23%, reduced $LVdP/dt_{max}$ by 32% (Van Woerkens et al. 1991). With UL-FS 49 we found that a 30% lowering of heart rate reduced $LVdP/dt_{max}$ by only 12% (Table 2). Taking into account that $LVdP/dt_{max}$ not only depends on myocardial contractility but also on heart rate and afterload, we like to conclude that the negative inotropic effects of UL-FS 49 are negligible at doses up to 300 $\mu g\ kg^{-1}$, which is most likely the major reason that UL-FS 49 and propranolol have opposite effects on stroke volume (Figure 3). As discussed by Indolfi et al. (1989), reduction of heart rate by UL-FS 49 has also the advantage over beta-adrenoceptor blockade because UL-FS 49 does not cause unmasking of alpha-adrenergic constriction in the large coronary vessels Kobinger, 1985 and Riley et al 1987).

We conclude that in light of absence of a depression of cardiovascular performance, the data presented in this study suggest that UL-FS 49 could be an attractive agent to selectively reduce heart rate during chronic mild left ventricular dysfunction. Further studies evaluating the effects of chronic treatment of UL-FS 49 therefore appears to be the logical next approach.

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References

Bache RJ. Effects of hypertrophy on the coronary circulation. *Prog Cardiovasc Dis* 31; 403-440, 1988.

Bouman LN, Jongsma HJ, Opthof T and Van Ginneken ACG. Does I_f contribute to the pace making in the rabbit sinoatrial node? *J Physiol* 358; 51P, 1985.

Canty JM, Giglia J and Kandath GJ. Effect of tachycardia on regional function and transmural myocardial perfusion during graded coronary pressure reduction in conscious dogs. *Circulation* 82; 1815-1825, 1990.

Drake-Holland AJ, Laird JD, Noble MIM, Spaan JAE and Vergroesen I. Oxygen and coronary vascular resistance during autoregulation and metabolic vasodilation in the dog. *J Physiol (London)* 348; 285-299, 1984.

Guth BB, Heusch G, Seitelberger R and Ross J. Elimination of exercise-induced regional myocardial dysfunction by a bradycardic agent in dogs with chronic coronary stenosis. *Circulation* 75; 661-669, 1987.

Hoffman JIE. Autoregulation and heart rate. *Circulation* 82; 1880-1881, 1990.

Indolfi C, Guth BD, Miura T, Miyazaki S, Schulz R and Ross J Jr. Mechanisms of improved ischemic regional dysfunction by bradycardia. *Circulation* 80; 983-993, 1989.

Kobinger W. Specific bradycardic agents, a new approach to therapy in angina pectoris. *Prog Pharmacol* 5; 89-100, 1985.

Kobinger W and Lillie C. Cardiovascular characterization of UL-FS 49, 1,3,5-Tetrahydro-7,8-dimethoxy-3-[[2-(3,4-dimethoxyphenyl)ethyl]methylimino]propyl]-2H-3-benzazepin-2-on-hydrochloride, a new "specific bradycardic agent". *Eur J Pharmacol* 104; 9-18, 1984.

Krumpl G, Winkler M, Schneider W and Raberger G. Comparison of the haemodynamic effects of the selective bradycardic agent UL-FS 49, with those of propranolol during treadmill exercise in dogs. *Br J Pharmacol* 94; 55-64, 1988.

Lillie C and Kobinger W. Investigations into the bradycardic effects of UL-FS 49 (1,3,5-Tetrahydro-7,8-dimethoxy-3-[[2-(3,4-dimethoxyphenyl)ethyl]methylimino]propyl]-2H-3-benzazepin-2-on-hydrochloride) in isolated guinea pig atria. *J Cardiovasc Pharmacol* 8; 791-797, 1986.

Millar JS and Vaughan Williams EM. Pacemaker selectivity: influence on rabbit atria of ionic environment and alinidine, a possible anion antagonist. *Cardiovasc Res* 15; 335-350, 1981.

Raff WK, Kosche F and Lochner W. Extravascular coronary resistance and its relation to microcirculation. *Am J Cardiol* 29; 598-603, 1972.

Riley DC, Gross GJ, Kampine JP and Warltier DC. Specific bradycardic agents, a new therapeutic modality for anesthesiology: Hemodynamic effects of UL-FS 49 and propanolol in conscious and isofluorane-anesthetized dogs. *Anesthesiology* 67; 707-716, 1987.

Schamhardt HC, Verdouw PD and Saxena PR. Improvement of perfusion and function of ischaemic porcine myocardium after reduction of heart rate by alinidine. *J Cardiovasc Pharmacol* 3; 728-738, 1981.

Scheffer MG and Verdouw PD. Decreased incidence of ventricular fibrillation after an acute coronary artery ligation in exercised pigs. *Basic Res Cardiol* 78; 298-309, 1983.

Snyders DJ and Van Bogaert PP. Mode of action of alinidine, a new bradycardic agent:

a voltage clamp study. *J Am Coll Cardiol* 5; 494, 1985.

Van der Giessen WJ, Van Woerkens LJ, Duncker DJ, Roelandt JRTC and Verdouw PD. Acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J Cardiovasc Pharmacol* 14; 653-658, 1989.

Van Woerkens LJ, Schotman SN, Van der Giessen WJ and Verdouw PD. Cardiovascular effects of elgodipine in conscious pigs with a normal coronary circulation and in conscious pigs with a healed myocardial infarction. *J Cardiovasc Pharmacol* 17; 976-982, 1991.

Verdouw PD, Bom HPA and Bijleveld RE. Cardiovascular responses to increasing plasma concentrations of AQ-A 39 Cl, a new compound with negative chronotropic effects. *Arzneim-Forsch/Drug Res* 33; 702-706, 1983.

Verdouw PD, Duncker DJ, Heere ThJM and Saxena PR. Changes in systemic haemodynamics, regional myocardial function and perfusion induced by alinidine in pigs with and without narrowed coronary arteries. *Eur Heart J* 8(suppl.L); 43-52, 1987.

Verdouw PD, Saxena PR, Schamhardt HC, Van der Hoek TM and Rutteman AM. The effects of alinidine, an N-allyl derivative of clonidine on regional myocardial perfusion and performance in the pig with or without atrial pacing. *Eur J Pharmacol* 64; 209-220, 1980.

Chapter 8

Evidence against a role for dopamine D_1 receptors in the porcine myocardium.

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Evidence against a role for dopamine D_1 receptors in the myocardium of the pig

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1 We investigated the presence of dopamine D_1 receptors in the myocardium of anaesthetized pigs using intravenous infusions of dopamine, alone and after α- and β-adrenoceptor blockade and intracoronary infusions of the selective D_1 receptor agonist, fenoldopam.

2 Intravenous infusion of dopamine (2.5, 5 and 10 $\mu g\,kg^{-1}\,min^{-1}$ for 10 min, $n = 6$) caused dose-dependent changes in heart rate (from 94 ± 6 to 132 ± 10 beats min^{-1}, $P < 0.05$), the maximal rate of rise of left ventricular pressure ($LVdP/dt_{max}$; from 2280 ± 170 to 4800 ± 410 mmHg s^{-1}, $P < 0.05$), mean arterial blood pressure (from 87 ± 5 to 62 ± 3 mmHg) and systemic vascular resistance (from 40 ± 4 to 28 ± 2 mmHg l^{-1} min, $P < 0.05$). The increases in heart rate and $LVdP/dt_{max}$ were abolished when dopamine was infused after α- and β-adrenoceptor blockade. The vasodilator response was, however, only minimally affected.

3 Intravenous infusions of dopamine decreased coronary vascular resistance from 0.90 ± 0.06 to 0.53 ± 0.07 mmHg ml^{-1} min 100 g ($P < 0.05$). This action of dopamine was not observed when dopamine was infused after blockade of the α- and β-adrenoceptors.

4 Pretreatment with α- and β-adrenoceptor blockade had no effect or only slightly attenuated the dopamine-induced decrease in vascular resistance of the brain, kidneys, adrenals and small intestine.

5 In 7 animals, intracoronary doses of 0.04, 0.1, 0.2 and 0.4 $\mu g\,kg^{-1}\,min^{-1}$ of fenoldopam had no effect on coronary venous oxygen content, local myocardial oxygen consumption, coronary blood flow or coronary vascular resistance. However, systemic effects were observed at the highest two doses, as manifested by a drop in mean arterial blood pressure from 82 ± 4 to 72 ± 4 mmHg ($P < 0.05$) due to peripheral vasodilatation (e.g. cerebral vascular bed). Heart rate, $LVdP/dt_{max}$, regional myocardial segment length shortening and left ventricular end-diastolic pressure were not affected at these doses. In 2 animals the infusion rate was increased to 4 $\mu g\,kg^{-1}\,min^{-1}$, but again there was no evidence for coronary vasodilatation.

6 We conclude that the intravenous infusion of dopamine after α- and β-adrenoceptor blockade and the intracoronary infusion of fenoldopam provided no evidence for a major role of D_1 receptors in the coronary circulation of pigs. The absence of any effect of the employed doses of fenoldopam on $LVdP/dt_{max}$ and on regional myocardial segment length shortening also indicates that fenoldopam does not exhibit any inotropic action in this species.

Keywords: Dopamine; fenoldopam; D_1 receptors; systemic haemodynamics; regional blood flows; coronary blood flow; inotropy; pig myocardium

Introduction

Dopamine D_1 receptors have been identified in a number of vascular beds, including the renal, mesenteric and cerebral vasculature (Ueda *et al.*, 1982; Toda, 1983; Hughes & Sever, 1989). However, evidence for their presence in the coronary circulation is limited. Kopia & Valocik (1989) demonstrated a specific D_1 receptor-mediated vasodilatation in the coronary vascular bed of pentobarbitone-anaesthetized dogs following intracoronary infusion of the specific D_1 receptor agonist, fenoldopam. Hieble *et al.* (1987) showed that fenoldopam, in both dogs and rats, increased blood flow to various vascular beds, but not to the heart. Recently, Zhao *et al.* (1990) concluded from their experiments in pentobarbitone-anaesthetized dogs that coronary vasodilatation after intracoronary infusion of fenoldopam was due to a positive inotropic effect of the drug rather than to a direct effect on the coronary vasculature. Furthermore, fenoldopam has failed to relax isolated conduit coronary arteries of humans precontracted by noradrenaline (Hughes & Sever, 1989).

Zhao *et al.* (1990) reported an increased inotropic state during infusion of fenoldopam in dogs, but Hieble *et al.* (1987) found that in the same species the drug reduced the maximal rate of rise of left ventricular pressure ($LVdP/dt_{max}$) and ascribed the latter to a negative inotropic action of the drug. Kopia & Valocik (1989), however, could not confirm either observation, when they infused fenoldopam directly into a coronary artery.

In view of these discrepancies, we used two approaches to investigate the role of D_1 receptors in the myocardium of pigs, a species frequently used in biomedical research. We first determined the responsiveness to intravenous infusions of dopamine alone and after α- and β-adrenoceptor blockade. We then used intracoronary infusions of fenoldopam to evaluate the direct effects of fenoldopam on coronary blood flow and myocardial contractility.

Methods

General

After an overnight fast, 25 cross-bred Landrace × Yorkshire pigs (H.V.C., Hedel, The Netherlands) of either sex and weighing from 22–28 kg were sedated with intramuscular 5 mg kg^{-1} azaperone (Stresnil, Janssen Pharmaceutica, Beerse, Belgium), anaesthetized with intravenous 6 mg kg^{-1} metomidate (Hypnodil, Janssen Pharmaceutica, Beerse, Belgium), 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

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keep arterial blood gases within the normal range: $7.35 < pH < 7.45$; $35\,mmHg < Pco_2 < 45\,mmHg$ and $120\,mmHg < Po_2 < 160\,mmHg$. 7F catheters were placed in the superior caval vein for administration of $150\,mg\,kg^{-1}$ α-chloralose (E. Merck, Darmstadt, Germany) followed by an infusion of a low dose ($5\,mg\,kg^{-1}\,h^{-1}$) of sodium pentobarbitone (Apharma, Arnhem, The Netherlands); for administration of 4 mg of the muscle relaxant pancuronium bromide (Organon Teknika BV, Boxtel, The Netherlands) prior to thoracotomy; and for administration of haemaccel (Behringwerke A.G., Marburg, Germany) to compensate for loss of intravascular volume. Catheters were also positioned in the descending aorta for withdrawal of blood samples and measurement of central aortic blood pressure. A Sensodyn MTC P7 catheter (B. Braun, Medical BV, Uden, The Netherlands) inserted via the left carotid artery was used to measure left ventricular pressure and its first derivative ($LVdP/dt$). After thoracotomy, an electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the ascending aorta, and the great cardiac vein was cannulated for subsequent collection of blood for determining haemoglobin concentration and oxygen saturation (OSM2, Radiometer, Copenhagen, Denmark). In 14 of the animals the proximal left anterior descending coronary artery was dissected free and a small cannula inserted for intracoronary infusions of fenoldopam or its solvent.

Regional blood flows

In order to determine regional blood flows, the left atrial appendage was cannulated for injection of a batch of $1–2 \times 10^6$ carbonized plastic microspheres [$15 \pm 1\,\mu m$ (s.d.) in diameter] labelled with ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce. Starting 15 s before the injection of microspheres, blood was withdrawn from a femoral artery at a rate of $10\,ml\,min^{-1}$ until 60–65 s after completion of the injection of the microspheres. In the animals which received the intracoronary infusions of fenoldopam, the area perfused by the left anterior descending coronary artery (LADCA) was identified by an intracoronary injection of patent blue violet (Sigma, St. Louis, MO, U.S.A.) at the end of the experiment. Animals were killed with an overdose of sodium pentobarbitone and the heart, the brain (divided into hemispheres, diencephalon, cerebellum and brainstem) adrenals, kidneys and aliquots of the skeletal muscle, skin and small intestine were excised. The heart was fixed in formaldehyde (10% v/v) for 48 h at which point of time the left ventricle was divided into three layers of equal thickness. From the radioactivity in the tissue, blood flows were determined by standard procedures (Saxena *et al.*, 1980).

Myocardial oxygen consumption and contractile function

Myocardial oxygen consumption ($M\dot{V}O_2$) of the LADCA perfused area was calculated as the product of coronary blood flow (microsphere measurements) and the difference in the oxygen contents of the arterial and coronary venous blood.

In the animals in which fenoldopam was infused, regional myocardial segment length shortening was assessed by sonomicrometry (Triton Technology Inc., San Diego, CA, U.S.A.) with a pair of ultrasonic crystals implanted approximately 10 mm apart in the subendocardial layers of the myocardium perfused by the LADCA. From the tracings, systolic segment length shortening (SLS) was calculated as:

$$SLS\ (\%) = 100 \times (EDL - ESL)/EDL,$$

in which EDL and ESL are the segment length at end-diastole and end-systole, respectively.

Experimental protocols

Two series of experiments were performed. In the first series of experiments the effects of dopamine on systemic haemodynamics and regional blood flows were evaluated. To this end 6 pigs received three consecutive 10 min intravenous infusions (2.5, 5 and $10\,\mu g\,kg^{-1}\,min^{-1}$) of dopamine and 5 other animals received four consecutive 10 min intravenous infusions (2.5, 5, 10 and $20\,\mu g\,kg^{-1}\,min^{-1}$) of dopamine after α- and β-adrenoceptor blockade with phentolamine ($1\,mg\,kg^{-1}$) and propranolol ($0.5\,mg\,kg^{-1} + 0.5\,mg\,kg^{-1}\,h^{-1}$), respectively. Systemic haemodynamics, arterial and coronary venous oxygen contents and regional blood flows were determined at baseline and at the end of each infusion rate. In the second series of experiments, the direct effects of fenoldopam on the coronary circulation and the myocardium were evaluated. Hitherto, four incremental doses of fenoldopam (0.04, 0.1, 0.2 and $0.4\,\mu g\,kg^{-1}\,min^{-1}$) were infused directly into the left anterior descending coronary artery for 10 min each in the experimental group ($n = 7$) and the results were compared with those of a control group ($n = 7$), which received equal volumes of the solvent. Systemic haemodynamics, regional myocardial function, arterial and coronary venous oxygen contents and coronary blood flow were determined again at baseline and at the end of each infusion rate.

Drugs

Dopamine hydrochloride (Department of Pharmacy, Academic Hospital Dijkzigt, Rotterdam, The Netherlands), propranolol hydrochloride (gift: ICI-Pharmaceuticals, Rotterdam, The Netherlands) and phentolamine methane-sulphonide (gift: Ciba-Geigy B.V., Arnhem, The Netherlands) were dissolved in physiological saline. Fenoldopam mesylate (gift: Dr F. Lippens, Smith, Kline and Beecham, Rijswijk, The Netherlands) was dissolved in physiological saline containing 1% v/v ethanol and the required doses were obtained by adjusting the infusion rate (from $0.13\,ml\,min^{-1}$ to $2\,ml\,min^{-1}$).

Statistical evaluation

All data are presented as arithmetic mean ± s.e.mean. Statistical analysis was performed by use of a parametric two-way of analysis of variance (random-block design), followed by the Duncan new multiple range test. Statistical significance was accepted for $P < 0.05$.

Results

Intravenous infusions of dopamine without and after α- and β-adrenoceptor blockade

Systemic haemodynamics Intravenous infusion of dopamine caused an increase in heart rate from 94 ± 6 to 132 ± 10 beats min^{-1} ($P < 0.05$) and a decrease in mean arterial blood pressure from 87 ± 5 to 62 ± 3 mmHg ($P < 0.05$); both changes were dose-dependent (Figure 1). Cardiac output did not change, which implies that the hypotensive effect was caused by a reduction of the systemic vascular resistance ($P < 0.05$). $LVdP/dt_{max}$ increased by more than 100%, while left ventricular end-diastolic pressure showed a decrease from 10 ± 1 to 7 ± 1 mmHg ($P < 0.05$).

The animals in which the α- and β-adrenoceptors were blocked had a 20% lower $LVdP/dt_{max}$ at baseline, but other systemic haemodynamic parameters were not significantly different from the animals without adrenoceptor blockade (Figure 1). When dopamine was infused in these animals, the increases in heart rate and in $LVdP/dt_{max}$ were inhibited, but the systemic vasodilator response was only minimally affected (Figure 1).

Myocardial blood flow and oxygen consumption During infusion of the lowest dose of dopamine ($2.5\,\mu g\,kg^{-1}\,min^{-1}$) there was a 10% decrease in left ventricular transmural blood flow, but during the infusion of 5 and $10\,\mu g\,kg^{-1}\,min^{-1}$, transmural blood flow was, respectively, 20% and 50% higher than the baseline flow (Figure 2). The subepicardial layers benefitted

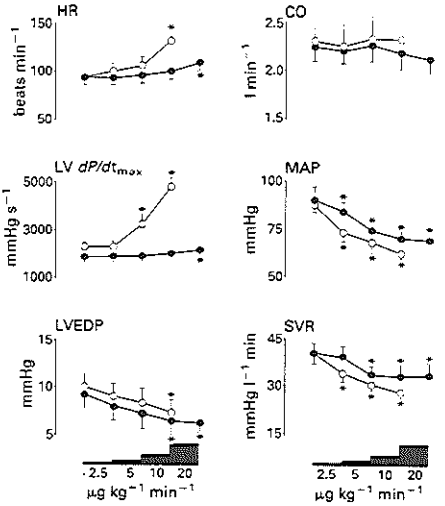

Figure 1 Systemic haemodynamic effects of dopamine alone (○, $n = 6$) and after α- and β-adrenoceptor blockade (●, $n = 5$) in anaesthetized pigs. HR = heart rate (beats min^{-1}); CO = cardiac output ($l\,min^{-1}$); MAP = mean arterial blood pressure (mmHg); $LVdP/dt_{max}$ = maximum rate of rise in left ventricular pressure ($mmHg\,s^{-1}$); LVEDP = left ventricular end-diastolic pressure (mmHg). SVR = systemic vascular resistance ($mmHg\,l^{-1}\,min$). Note that after α- and β-adrenoceptor blockade the dopamine-induced increases in heart rate and $LVdP/dt_{max}$ were almost completely abolished, but that dilatation of the systemic vascular bed was much less affected. *$P < 0.05$ vs baseline.

more from the increase in blood flow than the subendocardial layers as the ratio of the normalized subendocardial and subepicardial blood flows decreased from 1.07 ± 0.04 to 0.97 ± 0.06 ($P < 0.05$). Calculation of the coronary vascular

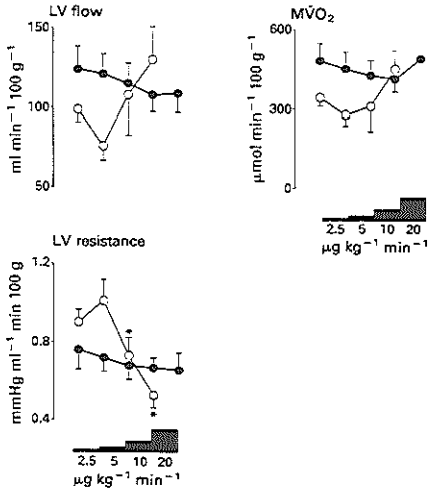

Figure 2 The effect of dopamine on transmural left ventricular blood flow (LV) and left ventricular vascular resistance (LV resistance) and left ventricular oxygen consumption ($M\dot{V}O_2$) in anaesthetized pigs. Data were obtained without (○, $n = 6$) and after α- and β-adrenoceptor blockade (●, $n = 5$). *$P < 0.05$ vs baseline.

resistance revealed no change at $2.5\,\mu g\,kg^{-1}\,min^{-1}$, but decreases of 30% and 50% were observed during the dopamine infusions of 5 and $10\,\mu g\,kg^{-1}\,min^{-1}$, respectively. Myocardial oxygen consumption decreased (by 20%) during infusion of the lowest dose, was not different from baseline during infusion of the middle dose and increased (by 30%) during infusion of the highest dose. The left ventricular systolic pressure-heart rate product, a measure of myocardial oxygen demand, followed a similar pattern.

After blockade of the α- and β-adrenoceptors, the dopamine-induced increases in coronary blood flow were abolished and the decrease in coronary vascular resistance was attenuated. Myocardial oxygen consumption tended to decrease slightly, but levels of statistical significance were not reached. The decrease in myocardial efficiency did not reach levels of statistical significance also.

Regional vascular beds Figure 3 demonstrates the existence of peripheral dopamine receptors in the pig as the decreases in vascular resistances of, in particular the kidneys, adrenals, brain and small intestine, were only minimally affected when dopamine was infused after α- and β-adrenoceptor blockade. The regional differences in the brain were relatively small as after α- and β-adrenoceptor blockade the dopamine-induced decreases in the vascular resistances of the diencephalon, hemispheres, cerebellum and brainstem were maximally $16 \pm 4\%$, $16 \pm 3\%$, $13 \pm 3\%$ and $11 \pm 7\%$, respectively.

Intracoronary infusions of fenoldopam

Systemic haemodynamics As shown in Table 1, intracoronary infusions of fenoldopam up to $0.4\,\mu g\,kg^{-1}\,min^{-1}$ had no effect on cardiac output, heart rate, $LVdP/dt_{max}$ and left ventricular end-diastolic pressure. Mean arterial blood pressure decreased from 82 ± 4 to 72 ± 4 mmHg (12%, $P < 0.05$) with an associated reduction in systemic vascular resistance from 41 ± 3 to $35 \pm 3\,mmHg\,l^{-1}$ min (15%, $P < 0.05$). In 2 of the experiments intracoronary infusions up to $4\,\mu g\,kg^{-1}\,min^{-1}$ were

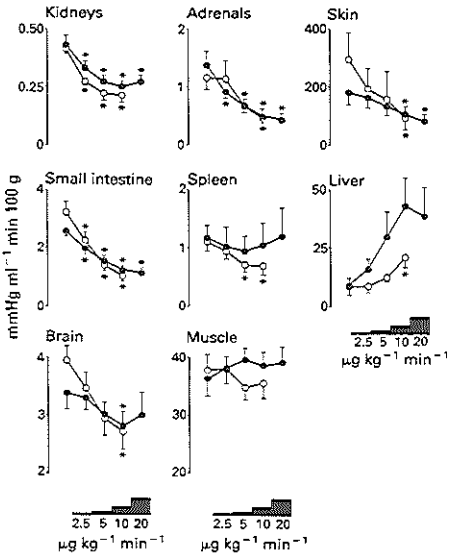

Figure 3 The effect of dopamine on the regional vascular resistance of regional vascular beds. Data were obtained without (○, $n = 6$) and after α- and β-adrenoceptor blockade (●, $n = 5$). *$P < 0.05$ vs baseline.

Table 1 Systemic haemodynamics after continuous intracoronary 10 min infusions of fenoldopam or its solvent in anaesthetized pigs

		Fenoldopam ($\mu g\,kg^{-1}\,min^{-1}$) *or equal volumes of its solvent*				
		0	0.04	0.1	0.2	0.4
HR	Solvent	112 ± 7	110 ± 6	110 ± 5	112 ± 5	113 ± 6
	Fenoldopam	118 ± 8	114 ± 9	114 ± 8	118 ± 9	115 ± 8
MAP	Solvent	89 ± 5	86 ± 9	84 ± 4	85 ± 4	85 ± 6
	Fenoldopam	82 ± 4	80 ± 5	79 ± 4	72 ± 4*	73 ± 6*
CO	Solvent	2.5 ± 0.3	2.5 ± 0.3	2.5 ± 0.3	2.5 ± 0.3	2.4 ± 0.3
	Fenoldopam	2.1 ± 0.2	2.0 ± 0.2	2.1 ± 0.2	2.1 ± 0.2	2.1 ± 0.1
SVR	Solvent	37 ± 3	36 ± 3	36 ± 3	36 ± 3	38 ± 4
	Fenoldopam	41 ± 3	41 ± 3	40 ± 3	35 ± 3*	35 ± 3*
$LVdP/dt_{max}$	Solvent	2360 ± 390	2330 ± 310	2260 ± 330	2430 ± 410	2400 ± 410
	Fenoldopam	2010 ± 320	1850 ± 260	1850 ± 270	1790 ± 280	1880 ± 280
LVEDP	Solvent	7.3 ± 1.1	6.4 ± 1.1	6.5 ± 1.1	6.4 ± 1.1	6.4 ± 1.0
	Fenoldopam	6.9 ± 0.8	7.1 ± 0.8	7.1 ± 0.8	6.8 ± 0.8	7.5 ± 1.0

Data are mean ± s.e.mean; $n = 7$ for the solvent-treated and $n = 7$ for the fenoldopam-treated animals. HR = heart rate (beats min^{-1}); MAP = mean arterial blood pressure (mmHg); CO = cardiac output (l min^{-1}); SVR = systemic vascular resistance (mmHg l^{-1} min); $LVdP/dt_{max}$ = maximal rate of rise of left ventricular blood pressure (mmHg s^{-1}); LVEDP = left ventricular end-diastolic blood pressure (mmHg).

