ASPECTS ON THE TREATMENT OF EXPERIMENTALLY INDUCED CORONARY ARTERY DISEASE

ASPECTEN VAN DE BEHANDELING VAN EXPERIMENTEEL OPGEWEKT CORONAIR VAATLIJDEN

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Aan mijn ouders,
en aan Maarten.....

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Chapter 1

General introduction and aim of the thesis

GENERAL INTRODUCTION

Coronary artery disease is caused by the systemic condition atherosclerosis and its ultimate consequence is ischemic heart disease. This disease is multifaceted not only in its origin but also in its pathological manifestations in anatomical, biochemical and physiological terms. Consequently, the treatment can also be looked upon in many different ways. In practice, the first choice of treatment of a patient with coronary artery disease is to prevent the development of ischemic heart disease by medicamentous means or surgical treatment. One can also attack the underlying coronary atherosclerosis itself, preferably before the manifestations of ischemic heart disease become overt, and one can even try to cause regression of established lesions.

Initiation and progression of atherosclerosis

According to the so-called "response-to-injury" hypothesis (Ross, 1986), the initial stage of the disease is characterized by some kind of injury to the endothelium, due to infections, trauma, high levels of LDL-C, hypertension, diabetes and/or smoking. The injurious event is followed by attachment of monocytes, which migrate into the intima, and are transformed into macrophages. During their differentiation into macrophages the cells acquire multiple metabolic activities contributing to the atherogenic process (e.g. the formation of the tumor necrosis factor, platelet-derived-growth-factor (Habenicht et al., 1984, 1985) and thromboxane (Goerig et al., 1987, 1988). Furthermore, circulating platelets adhere to the exposed connective tissue underlying the damaged endothelium and aggregate, which leads to release of platelet derived growth factor and thromboxane. The smooth muscle cells and the damaged endothelial cells also release growth factors contributing to the proliferative processes occurring in the newly formed lesion. The potent chemoattractants and mitogens cause migration of smooth muscle cells to the site of endothelial injury and proliferation. The primary proliferative lesions tend to regress and heal, and the arterial intima is histologically (but not necessarily functionally) restored to normal. However, when macrophages and smooth muscle cells ingest plasma lipids by means of the non-LDL-receptor scavenger pathway and become foam cells, the process of lesion formation becomes progressive. Because of the constant process of healing and remodelling of the plaque, the intimal surface remains thrombogenic, thereby sustaining the vicious cycle converting the early lesion (fatty streak) into advanced lesions consisting of intra- and extracellular fat deposits, collagen, necrotic material and calcium deposits.

Ca^{2+} antagonists and the progression of atherosclerosis. The rationale for using Ca^{2+} antagonists in the prevention or the treatment of atherosclerosis emerges from the significant role Ca^{2+} plays in many biological and pathophysiological processes that are

involved in atherosclerosis. Indeed, many different Ca^{2+} antagonists have been shown to prevent experimentally induced atherosclerosis (Blumlein et al., 1984; Willis et al., 1985; Sugano et al., 1986; Weinstein and Heider, 1987). These promising results already inspired researchers to start prospective clinical trials (MIDAS, Borhani, 1990; INTACT, Lichtlen et al., 1987; Jost et al., 1989; FIPS, Kober et al., 1989) in which the effect of this class of drug on the progression and regression of coronary artery disease is evaluated. In the animal experiments the antiatherosclerotic effect of Ca^{2+} antagonists seems to be independent of a blood pressure lowering action since at variance with verapamil, hydralazine appeared to ineffective as an antiatherogenic agent in cholesterol-fed rabbits (Blumlein et al., 1984). Some of the mechanisms that are held responsible for the antiatherogenic effects of Ca^{2+}-antagonists are listed in Table 1.

Table 1. *Some possible mechanisms for the antiatherosclerotic effects of Ca^{2+}-antagonists.*

↓	LDL cholesterol (Sugano et al., 1986)
↓	Smooth muscle cell proliferation (Nilsson et al., 1985; Saito et al., 1986)
↓	Platelet aggregation (Ono and Kimura, 1981; Mehta et al., 1983; Ware et al., 1986)
↑	Proliferation of LDL receptors (Stein et al., 1985)
↑	Lysosomal degradation due to enhanced lysosomal cholesteryl-ester hydrolase activity (Etingin and Hajjar, 1985)
↑	Production of matrix proteins such as collagen, elastin, glycosaminoglycan; Weinstein and Heider, 1987)

Regression of atherosclerosis

Atherosclerosis has been shown not only to be a preventable but also to be a reversible pathological process. Upon removal of the atherogenic stimulus, in primates regression begins within 4 weeks after serum cholesterol has returned to control levels (Stary and Strong, 1976; Stary, 1979) and most of the foam cells have disappeared by 16 weeks (Kokatnur et al., 1975). When the lesions are more severe than early fatty streak lesions, and contain both intra- and extracellularly abundant amounts of cholesterol, the loss of cholesterol is multiphasic with a rapid loss occurring within the first 6 months, correlating with the loss of foam cells, and a much slower removal rate of extracellular lipid in a later phase. In swine (Daoud et al., 1981) the time course of removal of cellular lipid is as rapid but the removal rate for extracellular lipid is much slower.

Necrotic material can be mobilized from the lesion during regression but extracellular accumulations of cholesterol can persist for many years, even under the most favorable conditions for regression (Clarkson et al., 1973). The mechanism responsible for the removal of extracellular debris and lipid from the arterial wall during regression could involve exiting of debris and lipid through lymphatic channels (Stary, 1978), or through phagocytosis by macrophages (Fritz et al., 1980; Daoud et al., 1981). Furthermore, the foam cells may migrate out of the plaque, carrying their load of lipid (Gerrity, 1981). Finally, cholesterol esterification and storage in macrophages may be followed by cholesterylester hydrolysis (Brown et al., 1980) and excretion in the presence of a suitable cholesterol acceptor (Ho et al., 1980) could also contribute. The fate of collagen in lesions during regression is still diverse; histologically there is an increase but whether this represents new synthesis or merely condensation of existing collagen remains unclear. With more complicated lesions it has been shown that collagen increases during regression (Wagner et al., 1980; Hollander et al., 1979), possibly due to increased synthesis (Langner et al., 1977). The accumulation of connective tissue can be so extensive that it can largely replace the space previously occupied by foam cells, resulting in the maintenance of intimal thickening (Clarkson et al., 1973; Hollander et al., 1979). In severe cases this increase in collagen can therefore even prevent a substantial decrease in intimal area with regression. Also Ca^{2+} has been shown to increase in the arterial wall (Daoud et al., 1981).

Fish oil and atherosclerosis

It was not until Bang et al. (1976) and Dyerberg et al. (1975) presented their epidemiological studies on the Eskimos that high intake of fish was associated with a lower mortality rate due to coronary artery disease. These studies initiated a number of experimental and clinical studies revealing that fish oil has potentially anti-atheromatous effects. Fish oil contains a considerable amount of n-3 fatty acids (e.g. eicosapentaenoic acid, EPA and docosahexaenoic acid, DHA), which are highly unsaturated fatty acids with the first double bond three atoms removed from the methyl end of the molecule (as compared to sixth atoms in the n-6 fatty acids). These n-3 fatty acids are synthesized by phytoplankton, then progress up in the food chain until they ultimately appear in cold-water fish, whale and seal, and are ingested by man. After ingestion of these fatty acids, they are rapidly incorporated into cell membranes (Hartog et al., 1986) and, by competing with the n-6 fatty acids (e.g. linoleic acid and arachidonic acid), they affect many biochemical reactions and thereby cellular functions (Lamers et al., 1986; Leaf and Weber, 1988).

Prostaglandin synthesis. At variance with linoleic acid, which serves as a cyclooxygenase substrate for the two-series of prostaglandins, EPA serves as a cyclooxygenase substrate for the three series of prostaglandin (Needleman et al., 1979; Fischer and Weber,

1983). However, the ability of TXA_3 to induce aggregation of platelets or constriction of blood vessels is less than that of TXA_2 (Needleman et al., 1979; Fischer and Weber, 1983). Furthermore, by competition of linoleic acid and EPA for the cyclooxygenase, the amount of TXA_2 is substantially reduced. An independent inhibitory effect of DHA on cyclooxygenase contributes as well (Goodnight, 1986). Similar effects of n-3 fatty acids on cyclooxygenase occur in cultured endothelial cells where PGI_3 is formed from EPA, which however has platelet-inhibitor and vasodilator potency, similar to PGI_2 (Fischer and Weber, 1984). It has also been shown that in the coronary venous blood the levels of PGI_2 and TXA_2 were lower in mackerel oil fed pigs than in lard fat fed pigs but that the decrease in PGI_2 was relatively less (Hartog et al., 1986).

Leukotriene synthesis. Through 5-lipoxygenase, arachidonic acid is metabolized to the 4-series of leukotrienes. Leukotrienes have a strong chemoattraction to circulating polymorphonuclear leukocytes and monocytes. These leukotrienes are involved in inflammatory, allergic and immune responses (Samuelsson, 1983), and in the vascular response to ischemia (Mullane et al., 1987). By competition of EPA and linoleic acid for the 5-lipoxygenase, fish oil also alters this leukotriene synthesis (Lee et al., 1985). Leukotriene B4 (LTB4) production is decreased, while LTB5 is formed by polymorphonuclear leukocytes (Strasser et al., 1985). The decreased chemotactic activity of neutrophils and reduced adhesion of neutrophils to vascular endothelium suggests that an alteration in leukotriene production is a potential mechanism by which n-3 fatty acids suppress inflammation.

Interleukin-1 and tumor necrosis factor production by monocytes. N-3 fatty acids are also incorporated into the cellular membranes of monocytes or macrophages resulting in a reduced production of interleukin-1 and tumor necrosis factor following stimulation of cells with endotoxin (Endres et al., 1987; Billiar et al., 1988). The reduction in the production of these cytokines may prove to be responsible for a reduction in inflammation and the propensity for thrombosis.

Lipoproteins. Although the earlier studies seemed promising, it is now generally agreed that the effects of fish oil on total and LDL-cholesterol (LDL-C) in humans are inconsistent (Harris, 1989). Either reduction or elevation of the levels of LDL-C have been found. The most obvious factor contributing to this variability in response is the dose given and the type of patient being studied. The choices of the control diet contributes too, especially those containing more saturated fat than present in the fish oil diet used. Another factor could be the differences in the EPA/DHA ratio. Besides the level of LDL-C, the atherogenicity of the lipoprotein particles may also be influenced by n-3 fatty acids. It has been demonstrated that due to increased density and reduced size of the lipoprotein particles, the transition temperature of its lipids is lowered, which condition may be associated with lowered atherogenicity (Glick et al., 1983; Kim et al., 1989; Parks and Bullock, 1987).

It has invariably been shown that n-3 fatty acids cause a marked decreases in the

level of triglycerides, already at low doses (Harris, 1989). This effect may be mediated by suppression of both hepatic triglyceride and apolipoprotein B synthesis (Nestel et al., 1984; Groot et al., 1989).

HDL-C levels normally increase with low doses of fish oil, but decrease after high doses (Illingworth et al., 1984; Hartog et al., 1987a, 1987b; Harris et al., 1988).
An important adverse effect of high intake of fish oil is the potential risk of antioxidant deficiency, ultimately leading to tissue damage. In pigs this can lead to the so-called yellow fat disease (Ruiter et al., 1978). The addition of natural antioxidants like selenium, vitamin E and C does not always lead to complete alleviation of this antioxidant deficiency (Lamers et al., 1988).

Animal models in the study of atherosclerosis

Risk factors for human atherosclerosis, in particular hyperlipidemia, have provided guidelines for the induction of atherosclerosis in the experimental animal. A schematic representation of the features of commonly used animal models of atherosclerosis is shown in Figure 1. In this thesis the domestic pig was used as the experimental animal.

Swine: Swine spontaneously develop atherosclerotic lesions in the abdominal aorta, coronary and cerebral arteries and other arterial sites (Luginbühl et al., 1965) from 6 months of age onwards. The lesions start as "musculoelastic thickening" (smooth muscle cells, elastic fibers and collagen within the intima) which progress into "elastic-hyperplastic" thickenings where intra- and extracellular fat appears but there is no evidence of complicated features like hemorrhage or ulceration. These naturally occurring lesions in pigs are said to closely resemble early lesions in human beings (Luginbühl et al., 1965). A marked exacerbation of atherosclerosis can be achieved by feeding a diet containing 2-3% cholesterol, which after 6-7 months causes a 10-20 % abdominal aortic coverage with raised lesions (Florentin et al., 1968). Morphologically, aortic and coronary artery lesions are similar. Cellular lesions, composed principally of lipid-filled cells, and true atheromatous lesions are present. In their most severe form the atheromatous lesions have both necrosis and calcification, but even after one year of cholesterol feeding, there is no thrombosis or myocardial infarction. The latter can be provoked when combining the hypercholesterolemia with some type of injury (Nam et al., 1973; Lee and Lee, 1975) such as balloon-catheter injury (Baumgarter and Spact, 1970) or X-irradiation. The synergistic effect of cholesterol feeding and endothelial injury results in the development of severe atheromatous lesions with large areas of necrosis, calcification, extracellular lipid, cholesterol crystals, foam cells, hemorrhage, surface ulceration and thrombus formation. In addition to the similarity of the location and the morphology of the atherosclerotic lesions the platelet and coagulation systems in pigs are also very similar to those in humans (Leach and Thornburn, 1982).

	Rabbit	Pigeon	Rat	Dog	Swine	Squirrel Monkey	Rhesus Monkey and other comparable macaques	Human
Spontaneous disease	±	++	–	–	+	++	–	+
Sensitivity to induced dietary disease	++++	++++	+	+	++	++++	++++	+++
Distribution in aorta								
Usual microscopic lesion								
Small artery involvement	++++	++++	+++	+	++	++	+	+
Loading of RES[1]	++++	+++	–	–	–	–	–	–

Figure 1 *Diagrammatic representation of some of the most notable variables in the responses of commonly used animal models which have been studied and reported. Aortic drawing: Parallel lines represent diffuse lesions, less severe than those indicated by fine stippling or focal plaques. Microscopic drawing: represents an attempt to differentiate between foam cell lesions (little round circles) and predominantly smooth muscle cell lesions (ellipsoid) with some of them showing a necrotic centre of the atheroma.* [1]*RES = Reticuloendothelial System. Adapted from the Annals of the New York Academy of Science, 1985, volume 454, Atherosclerosis ed. KT Lee. A new way to look at atherosclerotic involvement of the artery wall and the functional effects, by RW Wissler et al.*

Myocardial ischemia

Myocardial ischemia is caused by a disturbance of the balance between oxygen supply and demand. Decreasing oxygen supply by a superimposed spasm and/or thrombus on a plaque or increasing oxygen demand during exercise in a patient with coronary atherosclerosis can cause this imbalance. Myocardial oxygen demand is determined by the energy, required for basal metabolism, electrical activation (together 25%, Bonhoeffer, 1967) and for mechanical work (75%, Klocke et al., 1966). The three major determinants of myocardial work and therefore oxygen demand are heart rate, myocardial wall tension and the intrinsic contractile state of the myocardium, the two latter being represented by left ventricular volume or pressure and maximal rate of increase in systolic pressure ($LVdP/dt_{max}$). The subendocardium requires approximately 10-20% more energy relative to the subepicardium (Monroe et al., 1975; Weiss, 1979) since subendocardial wall tension exceed that of the subepicardium (Kirk and Honig, 1964; Baird and Ameli, 1971). Myocardial oxygen supply is generally determined by the local rate of capillary perfusion and the amount of oxygen extraction from the blood which is high (75%) under normal conditions and can increase to 90% in extreme situations. Since this increment is by far not enough to meet the changes in metabolic demand with varying myocardial work load, oxygen supply is mainly regulated by changes in local blood flow. Coronary blood flow is therefore tightly matched to myocardial oxygen needs: metabolic regulation of coronary blood flow, which is regulated by control of resistance of the capillary arterioles and of which either adenosine-receptor interaction (Berne, 1980) or K^+-movements (Belloni, 1979) may form the metabolic basis. Another process in regulating coronary blood flow is autoregulation, which is the capability of the myocardium to maintain constant coronary flow, despite variation in coronary perfusion pressure. This phenomenon is, however, limited: when maximal vasodilation has been achieved the microvasculature becomes dependent on the perfusion pressure, counterbalanced by extravascular compressive force (Archie, 1978). Arteriolar pressure is dependent on aortic pressure and the resistance across the large epicardial vessels. In Table 2 some of the cellular functions that can influence the balance between myocardial oxygen supply and demand, but also some intracellular biochemical processes that are generally believed to be involved in the development of myocardial cell damage, are presented. Furthermore, the potential beneficial actions of the classes of drugs, investigated in this thesis, are indicated.

Drugs in the treatment of myocardial ischemia

Ca^{2+}-antagonists.Ca^{2+}-antagonists are drugs that specifically inhibit the influx of Ca^{2+} through L-type Ca^{2+}-channels and receptor operated channels into both the myocardial and vascular smooth muscle cells (Fleckenstein, 1967) and therefore partially block the

excitation-contraction coupling in heart and smooth muscle. The various Ca^{2+}-antagonists differ in terms of their inhibitory effect on tension development in vascular relative to cardiac muscle, but generally the sensitivity of vascular smooth muscle outweighs that of cardiac muscle. Coronary vasodilation results in augmentation of the supply of oxygen and substrate to the area at risk and acceleration of the removal of H^+ and other byproducts of anaerobic glycolysis. The drugs also cause arterial vasodilation which may decrease myocardial oxygen demand. However, the use of arterial vasodilators during ischemia is not always beneficial since the resulting decrease in blood pressure may cause a reflex-mediated cardiostimulation (Warltier et al., 1984; Duncker et al., 1987a). This, in combination with the decreased perfusion pressure may be detrimental for myocardium, which, as a result of the coronary artery stenosis, is totally dependent on the perfusion pressure due to maximal vasodilation. The need for combination therapy with beta-blockers (Dargie et al., 1981; Fox et al., 1981) then emerges. However, some Ca^{2+}-antagonists have also proven to directly reduce heart rate, by which mechanism they not only decrease myocardial oxygen demand but may even increase myocardial oxygen supply by increasing diastole and thus coronary perfusion time. The specificity for coronary arteries relative to peripheral arteries is also important in the efficacy of the Ca^{2+}-antagonist as an ani-ischemic drug.

Beta-adrenoceptor antagonists: These drugs block the continuous, excitatory sympathetic influences on the heart leading to changes in cardiac function even during the basal state. Moreover, the response of the heart to exercise or other stress is markedly decreased and the increases in heart rate and cardiac output do not occur. Beta-adrenoceptor antagonists therefore reduce myocardial oxygen demand by decreasing contractility of the myocardium and by decreasing heart rate. The latter also results in a longer duration of diastole, increasing perfusion time of the myocardium, thereby increasing oxygen supply.

Nitrates: Nitrates act beneficially in myocardial ischemia especially by reducing preload, and to some extent, afterload (Williams et al., 1965; Burggraff and Parker, 1974). Consequently, ventricular dimension and wall tension are reduced, which results in decreased oxygen demand. By reducing the intraventricular diastolic pressure, nitrates improve the transmural distribution of myocardial perfusion by reducing extrinsic diastolic compression of the subendocardial vessels. Nitrates do not appear to dilate the coronary arteriolar resistance bed, but do cause vasodilation of diseased and undiseased epicardial vessels (Brown et al., 1981).

K^+ channel activators: K^+ channel activators are a new type of drugs that increase the conductance of the ATP-dependent K^+ channels and therefore cause hyperpolarization of the cell membrane (Weir and Weston, 1986), which reduces the Ca^{2+}-influx and thereby attenuates contractility in heart and smooth muscle (Hamilton et al., 1986; Cook, 1988). At variance with the usual nomenclature, for the ATP-dependent K^+ channels, ATP-dependency implies that opening of the channel is inhibited by

Table 2 *Some potential mechanisms of action of compounds, investigated in this thesis, leading to preservation of jeopardized myocytes in ischemic myocardium.*

INCREASE OF OXYGEN AND SUBSTRATE SUPPLY

Coronary vasodilation *(nitrates, K^+ch act, L-pr c?)*
↑ Perfusion pressure
↑ Collateral flow *(K^+ch act, Ca^{2+}-ant)*
↓ Platelet aggregation *(Ca^{2+}-ant)*
↓ Endothelial swelling *(L-pr c)*
↓ Endothelial deformation *(Ca^{2+}-ant)*
↓ Formation interendothelial gaps *(Ca^{2+}-ant)*
↓ Microvascular damage or obstruction *(L-pr c, Ca^{2+}-ant)*
↓ Heart rate *(ß-bl, some Ca^{2+}-ant)*

DECREASE OF OXYGEN AND SUBSTRATE DEMAND

↓ Preload *(nitrates, K^+ch act)*
↓ Afterload *(nitrates, K^+ch act. Ca^{2+}-ant,L-pr c?)*
↓ Contractility *(Ca^{2+}-antß-bl, K ch act)*
↓ Heart rate *(ß-bl, some Ca^{2+}-ant)*

INTRACELLULAR ACTIONS

↑ Cell membrane integrity *(L-pr c)*
↓ Osmotic load / cell swelling *(ß-bl, Ca^{2+}-ant)*
↓ Ca^{2+} overload during reperfusion *(Ca^{2+}-ant,K^+ch act?)*
↑ Ca^{2+} pump-activity sarcoplasmic reticulum *(Ca^{2+}-ant)*
↓ Accumulation of lipid intermediates *(L-pr c)*
↓ Phospholipid breakdown sarcolemma *(Ca^{2+}-ant)*
↓ Substrate and O_2-utilization

Ca^{2+}-ant= Ca^{2+}-antagonistß-bl = beta-adrenoceptor antagonist; K^+ch act = potassium channel activator; L pr c = L-propionylcarnitine

physiological levels of ATP. The physiologic role of the ATP-dependent K^+ channels is ambiguous but they have been shown to play a role the hypoxia-induced coronary vasodilation (Daut et al., 1990). K^+ channel activation results in predominantly arterial vasodilation (Clapham and Buckingham, 1988; Cook and Hof, 1988; Gotanda et al., 1989). Moreover, evidence has been presented that coronary vasodilation precedes systemic vasodilation in collateral-dependent myocardium of the dog (Maruyama et al., 1989), which seems promising with respect to the coronary selectivity of this type of drugs. However, data are lacking on the effect of K^+ channel activators on the large epicardial coronary arteries, which is critical for their anti-ischemic potential in patient without collaterals. K^+ channel activators can possibly also have direct anti-ischemic effects independent of their vasodilatory action (Grover et al., 1989, 1990). The increased K^+ flux may result in some degree of hyperpolarization to counteract the depolarizing effects associated with hypoxia or ischemia. The resulting reduction in Ca^{2+} influx through voltage-operated channels and the relatively decreased energy consumption could both result in a preservation of cardiac tissue (Noma and Shibasaki, 1988).

L-propionylcarnitine: L-propionylcarnitine is a natural derivative of L-carnitine and does not have pronounced systemic hemodynamic effects, although it has been suggested that it can exert a slight positive inotropic action (Paulson et al., 1986; Liedtke et al., 1988). The compound has been shown to protect ischemic myocardium in the isolated working rat heart preparation (Paulson et al., 1986) but also reversed stunning in the anesthetized pig (Liedtke et al., 1988). The beneficial effects during myocardial ischemia appear to occur at the cellular level. The effects seem analogous to L-carnitine, which is an essential cofactor in the transfer of activated long-chain fatty acids and repletes the ischemia-induced loss in L-carnitine. Furthermore, by converting them to acylcarnitine, L-carnitine is capable of attenuating the harmful accumulation of long-chain acylCoA. Indeed, L-propionylcarnitine stimulates the fatty acid oxidation. The propionate moiety in L-propionylcarnitine can deliver anaplerotic fuel to the Krebs-cycle, which also contributes in stimulating glucose and fatty acid oxidation.