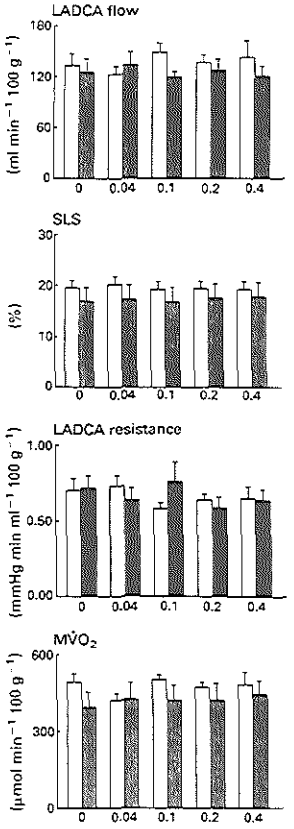

Figure 4 Intracoronary infusions of fenoldopam into the left anterior descending coronary artery (LADCA) had no effect on coronary blood flow, coronary vascular resistance, regional systolic segment length shortening (SLS) or regional myocardial O_2-consumption ($M\dot{V}O_2$); open columns, solvent infusion ($n = 7$); hatched columns, fenoldopam infusion ($n = 7$). Columns represent means and vertical bars represent the standard errors.

used. In these animals there was a further decrease in mean arterial blood pressure by 12 and 18 mmHg, respectively, with a further reduction in systemic vascular resistance. Cardiac output, heart rate and $LVdP/dt_{max}$ were not affected (data not shown in Table 1).

Resistance of the cerebral vascular bed decreased dose dependently from 2.93 ± 0.33 to 2.24 ± 0.23 mm Hg ml^{-1} min 100 g (25%, $P < 0.05$) during the fenoldopam infusions. The resistance of the renal vascular bed was 0.45 ± 0.10 mmHg ml^{-1} min 100 g at baseline and decreased gradually to 0.32 ± 0.04 mmHg ml^{-1} min 100 g at 0.4 $\mu g\,kg^{-1}\,min^{-1}$ fenoldopam. This decrement, however, was not significant compared to the changes in the solvent-treated animals.

Myocardial blood flow, oxygen consumption and contractile function Intracoronary infusions of fenoldopam did not alter transmural blood flow or vascular resistance in the LADCA-perfused region (Figure 4). Since coronary venous oxygen saturation (baseline value: 27 ± 3%) was also not affected, myocardial oxygen consumption was maintained (Figure 4). This observation is not surprising as global and regional parameters of myocardial contractile function, $LVdP/dt_{max}$ (Table 1) and segment length shortening (Figure 4), respectively, did not change.

In the two animals in which fenoldopam was infused at 4 $\mu g\,kg^{-1}\,min^{-1}$, coronary venous oxygen saturation and regional segment length shortening remained constant.

Discussion

The principal findings of this study are that intracoronary infusions of fenoldopam (up to a dose of 0.4 $\mu g\,kg^{-1}\,min^{-1}$) neither affected myocardial blood flow nor myocardial function and oxygen consumption in anaesthetized pigs. The doses used were high enough to cause changes in the peripheral vasculature as reflected by decreases in mean arterial blood pressure and systemic vascular resistance. Furthermore, in two animals the rate of the intracoronary infusions of fenoldopam was increased to 4 $\mu g\,kg^{-1}\,min^{-1}$ without causing any changes in regional myocardial wall motion and coronary venous oxygen saturation, which is a sensitive parameter to detect coronary vasodilatation provided myocardial oxygen consumption does not increase (Duncker *et al.*, 1986; Verdouw *et al.*, 1987; Sassen *et al.*, 1990a,b). These observations suggest that D_1 receptors are of minor importance in the coronary circulation of the anaesthetized pig.

The presence of D_1 receptors in the renal, mesenteric and cerebral vascular beds has now been well established in a

number of species including man, dogs and pigs (Goldberg, 1972; Ueda *et al.*, 1982; Gootman *et al.*, 1983; Toda, 1983; Hughes & Sever, 1989). In contrast, evidence for D_1 receptors in the coronary circulation is inconclusive. In dogs pretreated with α- and β-adrenoceptor antagonists, dopamine has been reported to elicit a D_1 receptor-mediated vasodilatation in coronary conductance vessels (Toda & Hatano, 1979) as well as in arterioles (Schuelke *et al.*, 1971; Kopia & Valocik, 1989). However, in the present experiments in pigs, dopamine-induced coronary vasodilatation was accompanied by increases in heart rate and myocardial contractility, and these effects were absent in animals with α- and β-adrenoceptor blockade. Similarly, fenoldopam-induced coronary vasodilatation has not been consistently observed. While Kopia & Valocik (1989) found coronary vasodilatation after intracoronary administration of $1\,\mu g\,kg^{-1}\,min^{-1}$ fenoldopam in the dog, no such effect was noticed with this drug after intravenous infusion in the same species (Hieble *et al.*, 1987) or after intracoronary infusion in the pig (present experiments). In human isolated coronary arteries precontracted with noradrenaline, fenoldopam failed to elicit a vasodilator response in 6 out of 9 artery segments (6 out of 7 patients) (Hughes & Sever, 1989), implying that the human conduit coronary vessels are devoid of D_1 receptors.

Another controversial point is the potential effects of fenoldopam on myocardial contractility. Hieble *et al.* (1987) suggested that fenoldopam exerts a negative inotropic action as shown by a 26% decrease in $LVdP/dt_{max}$ during intravenous infusion of the drug. The authors did not take into account that $LVdP/dt_{max}$ depends not only on myocardial contractility but also on heart rate, left ventricular filling pressure and arterial blood pressure (afterload). Since in their study arterial blood pressure decreased by approximately 25%, the effect on dP/dt_{max} cannot be assumed to be secondary to possible cardiodepressant actions of the drug only. In contrast, Zhao *et al.* (1990) observed that intracoronary infusion of fenoldopam at concentrations up to 5000 nM (equivalent to intracoronary infusions of $1–2\,\mu g\,kg^{-1}\,min^{-1}$ fenoldopam in our experiments) at constant coronary perfusion pressure increased $LVdP/dt_{max}$ in anaesthetized dogs. Kopia & Valocik (1989), using a similar experimental set-up found no change in myocardial function. We also did not observe change in $LVdP/dt_{max}$ or in regional myocardial segment length shortening indicating the absence of a negative inotropic action. Furthermore, to achieve equivalent intracoronary concentrations via intravenous administration one would require a fifty-fold higher dose of the drug. This may be unacceptable because of severe hypotension.

Finally, two points deserve comment. First, it is theoretically possible that in our model a vasodilator response of fenoldopam could not be observed because the coronary vascular bed was already maximally dilated. This seems very unlikely as we have repeatedly shown that in the same model a number of drugs increased coronary blood flow up to 100% (Duncker *et al.*, 1986; Verdouw *et al.*, 1987; Sassen *et al.*, 1990a,b) while in the present study intravenous infusions of dopamine reduced coronary vascular resistance to 60% of baseline. Secondly, it could be argued that the butyrophenone derivative azaperone (Niemegeers *et al.*, 1974), used by us as anaesthetic premedication, may have antagonized a possible vasodilator effect of fenoldopam. This drug has indeed been shown to exhibit antidopamine activity, but the inhibition is at the D_2 receptor site (Fukuchi *et al.*, 1988). Furthermore, intravenous infusion of dopamine in the presence of α- and β-adrenoceptor blockade revealed the existence of dopamine receptors in several peripheral vascular beds.

In conclusion, the lack of effect of intracoronary infusions of high doses of fenoldopam as well as of intravenous infusions of dopamine (after adrenoceptor blockade) on coronary blood flow and myocardial performance provides evidence that D_1 receptors do not play a major physiological role in the coronary circulation and myocardium of pigs. This observation should be taken into account in the study of the effects of agents acting on dopamine receptors.

References

DUNCKER, D.J., VAN DALEN, F.J., HARTOG, J.M., LAMERS, J.M.J., RENSEN, R.J., SAXENA, P.R. & VERDOUW, P.D. (1986). Usefulness of pimobendan in the treatment of heart failure. *Arzneim. Forsch.*, **36**, 1740–1744.

FUKUCHI, I., DAWASHIMA, K., MATSUOKA, Y. & ISHIDA, R. (1988). Neurochemical study of mafoprazine, a new phenylpiperazine derivative. *Jpn. J. Pharmacol.*, **47**, 51–61.

GOLDBERG, L.I. (1972). Cardiovascular and renal actions of dopamine: potential clinical applications. *Pharmacol. Rev.*, **24**, 1–29.

GOOTMAN, N., BUCKLEY, B.J., GOOTMAN, P.M., GRISWOLD, P.G., MELE, J.D. & NUDEL, D.B. (1983). Maturation-related differences in regional circulatory effects of dopamine infusion in swine. *Dev. Pharmacol. Ther.*, **6**, 9–22.

HIEBLE, J.P., OWEN, D.A.A., HARVEY, C.A., BLUMBERG, A.L., VALOCIK, R.E. & DEMARINIS, R.M. (1987). Hemodynamic effects of selective receptor agonists in the rat and dog. *Clin. Exp. Hypertension*, **9**, 889–912.

HUGHES, A.D. & SEVER, P.S. (1989). Action of fenoldopam. A selective dopamine (DA_1) receptor agonist, on isolated human arteries. *Blood Vessels*, **26**, 119–127.

KOPIA, G.A. & VALOCIK, R.E. (1989). Demonstration of specific dopamine-1 receptor-mediated coronary vasodilation in the anesthetized dog. *J. Pharmacol. Exp. Ther.*, **248**, 215–221.

NIEMEGEERS, C.J.E., VAN NUETEN, J.M. & JANSSEN, P.A.C. (1974). Azaperone, a sedative neuroleptic of the butyrophenone series with pronounced anti-aggressive and anti-shock activity. *Arzneim. Forsch.*, **24**, 1798–1806.

SASSEN, L.M.A., SOEI, L.K., KONING, M.M.G. & VERDOUW, P.D. (1990a). The central and regional cardiovascular responses to intravenous and intracoronary administration of the phenyldihydropyridine elgodipine in anaesthetized pigs. *Br. J. Pharmacol.*, **99**, 355–363.

SASSEN, L.M.A., DUNCKER, D.J.G.M., GHO, B.C.G., DIEKMANN, H.W. & VERDOUW, P.D. (1990b). Haemodynamic profile of the potassium channel activator EMD 52692 in anaesthetized pigs. *Br. J. Pharmacol.*, **101**, 605–614.

SAXENA, P.R., SCHAMHARDT, H.C., FORSYTH, R.P. & LOEVE, J. (1980). Computer programs for the radioactive microsphere technique. Determination of experimental circumstances. *Comp. Prog. Biomed.*, **12**, 63–84.

SCHUELKE, D.M., MARK, A.L., SCHMID, P.G. & ECKSTEIN, J.W. (1971). Coronary vasodilation produced by dopamine after adrenergic blockade. *J. Pharmacol. Exp. Ther.*, **176**, 320–327.

TODA, N. (1983). Dopamine vasodilates human cerebral arteries. *Experientia*, **39**, 1131–1132.

TODA, N. & HATANO, Y. (1979). Antagonism by droperidol of dopamine-induced relaxation in isolated dog arteries. *Eur. J. Pharmacol.*, **57**, 231–238.

UEDA, Y., YANO, S. & SAKANASHI, M. (1982). In vitro evidence for dopaminergic receptors in human renal artery. *J. Cardiovasc. Pharmacol.*, **4**, 76–81.

VERDOUW, P.D., SASSEN, L.M.A., DUNCKER, D.J., SCHMEETS, I.O.L., RENSEN, R.J. & SAXENA, P.R. (1987). Nicorandil-induced changes in the distribution of cardiac output and coronary blood flow in pigs. *Naunyn-Schmiedebergs Arch. Pharmacol.*, **336**, 352–358.

ZHAO, R., FENNELL, W.H. & ABEL, F.L. (1990). Effects of dopamine D_1 and dopamine D_2 receptor agonists on coronary and peripheral hemodynamics. *Eur. J. Pharmacol.*, **190**, 193–202.

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Chapter 9

Comparison of the cardiovascular effects of dopamine and epinine in conscious pigs.

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submitted for publication

Comparison of the cardiovascular effects of epinine and dopamine in conscious pigs

Summary

The effects of epinine or dopamine on systemic hemodynamics and plasma concentrations of catecholamines and prolactin were studied in conscious pigs before and after combined non-selective alpha- and beta- adrenoceptor blockade. Epinine increased cardiac output (CO, 24 ± 6%), which for doses less than 10 $\mu g.kg^{-1}.min^{-1}$ was due to an increase in heart rate (HR). At 10 $\mu g.kg^{-1}.min^{-1}$ HR decreased slightly (10 ± 3%, as compared to the value obtained at 5 $\mu g.kg^{-1}.min^{-1}$) and stroke volume increased up to 15% ($P<0.05$). Mean arterial pressure (MAP, 99 ± 3 mmHg at baseline) decreased dose dependently (14 ± 2%, $P<0.05$) up to the infusion rate of 5 $\mu g.kg^{-1}.min^{-1}$, but increased by 4.0 ± 1.8 mmHg during infusion of 10 $\mu g.kg^{-1}.min^{-1}$. Systemic vascular resistance (SVR) decreased up to 23 ± 3% for doses less than 10 $\mu g.kg^{-1}.min^{-1}$, but did not further change during infusion of the highest dose. $LVdP/dt_{max}$ increased during the two highest infusion rates up to 22 ± 6% ($P<0.05$). After the infusion was stopped there was an abrupt increase in HR (18 ± 4%, $P<0.05$) and a further decrease in SVR before all parameters returned to baseline. Adrenoceptor blockade inhibited all epinine-induced changes. Dopamine caused similar increases in CO (27 ± 3%) as epinine, the only difference being that HR continued to increase (32 ± 5%) and MAP (13 ± 3%) and SVR continued to decrease (31 ± 3%) over the entire dose range. The increase in $LVdP/dt_{max}$ at the highest dose (48 ± 4%, $P<0.05$) was more pronounced than with epinine. Adrenoceptor blockade did not affect the dopamine-induced changes in CO, SVR and MAP, but attenuated the increases in HR and $LVdP/dt_{max}$. Norepinephrine (NE) and epinephrine (E) concentrations did not change during infusion of epinine or dopamine, but NE increased by 50% within 2.5 min after stopping the infusion of epinine. After adrenoceptor blockade NE and E concentrations did not change during infusion of dopamine, but NE decreased by 55 ± 5 % of its baseline value ($P<0.05$) during infusion of epinine. Prolactin concentrations decreased gradually from 480 ± 40 $pg.ml^{-1}$ to 270 ± 50 $pg.ml^{-1}$ ($P<0.05$) during infusion of epinine, but did not change significantly during dopamine infusion. The differential effects of epinine and dopamine on MAP, SVR, plasma norepinephrine (before and after adrenoceptor blockade) and prolactin, leads us to conclude that in conscious pigs, epinine is a more potent *alpha*, *beta*-2 and *dopamine*-2-agonist, but a weaker *dopamine*-1-agonist than dopamine.

Introduction

Intravenous administration of dopamine has proved to be useful in some patients with congestive heart failure, but poor absorption after oral administration seriously limits its use in the chronic treatment of this disorder (Goldberg et al., 1974). Therefore, there has been a long-lasting search for dopaminergic drugs that are active after oral administration (Casagrande, 1986). This has led to the development of ibopamine, the di-iso-butyrylester of epinine (N-methyldopamine), for the chronic treatment of congestive heart failure. Ibopamine is a prodrug, which itself is devoid of pharmacological activity. It is rapidly hydrolyzed by plasma esterases to the active moity epinine (Randolph et al., 1983, Pocchiari et al., 1986 and Rajfer et al., 1986). The cardiovascular actions of epinine have been studied and compared to those of dopamine, but the investigations have exclusively been performed in anesthetized animals (Itoh et al., 1985, Nichols et al, 1987a,b, Shebuski et al., 1987 and Kopia et al., 1988). Epinine stimulates non-selectively *alpha*-, *beta*- and *dopamine* (DA) receptors. Stimulation of *dopamine*-2-receptors inhibits ganglionic and noradrenergic neurotransmission, preferentially at high levels of sympathetic activity. Because during anesthesia there may be high sympathetic activity, we studied the cardiovascular actions of epinine and dopamine in conscious animals, thereby avoiding the possible interference of anesthetic agents. The pig was chosen as the experimental animal, because of its increasing use in the study of chronic left ventricular dysfunction (Van der Giessen et al., 1989 and Van Woerkens et al., 1991b,c,d).

To elucidate its mechanisms of action, we studied the cardiovascular effects of infusions of epinine and those of dopamine before and after non-selective blockade of the alpha- and beta-adrenoceptors, in order to delineate the contribution of *dopamine*-1-, *beta*-2- and alpha-adrenoceptor stimulation to the overall vasodilator response to these drugs. We also determined arterial plasma concentrations of catecholamines, since dopamine and epinine may inhibit noradrenergic neurotransmission and norepinephrine release by presynaptic *alpha*-1- and *dopamine*-2- receptor stimulation. Furthermore, plasma prolactin was measured as a specific *dopamine*-2-receptor mediated response, since stimulation of *dopamine*-2 receptors in the pituitary gland inhibits release of prolactin (Moore and Bloom, 1978).

Materials and Methods

The experimental procedures were approved by the Committee on Animal Experimentation of the Erasmus University Rotterdam, and complied with the Guide for the Care and Use of Laboratory Animals as adopted by the National Institutes of Health.

Surgical procedures.

After an overnight fast, cross-bred Landrace x Yorkshire pigs of either sex (19-21 kg at the time of surgery), pretreated with 600 mg of a mixture of procaine penicillin-G and benzathine penicillin-G intramuscularly (Duplocillin, Gist-Brocades NV, Delft, The Netherlands), were sedated with an intramuscular injection of 30 $mg.kg^{-1}$ ketamine HCL (Aeskoket, Aesculaap BV, Boxtel, The Netherlands). Subsequently the animals were intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1:2) to which 1-4% (v/v) enflurane was added. Under sterile conditions, a jugular vein and a common carotid artery were cannulated for infusion of drugs or solvent and measurement of arterial blood pressure, respectively. The chest was opened via the third left intercostal space and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for the measurement of aortic blood flow. The heart was exposed via the fifth intercostal space and a pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) 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. The wires were than tunnelled to the back, the chest was closed and the animals were allowed to recover.

Post-surgical period

The animals received daily intravenous doses of 500 mg amoxicillin (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and, during the first week only, 500 mg kanamicine 10% (Alfasan, Woerden, The Netherlands) to prevent infection. Catheters were flushed daily with an isotonic saline solution containing 500 IU ml^{-1} heparin. During the first weeks of the post-operative recovery period the animals were daily adapted to the laboratory facilities (8 to 10 sessions), while hemodynamic parameters were monitored. The experimental protocols were executed when systemic hemodynamics remained stable for at least one hour, usually 2-3 weeks after instrumentation.

Experimental protocol

On the day of the experiment 4 doses of either epinine or dopamine were infused at incremental rates of 1, 2.5, 5 and 10 $\mu g.kg^{-1}.min^{-1}$ for 10 minutes each. At the end of each infusion step and at 2.5, 5, 10 and 15 min of the post-infusion period, systemic hemodynamic parameters were recorded and 4 ml of arterial blood was withdrawn for determination of plasma concentrations of catecholamines and prolactin. Experiments were performed before and after combined blockades of alpha- adrenoceptors with phentolamine (1 $mg.kg^{-1}$) and of beta- adrenoceptors with propranolol (0.5 $mg.kg^{-1}$ + 0.5 $mg.kg^{-1}.h^{-1}$, Duncker et al., 1987). The stability of the cardiovascular condition of each animal was evaluated by infusion of the solvent at identical volumes (0.2, 0.5, 1.0 and 2.0 $ml.min^{-1}$ for 10 min) as used during the infusion of the drugs. Infusions of the drugs or solvent were in random order and experiments in the same animals were separated by at least 24 hours. All measurements were done while the animals were quietly resting in a constraining jacket.

Plasma catecholamine determination

Catecholamines (norepinephrine, epinephrine and dopamine) and epinine were measured simultaneously by HPLC with gradient elution and with fluorimetric detection (Van der Hoorn et al., 1989 and Boomsma et al., 1991). Arterial plasma porcine prolactin concentrations were estimated by a specific, homologous double-antibody radioimmunoassay (Bevers et al., 1978). The intra- and interassay coefficients of variation were 1.8 % and 15.1 % respectively. The sensitivity of the assay was 0.2 $\mu g.l^{-1}$

Drugs

Epinine was a gift from Dr. C. Casagrande (Zambon Research, Milan, Italy) and prepared daily in physiological saline (0.9% NaCl) and infused in concentrations of 5 $\mu g.ml^{-1}$. Epinine appeared to be contaminated with dopamine by 0.66%. Dopamine, propranolol and phentolamine were obtained from the Pharmacy of the Academic Hospital Rotterdam "Dijkzigt".

Statistical analysis

All data are presented as the mean ± SEM. The significance of the effects of the drugs has been evaluated by comparing the changes from baseline induced by the drugs to the changes from baseline during infusion of the solvent, using analysis of variance. Significance was accepted for $P<0.05$. A Bonferroni correction was used because of comparison for multiple measurements.

Results

Stability of animal preparation

Infusion of the solvent did not affect any of the cardiovascular parameters or arterial plasma catecholamine levels (table 1).

Cardiovascular changes during infusion of epinine before and after adrenoceptor blockade

Infusion of epinine increased cardiac output (CO) dose dependently up to 22% (Figure 1). At the lower dose range (1-5 $\mu g.kg^{-1}.min^{-1}$), the increase was exclusively due to an increase in heart rate (HR, up to 17%, $P<0.05$), but during infusion of 10 $\mu g.kg^{-1}.min^{-1}$, HR decreased from 132 ± 8 beats.min^{-1} to 125 ± 5 beats.min^{-1} ($P<0.05$) and stroke volume increased from 23.7 ± 1.7 ml to 27.6 ± 2.5 ml ($P<0.05$). Mean arterial blood pressure (MAP) decreased dose dependently during infusions up to 5 $\mu g.kg^{-1}.min^{-1}$, but increased by 4.0 ± 1.8 mmHg ($P<0.05$) during infusion of the highest dose. Calculation of the arterial pulse pressure (difference between systolic and diastolic arterial blood pressure) revealed no change for doses less than 5 $\mu g.kg^{-1}.min^{-1}$ (46 ± 4 mmHg), but an increase to 51 ± 3 mmHg ($P<0.05$) during infusion of 10 $\mu g.kg^{-1}.min^{-1}$, as systolic arterial blood pressure increased (4.1 ± 2.2 mmHg), but diastolic arterial blood pressure did not change. In view of the changes in CO and MAP it can be calculated that systemic vascular resistance (SVR) decreased up to approximately 25% in the dose range of 1-5 $\mu g.kg^{-1}.min^{-1}$, but did not further change during infusion of the highest dose. $LVdP/dt_{max}$ increased slightly (6 ± 2%, $P<0.05$) during infusion of 5 $\mu g.kg^{-1}.min^{-1}$ but rose markedly (22 ± 6%, $P<0.05$) during infusion of the highest dose. Left ventricular end-diastolic pressure was not influenced at any dose.

After *alpha*- and *beta*-adrenoceptor blockade, infusion of epinine had no significant effect on any of the cardiovascular parameters (figure 1).

Table 1. *Effect of solvent on cardiovascular parameters and plasma catecholamines in 10 conscious pigs.*

	Baseline	Solvent ($ml.min^{-1}$ for 10 min) 0.2	0.5	1	2
Systemic hemodynamics					
CO ($l.min^{-1}$)	2.61 ± 0.15	2.60 ± 0.15	2.60 ± 0.14	2.58 ± 0.14	2.61 ± 0.13
MAP (mmHg)	98 ± 3	99 ± 4	98 ± 3	98 ± 3	98 ± 3
HR ($beats.min^{-1}$)	111 ± 4	111 ± 5	111 ± 4	109 ± 4	111 ± 4
$LVdP/dt_{max}$ ($mmHg.s^{-1}$)	3140 ± 120	3100 ± 120	3060 ± 120	3080 ± 130	3150 ± 150
LVEDP ($mmHg.s^{-1}$)	8.7 ± 0.4	8.8 ± 0.4	8.8 ± 0.4	8.8 ± 0.4	8.9 ± 0.5
SVR ($mmHg.min.l^{-1}$)	37.4 ± 2.2	37.7 ± 2.3	37.8 ± 2.3	38.0 ± 2.2	37.9 ± 2.2
SV (ml)	24.0 ± 1.7	23.7 ± 1.5	23.9 ± 1.5	24.2 ± 1.6	23.9 ± 1.5
Arterial plasma catecholamines					
Dopamine ($pg.ml^{-1}$)	19 ± 5	16 ± 4	15 ± 3	15 ± 3	29 ± 6
Norepinephrine ($pg.ml^{-1}$)	211 ± 31	186 ± 21	191 ± 20	182 ± 17	268 ± 69
Epinephrine ($pg.ml^{-1}$)	130 ± 41	123 ± 24	147 ± 24	115 ± 25	131 ± 29

CO = cardiac output; MAP = mean arterial blood pressure; HR = heart rate; $LVdP/dt_{max}$ = maximal rate of rise in left ventricular pressure; LVEDP = left ventricular end-diastolic pressure; SVR = systemic vascular resistance; SV = stroke volume. All data have been presented as mean ± SEM.

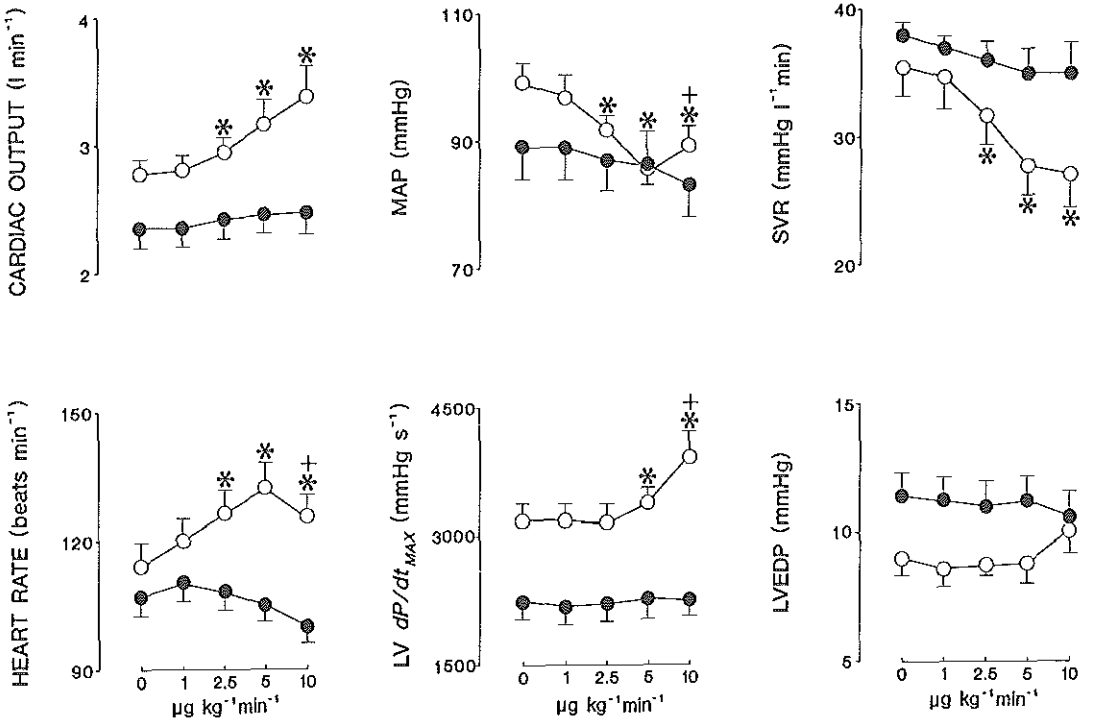

Fig. 1. *Cardiovascular effects of consecutive 10 min infusions of epinine in conscious pigs. Infusions were performed before (○, n = 10) and after combined non-selective alpha- and beta-adrenoceptor blockade (●, n = 5). All data have been presented as mean ± SEM. * P<0.05 versus pre-drug value (0 µg.kg⁻¹.min⁻¹). + P<0.05 versus value obtained after 5 µg.kg⁻¹.min⁻¹.*

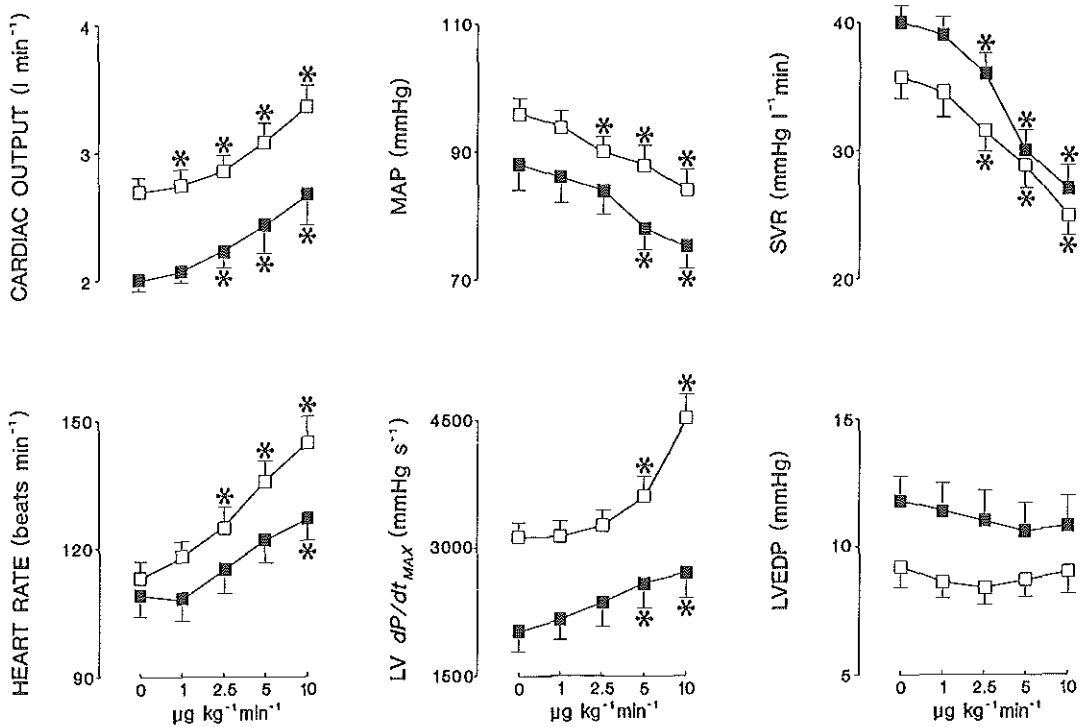

Fig. 2. *Cardiovascular effects of consecutive 10 min infusions of dopamine in conscious pigs. Infusions were performed before (□, n = 10) and after combined non-selective alpha- and beta- adrenoceptor blockade (■, n = 5). All data have been presented as mean ± SEM. * P<0.05 versus pre-drug value (0 µg.kg^{-1}.min^{-1}). + P<0.05 versus value obtained after 5 µg.kg^{-1}.min^{-1}.*

Dopamine exerted very similar cardiovascular effects as compared to epinine in the dose range of 1-5 $\mu g.kg^{-1}.min^{-1}$, but during infusion of 10 $\mu g.kg^{-1}.min^{-1}$ there were some differences: HR continued to increase and SVR continued to decrease (figure 2). Finally, at the highest dose the dopamine-induced increase in $LVdP/dt_{max}$ (1440 ± 110 $mmHg.s^{-1}$) was considerably larger than after epinine (680 ± 210 $mmHg.s^{-1}$, $P<0.05$). Blockade of the alpha- and beta- adrenoceptors neither affected the dopamine-induced increase in CO, nor the dopamine-induced decreases in MAP and SVR (Figure 2). HR still increased, but this was significant only at the highest dose. $LVdP/dt_{max}$ increased, but the effect (690 ± 160 $mmHg.s^{-1}$) was attenuated compared to the increment without blockade (1440 ± 110 $mmHg.s^{-1}$, $P<0.05$).

Cardiovascular effects of epinine and dopamine during the post-infusion period

Immediately after the epinine infusions were stopped there was an immediate increase in HR of 49 ± 5 $beats.min^{-1}$ (40 ± 5%), which was accompanied by a further increase of 680 ± 170 $mmHg.s^{-1}$ (18 ± 5%) in $LVdP/dt_{max}$ (table 2). The tachycardia was most likely due to a reflex mechanism which became operative to prevent severe hypertension, because of a further fall in SVR. CO and HR remained elevated, but all other parameters returned to baseline values in the subsequent recovery period. Adrenoceptor blockade prevented the tachycardia in the post-infusion period.
After the dopamine infusions had been stopped, all parameters returned gradually towards baseline values (table 2). HR was still elevated after 15 min. A similar pattern of changes was observed when the dopamine infusions were performed after adrenoceptor blockade (table 2).

Arterial plasma concentrations of catecholamines during infusion of epinine before and after adrenoceptor blockade

After infusion of epinine plasma concentrations of epinine increased in a dose-dependent way up to 6,2020 ± 3,980 $pg.ml^{-1}$ (figure 3). During the subsequent washout period there was a very rapid decline in plasma levels to 3,690 ± 410 $pg.ml^{-1}$ after 2.5 min and 430 ± 90 $pg.ml^{-1}$ after 15 min (table 2). A very similar pattern, was found when the epinine infusions were repeated after adrenoceptor blockade (figure 3).
Dopamine plasma concentrations increased dose dependently from 42 ± 24 $pg.ml^{-1}$ to 620 ± 226 $pg.ml^{-1}$ with increasing infusion rates of epinine, and fell rapidly after the infusions were stopped. These changes in dopamine can be fully accounted for by the contamination of epinine with 0.66% of dopamine. A similar pattern of changes in

Table 2. *Cardiovascular effects of epinine and dopamine during the post-infusion period*

		Baseline	After	Post-infusion period (min)			
			10 $\mu g.kg^{-1}.min^{-1}$	2.5	5	10	15
CO	E	2.77 ± 0.11	3.38 ± 0.25*	3.56 ± 0.23*+	3.29 ± 0.24*	3.07 ± 0.08*	2.84 ± 0.09*
	D	2.69 ± 0.11	3.36 ± 0.17*	3.02 ± 0.17*+	2.70 ± 0.07*+	2.64 ± 0.11+	2.57 ± 0.11+
	αßE	2.35 ± 0.16	2.47 ± 0.17	2.40 ± 0.15	2.38 ± 0.14	2.33 ± 0.15	2.28 ± 0.12
	αßD	2.00 ± 0.12	2.67 ± 0.26*	2.33 ± 0.14*+	2.30 ± 0.14+	2.15 ± 0.14+	2.12 ± 0.15+
MAP	E	99 ± 3	89 ± 4*	85 ± 3*	92 ± 4	97 ± 4+	100 ± 4+
	D	96 ± 2	84 ± 3*	95 ± 5+	99 ± 4+	101 ± 4+	89 ± 7
	αßE	89 ± 8	83 ± 6*	84 ± 6	88 ± 6	89 ± 7	89 ± 7
	αßD	88 ± 6	75 ± 4*	88 ± 5+	91 ± 3+	96 ± 5*+	95 ± 5*+
HR	E	114 ± 7	125 ± 5*	174 ± 6*+	158 ± 6*+	144 ± 7*+	137 ± 8*+
	D	113 ± 5	145 ± 8*	136 ± 9*+	130 ± 9*+	126 ± 9*+	123 ± 9*+
	αßE	107 ± 5	100 ± 4*	107 ± 3	104 ± 3	102 ± 6	100 ± 6
	αßD	109 ± 6	127 ± 7*	106 ± 2	111 ± 7+	106 ± 6+	103 ± 6+
$LVdP/dt_{max}$	E	3190 ± 210	3930 ± 300*	4620 ± 350*+	4280 ± 320*	3810 ± 320*	3670 ± 350
	D	3120 ± 200	4560 ± 250*	4220 ± 190*	3970 ± 350*+	3820 ± 450*+	3480 ± 360+
	αßE	2250 ± 210	2250 ± 170	2250 ± 160	2230 ± 150	2290 ± 200	2250 ± 180
	αßD	2020 ± 250	2710 ± 330*	2370 ± 310	2490 ± 310	2450 ± 300+	2390 ± 290+

LVEDP	E	9.0	±	0.7	10.1	±	1.0	9.1	±	0.9^{+}	8.4	±	0.9^{+}	9.0	±	1.0	9.1	±	1.0
	D	9.2	±	0.8	9.0	±	0.9	8.8	±	1.1	8.3	±	1.1	8.3	±	0.9	9.0	±	1.0
	αßE	11.4	±	0.9	10.6	±	1.1	11.0	±	1.4	10.2	±	1.2	10.2	±	1.2	10.0	±	1.2
	αßD	11.8	±	1.0	10.8	±	1.2	10.8	±	1.3	11.6	±	1.2	11.4	±	1.2	11.2	±	1.1
SVR	E	35.5	±	2.3	27.1	±	2.6*	24.3	±	2.3*	28.0	±	2.6*	30.5	±	2.0*$^{+}$	33.8	±	2.0^{+}
	D	35.7	±	1.8	24.9	±	1.5*	29.9	±	1.8*$^{+}$	35.0	±	0.8^{+}	36.2	±	1.4^{+}	37.0	±	1.5^{+}
	αßE	37.7	±	0.7	34.5	±	2.7	36.5	±	2.6^{+}	38.4	±	3.0^{+}	40.1	±	2.8^{+}	40.7	±	2.4^{+}
	αßD	41.3	±	0.6	27.0	±	2.4*	36.3	±	2.2^{+}	38.6	±	2.1^{+}	42.9	±	2.3^{+}	43.1	±	2.7^{+}
SV	E	24.2	±	1.8	27.6	±	2.5*	21.4	±	1.9^{+}	21.5	±	1.8^{+}	22.1	±	1.3^{+}	21.9	±	1.4^{+}
	D	24.1	±	1.7	23.4	±	1.4	23.1	±	2.0	22.8	±	2.1	23.1	±	2.3	22.6	±	2.4
	αßE	22.4	±	1.1	25.9	±	2.7	22.6	±	0.6	23.4	±	1.9	24.4	±	2.8	24.1	±	1.9
	αßD	19.1	±	0.7	22.0	±	0.4	22.2	±	1.3	22.4	±	1.6	21.2	±	0.5	21.8	±	1.0
Norepinephrine	E	261	±	64	235	±	22	345	±	35^{+}	290	±	24	247	±	22	308	±	82
	D	326	±	142	417	±	46	278	±	33^{+}	301	±	45^{+}	253	±	38^{+}	251	±	49^{+}
	αßE	1170	±	250	570	±	120*	650	±	120*	570	±	110*	520	±	90*	550	±	120*
	αßD	1260	±	230	1230	±	240	840	±	240^{+}	860	±	120*$^{+}$	800	±	170*	710	±	180*$^{+}$
Epinephrine	E	109	±	19	103	±	8	118	±	19	124	±	13	139	±	23	159	±	38
	D	92	±	12	80	±	15	117	±	21	98	±	29	103	±	28	121	±	17^{+}
	αßE	262	±	81	457	±	195	497	±	172	441	±	152	520	±	277	641	±	366
	αßD	279	±	59	218	±	52*	186	±	64*	220	±	52	194	±	63	224	±	85

CO = cardiac output ($l.min^{-1}$); MAP = mean arterial blood pressure (mmHg); HR = heart rate ($beats.min^{-1}$); SV = stroke volume (ml); $LVdP/dt_{max}$ = maximum rate of rise of left ventricular end-diastolic pressure ($mmHg.s^{-1}$); LVEDP = left ventricular end-diastolic pressure (mmHg); SVR = systemic vascular resistance ($mmHg.l^{-1}.min$); SV = stroke volume (ml); Norepinephrine and epinine concentrations are in $pg.ml^{-1}$. Data have been presented as mean ± SEM. $P<0.05$ versus Baseline; $^{+}$ $P<0.05$ versus 10 $\mu g.kg^{-1}.min^{-1}$*

dopamine was seen when the epinine infusions were repeated after adrenoceptor blockade (not shown).

Plasma norepinephrine concentrations (NE) did not change during infusion of epinine (Fig. 4), but increased from 235 ± 22 pg.ml^{-1} to 345 ± 35 pg.ml^{-1} ($P<0.05$) (table 2) during the first 2.5 min of the post-infusion period and returned to baseline in the following minutes. NE increased sharply from 160 ± 30 pg.ml^{-1} to 1040 ± 170 pg.ml^{-1} ($P<0.05$) after alpha-adrenoceptor blockade, and rose further to 1360 ± 230 μg.ml^{-1} after additional beta-adrenoceptor blockade (figure 4). During the additional infusion of epinine there was a decrease in NE which continued during the postinfusion period to 550 ± 120 pg.ml^{-1}, $P<0.05$ versus baseline) (table 2). Now, the increase previously seen in the early post-infusion period before adrenoceptor-blockade (from 570 ± 120 pg.ml^{-1} to 650 ± 120 pg.ml^{-1}) was not significant. Epinephrine (E) was not affected by infusion of epinine, neither before nor after adrenoceptor blockade (table 2, figure 4).

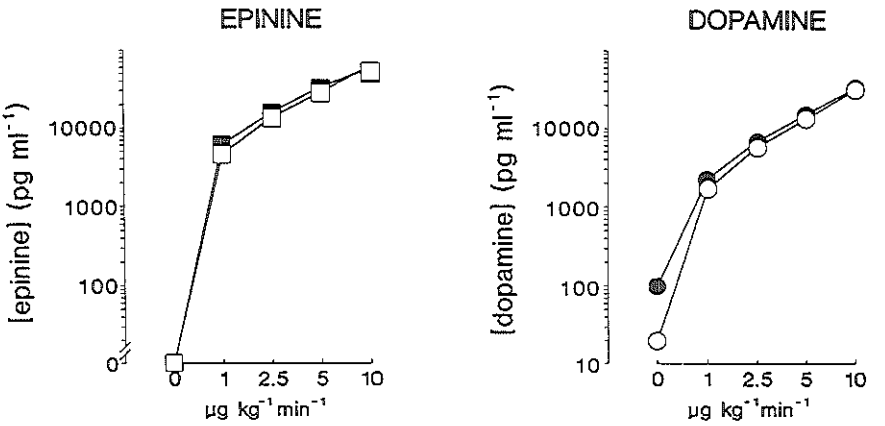

Fig. 3. *Arterial plasma levels of epinine and dopamine during intravenous infusion of epinine and of dopamine in conscious pigs, respectively. Infusions were performed before (open symbols) and after (closed symbols) adrenoceptor blockade.*

Arterial plasma concentrations of catecholamines during infusion of dopamine before and after adrenoceptor blockade.

Dopamine concentrations increased up to 1080 ± 150 pg.ml^{-1} during infusion of dopamine. The same dopamine concentrations were observed when the infusions were repeated after adrenoceptor blockade (figure 3).

During the post-infusion period the dopamine concentration gradually declined, but it was still elevated after 15 min (table 2). During the dopamine infusions, both before and after adrenoceptor blockade, NE and E were similar to the concentrations seen during the epinine infusions. The same was true in the post-infusion period, however, in contrast to what was observed after stopping epinine norepinephrine did not rise during the early washout period (table 2).

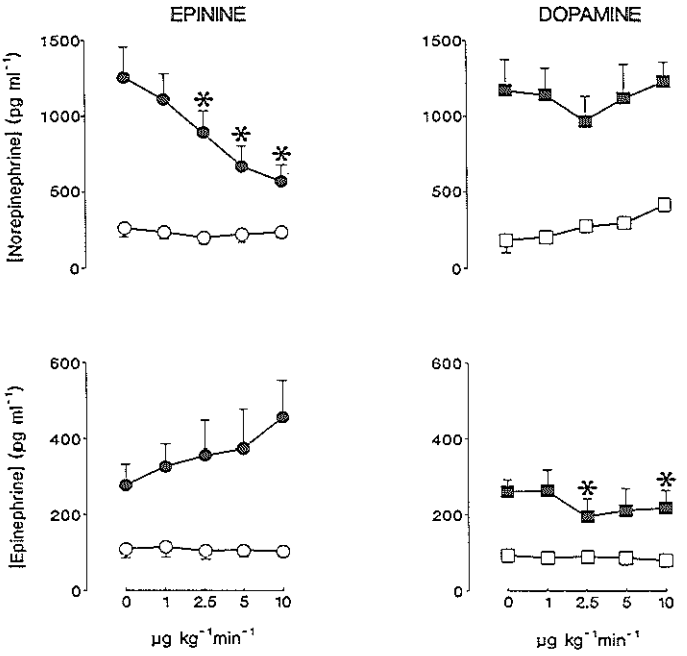

Fig. 4. *Arterial plasma catecholamine concentrations during intravenous infusion of epinine and of dopamine, respectively. Infusions were performed before (open symbols, n = 10) and after (closed symbols, n = 5) combined non-selective alpha- and beta- adrenoceptor blockade. All data have been presented as mean ± SEM. * P<0.05 versus pre-drug value (0 µg.kg^{-1}.min^{-1}). + P<0.05 versus value obtained after 5 µg.kg^{-1}.min^{-1}.*

Arterial plasma concentrations of prolactin during infusion of epinine and dopamine

Figure 5 shows that the arterial concentrations of prolactin decreased gradually ($p < 0.05$) during the infusion of epinine, but did not change significantly during infusion of dopamine (figure 5).

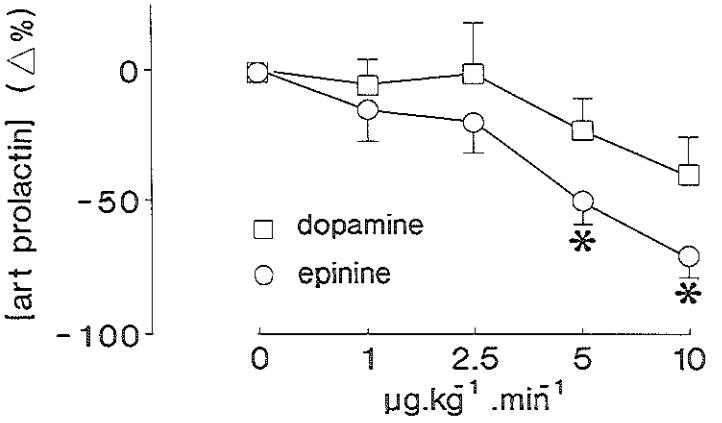

Fig. 5. *Changes in arterial plasma concentrations of prolactin during infusion of epinine and of dopamine. All data have been presented as mean ± SEM. * $P < 0.05$ versus pre-drug value (0 $\mu g.kg^{-1}.min^{-1}$).*

Discussion

In several studies the cardiovascular effects of epinine and dopamine have been compared (Itoh et al., 1985, Nichols et al., 1987a,b, Shebuski et al., 1987 and Kopia et al., 1988). All of these studies, however, have been performed in animals under anesthesia and this condition may have influenced the results. For instance, Itoh et al. (1985) reported that heart rate decreased and Kopia et al. (1988) that heart rate increased, when epinine was infused in anesthetized dogs in a dose range similar to that used in the present study. In the present investigation the animals were conscious, thus avoiding any possible interference of anesthetic agents with the hemodynamic effects of the drugs under study. The animals had been carefully adapted to the laboratory conditions, as reflected by the stability of the cardiovascular condition and arterial plasma cathecholamines concentrations during infusion of the solvent. Moreover, the plasma concentrations of norepinephrine and epinephrine in our instrumented animals

were very similar to those reported by Barrand et al. (1981) for conscious, not-instrumented pigs of the same age and weight. This strongly suggests that the stability of the hemodynamic status and the catecholamine concentrations indeed reflects that the animals were not disturbed.

Changes in $LVdP/dt_{max}$ are frequently interpreted as changes in inotropy, but the dependency of $LVdP/dt_{max}$ on heart rate, pre- and afterload, limits the value of $LVdP/dt_{max}$ as an index of myocardial contractility. In the range of 1-5 $\mu g.kg^{-1}.min^{-1}$ epinine and dopamine caused similar changes in $LVdP/dt_{max}$, heart rate, left ventricular end-diastolic pressure and arterial blood pressure. Similar changes in $LVdP/dt_{max}$ after both agents therefore suggests that epinine and dopamine had equal effects on myocardial contractility. This positive inotropic effect was most likely also the reason that stroke volume increased at this dose as systemic vascular resistance and left ventricular end-diastolic pressure were not affected and mean arterial blood pressure even slightly increased.

Infusion of epinine, after combined non-selective alpha- and beta-adrenoceptor blockade did not change any of the cardiovascular parameters, which suggests that stimulation of alpha- and beta-adrenoceptors was the cause of its cardiovascular effects. This conclusion is premature, however, as both arterial prolactin and, norepinephrine concentrations after alpha- and beta-adrenoceptor blockade progressively decreased during infusion of epinine. Both effects can be explained by stimulation of *dopamine*-2-receptors. Prolactin release from the pituitary gland is directly inhibited through stimulation of *dopamine*-2-receptors (Moore and Bloom, 1978). Noradrenergic neurotransmission is inhibited by *dopamine*-2-receptors located either on gangliomic cells or located prejunctionally on post-ganglionic sympathetic nerve fibers, thus diminishing noradrenaline release and spill-over into plasma. The latter process mainly comes into action at higher levels of sympathetic nerve traffic as is the case during non-selective alpha-adrenoceptor blockade (Lokhandwala and Barratt, 1982). The unchanged systemic vascular resistance, during epinine infusion, after non-selective adrenoceptor blockade, indicates that the vasodilator capacity of epinine via *dopamine*-1- receptor activation is limited. Under the experimental conditions of the current protocol a *dopamine*-2-receptor mediated contribution to the overall vasodilator effect of epinine is difficult to demonstrate for two reasons: Firstly when sympathetic tone is not elevated, such a mechanism probably contributes little to the vasodilator effect. Secondly, effect of epinine during combined alpha- and beta-adrenergic blockade a decreased alpha-adrenoceptor mediated vasoconstrictor tone, as witnessed by a fall in plasma noradrenaline after infusion of epinine, would not have produced vasodilatation, since alpha-adrenoceptors were blocked. Therefore, our data do not exclude the possibility that epinine exerts a

vasodilator action through *dopamine*-2-receptors when, sympathetic tone is elevated, as is the case in severe forms of congestive heart failure.