In vivo animal models of myocardial ischemia

The ideal animal model, mimicking most closely the human situation, would be an animal with coronary atherosclerosis, in which the balance between myocardial oxygen supply and demand is disturbed by inducing a spasm and/or a thrombus at the site of an atherosclerotic plaque, possibly in combination with exercise, thereby causing myocardial ischemia. Since it is too costly to use this type of model only for the study of anti-ischemic drugs and because the model is too variable with respect to the type (concentric versus eccentric stenosis) and degree of the stenosis, several models have been developed to mimic the events causing and associated with ischemic heart disease.

Species: The choice of the species being used in the study of the treatment of myocardial ischemia greatly depends on the questions being asked. Pigs (Fedor et al., 1978; Verdouw et al., 1983; McDonough et al., 1984) and non-human primates (Weisse et al., 1976; Crozatier et al., 1978), like humans, have very few collateral anastomoses and therefore an acute occlusion causes severe transmural ischemia, imitating the younger patient with acute myocardial infarction without preceding ischemic events.

Conscious animals: Inducing myocardial ischemia in conscious animals gives the opportunity to investigate the consequences of myocardial ischemia in the setting of intact coronary physiology, including sympathetic and parasympathetic reflexes. The high cost in time, limit the use of such models. Another way of inducing myocardial ischemia is by catheterization, which however requires fluoroscopic equipment. One of the advantages is that damage to the coronary vessel and adjacent nerves is avoided. In conscious animals the analgesics, needed to minimize discomfort, may however also blur the cardiovascular effects of the investigated drugs.

Anesthetized animals: Anesthesia and the open-thorax-condition can change the pathophysiologic responses to ischemic events and the cardiovascular responses to drugs, but are sometimes needed to answer questions that require more extensive and advanced techniques and measurements. In the present thesis myocardial ischemia is induced by inflating a balloon, that is placed around the coronary artery of the anesthetized pig. The partial occlusion causes severe ischemia in the porcine heart with few collaterals and can be relieved at any desired moment; the drug-induced changes during reperfusion can therefore also be studied. The results of those studies are most precise if onset, severity and duration of the ischemic injury are clearly defined.

Aim of this thesis

In this thesis some therapeutic aspects of experimentally induced coronary artery disease are being highlighted. In chapter 2 the effects of the Ca^{2+} antagonist diltiazem on the progression of coronary and aortic atherosclerosis in pigs is being studied. So far, studies on the anti-atherosclerotic effects of this drug (Ginsburg et al., 1983; Naito et al., 1984; Sugano et al., 1986; Dicciani et al., 1987; Sugano et al., 1988) have only been performed in the hypercholesterolemic rabbit and in only one study (Ginsburg et al., 1983) also the effect on coronary atherosclerosis was investigated. However, hypercholesterolemic rabbits develop atherosclerosis in the small intramyocardial branches rather than in the large epicardial coronary arteries, as observed in man and in swine. Furthermore, in these studies the doses used were so high that they would never be tolerated by man.

Besides a medicamentous regimen, dietary intervention may also present a possible

way of dealing with atherosclerosis. In chapter 3 the regression of porcine atherosclerotic lesions and the effects of dietary fish oil on this process have been investigated.

Myocardial ischemia has been mimicked in anesthetized pigs by creating a concentric stenosis by inflating a balloon placed around the vessel. Several therapeutic approaches have been explored (chapters 4, 6, 8 and 10). The effect of a low dose of the Ca^{2+} antagonist nisoldipine (chapter 4) and L-propionylcarnitine (chapter 6) on ischemic and postischemic blood flow and function have been investigated. Furthermore, an attempt has been made to predict long term outcome of stunned myocardium by means of two markers for long term recovery: the post systolic wall thickening (Takayama et al., 1988) and Ca^{2+} uptake and phospholamban phosphorylation of the sarcoplasmic reticulum (Schoutsen et al., 1989).

The reflex-mediated tachycardia, as observed in conscious (Duncker et al., 1987a), but also in anesthetized animals (Duncker et al., 1986) may, in myocardial ischemia, render a potential detrimental effect of vasodilators such as the Ca^{2+}-antagonists. In chapter 5 the cardiovascular profile of the newly developed Ca^{2+}-antagonists elgodipine has been surveyed.

In chapter 7 the global and regional cardiovascular effects of nicorandil were investigated while in chapter 8 the drug was tested for its anti-ischemic potential. As indicated, nicorandil is a compound with nitrate-like actions, but it has also been shown that the cardiac effects and possibly in part the vasodilating property, are mediated by K^+ channel activation. In chapter 9 we investigated the cardiovascular effects of a selective K^+ channel activator for comparison with those of nicorandil.

Despite new developments, ß-adrenoceptor-antagonists remain important for the treatment of myocardial ischemia. In this respect the cardioselective type of ß-adrenergic antagonists are of particular interest. One such drug is bisoprolol (Harting et al., 1986), a compound that has been shown to exert pronounced cardiovascular actions at doses considerably lower than required to inhibit the isoproterenol-induced increases in heart rate and contractility (Duncker et al., 1987b). In chapter 10 its effects on contractile function and myocardial flow in ischemic porcine myocardium are described.

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Chapter 2

Failure of diltiazem to suppress cholesterol-induced atherogenesis of endothelium-denudated arteries in pigs

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Summary

To investigate the effect of diltiazem on the development of atherosclerosis, 15 pigs were fed a fat-poor basal diet to which 8% (w/w) lard fat and 2% (w/w) cholesterol were added for 8 months. To enhance the formation of atherosclerotic plaques endothelium of the aorta and the left anterior descending coronary artery was removed after 1 month and 0.5% (w/w) bile acids were added to the diet after 3 months. Seven animals served as control, while 8 animals also received diltiazem (the first 2 months 10 mg/kg twice daily and during the remainder of the dietary period 5 mg/kg twice daily). The diet-induced increases in plasma level of total cholesterol were not affected by diltiazem. Triglyceride levels did not change in the control group but decreased significantly in the diltiazem-treated animals. Collagen-induced (1 μg/ml) platelet aggregation was increased by diltiazem. The sum of free and esterified cholesterol was increased in the lesions of the aortic wall in the diltiazem-treated animals (9.8 ± 1.3 μg/g wet weight vs. 6.3 ± 1.0 μmol/g wet weight in the untreated animals), but coverage of the aorta with sudanophilic lesions was similar for both groups (40 ± 4% for the treated and 34 ± 9% for the control animals). Narrowing of the previously abraded coronary arteries was similar for the diltiazem-treated (median 7.1%, ranges 2.6–29.0%) and the control group (median 10.0%, ranges 2.3–24.1%). It is concluded that the dose range of diltiazem of 5–10 mg/kg twice daily, which is close to that used in the clinical setting, had no effect on the experimentally induced atherogenesis in pigs.

Key words: Atherosclerosis; Ca^{2+} entry blocker; Platelet aggregation; Blood lipids; Coronary intimal thickening; Aortic lipids; Swine

Introduction

Since Blumenthal et al. [1] pointed out that in the human aorta medial calcification always pre-

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ceded the development of intimal plaques, the role of calcium, both within and outside the arterial wall, in provoking atherogenesis has received wide attention [2–6]. Wartman et al. already noted that a divalent cation chelating agent suppressed plaque formation in cholesterol-fed rabbits [7]. Drugs which specifically block the calcium-influx into the cell can potentially retard the development of atherosclerotic plaques because several factors (arterial blood pressure, heart rate, smooth muscle cell migration and proliferation, platelet-vessel wall interaction, endothelial cell contraction, platelet aggregation, receptor-mediated endocytosis of LDL by aortic cells) involved in atherogenesis are modulated by Ca^{2+} influx [8,9]. Indeed, a number of studies employing nifedipine, verapamil and diltiazem reported a beneficial effect on the development of atherosclerosis in hypercholesterolemic rabbits [10–14]. On the other hand, some negative reports exist as well [15–19].

Considerable criticism has been raised regarding the suitability of the rabbit in experimental atherogenesis [9,22,23]. In particular the excessive plasma levels of cholesterol (30–50 mmol/l) needed for the formation of the plaques and the location of these plaques in the arterial tree have been widely discussed. As opposed to man, rabbits develop lesions in the intramural rather than in the large epicardial coronary arteries. Therefore to date no data are available on the effects of drugs on the formation of atherosclerotic plaques in the coronary vessels which are most relevant for the development of ischemic heart disease in man [23–26]. Domestic pigs on atherogenic diets develop lesions at cholesterol levels and at locations similar to those found in man [23].

In view of the controversial results with diltiazem in rabbits [11,13–15,19], we studied its possible anti-atherosclerotic actions in pigs fed an atherogenic diet. Since the effects on lesions in the aorta are not necessarily accompanied by similar effects on lesions in the coronary arteries [13,26] we studied both vascular beds. The dose of diltiazem used was close to the one that is clinically relevant.

Materials and methods

Induction of atherosclerosis

Young castrated male cross-bred (Yorkshire × Landrace) pigs ($n = 21$, 7–10 kg) were fed a low fat (< 2%, w/w) basal diet (Hope Farms, B.V., Woerden, The Netherlands) to which 8% (w/w) lard fat and 2% (w/w) cholesterol (GEBRO, Smilde, B.V., Heerenveen, The Netherlands) were added. The animals were fed twice a day and the portions were consumed within 1 h. Initially the portions were 200 g daily but, since the animals gain weight, the portions were gradually increased to 1500 g daily (in the 8th month). No diarrhea occurred during the experimental feeding period.

At 1 month the overnight fasted pigs were pretreated with 300 000 U procaine penicillin-G and 300 000 U benzathin penicillin-G (Duplocilline®, Gist Brocades, Delft, The Netherlands). After the animals were anesthetized with 1% halothane, they were intubated and connected to a respirator for artificial ventilation with a mixture of oxygen and nitrous oxide (1 : 2). After an incision was made to expose the left carotid artery, a 4 F Fogarty balloon catheter (American Edwards Laboratory, Santa Ana, CA, U.S.A.) was introduced and passed into the left anterior descending coronary artery under fluoroscopy. The balloon was inflated with 0.7 ml air, pulled back and deflated. This procedure was repeated twice. Then a 7 F Fogarty catheter was introduced and passed into the abdominal aorta until the iliac bifurcation. The balloon was inflated with 3 ml air and pulled back quickly. This aortic endothelial denudation was also repeated twice. After these procedures the animals were allowed to recover. In the following 24 h 6 of the 21 piglets died suddenly. Two months later bile acids (0.5%, w/w) were added to the diet to further raise the plasma level of cholesterol.

Dose and plasma levels of diltiazem

From the start of the dietary period 11 animals were randomly assigned to treatment with 10 mg/kg of diltiazem twice daily. After 2 months plasma levels of diltiazem in these animals were 1089 ± 140 μg/l (therapeutic levels in man 50–200 μg/l). The dose of diltiazem was lowered to 5 mg/kg twice daily. At 5 months the plasma levels of diltiazem were 122 ± 40 μg/l.

Platelet aggregation and lipids

After 0, 1, 2, 4, 6 and 8 months the animals were fasted for 24 h, the subclavian vein punctured and blood samples drawn for the measure-

ment of total cholesterol, HDL-cholesterol and triglycerides [27]. ADP (1 μmol/l) and collagen (1 μg/ml) induced platelet aggregation in whole blood was measured with an electrical aggregometer [28] and the number of platelets was estimated after 1 month and at the end of the dietary period.

Lipid infiltration of aortic wall

After 8 months the animals were killed with an overdose of pentobarbitone sodium and the aorta, from the level of the aortic valve down to the iliac bifurcation, was removed, dissected free of adventitia and split longitudinally. Intima-media preparations were excised from the macroscopically visible fatty streaks in the abdominal part of the aorta. In these samples lipid extraction was performed using a slightly modified version of the method described by Bligh and Dyer [29]. Briefly, the sample (40–200 mg) was homogenized in $CHCl_3/CH_3OH/H_2O$ (4 : 10 : 5, v/v) with a Polytron PT 10 (half setting). After centrifugation at $1550 \times g_{max}$ for 5 min and washing the pellet by homogenization in 1.9 ml of the same medium, the 2 supernatants were combined and supplemented with 1.5 ml $CHCl_3$ and 1.5 ml H_2O. After mixing and centrifugation, the upper phase was discarded and the remaining solid material dissolved by dropwise addition of methanol. Subsequently, the mixture was dried under N_2 at 37 °C and the residue dissolved in 0.2 ml 2-propanol. Phospholipid (PAP 150 of Biomérieux, Cherbourieres, Les Bains, France), cholesterol, cholesterol ester (CHOD-PAP, Boehringer, Mannheim, F.R.G.) and triglycerides (GPO-PAP, Boehringer, Mannheim, F.R.G.) were measured enzymatically in the organic extract.

Sudanophilia of the aortic wall

Subsequently the aorta was stained for lipid using Sudan IV [30]. Sudanophilia of the aorta was determined by projecting the aortic image onto a monitor and using an integrated image analysis system (IBAS-2000, Kontron, Oberkochen, F.R.G.) the area of the Sudan-positive surface was calculated. Since the aorta had to be cut longitudinally for these measurements we could not determine the intimal thickening of this vessel as this requires transversal slides.

Intimal thickening of the coronary arteries

The hearts were excised and the coronary arteries dissected free. Every 10 mm, transversal sections were cut from the left anterior descending coronary artery, the left circumflex coronary artery and the right coronary artery. The sections were stained routinely with haematoxylin–azophloxine and resorcin–fuchsin (RF) after paraffin sections were made. The RF stain colours elastin black and the background yellow, allowing an easy discrimination of the internal elastic lamina, which is necessary for the determination of the intimal thickening. The latter was measured using a computer-assisted morphometric analysis technique [25,31].

Statistical analysis

Data are presented as mean ± SEM, except for the data on luminal encroachment of the coronary arteries. These data were not equally distributed and have therefore been expressed as the median with the ranges. The Student *t*-test was used for determining the statistical significance of the differences. For the data on luminal encroachment the nonparametric Mann-Whitney U-test was used and confidence intervals were calculated for the difference in medians [32]. Statistical significance was accepted for $P < 0.05$ (two-tailed).

Results

Because 6 animals died suddenly after the endothelial denudation procedure, 7 animals of the control group and 8 animals of the diltiazem-treated group completed the study. During the dietary period the control animals increased their weight to 64 ± 5 kg and the diltiazem-treated animals to 70 ± 2 kg ($P < 0.05$).

Blood lipids

In the control group plasma levels of total cholesterol increased gradually from 2.24 ± 0.20 to 4.21 ± 0.33 mmol/l during the first 2 months of high cholesterol feeding (Fig. 1). Previously we have demonstrated that plasma cholesterol levels do not further change after this time [26]. To further increase the level of plasma cholesterol, bile acids were supplemented to the diets [25] after 3 months. The addition of the bile acids led to a

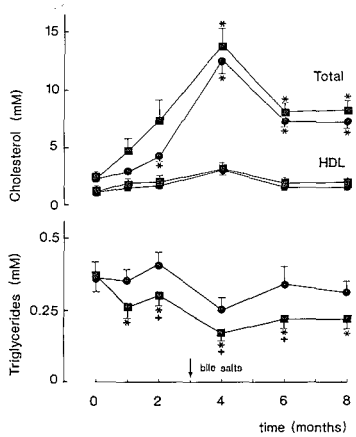

Fig. 1. Effect of diltiazem on the plasma levels of total cholesterol, HDL-cholesterol and triglycerides in pigs fed a high cholesterol diet for 8 months to which bile acids were added during the last 5 months. ■, diltiazem-treated group; ●, control group. * $P < 0.05$ vs. $t = 0$; $^{+}$ $P < 0.05$ diltiazem treated vs. control group.

further increase of plasma cholesterol to 12.7 ± 1.1 mmol/l (Fig. 1). During the last months plasma levels of total cholesterol remained at 7 mmol/l. Diltiazem had no effect on the diet-induced increases in total cholesterol (Fig. 1). HDL-cholesterol was not affected in either group. Triglyceride levels did not change in the control group but decreased to 60% of the pre-dietary value in the animals which also received diltiazem (Fig. 1).

Platelet aggregation

The diet had no effect on platelet aggregability as the ADP (1 μmol/l)- and collagen (1 μg/ml)-induced platelet aggregation and the number of platelets in the control group were similar after 1 and 8 months (Table 1). Diltiazem had no effect on the ADP-induced, but significantly increased the collagen-induced platelet aggregation (Table 1).

TABLE 1

EFFECT OF DILTIAZEM ON THE NUMBER OF PLATELETS AND THE ADP- AND COLLAGEN-INDUCED PLATELET AGGREGATION IN PIGS FED A HIGH CHOLESTEROL DIET FOR 8 MONTHS TO WHICH BILE ACIDS WERE ADDED DURING THE LAST 5 MONTHS

	1 month		8 months	
	Control ($n = 5$)	Diltiazem ($n = 6$)	Control ($n = 5$)	Diltiazem ($n = 6$)
Thrombocytes ($\times 10^3$/l)	294 ± 25	300 ± 39	271 ± 48	318 ± 42
Platelet aggregation (Ω)				
ADP-induced (1 μmol/l)	33 ± 3	39 ± 3	27 ± 7	35 ± 5
collagen (1 μg/ml)	34 ± 3	33 ± 1	33 ± 2	45 ± 4 *

* $P < 0.05$ vs. 1 month. Platelet aggregation was measured with an electrical aggregometer [28], and an increase in platelet aggregation was measured as an increase in resistance (Ω).

TABLE 2

EFFECT OF DILTIAZEM ON LIPID CONTENT OF THE NON-ABRADED (ASCENDING) AND ABRADED (ABDOMINAL) AORTA OF PIGS FED A HIGH CHOLESTEROL DIET FOR 8 MONTHS TO WHICH BILE ACIDS WERE ADDED DURING THE LAST 5 MONTHS

	Ascending aorta		Abdominal aorta	
	Control ($n = 7$)	Diltiazem ($n = 7$)	Control ($n = 7$)	Diltiazem ($n = 7$)
Cholesterol [a] (μmol/g wet weight)	3.8 ± 0.4	4.4 ± 0.5	6.3 ± 1.0 *	9.8 ± 1.3 *$^{+}$
Triglycerides (μmol/g wet weight)	0.7 ± 0.3	0.8 ± 0.2	0.9 ± 0.4	2.4 ± 0.9 *
Phospholipids (μmol/g wet weight)	2.7 ± 0.3	2.8 ± 0.3	4.0 ± 0.5 *	4.7 ± 0.5 *

[a] Cholesterol represents the sum of cholesterol ester and free cholesterol content of the vessel wall. * $P < 0.05$ vs. ascending aorta; $^{+}$ $P < 0.05$ vs. control.

TABLE 3

EFFECT OF DILTIAZEM ON THE MEAN LUMINAL ENCROACHMENT OF THE ABRADED AND NON-ABRADED CORONARY ARTERIES OF PIGS FED A HIGH-CHOLESTEROL DIET FOR 8 MONTHS TO WHICH BILE ACIDS WERE ADDED DURING THE LAST 5 MONTHS

	Control ($n = 7$)	Diltiazem ($n = 8$)	P	95% confidence interval of difference * in medians
LADCA (%) abraded	10.0 (2.3–24.1)	7.1 (2.6–29.0)	0.87	−15.4/8.4
LCXCA (%) non-abraded	1.5 (0.1–14.2)	4.4 (1.1–10.9)	0.09	−6.4/0.3
RCA (%) non-abraded	2.0 (0.7– 7.8)	5.2 (0.4–10.4)	0.07	−8.0/1.1

Data given are medians. Ranges are given in parentheses; *, control − diltiazem; LADCA: left anterior descending coronary artery; LCXCA: left circumflex coronary artery; RCA: right coronary artery.

Lipid infiltration of the aortic wall

In both the control and the diltiazem-treated animals there were no macroscopically visible lesions in the non-abraded ascending aorta. Diltiazem had no effect on lipid content of samples from this part of the aorta (Table 2). In both the control and diltiazem-treated animals, free plus esterified cholesterol and phospholipid contents in the previously abraded abdominal aorta were higher than in the ascending aorta. In the diltiazem-treated animals the sum of free and esterified cholesterol of the abraded aorta was higher than that of the control animals ($P < 0.05$).

Sudanophilia of the aortic wall

Coverage of the intimal surface with Sudan-positive lesions was $34 \pm 9\%$ in the 7 control animals and $40 \pm 4\%$ in the 8 diltiazem-treated animals ($P > 0.05$).

Intimal thickening of the coronary arteries

Mean luminal encroachment in the abraded left anterior descending coronary artery in the control group was significantly higher than in the non-abraded left circumflex and right coronary arteries (Table 3). Diltiazem treatment did not suppress atherogenesis of the abraded vessels and even tended to increase intimal thickening in the non-abraded coronary arteries, but levels of significance were not reached (Table 3).

Discussion

In the present study we failed to demonstrate an effect of diltiazem on intimal thickening of coronary arteries, which had similar mean cross sectional areas (e.g., 1.1 ± 0.2 mm^2 for the LADCA of both groups). It must be noted, however, that diltiazem exerts a mild vasodilatory action on the epicardial arteries and that the diameter of the arteries may have been larger in vivo than in vitro after immersion fixation. This would result in lower values for intimal thickening, but does not imply that the development of atherosclerosis would have been less in the diltiazem-treated animals. Additionally, no beneficial effect of the drug was seen on lipid infiltration and sudanophilia of the abdominal aorta. Intimal thickening of the abdominal aorta could not be determined reliably because of reasons described before (see Methods, section on sudanophilia of the aorta) and we can therefore not be entirely sure that diltiazem had not affected intimal thickening of this vessel.

In trying to elucidate the lack of effect of diltiazem on these parameters reflecting atherogenesis, one must consider the many factors contributing to the formation of atherosclerotic lesions such as high plasma levels of cholesterol, platelet aggregability and endothelial and smooth muscle cell proliferation. It is unlikely that the failure of diltiazem to lower plasma cholesterol explains the ineffectiveness of the drug as in several studies the beneficial effect of calcium antagonists was not accompanied by changes in cholesterol [4,6,10,13,33].

In the model we used, a predominant role can be ascribed to reactions like platelet aggregation, platelet vessel wall interaction and mural throm-

TABLE 4

DILTIAZEM AND THE PROGRESSION OF ATHEROSCLEROSIS

	Experimental animal	Duration (weeks)	Dose (mg/kg per day)	Blood lipids	Aortic coverage	Aortic lipid content	Coronary arteries
Ginsburg et al. (13)	rabbit	10	103	–	↓	nm	–
Naito et al. (15)	rabbit	14	70	–		nm	nm
Sugano et al. (11)	rabbit	10	25 (ip)	tot. chol. ↓, TG–	↓	↓	nm
Dicciani et al. (19)	rabbit	14	60	–	–	nm	nm
Sugano et al. (14)	rabbit	10	15 (ip)	tot. chol.–, HDL↑, LDL↓, TG–	–	↓	nm
Present study	pig	35	10–20	–	–	–	–

nm = not measured; ip = intraperitoneally; TG = triglycerides; tot. chol. = total cholesterol; HDL = HDL-cholesterol; LDL = LDL-cholesterol; – = no effect.

bosis-induced intimal proliferation [25]. Diltiazem did not reduce platelet aggregation, rather it increased collagen- but not ADP-induced platelet aggregation. While some Ca^{2+} entry blockers, such as verapamil and nifedipine [34,35], may decrease platelet aggregability in normolipidemic mammals, diltiazem appears to be ineffective [33,35].

Flunarizine [37] and nifedipine [38] have been shown to decrease proliferation of cultured smooth muscle cells. Jackson et al. [39] showed that high doses of several Ca^{2+} entry blockers including diltiazem (100 mg/kg/day) suppressed arterial smooth muscle cell proliferation in vivo. The use of a lower but clinically more relevant dose of diltiazem (10–20 mg/kg) and the longer exposure of the pigs to the atherogenic diet may have masked the anti-proliferative effect in the present study.