From the decrease in prolactin levels we may conclude that epinine, in the dose range tested (1-10 $\mu g.kg^{-1}.min^{-1}$), is a more potent *dopamine*-2-agonist, than dopamine. Since the decrease in systemic vascular resistance by dopamine was not affected by combined alpha- and beta-adrenoceptor blockade, and dopamine is a weaker *dopamine*-2-agonist than epinine, it must follow that dopamine is a more potent *dopamine*-1-agonist than epinine. Given the magnitude of the changes in $LVdP/dt_{max}$ and heart rate after calcium channel blockers in conscious pigs (Duncker et al., 1988), the *beta*-1-agonistic activity of epinine and dopamine, if any, appears to be rather weak from a quantitative point of view, in the dose-range tested. At 10 $\mu g.kg^{-1}.min^{-1}$ the alpha-adrenoceptor agonistic activity of epinine appears to exceed that of dopamine, because during infusion of that dose arterial blood pressure and vascular resistance started to increase and heart rate decreased. Additional evidence here is the large increase in heart rate and the fall in arterial blood pressure, due to a decrease in systemic vascular resistance, immediately after the infusion of epinine had been stopped. The latter was, most likely, due to an abrupt loss of its alpha-adrenoceptor agonistic activity at lower plasma concentrations of epinine. With dopamine such a sudden increase in heart rate and decrease in arterial blood pressure were not seen, providing evidence that a sudden loss of alpha-adrenoceptor agonistic activity after stopping the infusion of dopamine did not occur. Furthermore, plasma norepinephrine abruptly increased after stopping epinine and not after cessation of the dopamine infusion, which is further evidence for the baroreflex-mediated nature of the hemodynamic changes as seen after the former. Finally, considering the facts that 1) the observed vasodilation with epinine was due to *beta*-2-adrenoceptor activation (alpha- and beta-adrenoceptor blockade inhibited the epinine-induced vasodilation), 2) that alpha- and beta-adrenoceptor blockade did not affect the vasodilator response to dopamine, and 3) that dopamine is the weaker alpha-adrenoceptor agonist, it also follows that epinine is a more potent *beta*-2-adrenoceptor agonist than dopamine.

These data lead us to conclude that in the conscious pigs and in the dose-range tested (1-10 $\mu g.kg^{-1}.min^{-1}$), epinine is a weaker *dopamine*-1-agonist, but a more potent *dopamine*-2, *beta*-2- and alpha-agonist than dopamine, while the *beta*-1-agonistic potency of both compounds is rather weak, if present at all. Further studies are required to delineate the role of these receptors in the hemodynamic profile of epinine or its prodrug ibopamine in healthy man and in patients with congestive heart failure.

References

Boomsma F, Alberts G, Hoorn FAJ, Man in 't Veld AJ and Schalekamp MADH. Simultaneous determination of free cathecholamines and epinine and estimation of total epinine and dopamine in plasma and urine by HPLC with fluorimetric detection. *J Chromatography* 1991, in press.

Bevers MM, WILLEMSE AH and KRUIP ThAM. Plasma prolactin levels in the sow during lactation and the postweaning period as measured by radioimmunoassay. *Biol Reprod* 19; 628-634, 1978.

Casagrande C. Pharmacology and clinical applications of ibopamine, a pro-drug acting on dopamine receptors. *Arzneimittel Forschung* 36; 285-408, 1986.

Duncker DJ, Saxena PR and Verdouw PD. Systemic hemodynamic and beta-adrenoceptor antagonistic effects of bisoprolol in conscious pigs: a comparison with propranolol. *Arch Int Pharmacodyn Ther* 290; 54-63, 1987.

Duncker DJ, Saxena PR and Verdouw PD. Systemic hemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *Eur J Pharmacol* 156; 401-409, 1988.

Goldberg LI. Dopamine - Clinical uses of an endogenous catecholamine. *N Engl J Med* 291; 707-710, 1974.

Holubarsch CH, Walde T, Hasenfuss G, Pieske B and Just H. Concentration-dependent separation between vasodilating and positive inotropic effect of ibopamine/epinine. *Eur Heart Journal* 12 (abstract suppl.): 183, 1991.

Itoh H, Hohli J, Rajfer S and Goldberg L. Comparison of the cardiovascular actions of dopamine and epinine in the dog. *J Pharmacol Exp Ther* 233; 87-93, 1985.

Kopia G, Ohlstein E and Ruffolo R. Systemic and coronary hemodynamic actions of the novel inotropic agent, ibopamine, and the de-esterified metabolic and active form, epinine: Relationship to left ventricular performance in the dog. *J Pharmacol Exp Ther* 246; 434-440, 1988.

Lo Khandwala MF and Barrett RJ. Review of cardiovascular dopamine receptors: physiological, pharmacological and therapeutic implications. *J Autonom Pharmacol* 3; 189-215, 1982.

More RY and Bloom FE. Central catecholamine neuron systems: anatomy anphysiology of the dopamine systems. *Ann Rev Neurosci* 1; 129-169, 1978.

Nihols A and Ruffolo R. Evaluation of the alpha and beta adrenoceptor-mediated activities of the novel, orally active inotropic agent, ibopamine, in the cardiovascular system of the pithed rat: Comparison with epinine and dopamine. *J Pharmacol Exp Ther* 242; 455-463, 1987.

Nichols A, Smith J, Shebuski R and Ruffolo R. Comparison of the effects of the novel inotropic agent, ibopamine, with epinine, dopamine and fenoldopam on renal vascular dopamine receptors in the anaesthetized dog. *J Pharmacol Exp Ther* 242; 573-578, 1987.

Pocchiari F, Pataccini R, Castelnovo P, Longo A, Paro M and Casagrande C. Ibopamine, an orally active dopamine-like drug: Metabolism and pharmacokinetics in dogs. *Arzneim Forsch* 36; 341-344, 1986.

Randolph WC, Swagzdis JE, Joseph GL and Gifford R. Circulating levels of epinine and epinine conjugates in rat, dog and man following oral administration of ibopamine. *Pharmacologist* 25; 117, 1983.

Rajfer SL, Rossen JD, Douglas FL, Goldberg LI and Karrison T. Effects of long-term therapy with oral ibopamine on resting hemodynamics and exercise capacity in patients with heart failure: Relationship to the generation of N-methyldopamine and to plasma norepinephrine levels. *Circulation* 73; 740-749, 1986.

Rousseau MF, Raigoso J, Ahn S, Musso NR, Lotti G and Pouleur H. Modulation of the neuro-humoral activation in ischemic heart failure by dopaminergic receptors stimulation. *Eur Heart J* 12 (abstract suppl.); 184, 1991.

Shebuski R, Fujita T, Smith J and Ruffolo R. Comparison of the alpha adrenoceptor activity of dopamine, ibopamine and epinine in the pulmonary circulation of the dog. *J Pharmacol Exp Ther* 241; 6-12, 1986.

Van der Giessen WJ, Van Woerkens LJ, Duncker DJ, Roelandt JRTC and Verdouw PD. The acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J Cardiovasc Pharmacol* 14; 653-658, 1989.

Van der Hoorn FAJ, Boomsma F, Man in 't Veld AJ and Schalekamp MADH. Determination of catecholamines in human plasma by high-performance liquid chromatography: caparison between a new method with fluorescence determination and an established method with electrochemical determination. *J Chromatograph* 487; 17-28, 1989.

Van Woerkens LJ, Duncker DJ, Den Boer MO, McFalls EO, Sassen LMA, Saxena PR and Verdouw PD. Evidence against a role for dopamine D_1 receptors in the porcine myocardium. *Br J Pharmacol* 104: 246-250, 1991a.

Van Woerkens LJ, Schotman SN, Van der Giessen WJ and Verdouw PD. Cardiovascular effects of elgodipine in conscious pigs with a normal coronary circulation and in conscious pigs with a healed myocardial infarction. *J Cardiovasc Pharmacol* 17; 976-982, 1991b.

Van Woerkens LJ, Van der Giessen WJ and Verdouw PD. Cardiovascular profile of 5 novel nitrate-esters: A comparative study with nitroglycerin in conscious pigs with

and without left ventricular dysfunction. *Br J Pharmacol* 104; 7-14, 1991c.

Van Woerkens LJ, Van der Giessen WJ and Verdouw PD. The selective bradycardic effects of UL-FS 49 do not adversely affect left ventricular function in conscious pigs with chronic coronary artery occlusion. *Cardiovasc Drugs Ther* 1991d, in press.

Chapter 10

Effect of epinine on systemic hemodynamics and regional blood flows in conscious pigs.

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Effect of Epinine on Systemic Hemodynamics and Regional Blood Flows in Conscious Pigs

Summary

Intravenous infusions (1, 2.5, 5 and 10 $\mu g.kg^{-1}.min^{-1}$ for 10 min) were used to evaluate the cardiovascular effects of epinine (N-methyl-dopamine) in 8 conscious pigs. Epinine is a non-selective and non-specific dopamine (DA) agonist, that also stimulates alpha- and beta-adrenoceptors. Epinine (1-5 $\mu g.kg^{-1}.min^{-1}$) increased cardiac output up to 15 ± 5% ($P<0.05$), due to an increase in heart rate (24 ± 6%), but an increase in stroke volume (16 ± 4%) caused the further increase in cardiac output at 10 $\mu g.kg^{-1}.min^{-1}$. Mean arterial blood pressure decreased gradually from 100 ± 5 mmHg to 84 ± 4 mm Hg during infusions up to 5 $\mu g.kg^{-1}min^{-1}$, but increased to 89 ± 4 mm Hg during infusion of 10 $\mu g.kg^{-1}.min^{-1}$ ($p<0.05$). Systemic vascular resistance had decreased from 36.5 ± 2.8 $mmHg.min.l^{-1}$ to 27.5 ± 3.0 mm $Hg.min.l^{-1}$ after infusion of 5 $\mu g.kg^{-1}.min^{-1}$, but did not change further during infusion of 10 $\mu g.kg^{-1}.min^{-1}$. LV dP/dt_{max} increased only at 10 $\mu g/kg/min$. Myocardial blood flow did not change at any dose, due to metabolically regulated coronary vasodilatation (myocardial work did not change). Flow to the adrenals (up to 110 ± 37%) and the spleen (up to 95 ± 13%) increased dose dependently. Cerebral blood flow increased only at the highest dose (15 ± 5%, $P<0.05$), while flow to the kidneys, liver, small intestine and skeletal muscle did not change. Flow decreased to the stomach (21 ± 5%) and skin (for doses less than 2.5 $\mu g.kg^{-1}.min^{-1}$). Further studies are needed to evaluate the contribution of activation of DA receptors and $beta_2$-adrenoceptors to the epinine-induced vasodilation of the various regional vascular beds.

Introduction

Ibopamine is a novel orally active drug for the treatment of chronic congestive heart failure (Casagrande et al., 1985, Rajfer et al., 1986 and Taylor and Cicchetti, 1990). Ibopamine is a prodrug which is pharmacologically inactive, but it is rapidly hydrolyzed to the active compound epinine (Randolph et al., 1983, Pocchiari et al., 1986 and Lodola et al., 1986). Epinine is a non-selective and non-specific dopamine (DA) agonist, that also stimulates alpha- and beta-adrenoceptors. The cardiovascular effects of epinine, however, have only been characterized in anesthetized animals (Itoh et al., 1985, Shebuski et al., 1986 and Nichols and Ruffolo, 1987) and the data are inconclusive. For instance, Itoh et al. (1985) reported a decrease in heart rate, while Kopia et al. (1988)

reported an increase in heart rate, when epinine was administered to anesthetized dogs in the range of 1-10 $\mu g.kg^{-1}.min^{-1}$. Furthermore, only one study, also performed in anesthetized dogs, has addressed the effects of epinine on the coronary circulation, but due to the small number of animals no conclusive data were obtained (Kopia et al., 1988). Epinine is active at DA_1- as well as at DA_2-receptors. Stimulation of DA_2-receptors inhibits ganglionic and noradrenergic neurotransmission, preferentially at high levels of sympathetic activity. Since sympathetic activity may be high during anesthesia the hemodynamic profile of epinine may be affected by this procedure. In order to avoid any possible interference of anesthetic agents with the actions of epinine, we now describe the effects of epinine on cardiovascular dynamics in healthy conscious pigs, which had been instrumented 2-3 weeks before the studies and which were adapted to the laboratory conditions. In addition we report on the distribution of cardiac output during infusion of epinine, by measuring regional blood flows using radioactive labelled microspheres.

Material and methods

The experimental procedures were approved by the Committee on animal experiments of the Erasmus University Rotterdam, and complied to the guidelines for the use and care of experimental animals as put forward by the council of the American Physiological Society (DHEW Publication No. (NIH) 80-23, 1980).

Surgical procedures

After an overnight fast, 8 cross-bred Landrace x Yorkshire pigs of either sex (19-21 kg at the time of surgery), pretreated with 600 mg of a mixture of procaine penicillin-G and benzathine penicillin-G intramuscularly (Duplocillin, Gist-Brocades NV, Delft, The Netherlands), were sedated with an intramuscular injection of 30 $mg.kg^{-1}$ ketamine HCL (Aeskoket, Aesculaap BV, Boxtel, The Netherlands). Subsequently the animals were intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1:2) to which 1-4% (v/v) enflurane was added. Under sterile conditions, a jugular vein and a common carotid artery were cannulated for infusion of drugs or solvent and measurement of arterial blood pressure, respectively. The chest was opened via the third left intercostal space and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for the measurement of aortic blood flow. The heart was exposed via the fifth intercostal space and a pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) 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. In addition the left atrial canula was used for the injection of radioactive microspheres to determine regional blood flows. After instrumentation was completed, and the wires tunnelled to the back, the chest was closed and the animals were allowed to recover from surgery.

Post-surgical period

The animals received daily intravenous doses of 500 mg amoxicillin (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and, during the first week only, 500 mg kanamycin (Kanamicine 10%, Alfason, Woerden, The Netherlands) to prevent infection. Catheters were flushed daily with an isotonic saline solution containing 500 IU/ml heparin. During the first weeks of the post operative recovery period the animals were adapted to the laboratory facilities (8 to 10 daily sessions), while hemodynamic parameters were monitored. The experimental protocols were executed when systemic hemodynamics remained stable for at least one hour, usually 2-3 weeks after instrumentation.

Experimental protocol

On the day of the experiment 4 doses of epinine were infused at incremental rates of 1, 2.5, 5 and 10 $\mu g.kg^{-1}.min^{-1}$ for 10 min or solvent (0.2, 0.5, 1 and 2 $ml.min^{-1}$ for 10 min). At baseline and at the end of each infusion step, systemic hemodynamic parameters were recorded. Experiments with epinine or solvent were performed in random order and consecutive experiments in the same animal were separated by at least 24 hours. All measurements were done while the animals were quietly resting in a constraining jacket. Regional organ blood flows were also determined at baseline and after each infusion rate of epinine, by injecting a batch of $1\text{-}2x10^6$ carbonized plastic microspheres [15 ± 1 μm (SD) in diameter] labelled with either ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce (NEN Chemicals GmbH, Dreieich, Germany) into the left atrium. To calculate regional blood flows a reference blood sample was withdrawn from the cannula in the carotid artery at a rate of 10 $ml.min^{-1}$, starting 15 s before the injection of microspheres, until 90 s after completion of the injection of the microspheres. At the end of the experiments animals were killed with an overdose of sodium pentobarbitone. From the animals various organs

(adrenals, liver, spleen, stomach, small intestine, brain and kidneys) and representative aliquots of abdominal skin and skeletal muscle (gluteus) were excised, weighed and put into vials. The hearts were fixed in formaldehyde (10% v/v) and 48 hours later the atria and right ventricle were cut off the left ventricle. The myocardium of the left ventricle was divided into three layers of equal thickness: subepicardium, mid-myocardium and subendocardium.

The radioactivity was counted and the amount of blood flow to the various tissues ($\dot{Q}_{tis}$) was calculated as:

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

where I_{tis} and I_{art} are the radioactivity in a particular tissue and that of the arterial blood sample, while $\dot{Q}_{art}$ is the rate of withdrawal of the blood sample, respectively (Saxena and Verdouw, 1985).

Drugs

Epinine (N-methyldopamine) was a gift from Dr. C. Casagrande (Zambon Research Laboratories, Milan, Italy) and prepared daily in physiological saline (0.9% NaCl) in a concentration of 5 $\mu g.kg^{-1}.ml^{-1}$ which allowed infusion of volumes of 0.2, 0.5, 1.0 and 2.0 $ml.min^{-1}$, respectively.

Data presentation and statistical analysis

Arterial pulse pressure was taken as the difference between the systolic and the diastolic arterial blood pressures. Cardiac output was taken as the sum of the ascending aortic blood flow (electromagnetic flow meter) and myocardial blood flow (radioactive microspheres). Systemic vascular resistance was calculated as the ratio of mean arterial blood pressure and cardiac output, while the regional vascular resistances were calculated by dividing the mean arterial blood pressure by the respective regional blood flows. Finally left ventricular work was calculated as the product of cardiac output and mean arterial blood pressure.

All data are presented as the means ± S.E.M. The significance of the effects of epinine have been evaluated by comparing the epinine-induced changes from baseline to the solvent-induced changes from baseline of the solvent, using analysis of variance. Significance was accepted for $p<0.05$ (two tailed). A Bonferroni correction was used because of comparison for multiple measurements.

Results

Effect of solvent on systemic hemodynamics

Infusion of the solvent had no effect on any of the systemic hemodynamic parameters (Fig. 1).

Effect of epinine on systemic hemodynamics

In doses up to 5 $\mu g.kg^{-1}.min^{-1}$, cardiac output increased dose dependently (up to 15 ± 5%, $p<0.05$), but mean arterial blood pressure fell by as much as 16 ± 2%. The hypotensive action of epinine was therefore due to systemic vasodilatation, as systemic vascular resistance decreased by as much as 26 ± 4% ($p<0.05$) after infusion of 5 $\mu g.kg^{-1}.min^{-1}$ epinine (Fig. 1). An increase in heart rate (up to 24 ± 5%) was responsible for the increase in cardiac output as stoke volume did not change (Fig. 1). LV dP/dt_{max} increased only minimally (7 ± 3%, $p<0.05$), while arterial pulse pressure and left ventricular end-diastolic blood pressure were not affected.
During infusion of 10 $\mu g.kg^{-1}.min^{-1}$ epinine, there was a further increase in cardiac output (25 ± 7%), but heart rate decreased from 141 ± 8 $beats.min^{-1}$ to 124 ± 5 $beats.min^{-1}$ ($p<0.05$). The latter implies that an increase in stroke volume from 23.0 ± 1.9 ml to 28.3 ± 2.7 ml ($p<0.05$) was now responsible for the increase in cardiac output. Mean arterial blood pressure increased by 5 ± 2 mm Hg ($p<0.05$) over the value measured after 5 $\mu g.kg^{-1}.min^{-1}$, due to an increase in systolic arterial blood pressure as diastolic arterial blood pressure did not change (Fig. 1). Consequently arterial pulse pressure increased from 45 ± 3 mm Hg to 53 ± 3 mm Hg ($p<0.05$) during infusion of 10 $\mu g.kg^{-1}.min^{-1}$. Systemic vascular resistance remained unchanged compared to the value determined at 5 $\mu g.kg^{-1}.min^{-1}$, but LV dP/dt_{max} increased to 24 ± 7% ($p<0.05$) above its baseline value.

Effect of epinine on regional blood flows

Myocardial blood flow. In the dose range tested, epinine had no effect on left ventricular transmural blood flow or its distribution over the subendocardium and subepicardium (Fig. 2). Since mean arterial blood pressure had decreased, it follows that coronary vascular resistance had decreased. At variance with the observations on the systemic vascular resistance, coronary vascular resistance continued to decrease (26 ± 3%, $p<0.05$) during infusion of the highest dose. Myocardial work was not affected at any dose (Fig. 2). Right ventricular blood flow tended to increase from 124 ± 11

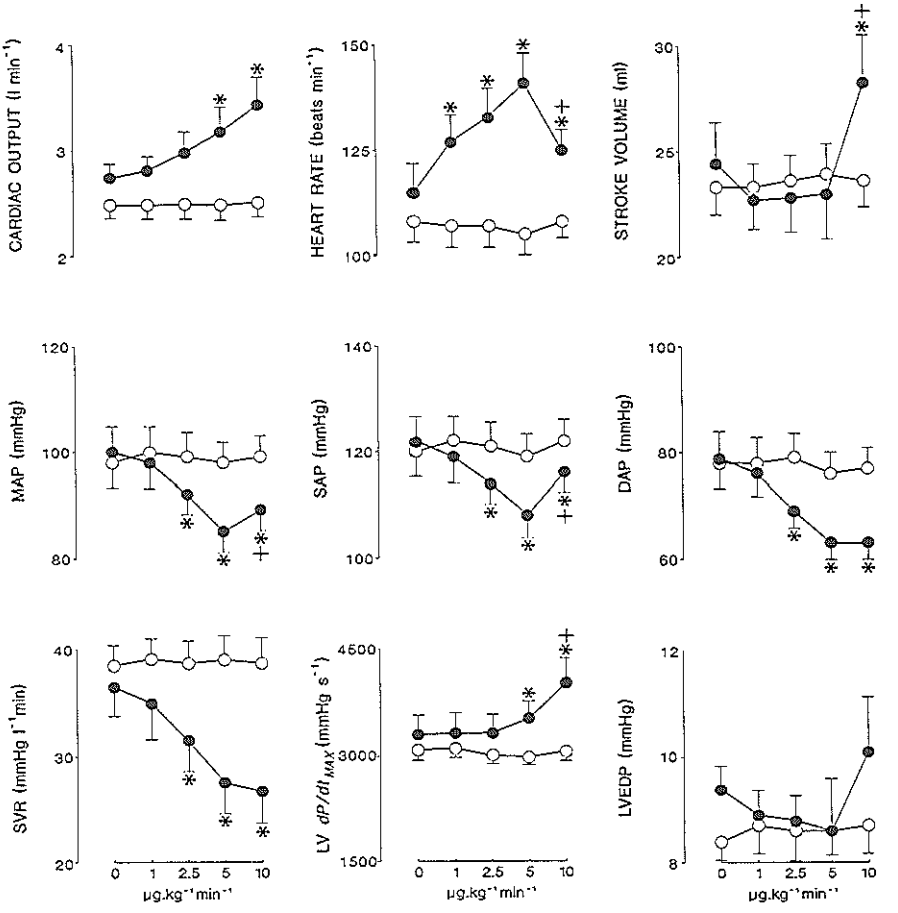

Figure 1. *Systemic hemodynamic effects of epinine (●) and its solvent (○) in 8 conscious pigs. MAP, SAP and DAP are mean, systolic and diastolic arterial blood pressure, respectively. SVR = systemic vascular resistance; LVEDP = left ventricular end-diastolic blood pressure. Data are means ± S.E.M.; * The epinine-induced changes from baseline (0 µg/kg/min) are significantly different ($p < 0.05$) from the solvent-induced changes from baseline; + Values obtained after 10 µg/kg/min are significantly different ($p < 0.05$) from the values obtained after 5 µg/kg/min.*

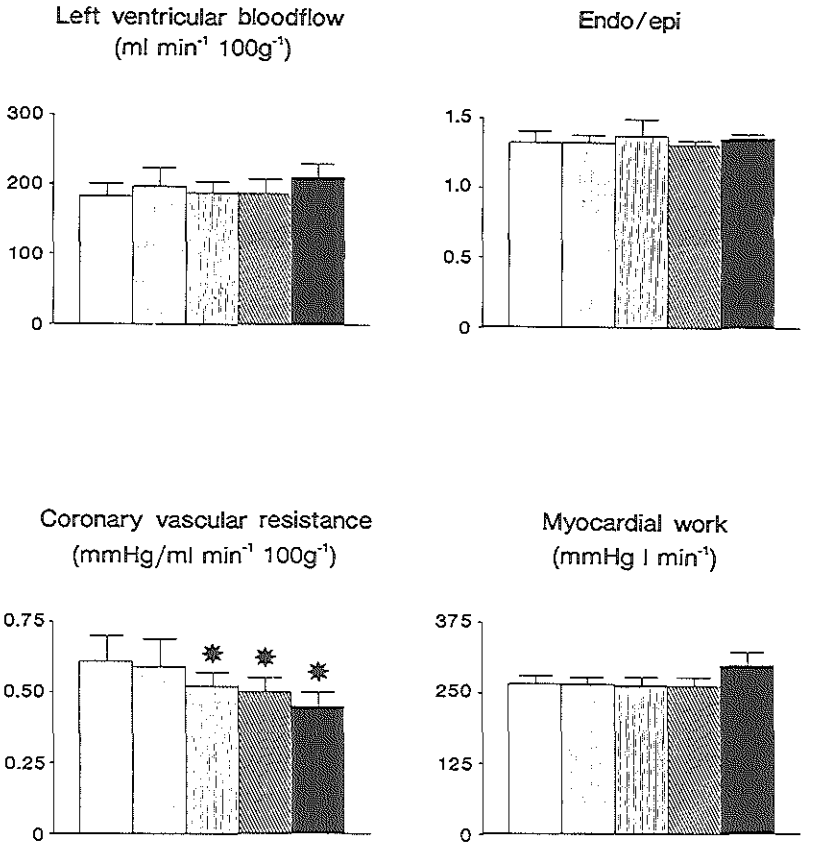

Figure 2. *Effect of cumulative 10 min intravenous infusions of epinine on transmural left ventricular blood flow and its distribution (Endo/epi), coronary vascular resistance and left ventricular work in 8 conscious pigs (□ = baseline; ▨ , ▨ , ▨ , and ■ denote infusion rates of 1, 2.5, 5 and 10 μg/kg/min, respectively). Data are means ± S.E.M. * $p<0.05$ compared to baseline.*

$ml.min^{-1}.100g^{-1}$ at baseline to 155 ± 19 $ml.min^{-1}.mg^{-1}$ after infusion of 10 $\mu g.kg^{-1}.min^{-1}$, but levels of statistical significance were not achieved (not shown). The decrease in coronary vascular resistance of the right ventricle from 0.87 ± 0.08 mm $Hg(ml.min^{-1}.100g^{-1})^{-1}$ at baseline became already significant during infusion of 1 $\mu g.kg^{-1}.min^{-1}$ (0.70 ± 0.08 mm $Hg(ml.min^{-1}.100g^{-1})^{-1}$, $p<0.05$). With the higher doses there was only a small additional effect (0.65 mm $Hg(ml.min^{-1}.100g^{-1})^{-1}$ after 10 $\mu g.kg^{-1}.min^{-1})^{-1}$.

Cerebral blood flow

Except for the cerebellum, none of the other parts of the brain showed any increase in flow at doses less than 10 $\mu g.kg^{-1}.min^{-1}$. With the highest dose, all parts of the brain showed an increase of 10% to 20%. Except for the hemispheres, the decreases in vascular resistances became in all parts already significant during infusion of 2.5 $\mu g.kg^{-1}.min^{-1}$ of epinine (Fig. 3).

Renal blood flows

Flow remained unaffected at all doses. In view of the decrease in arterial blood pressure it follows that vasodilatation had occurred (in both the cortex and medulla) during infusion of 5 and 10 $\mu g.kg^{-1}.min^{-1}$ (Fig. 4).

Other regional blood flows

Table 1 shows that flow increased dose dependently in the adrenals (110 ± 37%, $p<0.05$) and spleen (95 ± 13%, $p<0.05$), did not change in the small intestine, skeletal muscle and liver, but decreased in the stomach (by 29 ± 4% at 5 $\mu g.kg^{-1}.min^{-1}$) and in the skin (abdomen) at doses less than 2.5 $\mu g.kg^{-1}.min^{-1}$. Vasodilatation had therefore occurred in the adrenals and spleen and in the liver (30% at 10 $\mu g.kg^{-1}.min^{-1}$, $p<0.05$), while vasoconstriction was observed in the skin (15% at 1 $\mu g.kg^{-1}.min^{-1}$, $p<0.05$) and stomach (19% and 20% at 2.5 and 5 $\mu g.kg^{-1}.min^{-1}$, respectively, $p<0.05$).

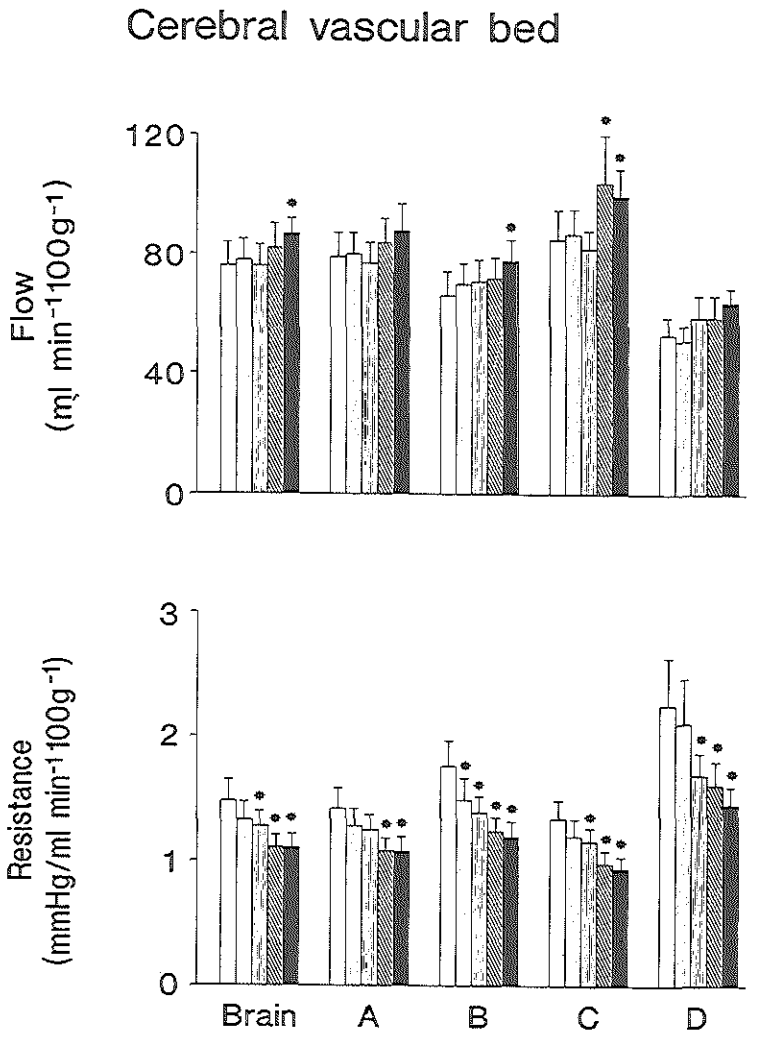

Figure 3. *Effect of cumulative 10 min intravenous epinine on cerebral blood flow and vascular resistance and their distribution (A = hemispheres; B = diencephalon; C = cerebellum; D = brainstem) in 8 conscious pigs (□ = baseline; ▨, □, ▨ and ■ denote infusion rates of 1, 2.5, 5 and 10 μg/kg/min, respectively). Data are means ± S.E.M. * $p<0.05$ compared to baseline.*

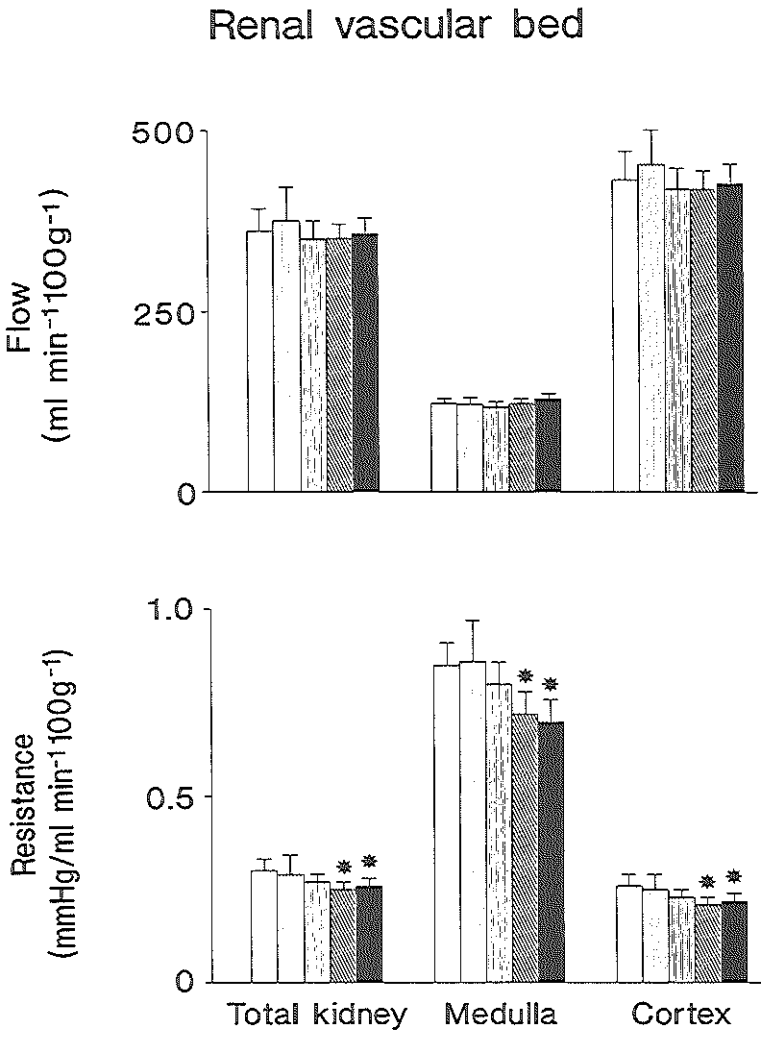

Figure 4. *Effect of cumulative 10 min intravenous epinine on total renal blood flow and vascular resistance and their distribution in 8 conscious pigs (□ = baseline; □, ▨, ▩, and ■ denote infusion rates of 1, 2.5, 5 and 10 μg/kg/min, respectively). Data are means ± S.E.M. * $p<0.05$ compared to baseline.*

Table 1 *Regional blood flows and vascular resistances at baseline and during cumulative 10 min intravenous infusions of epinine in 8 conscious pigs.*

	Baseline	Epinine (μg/kg/min for 10 min)			
		1	2.5	5	10
Flows					
Adrenals	161 ± 64	171 ± 59	184 ± 71	239 ± 92[a]	319 ± 148[a]
Stomach	49 ± 5	46 ± 9	38 ± 4[a]	34 ± 3[a]	38 ± 3[a]
Small intestine	69 ± 7	68 ± 9	59 ± 5	60 ± 7	68 ± 5
Skin (abdomen)	9.2 ± 1.1	7.4 ± 0.8[a]	7.3 ± 0.9[a]	8.2 ± 0.9	8.1 ± 0.9
Skeletal muscle (gluteus)	3.7 ± 0.3	3.8 ± 0.4	4.3 ± 1.1	3.3 ± 0.5	4.1 ± 0.6
Spleen	220 ± 40	241 ± 42	274 ± 51[a]	312 ± 54[a]	405 ± 63[a]
Liver	13 ± 3	13 ± 3	14 ± 3	15 ± 3	17 ± 4
Vascular resistances					
Adrenals	1.10 ± 0.30	0.82 ± 0.14[a]	0.75 ± 0.12[a]	0.56 ± 0.09[a]	0.48 ± 0.08[a]
Stomach	2.21 ± 0.22	2.62 ± 0.47	2.68 ± 0.35[a]	2.65 ± 0.29[a]	2.53 ± 0.32
Small intestine	1.67 ± 0.27	1.71 ± 0.31	1.73 ± 0.23	1.57 ± 0.19	1.39 ± 0.15
Skin (abdomen)	12.3 ± 1.3	14.3 ± 1.5[a]	13.8 ± 1.2	11.3 ± 1.1	12.0 ± 1.4
Skeletal muscle (gluteus)	29.5 ± 2.5	28.5 ± 2.7	29.6 ± 5.0	29.0 ± 4.1	25.2 ± 3.8
Spleen	0.59 ± 0.09	0.49 ± 0.07	0.42 ± 0.06[a]	0.32 ± 0.05[a]	0.25 ± 0.03[a]
Liver	14.6 ± 4.6	12.8 ± 3.8	13.2 ± 4.2	10.9 ± 3.2	9.2 ± 3.1[a]

Units are ml/min/100g for flows and mm Hg/(ml/min/100g) for resistances; data are means ± S.E.M. [a] *$p < 0.05$ compared with baseline*

Discussion

This is the first study that presents a detailed description of the effects of epinine on cardiovascular dynamics and regional organ and tissue perfusion in conscious animals. In the dose range tested (1-10 $\mu g.kg^{-1}.min^{-1}$) epinine increased cardiac output dose dependently but lowered mean arterial blood pressure, although the latter started to return to predrug levels during infusion of 10 $\mu g.kg^{-1}.min^{-1}$. The hypotensive action of epinine was the consequence of arterial vasodilatation, which was most pronounced during infusion of 5 $\mu g.kg^{-1}.min^{-1}$. Our results differ from those reported for anesthetized dogs by Itoh et al. (1985) and Kopia et al. (1988), as both groups of investigators described a pressor response due to arterial vasoconstriction, when epinine was infused in the same dose range as used in the present study. The reason for the absence of an increase in arterial blood pressure in our pigs is most likely caused by the dominance of vasodilation either through $beta_2$-adrenoceptor or DA-receptor stimulation over the alpha-adrenoceptor-induced vasoconstriction in pigs. Only at the highest dose, we noticed that arterial blood pressure and especially the systolic arterial blood pressure started to increase and that systemic vascular resistance did not further decrease, which is most likely due to an increased alpha-adrenoceptor stimulation. In view of the discrepancy between the observation in the animal studies it is of interest that in man an infusion of 1 $\mu g.kg^{-1}.min^{-1}$ decreases arterial blood pressure due to systemic vasodilation (Rousseau et al., 1991).

In both the study of Kopia et al. (1988) and ours, LV dP/dt_{max} increased during infusion of 10 $\mu g/kg/min$, but the four fold increase in the anesthetized dogs (Kopia et al., 1988) was much larger than the 24% increase in the conscious pigs. Because heart rate and arterial blood pressure (from 106 mm Hg to 181 mm Hg) also increased, the contribution of a positive inotropic effect to the increase in LV dP/dt_{max} in the anesthetized dogs is difficult to assess. Based on these data, it is tempting to argue that the inotropic reserve in the pig is much less than in the dog. However, in earlier studies we have shown that LV dP/dt_{max} can, at similar heart rates, reach values as high as those reported for the dog (14,15). In view of the finding that LV dP/dt_{max} increased during infusion of 10 $\mu g.kg^{-1}.min^{-1}$, while heart rate decreased and mean arterial blood pressure increased, we may assume that the increase in LV dP/dt_{max} at this dose represented a weak positive inotropic effect of epinine, probably caused by stimulation of the $beta_1$-adrenoceptors (Kopia et al., 1988). Our findings are consistent with the observations by Holubarsch et al. (1991), who showed that in clinically relevant concentrations the positive inotropic properties of epinine in isometrically contracting papillary muscle from guinea pigs are negligible. Infusion of 1 $\mu g.kg^{-1}.min^{-1}$ epinine did also not lead to changes in LV dP/dt_{max}

in patients with ischemic heart failure (Rousseau et al., 1991).

Because of the limited number of radioactive microspheres which can be used, we have no data on regional blood flow during infusion of the solvent. However, we have earlier shown that under conditions in which systemic hemodynamics are not affected by the solvent, regional blood flows do also not change (Van der Giessen et al., 1990). In the present study, coronary blood flow was maintained in spite of the hypotensive effect of epinine due to coronary vasodilatation. Since left ventricular work did not change, it may be concluded that the epinine-induced vasodilatation was not a specific property of the drug, but was metabolically regulated. In view of the changes in heart rate and mean arterial blood pressure, it is also important to note that the transmural distribution of the coronary blood flow was not adversely affected (Domenech and Goich, 1976). Kopia et al. (1988) observed an increase in coronary blood flow of approximately 250%, which was related to the increase in left ventricular work. However, due to the small number of observations, the changes in coronary vascular resistance did not reach levels of statistical significance.

The presence of dopamine receptors in the human cerebral arteries has been demonstrated (Toda, 1983) and we have shown that in pigs during infusion of the selective DA_1 agonist, fenoldopam, vasodilation persisted after alpha- and beta-adrenoceptor blockade (Van Woerkens et al., 1991). Further studies are, however, needed to determine whether the epinine-induced increase in cerebral blood is related to stimulation of DA_1 receptors.

Renal blood flow did not change in our experiments, which is in agreement with the observation by Itoh et al. (1985), who used doses of 3 and 6 $\mu g.kg^{-1}.min^{-1}$ in anesthetized dogs. It should be pointed out, however, that, while Itoh et al. found a 15% increase in renal vascular resistance, we found a 15% decrease at doses of 5 $\mu g.kg^{-1}.min^{-1}$. Itoh et al. (1985) concluded that a consistent increase in renal blood flow was prevented because the DA_1 activity of epinine was offset by the alpha-adrenergic activity. On the other hand, Nichols et al. (1987) showed that in anesthetized dogs, pretreated with phenoxybenzamine and propranolol, epinine was still capable of reducing renal vascular resistance, an action which was antagonized after pretreatment with the DA_1 antagonist SK&F R-83566. We have demonstrated the presence of DA_1 receptors in the kidneys of the pig, using infusions with the selective DA_1 agonist fenoldopam (Van Woerkens et al., 1991). Further studies are needed to evaluate the contribution of activation of DA receptors and $beta_2$-adrenoceptors to the epinine-induced vasodilation of the various regional vascular beds.

References

Casagrande C, Ghirardi P and Marchetti G. Ibopamine. New Drugs Ann: *Cardiovasc Drugs* 3; 173-196, 1985.

Domenech RJ and Goich J. Effect of heart rate on regional coronary blood flow. *Cardiovasc Res* 10; 224-231, 1976.

Duncker DJ, Saxena PR and Verdouw PD. Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *Eur J Pharmacol* 156; 401-409, 1988.

Holubarsch Ch, Walde T, Hasenfuss G, Pieske B and Just H. Concentration-dependent separation between vasodilating and positive inotropic effect of ibopamine/epinine. *Eur Heart Journal* 12 (Abstract Suppl. August); 183, 1991.

Itoh H, Hohli J, Rajfer S and Goldberg L. Comparison of the cardiovascular actions of dopamine and epinine in the dog. *J Pharmacol Exp Ther* 233; 87-93, 1985.

Kopia G, Ohlstein E and Ruffolo R. Systemic and coronary hemodynamic actions of the novel inotropic agent, ibopamine, and the de-esterified metabolic and active form, epinine: Relationship to left ventricular performance in the dog. *J Pharmacol Exp Ther* 246; 434-440, 1988.

Lodola E, Borgia M, Longo A, Pocchiari F, Pataccini R and Sher S. Ibopamine kinetics after a single oral dose in healthy volunteers. *Arzneim Forsch* 36; 345-348, 1986.

Nichols A and Ruffolo R. Evaluation of the alpha and beta adrenoceptor-mediated activities of the novel, orally active inotropic agent, ibopamine, in the cardiovascular system of the pithed rat: Comparison with epinine and dopamine. *J Pharmacol Exp Ther* 242:455-463, 1987.

Nichols A, Smith J, Shebuski R and Ruffolo R. Comparison of the effects of the novel inotropic agent, ibopamine, with epinine, dopamine and fenoldopam on renal vascular dopamine receptors in the anaesthetized dog. *J Pharmacol Exp Ther* 242; 573-578, 1987.

Pocchiari F, Pataccini R, Castelnovo P, Longo A, Paro M and Casagrande C. Ibopamine, an orally active dopamine-like drug: Metabolism and pharmacokinetics in dogs. *Arzneim Forsch* 36; 341-344, 1986.

Rajfer SL, Rossen JD, Douglas FL, Goldberg LI and Karrison T. Effects of long-term therapy with oral ibopamine on resting hemodynamics and exercise capacity in patients with heart failure: Relationship to the generation of N-methyldopamine and to plasma norepinephrine levels. *Circulation* 73; 740-749, 1986.

Randolph WC, Swagzdis JE, Joseph GL and Gifford R. Circulating levels of epinine and epinine conjugates in rat, dog and man following oral administration of

ibopamine. *Pharmacologist* 25; 117, 1983.

Rousseau MF, Raigoso J, Ahn S, Musso NR, Lotti G and Pouleur H. Modulation of the neuro-humoral activation in ischemic heart failure by dopaminergic receptors stimulation. *Eur Heart J* 12 (Abstract Suppl. August); 184, 1991.

Saxena PR and Verdouw PD. 5-Carboxamide tryptamine, a compound with high affinity for 5-hydroxytryptamine$_1$ binding sites, dilates arterioles and constricts arteriovenous anastomoses. *Br J Pharmacol* 84; 533-544, 1985.

Shebuski R, Fujita T, Smith J and Ruffolo R. Comparison of the alpha adrenoceptor activity of dopamine, ibopamine and epinine in the pulmonary circulation of the dog. *J Pharmacol Exp Ther* 241; 6-12, 1986.

Taylor S and Cicchetti V. Efficacy of ibopamine in the treatment of heart failure. *Am Heart J* 120; 1583-1590, 1990.

Toda N. Dopamine vasodilates human cerebral arteries. *Experienta* 39; 1131-1132, 1983.

Van der Giessen WJ, Duncker DJ, Saxena PR and Verdouw PD. Nimodipine has no effect on the cerebral circulation in conscious pigs, despite an increase in cardiac output. *Br J Pharmacol* 100; 277-282, 1990.

Van Woerkens LJ, Schotman SN, Van der Giessen WJ and Verdouw PD. Cardiovascular effects of elgodipine in conscious pigs with a normal coronary circulation and in conscious pigs with a healed myocardial infarction. *J Cardiovasc Pharmacol* 17; 976-982, 1991a.

Van Woerkens LJ, Duncker DJ, Den Boer MO, McFalls EO, Sassen LMA, Saxena PR and Verdouw PD. Evidence against a role for dopamine D_1 receptors in the porcine myocardium. *Br J Pharmacol* 104; 246-250, 1991b.

Chapter 11

Cardiovascular effects of dopamine and dobutamine in conscious pigs with chronic heart failure.

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Cardiovascular effects of dopamine and dobutamine in conscious pigs with chronic heart failure

Summary

The hemodynamic effects of dopamine and dobutamine (1-25 $\mu g.kg^{-1}.min^{-1}$) were studied in conscious pigs with chronic left ventricular dysfunction and compared to those of dopamine (1-10 $\mu g.kg^{-1}.min^{-1}$) in normal conscious pigs. Left ventricular dysfunction induced by occluding a coronary artery 3-4 weeks before measurements were taken, was characterized by a lower cardiac output (23%), stroke volume (34%) and $LVdP/dt_{max}$ (15%) and a higher heart rate (16%), peripheral resistance (41%) and left ventricular end-diastolic pressure (96%). In the failing animals, dopamine increased cardiac output similarly as in the normal animals, but only in the failing animals stroke volume increased. The vasodilatory response was also not affected but the increase in $LVdP/dt_{max}$ was attenuated compared to normal animals. In the animals with heart failure, dobutamine showed similar cardiovascular effects as dopamine. Left ventricular work, depressed at baseline, increased more rapidly during infusion of dopamine (or dobutamine) in the animals with heart failure than in normal animals. Thus, in contrast to in normal pigs, stroke volume increases in pigs with chronic heart failure during infusion of dopamine or dobutamine, in spite of an attenuated response of $LVdP/dt_{max}$.

Introduction

The cardiovascular effects of dopamine in normal pigs have been characterized extensively (Vane et al., 1982, Gootman et al., 1983, Buckley et al., 1983, Fiser et al., 1988 and Van Woerkens et al., 1991a). It has been well established, however, that the cardiovascular responses to pharmacological agents in animals with a failing cardiovascular system might be different from those in healthy normal animals. We have, for instance, shown that in conscious pigs with chronic left ventricular dysfunction the positive inotropic response of the phosphodiesterase inhibitor pimobendan is attenuated (Van der Giessen et al., 1989), the dihydropyridine calcium antagonists nisoldipine and elgodipine, in contrast to their actions in normal animals, lowered left ventricular filling pressure (Van der Giessen et al., 1989 and Van Woerkens et al., 1991b) and that the depression of cardiac function by propranolol was more pronounced (Van Woerkens et al., 1991b). For this reason we studied the cardiovascular effects of dopamine and dobutamine in young pigs with chronic left ventricular heart failure, induced by a

permanent occlusion of the left circumflex coronary artery 3-4 weeks before the measurements were taken. Although the cardiovascular effects of dopamine an dobutamine in conscious pigs have been well described (Fiser et al., 1988), we have included our own data in normal conscious pigs for comparison. All measurements were performed after the animals had been adapted to the laboratory facilities, while solvent infusions were used to evaluate the stability of the hemodynamic condition of the animals at the time of the measurements.

Material and methods

The experimental procedures were approved by the Committee on animal experiments of the Erasmus University Rotterdam, and complied to the guidelines for the use and care of experimental animals as put forward by the council of the American Physiological Society (DHEW Publication No. (NIH) 80-23, 1980).

Surgical procedures

After an overnight fast, cross-bred Landrace x Yorkshire pigs of either sex (18-20 kg at the time of surgery), pretreated with 600 mg of a mixture of procaine penicillin-G and benzathine penicillin-G intramuscularly (Duplocillin, Gist-Brocades NV, Delft, The Netherlands), were sedated with an intramuscular injection of 30 mg kg^{-1} ketamine HCL (Aeskoket, Aesculaap BV, Boxtel, The Netherlands). Subsequently the animals were intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1:2) to which 1-4% (v/v) enflurane was added. Under sterile conditions, a jugular vein and a common carotid artery were cannulated for infusion of drugs or solvent and measurement of arterial blood pressure, respectively. The chest was opened via the third left intercostal space and an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for the measurement of aortic blood flow. The heart was exposed via the fifth intercostal space and a pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) 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.
In 12 of the 24 animals, the proximal segment of the left circumflex coronary artery (LCXCA) was permanently ligated. Causing approximately 14 ± 2% of the left ventricle weight become fibrotic within 3-4 weeks (Van der Giessen et al., 1989). After instrumentation was completed, a period of 30 min was allowed before closure of the

chest. During this period ventricular tachycardia and ventricular fibrillation (occurring exclusively in the group with coronary artery ligation) were treated with DC-countershock. The chest was then closed and the wires tunnelled to the back, and the animals were allowed to recover from surgery.

Post-surgical period

The animals received daily intravenous doses of 500 mg amoxicillin (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and, during the first week only, 500 mg kanamycin (Kanamycine 10%, Alfasan, Woerden, The Netherlands) to prevent infection. Catheters were flushed daily with an isotonic saline solution containing 500 IU ml^{-1} heparin. During the first month of the post operative recovery period the animals were adapted to the laboratory facilities (8 to 10 sessions), while hemodynamic parameters were monitored.
Two animals with an intact coronary circulation and one animal with a ligated circumflex coronary artery were excluded from further study because of failure of the electrical signals. From the 12 pigs in which the left circumflex coronary artery was occluded, 3 animals died suddenly during the early post-operative period, most likely due to a ventricular arrhythmia.

Experimental protocols

The experimental protocols were executed when systemic hemodynamics remained stable for at least one hour, usually 3-4 weeks after instrumentation. All measurements were done while the animals were quietly resting in a constraining jacket.
In the animals with the intact coronary circulation 4 consecutive 10 min intravenous infusions with increasing doses of dopamine (1, 2.5, 5 and 10 $\mu g.kg^{-1}.min^{-1}$ or equal volumes of solvent were administered during separate runs of the protocol. Heart rate, arterial blood pressure, mean aortic blood flow, left ventricular pressure and its first derivative (LVdP/dt) were recorded at the end of each infusion period. In all experiments infusions dopamine or solvent in the same animals were separated by at least 24 hours.
In the animals with the infarction 5 consecutive 10 min intravenous infusions of dopamine or dobutamine increasing doses of 1, 2.5, 5, 10 and 25 $\mu g.kg^{-1}.min^{-1}$ were used.

Drugs

Dopamine was obtained from the Pharmacy Department of the Academic Hospital Rotterdam "Dijkzigt", while dobutamine (Dobutex) was bought from Eli Lilly, Amsterdam, The Netherlands. Both compounds were dissolved in physiological saline (0.9% NaCl) until concentrations were reached which allowed infusion in volumes of 0.1, 0.25, 0.5, 1 and 2 $ml.min^{-1}$.