So far 5 studies reported on the effects of diltiazem on the progression of atherosclerosis [11,13–15,19]. Table 4 emphasizes the differences in species, dose of diltiazem and duration of the study. From these data it is premature to suggest that a higher dose of diltiazem, provided that the animals could tolerate such as dose, would be effective in pigs. The information on the concentrations of diltiazem in plasma after daily administration is controversial. Ginsburg et al. [13] reported on plasma concentration of diltiazem considered to be 1000 times the therapeutic levels in man, while the intraperitoneally administered dose of Sugano et al. [11] corresponds to an oral dose twice as high as in the former study [13]. In our own experience in man and pigs similar doses of drugs can be employed to evoke the desired cardiovascular responses [40,41]. We started with 10 mg/kg twice daily, which is about twice the maximal clinical dose. After 2 months plasma levels of diltiazem proved to be above therapeutic levels, and the dose was halved for the remaining period. Administration of 5 mg/kg diltiazem to normotensive conscious pigs lowers mean arterial blood pressure by 10% after 3 h (unpublished results). Since in the present study diltiazem at a dose of 5 mg/kg twice daily resulted in peak plasma levels within the normal range, we maintained this dose during the remainder of the dietary period.

From the data presented in this study we conclude that diltiazem at this clinically relevant dose did not protect the pigs against experimental atherosclerosis.

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Chapter 3

Mackerel oil and atherosclerosis in pigs

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Mackerel oil and atherosclerosis in pigs

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KEY WORDS: Atherosclerosis, cholesterol, endothelial denudation, luminal encroachment, aortic wall lipid content, platelet aggregation, prostaglandins, mackerel oil, pig.

In 35 pigs atherosclerosis was induced by balloon abrasion and a diet containing 2% (w/w) cholesterol and 7% (w/w) lard fat. After 4 months of induction nine animals were killed (I) for analysis of the extent of atherosclerosis, while the diet of the other 26 pigs was changed to a low cholesterol diet containing either 9% (w/w) lard fat (L), 9% (w/w) fish oil (F) or 4·5% (w/w) lard fat and 4·5% (w/w) fish oil (LF). This diet was continued for 3 months to induce regression of atherosclerosis. The cholesterol-rich diet increased plasma total cholesterol, but did not affect plasma triglycerides. Low-cholesterol feeding decreased plasma total cholesterol in all three groups, but triglycerides only in LF and F. Lipid infiltration of the aortic wall was similar in I, L, LF and F. In the denudated coronary arteries of I mean luminal encroachment was 11 ± 2%. This was similar in L (13 ± 4%) but significantly lower ($P < 0·05$) in LF (6 ± 2%) and in F (3 ± 1%). In the non-abraded coronary arteries of I mean luminal encroachment was 1·3 ± 0·3%. For F and LF similar values were found, but in L there was an increase to 11 ± 3% during low-cholesterol feeding. ADP-induced platelet aggregation was lower in LF and F than in L. Thromboxane A_2 production was only reduced in F, while the production of the weak thromboxane A_3 agonist was larger in F than in LF. It is concluded that fish oil retards the progression of and causes regression of coronary atherosclerosis.

Introduction

Anatomical evidence of regression of coronary atherosclerosis has been demonstrated after combined colestipol-niacin therapy in non-smokers who have undergone coronary bypass surgery[1]. Lowering of LDL-cholesterol and the concomitant increase in HDL-cholesterol appear to be the two major factors associated with the reduction in the number of lesions in the native coronary arteries of these patients. Armstrong *et al.* showed that in non-human primates, coronary atherosclerosis regressed both morphologically[2] and biochemically[3] after 40 months of low-cholesterol feeding following an induction period of 17 months. Clarkson *et al.*[4] found that in rhesus monkeys cholesterol-induced coronary atherosclerosis regressed after total cholesterol levels were decreased from 15 mM to 5 mM, but still progressed in half of the animals in which cholesterol was reduced to only 7·5mM (normal values 3·4–5·4 mM[5]). Regression of atherosclerosis has also been shown in pigs on removal of cholesterol from the diet[6–8]. Weiner *et al.*[9] have shown that fish oil added to an atherogenic diet can retard the formation of plaques in coronary arteries of pigs but it is not known whether marine oil can also cause regression of atherosclerosis.

The purpose of the present study was to assess the effect of regular intake of mackerel oil on the regression of atherosclerosis. We have previously shown that the amounts of fish oil used in the present study markedly reduce levels of plasma cholesterol and triglycerides in normolipidaemic pigs, whereas lard fat did not exert these effects.[10,11]. In the mackerel oil fed pigs prostanoid formation and aggregation of platelets were also lower than in the lard fat fed pigs[10,11].

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Materials and methods

INDUCTION OF ATHEROSCLEROSIS

Blood samples for measurement of blood lipids were obtained from overnight fasted castrated

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Table 1 Composition of the atherosclerotic induction diet and the three low-cholesterol diets

	Induction diet	Post-induction diets		
Ingredients	I	L	LF	F
Corn (extruded)	32	32	32	32
Wheat (extruded)	18	18	18	18
Soybean meal	14	14	14	14
Wheat middling	9	9	9	9
Dehydrated skimmed milk powder	14	14	14	14
$CaHPO_4.H_2O$	1·3	1·3	1·3	1·3
$CaCOO_3$	1·1	1·1	1·1	1·1
NaCl, iodinized	0·3	0·3	0·3	0·3
MgO	0·05	0·05	0·05	0·05
$MgSO_4$	0·05	0·05	0·05	0·05
$KH_2PO_4.2H_2O$	0·36	0·36	0·36	0·36
Choline chloride 50% (w/w)	0·18	0·18	0·18	0·18
Vitamin and trace element mixes[1]	0·7	0·7	0·7	0·7
Lard fat	7·27	9·10	4·55	—
Fish oil	—	—	4·55	9·10
Mixed tocopherols	0·03	0·03	0·03	0·03
Cholesterol	1·83	0·01	0·02	0·03

[1]Vitamin and trace element mixes supply the following per 100 g diet: retinol 1400 IU; cholecalciferol 140 IU; alphatocopherol 8 mg; menadione 0·2 mg; thiamine hydrochloride 1·8 mg; riboflavin 1·8 mg; pyridoxine HCl 1·4 mg; niacin 3·6 mg; vitamin C coated 20 mg; D-calcium pantothenate 3·6 mg; folic acid 0·4 mg; cyanocobalamin 0·004 mg; biotin 0·1 mg; inositol 4·5 mg; iron subcarbonate (57% Fe) 9·1 mg; $FeSO_4.H_2O$ (30% Fe) 14 mg; $Cu_2(OH)_2CO_3$ (55% Cu) 2·3 mg; ZnO (78% Zn) 11 mg; MnO (62% Mn) 9·1 mg; $Na_2Se_3.5H_2O$ (45% Se) 0·08 mg; $Ca(IO_3)_2$ (65% I) 0·2 mg; $CoCO_3$ (47% Co) 0·09 mg; The composition $(g\,(100\,g)^{-1})$ is on an as-fed basis.

male Yorkshire piglets (5 weeks of age, 9·4 ± 0·2 kg, n = 48). The animals were then started on a diet containing 7% (w/w) lard fat and 2% (w/w) cholesterol (Hope Farms, Woerden, The Netherlands, see Tables 1 and 2). Initially the animals received 200 g daily, but the portions were increased by 200 g each month as the animals gained weight. After 2 weeks the endothelium of the left anterior descending coronary artery and descending aorta was removed by the balloon abrasion technique[6,7,9]. Hitherto the overnight fasted piglets (now weighing 11·0 ± 0·2 kg) were pretreated with an intramuscular injection of 300 000 U procain-penicillin-G and 300 000 U benzathin-penicillin-G (Duplocilline^R, Gist Brocades, Delft, The Netherlands), anaesthetized by inhalation of a mixture of oxygen and nitrous oxide (1:2) and 1% halothane (Fluothane^R, Macclesfield, UK) and connected to a respirator for artificial ventilation. Following an intravenous injection of 5000 IU of heparin (Thromboliquine^R, Organon Teknika B.V., Boxtel, the Netherlands) the animals were catheterized via the left external carotid artery. For denudation of the left anterior descending coronary artery a 4F Fogarty embolectomy catheter (Edwards Laboratories, Santa Ana, California, USA) was introduced under the

Table 2 Fatty acid composition (mole %) of the induction diet and the three post-induction diets

	Induction diet	Post-induction diets		
Fatty acid	I	L	LF	F
14:0	2	2	5	7
16:0	24	24	21	18
16:1	2	3	6	8
18:0	9	10	6	1
18:1	41	42	29	17
18:2 n–6	16	15	11	8
18:3	1	1	1	1
20:1	1	1	4	6
20:5 n–3	—	—	8	17
22:1	—	—	2	4
22:6 n–3	—	—	5	9
24:1	—	—	1	1
others	4	2	1	3

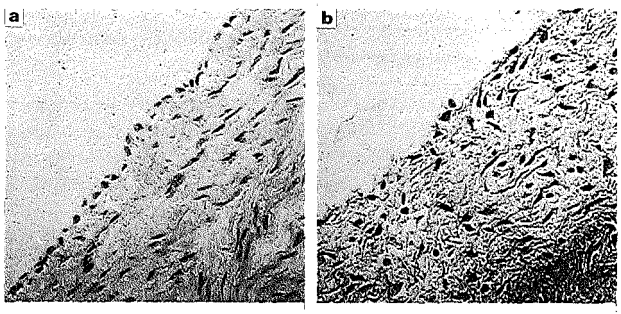

Figure 1 The efficacy of the abrasion technique. An example of a coronary artery with intact endothelium is shown on the left hand side (a), while the example on the right (b) was taken from one of the animals that died suddenly 1 day after the procedure.

guidance of fluoroscopy. The balloon was inflated with 0·3 ml air and pulled back quickly[12]. This procedure was repeated twice to ensure effective denudation of the coronary artery. The descending aorta was catheterized with a 7F Fogarty embolectomy catheter and the balloon was subsequently inflated with 3 ml air and pulled back to the aortic arch. This procedure was also repeated twice. After the carotid artery was stitched, the incision was closed in one layer, the animals were allowed to recover and the dietary period was continued. The efficacy of this abrasion technique was evaluated in a few animals prior to the study (Fig. 1).

Thirteen animals encountered sudden death during the immediate postoperative period. Autopsy showed ruptures of the coronary artery in five animals and perforation of the left ventricle in two animals. No coronary artery lesions or thrombosis were found in the six other animals.

EXTENT OF ATHEROSCLEROSIS AFTER 4 MONTHS OF HIGH-CHOLESTEROL FEEDING

14 weeks after balloon denudation (total dietary period 4 months), nine randomly chosen animals (I) were sacrificed to establish the severity of atherosclerosis (see below).

DIETS AFTER 4 MONTHS OF HIGH-CHOLESTEROL FEEDING

The remaining animals (n = 26) were arbitrarily divided into three groups and their diet was changed to low cholesterol but with a different fatty acid composition for each group. Nine animals (L) received 9% (w/w) lard fat, nine animals (LF) 4·5% (w/w) lard fat and 4·5% (w/w) mackerel oil (AS Johan C Martens and Co, Bergen, Norway) and eight animals received 9% (w/w) of mackerel oil (F) for 3 months. These diets will be called post-induction diets (Tables 1 and 2). For convenience the experimental groups have been schematically presented in Fig. 2.

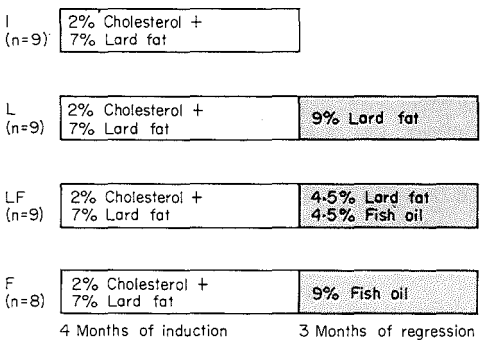

Figure 2 Schematic overview of the dietary groups. Each group received a low fat basal diet to which 9% (w/w) fat was added. The 13 animals which died immediately following the endothelial denudation are not included.

PLASMA LIPIDS

Plasma levels of triglycerides[13], total[14] and HDL-cholesterol[15] were determined before the start of, and at 1-month intervals during the dietary period. Collection of blood samples was performed after an overnight fast by puncturing the jugular vein. During the first 2 months, samples were taken from conscious animals, but after the third month the animals were first sedated with 30 mg kg^{-1}

ketamine i.m. (Aescoket^{R}, Aesculaap BV, Boxtel, the Netherlands).

PLATELET AGGREGATION

After 7 months (4 months of induction and 3 months of post-induction) a 5 ml blood sample was collected and placed in tubes containing 1000 IU heparin. After a 15 min incubation time at room temperature, whole blood aggregation tests were performed after the addition of 2 $\mu g\,ml^{-1}$ collagen or 5 μM ADP for 10 min in an electrical aggregometer[16]. For measurement of the prostanoid production the aggregation reaction was stopped after 10 min of stimulation with 2 $\mu g\,ml^{-1}$ collagen by placing the blood sample on ice. After centrifugation (at 2000 g_{max} for 5 min) the plasma was stored at -80 °C. The levels of the thromboxane A (TXA) derivatives, HHT and HHTE, were determined by high pressure liquid chromatography[17]. HHT (12 L-hydroxy-5,8,10-heptadecatrienoic acid) and HHTE (12 L-hydroxy-5,8,10,14- heptadecatetraenoic acid) are stable products which are, through the cyclo-oxygenase pathway, derived from arachidonic (20:4 n–6) and eicosapentaenoic acid (20:5 n–3), respectively, and reflect TXA_2 and TXA_3 formation[17–20]. The platelets were counted and TXA production was calculated on the basis of platelet concentration.

CHEMICAL ANALYSIS OF THE AORTIC VESSEL WALL

Samples (40–200 mg) were excised from lesion (abdominal) and non-lesion (ascending) areas of the aorta, dissected free of adventitia and directly frozen in liquid nitrogen and stored at -80 °C until analysis. In principle the method of Bligh and Dyer[21] was used for lipid extraction. Briefly, tissue samples were homogenized in chloroform/methanol/distilled water (4:10:5, v/v/v) with a polytron PT 10 (half-setting). After centrifugation at 1500 g_{max} for 5 min and washing the pellet by rehomogenization in 1·9 ml of the same solvent, the two supernatants were combined and supplemented with 1·5 ml chloroform and 1·5 ml water. After vigorous mixing the upper phase was discarded and, when necessary, the intermediate solid material dissolved by dropwise addition of methanol. Subsequently the mixture was dried under nitrogen at 37 °C and the residue dissolved in 0·2 ml 2-propranolol. Cholesterol, triglyceride and phospholipid contents were measured enzymatically (GHOD-PAP and GPO-PAP, Boehringer, Mannheim, FRG; and PAP 150 of Biomerieux, Cherbouriéres, Les Bains, France). In the delipidized extracts protein[22] and DNA[23] contents were measured.

GRADING OF CORONARY ARTERY ATHEROSCLEROSIS

From the coronary arteries biopsies were taken at 5 mm intervals. All biopsies from the heart and aorta were immediately fixed in 10% formalin. Tissue samples were routinely processed for light microscopy: they were embedded in paraffin, cut 7 µm thick and stained with haematoxylin azophloxine and recorsine fuchsine. The sections were projected on a video screen and the outer contours, external and internal elastic lamina, and endothelial lining of the vessels were traced using an integrated image analysis system (IBAS-2000, Kontron, Oberkochen, FRG). The surface between the endothelial lining of the lumen and the internal elastic lamina was taken as the lesion area[24]. The encroachment was defined as the ratio ($\times 100\%$) of the surface of the lesion area and the corrected (by circular shape factor) surface of the area surrounded by the internal elastic lamina[24,25]. The media area was the difference between the surfaces circumscribed by the internal and external elastic lamina.

STATISTICAL ANALYSIS

All data are described as mean $\pm$ standard error of the mean (SEM). The data were analysed statistically using paired and unpaired Student's *t*-tests and a one-way analysis of variance with repeated measurements followed by the Newman-Keul procedure for multiple comparisons of mean values. Statistical significance was accepted at $P < 0{\cdot}05$.

Results

EXPERIMENTAL ANIMALS

After 4 months the animals weighed 55 ± 1 kg. During the 3 months of low-cholesterol feeding the animals increased their weight to 91 ± 3 kg(L), 88 ± 4 kg(LF) and 94 ± 3 kg(F). There were no differences in the weight gain of the three groups during these 3 months.

PLASMA LIPIDS

During the 4 months of 2% (w/w) cholesterol feeding plasma levels of total cholesterol doubled, primarily due to a tripling of the VLDL- + LDL-cholesterol content as HDL-cholesterol levels increased by less than 30% (Fig. 3). A consequence of these changes was that the ratio between HDL-cholesterol and total cholesterol decreased from

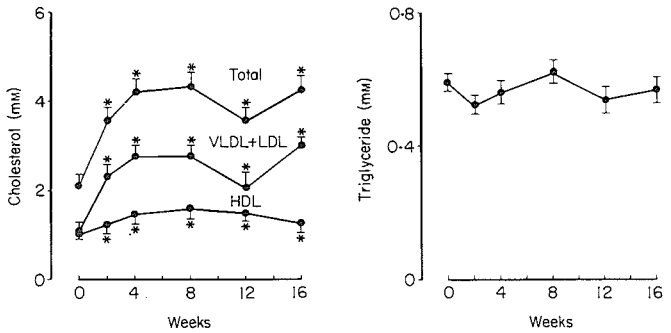

Figure 3 Plasma lipid levels during the 4-month induction period. * = $P < 0.05$ vs preatherogenic diet value.

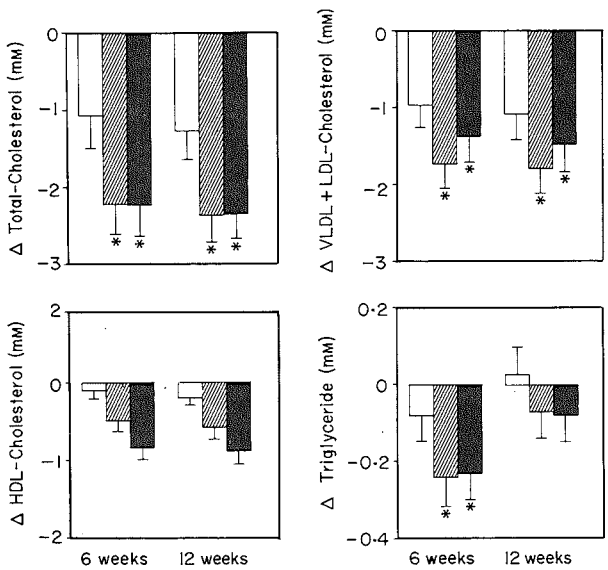

Figure 4 Changes in plasma lipid levels during the 3-month post-induction period on low-cholesterol diets. □ L = 9% lard fat, (n = 8); ▨ LF = 4·5% lard fat and 4·5% fish oil, (n = 9); ■ F = 9% fish oil, (n = 9). * = $P < 0.05$ vs L.

0·50 ± 0·01 to 0·31 ± 0·01 ($P < 0.05$). Levels of plasma triglycerides (0·59 ± 0·03 mM) were not affected by this diet (Fig. 3).

During the 3 months of low-cholesterol feeding total cholesterol decreased in all three groups, the changes being the most pronounced in the fish oil fed animals (−1·28 ± 0·33 mM in L, −2·37 ± 0·34 mM in LF and −2·35 ± 0·35 mM in F, Fig 4). The largest changes occurred during the first few weeks after the animals had changed from the high- to the low-cholesterol diet. Although HDL-cholesterol also decreased in all three groups, there was an increase in the HDL-total cholesterol ratio (0·48 ± 0·03 in L, 0·44 ± 0·02 in LF and 0·40 ± 0·01

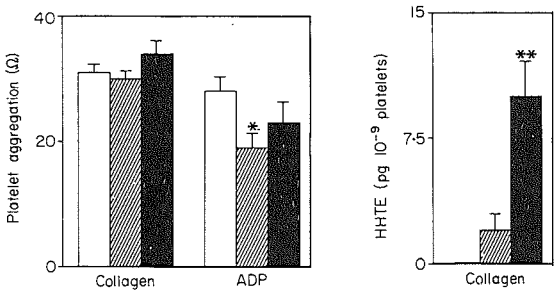

Figure 5 Collagen (2 μg ml)$^{-1}$- and ADP (5 μM)-induced platelet aggregation in the three post-induction groups after 3 months of low-cholesterol feeding. For measurement of the prostanoid production the collagen-induced aggregation was stopped after 10 min. HHTE was not produced in the animals which received only lard fat. □ L = 9% lard fat, (n = 8); ▨ LF = 4·5% lard fat and 4·5% fish oil, (n = 9); ■ F = 9% fish oil, (n = 9). * = $P < 0.05$ vs L; ** = $P < 0.05$ vs LF.

Table 3. Lipids, protein and DNA contents of the aortic vessel wall

		Induction group	Post-induction groups		
		I (n = 6)	L (n = 6)	LF (n = 7)	F (n = 7)
Abdominal Aorta (abraded)					
cholesterol	(μmol g^{-1})[a]	4·07 ± 0·46*	5·10 ± 0·60*	5·54 ± 0·66*	4·83 ± 0·47*
triglyceride	(μmol g^{-1})	3·05 ± 0·64*	1·92 ± 0·45	2·19 ± 0·34	2·05 ± 0·31
phospholipids	(μmol g^{-1})	3·71 ± 0·27*	4·56 ± 0·30*	4·37 ± 0·50*	4·06 ± 0·17*
protein	(mg g^{-1})	160 ± 14	168 ± 15	185 ± 20	208 ± 30
DNA	(mg g^{-1})	1·91 ± 0·25	2·45 ± 0·20	2·03 ± 0·14	2·15 ± 0·10
Ascending Aorta (non-abraded)					
cholesterol	(μmol g^{-1})	2·82 ± 0·36	2·63 ± 0·13	2·60 ± 0·12	2·82 ± 0·11
triglyceride	(μmol g^{-1})	1·35 ± 0·28	1·38 ± 0·23	1·68 ± 0·43	1·67 ± 0·73
phospholipids	(μmol g^{-1})	2·92 ± 0·07	2·91 ± 0·10	2·91 ± 0·17	2·92 ± 0·19
protein	(mg g^{-1})	166 ± 3	235 ± 42	222 ± 23	228 ± 31
DNA	(mg g^{-1})	2·45 ± 0·13	2·18 ± 0·29	2·71 ± 0·40	2·67 ± 0·52

For the composition of the diets see Tables 1 and 2. a = All units per g wet weight. * = $P < 0.05$ vs ascending aorta in the same group.

in F). The plasma triglyceride levels were significantly reduced in the first 6 weeks in LF and F, but returned to those measured in L after 12 weeks.

PLATELET AGGREGATION AND PROSTAGLANDIN PRODUCTION

Collagen-stimulated platelet aggregation was similar in L, LF and F (not shown), but the ADP-stimulated aggregation was lower in LF and F than in the animals which received lard fat only (Fig. 5).

The total amount of thromboxane A derivatives, measured after 10 min of stimulation with collagen, was similar in L (62 ± 5 pg (10^9 platelets)$^{-1}$), LF (67 ± 6 pg (10^9 platelets)$^{-1}$) and F (58 ± 7 pg (10^9 platelets)$^{-1}$). HHT production was lower in F (49 ± 6 pg (10^9 platelets)$^{-1}$) than in LF (55 ± 6 pg (10^9 platelets)$^{-1}$) and L (62 ± 5 pg (10^9 platelets)$^{-1}$).

Table 4 Luminal encroachment of the abraded and non-abraded coronary arteries

		Induction group	Post-induction groups		
		I (n=9)	L (n=8)	LF (n=9)	F (n=9)
LADCA (abraded)					
lesion area	(mm^2)	0·22±0·03*	0·27±0·03	0·10±0·02*+×	0·09±0·02×+
encroachment	(%)	11±2*	13±2	6±1*+×	3±1+×o
media	(mm^2)	0·86±0·06	0·90±0·08	0·83±0·08	0·97±0·05
LCXCA (non-abraded)					
lesion area	(mm^2)	0·03±0·01	0·30±0·06+	0·02±0·01×	0·05±0·02×
encroachment	(%)	1·3±0·3	11±3+	0·9±0·3×	1·1±0·4×
media	(mm^2)	0·94±0·08	1·08±0·11	0·84±0·08	0·82±0·12

For the composition of the diets see Tables 1 and 2. * = $P<0·05$ vs non-abraded vessel of the same group; + = $P<0·05$ vs I; × = $P<0·05$ vs L; o = $P<0·05$ vs LF; LADCA = left anterior descending coronary artery; LCXCA = left circumflex coronary artery.