Data presentation and statistical analysis

Stroke volume was calculated by dividing mean aortic blood flow and heart rate, while systemic vascular resistance was determined by dividing mean arterial blood pressure and mean aortic blood flow. Left ventricular work was calculated as the product of cardiac output and mean arterial blood pressure, while left ventricular stroke work was obtained by dividing left ventricular work and heart rate.
All data are presented as the means ± s.e. mean. The significance of the effects of the drugs have been evaluated by comparing the changes from baseline induced by the drugs to the changes from baseline during infusion of the solvent, using analysis of variance. Significance was accepted for $P<0.05$. A Bonferroni correction was used because of comparison for multiple measurements.

Results

Cardiovascular effects of solvent

Baseline values of the two groups of animals are shown in Table 1. In the animals with the permanent left circumflex coronary artery occlusion left ventricular dysfunction was characterized by a lowering of cardiac output (23%) and $LVdP/dt_{max}$ (15%) and an elevation of heart rate (16%), systemic vascular resistance (41%) and left ventricular end-diastolic pressure (96%), which is in concord with earlier observations (Van der Giessen et al., 1989 and Van Woerkens et al., 1991b). Left ventricular stroke work was lowered by 30%, but left ventricular work was less affected (19%) due to the tachycardia in the animals with heart failure (Table 1).
All systemic hemodynamic parameters remained unchanged in either group of animals during infusion of the solvent, indicating that the animals were well adapted to the laboratory conditions for the duration of the experiment (Table 1).

Table 1. *Cardiovascular effects of solvent in conscious pigs without and with chronic left ventricular heart failure*

		Baseline	Solvent (ml.min^{-1}) 0.2	0.5	1	2
CO	N	2.70 ± 0.19	2.71 ± 0.19	2.69 ± 0.17	2.68 ± 0.17	2.69 ± 0.16
	LVH	2.07 ± 0.12*	1.99 ± 0.13	1.99 ± 0.13	2.01 ± 0.13	2.12 ± 0.12
HR	N	112 ± 4	113 ± 5	113 ± 4	110 ± 5	112 ± 4
	LVH	130 ± 6*	124 ± 6	127 ± 6	127 ± 6	127 ± 6
MAP	N	98 ± 4	99 ± 5	98 ± 4	98 ± 3	99 ± 3
	LVH	101 ± 4	100 ± 3	100 ± 3	103 ± 3	103 ± 3
$LVdP/dt_{max}$	N	3220 ± 140	3150 ± 140	3150 ± 130	3160 ± 150	3220 ± 170
	LVH	2720 ± 70*	2730 ± 70	2830 ± 90	2870 ± 70	2900 ± 90
LVEDP	N	9.0 ± 0.5	9.1 ± 0.5	9.3 ± 0.4	9.1 ± 0.4	9.3 ± 0.5
	LVH	17.6 ± 1.9*	17.3 ± 1.3	17.1 ± 2.3	17.9 ± 1.7	18.3 ± 1.8
SVR	N	35.9 ± 2.7	35.9 ± 2.5	36.0 ± 2.5	36.4 ± 2.5	36.5 ± 2.6
	LVH	50.7 ± 4.9*	52.4 ± 5.0	53.4 ± 5.3	53.4 ± 5.3	49.8 ± 4.3
SV	N	24.3 ± 2.0	24.1 ± 1.9	24.2 ± 1.8	24.8 ± 2.0	24.4 ± 1.9
	LVH	16.1 ± 1.1*	16.1 ± 1.2	15.8 ± 1.2	16.1 ± 1.3	16.9 ± 1.1
LVW	N	255 ± 19	257 ± 20	256 ± 18	255 ± 16	258 ± 15
	LVH	206 ± 9*	198 ± 11	199 ± 11	204 ± 10	215 ± 10
LVSW	N	2.30 ± 0.21	2.28 ± 0.19	2.29 ± 0.18	2.36 ± 0.18	2.34 ± 0.17
	LVH	1.60 ± 0.07*	1.60 ± 0.09	1.58 ± 0.08	1.63 ± 0.09	1.71 ± 0.09

*N = normal animals (n = 10); LVH = animals with chronic left ventricular heart failure (n = 8); CO = cardiac output (l.min^{-1}); HR = heart rate (beats.min^{-1}); MAP = mean arterial blood pressure (mmHg); $LVdP/dt_{max}$ = maximum rate of rise of left ventricular pressure (mmhg.s^{-1}); LVEDP = left ventricular end-diastolic pressure (mmHg); SVR = systemic vascular resistance (mmHg.min.l^{-1}); SV = stroke volume (ml); LVW = left ventricular work (MAP x CO, mmHg.l.min^{-1}); LVSW = left ventricular stroke work (LVW/HR, mmHg.l^{-1}). Data are means ± S.E.M. * $P<0.05$ compared to N (presented for Baseline only).*

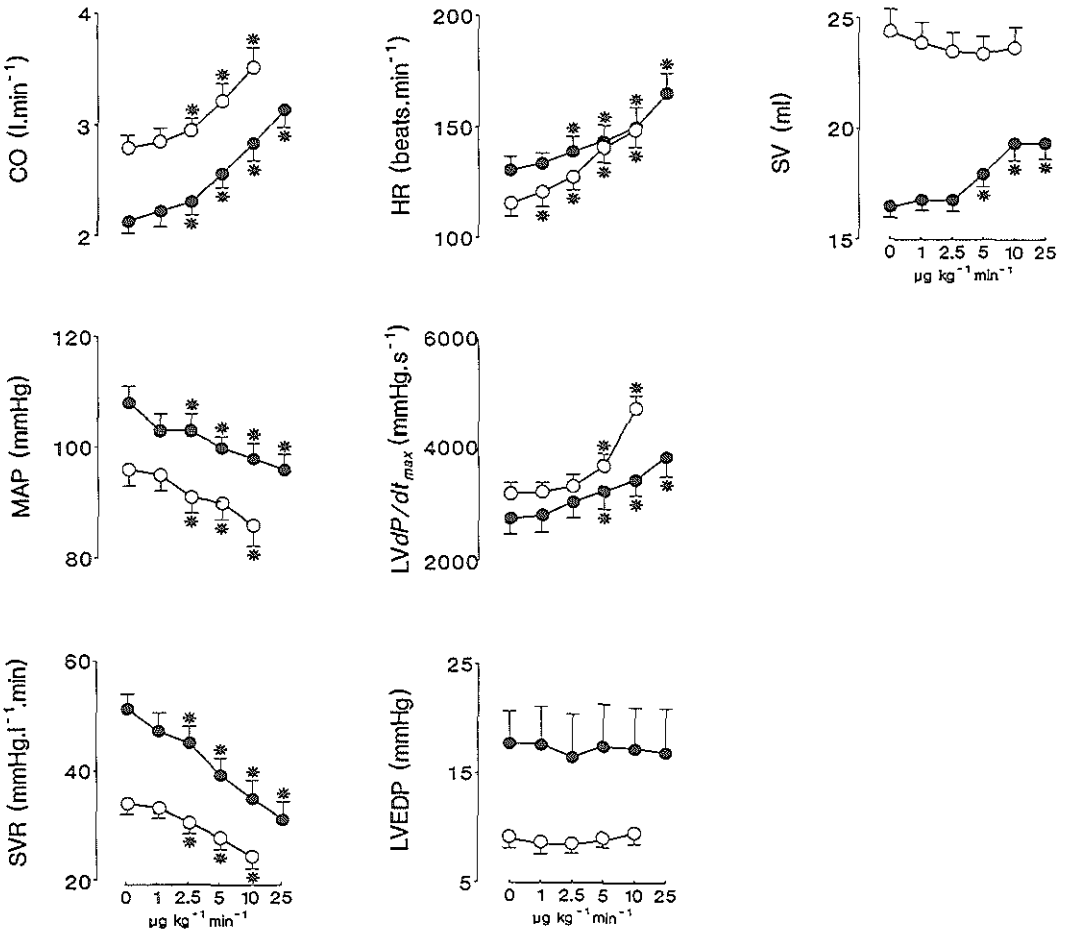

Figure 1. *Cardiovascular effects of dopamine in normal conscious pigs (○, n = 10) and in conscious pigs with chronic left ventricular heart failure (●, n = 8). CO = cardiac output; HR = heart rate; MAP = mean arterial blood pressure; LVdP/dt$_{max}$ = maximal rate of rise of left ventricular pressure; SVR = systemic vascular resistance; LVEDP = left ventricular end-diastolic pressure; SV = stroke volume. Data are means ± S.E.M. * P<0.05 compared to baseline (0 µg.kg^{-1}.min^{-1}).*

Systemic hemodynamic *effects of dopamine in normal pigs*

Dopamine caused a dose dependent increase in cardiac output (up to 27%), due to a similar increase in heart rate (up to 28%), while stroke volume remained unchanged (Fig. 1). Arterial blood pressure decreased (by 13%), as the decrease in systemic vascular resistance (by 31%) was more pronounced than the increase in cardiac output. $LVdP/dt_{max}$ increased (up to 48%), but left ventricular end-diastolic pressure did not change.

Systemic hemodynamic effects of dopamine in pigs with chronic heart failure

Fig. 1 shows that the increase in cardiac output was not affected in the animals with chronic heart failure. However, in contrast to the normal animals, in the animals with heart failure, for doses higher than 5 $\mu g.kg^{-1}.min^{-1}$ also an increase in stroke volume (up to 18%, $P<0.05$) contributed to the increase in cardiac output. The vasodilatory capacity of dopamine was not affected, which resulted in similar decreases in arterial blood pressure as in the normals. $LVdP/dt_{max}$ increased, but the response was significantly attenuated (690 ± 170 $mmHg.s^{-1}$ after 10 $\mu g.kg^{-1}.min^{-1}$ compared to 1500 ± 290 $mmHg.s^{-1}$ in the normal animals, $P<0.05$). Left ventricular end-diastolic pressure remained again unchanged.

Systemic hemodynamic effects of dobutamine in pigs with chronic heart failure

Dobutamine increased cardiac output dose dependently (up to 81% during infusion of the highest dose) both due to an increase in heart rate (up to 48%) and stroke volume (up to 25%). The increases in cardiac output were slightly but consistently larger than during administration of the same doses of dopamine. Mean arterial blood pressure decreased only at the two highest doses. The decrease in systemic vascular resistance at these doses was, however, more pronounced than at the same doses of dopamine. $LVdP/dt_{max}$ increased gradually to values well beyond those measured during baseline conditions of normal animals, but left ventricular end-diastolic pressure was not affected (Fig. 2).

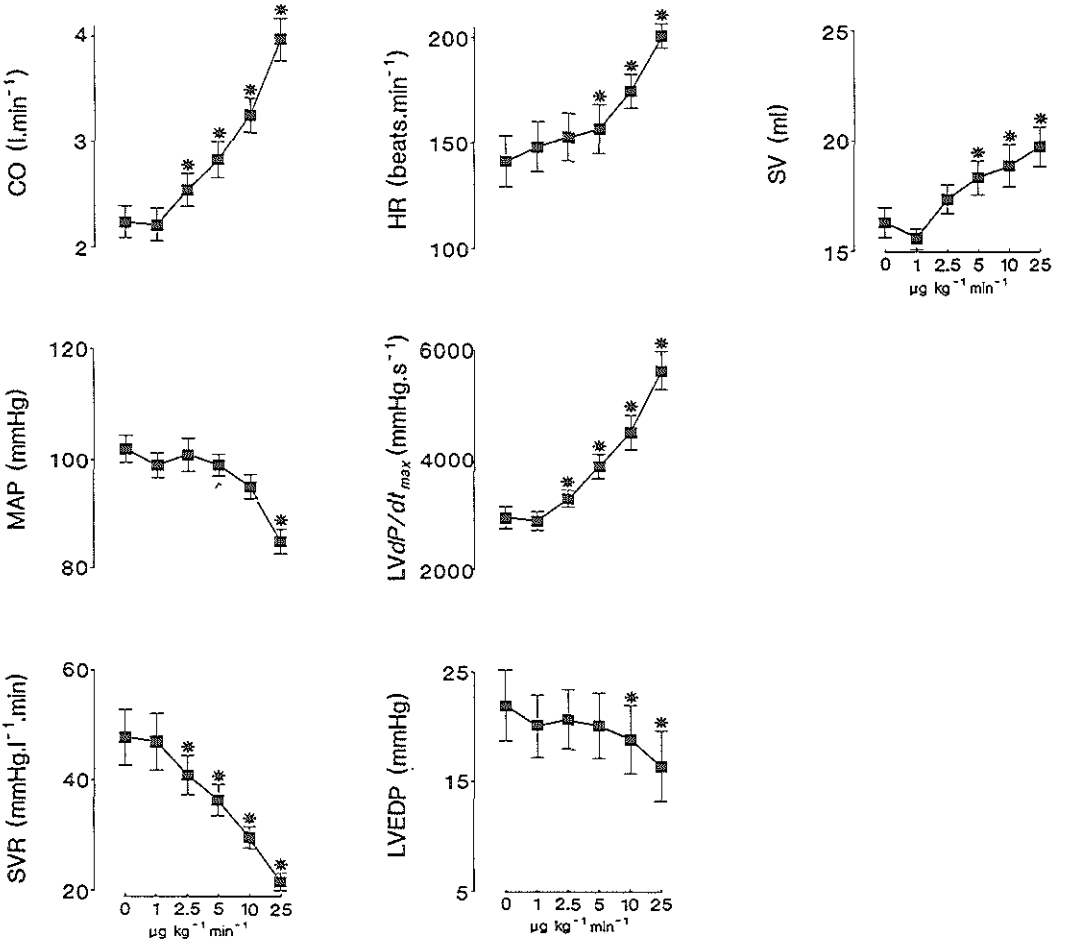

Figure 2. *Cardiovascular effects of dobutamine (■) in 8 conscious pigs with chronic left ventricular heart failure. See legends to figure 1 for details.*

Effects of dopamine and dobutamine on left ventricular work and left ventricular stroke work

Figure 3 shows that dopamine caused only a minor increase in left ventricular work in the normal pigs, while left ventricular stroke work decreased. In the animals with heart failure the left ventricular work was depressed at baseline, but increased more rapidly than in the normal animals during infusion of dopamine. Left ventricular stroke work, which was also depressed at baseline in these animals did not change and was not different from that of the normal animals after infusion of 10 $\mu g.kg^{-1}.min^{-1}$. Dobutamine tended to decrease left ventricular work during infusion of 1 $\mu g.kg^{-1}.min^{-1}$ in the animals with heart failure, but with the higher doses there was a more pronounced increase than with dopamine (fig. 3), left ventricular stroke work did not change.

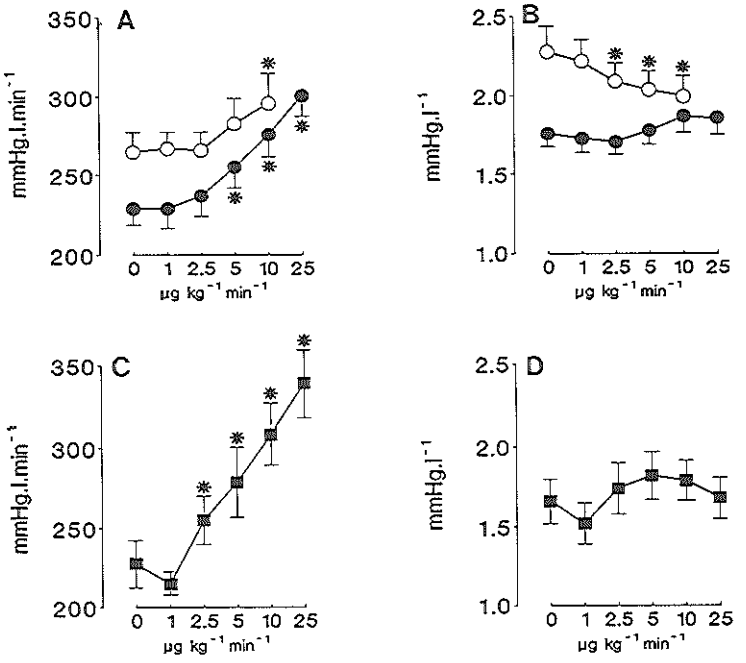

Figure 3. *Effect of dopamine on left ventricular work (a) and stroke work (b) in normal conscious pigs (○, n = 10) and in conscious pigs with chronic heart failure (●, n = 8). In panels c and d the effects of dobutamine in conscious pigs with chronic heart failure have depicted. Data are means ± S.E.M. * P<0.05 compared to baseline (10 $\mu g.kg^{-1}.min^{-1}$).*

Discussion

This is the first study that has evaluated the effects of dopamine and dobutamine on systemic hemodynamics in conscious pigs with chronic left ventricular heart failure, induced by a permanent occlusion of the left circumflex 3-4 weeks before the measurements were taken. The changes in cardiovascular performance induced by the permanent coronary artery occlusion in the present study are in good agreement with those reported earlier (Van der Giessen et al., 1989 and Van Woerkens et al., 1991b). In this model, arterial blood pressure is maintained in spite of the decrease in cardiac output by a compensating increase in peripheral resistance. The decreases in myocardial contractility, reflected by the decrease in $LVdP/dt_{max}$, stroke volume and left ventricular stroke work and the doubling of left ventricular end-diastolic pressure and are other indications of left ventricular dysfunction and the chronic presence of these changes make the model very suitable for the evaluation of the effects of pharmacological agents in a setting often encountered clinical practice. The observation that, after adaptation to the laboratory facilities, the animals are in a stable hemodynamic condition for the duration of the measurements is an additional attractive (Table 1).

The systemic hemodynamic effects of dopamine and dobutamine in normal conscious animals, both pigs (Fiser et al., 1988) and dogs (Vatner et al., 1974 and Liang and Hood, 1979) have been studied are in good agreement. We therefore studied only the effects of dopamine in conscious normal pigs as a reference for the study in the animals with chronic heart failure. Indeed, in the normal animals dopamine increased cardiac output due to an increase in heart rate as stroke volume did not change. Similar to the results described by Fiser et al. (1988), arterial blood pressure decreased by approximately 10%, because of systemic vasodilation and left ventricular end-diastolic pressure was not affected. $LVdP/dt_{max}$ is often used as an index of myocardial contractility, but its value is limited by its dependency on heart rate and arterial blood pressure. The latter changes in the present experiments, but the magnitude of these changes suggest that a positive inotropic effect must have at least contributed to the increase in $LVdP/dt_{max}$ (Scheffer and Verdouw, 1983).

The cardiovascular effects of dopamine and dobutamine in the animals with heart failure proved also to be very similar and at first glance they compare to the effects in the normal animals. There are, however, some remarkable differences. Although the magnitude of the increase in cardiac output was very similar, there was a marked difference in the mechanism. In the normal animals, tachycardia was the only factor responsible for the increase in cardiac output, but in the animals with heart failure an increase in stroke volume also contributed significantly. As a matter of fact, the increase

in heart rate during dopamine infusion was slightly less than in the normal animals. Although stroke volume increased, baseline values of the normal animals were not achieved. A major factor may have been the attenuated increase in $LVdP/dt_{max}$, which most likely reflects a smaller positive inotropic effect, due to down regulation of beta-adrenoceptors. This explanation is consistent with our earlier observations that in pigs the positive inotropic effects of dopamine are almost exclusively due to beta-adrenoceptor stimulation (Van Woerkens et al., 1991a). As also shown for the dihydropyridine calcium antagonists nisoldipine and elgodipine (Van Woerkens et al, 1991c) the vasodilatory response of dopamine was not affected in this model. In contrast to these last drugs, we did not observe a lowering of left ventricular filling pressure in the animals with heart failure.

In the dose range of 10-25 $\mu g.kg^{-1}.min^{-1}$ the increments in cardiac output were larger with dobutamine than with dopamine, probably because dobutamine unlike dopamine acts exclusively on alpha- and beta-adrenoceptors, which allows for larger increases in cardiac output than dopamine receptors (Horwitz et al., 1962).

The lack of effects of both drugs to lower left ventricular filling pressure lends further support to a combination with agents which act primarily on left ventricular preload. The design of the study does not allow any conclusions on the superiority of one drug over the other in the treatment of patients. In order to arrive at such a conclusion it would be necessary to determine regional blood flows and to what extent the drugs activate the neurohumoral system.

References

Buckley NM, Brazeau P and Frasier ID. Cardiovascular effects of dopamine in developing swine. *Biol Neonate* 43; 50-60, 1983.

Fiser DH, Fewell JE, Hill DE and Brown AL. Cardiovascular and renal effects of dopamine and dobutamine in healthy, conscious piglets. *Crit Care Med* 16; 340-345, 1988.

Gootman N, Buckley BJ, Gootman PM, Griswold PG, Mel JD and Nudel DB. Maturation-related differences in regional circulatory effects of dopamine infusion in swine. *Dev Pharmacol Ther* 6; 9-22,1983.

Liang C and Hood WB. Dobutamine infusion in conscious dogs with and without autonomic nervous system inhibition: effects on systemic hemodynamics, regional blood flows and cardiac metabolism. *J Pharmacol Exp Ther* 211; 698-705, 1979.

Scheffer MG and Verdouw PD. Decreased incidence of ventricular fibrillation after an acute coronary artery ligation in exercised pigs. *Basic Res Cardiol* 78; 298-309,

1983.

Van der Giessen WJ, Van Woerkens LJ, Duncker DJ, Roelandt JRTC and Verdouw PD. The acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J Cardiovasc Pharmacol* 14; 653-658, 1989.

Vane SW, Weber TR, Careskey J and Grosfeld JL. Systemic and renal effects of dopamine in the infant pig. *J Surg Res* 32; 477-483, 1982.

Van Woerkens LJ, Duncker DJ, Den Boer MO, McFalls EO, Sassen LMA, Saxena PR and Verdouw PD. Evidence against a role for dopamine D_1 receptors in the porcine myocardium. *Br J Pharmacol* 104; 246-250, 1991a.

Van Woerkens LJ, Van der Giessen WJ and Verdouw PD. Cardiovascular profile of 5 novel nitrate-esters: A comparative study with nitroglycerin in conscious pigs with and without left ventricular dysfunction. *Br J Pharmacol* 104; 7- 14, 1991b.

Van Woerkens LJ, Schotman SN, Van der Giessen WJ and Verdouw PD. Cardiovascular effects of elgodipine in conscious pigs with a normal coronary circulation and in conscious pigs with a healed myocardial infarction. *J Cardiovasc Pharmacol* 17; 976-982, 1991c.

Vatner SF, McRitchie RJ and Braunwald E. Effects of dobutamine on left ventricular performance, coronary dynamics, and distribution of cardiac output in consciou dogs. *J Clin Invest* 53; 1265-1273, 1974.

Horwitz D, Fox SM and Goldberg LI. Effects of dopamine in man. *Circ Res* 10; 237-243, 1962.

Chapter 12

Redistribution of cardiac output caused by opening of arteriovenous anastomoses by a combination of azaperone and metomidate.

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REDISTRIBUTION OF CARDIAC OUTPUT CAUSED BY OPENING OF ARTERIOVENOUS ANASTOMOSES BY A COMBINATION OF AZAPERONE AND METOMIDATE

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SUMMARY

The effects of the butyrophenone, azaperone 5 mg kg^{-1} i.m. alone and after addition of the imidazole derivative metomidate 6 mg kg^{-1} i.v. were studied in eight conscious pigs. Fifteen minutes after administration of azaperone, systemic arterial pressure was reduced by 35% as a result of a 45% increase in systemic vascular conductance and 10% decrease in cardiac output ($\dot{Q}$). After azaperone, 23% of the radioactive labelled microspheres (15 (SD 1) μm) injected into the left atrium were detected in the lungs as a result of opening of arteriovenous anastomoses (baseline 3%). The increase in arteriovenous anastomotic blood flow was at the expense of the nutritional (= capillary) channels. Flow to the brain was maintained, but that to the left ventricle decreased in parallel with the reduction in arterial pressure. Vascular conductance of most other organs, except the skin, increased or was maintained. The addition of metomidate had no effect on $\dot{Q}$ because an increase in stroke volume (by 30%) compensated for the decrease in heart rate. Systemic vascular conductance decreased, most noticeably in the brain, left ventricle and skeletal muscle. We conclude that azaperone alone and in combination with metomidate had only a moderate effect on $\dot{Q}$, but caused a redistribution in favour of arteriovenous anastomoses.

KEY WORDS

Heart: cardiac output, circulatory effects. Hypnotics: butyrophenones, azaperone, metomidate.

Butyrophenone compounds are used both in human (droperidol) and animal (azaperone) practice. Azaperone (4′-fluoro-4(2-pyridyl-1-piperazinyl)-butyrophenone) is used widely in pigs as a sedative when physical restraint or diagnostic procedures are required [1–4]. In addition, this drug is used frequently in combination with the veterinary analogue of etomidate, the imidazole derivative metomidate (methyl-1-(α-methylbenzyl)imidazol-5-carboxyl-hydrochloride), to provide anaesthesia for short-term surgical procedures in pigs [5]. In recent years several investigators have used this combination for both induction and maintenance of anaesthesia (with or without the addition of nitrous oxide) for physiological studies [6–8].

Although it has been demonstrated that the combination reduces systemic arterial pressure and cardiac output ($\dot{Q}$) [5, 9] detailed knowledge of regional cardiovascular responses is lacking. In the present study we investigated changes in central haemodynamics and regional blood flow (measured with radioactive microspheres) occurring in conscious pigs after sedation with azaperone and additional administration of metomidate. It is known that, in anaesthetized, but not conscious, animals a large fraction of the microspheres is trapped in the lungs because of shunting by arteriovenous anastomoses [7,

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10–13]. We therefore paid special attention to the occurrence of this phenomenon.

MATERIALS AND METHODS

After an overnight fast, cross-bred Landrace × Yorkshire pigs (20–25 kg, $n = 8$) pretreated with a mixture of procaine penicillin-G 300 000 u. i.m. and benzathine-penicillin-G 300 000 u. i.m. (Duplocillin, Gist-Brocades N.V., Delft, The Netherlands) were anaesthetized with ketamine 30 mg kg^{-1} i.m. (Aescoket, Aesculaap B.V., Boxtel, The Netherlands) [3]. The trachea was intubated and the lungs ventilated with a mixture of 66% nitrous oxide and 1% halothane in oxygen. A jugular vein and common carotid artery were cannulated for administration of drugs and measurement of systemic arterial pressure, acid–base state and blood-gas tensions (ABL-3 and OSM_2, Radiometer, Copenhagen, Denmark).