None of the animals belonging to L produced HHTE (Fig. 5), which is in accordance with the absence of eicosapentaenoic acid in the platelet membrane[26]. Not all fish oil fed animals produced HHTE (Fig. 5). A large production of HHTE was found in four of the seven animals of F but only two of nine animals of LF produced some HHTE.

LIPID INFILTRATION IN THE ABRADED AND THE NON-ABRADED SEGMENTS OF THE AORTIC WALL

After 4 months of cholesterol feeding (I), all lipid concentrations in the abraded segments of the abdominal aorta were higher than in the non-abraded segments of the ascending aorta (Table 3). During the following 3 months of low-cholesterol feeding, there were no changes in the lipid content of the non-abraded segments in any of the three post-induction groups. Cholesterol and phospholipid infiltration into the lesions tended to increase in all three groups during low-cholesterol feeding. On the other hand, triglyceride content of the abraded and the non-abraded areas did not differ at the end of the post-induction period. Expression of lipid contents as μmol (g protein)$^{-1}$ and μmol (mg DNA)$^{-1}$ gave similar differences between the abraded and the non-abraded areas of I and the three post-induction groups (not shown).

LUMINAL ENCROACHMENT OF THE ABRADED AND NON-ABRADED CORONARY ARTERIES

Mean luminal encroachment of the abraded left anterior descending coronary artery was 11±2% and of the non-abraded left circumflex coronary artery 1·3±0·3% after 4 months of 2% (w/w) cholesterol feeding (I, Table 4). During the post-induction period, feeding with only lard fat had no effect on the mean luminal encroachment of the abraded coronary arteries, but addition of fish oil caused a dose-dependent decrease. In the non-abraded left circumflex coronary artery mean luminal encroachment increased to 11±3% in L. Addition of fish oil to the low-cholesterol diet inhibited this increase (Table 4).

Discussion

In the present study addition of 2% (w/w) cholesterol to the 21 energy% fat (15 mol% linoleic acid) containing diet increased plasma cholesterol from 2 mM to 4 mM. Weiner *et al.* added 2% (w/w) cholesterol *and* 1% sodium cholate to a 30 energy% fat (mainly saturated fatty acids) containing diet causing increases up to 14 mM[24]. This partly explains the lower mean luminal encroachment (11%) in the abraded coronary arteries of the present study compared with that found by Weiner *et al.* (44%).[24] The total cholesterol content of the abdominal aortic lesions in the present study was 4·1 μmol (g wet weight)$^{-1}$, which is about 27 μmol (g dry weight)$^{-1}$. Fritz *et al.*[6] found a cholesterol content of about 38 μmol (g dry weight)$^{-1}$ in the aortic wall with mean plasma levels of cholesterol of 14 mM. Other factors contributing to the

difference in luminal encroachment in the coronary arteries and lipid infiltration in the aortic wall may be the difference in the duration of the induction period and performance of the abrasion technique.

Three months of low-cholesterol feeding lowered the plasma cholesterol levels (Fig. 4). In the animals with fish oil added to the diet (F and LF) these decreases were more pronounced than in the animals in which only lard fat (L) was added, which is consistent with earlier observations in normolipidaemic pigs[10,11,19]. The reduction in ADP-induced platelet aggregation was less in F than in LF. The data on platelet aggregation in F are not in agreement with the changes in HHT and HHTE production, which were measured in vitro after the aggregation reaction. However in the in vivo situation the effects of fish oil on the synthesis of prostacyclin I_2 and I_3 by endothelial cells should also be taken into account[19,27–29].

At the end of the induction period the abraded abdominal aorta contained more cholesterol than the non-abraded ascending aorta. Three months of low-cholesterol feeding did not affect the lipid content of the aortic wall in either group.

Further studies are needed on the localization (intra- or extracellularly) and precipitation forms of cholesterol(ester) for definite proof of lack of regression[30].

Addition of fish oil to the low-cholesterol diet decreased luminal encroachment in the abraded coronary arteries, whereas addition of lard fat alone only inhibited further intimal proliferation. In the non-abraded coronary arteries intimal proliferation was negligible after 4 months, but developed during the following 3 months when only lard fat was added to the low-cholesterol diet. Although in L cholesterol levels were lower during lard fat feeding than during high-cholesterol feeding they were still higher than in low-cholesterol fed pigs of the same age[31]. This is consistent with the findings of Clarkson *et al.*[4], who also found progression of lesions in half of the animals whose cholesterol levels remained elevated compared with baseline values whereas in the animals with normal levels of cholesterol, lesions did regress. Lesions did not develop in the non-abraded arteries of the pigs with fish oil added to the diet, proving that fish oil can also prevent progression of coronary atherosclerosis.

In LF and F the return to normal cholesterol levels is most likely responsible for the regression of coronary artery sclerosis, but it cannot explain the dose-dependent effect of fish oil. The results on platelet aggregation cannot elucidate this dose-dependency either, because platelet aggregation was only reduced in LF. Several factors such as leukotrienes, tissue plasminogen activator, platelet-derived growth factor, interleukin-1 and tumour necrosis factor, have also been implicated in the development (or regression) of atherosclerosis and are also modulated by fish oil (for review see Refs. 32,33).

Lipid infiltration could not be determined in the coronary arteries, as the samples were used for measurement of luminal encroachment. If the results obtained in the aorta can be extrapolated to the smaller blood vessels there appears to be a discrepancy between these two variables, which are both frequently used for the grading of the severity of atherosclerosis. An additional complication is that widening of a lumen at the site of the lesions does not necessarily imply a regression of the atherosclerotic process[8]. Further studies on the constitution of the plaques are needed to assess whether the data on intimal thickening in the present investigation represent a true regression of the atherosclerotic process.

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Chapter 4

Effects of nisoldipine on recovery of coronary blood flow, sarcoplasmic reticulum function and other biochemical parameters in post-ischaemic porcine myocardium

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Summary

The effects of nisoldipine (0.1 μg/kg/min; n=9) or its solvent (n=9) were studied in pigs, in which left anterior descending coronary artery (LADCA) blood flow in both groups was reduced to 80% of baseline for 60 min and reperfused for 2 h. Infusions were started at 30 min of ischaemia and lasted throughout reperfusion. In both groups, flow reduction abolished regional contractile function and caused similar decreases in the level of creatine phosphate (CP; by 70%) and the energy charge (from 0.91 to 0.69), mean arterial blood pressure (by 25%), $LVdP/dt_{max}$ (by 30%) and cardiac output (by 30%). During ischaemia LADCA blood flow slightly increased (from 14 ± 8 to 24 ± 6 ml/min/100g; $P>0.05$) in the nisoldipine-treated animals, resulting in an increase in CP to 91 ± 24% of baseline and preventing further decreases in energy charge, as observed in the solvent-treated animals. After 2 h of reperfusion in neither group return of contractile function of the post-ischaemic myocardium was observed. Post-ischaemic blood flow in the nisoldipine-treated pigs increased from 24 ± 6 ml/min/100g to 76±14 ml/min/100g and from 19 ± 6 ml/min/100g to 41±6 ml/min/100g in the solvent-treated animals ($P<0.05$) after 2 h of reperfusion. Myocardial work was significantly higher in the nisoldipine-treated animals (111 ± 15 mmHg.(L/min) as compared to 69 ± 14 mmHg.(L/min) in the solvent-treated pigs after 2 h of ischaemia). The energy charge of the post-ischaemic myocardium was similar for both groups (0.84 ± 0.02 for the nisoldipine-treated and 0.83 ± 0.03 for the solvent-treated animals). The rate of sarcoplasmic reticular Ca^{2+} uptake of the non-ischaemic segment of the nisoldipine-treated animals was 61% higher ($P<0.05$) than that of the solvent-treated animals. In the post-ischaemic myocardium similar rates of Ca^{2+} uptake were found in both groups, but the activities were markedly lower as compared to the non-ischaemic myocardium. It is concluded that nisoldipine increases blood flow during reperfusion, which may have been caused by coronary vasodilatation. However, attenuation of the "no-reflow" phenomenon also contributed, since more rapid rephosphorylation of ADP leading to an increase in CP during ischaemia may have preserved jeopardized cells. Moreover, nisoldipine increases the sarcoplasmic reticular Ca^{2+} pump activity independent of ischaemia, which may have contributed in reducing the Ca^{2+} overload.

Abbreviations: CP, creatine phosphate; ATP, adenosine-5'-triphosphate; ADP, adenosine-5'-diphosphate; HPLC, high performance liquid chromatography; SDS, sodium dodecylsulphate; SR, sarcoplasmic reticulum; LADCA, left anterior descending coronary artery; $LVdP/dt_{max}$, maximum rate of rise of left ventricular blood pressure; EDT, end-diastolic wall thickness; EST, end-systolic wall thickness; SWT, systolic wall thickening; PSWT, post-systolic wall thickening; Max T, maximal wall thickness

Introduction

The rapid and uncontrolled increase in cytosolic Ca^{2+} that occurs upon reperfusion of ischaemic myocardial tissue is believed to play a central role in the subsequent loss in cell viability. The Ca^{2+} overload triggers a chain of destructive events. Activation of Ca^{2+}-dependent reactions such as ATPases, proteases and phospholipases result in energy waste and membrane disruption. The cytosolic free Ca^{2+} concentration can increase by entrance via the Na^{+}:Ca^{2+} antiporter [1], via receptor- and voltage-operated Ca^{2+} channels and, as the cell membrane disintegrates via passive diffusion [2]. The loss in ability of the sarcolemma and sarcoplasmic reticulum (SR) to remove Ca^{2+} from the cytosol could also contribute to cytosolic Ca^{2+} overload. Ischaemia causes a reduction in active Ca^{2+} transport rate of sarcoplasmic reticulum [3] and sarcolemma [4]. Ca^{2+} entry blockers are specific blockers of the voltage-operated Ca^{2+} channels [5] and therefore have the potential to protect the myocardium against Ca^{2+} overload [6]. Moreover, some dihydropyridines (nitrendipine and felodipine) have been shown to directly stimulate the *in vitro* Ca^{2+} uptake rate of canine sarcoplasmic reticulum, which may present another potential action site to prevent myocardial Ca^{2+} overload [7].

The activity of the sarcoplasmic reticulum Ca^{2+} pump is modulated by phospholamban phosphorylation [8, 9]. Experiments conducted with intact hearts indicate that cyclic AMP- and Ca^{2+}-induced phosphorylation of phospholamban is involved in the ß-adrenergic activation of the calcium pump of the SR [10]. We obtained evidence that the ischaemia-induced inactivation of the calcium pump is a consequence of the altered characteristics of phospholamban [3].

In addition to their vasodilatory potential, leading to increases in blood flow to the area at risk and sparing of energy, Ca^{2+} entry blockers can also preserve myocardium by avoiding the loss of adenosine precursors [11], protect the vascular endothelial cells independent of vasodilatation [12], beneficially interact with the sarcolemma membrane [13] and mitochondria [14] of the myocardial cell. By protecting the myocardium via these mechanisms, Ca^{2+} antagonists may also indirectly prevent the reduction in the Ca^{2+} uptake and phospholamban phosphorylation of sarcoplasmic reticulum of post-ischaemic myocardium.

The present study was conducted to investigate the effects of nisoldipine, given 30 min prior to and throughout reperfusion, on the Ca^{2+} transport activity and *in vitro* ^{32}P incorporation into phospholamban of SR, isolated from large transmural myocardial segments of pigs subjected to 1 h of ischaemia followed by 2 h of reperfusion. In view of the relative lack of data on the effects of nisoldipine on energy metabolism in an *in vivo* model, other biochemical parameters such as the energy charge and levels of creatine phosphate (CP) were measured in small transmural myocardial biopsies collected during ischaemia and reperfusion. Additionally, the effects of the drug on systemic haemodynamics, regional contractile function and coronary blood flow were investigated.

Materials and methods

General. After an overnight fast cross-bred Landrace x Yorkshire pigs of either sex (n=18, 25-42 kg) were sedated with 120 mg azaperone (Janssen Pharmaceutica, Beerse, Belgium) i.m., anaesthetized with 150 mg metomidate (Janssen Pharmaceutica, Beerse, Belgium) i.v. and intubated for artificial ventilation with a mixture of oxygen and nitrous oxide (1:2). Respiratory rate and tidal volume were adjusted to keep arterial blood gases within the normal range: pH between 7.37 and 7.49; pCO_2 between 34 and 48 mmHg; pO_2 between 120 and 180 mmHg. A catheter was positioned into the superior caval vein for the administration of 160 mg/kg alpha-chloralose (Merck, Darmstadt, F.R.G.) followed by an infusion of 5 mg/kg/h pentobarbitone sodium (Sanofi, Paris, France) and for the administration of the muscle relaxant pancuronium bromide (4 mg) prior to thoracotomy. Haemaccel (Behringwerke A.G., Marburg, F.R.G.) was administered to replace the blood, withdrawn during sampling (see below). An 8F micromanometer-tipped catheter (Millar, Houston, Texas, U.S.A.) was inserted into the left ventricle by way of the left carotid artery, for the measurement of left ventricular blood pressure and its first derivative (LVdP/dt). Catheters were also inserted into the aorta, for measurement of central aortic blood pressure (50 AD pressure transducer, Spectramed, Bilthoven, the Netherlands), the collection of blood samples for the determination of blood gases and for the withdrawal of reference samples necessary for calibration of the radioactive microsphere flow measurements. After thoracotomy, an electromagnetic flow probe (Skalar, Delft, the Netherlands) was placed around the ascending aorta for the measurement of aortic blood flow. The left anterior descending coronary artery (LADCA) was dissected free just distal from its first diagonal branch and an inflatable balloon (R.E. Jones, Silver Spring, Maryland, U.S.A.) was placed around the LADCA and connected to a 1 ml syringe (Hamilton Bonaduz, Bonaduz, Switzerland) driven by a micrometer (Hamilton Co., Reno, Nevada, U.S.A.). The vein accompanying the LADCA was cannulated for the withdrawal of blood samples for the determination of coronary venous blood gases.

Regional blood flow. To determine regional blood flows the left atrial appendage was catheterized for the injection of a batch of $1\text{-}2.10^6$ radioactive microspheres, 15 ± 1 (SD) μm in diameter (NEN Company, Dreieich, F.R.G.), labelled with either ^{95}Nb, ^{103}Ru, ^{113}Sn, ^{46}Sc or ^{141}Ce. Full details of the procedures and the calculation of flow data using the reference sample technique have been reported earlier [15, 16].

Regional myocardial function. Regional myocardial function was estimated from recordings of myocardial wall thickness obtained with a 5 MHz ultrasonic transducer (Krautkamer-Branson, Lewistown, PA, U.S.A.) sutured onto a part of the epicardial surface perfused by the LADCA. From the tracings end-diastolic (EDT), end-systolic (EST) and maximal post-systolic (maxT) wall thickness were measured. Systolic wall thickening (SWT, %) was calculated as 100 x (EST-EDT)/EDT, while post-systolic wall thickening (PSWT, %) was defined as 100 x (maxT-EST)/EDT

Experimental protocol. After systemic haemodynamics had been stable for at least 30 min following completion of the instrumentation, baseline values of systemic haemodynamics, regional myocardial function and arterial and coronary venous blood gases were obtained while

a batch of microspheres was injected for the measurement of distribution of myocardial blood flow. Furthermore, transmural needle biopsies (processed as described below) were taken from the myocardium nourished by the distal part of the LADCA and from a segment of the anterior wall of the heart, which was not supplied by the LADCA. In all pigs the flow in the LADCA was then gradually reduced by slowly inflating the balloon, until complete loss of regional contractile function. After 30 min of ischaemia, the measurements of systemic haemodynamics and regional blood flows were repeated and a transmural biopsy was taken from the ischaemic area. In nine animals an infusion of nisoldipine (0.1 μg/kg/min) was started and in the other animals solvent was administered at a similar infusion rate (1 ml/min). If ventricular fibrillation occurred the animal was promptly (within 30 seconds) defibrillated with DC-countershock. After 60 min, when all measurements had been repeated and biopsies were obtained from both the ischaemic and the non-ischaemic area, the balloon was deflated. Two hours later, the last haemodynamic measurements were taken and biopsies were collected. The heart was then excised and immediately cooled on ice and transmural myocardial samples (5-7 g) were obtained from the post-ischaemic segment and from the posterior wall of the heart (non-ischaemic segment). The segments were homogenized for isolation of SR membrane vesicles (see below). Furthermore, the radioactive label present in the pellet after the first centrifugationstep (10,000 g, 20 min) for SR isolation was counted in order to obtain myocardial blood flow data.

Adenine nucleotides, creatine phosphate and creatine. The transmural myocardial biopsies, taken with a Tru-Cut needle (Travenol Laboratories Inc., Deerfield, Illinois, USA) from the ischaemic area and the adjacent non-ischaemic area were dipped into 0.9% NaCl of 0 °C to remove adherent blood, and immediately (within 10 s) frozen in liquid nitrogen. The biopsies were stored at -80 °C until analysis. The biopsies (5-20 mg) were homogenized in 0.5 ml 0.42 M $HClO_4$ at liquid nitrogen temperature with the Braun micro-dismembrator (B. Braun, Melsungen, FRG), thawed, shaken and centrifuged. After neutralization of the supernatant the adenine nucleotides, CP and creatine were separated and the concentrations estimated with an isocratic ionpairing high performance liquid chromatography (HPLC) [17], except that 175 mM potassium phosphate, 2.3 mM tetrabutylammonium hydrogensulfate, 2.5% acetonitrile, pH 6.25 was used as a running buffer. With this HPLC method we also checked the purity of the [gamma-^{32}P]ATP used in the phospholamban phosphorylation assays.

Isolation of sarcoplasmic reticulum. The transmural myocardial tissue samples were homogenized 3 times during 10 s in 4 volumes of 10 mM $NaHCO_3$, pH 7.0 at 0 °C with a Polytron PT 10 (Kinematica Gmbh, Luzern, Switzerland). The SR was isolated as described [3]. Immediately after isolation, the SR suspension was frozen in liquid nitrogen and stored at -80 °C until analysis of Ca^{2+} uptake and phospholamban phosphorylation activities.

Phosphorylation of phospholamban and Ca^{2+}uptake activity. The cyclic AMP-dependent phosphorylation of SR vesicles (5-10 μg protein) was determined as described [3] with 5 μM cyclic AMP to activate the endogenous protein kinase and 300 U/ml exogenous catalytic subunit of cyclic AMP-dependent protein kinase (Sigma Chem. Co, St Louis, USA). The samples were preincubated for 2 min at 25 °C and the phosphorylation reaction was started by the addition of 200 μM (final concentration) [gamma-^{32}P]ATP (150 TBq/mol). After 5 min the reaction was stopped with a mixture of sodiumdodecylsulphate (SDS), ß-mercaptethanol

and glycerol [18, 3]. The samples were then heated at 95 °C to dissociate phospholamban into monomers, and subjected to SDS-polyacrylamide gelelectrophoresis [18]. Phosphorylated phospholamban was located on dried gels by autoradiography and ^{32}P content in the pieces, excised from the gel, was estimated by liquid scintillation counting. Ca^{2+} uptake activity was measured by incubation of SR with 50 μM ^{45}Ca in the presence of 1 mM ATP [3]. After the incubation the ^{45}Ca-containing SR vesicles were filtered through Millipore filters (0.45 μm). The ^{45}Ca content of the SR vesicles remaining on the filters was estimated by liquid scintillation counting [3]. Ca^{2+} uptake activity in blank reactions, obtained by omitting ATP from the reaction mixture, were subtracted.

Protein. The protein content of SR was estimated with the method of Lowry et al. [19]. In the needle biopsies, protein was estimated by another procedure than in the crude pellet obtained after centrifugation of the homogenate acidified with $HClO_4$. After dissolution in 0.1 M KOH, protein was estimated with the Coomassie Brilliant Blue assay [20] obtained from Bio-Rad (Bio-Rad Laboratories, Munich, FRG). For both methods bovine serum albumin was used as the standard.

Drugs. Nisoldipine (Bayer A.G., Wuppertal, F.R.G.) was dissolved in a mixture of polyethylene glycol 400, glycerol and distilled water. The nisoldipine solution (0.1 mg/ml) was diluted with 0.9% w/v NaCl immediately before use. Preparation of the solution and administration of nisoldipine occurred while the drug was protected from light.

Statistical analysis. All data have been presented as means ± SE. The significance of the changes produced by the LADCA flow reduction in the animals was evaluated by Duncan's new multiple range test once on analysis of variance had revealed that the samples represented different populations. The significance of the nisoldipine-induced changes was determined by comparing these changes with those observed in the solvent-treated animals at corresponding times. Statistical significance was accepted for $P<0.05$.

Results

Ventricular arrhythmias In both groups 7 out of 9 animals (78%) encountered an episode of ventricular fibrillation during the ischaemic period. All animals were defibrillated within 25 s and resumed pre-fibrillation values. Ventricular fibrillation was not observed during reperfusion in any animal.

Systemic haemodynamics During the first 30 min of LADCA flow reduction in the solvent-treated animals mean arterial blood pressure, cardiac output, stroke volume and $LVdP/dt_{max}$ had decreased ($P<0.05$) by 23%, 30%, 36% and 32%, respectively, left ventricular end diastolic pressure increased from 8 to 13 mmHg ($P<0.05$), while heart rate was not affected (Table 1). During the 2 h of reperfusion there was a further decline in mean arterial blood pressure (to 59% of baseline), $LVdP/dt_{max}$ (to 54% of baseline), cardiac output (to 52% of baseline) and stroke volume (to 56% of baseline), while left ventricular end diastolic pressure further increased to 14 ± 2 mmHg. Vasoconstriction (increase in systemic vascular resistance to 35%

Table 1 *Systemic haemodynamics in solvent-treated and nisoldipine-treated open-chest anaesthetized pigs at baseline (BL), 30 and 60 minutes of ischaemia (I) and 120 minutes of reperfusion (R). Nisoldipine (0.1 μg/kg/min) was started at 30 min of ischaemia and lasted throughout reperfusion.*

		BL	30 I	60 I	120 R
MAP	S	97 ± 5	75 ± 5*	72 ± 3*	57 ± 4*
	N	90 ± 4	66 ± 5*	65 ± 3*	65 ± 6*••
CO	S	2.3 0.1	1.6 ± 0.1*	1.6 ± 0.1*	1.2 ± 0.2*
	N	2.5 ± 0.2	1.8 ± 0.1*	1.8 ± 0.1*	1.6 ± 0.2*•
HR	S	94 ± 5	98 ± 4	95 ± 7	88 ± 8
	N	95 ± 4	100 ± 6	97 ± 4	103 ± 8
$LVdP/dt_{max}$	S	2590 ± 130	1760 ± 120*	1850 ± 140*	1390 ± 230*
	N	2390 ± 160	1690 ± 90*	1550 ± 120*	1900 ± 240*+•
LVEDP	S	8 ± 1	13 ± 1*	12 ± 1*	14 ± 2*
	N	10 ± 1	15 ± 1*	14 ± 1*	13 ± 1*•
SV	S	25 ± 2	17 ± 2*	18 ± 2*	14 ± 2*
	N	27 ± 2	18 ± 2*	19 ± 1*	16 ± 2*
SVR	S	43 ± 3	48 ± 4	46 ± 3	58 ± 10
	N	38 ± 2	40 ± 3	37 ± 2	40 ± 4
MW	S	225 ± 16	121 ± 16	118 ± 14*	69 ± 14*
	N	222 ± 18	130 ± 13	124 ± 11*	111 ± 15*••

*Data are presented as means ± SE; n=9 for both groups; S = solvent-treated, N = nisoldipine-treated; MAP = mean arterial blood pressure (mmHg); CO = cardiac output (l/min); HR = heart rate; $LVdP/dt_{max}$ = maximum rise in left ventricular pressure (mmHg/s); LVEDP = left ventricular end diastolic blood pressure (mmHg); SV = stroke volume (ml); SVR = systemic vascular resistance (mmHg/(L/min)); MW = myocardial work (mmHg.(L/min)); * P<0.05 versus baseline; + nisoldipine-induced changes versus baseline are significantly different from changes in the solvent-treated animals; • nisoldipine-induced changed versus 30 min of ischaemia are significantly different from changes in the solvent-treated animals; •• nisoldipine-induced changes versus 60 min of ischaemia are significantly different from changes in the solvent-treated animals.*

above baseline) prevented a more severe fall in mean arterial blood pressure (Table 1). During ischaemia there were similar decreases in mean arterial blood pressure, cardiac output, stroke volume and left ventricular dP/dt_{max} in the nisoldipine-treated animals (Table 1). During reperfusion cardiac output further declined; the decrease *versus* baseline was, however, significantly smaller than in the solvent-treated animals (Table 1). Mean arterial blood pressure did not further decrease and $LVdP/dt_{max}$ even increased ($P<0.05$ *versus* changes in solvent-treated animals). Left ventricular end diastolic pressure decreased from 14 ± 1 to 13 ± 1 mmHg, which was significantly different from the increase, observed in the solvent-treated animals. Heart rate was not affected and stroke volume decreased similarly as in the solvent-treated animals.

Regional myocardial perfusion and myocardial oxygen consumption The microsphere data revealed that inflation of the balloon had caused similar decreases in transmural myocardial blood flow in the solvent-treated (from 82 ± 5 ml/min/100g to 19 ± 9 ml/min/100g) and the nisoldipine-treated animals (from 87 ± 12 to 14 ± 8 ml/min/100g, Fig. 1) during the first 30 min of ischaemia. There were no further changes in the solvent-treated animals during the following 30 min of LADCA stenosis (LADCA blood flow was 19 ± 6 ml/min/100g at 30 min of ischaemia). In the nisoldipine-treated animals myocardial blood flow increased from 14 ± 8 ml/min/100g to 24 ± 6 ml/min/100g. After 2 h of reperfusion transmural myocardial blood flow of the LADCA-perfused area had increased to 41 ± 6 ml/min/100g and 76 ± 14 ml/min/100g in the solvent-treated and the nisoldipine-treated animals, respectively ($P<0.05$ *versus* 60 min of ischaemia; Fig. 1).

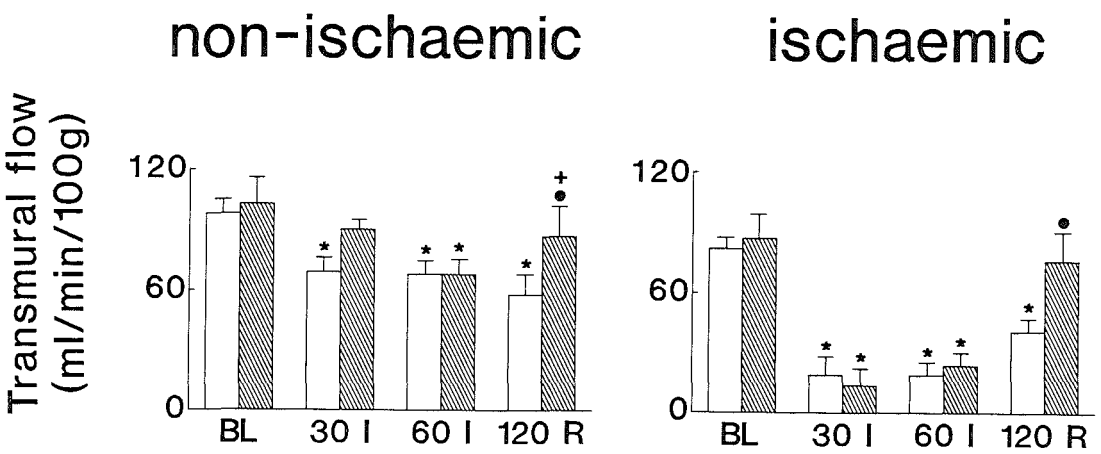

Figure 1 *Transmural myocardial blood flow (ml/min/100 g) of non-ischaemic and ischaemic myocardium in solvent-treated (open bars, n=9) and nisoldipine-treated (hatched bars, n=9) pigs at baseline (BL), after 30 and 60 min of ischaemia (I), and 120 min of reperfusion (R). * P<0.05 versus baseline, + changes versus baseline in the nisoldipine-treated animals are significantly different from the changes versus baseline in the solvent-treated animals; • changes versus 60 min of ischaemia in the nisoldipine-treated animals are significantly different from the changes versus 60 min of ischaemia in the solvent-treated animals.*

In both the solvent- and the nisoldipine-treated animals transmural blood flow to the non-ischaemic area decreased slightly during ischaemia, secondary to the fall in arterial blood pressure. However, during reperfusion, vasodilatation prevented perfusion to decrease parallel to the fall in perfusion pressure in the nisoldipine-treated but not in the solvent-treated pigs.

Baseline values of myocardial oxygen consumption of the LADCA-perfused myocardium were 2.9 ± 0.3 ml O_2/min/g and 3.5 ± 0.5 ml O_2/min/g in the solvent-treated and the nisoldipine-treated animals, respectively. After 2 h of reperfusion these respective values were 0.56 ± 0.06 and 1.18 ± 0.30 ml O_2/min/g ($P>0.05$).

Regional myocardial wall function. Reduction of coronary blood flow totally abolished systolic wall thickening of the segment perfused by the LADCA in both groups (baseline values in the nisoldipine-treated and the solvent-treated animals were 31 ± 4% and 33 ± 4%, respectively), which did not change in either group neither during the remainder of the ischaemic episode nor during the reperfusion period. PSWT of the ischaemic myocardium after 30 and 60 min of ischaemia was 11 ± 1% and 8 ± 1% in the solvent-treated, and 10 ± 2% and 10 ± 2% in the nisoldipine-treated animals, respectively.

High energy phosphates. During ischaemia and reperfusion there were no changes in the levels of ATP, adenine nucleotides and the energy charge in the non-ischaemic myocardium of both the solvent- and the nisoldipine-treated animals (Figs. 2 and 3). The concentrations of CP and total creatine decreased in the nisoldipine-treated animals while there was a slight increase in the solvent-treated animals ($P<0.05$; Figs. 2 and 3). After 2 h of reperfusion the differences between groups in concentrations of CP and total creatine were no longer present.

In the LADCA-perfused myocardium of the solvent- and the nisoldipine-treated animals 30 min of flow reduction caused decreases in ATP (by 66% and 68%, respectively), CP (by 70% and 73%, respectively) and total adenine nucleotides (47% and 48%, respectively), while the energy charge decreased from 0.90 ± 0.01 and 0.91 ± 0.01 in the nisoldipine-and solvent-treated animals, respectively to 0.69 ± 0.04 in both groups. During the following 30 min of ischaemia in the solvent- but not in the nisoldipine-treated animals the level of ATP and the energy charge further decreased. Furthermore, the increase in CP to 92 ± 24% of baseline observed in the nisoldipine-treated animals was absent in the solvent-treated pigs. The total creatine pool showed no changes in the solvent-treated animals during ischaemia, while a slight but significant decrease was observed in the nisoldipine-treated animals after 30 min of ischaemia. After 2 h of reperfusion no recovery of the levels of ATP and total adenine nucleotides was observed in either group, while those of CP were at baseline and the energy charge had recovered similarly in both groups to 0.83 ± 0.03. The level of total creatine remained 25% and 35% below baseline in the nisoldipine- and the solvent-treated animals, respectively.

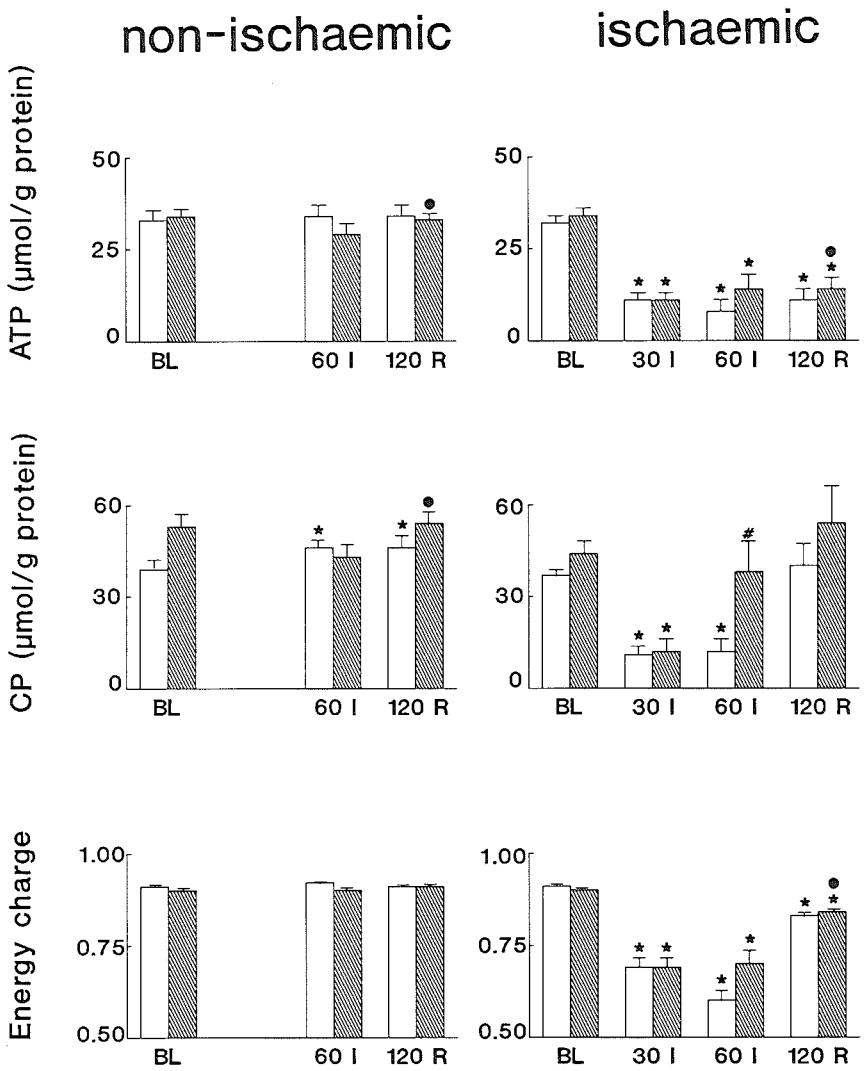

Figure 2 *ATP and CP (µmol/g protein) and energy charge ((ATP + ½ADP) / (ATP + ADP + AMP)) of the ischaemic and non-ischaemic myocardium of solvent-treated (open bars, n=9) and nisoldipine-treated pigs (hatched bars, n=9) at baseline (BL), at 30 min (only for the ischaemic segment) and 60 min of ischaemia (I) and after 120 min of reperfusion (R). * P<0.05 versus baseline; + changes versus baseline in the nisoldipine-treated animals are significantly different from the changes versus baseline in the solvent-treated animals; # changes versus 30 min of ischaemia in the nisoldipine-treated animals are significantly different from changes versus 30 min of ischaemia in the solvent-treated animals; • changes versus 60 min of ischaemia in the nisoldipine-treated animals are significantly different from the changes versus 60 min of ischaemia in the solvent-treated animals.*

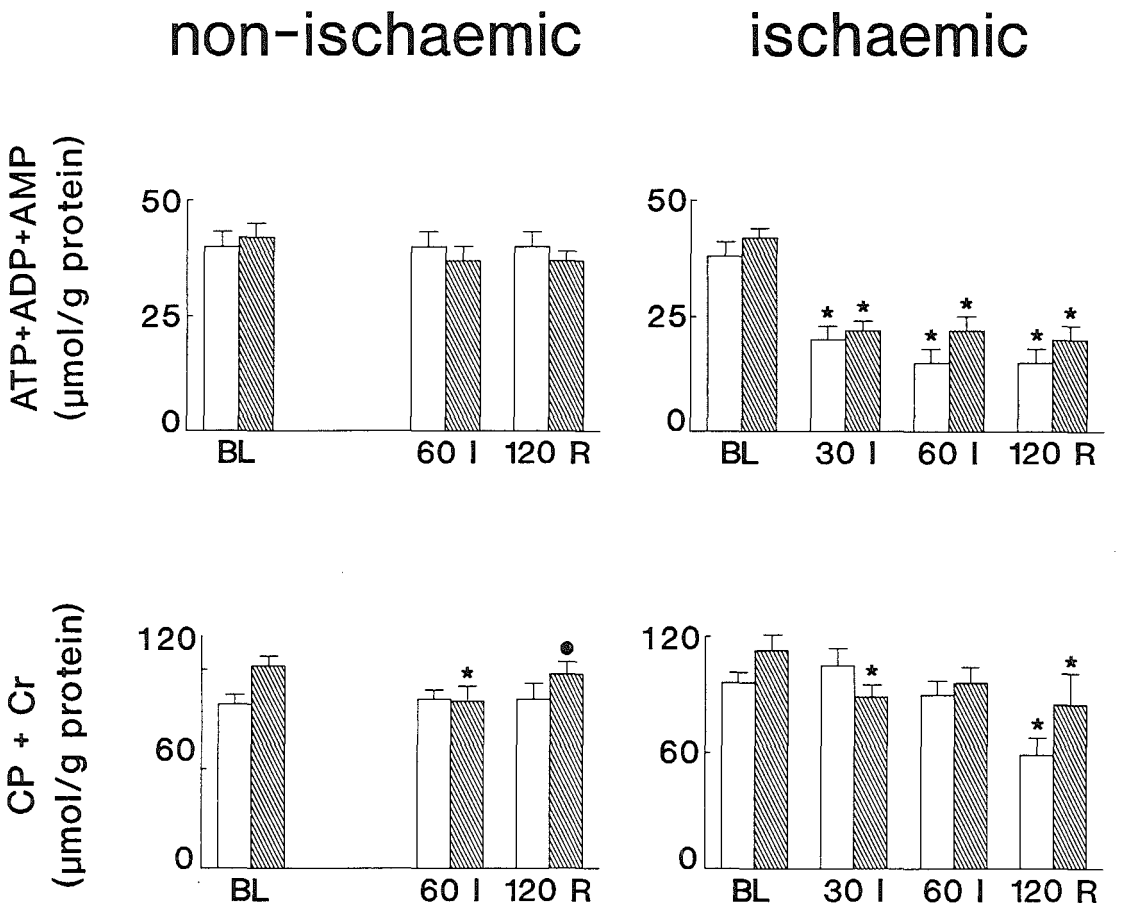

Figure 3 *Total adenine nucleotides (ATP + ADP + AMP) and creatine (CP + creatine) of the non-ischaemic and ischaemic myocardium in solvent-treated (open bars, n=9) and nisoldipine-treated (hatched bars, n=9) pigs at baseline (BL), at 30 min (only for the ischaemic segment) and 60 min of ischaemia (I) and after 120 min of reperfusion ((R). * P<0.05 versus baseline; + changes versus baseline in the nisoldipine-treated animals are significantly different from the changes versus baseline in the solvent-treated animals; • changes versus 60 min of ischaemia in the nisoldipine-treated animals are significantly different from the changes versus 60 min of ischaemia in the solvent-treated animals.*

Ca^{2+}uptake and phospholamban phosphorylation

Ca^{2+} uptake and *in vitro* phosphorylation of phospholamban could only be determined at the end of reperfusion because of the amount of myocardium (5 g), needed for the isolation of

sufficient amounts of SR membrane vesicles. The rate of Ca^{2+} uptake of the non-ischaemic segment of the nisoldipine-treated animals was 61% higher ($P<0.05$) than that of the solvent-treated animals, and phospholamban phosphorylation, on the other hand, was 33% lower ($P<0.05$; Table 2). The rates of Ca^{2+} uptake of SR vesicles isolated from post-ischaemic segments were not different: 531 ± 94 nmol/min/mg and 727 ± 212 nmol/min/mg in the solvent-treated and the nisoldipine-treated animals, respectively (Table 2). The *in vitro* ^{32}P incorporation remained 43% lower in the nisoldipine-treated (765 ± 96 pmol/mg) compared to the solvent-treated animals (1336 ± 263 pmol/mg; Table 2).Ca^{2+} uptake and phospholamban phosphorylation.

Table 2 *Effect of nisoldipine or its solvent on Ca^{2+}uptake and in vitro ^{32}Pincorporation into phospholamban of sarcoplasmic reticulum isolated from post-ischaemic myocardium in open-chest anaesthetized pigs*

		Ca^{2+}-uptake (nmol/min/mg)	^{32}P incorporation (pmol/mg)
Non-ischaemic myocardium	S	830 ± 117	1605 ± 170
	N	1335 ± 107*	1083 ± 48*
Post-ischaemic myocardium	S	531 ± 94	1336 ± 263
	N	727 ± 212	765 ± 96*

*Data are presented as means ± SE; S = solvent-treated, (n = 7); N = nisoldipine-treated, (n = 9); * $P<0.05$ versus solvent-treated animals*

Discussion

The present study demonstrates that nisoldipine increased post-ischaemic myocardial blood flow. This could be due to a direct coronary vasodilatory action of the drug, since vascular resistance of the non-ischaemic myocardium decreased (from 0.91 ± 0.09 mmHg/(ml/min/100g) to 0.74 ± 0.10 mmHg/(ml/min/100 g); $P<0.05$ *versus* baseline) in the nisoldipine-treated animals, whereas there was no change in the solvent-treated animals (baseline 1.02 mmHg/ml/min/100 g). However, the higher myocardial oxygen demand reflected by the higher double-product (heart rate times systolic arterial pressure) in the nisoldipine-treated animals (94.10^2 ± 11.10^2 mmHg.beats/min *versus* 71.10^2 ± 11.10^2 mmHg.beats/min in the solvent-treated pigs) also contributes to the vasodilatation of the

coronary bed of the non-ischaemic myocardium.

The failure of tissue to reperfuse after a transient ischaemic period has been called the no-reflow phenomenon. The higher blood flow in the post-ischaemic myocardium in the nisoldipine-treated animals could also be secondary to attenuation of this phenomenon by nisoldipine. The mechanisms by which nisoldipine can preserve cardiomyocytes and endothelial cells are diverse. Takahashi and Kako [21] demonstrated that nisoldipine was capable of suppressing the ischaemia-induced increase in phospholipid breakdown of canine cardiac sarcolemma. It has also been shown that nisoldipine decreased transcoronary extravasation [12] and the authors postulated that secondary to a reduction in Ca^{2+} uptake during reperfusion, nisoldipine prevented endothelial deformation and formation of interendothelial gaps. We found that nisoldipine caused a significant increase in CP already during ischaemia. The further decreases in the level of ATP and the energy charge, as observed in the saline-treated animals during the last 30 min of ischaemia, were prevented in the nisoldipine-treated pigs, albeit that these differences were not significant. Nevertheless, since the increase in CP must be a consequence of a more rapid rephosphorylation of ADP, the energy metabolism in the nisoldipine-treated animals during ischaemia must have been more favourable. This might have led to preservation of jeopardized cells contributing to the maintenance of the microvascular integrity.

Recently, Gross et al [22] investigated the effect of the dihydropyridine amlodipine on subendocardial segment length shortening, regional blood flow and myocardial high energy phosphate levels in coronary ligated (45 min of total occlusion) dogs followed by 2 h of reperfusion). LADCA blood flow during ischaemia and systemic haemodynamics in that study were similar as those in the present study. However, at variance with the present study, Gross *et al.* observed a marked and sustained improvement in systolic wall function at the end of the reperfusion period. The authors postulated that a positive inotropic action, as also observed for the Ca^{2+} antagonist felodipine [23], might have reversed myocardial stunning, thereby attenuating the rebound in CP.

In the present study the higher post-ischaemic blood flow in the nisoldipine-treated animals was not accompanied by a return of systolic contractile function during early reperfusion. The explanation for this can be fourfold. Firstly, a negative inotropic action of the drug prevented return of function. This is highly unlikely as in pigs this dose of nisoldipine does not cause negative inotropy [24]. Secondly, the myocardial tissue is irreversibly injured. Garcia-Dorado et al. [25] have shown (by tetrazolium-staining) that in pigs 30-40% of the myocardium at risk was still viable after 1 h of total coronary artery occlusion. In the present study we reduced coronary blood flow to 15-20% of baseline during the 60 min of ischaemia and a significant fraction of the affected myocardium must therefore ultimately (even after days) recover in function. Thirdly, the low energy charge of the post-ischaemic myocardium prevented contractile function. However, it has repeatedly been demonstrated that enhanced recovery of function occurs while the ATP-levels are still low [26-28]. Finally, Krause et al. [29] have suggested that the inability of the stunned myocardium to function normally is due to a slight reduction of the activity of the Ca^{2+} pump.

Ca^{2+}-uptake in the non-ischaemic myocardium of the nisoldipine-treated pigs was higher than that of the solvent-treated animals. A direct stimulation of the pump by nisoldipine could

be involved since it has been demonstrated that dihydropyridines are capable of stimulating Ca^{2+} uptake of canine cardiac sarcoplasmic reticulum [7] and interestingly also of porcine cardiac sarcolemma [31]. On the other hand, this mechanism of action is expected to be masked by the SR isolation procedure, during which drugs can be removed from their target proteins or phospholipids. However, due to the lipophilic nature of the dihydropyridines [30], their removal from the phospholipid bilayer may have been retarded. The activation of the Ca^{2+} pump could also be secondary to a ß-adrenoceptor-mediated mechanism. In the nisoldipine-treated animals the $LVdP/dt_{max}$ was significantly increased and there was a tendency for the heart rate to increase, both suggesting a higher level of ß-adrenoceptor activity. The nisoldipine-induced decrease in *in vitro* ^{32}P incorporation of phospholamban, found in the non-ischaemic myocardium, is in agreement with an increased ß-adrenoceptor activity causing an *in vivo* phosphorylation of the protein, although some other facts are not in agreement with such an explanation: In ischaemic myocardium the cyclic AMP and ATP levels are reduced so that endogenous phosphorylation of the protein most likely occurs at a much slower rate. Furthermore, dephosphorylation of sarcolemmal phospholamban has been shown to occur during the homogenization and fractionation of the myocardial tissue that was needed for isolation of the membrane vesicles. Therefore, to definitely prove that the increase of Ca^{2+} uptake rate and decrease of *in vitro* ^{32}P incorporation in myocardium by nisoldipine is due to increased ß-adrenoceptor activity, the phosphorylation of phospholamban should be measured *in vivo*.

A lower *in vitro* ^{32}P incorporation into phospholamban in ischaemic myocardium has been associated with a modification of the structure or membrane component of the protein [3,18,32]. Evidence has been obtained that the reduction in sarcoplasmic reticulum Ca^{2+} pump is causally related with the modified properties of phospholamban [3]. We found a reduced Ca^{2+} uptake and *in vitro* ^{32}P incorporation into phospholamban of the ischaemic segments of both the solvent- and the nisoldipine-treated animals. However, the effects of nisoldipine on Ca^{2+} uptake and *in vitro* ^{32}P-incorporation into phospholamban observed in the ischemic myocardium tend to be similar to those in the non-ischaemic myocardium. Therefore, the interpretation of the nisoldipine-induced effects on SR function during ischaemia-reperfusion are complicated by a possible direct or ß-adrenoceptor-mediated action of the drug.

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Chapter 5

The central and regional cardiovascular responses to intravenous and intracoronary administration of the phenyldihydropyridine elgodipine in anaesthetized pigs.