The chest was opened through the left fifth intercostal space to expose the heart. The left atrium was cannulated for injection of radioactive microspheres (15 (SD 1) μm diameter), labelled with cerium-141, ruthenium-103 or niobium-95 (NEN Chemicals GmbH, Dreieich, West Germany) and suspended in saline containing one drop of Tween 80. Via the third intercostal space, an electromagnetic flowprobe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta. $\dot{Q}$ was calculated by adding myocardial blood flow (determined with radioactive microspheres) to the electromagnetic flow readings. Catheters and wires were tunnelled subcutaneously to the back, the chest was closed and the animals allowed to recover. After operation, the animals received bolus injections of amoxicillin 500 mg i.v. daily (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and, during the first week, kanamycin 500 mg (Kamynex, Gist-Brocades N.V., Delft, The Netherlands) to prevent infection. The catheters were flushed daily with an isotonic saline solution containing heparin 500 iu ml^{-1}. After 1 week of post-surgical recovery, at least eight to 10 sessions were held to adapt the animals to the laboratory facilities and experimental conditions; when animals are adapted to experimental conditions, systemic haemodynamic parameters and regional blood flows change less than 5% over 1 h [14, and unpublished data from our laboratory]. The experimental study was performed 3–4 weeks after instrumentation. Antibiotics were withheld at least 24 h before the experiment. Neuromuscular blocking agents were not administered.

Experimental procedure

After baseline systemic haemodynamic and arterial blood-gas data had been recorded, 1–2 × 10^6 microspheres were injected i.v. while an arterial reference sample was withdrawn (10 ml min^{-1}) for calibration of regional blood flow measurements [10]. Subsequently, the animals received azaperone 5 mg kg^{-1} i.m. and 15 min later, after all measurements had been repeated, metomidate 6 mg kg^{-1} i.v. was administered. Immediately thereafter, the trachea was intubated and the lungs ventilated with 66% nitrogen in oxygen to maintain arterial blood-gas values at baseline. The last set of values was obtained 10 min after i.v. injection of metomidate, while the animals were in a stable haemodynamic condition.

At the end of each experiment the animal was killed with an overdose of pentobarbitone sodium. Tissue was dissected, weighed and placed in vials. The brain was divided into left and right cerebral hemispheres, diencephalon, brain stem and cerebellum. The heart was stored in 10% formaldehyde for 48 h and subsequently the left ventricle was dissected into three layers of equal thickness. The radioactivity in the vials was counted for 10 min in a gamma-scintillation counter (Packard Minaxi Autogamma 5000) with a multichannel pulse height analyser using suitable windows for discrimination of the different isotopes. The amount of blood distributed to the various tissues ($\dot{Q}$tis) was calculated as:

$$\dot{Q}\text{tis (ml min}^{-1}) = (I\text{tis}/I\text{art}) \times \dot{Q}\text{art}$$

Where Itis and Iart 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 the blood sample. Vascular conductances were calculated by dividing blood flow by mean arterial pressure.

Statistical analysis and data presentation

Statistical analysis was performed using the Duncan New Multiple Range test after parametric two-way analysis had revealed that the samples represented different populations. Statistical significance was accepted at $P < 0.05$. All values are presented as mean (SEM).

RESULTS

Arterial acid–base and blood-gas state

Arterial blood-gas values did not change from respective baseline values (pH 7.46 (0.03); Pa_{CO_2} 4.9 (0.1) kPa; Pa_{O_2} 10.1 (0.5) kPa; oxygen saturation 95 (1) %; haemoglobin 5.0 (0.2) mmol litre^{-1}), either during spontaneous ventilation after azaperone or during controlled mechanical ventilation after metomidate.

Systemic haemodynamics

Administration of azaperone decreased $\dot{Q}$ by 10 % and mean arterial pressure (MAP) by 35 % (fig. 1). Because the decrease in MAP considerably exceeded the decrease in $\dot{Q}$, calculated systemic vascular conductance increased by 45 %. The decrease in $\dot{Q}$ was secondary to a reduction in stroke volume by 13 % as heart rate remained unaffected. After the i.v. administration of metomidate, $\dot{Q}$ was maintained, as the increase in stroke volume of 30 % counterbalanced the drug-induced bradycardia. In view of the slight increase in arterial pressure and unchanged cardiac output, systemic vascular conductance was attenuated.

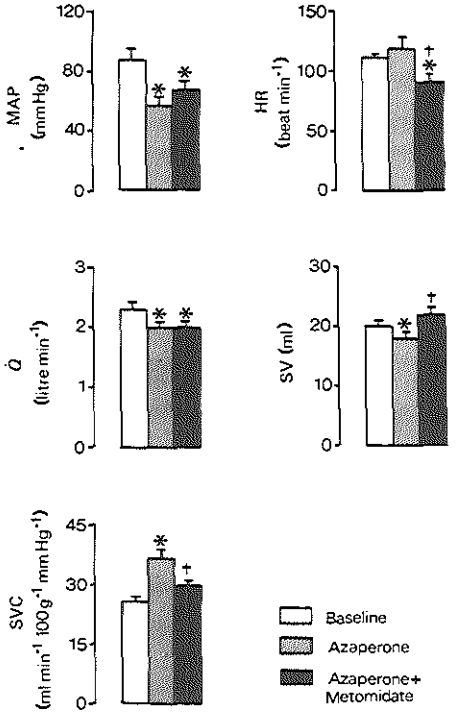

FIG. 1. Effects of azaperone 5 mg kg^{-1} i.m. alone and after addition of metomidate 6 mg kg^{-1} i.v. on systemic haemodynamics in eight pigs (mean (SEM)). MAP = Mean arterial pressure; HR = heart rate; $\dot{Q}$ = cardiac output; SV = stroke volume; SVC = systemic vascular conductance. *$P < 0.05$ *vs* baseline; †$P < 0.05$ *vs* azaperone.

Distribution of cardiac output to arteriovenous anastomotic and nutritional channels

During baseline conditions only 3 % of the microspheres were trapped by the lungs (mainly bronchial arterial blood flow [15]). After administration of azaperone, blood flow was redistributed in favour of arteriovenous anastomoses, as 23 % of the microspheres were trapped in the lungs, while the nutritional capillary blood flow decreased in parallel with arterial pressure (fig. 2). Consequently the vascular conductance of the nutritional vasculature was not affected. After administration of metomidate there were no significant changes in nutritional and arteriovenous blood flows. Arteriovenous conductance was not affected, but nutritional vascular conductance decreased and was responsible, therefore, for the attenuation of systemic vascular conductance.

Distribution of nutritional blood flow

Azaperone decreased left ventricular myocardial blood flow (fig. 3), which was distributed

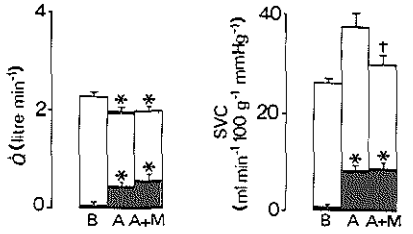

FIG. 2. Effects of azaperone 5 mg kg^{-1} i.m. alone (A) and after addition of metomidate 6 mg kg^{-1} i.v. (A+M) on the contribution of the arteriovenous anastomoses (▩) and the nutritional vascular bed (□) to cardiac output ($\dot{Q}$) and systemic vascular conductance (SVC). The contribution of the nutritional vessels has been superimposed on that of the arteriovenous anastomoses such that the sum reflects $\dot{Q}$ and SVC. The symbols for statistically significant changes and the SEM bars are those for the two fractions. *$P < 0.05$ *vs* baseline (B); †$P < 0.05$ *vs* azaperone (A).

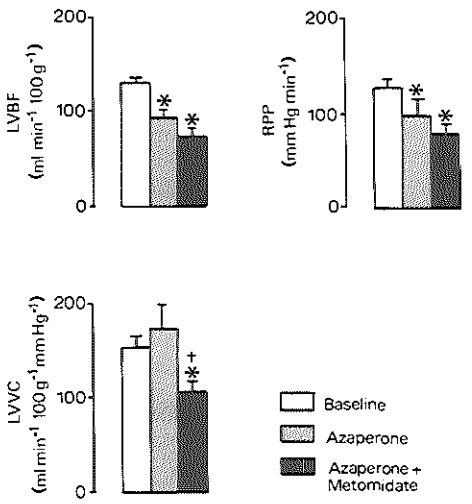

FIG. 3. Effect of azaperone 5 mg kg^{-1} i.m. alone and after addition of metomidate 6 mg kg^{-1} i.v. on transmural left ventricular blood flow (LVBF), left ventricular oxygen demand estimated by the rate–pressure product (RPP) and left ventricular vascular conductance (LVVC) (mean (SEM)). *$P < 0.05$ *vs* baseline; †$P < 0.05$ *vs* azaperone.

homogeneously over the myocardial layers, as the endocardial:epicardial ratio did not change from its baseline value (1.39 (0.05); not shown). A 27 % decrease in transmural blood flow accompanied a 22 % decrease in oxygen demand (estimated by the rate–pressure product). As the decrease in left ventricular blood flow was of a magnitude similar to the decrease in mean arterial pressure, there was no change in left ventricular vascular conductance. Addition of metomidate decreased further left ventricular blood flow but, as the oxygen demand changed similarly, there was no detrimental effect on myocardial oxygen supply:demand ratio. Whereas there was no change in left ventricular conductance with azaperone, there was a marked decrease in conductance after metomidate was added.

In the conscious animals, the brain stem received 30–35 % less blood than other parts of the brain (table I). Azaperone caused marked vasodilatation, as blood flows to all cerebral regions were maintained despite the 35 % decrease in arterial pressure. However, after addition of metomidate, blood flows to all parts were reduced by 40–55 %, despite the slight increase in arterial pressure. From the changes in arterial pressure and flow it follows that the conductance of all regions of the brain decreased to 60–80 % of baseline values.

Following azaperone, blood flow to some organs was maintained (liver, spleen, right ventricle and skeletal muscle) or even increased (adrenals). Blood flow to the kidneys, stomach, small intestine and skin were reduced (table II). Vascular conductance was diminished in the skin, did not change in kidneys and skeletal muscle, stomach and small intestine, and increased in liver, spleen and adrenals. The addition of metomidate had no effect on blood flow to some organs (liver, spleen, small intestine, stomach and kidneys), but largely abolished the azaperone-induced increase in blood flow to the adrenals, while it reduced flow to the

TABLE I. *Regional cerebral haemodynamics after azaperone 5 mg kg^{-1} i.m. and addition of metomidate 6 mg kg^{-1} i.m. in eight pigs (mean (SEM)).* *P < 0.05 vs *baseline*; †P < 0.05 vs *azaperone*; §P < 0.05 vs *all other brain regions*

	Baseline	Azaperone	Azaperone + metomidate
Blood flows (ml min^{-1} 100 g^{-1})			
Right hemisphere	77 (5)	79 (6)	35 (3)*†
Left hemisphere	76 (6)	76 (4)	36 (2)*†
Diencephalon	77 (7)	81 (9)	38 (2)*†
Cerebellum	81 (5)	80 (5)	49 (3)*†§
Brain stem	53 (5)§	56 (3)§	33 (3)*†
Total brain	74 (4)	74 (4)	35 (3)*†
Conductances (10^{-2} ml min^{-1} 100 g^{-1} mm Hg^{-1})			
Right hemisphere	90 (9)	154 (29)*	53 (5)*†
Left hemisphere	92 (6)	146 (14)*	54 (3)*†
Diencephalon	95 (7)	158 (23)*	57 (6)*†
Cerebellum	100 (5)	162 (27)*	73 (7)†§
Brain stem	65 (5)§	113 (18)*§	51 (7)†
Total brain	86 (7)	141 (20)*	54 (5)*†

TABLE II. *Regional haemodynamics after azaperone 5 mg kg^{-1} i.m. and addition of metomidate 6 mg kg^{-1} i.m. in eight pigs (mean (SEM)). *P < 0.05 vs baseline; †P < 0.05 vs azaperone*

	Baseline	Azaperone	Azaperone + metomidate
Blood flows (ml min^{-1} 100 g^{-1})			
Liver	19 (4)	18 (3)	14 (3)
Spleen	160 (10)	144 (17)	123 (18)
Kidneys	366 (36)	244 (40)*	252 (34)*
Adrenals	127 (5)	276 (40)*	191 (27)*†
Stomach	42 (11)	19 (5)*	21 (5)*
Small intestine	50 (6)	32 (4)*	36 (6)*
Right ventricle	88 (5)	80 (13)	51 (6)*†
Skeletal muscle (iliopsoas)	13.6 (4.7)	8.3 (2.6)	3.2 (0.3)*
Skin	6.8 (1.0)	2.0 (0.3)*	1.1 (0.3)*
Conductances (10^{-2} ml min^{-1} 100 g^{-1} mm Hg^{-1})			
Liver	22 (5)	37 (11)*	22 (5)†
Spleen	185 (14)	261 (28)*	179 (20)†
Kidneys	424 (48)	434 (63)	366 (36)
Adrenals	146 (9)	494 (58)*	301 (57)*†
Stomach	51 (14)	36 (10)	29 (6)*
Small intestine	59 (8)	65 (14)	52 (7)
Right ventricle	102 (9)	143 (20)*	77 (9)†
Skeletal muscle (iliopsoas)	15.3 (4.8)	15.3 (4.5)	4.9 (0.4)*†
Skin	7.6 (0.8)	3.6 (0.6)*	1.7 (0.3)*†

right ventricle, skeletal muscle and skin. Consequently, metomidate abolished (adrenals) or attenuated (liver, spleen, right ventricle) the azaperone-induced vasodilatation in some organs, while in others the reduction in vascular conductance was enhanced (skin) or became apparent (stomach, skeletal muscle).

DISCUSSION

Azaperone

Azaperone-induced hypotension has been reported by others [4, 5, 16]. Lees and Serrano [16] also found that this was caused by systemic vasodilatation rather than a decrease in $\dot{Q}$. Although $\dot{Q}$ was affected only minimally, there was profound redistribution of blood flow, implying an opening of arteriovenous anastomoses [7, 10–13]. In the pig, these anastomoses are abundantly present in the skin [7] and play a major role in the regulation of body temperature. The opening of these anastomoses by azaperone may, therefore, explain the decrease in body temperature described by others [1, 2]. The increase in conductance of the arteriovenous anastomoses was responsible also for the increase in systemic vascular conductance, as the changes in conductance of the nutritional vessels counterbalanced each other. These results suggest that the mechanism responsible for opening of the arteriovenous anastomoses is the same as the mechanism for the hypotensive action. However, both the mechanisms for opening of the arteriovenous anastomoses in general and that by which azaperone decreases arterial pressure are largely unknown. It has been suggested that azaperone possesses α-adrenoceptor antagonistic activities [17–19]. As the physiological regulation of arteriovenous anastomotic blood flow is mediated in part by the sympathetic nervous system [20], it may be amenable to α-adrenoceptor antagonism. However, other mechanisms such as active neurogenic vasodilatation, for which no neurotransmitter has yet been identified [18], may be involved in the control of arteriovenous anastomotic blood flow. Also, as with noradrenaline, the arteriovenous anastomoses are constricted by 5-hydroxytryptamine [21]. In addition, butyrophenones possess antidopaminergic properties and dopamine is believed to play a role in central regulation of body temperature [22], so that the opening of arteriovenous anastomoses by azaperone could conceivably be caused by a mechanism not related to α-adrenoceptor antagonism. The clinical analogue of azaperone, the butyrophenone droperidol, induces hypotension in man by systemic vasodilatation, which is probably secondary to the combination of

central nervous effects and block of vascular α-adrenoceptors [23]. The opening of arteriovenous anastomoses could also be involved, but this may be difficult to assess in man.

Left ventricular blood flow decreased in accord with the reduction in myocardial oxygen demand. As the reduced arterial pressure was responsible for the reduced oxygen demand, left ventricular blood flow was reduced without affecting coronary vascular conductance. On the other hand, cerebral flow was well maintained in spite of the hypotensive action of azaperone. Consequently, there was a marked increase in the vascular conductance of all regions of the brain. From the present studies, it cannot be concluded whether cerebral vasodilatation was caused by a metabolic regulation or by a direct action of the drug.

Azaperone and metomidate

In the present study, the addition of metomidate attenuated the azaperone-induced decrease in arterial pressure, by decreasing the systemic vascular conductance. From figure 2 it is clear that the decrease in vascular conductance was restricted to the nutritional channels, as conductance of the arteriovenous anastomoses remained unchanged. The decreases in vascular conductance were most noticeable in the brain, left ventricle and skeletal muscle. In the brain this was probably secondary to a decrease in cerebral metabolism similar to that shown for its clinical analogue etomidate [24, 25]. From the present study we cannot conclude if cerebral metabolism decreased. In order to measure oxygen consumption, knowledge of the oxygen content of cerebral venous blood is necessary, but this cannot be readily obtained. Sampling from the internal jugular vein does not provide reliable information because in pigs it does not selectively drain blood from the cerebral circulation [26].

The addition of metomidate caused further reduction in left ventricular oxygen demand. In contrast with azaperone, this occurred by decrease in heart rate rather than decrease in arterial pressure. The reduced metabolic needs were accompanied by vasoconstriction, as arterial perfusion pressure was not significantly affected. These results are in agreement with those reported for etomidate in animals [24] and in man [25, 27].

Lees and Serrano [16] have shown that the actions of azaperone in ponies persist for at least 40–60 min. Our observations with metomidate were made within 30 min of administration of azaperone. Nevertheless, it could be argued that, because of differences in kinetics, our observations in pigs could be explained by washout of azaperone. However, the nature of the changes does not support such a hypothesis.

Clinical implications

Azaperone is used widely in veterinary practice, alone or in combination with metomidate. It has been claimed to have minor effects on cardiovascular function, despite reports of its hypotensive action [2, 3]. In view of our findings, such a claim is not warranted. Furthermore, although cardiac output was well maintained, large changes occurred in distribution. Opening of arteriovenous anastomoses shown in the present study and the earlier reports on severe hypothermia [1], suggest that the drug interferes with thermoregulation and that care must be taken to maintain body temperature, both in veterinary practice and in physiological preparations. To our knowledge, no reports on effects of droperidol on body temperature of man are available.

Azaperone and metomidate are not used in human practice, but their clinical analogues, droperidol and etomidate, respectively, have been shown to exert similar central haemodynamic actions [28]. This may also be true for regional blood flow.

REFERENCES

1. Green CJ. *Animal Anaesthesia*, 2nd Edn. London: Laboratory Animals Ltd, 1982; 163–198.
2. Hall LW, Clarke KW. *Veterinary Anaesthesia*, 8th Edn. London: Baillière Tindall, 1983; 287–304.
3. Thurmon JC, Tranquilli, WJ. Anesthesia for cardiovascular research. In: Stanton HC, Mersmann HJ, eds. *Swine in Cardiovascular Research*. Boca Raton, Florida: CRC Press, Inc., 1986; 39–60.
4. Clarke KW. Effect of azaperone on the blood pressure and pulmonary ventilation in pigs. *Veterinary Research* 1969; 85: 649–651.
5. Dimingen J, Reetz I. Trials with the neuroleptic azaperone and the hypnotic metomidate for anaesthesia in pigs. *Deutsche Tierärztliche Wochenschrift* 1970; 77: 470–473.
6. de Graeff PA, van Gilst WH, Bel K, de Langen CDJ, Kingma JH, Wesseling H. Concentration-dependent protection by captopril against myocardial damage during ischemia and reperfusion in a closed chest pig model. *Journal of Cardiovascular Pharmacology* 1987; 9: S37–S42.
7. Saxena PR, Verdouw PD. Tissue blood flow and localization of arteriovenous anastomoses in pigs with microspheres of four different sizes. *Pflügers Archiv, European Journal of Physiology* 1985; 403: 128–135.

8. Klein HH, Schubothe M, Nebendahl K, Kreuzer H. The effect of two different diltiazem treatments on infarct size in ischemic, reperfused porcine hearts. *Circulation* 1984; 69: 1000–1005.
9. Orr JA, Manohar M, Will JA. Cardiopulmonary effects of the combination of neuroleptic azaperone and hypnotic metomidate in swine. *American Journal of Veterinary Research* 1968; 11: 1305–1308.
10. Kaihara S, Van Heerden PD, Migita T, Wagner HN. Measurement of distribution of cardiac output. *Journal of Applied Physiology* 1968; 25: 696–700.
11. Warren DJ, Ledingham JGG. Measurement of cardiac output distribution using microspheres. Some practical and theoretical considerations. *Cardiovascular Research* 1974; 8: 570–581.
12. Hales JRS, Fawcett AA, Bennett JW. Radioactive microsphere partitioning of blood flow between capillaries and arteriovenous anastomoses in skin of conscious sheep. *Pflügers Archiv, European Journal of Physiology* 1978; 376: 87–91.
13. Hof RP, Hof A. Differential effects of antihypertensive drugs on nutritive and nonnutritive blood flow in anaesthetized rabbits. *Journal of Cardiovascular Pharmacology* 1989; 13: 565–571.
14. Duncker DJ, Saxena PR, Verdouw PD. Systemic haemodynamics of dihydropyridine derivatives in conscious pigs with or without propranolol. *European Journal of Pharmacology* 1988; 157: 401–409.
15. Baile EM, Nelems JMB, Schulzer M, Paré PD. Measurement of regional bronchial arterial blood flow and bronchovascular resistance in dogs. *Journal of Applied Physiology* 1982; 53: 1044–1049.
16. Lees P, Serrano L. Effects of azaperone on cardiovascular and respiratory functions in the horse. *British Journal of Pharmacology* 1976; 56: 263–269.
17. Marsboom R. On the pharmacology of azaperone, a neuroleptic used for restraint in wild animals. *Acta Zoologica et Pathologica Antverpiensia* 1969; 48: 155–161.
18. Marsboom R. The development and pharmacology of butyrophenones in human and veterinary medicine. *Proceedings of the Association of Veterinary Anaesthetists of Great Britain and Ireland* 1971; 2: 81–98.
19. Hapke VJH, Prigge E. Herz- und Kreislauf Wirkungen von Azaperone (Stresnil). *Deutsche Tierärztliche Wochenschrift* 1972; 79: 500–504.
20. Hales JRS. Skin arteriovenous anastomoses, their control and role in thermoregulation. In: Johansen K, Burggren W, eds. *Cardiovascular Shunts*. Copenhagen: Munksgaard, 1984; 433–448.
21. Saxena PR, Verdouw PD. Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow. *Journal of Physiology (London)* 1982; 332: 501–520.
22. Cox B. Dopamine. In: Lomax P, Schönbaum E, eds. *Body Temperature-Regulation, Drug Effects and Therapeutic Implications*. New York: Marcel Dekker, 1979; 231–255.
23. Muldoon SM, Janssens WJ, Verbeuren JJ, Vanhoutte PM. Alpha-adrenergic blocking properties of droperidol on the isolated blood vessels of the dog. *British Journal of Anaesthesia* 1977; 49: 211–216.
24. Renou AM, Vernhiet J, Macrez P, Constant P, Billerey J, Khadaroo MY, Caille JM. Cerebral blood flow and metabolism during etomidate anaesthesia in man. *British Journal of Anaesthesia* 1978; 50: 1047–1051.
25. Kettler D, Sonntag H, Donath U, Regensburger D, Schenk HD. Hämodynamik, Myocardmechanik, Sauerstoffbedarf und Sauerstoffversorgung des menschlichen Herzens unter Narkoseeinleitung mit Etomidate. *Anaesthesist* 1974; 23: 116–121.
26. Saxena PR, Verdouw PD. Redistribution by 5-hydroxytryptamine of carotid arterial blood at the expense of arteriovenous anastomotic blood flow. *Journal of Physiology (London)* 1982; 332: 501–520.
27. Patschke D, Bruckner JB, Eberlein HJ, Hess W, Tarnow J, Weymar A. Effects of Althesin, etomidate and fentanyl on haemodynamic and myocardial oxygen consumption in man. *Canadian Anaesthetists Society Journal* 1977; 24: 57–69.
28. Dundee JW, Wyant GM, Clarke RSJ, McKay AC, Bovill JG. In: *Intravenous Anaesthesia*. Edinburgh, London, Melbourne and New York: Churchill Livingstone, 1988; 206–247.

Chapter 13

Epicrisis

Epicrisis

Chronic effects of permanent left circumflex coronary artery occlusion in pigs.

Mortality

During the instrumentation for our conscious pig model in preparation for the several studies, coronary artery occlusion resulted in ventricular fibrillation approximately 25 % of the animals within the first 30 minutes after the occlusion. Electrical defibrillation was successful in all animals. Mortality, most likely due to ventricular arrhythmias, varied between 10 and 30 % during the first 7 postoperative days. After that time no sudden deaths occurred, but some animals were sacrificed because of an infection, or failure of the eltrical catheters.

Infarct size

Brooks et al. (1977) have shown that the left circumflex coronary artery in normal pigs perfuses approximately 22 % of the left ventricle. Post mortem examination during our studies revealed a fibrotic area weighing 13 ± 2 % of the total left ventricular mass. The major reasons for the small value are the development of scar tissue, the growth of young animals and the development of left ventricular hypertrophy during the postoperative period.

Systemic hemodynamics

Systemic hemodynamic parameters reached a steady state and differed significantly ($p < 0.05$) compared to conscious pigs with a non-occluded coronary circulation 2-3 weeks after infarction, when the actual experiments were performed (see also table 1; chapter 3). During this period a total number of 6 to 8 sessions were held, for adaptation of the animal to the laboratory facilities.