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The central and regional cardiovascular responses to intravenous and intracoronary administration of the phenyldihydropyridine elgodipine in anaesthetized pigs

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1 The central and regional cardiovascular responses to intravenous (0.3, 1.0, 3.0 and 10.0 $\mu g\,kg^{-1}\,min^{-1}$) and intracoronary (0.3, 0.9, 3.0 and 4.5 $\mu g\,kg^{-1}\,min^{-1}$) infusions of elgodipine, a phenyldihydropyridine, and its solvent were studied in anaesthetized pigs.

2 Elgodipine (i.v.) caused dose-dependent decreases in arterial blood pressure (up to 44%) and systemic vascular resistance (up to 48%), whereas heart rate, LV dP/dt_{max}, left ventricular filling pressure, cardiac output and segment length shortening did not change. The absence of a negative inotropic effect with the employed doses was confirmed by the intracoronary infusions; with the lowest dose (0.3 $\mu g\,kg^{-1}\,min^{-1}$) both LV dP/dt_{max} and segment length shortening decreased by less than 10%. With 0.9 $\mu g\,kg^{-1}\,min^{-1}$ (intracoronary) the negative inotropic properties of the drug became apparent as LV dP/dt_{max} and segment length shortening decreased by 20% and 33%, respectively, whereas heart rate and left ventricular filling pressure were not affected.

3 Transmural myocardial blood flow did not change during intravenous infusion of elgodipine, as vasodilatation, more pronounced in the subepicardial than in the subendocardial layers, compensated for the decrease in arterial perfusion pressure. The intracoronary infusions revealed that the decrease in normalized subendocardial/subepicardial blood flow ratio was not secondary to the fall in arterial blood pressure.

4 Myocardial oxygen consumption decreased during both the i.v. and the intracoronary administration of elgodipine. With the i.v. administration the decrease was secondary to the hypotensive action of the drug, whereas with the intracoronary administration the negative inotropic properties played the dominant role.

5 Elgodipine (i.v.), although not affecting total cardiac output, caused a redistribution in favour of the nutritional blood flow at the expense of the arteriovenous anastomotic (AVA) blood flow. Up to an infusion rate of 3.0 $\mu g\,kg^{-1}\,min^{-1}$ the decrease in AVA-flow was due to a fall in arterial blood pressure, but at the highest infusion rate both the decrease in arterial perfusion pressure and an increase in their resistance contributed to a further decrease in AVA blood flow.

6 The skeletal muscles benefited most from the elgodipine(i.v.)-induced increase in nutritional blood flow, but vasodilatation was not uniform for all muscle groups. Up to an infusion rate of 3 $\mu g\,kg^{-1}\,min^{-1}$ the vasodilatation in the renal vascular bed was more pronounced in the inner than in the outer cortex, but, at 10 $\mu g\,kg^{-1}\,min^{-1}$, vascular resistances of both cortical layers returned to baseline values. In all regions of the brain, blood flow was maintained until the highest infusion rate was given. With 10 $\mu g\,kg^{-1}\,min^{-1}$ only flow to the vital parts of the brain (diencephalon and brain stem) was maintained. Blood flows to the skin and various abdominal organs were well maintained up to 3 $\mu g\,kg^{-1}\,min^{-1}$ but, at the highest dose, a decrease was observed in blood flow to the adrenals and spleen. Vascular resistances of all these organs and tissues decreased dose-dependently.

7 The potent systemic and coronary vasodilator actions of elgodipine during i.v. administration, which were not accompanied by negative inotropic and positive chronotropic properties or decreases in the perfusion of vital organs, warrant further study as this compound could be useful in the treatment of essential hypertension, myocardial ischaemia and, possibly, moderate chronic heart failure.

Introduction

The effectiveness of the first generation of calcium antagonists (nifedipine, verapamil and diltiazem) in the treatment of a large number of cardiovascular disorders has led to the development of a new generation of calcium entry blockers, with more selective properties. Elgodipine (IQB-875) is a newly synthesized drug with calcium entry blocking properties (Tejerina *et al.*, 1989), belonging to the phenyldihydropyridine group (Figure 1). *In vitro* the drug proved to be 100 fold more potent in causing vasodilatation than in causing negative inotropic and chronotropic effects (Tejerina *et al.*, 1989). The *in vivo* effects of elgodipine on the cardiovascular system have not yet been evaluated and, thus, the aim of the present study was two fold.

Firstly, we studied the effects of elgodipine on systemic haemodynamics and on regional blood flows in anaesthetized pigs. Apart from the effects of elgodipine on the various layers of the myocardium, we also studied the effects of this drug on the several parts of the brain and the different layers of the kidney. As a calcium antagonist may have differential effects on skeletal muscles of different regions (Duncker *et al.*, 1986a), we also estimated elgodipine-induced changes in blood flow in six different muscle groups. Since it has been well established that, in anaesthetized animals, a large fraction of the cardiac output is shunted through arteriovenous anastomoses (AVA: Kaihara *et al.*, 1968; Verdouw *et al.*, 1980; 1982; Saxena & Verdouw, 1985a), which are abundantly present in the skin (Hales & Cliff, 1977; Saxena & Verdouw, 1985b), and that antihypertensive drugs may exert differential effects on the flow through these anastomoses (Hof & Hof, 1989), we paid

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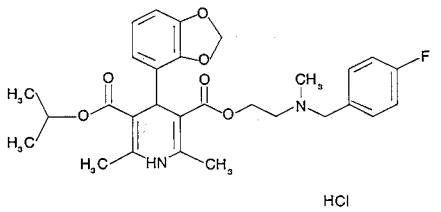

Figure 1 Chemical structure of elgodipine (IQB 875) a phenyldihydropyridine derivative with a molecular weight of 561.06. Isopropyl (2-(N-methyl-N-(4-fluorobenzyl)-amine)-ethyl,2,6-dimethyl-4-(2',3'-methylenedioxyphenyl)-1.5-dihydropyridine-3,5-dicarboxylate, monohydrochloride).

special attention to the effects of elgodipine on the division of cardiac output into its nutritional and non-nutritional fractions using the radioactive microsphere technique. As the microspheres (of 15 μm diameter), which pass through the AVA, will be trapped by the lungs, this non-nutritional fraction can be determined by counting the radioactivity in the lungs (Saxena & Verdouw, 1982; Hof & Hof, 1989).

Secondly an attempt was made to separate the direct from the indirect (that is, those secondary to changes in systemic haemodynamics) effects of elgodipine on myocardial contractility and on distribution of coronary blood flow. This was done by infusing the drug directly into a coronary artery.

Methods

After an overnight fast, cross-bred Landrace × Yorkshire pigs of either sex (22–28 kg, $n = 27$) were sedated with 120 mg azaperone (Stresnil) i.m., anaesthetized with 150 mg metomidate (Hypnodil) i.v., intubated and connected to a respirator for intermittent positive pressure ventilation with a mixture of oxygen and nitrous oxide (1:2). Respiratory rate and tidal volume were set to keep arterial blood gases within the normal range: $7.35 < pH < 7.45$; $35\,mmHg < PCO_2 < 45\,mmHg$ and $110\,mmHg < PO_2 < 180\,mmHg$. Catheters (7F) were placed in the superior vena cava for administration of 160 mg kg^{-1} α-chloralose followed by an infusion of a low dose of sodium pentobarbitone (5 mg kg^{-1} h^{-1}); for administration of the muscle relaxant pancuronium bromide (4 mg) before thoracotomy; and for administration of haemaccel (Behringwerke A.G., Marburg, F.R.G.) to replace blood loss. Catheters were also positioned in the descending aorta for withdrawal of blood samples and measurement of central aortic blood pressure. A microtipped catheter (7F Millar), inserted via the left carotid artery, was used to measure left ventricular pressure and its first derivative (LV dP/dt). After thoractomy, an electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the ascending aorta, while the great cardiac vein was cannulated for collection of blood in which the haemoglobin concentration and oxygen saturation were determined (by an OSM2 system, Radiometer, Copenhagen, Denmark). In 13 of the animals the proximal left anterior descending coronary artery was dissected free for placement of an electromagnetic flow probe around the vessel, while a cannula was inserted for intracoronary infusions of elgodipine.

In order to determine regional blood flows, the left atrial appendage was cannulated for injection of a batch of 1–2 × 10^6 carbonized plastic microspheres (15 ± 1 μm (s.d.) in diameter) labelled with either ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce. Fifteen seconds 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. At the end of each experiment in the animals which received intracoronary infusions of drug or solvent, the area perfused by the left anterior descending coronary artery was identified by intracoronary injection of patent blue violet (Sigma, St. Louis, MO, U.S.A.). After the i.v. treated animals had been killed with an overdose of sodium pentobarbitone, various organs (adrenals, bladder, liver, stomach, small intestine, brains and kidneys) and tissues (abdominal skin, various muscle groups) were excised, weighed and put into vials. In the animals given intracoronary infusions only the heart was excised. The hearts were fixed with formaldehyde (10% v/v) and 48 h later divided into atria, right and left ventricle. The myocardium of the left ventricle was divided into 3 layers of equal thickness: subepicardium, mesocardium and subendocardium. The kidneys were divided into the medulla and 3 cortical layers of equal thickness. Of the brains, the cerebral hemispheres, the cerebellum, the diencephalon and the brain stem were removed in order to obtain blood flow data for the different parts of the brain.

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

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

where I_{tis} and I_{art} are, respectively, the radioactivity (c.p.m.) in a particular tissue and that of the arterial blood sample, while Q_{art} is the rate of withdrawal of the blood sample. The resistance of a particular tissue was calculated as the ratio between the mean arterial blood pressure and the Q_{tis}. Full details of the procedures and the calculation of flow data with this technique have been described previously (Duncker *et al.*, 1986a).

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

Regional myocardial segment length shortening was measured by sonomicrometry (Triton Technology Inc., San Diego, CA, U.S.A.) by a pair of ultrasonic crystals implanted approximately 10–15 mm apart in the subepicardial layer. From the tracings, 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

Four consecutive 10 min i.v. infusions of elgodipine (0.3, 1.0, 3.0 and 10 μg kg^{-1} min^{-1}; $n = 7$) and equal volumes of the solvent ($n = 7$) or 4 consecutive 10 min intracoronary infusions of elgodipine (0.3, 0.9, 3.0 and 4.5 μg kg^{-1} min^{-1}; $n = 7$) or equal volumes of the solvent ($n = 6$) were administered. Systemic haemodynamics, regional myocardial function and the distribution of coronary blood flow were determined in all 4 series of experiments, but the distribution of cardiac output was only determined during the i.v. infusions of elgodipine or its solvent.

Statistical analysis

All data have been presented as the arithmetic mean ± s.e.mean. The significance of the effects of the solvent or elgodipine on the different variables was evaluated by Duncan's new-multiple range-test once an analysis of variance (randomized block design) had revealed that the samples represented different populations. The baseline values and the effects of the solvent were compared similarly but, in this case, one-way analysis of variance was used. Statistical significance was defined as $P < 0.05$ (two-tailed).

Drugs

Except for the anaesthetics given during surgery the only drugs used in this study were elgodipine (by courtesy of Dr A. Galiano, Instituto de Investigacion y Desarrollo, Madrid, Spain). For the i.v. infusions in the anaesthetized pigs, elgodipine was dissolved in 3% (v/v) ethanol for the highest

Table 1 Systemic haemodynamics after cumulative 10 min intravenous infusions of elgodipine or its solvent in anaesthetized pigs

			Elgodipine ($\mu g\,kg^{-1}\,min^{-1}$) or equal volumes of its solvent ($2\,ml\,min^{-1}$)				
		Baseline values	*0.3*	*1.0*	*3.0*	*10.0*	*Recovery*
HR	Solvent	92 ± 7	91 ± 7	92 ± 7	92 ± 7	89 ± 7	
	Elgodipine	102 ± 7	101 ± 7	100 ± 9	103 ± 9	106 ± 11	112 ± 9†
MAP	Solvent	88 ± 5	86 ± 4	85 ± 5	85 ± 5	84 ± 5†	
	Elgodipine	91 ± 3	88 ± 4	80 ± 5*	63 ± 5*	51 ± 3*	72 ± 4†
CO	Solvent	2.4 ± 0.2	2.4 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	2.3 ± 0.2	
	Elgodipine	2.5 ± 0.3	2.4 ± 0.3	2.4 ± 0.3	2.5 ± 0.3	2.7 ± 0.4	2.6 ± 0.3
SVR	Solvent	38 ± 3	37 ± 3	38 ± 3	38 ± 3	38 ± 2	
	Elgodipine	40 ± 5	41 ± 5	38 ± 4*	28 ± 2*	21 ± 2*	32 ± 3†
LV dP/dt_{max}	Solvent	2230 ± 180	2240 ± 190	2230 ± 190	2180 ± 180	2180 ± 170	
	Elgodipine	2780 ± 350	2880 ± 330	2820 ± 340	2640 ± 360	2540 ± 470	3220 ± 410
LVEDP	Solvent	7 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1	
	Elgodipine	8 ± 1	7 ± 1	8 ± 1	7 ± 1	7 ± 1	6 ± 1

Data are presented as means ± s.e.mean, $n = 7$ for both groups. HR = heart rate (beats min^{-1}); MAP = mean arterial blood pressure (mmHg); CO = cardiac output ($l\,min^{-1}$); SVR = systemic vascular resistance ($mmHg\,min\,l^{-1}$); LV dP/dt_{max} = maximal rate of rise of left ventricular blood pressure ($mmHg\,s^{-1}$); LVEDP = left ventricular end-diastolic blood pressure (mmHg); Recovery = data obtained 45 min after the highest infusion rate had been stopped.

* Drug-induced changes from baseline significantly different ($P < 0.05$) from the solvent-induced changes from baseline.

† $P < 0.05$ vs baseline values (only presented for solvent and Recovery).

($10\,\mu g\,kg^{-1}\,min^{-1}$) infusion rate and subsequently further diluted with distilled water to give the lower doses. The infusion rate was $2\,ml\,min^{-1}$ for all doses. For the intracoronary infusions the elgodipine was dissolved in 1% (v/v) ethanol and the required doses were reached by adjusting the infusion rate (from $0.13\,ml\,min^{-1}$ to $2\,ml\,min^{-1}$). Preparation of the solutions, and infusion of the drug, took place while the drug was protected from light.

Results

Intravenous infusions of elgodipine

Systemic haemodynamics No significant changes occurred during infusion of the solvent in any of the systemic haemodynamic variables, except for a slight decrease (<4%) in arterial blood pressure (Table 1). The most striking effect of elgodipine was a dose-dependent lowering of the arterial blood pressure, which was already significant during infusion of $1\,\mu g\,kg^{-1}\,min^{-1}$. The fall in arterial blood pressure (up to 44%) was caused by systemic vascular dilatation as cardiac output was not affected. Heart rate, left ventricular dP/dt_{max} and left ventricular end-diastolic blood pressure were also unaffected. Forty-five min after the infusions had been stopped, arterial blood pressure and systemic vascular resistance showed signs of recovery, but were still lower (21% and 20%, respectively) than baseline.

Myocardial blood flow and performance The solvent had no effect either on the transmural blood flow and its distribution over the subendocardial and the subepicardial layers or on coronary vascular resistance during the course of the experiments (Figure 2). Elgodipine had a minor effect on left ventricular myocardial blood flow; only the increases (up to 30%) in the subepicardial layers during the two highest infusion rates reached levels of statistical significance (Figure 2). In view of the decrease in arterial perfusion pressure, it can be calculated that vasodilatation occurred in all layers of the myocardium since there were decreases in the coronary vascular resistance (that is, the ratio of mean arterial blood pressure and local blood flow); the decrease in the subepicardium (up to 50%) was more pronounced than the decrease (up to 25%) in the subendocardium (Figure 2). Forty-five min after termination of the highest infusion rate, overall coronary vascular resistance was still 35% lower than during baseline.

In the solvent-treated animals there was a minor decrease (5%, $P < 0.05$) in myocardial oxygen extraction, reflected by the small increase (from 22 ± 1% to 27 ± 2%, $P < 0.05$) in the oxygen saturation in the great cardiac vein. With elgodipine, the decrease (35% after the highest infusion rate) in oxygen extraction was considerably larger as the coronary venous oxygen saturation more than doubled (from 22 ± 3% to 48 ± 4%, $P < 0.05$). The decrease in myocardial oxygen extraction was secondary to the decrease in oxygen demand (Figure 3).

Myocardial segment length shortening did not change either during infusion of the solvent (19 ± 1%, 18 ± 1%, 19 ± 1%, 19 ± 1% and 20 ± 2% at baseline and after 10, 20, 30 and 40 min, respectively) or during infusion of the drug (19 ± 1%, 19 ± 2%, 20 ± 1%, 19 ± 2%, 18 ± 1% and 19 ± 2% at baseline, during 0.3, 1.0, 3.0, 10.0 $\mu g\,kg^{-1}\,min^{-1}$ of elgodipine and after 45 min of recovery).

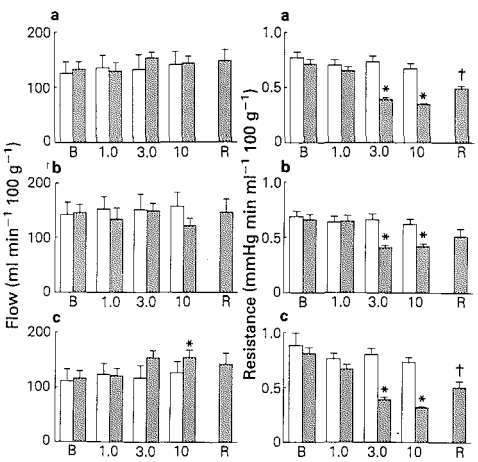

Figure 2 Effect of cumulative 10 min infusions of elgodipine (hatched columns, $n = 7$; 1, 3 and $10\,\mu g\,kg^{-1}\,min^{-1}$) and equal volumes ($2\,ml\,min^{-1}$) of its solvent (open columns, $n = 7$) on (a) transmural left ventricular, (b) subepicardial and (c) subendocardial blood flows and resistances in anaesthetized pigs. Data are presented as mean and the bars show s.e.mean.* The drug-induced changes from baseline are significantly different from the solvent-induced changes from baseline. †$P < 0.05$ vs baseline (determined only for the solvent-treated animals and after 45 min of recovery in the elgodipine-treated animals). B = baseline. R = data obtained 45 min after the infusions had been stopped.

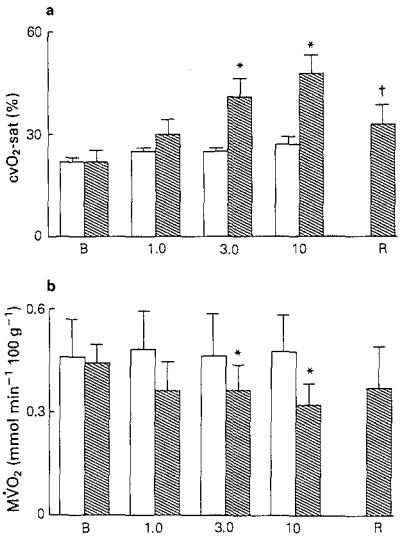

Figure 3 Effect of cumulative 10 min infusions of elgodipine (hatched columns, $n = 7$) or equal volumes (2 ml min^{-1}) of its solvent (open columns, $n = 7$) on (a) coronary venous oxygen saturation (cvO_2-sat) and (b) left ventricular oxygen consumption ($M\dot{V}O_2$). Data are presented as mean and the bars show s.e.mean.* The drug-induced changes from baseline are significantly different from the solvent-induced changes from baseline. †$P < 0.05$ vs baseline (determined only for the solvent-treated animals and after 45 min of recovery in the elgodipine-treated animals). B = baseline. R = data obtained 45 min after the infusions had been stopped.

Distribution of cardiac output

Fractionation of cardiac output into nutrient and non-nutrient flows Figure 4 illustrates that, under baseline conditions, a large fraction of the cardiac output was shunted through AVA. This fraction did not change during infusion of the solvent. Although elgodipine did not affect cardiac output, there was a redistribution in favour of the nutritional fraction. Forty-five min after the infusions were stopped, AVA-flow and resistance had returned to values close to baseline. At 1 and 3 $\mu g\,kg^{-1}\,min^{-1}$ elgodipine, the decrease in the non-nutrient, AVA, flow paralleled the decrease in mean arterial blood pressure, but at the highest infusion rate (10 $\mu g\,kg^{-1}\,min^{-1}$) constriction of the AVA also contributed to the lower AVA-flow (Figure 5).

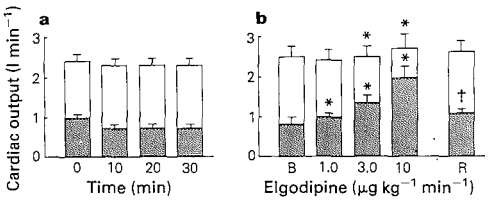

Figure 4 Fractionation of cardiac output in arteriovenous anastomotic (open column) and capillary (hatched column) blood flow during cumulative 10 min infusions of (b) elgodipine ($n = 7$) or (a) equal volumes (2 ml min^{-1}) of its solvent ($n = 7$) in anaesthetized pigs. Data are presented as mean and the bars show s.e.mean. *The drug-induced changes from baseline are significantly different from the solvent-induced changes from baseline. †$P < 0.05$ vs baseline (determined only for the solvent-treated animals and after 45 min of recovery in the elgodipine-treated animals). B = baseline. R = data obtained 45 min after the infusions had been stopped.

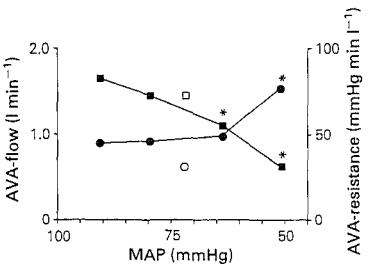

Figure 5 Arteriovenous anastomotic (AVA) blood flow (■, □) and resistance (●, ○) of the elgodipine-treated animals ($n = 7$) are plotted against the mean arterial blood pressure (MAP), measured at the end of each infusion rate. The open symbols are the data obtained 45 min after the infusions had been stopped. *$P < 0.05$ vs baseline.

Distribution of nutritional blood flow Table 2 shows that, in the kidneys, the normalized flow both to the inner cortex and to the outer cortex, in which it was twice that of the inner cortex, did not change during solvent infusion. No effect of elgodipine on cortical flows was observed until the highest dose was reached. With the highest infusion rate (i.e. at lowest renal perfusion pressure) both cortical flows decreased, the largest effect being observed in the outer cortex. Medullary blood flow was not affected by any rate of infusion of elgodipine. Lowering of the renal vascular resistances with the two lower infusion rates prevented a fall in blood flow due to the lower perfusion pressure. During infusion of 10 $\mu g\,kg^{-1}\,min^{-1}$ elgodipine, renal vascular resistances started to increase and this, together with the lower perfusion pressure, caused a decrease in renal blood flow at this dose. All renal vascular resistances had returned to pre-drug values when the measurements were repeated after the 45 min recovery period. The outer cortical flow was, however, still significantly lower than the pre-drug values due to the lower perfusion pressure.

Elgodipine had no effect on regional cerebral blood flows until the highest dose was reached (Table 3). With the highest infusion rate there was a 10–15% decrease ($P < 0.05$) in blood flow to all regions of the brain. All regional cerebral blood flows except that in the cerebellum returned to baseline during the recovery period, although mean arterial blood pressure remained 20 mmHg below baseline.

Flows to a number of other organs, except for the adrenals and spleen, were also well maintained until the highest infusion rate of elgodipine (Table 4). The blood flow to the abdominal skin tended to increase, but the increase did not reach levels of significance.

The skeletal muscles benefited more from the increase in nutritional blood flow than any other tissue or organ. Figure 6 illustrates that elgodipine did not exert the same effect on all muscle groups. With the highest dose the increases in blood flow to the masseter (10 fold), to the iliopsoas (7 fold) and to the diaphragm (5 fold) were all significantly higher than to the abdominal muscle (2 fold). Forty-five min after the infusion had stopped, all muscle flows had decreased by approximately 50%. As the increases in muscle flows occurred in spite of the lower mean arterial blood pressures, the decreases in the resistances were even more pronounced than the increases in flow (Figure 7).

Intracoronary infusions of elgodipine

Systemic haemodynamics Infusion of the solvent had no effect on systemic haemodynamics (Table 5). The lower infusion rates of elgodipine (up to 0.9 $\mu g\,kg^{-1}\,min^{-1}$) only affected left ventricular dP/dt_{max} (−20%, $P < 0.05$). At the higher infusion

Table 2 Effect of cumulative 10 min infusions of elgodipine or its solvent on renal blood flow and resistance in anaesthetized pigs

		Elgodipine ($\mu g\,kg^{-1}\,min^{-1}$) *or equal volumes of its solvent* ($2\,ml\,min^{-1}$)				
		Baseline values	*1.0*	*3.0*	*10.0*	*Recovery*
Blood flow ($ml\,min^{-1}\,100\,g^{-1}$)						
Kidneys	Solvent	235 ± 25	232 ± 16	223 ± 19	226 ± 16	
	Elgodipine	231 ± 19	246 ± 28	256 ± 25	151 ± 24*	192 ± 23
Outer Cortex	Solvent	362 ± 33	360 ± 21	354 ± 25	357 ± 23	
	Elgodipine	349 ± 28	350 ± 38	347 ± 31	201 ± 32*	278 ± 35†
Inner Cortex	Solvent	207 ± 24	206 ± 18	194 ± 23	194 ± 20	
	Elgodipine	206 ± 19	226 ± 26	240 ± 25	142 ± 22*	174 ± 22
Medulla	Solvent	63 ± 6	61 ± 4	56 ± 5	55 ± 4	
	Elgodipine	66 ± 5	69 ± 7	79 ± 7	51 ± 6	58 ± 7
Resistance ($mmHg\,min\,ml^{-1}\,100\,g$)						
Kidneys	Solvent	0.40 ± 0.05	0.38 ± 0.04	0.40 ± 0.04	0.39 ± 0.04	
	Elgodipine	0.41 ± 0.04	0.36 ± 0.05	0.26 ± 0.03*	0.38 ± 0.06*	0.43 ± 0.09
Outer Cortex	Solvent	0.25 ± 0.02	0.24 ± 0.02	0.25 ± 0.02	0.24 ± 0.02	
	Elgodipine	0.28 ± 0.02	0.25 ± 0.03	0.19 ± 0.02*	0.29 ± 0.04*	0.31 ± 0.08
Inner Cortex	Solvent	0.47 ± 0.07	0.44 ± 0.05	0.47 ± 0.06	0.47 ± 0.06	
	Elgodipine	0.47 ± 0.04	0.39 ± 0.05	0.28 ± 0.03*	0.40 ± 0.05	0.47 ± 0.09
Medulla	Solvent	1.55 ± 0.28	1.47 ± 0.18	1.63 ± 0.26	1.61 ± 0.21	
	Elgodipine	1.45 ± 0.11	1.26 ± 0.14	0.87 ± 0.13*	1.07 ± 0.11*	1.46 ± 0.32

Data are presented as means ± s.e.mean, $n = 7$ for both groups.
Recovery = data obtained 45 min after the highest rate of infusion was stopped.
* Drug-induced changes from baseline are significantly different ($P < 0.05$) from the solvent-induced changes from baseline.
† $P < 0.05$ vs baseline values (only presented for solvent and Recovery).

Table 3 Effect of cumulative 10 min intravenous infusions of elgodipine or solvent on cerebral blood flow in anaesthetized pigs

		Elgodipine ($\mu g\,kg^{-1}\,min^{-1}$) *or equal volumes of its solvent* ($2\,ml\,min^{-1}$)				
		Baseline values	*1.0*	*3.0*	*10.0*	*Recovery*
Total brain	Solvent	29 ± 2	30 ± 2	30 ± 2	30 ± 2	
	Elgodipine	32 ± 1	31 ± 2	31 ± 2	27 ± 2*	28 ± 2†
Cerebral hemispheres	Solvent	29 ± 2	30 ± 2	29 ± 2	30 ± 1	
	Elgodipine	32 ± 1	31 ± 2	31 ± 2	27 ± 2*	28 ± 2
Diencephalon	Solvent	28 ± 2	30 ± 3	28 ± 2	28 ± 2	
	Elgodipine	27 ± 1	27 ± 2	28 ± 2	25 ± 1	26 ± 1
Cerebellum	Solvent	33 ± 2	35 ± 2	35 ± 3	34 ± 2	
	Elgodipine	38 ± 2	38 ± 3	37 ± 3	33 ± 3*	33 ± 2†
Brain stem	Solvent	26 ± 2	26 ± 2	26 ± 2	27 ± 2	
	Elgodipine	28 ± 2	25 ± 3	28 ± 2	26 ± 2	25 ± 2

Data are presented as mean ± s.e.mean, $n = 7$ for both groups. Flow data are presented in $ml\,min^{-1}\,100\,g^{-1}$. Recovery = data obtained 45 min after the highest rate of infusion was stopped.
* Drug-induced changes from baseline are significantly different ($P < 0.05$) from the solvent-induced changes from baseline.
† $P < 0.05$ vs baseline values (only presented for solvent and Recovery).

Table 4 Effect of cumulative 10 min intravenous infusions of elgodipine or solvent on regional blood flows in anaesthetized pigs

		Elgodipine ($\mu g\,kg^{-1}\,min^{-1}$) *or equal amounts of its solvent* ($2\,ml\,min^{-1}$)				
		Baseline values	*1.0*	*3.0*	*10.0*	*Recovery*
Adrenals	Solvent	135 ± 15	149 ± 11	147 ± 16	154 ± 21	
	Elgodipine	184 ± 14	159 ± 8	178 ± 18	134 ± 18*	209 ± 29
Skin	Solvent	1.1 ± 0.4	1.1 ± 0.5	0.7 ± 0.1	0.9 ± 0.4	
	Elgodipine	1.1 ± 0.3	1.1 ± 0.3	1.4 ± 0.3	1.7 ± 0.5	0.9 ± 0.2
Bladder	Solvent	4.1 ± 0.4	4.6 ± 0.3	4.6 ± 0.4	4.9 ± 0.5	
	Elgodipine	4.7 ± 0.5	4.9 ± 0.4	5.1 ± 0.7	3.7 ± 0.6	3.7 ± 0.8
Liver	Solvent	43 ± 4	34 ± 7	28 ± 7	27 ± 6	
	Elgodipine	39 ± 9	24 ± 7	17 ± 6	10 ± 2	14 ± 6†
Stomach	Solvent	20 ± 3	22 ± 3	20 ± 3	20 ± 3	
	Elgodipine	19 ± 2	23 ± 5	26 ± 6	21 ± 3	26 ± 2
Small intestine	Solvent	38 ± 5	35 ± 3	34 ± 4	35 ± 3	
	Elgodipine	39 ± 3	38 ± 3	35 ± 3	32 ± 3	38 ± 2
Spleen	Solvent	247 ± 45	241 ± 48	222 ± 48	246 ± 49	
	Elgodipine	273 ± 25	271 ± 27	257 ± 45	144 ± 31*	251 ± 51

Data are presented as mean ± s.e.mean, $n = 7$ for both groups. Flow data are presented in $ml\,min^{-1}\,100\,g^{-1}$. Recovery = data obtained 45 min after the highest dose was stopped.
* Drug-induced changes from baseline are significantly different ($P < 0.05$) from the solvent-induced changes from baseline.
† $P < 0.05$ vs baseline values (only presented for solvent and Recovery).

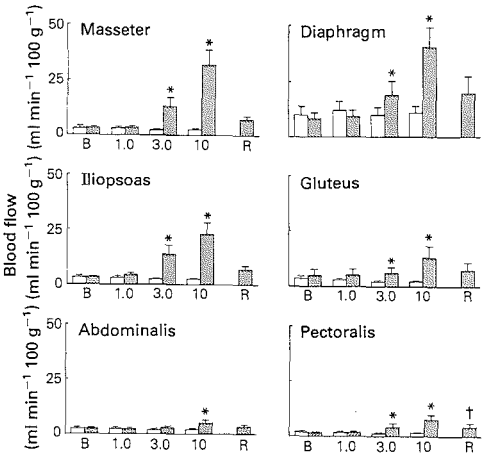

Figure 6 Effect of cumulative 10 min infusions of elgodipine (hatched columns, $n = 7$) or equal volumes (2 ml min^{-1}) of its solvent (open columns, $n = 7$) on blood flow to different muscle groups in anaesthetized pigs. Data are presented as mean and the bars show s.e.mean. *The drug-induced changes from baseline are significantly different from the solvent-induced changes from baseline. †$P < 0.05$ vs baseline (determined only for the solvent-treated animals and after 45 min of recovery in the elgodipine-treated animals). B = baseline. R = data obtained 45 min after the infusions had been stopped.

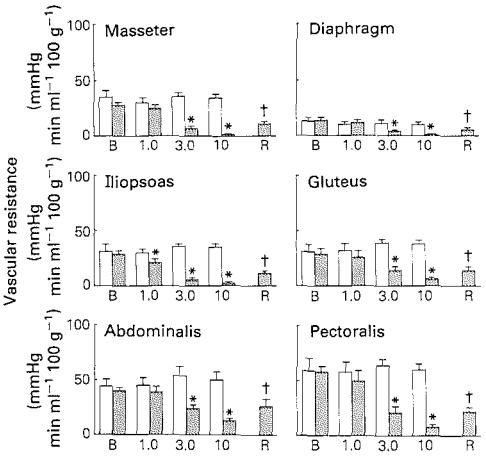

Figure 7 Effect of cumulative 10 min infusions of elgodipine (hatched columns, $n = 7$) or equal volumes (2 ml min^{-1}) of its solvent (open columns, $n = 7$) on the resistances of different muscle groups in anaesthetized pigs. Data are presented as mean and the bars show s.e.mean. *The drug-induced changes from baseline are significantly different from the solvent-induced changes from baseline. †$P < 0.05$ vs baseline (determined only for the solvent-treated animals and after 45 min of recovery in the elgodipine-treated animals). B = baseline. R = data obtained 45 min after the infusions had been stopped.

rates there were not only further decreases in LV dP/dt_{max} (−30%, $P < 0.05$), but also cardiac output (−15%), mean arterial blood pressure (−35%) and systemic vascular resistance (−25%) were significantly affected (Table 5). Sixty min after the highest rate of elgodipine infusion had stopped, all parameters had returned to baseline except for mean arterial blood pressure, which was still depressed (−10%).

Myocardial blood flow Intracoronary infusions of the solvent had neither an effect on myocardial perfusion (Figure 8), nor on myocardial resistance. Elgodipine increased transmural blood flow of the myocardium perfused by the left anterior descending coronary artery by up to 200% during infusion of 0.9 μg kg^{-1} min^{-1}. When the infusion rate was increased to 3 μg kg^{-1} min^{-1}, blood flow started to decrease and, at 4.5 μg kg^{-1} min^{-1}, transmural blood flow was not different from baseline (Figure 8). Although all layers benefited from the initial increase in transmural flow, the increments in the subepicardium (140 ml min^{-1} 100 g^{-1}) were considerably larger than in the subendocardium (100 ml min^{-1} 100 g^{-1}). The decreases in subendo- and subepicardial blood flows at the higher infusion rates were very similar. In the segment adjacent to the myocardium perfused by the left anterior descending coronary artery, the only significant effect was a 25% decrease in subendocardial blood flow at the highest infusion rate of elgodipine.

Table 5 Systemic haemodynamics after cumulative 10 min intracoronary infusions of elgodipine or its solvent in anaesthetized pigs

			Elgodipine (μg kg^{-1} min^{-1}) or equal volumes of its solvent (0.13, 0.39, 1.3, 2.0 ml min^{-1})				
		Baseline values	*0.3*	*0.9*	*3.0*	*4.5*	*Recovery*
HR	Solvent	103 ± 5	102 ± 4	102 ± 4	102 ± 4	106 ± 3	104 ± 3
	Elgodipine	102 ± 7	103 ± 8	104 ± 9	108 ± 9	108 ± 10	98 ± 9
MAP	Solvent	85 ± 6	83 ± 6	81 ± 5	81 ± 7	86 ± 7	83 ± 7
	Elgodipine	86 ± 5	83 ± 4	76 ± 6	63 ± 4*	57 ± 4*	77 ± 3*
CO	Solvent	2.8 ± 0.4	2.8 ± 0.3	2.7 ± 0.3	2.7 ± 0.3	2.8 ± 0.3	2.5 ± 0.4
	Elgodipine	2.8 ± 0.1	2.7 ± 0.2	2.5 ± 0.2	2.4 ± 0.2	2.4 ± 0.2*	2.6 ± 0.2
SVR	Solvent	32 ± 3	30 ± 3	31 ± 3	31 ± 3	32 ± 4	38 ± 5
	Elgodipine	31 ± 2	31 ± 3	32 ± 3	26 ± 2*	24 ± 2*	32 ± 3
SV	Solvent	27 ± 3	28 ± 3	27 ± 3	26 ± 3	27 ± 3	24 ± 4
	Elgodipine	28 ± 2	27 ± 3	25 ± 2	24 ± 2	22 ± 2*	27 ± 3
LV dP/dt_{max}	Solvent	2660 ± 280	2570 ± 260	2700 ± 360	2830 ± 440	3000 ± 460	2790 ± 330
	Elgodipine	2490 ± 329	2330 ± 280	2000 ± 290*	1970 ± 230*	1750 ± 240*	2460 ± 400
LVEDP	Solvent	6 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1	5 ± 1
	Elgodipine	8 ± 1	8 ± 1	9 ± 1	10 ± 1	10 ± 1	8 ± 1

Data are presented as mean ± s.e.mean, $n = 7$ for the elgodipine group and 6 for the solvent group.
HR = heart rate (beats min^{-1}); MAP = mean arterial blood pressure (mmHg); CO = cardiac output (l min^{-1}); SVR = systemic vascular resistance (mmHg min l^{-1}); SV = stroke volume (ml); LV dP/dt_{max} = maximal rate of rise of left ventricular blood pressure (mmHg s^{-1}); LVEDP = left ventricular end-diastolic blood pressure (mmHg); Recovery = data were obtained 60 min after infusion at the highest rate had been stopped.
* Drug-induced change from baseline significantly different ($P < 0.05$) from the solvent-induced change from baseline.

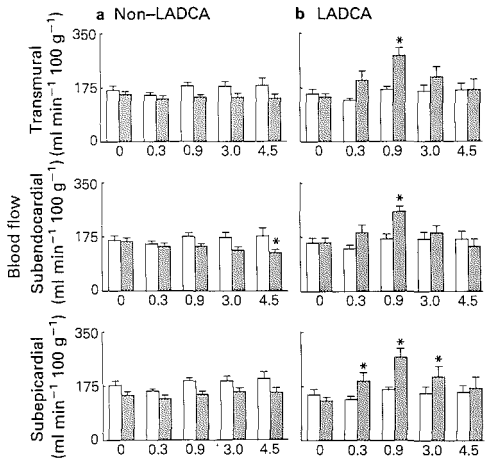

Figure 8 Myocardial blood flow after cumulative (0.3, 0.9, 3.0, 4.5 $\mu g\,kg^{-1}\,min^{-1}$) intracoronary infusions of elgodipine (hatched columns, $n = 7$) or its solvent (open columns, $n = 6$) directly into the left anterior descending coronary artery (LADCA) (b). (a) Non-LADCA = area adjacent to the LADCA-perfused myocardium. Data have been presented as mean and the bars show s.e.mean. *Elgodipine-induced changes from baseline are significantly different from solvent-induced changes from baseline.

Oxygen consumption and function of the myocardium perfused by the left anterior descending coronary artery The solvent had no effect on either the myocardial oxygen consumption (515 ± 26, 432 ± 27, 510 ± 36, 463 ± 25, $507 \pm 67\,\mu mol\,min^{-1}\,100\,g^{-1}$ at baseline and after 10, 20, 30 and 40 min of solvent infusion, respectively) or regional segment length shortening ($22 \pm 1\%$, $22 \pm 1\%$, $21 \pm 1\%$, $21 \pm 1\%$ and $21 \pm 1\%$ at baseline and after 10, 20, 30 and 40 min of solvent infusion). The oxygen saturation in the great cardiac vein was $23 \pm 2\%$, $49 \pm 4\%$, $66 \pm 3\%$, $71 \pm 2\%$ and $78 \pm 3\%$ (all $P < 0.05$ versus baseline) at baseline and after 0.3, 0.9, 3.0 and $4.5\,\mu g\,kg^{-1}\,min^{-1}$ of elgodipine, respectively, and returned to $29 \pm 3\%$ 60 min after the infusions had been stopped. The decrease in myocardial oxygen extraction was caused by a decrease in myocardial oxygen consumption (470 ± 48, 410 ± 42, 366 ± 46, 274 ± 47 and $200 \pm 44\,\mu mol\,kg^{-1}\,min^{-1}$ at baseline and after 0.3, 0.9, 3.0 and $4.5\,\mu g\,kg^{-1}\,min^{-1}$ of elgodipine, respectively). A representative example of the effects of elgodipine on segment length shortening is shown in Figure 9. At the lowest dose there was a slight but significant decrease in segment length shortening (from $18 \pm 2\%$ to $16 \pm 2\%$, $P < 0.05$). With increasing infusion rates there were further dose-dependent decreases (to $12 \pm 2\%$ and $7 \pm 3\%$ after 0.9 and $3.0\,\mu g\,kg^{-1}\,min^{-1}$ of elgodipine, respectively) and, after the highest dose, segment length shortening was almost completely abolished ($3 \pm 2\%$). Figure 10 illustrates that the reduction in myocardial oxygen consumption was closely related to the reduction in segment length shortening ($r = 0.99$, $P < 0.05$). During the wash-out period segment length shortening recovered gradually ($10 \pm 2\%$ and $15 \pm 3\%$ after 15 and 30 min, respectively) and had returned to pre-drug values ($18 \pm 2\%$) after 60 min.

Discussion

Systemic haemodynamics

The present study shows that elgodipine is a potent arterial vasodilator. With the same model, we have previously evaluated the effects not only of a number of other dihydropyridines such as nifedipine (Wolffenbüttel & Verdouw, 1983), nimodipine (Duncker *et al.*, 1986a), nisoldipine (Duncker *et al.*, 1986b) and bepridil (Verdouw & Scheffer, 1984) but also of vasodilators such as nicorandil (Verdouw *et al.*, 1987a), pimobendan (Verdouw *et al.*, 1986) and its *O*-demethyl metabolite UD-CG 212 Cl (Verdouw *et al.*, 1987b). Comparison of these studies reveals that the potency of elgodipine in reducing sys-

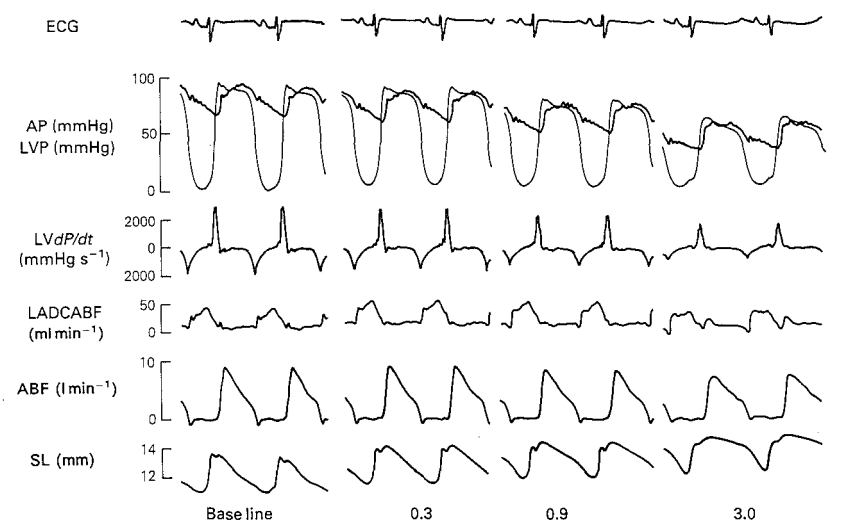

Figure 9 Representative tracing of the effects of intracoronary (left anterior descending coronary artery) infusions of elgodipine on systemic haemodynamics and regional myocardial blood flow and function in an anaesthetized pig. AP and LVP are central aortic and left ventricular blood pressure, respectively; LV dP/dt first derivative of LVP; LADCABF = left anterior descending coronary artery blood flow; ABF = ascending aortic blood flow; SL = segment length. The infusion rates of elgodipine ($\mu g\,kg^{-1}\,min^{-1}$) are given at the bottom of the figure.

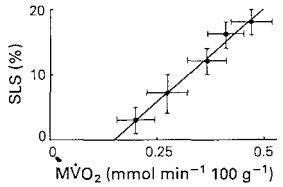

Figure 10 During the intracoronary infusion of elgodipine the reduction in local myocardial oxygen consumption ($M\dot{V}O_2$) is closely related ($r = 0.99$) to the decrease in segment length shortening (SLS). Data are presented as mean and the bars show s.e.mean.

temic vascular resistance is slightly less than that of nifedipine, nisoldipine and nimodipine, but considerably larger (10–50 fold) than that of bepridil, nicorandil, pimobendan and UD-CG 212 Cl. As mean arterial blood pressure was considerably lowered by elgodipine, a reflex mediated tachycardia would be expected to compensate for the hypotensive action of the drug. The absence of this tachycardia can partially be explained by the anaesthetic regimen used. It must, however, be kept in mind that, in the same preparation, nisoldipine, while causing similar decreases in arterial blood pressure, increased heart rate by up to 25% (Duncker *et al.*, 1986b). It is therefore likely that, as observed with nimodipine (Duncker *et al.*, 1986a), elgodipine possesses some negative chronotropic actions (Tejerina *et al.*, 1989) which counterbalance the reflex tachycardia. Interestingly, LV dP/dt_{max} was not affected by elgodipine despite the fall in arterial pressure. As heart rate and left ventricular filling pressure were also not affected it can be concluded that, in doses up to $10\,\mu g\,kg^{-1}\,min^{-1}$ *in vivo*, the compound does not exert negative inotropic actions. The absence of a negative inotropic effect of elgodipine became even more evident during the intracoronary infusions, as $0.3\,\mu g\,kg^{-1}\,min^{-1}$ substantially dilated the vascular bed of the left anterior descending coronary artery but had only a minor effect on regional contractile function, as reflected by the changes in local segment length shortening and LV dP/dt_{max}. With intracoronary administration of $0.9\,\mu g\,kg^{-1}\,min^{-1}$, the negative inotropic properties of elgodipine became apparent. In view of the small fraction (3–4%) of the cardiac output used for myocardial perfusion, a 30 times higher dose (approximately $30\,\mu g\,kg^{-1}\,min^{-1}$) may be needed to observe a negative inotropic effect with i.v. infusions. These doses will most likely not be clinically applicable because of severe hypotension.

Myocardial blood flow and function

Despite the decrease in arterial blood pressure, left ventricular blood flow was well maintained during the elgodipine infusions as a result of a direct vasodilator response to the drug, the effect being slightly more pronounced in the subepicardial layer (45% decrease in vascular resistance) than in the subendocardial layer (30% decrease in the vascular resistance). The preferential vasodilatation of the subepicardial layers by antihypertensive drugs, in particular when the fall in blood pressure is accompanied by a tachycardia, has been well established (Domenech & Goich, 1976; Feigl, 1983). The observation that intracoronary infusion of $0.3\,\mu g\,kg^{-1}\,min^{-1}$ elgodipine, which did not affect arterial blood pressure, exclusively increased blood flow to the subepicardial layers of the area perfused by the left anterior descending coronary artery indicates a preferential effect of the drug to give subepicardial vasodilatation.

With the i.v. infusions, the decrease in myocardial oxygen consumption was secondary to the hypotensive action of elgodipine. The much larger decrease in myocardial oxygen consumption with the intracoronary infusions was caused by the additional decrease in regional contractile function (Figure 10). Intracoronary administration of drugs, which locally decrease myocardial oxygen consumption while maintaining a global left ventricular function, may be useful during coronary angioplasty as they improve ischaemic tolerance during this procedure (Amende *et al.*, 1987). The data from the present study indicate that elgodipine can be a useful addition to the armament for such interventions.