Compared to conscious animals without LCX-occlusion there was a slight increase in heart rate but this never reached levels of statistical significance. Mean arterial blood pressure was not affected, but the decrease in cardiac output varied between 5 and 25 %. Calculated systemic vascular resistance was only slightly affected in some groups, but increased significantly up to 34 % in others. The decrease in stroke volume varied between 5 and 30 %, but was only significant in the study with nisoldipine (chapter 2 and 4). Left ventricular end-diastolic pressure increased in all studies after coronary artery

occlusion (50-110 %, $p < 0.05$). Left ventricular dP/dt_{max} showed a decrease, which ranged between 12 and 30 %. These hemodynamic changes after coronary occlusion are comparable to those published for other species (Leddy et al., 1983, Tikkanen et al., 1987 and Sabbah et al., 1991).
The growth of the animals during the study period tended to be less compared to chronically instrumented pigs without a myocardial infarction, but this did not reach levels of statistical significance, probably because of the wide range of severity of left ventricular dysfunction and the occurrence of organomegaly.

Table 1. *Systemic hemodynamic effects in normal conscious pigs at a decrease in systemic vascular resistance by 25%.*

	HR	CO	EDP	MAP	LVdP/dt
nisoldipine	↑	↑	↓	↓	↑
elgodipine	↑	↑	-	↓	↑
pimobendan	↑↑↑	↑	↓↓↓	↓	↑↑↑
bimakalim	↑↑	↑	-	↓	↑
nicorandil	↑↑	↑	↓↓	↓	↑
glyceryl trinitrate	↑↑	↑	↓↓↓	↓↓	↑↑
CEDO 8956	↑	↑	↓	↓	↑

HR = heart rate; CO = cardiac output; EDP = left ventricular end-diastolic pressure; MAP = mean arterial blood pressure; LVdP/dt = maximun rate of rise of left ventricular pressure.

Effects of dihydropyridine calcium antagonist in conscious pigs.

In normal conscious animals nisoldipine and elgodipine exerted a similar cardiovascular profile but nisoldipine proved to be more potent. Nisoldipine decreased, however, left ventricular end-diastolic pressure, which did not occur after infusion of elgodipine (table 1).
In the animals with a 3-4 weeks old infarction both compounds increased cardiac output more after a similar decrease in systemic vascular resistance, than occurred in the normal

animals (table 2). Nisoldipine decreased left ventricular end-diastolic pressure to the same extent as in the normal animals, but elgodipine now decreased left ventricular end-diastolic pressure more compared to the effects in normal animals. These responses were however only obtained at higher infusion rates. The overall cardiovascular hemodynamic response after administration of the calcium antagonists nisoldipine and elgodipine in this model for mild heart failure is systemic vasodilatation, resulting in a enhanced cardiac output. This increase in cardiac output serves mainly for improvement of the skeletal muscle flow, as there were no changes in flow to kidneys, brain, liver or skin (chapter 2).

Effects of the phosphodiesterase-inhibitor pimobendan.

In normal animals pimobendan , while inducing the same decrease in systemic vascular resistance increases heart rate and left ventricular dP/dt_{max} and decreases left ventricular end-diastolic pressure more than nisoldipine or elgodipine (table 1).
After myocardial infarction a similar decrease in systemic vascular resistance produced a larger rise in cardiac output, while there was less effect on left ventricular end-diastolic pressure, mean arterial blood pressure and left ventricular dP/dt_{max} compared to the normal animals (table 2). We concluded from these data that pimobendan which behaves as an "inodilator" in normal animals, becomes more of a vasodilator during left ventricular dysfunction (chapter 4).

Effects of the potassium channel activator bimakalim

Although bimakalim is more potent than nicorandil, both showed a similar cardiovascular pattern in normal conscious pigs, except for a decrease in left ventricular end-diastolic pressure after the infusion of nicorandil (table 1).
Not surprisingly, reflex mediated tachycardia was increased in this group with myocardial infarction at the same extent of systemic vasodilatation after bimakalim. All other cardiovascular responses were similar to these in the normal group of animals (table 2). This was also true for nicorandil. However, the distinct lowering of left ventricular end-diastolic pressure after nicorandil in the normal animals was less in the animals with coronary artery occlusion.
Also in this class of drugs, therefore, it is concluded that both bimakalim and nicorandil behave more like systemic vasodilator drugs in animals with a myocardial infarction. In this condition, the nitrate-like actions of nicorandil could hardly be observed.

Table 2 *Changes in hemodynamic effects in conscious pigs with left ventricular dysfunction by a 3-4 weeks old infarction compared to the effects in in normal conscious pigs.*

	HR	CO	EDP	MAP	LVdP/dt
nisoldipine	-	↑>	-	↓<	-
elgodipine	-	↑>	↓>	-	-
pimobendan	-	↑>	↓<	↓<	-
bimakalim	↑<	-	-	-	-
nicorandil	↑<	-	↓<	-	↑<
glyceryl trinitrate	-	-	↓<	-	↑<
CEDO 8956	↑>	-	↓>	-	-

HR = heart rate; CO = cardiac output; EDP = left ventricular end-diastolic pressure; MAP = mean arterial blood pressure; LVdP/dt = maximun rate of rise of left ventricular pressure.

- ; no changes in effect in conscious animals with a 3-4 weeks old myocardial infaction compared to the systemic haemodynamic effects in normal conscious pigs at a decrease in sytemic vascular resistance by 25%.

↑> or ↓> ; more effect compared to normal conscious pigs

↑< or ↓< ; less effect compared to normal conscious pigs

Effects of nitrate-esters and glyceryl trinitrate.

Of the 5 nitrate-esters CEDO 8956 was the most potent vasodilator, exhibiting a similar cardiovascular profile as nitroglycerin, except for a lesser increase in heart rate and left ventricular dP/dt_{max} (Table 1, chapter 6). In normal animals for the same decrease in systemic vascular resistance there were larger decreases in left ventricular end-diastolic pressure and mean arterial blood pressure after administration of glyceryl trinitrate. Probably related, the increase in heart rate after glyceryl trinitrate was more pronounced. In the animals with left ventricular dysfunction for the same decrease in systemic vascular

resistance CEDO 8956 decreased left ventricular end-diastolic pressure more compared to normal animals, while the decrease in left ventricular end-diastolic pressure after glyceryl trinitrate was less pronounced compared to normal animals.
The responses after both drugs, therefore, became more uniform during left ventricular dysfunction. In this condition, both drugs lower left ventricular filling pressure and dilatate peripheral resistance vessels. The increase in cardiac output were, however, less compared to the calcium antagonists. In an attempt to combine the effects of nitrates and the dihydropyridines we have also studied hybrid drugs of both classes. In a preliminary study we investigated at the effects of 8 compounds which structurally possessed one to three nitrite groups and one dihydropyridine-group in normal conscious pigs. In all experiments, the compounds caused tachycardia without increases in left ventricular dP/dt_{max} or decrease in left ventricular end-diastolic pressure (unpublished data from our laboratory). These studies suggests that these compounds in fact possessed negative inotropic properties most likely because in the used dose range (0.5-2.0 $mg.min^{-1}$ (20-23 kg animals), which is indeed rather high for calcium antagonist) the negative inotropic effects of the dihydropyridine part of the compound prevailed.

Effects of UL-FS 49

There was no difference in the ability of Ul-FS 49 to reduce heart rate in the control animals and the infarcted animals (table 1 and 2 , chapter 7). In a dosage up to 100 $\mu g.kg^{-1}$ UL-FS 49 had no effect on arterial blood pressure while cardiac output and left ventricular dP/dt_{max} decreased by less than 10 % in either groups. UL-FS 49 had no effect on the duration of systole, but prolonged the duration of diastole which is in favour of myocardial perfusion. Because of the absence of depression of cardiovascular performance by UL-FS 49, this drug might be of interest for selective reduction of heart rate in patients with mild left ventricular dysfunction due to coronary artery disease. UL-FS 49 while inducing a similar decrease in heart rate caused less decrease in left ventricular dP/dt_{max} compared to the non-selective beta-adrenoceptor antagonist propranolol, and may therefore, prove to be useful in those patients with compromised left ventricular function, which have unwanted tachycardia due to other medication. It must be remarked however, that in man the specific bradycardic agents are usually less effective in lowering heart rate.

DA_1 receptors in the porcine coronary circulation

Intracoronary infusion of the specific DA_1 agonist fenoldopam in anaesthetized pigs had neither an effect on coronary blood flow and coronary vascular resistance, nor on coronary venous oxygen content and local myocardial oxygen consumption. The dosages studied were high enough, as reflected by the occurrence of systemic effects (decrease in mean arterial blood pressure and systemic vascular resistance). Infusion of dopamine after alpha- and beta-adrenoceptor blockade induced no changes in left ventricular blood flow, coronary vascular resistance or myocardial oxygen consumption. These data suggest that DA_1-receptors are of minor importance in the coronary circulation of anesthetized pigs.

Effects of epinine, dopamine and dobutamine in conscious pigs

Epinine stimulates dopaminergic as well as alpha- and beta-adrenoceptors in the cardiac and vascular tissues (Chapter 9 and 10). In normal conscious pigs epinine caused a dose dependent increase in cardiac output and left ventricular dP/dt_{max} and a decrease in systemic vascular resistance, while there were no changes in left ventricular end-diastolic pressure. In dosages up to 5 $\mu g.kg^{-1}.min^{-1}$ epinine decreased mean arterial blood pressure and increased heart rate, but during infusion of 10 $\mu g.kg^{-1}.min^{-1}$ these parameters started to return to predrug values. There were no changes in left ventricular and renal blood flow, but flow to some parts of the brain was increased during the highest dose. Vascular resistance decreased in brain, renal and myocardial vasculature. After alpha- and beta-adrenoceptor blockade, there was no significant decrease in systemic vascular resistance during the infusion of epinine. This does not necessarily imply that dopaminergic receptors do not play a role in the epinine induced vasodilatation, as it is feasible that a decrease in vascular resistance in a particular regional vascular bed (renal, cerebral or mesenteric) does not lead to significant decrease in the systemic vascular resistance, due to limitation of the used measurement techniques. For instance, kidney flow in conscious pigs without alpha- and beta-adrenoceptor blockade was 450 $ml.min^{-1}$ at baseline , which is 16 % of the cardiac output. If an increase in flow by 20 % in the kidney flow was the only effect, this would result in a flow of 540 $ml.min^{-1}$, which is an increase in cardiac output by only 2.8 %.

Compared to dopamine, dobutamine caused for the same increase in heart rate a larger increase in cardiac output and left ventricular dP/dt_{max}. Both drugs had no effect on left ventricular end-diastolic pressure in the infarcted animals.

In summary, dopaminergic drugs may be useful in the lower dose range in experimental

heart failure, although they lack an effect on filling pressure. The absence of DA_1 receptors in the coronary circulation of pigs, may make this animal less suited for the study of the myocardial and coronary effects of dopaminergic compounds.

In this conscious animal model we have not systematically followed the time course of the changes post operatively as we feel that adaptation of the animals would be a confounding factor, which would make a explanation speculative. We feel that when one wants to study the hemodynamic changes and also the neurohumoral changes during the early infarction period, the occlusions should be performed in conscious animals as described by Skinner et al. (1975a and 1975b). This last group of investigators first adapted the animals to the laboratory facilities before occluding the left anterior descending coronary artery in order to study the effect of adaptation on the incidence of ventricular fibrillation. The finding that ventricular fibrillation occurred much less in the adapted animals, also implies that the loss of animals due to sudden death will be relatively low.

References

Brooks H, Holland R and Al-Sadir. Right ventricular performance during ischemia: An anatomic and hemodynamic analysis. *Am J Physiol* 233 (*Heart Circ Physiol* 2); H500-513, 1977.

Leddy CL, Willen M and Franciosa JA. Effects of a new angiotensin converting enzyme inhibitor, enalapril, in acute and chronic left ventricular failure in dogs. *J Clin Pharmacol* 23; 189-198, 1983.

Sabbah HN, Stein PD, Kono T, Gheorghiada M, Levine TB, Jafri S, Hawkins ET and Goldstein S. A canine model of chronic heart failure produced by multiple sequential coronary microembolizations. *Am J Physiol* 260 (*Heart Circ Physiol* 29); H1379-1384, 1991.

Skinner JE, Lie JT and Entman ML. Modification of ventricular fibrillation latency following coronary artery acclusion in the conscious pig: The effect of psychological stress and beta-adrenergic blackade. *Circulation* 51; 656-667, 1975.

Tikkanen T, Svartström-Fraser M, Tikkanen I, Sariola H, Fyhrquist F. Haemodynamic effects of atrial natriuretic peptide in rats with heart failure. *Eur J Pharmacol* 140; 187-193, 1987.

Samenvatting

Hartfalen komt voor bij ongeveer 1 % van de mensen boven de 50 jaar en dit percentage neemt toe tot 10 % bij mensen ouder dan 80 jaar. Hartfalen wordt vaak gedefinieerd als een toestand waarin de pompcapaciteit van het hart niet groot genoeg is om aan de metabole behoeften van de diverse organen en weefsels te voldoen. Men kan ook stellen dat hartfalen een toestand is waarbij het systeem van hart-en-bloedvaten compensatiemechanismen nodig heeeft om de bloeddruk in de aorta op peil te houden zodat de vitale organen zoals hart en hersenen voldoende doorbloed worden. Indien deze compensatiemechanismen tekort gaan schieten worden de klinische symptomen van hartfalen duidelijk.
In dit proefschrift wordt de cardiovasculaire werking beschreven van een aantal soorten farmaca in wakkere (niet-genarcotiseerde) chronisch geinstrumenteerde varkens waarbij hartfalen was verkregen door het afsluiten van een coronairarterie die 3-4 weken voordat de experimenten plaatsvonden werd uitgevoerd.

Chronische effecten van permanente afsluiting van de ramus circumflex van de linker coronair arterie bij varkens

Mortaliteit
Gedurende de instrumentatie van de varkens voor de diverse studies van het wakkere varkensmodel resulteerde het afsluiten van de coronairarterie in ongeveer 25 % van de dieren in een ventrikelfibrillatie in de eerste 30 minuten na de afsluiting. Alle dieren konden met succes gedefibrilleerd worden. De mortaliteit gedurende de eerste week na de operatie bedroeg 10 tot 30 %.

Infarctgrootte
In varkens voorziet de ramus circumflex van de linker coronairarterie ongeveer 22 % van de linker ventrikel van bloed. In onze studie werd post mortem een fibrotisch gebied gevonden van 13 ± 3 % van het totale gewicht van de linker vertrikel.

Chronisch effect van myocardinfarct op de circulatie
De hemodynamische parameters van de varkens met een infarct bereikten na 2 tot 3 weken een stabiel stadium en verschilden dan significant ($p<0.05$) met de chronisch geinstrumenteerde varkens die geen afsluiting van een coronair arterie hadden. (zie ook tabel 1, hoofdstuk 1).

De dieren met een afgesloten coronairarterie hadden in vergelijking met dieren zonder een infarct een iets hogere hartfrequentie, maar dit verschil was in geen van de studies significant. Er was geen verschil in de gemiddelde bloeddruk, maar het hartminuutvolume was tussen de 5 en 25 % lager bij de dieren met een infarct. De berekende systemische vaatweerstand was in sommige studies maar nauwelijk veranderd, maar in andere significant toegenomen met 34 %. De vermindering van het slagvolume varieerde tussen de 5 en 30 %, maar was slechts significant in de studie met nisoldipine (hoofdstuk 2 en 4). De eind-diastolische druk van de linker ventrikel steeg na coronair afsluiting in alle studies (50-110 %, $p < 0.05$). De linker ventrikel dP/dt_{max} was tussen de 12 en 30 %.

De efffecten van dihydropyridine calcium-antagonisten in varkens

De cardiovasculaire hemodynamische effecten van de calcium-antagonisten nisoldipine en elgodipine in dit model voor matig hartfalen zijn : systemische vaatverwijding, resulterend in een toegenomen hartminuutvolume (hoofsdtuk 2-4). De toename in hartminuutvolume zorgt vooral voor een toename van de doorbloeding van de skeletspieren, terwijl er geen veranderingen waren in de doorbloeding van de nieren, hersenen, lever en huid (hoofdstuk 2).

De effecten van de phosphodiesterase-remmer pimobendan

Pimobendan gedraagt zich in normale biggen als een "inodilator". Bij toediening aan dieren met linker ventrikeldysfunctie konden wij geen inotrope werking meer aantonen.

De effecten van de kalium-kanaal opener bimakalim

Met betrekking tot kalium-kanaal openers kan geconcludeerd worden dat bimakalim en nicorandil in dieren met een infarct werken als een systemische vaatverwijder. De nitraat-achtige eigenschappen van nicorandil werden in de dieren met een infarct in veel mindere mate waargenomen dan bij de normale dieren.

De effecten van nitraat-esters en glyceryl trinitraat

De effecten van de nitraat-esters CEDO 8956 en glyceryl trinitraat waren in de dieren met linker ventrikel dysfunctie meer uniform dan in de normale biggen. Beide farmaca verlagen de eind-diastolische druk van de linker ventrikel en dilateren de perifere vaten.

Deze toename van het hartminuutvolume was echter minder dan de toename na toediening van calciumantagonisten.

De effecten van UL-FS 49

Er was geen verschil in de effecten van UL-FS 49 op de hartfrequentie bij normale dieren en de varkens met een infarct (tabel 1 en 2, hoofdstuk 7). UL-FS 49 had geen effect op de tijdsduur van de systole, maar verlengde de tijdsduur van de diastole , hetgeen positief is voor de myocarddoorbloeding. Vanwege de afwezigheid van depressie van de cardiovasculaire prestatie door UL-FS 49, is het gebruik van deze stof mogelijk bruikbaar voor het selectief verlagen van de hartfrequentie bij patiënten met matig linker ventrikelfalen ten gevolge van coronaire hart ziekten.

DA_1 receptoren in de coronaire circulatie van het varken

Intracoronaire infusie van de specifieke DA_1 agonist fenoldopam in varkens onder narcose had noch effect op de coronaire doorbloeding en coronaire vaatweerstand, noch op het coronairveneuze zuurstofgehalte en het locale zuurstofverbruik van het myocard. De doseringen die gebruikt werden in deze studie waren hoog genoeg, daar systemische effecten optraden (daling gemiddelde bloeddruk en systemische vaatweerstand). Ook infusie van dopamine na alpha- en beta-receptor blokkade gaf geen veranderingen in de doorbloeding van de linker ventrikel, de coronaire vaatweerstand en het zuurstof verbruik van het myocard. Deze gegevens gevel wel aan dat DA_1-receptoren van minimaal belang zijn in de coronaire circulatie van het genarcotiseerde varken.

De effecten van epinine, dopamine en dobutamine in wakkere varkens

Met betrekking tot dopaminerge farmaca kan gesteld worden, dat ze effectief zijn in lage doseringen bij experimenteel hartfalen, alhoewel effecten op vullingsdruk ontbreken. De afwezigheid van DA_1-recptoren in de coronaire circulatie van biggen maken dit dier minder geschikt voor onderzoek naar effecten van dopaminerge stoffen op het myocard en het coronaire vaatbed.

List of Publications

1. P.D. Verdouw, D.J. Duncker, I.O.L. Schmeets, L.J. van Woerkens and P.R. Saxana. Changes in regional blood flows and myocardial performance after administration of bisoprolol in pigs. *Eur Heart J* 8 (suppl.M): 71-79, 1987.

2. D.J. Duncker, L.J. van Woerkens en P.D. Verdouw. Nisoldipine en myocardischemie: een onderzoek bij varkens met een vernauwing in een kransslagader. *Ned Tijdschrift voor Cardiologie* 3: 20-32, 1989.

3. D.J. Duncker, L.J. van Woerkens, P.W. Serruys, J.R.T.C. Roelandt, P.G. Hugenholtz and P.D. Verdouw. Actions of nisoldipine in cardiovascular disease. *Can J Cardiol* 5: 266-274, 1989.

4. G.J. van Daal, B. Lachmann, W. Schairer, R. Tenbrinck, L.J. van Woerkens, P.D. Verdouw and W. Erdmann. The influence of different anesthetics on the oxygen delivery to and consumption of the heart. *Advances in Experimental Medicine and Biology* 248: 527-532, 1989.

5. L.J. van Woerkens, B. Lachmann, G.J. van Daal, W. Schairer, R. Tenbrinck, P.D. Verdouw and W. Erdmann. Influences of different routinely used muscle relaxants on oxygen delivery to and oxygen consumption by the heart during xenon-anesthesia. *Advances in Experimental Medicine and Biology* 248: 673-678, 1989.

6. L.M.A. Sassen, J.M. Hartog, J.M.J. Lamers, M. Klompe, L.J. van Woerkens and P.D. Verdouw. Mackerel oil and atherosclerosis in pigs. *Eur Heart J* 10: 838-846, 1989.

7. W.J. van der Giessen, L.J. van Woerkens, D.J. Duncker, J.R.T.C. Roelandt and P.D. Verdouw. The acute hemodynamic effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure. *J Cardiovasc Pharmacol* 14: 653-658, 1989.

8. P.D. Verdouw, L.M.A. Sassen, J.M. Hartog, L.J. van Woerkens and J.M.J. Lamers. Intimal proliferation in coronary arteries of normolipidemic pigs with a fixed stenosis: The effects of fish oil. *Eur Heart J* 10(suppl.F): 168-172, 1989.

9. L.M.A. Sassen, Th.J.M. Heere, L.J. van Woerkens, P.R. Saxena and P.D. Verdouw. Nicorandil and cardiovascular performance in anaesthetized pigs with a concentric coronary artery stenosis. *Naunyn-Schmiedeberg's Arch Pharmacol* 340: 733-739, 1989.

10. W.J. van der Giessen, L.J. van Woerkens, P.R. Saxena, J.R.T.C. Roelandt and P.D. Verdouw. Nisoldipine improves blood flow to skeletal muscles in conscious pigs with chronic heart failure. *Eur Heart J* 11: 552-559, 1990.

11. W.J. van der Giessen, P.W. Serruys, L.J. van Woerkens, K.J. Beatt, P.J. Visser, J.F. Jongkind, R.H. van Bremen, E. Ridderhof, H. van Loon, L.K. Soei, H.M.M. van Beusekom and P.D. Verdouw. Arterial stenting with self expandable and balloon expandable endoprothesis. *Intinternal J of Cardiac Imaging* 5: 163-171, 1990.

12. L.J. van Woerkens, R.J. Huigen, D.J. Duncker, W.J. van der Giessen and P.D. Verdouw. Redistribution of cardiac output to opening of arteriovenous anatomoses by a combination of azaperone and metomidate. *Br J Anesth* 65: 393-399, 1990.

13. W.J. van der Giessen, L.J. van Woerkens, D.J. Duncker, J.R.T.C. Roelandt and P.D. Verdouw. The cardiovascular effects of nisoldipine and pimobendan in conscious pigs with chronic heart failure: a comparison between a vasodilator and an inodilator. In: Lichtlen P.R. and Krayenbühl. New aspacts on nisoldipine. Schattauer mbH, Stuttgart. 107-117, 1990.

14. L.J. van Woerkens, D.J.G.M. Duncker, W.J. van der Giessen and P.D. Verdouw. Redistribution of cardiac output by azaperone and metomidate in conscious pigs. *Eur J Pharmacol* 183: 2235, 1990.

15. W.J. van der Giessen, P.W. Serruys, H.M.M. Beusekom, L.J. van Woerkens, H. van Loon, L.K. Soei, B.H. Strauss, K.J. Beatt and P.D. Verdouw. Coronary stenting with a new , radiopaque, balloon-expandable endoprosthesis in pigs. *Circulation* 83: 1788-1798, 1991 .

16. L.J. van Woerkens, S.N. Schotman, W.J. van der Giessen and P.D. Verdouw. Cardiovascular effects of elgodipine in conscious pigs with a normal coronary circulation and in conscious pigs with a healed myocardial infarction. *J Cardiovasc Pharmacol* 17: 976-982, 1991.

17. L.J. van Woerkens, W.J. van der Giessen and P.D. Verdouw. Cardiovascular profile of 5 novel nitrate-esters: A comparitive study with nitroglycerin in conscious pigs with and without left ventricular dysfunction. *Br J Pharmacol* 104; 7-14, 1991.

18. L.J. van Woerkens, D.J. Duncker, M.O. den Boer, E.O. McFalls, L.M.A. Sassen, P.R. Saxena and P.D. Verdouw. Evidence agaonst dopamine D_1 receptors in the porcine myocardium. *Br J Pharmacol* 104; 246-250, 1991.

19. L.J. van Woerkens, W.J. van der Giessen and P.D. Verdouw. The selctive bradycardic effects of UL-FS 49 are not affected by left ventricular dysfunction; a study in conscious pigs with chronic coronary artery occlusion. *Cardiovasc Drugs and Ther* 1991 (in press).

20. L.J. van Woerkens, A. Man in 't Veld, W.J. van der Giessen, J. van Meegen, F. Boomsma and P.D. Verdouw. Effect of epinine on systemic hemodynamics and regional blood flows in conscious pigs. (submitted)

21. L.J. van Woerkens, N.R.A. Baas, W.J. van der Giessen and P.D. Verdouw. Cardiovascular effects of the novel potassium channnel opener bimakalim in conscious pigs with and without myocardial infarction. A comparitive study with nicorandil. (submitted)

22. L.J.van Woerkens, W.J. van der Giessen and P.D.Verdouw. Cardiovascular effects of dopamine and dobutamine in conscious pigs with chronic heart failure. (submitted)

23. L.J. van Woerkens, F. Boomsma, M.Bevers, A.J. Man in 't Veld and P.D. Verdouw. Comparison of the cardiovascular effects of epinine and dopamine in conscious pigs. (submitted)

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Curriculum vitae

De schrijver van dit proefschrift werd geboren op 23 juli 1962 te Rotterdam. In 1981 behaalde hij het diploma HAVO en in 1982 het diploma VWO aan het Emmaus College Rotterdam. In 1982 begon hij met de studie Geneeskunde aan de Erasmus Universiteit Rotterdam, waar hij in 1988 het doctoraalexamen behaalde. Vanaf 1986 vervulde hij een student-assistentschap op het Laboratorium voor Experimentele Cardiologie onder leiding van Prof. Dr. P.D. Verdouw. In 1988 trad hij als AIO in dienst van de Erasmus Universiteit Rotterdam om in het Thoraxcentrum, hoofd Prof. Dr. J.R.T.C. Roelandt, op het Laboratorium voor Experimentele Cardiologie onder leiding van Prof. Dr. P.D. Verdouw dit proefschrift te bewerken.