Arteriovenous anastomotic blood flow

More than 20 years ago, Kaihara *et al.* (1968) showed that anaesthesia may open AVA, but relatively little attention has been paid to the effects of pharmacological agents on the fractionation of cardiac output into arteriovenous anastomotic and capillary blood flow (Verdouw *et al.*, 1980; Hof & Hof, 1989). The latter group studied the effect of several antihypertensive drugs on nutritional and non-nutritional blood flow and concluded that isradipine, a related compound to elgodipine, which decreased mean arterial blood pressure from 75 to 50 mmHg, also decreased AVA blood flow, but slightly increased the resistance of the AVA, probably due to sympathetic reflex activation (Hof & Hof, 1989). In the present study elgodipine had no effect on cardiac output but the fraction shunted through the anastomoses decreased dose-dependently. Up to $3\,\mu g\,kg^{-1}\,min^{-1}$ this was entirely due to a decrease in arterial blood pressure (Figure 4) as the resistance of the anastomoses was not affected. However, at the highest infusion rate, a doubling of the resistance of the AVA also contributed to a further fall in AVA-flow. As suggested by Hof & Hof (1989), this increase in resistance may be reflex-mediated, but as this increase occurs at the lowest mean arterial blood pressure (Figure 5), we cannot entirely exclude the possibility that the lumen diameter of the AVA decreased due to hypotension.

Regional blood flows

The largest increases in capillary blood flow were found in the skeletal muscle although, as with nimodipine (Duncker *et al.*, 1986a), we observed some regional differences.

With similar decreases (25–30%) in perfusion pressure elgodipine, like nisoldipine (Duncker *et al.*, 1986b) did not affect cerebral blood flow, whereas nimodipine caused a 20% decrease and nicorandil doubled cerebral blood flow. When, after elgodipine infusions, mean arterial blood pressure fell below 60 mmHg, there was a decrease in cerebral blood flow which was limited to the evolutionary higher parts of the brain (the cerebellum and the cerebral hemispheres), while sparing the more vital parts of the brain.

Nimodipine (Duncker *et al.*, 1986a), nisoldipine (Duncker *et al.*, 1986b) and nicorandil (Verdouw *et al.*, 1987b), while inducing similar hypotensive effects, decreased total renal blood flow by 20–50%, whereas no change was observed with elgodipine, demonstrating a renal vasodilator action by the latter drug. To our knowledge the effects of calcium antagonists on regional renal blood flows have not yet been investigated. Elgodipine (up to $3\,\mu g\,kg^{-1}\,min^{-1}$) caused vasodilatation in both the inner and outer cortex, the effect being more pronounced in the inner cortex. This is in accordance with earlier studies on other vasodilator drugs (Stein *et al.*, 1971; De Bermudez & Haysleet, 1972; Hardaker & Wechsler, 1973; Kirschenbaum *et al.*, 1974). Although it has been suggested that the diminution in perfusion pressure in itself, by causing intrarenal production and liberation of angiotensin (Lachance *et al.*, 1974), might be responsible for the intrarenal blood flow changes (Carrière & Daigenault, 1970), the importance of redistribution of renal blood flow with elgodipine cannot yet be assessed.

The potent vasodilator actions of i.v. administered elgodipine, which were not accompanied by negative inotropic and positive chronotropic properties or decreases in the perfusion of vital organs, warrant further study as this compound could be useful in the treatment of essential hypertension, myocardial ischaemia and, possibly, moderate chronic heart failure.

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Chapter 6

L-propionylcarnitine increases post-ischemic blood flow but does not affect recovery of the energy charge

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Summary

The effects of pretreatment with L-propionylcarnitine (50 mg/kg, n = 9) or saline (n = 10) were studied in open-chest anesthetized pigs, in which ischemia was induced by decreasing left anterior descending coronary artery blood flow to 20% of baseline. After 60 min of ischemia the myocardium was reperfused for 2 hours. In both groups, flow reduction abolished contractile function of the affected myocardium and caused similar decreases in ATP (by 55%) and energy charge (decrease from 0.91 to 0.60), mean arterial blood pressure (by 10-24%), the maximum rate of rise in left ventricular pressure (by 26-32%) and cardiac output (by 20-30%). During reperfusion "no-reflow" was attenuated by L-propionylcarnitine, since myocardial blood flow returned to 51% and 82% of baseline in the saline- and the L-propionylcarnitine-treated animals, respectively. Cardiac output of the saline-treated animals further decreased (to 52% of baseline) and an increase in systemic vascular resistance (from 46 ± 3 to 61 ± 9 mmHg.min/L) was necessary to maintain arterial blood pressure. In the L-propionylcarnitine-treated pigs cardiac output remained at 75% of baseline and systemic vascular resistance decreased from 42 ± 3 to 38 ± 4 mmHg.min/L. In both groups, energy charge, but not the ATP-level of the ischemic-reperfused myocardium tended to recover, while the CP level showed significantly more recovery in the saline-treated animals. We conclude that L-propionylcarnitine partially preserved vascular patency in ischemic-reperfused porcine myocardium, but had no immediate effect on "myocardial stunning". Potential markers for long-term recovery were not affected by L-propionylcarnitine.

Introduction

L-carnitine is an essential cofactor in the mitochondrial transfer of activated long-chain fatty acids. Administration of this compound has beneficial effects in ischemic myocardium of dog (7), swine (20) and man (6, 31). Several mechanisms have been suggested to explain this beneficial effect. Firstly, repletion of the ischemia-induced loss of the myocardial carnitine pool restores the transfer of activated long-chain fatty acids through the mitochondrial inner membrane (19). Secondly, L-carnitine, by extraction of the acylesters from coenzyme A (CoA), attenuates harmful accumulation of long-chain acylCoA caused by the reduction in fatty acid oxidation. The excess of acylcarnitine may then be transported out of the mitochondria and myocytes (26).

L-propionylcarnitine, the naturally occurring derivative of L-carnitine, enters the myocardial cell more rapidly than L-carnitine (24), enhances fatty acid utilization and has been shown to protect ischemic myocardium in the isolated working rat heart preparation (24). Moreover, Liedtke et al. (21) demonstrated reversal of myocardial stunning by L-propionylcarnitine in a porcine model of mild myocardial ischemia and suggested that a positive inotropic action of L-propionylcarnitine might be responsible

for this beneficial effect.

Liedtke et al. (21) used an extracorporeal coronary circulation, which allowed control of coronary blood flow not only during ischemia but also during reperfusion. Reperfusion of myocardium, which has been severely ischemic for a prolonged period of time, does not always lead to a complete recovery of myocardial blood flow (the "no-reflow phenomenon",13, 14). In the study by Liedtke et al. (21) no-reflow may not have been relevant because of the duration and degree of ischemia and the use of the extracorporeal circulation.

In the present study we therefore investigated the effect of L-propionylcarnitine during 2 hours of reperfusion after one hour of severe (80% flow reduction) ischemia. In addition to systemic hemodynamics, regional myocardial contractile function and blood flow, we also determined the myocardial levels of high energy phosphates. Furthermore we evaluated the effect of L-propionylcarnitine on post-systolic wall thickening during ischemia (30) and the reduction in Ca^{2+} uptake and *in vitro* phospholamban phosphorylation activity of sarcoplasmic reticulum (SR) after 2 hours of reperfusion (16, 28). These parameters have been shown to be related to long term recovery of ischemic myocardium (18, 30, 33).

Materials and methods

General. After an overnight fast cross-bred Landrace x Yorkshire pigs of either sex (n=19, 22-40 kg) were sedated with 120 mg azaperone (Janssen Pharmaceutica, Beerse, Belgium) i.m., anesthetized with 150 mg metomidate (Janssen Pharmaceutica, Beerse, Belgium) i.v. and intubated for artificial ventilation with a mixture of oxygen and nitrous oxide (1:2). Respiratory rate and tidal volume were adjusted to keep arterial blood gases within the normal range: pH between 7.35 and 7.45; pCO_2 between 35 and 45 mmHg; pO_2 between 120 and 180 mmHg. A catheter was positioned into the superior caval vein for the administration of 160 mg/kg alpha-chloralose (Merck, Darmstadt, F.R.G.) followed by an infusion of 5 mg/kg/h pentobarbitone sodium (Sanofi, Paris, France) and for the administration of the muscle relaxant pancuronium bromide (4 mg) prior to thoracotomy. Haemaccel (Behringwerke A.G., Marburg, F.R.G.) was administered to replace the blood, withdrawn during sampling (see below). An 8F micromanometer-tipped catheter (Millar, Houston, Texas, U.S.A.) was inserted into the left ventricle by way of the left carotid artery, for the measurement of left ventricular blood pressure and its first derivative (LVdP/dt). Catheters were also inserted into the aorta, for measurement of central aortic blood pressure (50 AD pressure transducer, Spectramed, Bilthoven, the Netherlands), the collection of blood samples for the determination of blood gases and for the withdrawal of reference samples necessary for calibration of the radioactive microsphere flow measurements. After thoracotomy, an electromagnetic flow probe (Skalar, Delft, the Netherlands) was placed around the ascending aorta for the measurement of aortic blood flow. The left anterior descending coronary artery

(LADCA) was dissected free just distal from its first diagonal branch and an inflatable balloon (R.E. Jones, Silver Spring, Maryland, U.S.A.) was placed around the LADCA and connected to a 1 ml syringe (Hamilton Bonaduz, Bonaduz, Switzerland) driven by a micrometer (Hamilton Co., Reno, Nevada, U.S.A.). The vein accompanying the LADCA was cannulated for the withdrawal of blood samples for the determination of coronary venous blood gases.

Regional blood flows. To determine regional blood flows the left atrial appendage was catheterized for the injection of a batch of $1\text{-}2.10^6$ radioactive microspheres, 15 ± 1 (SD) μm in diameter (NEN Company, Dreieich, F.R.G.), labelled with either ^{95}Nb, ^{103}Ru, ^{113}Sn, ^{46}Sc or ^{141}Ce. Full details of the procedures and the calculation of flow data using the reference sample technique have been reported earlier (27).

Regional myocardial function. Regional myocardial function was estimated from recordings of myocardial wall thickness obtained with a 5 MHz ultrasonic transducer (Krautkamer-Branson, Lewistown, PA, U.S.A.) sutured onto a part of the epicardial surface perfused by the LADCA. From the tracings end-diastolic (EDT), end-systolic (EST) and maximal post-systolic (maxT) wall thickness were measured. Systolic wall thickening (SWT, %) was calculated as 100 x (EST-EDT)/EDT, while post-systolic wall thickening (PSWT, %) was defined as 100 x (maxT-EST)/EDT

Experimental protocol. After systemic hemodynamics had been stable for at least 30 min following completion of the instrumentation, baseline values for systemic hemodynamics, regional myocardial function and arterial and coronary venous blood gases were obtained while a batch of microspheres was injected for the measurement of organ blood flows. Furthermore, transmural needle biopsies (processed as described below) were taken from the myocardium nourished by the distal part of the LADCA and from a segment of the anterior wall of the heart, which was not supplied by the LADCA. In order to minimize trauma, biopsies for myocardial carnitine and short-chain fattyacylcarnitine levels were obtained from only 7 animals. Subsequently, the 19 animals were divided into two groups; nine animals received 50 mg/kg L-propionylcarnitine over a period of 10 min, and 10 animals an equal volume (20 ml) of physiological saline. After administration of L-propionylcarnitine, global and regional hemodynamic parameters were again determined. In all pigs the flow in the LADCA was then gradually reduced by slowly inflating the balloon, until complete loss of regional contractile function. After 30 min of ischemia, systemic hemodynamics and regional blood flows were again determined and a transmural biopsy was taken from the ischemic area. In case of ventricular fibrillation the animal was promptly (within 30 seconds) defibrillated. After 60 min, when all measurements had been repeated and biopsies were obtained from both the ischemic and the non-ischemic area, the balloon was deflated. Two hours later the last set of global and regional hemodynamic measurements were taken and biopsies were collected for measurements of adenine nucleotides and creatine (phosphate) in all animals and for carnitine and short-chain fattyacylcarnitine in 3 of the saline-treated and 4 of the L-propionylcarnitine-treated animals. At the observation period the heart was excised and immediately cooled on ice and transmural myocardial samples (5-7 g) were obtained from the ischemic-reperfused segment and from the

posterior wall of the heart (non-ischemic segment). The segments were homogenized for isolation of SR membrane vesicles (see below). Furthermore, the radioactive label present in the pellet after the first step of the centrifugations (10,000 g, 20 min) for SR isolation was counted in order to obtain myocardial blood flow data. Several other organs and tissues (kidneys, small intestines, total brain, the iliopsoas muscle and a part of the abdominal skin) were also excised, weighed, put into vials and the radioactivity was counted to determine regional blood flow data.

Adenine nucleotides, creatine phosphate and creatine. The transmural myocardial biopsies, taken with a Tru-Cut needle (Travenol Laboratories Inc., Deerfield, Illinois, USA) from the ischemic area and the adjacent non-ischemic area were dipped into 0.9% NaCl of 0 °C to remove adherent blood, and immediately (within 10 s) frozen in liquid nitrogen. The biopsies were stored at -80 °C until analysis. The biopsies (5-20 mg) were homogenized in 0.5 ml 0.42 M $HClO_4$ at liquid nitrogen temperature with the Braun micro-dismembrator (B. Braun, Melsungen, FRG), thawed, shaken and centrifuged. After neutralization of the supernatant the adenine nucleotides, creatine phosphate (CP) and creatine were separated and the concentrations estimated with an isocratic ionpairing HPLC (29), except that 175 mM potassium phosphate, 2.3 mM tetrabutylammonium hydrogensulfate, 2.5% acetonitrile, pH 6.25 was used as a running buffer. With this HPLC method we also checked the purity of the [gamma-^{32}P]ATP used in the phospholamban phosphorylation assays.

Isolation of sarcoplasmic reticulum. The transmural myocardial tissue samples were homogenized 3 times during 10 s in 4 volumes of 10 mM $NaHCO_3$, pH 7.0 at 0 °C with a Polytron PT 10 (Kinematica Gmbh, Luzern, Switzerland). The SR was isolated as described (27). Immediately after isolation, the SR suspension was frozen in liquid nitrogen and stored at -80 °C until analysis of Ca^{2+} uptake and phospholamban phosphorylation activities.

Ca^{2+} uptake and phosphorylation of phospholamban. The cyclic AMP-dependent phosphorylation of SR vesicles (5-10 μg protein) was determined as described (28) with 5 μM cyclic AMP to activate the endogenous protein kinase and 300 U/ml exogenous catalytic subunit of cyclic AMP-dependent protein kinase (Sigma Chem. Co, St Louis, USA). The samples were preincubated for 2 min at 25 °C and the phosphorylation reaction was started by the addition of 200 μM (final concentration) [gamma-^{32}P]ATP (150 TBq/mol). After 5 min the reaction was stopped with a mixture of sodiumdodecylsulphate (SDS), ß-mercaptethanol and glycerol (16, 28). The samples were then heated at 95 °C to dissociate phospholamban into monomers, and subjected to SDS-polyacrylamide gelelectrophoresis (16). Phosphorylated phospholamban was located on dried gels by autoradiography and ^{32}P content in the pieces, excised from the gel, was estimated by liquid scintillation counting. Ca^{2+} uptake activity was measured by incubation of SR with 50 μM $^{45}CaCl_2$ in the presence of 1 mM ATP (28). After the incubation the SR vesicles were filtered through Millipore filters (0.45 μm). The ^{45}Ca content of the SR vesicles remaining on the filters was estimated by liquid scintillation counting (28). Ca^{2+} uptake activity in blank reactions, obtained by omitting ATP from the reaction mixture, were subtracted.

Carnitine and short-chain fattyacylcarnitine levels. Left ventricular biopsies were homogenized in $HClO_4$, centrifuged, and the supernatant neutralized as described above for the determination of carnitine and short-chain acylcarnitine levels. The neutralized $HCLO_4$ extracts and saline-treated plasma's were directly analyzed for free carnitine and short-chain fatty acyl carnitine by the radioisotope procedure (2, 17). Briefly, for determination of total carnitine, in one aliquot of the extract, short-chain fatty acylcarnitine was first hydrolyzed to free carnitine by incubating in 0.1 M KOH at 37°C for 30 min and subsequently neutralized with 0.5 2-[4-(-Hydroxyethyl)-1-piperazinyl ethansulfonic acid (HEPES). The other aliquot (not hydrolyzed) was used for determination of free carnitine. Carnitine was converted enzymatically with [^{14}C-]acetylCoA to [^{14}C-]acetylcarnitine. Unreacted [^{14}C-]acetylCoA was separated from the labelled carnitine derivative by passing through a Dowex AG1-X8 anion exchange resin (BioRad Laboratories, Richmond, VA). A sample of the [^{14}C]acetylcarnitine containing eluate was mixed with 8 ml Instagel and counted for radioactivity.

Protein. The protein content of SR was estimated according the method described by Lowry et al. (23). In the needle biopsies, protein was estimated in the crude pellet obtained after centrifugation of the homogenate acidified with $HClO_4$. After dissolution of this pellet in 0.1 M KOH, protein was estimated with the Coomassie Brilliant Blue assay (4) obtained from Bio-Rad (Bio-Rad Laboratories, Munich, FRG). For both methods bovine serum albumin was used as the standard.

Drugs. L-propionylcarnitine HCl (50 mg/kg) and $NaHCO_3$, in an equimolar ratio, were dissolved in 20 ml distilled water (pH of the final solution 6.8-7.3).

Statistical analysis. All data have been presented as means ± SE. The significance of the changes produced by the LADCA flow reduction in the animals was evaluated by Duncan's new multiple range test once on analysis of variance had revealed that the samples represented different populations. The significance of the L-propionylcarnitine-induced changes was determined by comparing these changes with those observed in the saline-treated animals at corresponding times. Statistical significance was accepted for $P<0.05$.

Results

Effect of L-propionylcarnitine before LADCA-flow reduction L-propionylcarnitine did not affect systemic hemodynamics as the measurements obtained before administration were not different from those determined immediately after administration (heart rate 98 ± 4 beats/min and 96 ± 4 beats/min, mean arterial blood pressure 93 ± 4 mmHg and 87 ± 6 mmHg, left ventricular filling pressure 8 ± 1 mmHg and 8 ± 1 mmHg, left ventricular dP/dt_{max} 2310 ± 130 mmHg/s and 2290 ± 110 mmHg/s, cardiac output 2.0 ± 0.1 L/min and 2.0 ± 0.1 L/min, respectively). The data determined after administration have been

presented as baseline before the flow reduction (Fig. 1). Regional blood flows were also not different before and after administration of L-propionylcarnitine. The post-administration values have again been presented as baseline (Fig. 2).

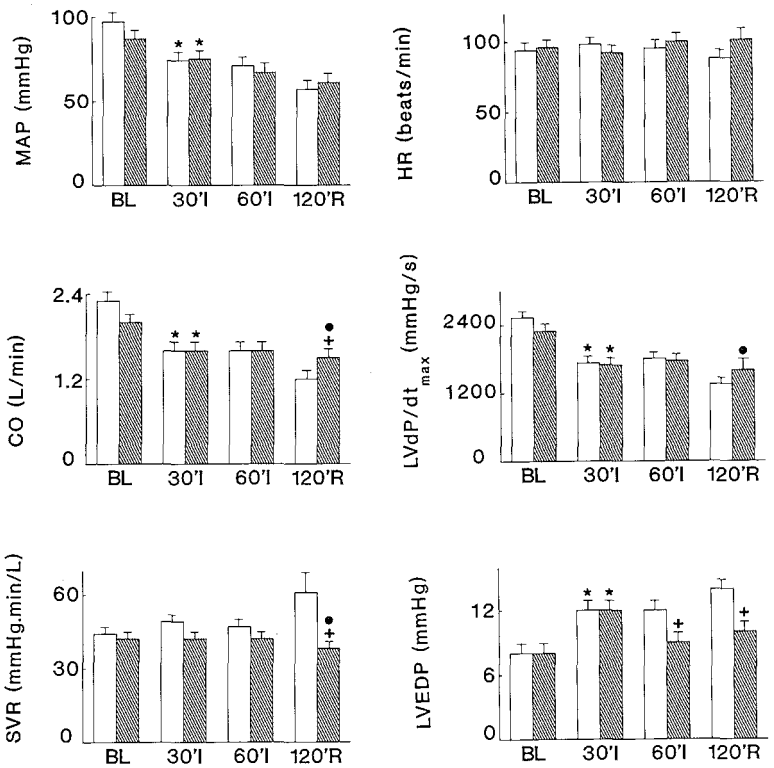

Figure 1 *Systemic hemodynamic effects in saline-treated (open bars, n=10) and L-propionylcarnitine-treated (hatched bars, n=9) pigs at baseline (BL), after 30 and 60 min of ischemia (I) and 120 min of reperfusion (R). * P<0.05 vs baseline, only presented for 30 min and 60 min of ischemia; + changes vs baseline in the L-propionylcarnitine-treated animals are significantly different from the changes vs baseline in the saline-treated animals; • changes vs 60 min of ischemia in the L-propionylcarnitine-treated animals are significantly different from the changes vs 60 min of ischemia in the saline-treated animals. MAP = mean arterial blood pressure, HR = heart rate, CO = cardiac output, LVdPdt$_{max}$ = maximal rate of rise of left ventricular pressure, SVR = systemic vascular resistance, LVEDP = left ventricular end diastolic pressure.*

Effects of L-propionylcarnitine during ischemia and reperfusion.

Ventricular arrhythmias. Seven L-propionylcarnitine-treated (78%) and 8 saline-treated (80%) animals had an episode of ventricular fibrillation during the ischemic period. All animals were defibrillated within 25 s and resumed pre-fibrillation values. Ventricular

fibrillation was not observed during reperfusion in any animal.

Systemic hemodynamics. During the first 30 min of LADCA flow reduction in the saline-treated animals mean arterial blood pressure, cardiac output, stroke volume and left ventricular dP/dt_{max} had decreased ($P<0.05$) by 23%, 30%, 36% and 32%, respectively, left ventricular end diastolic pressure increased from 8 to 12 mmHg ($P<0.05$, Fig. 1). During the 2 hours of reperfusion there was a further decline in mean arterial blood pressure (by 20%), left ventricular dP/dt_{max} (by 25%), cardiac output (by 25%) and stroke volume (by 24%), while left ventricular end diastolic pressure further increased to 14 ± 1 mmHg. Vasoconstriction (increase in systemic vascular resistance of 30%) prevented a more severe fall in mean arterial blood pressure (Fig. 1).

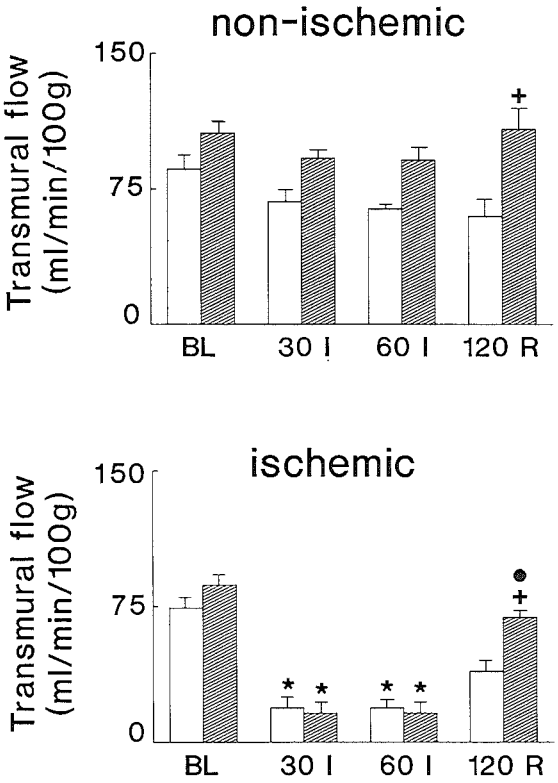

Figure 2 *Transmural myocardial blood flow (ml/min/100 g) of non-ischemic and ischemic myocardium in saline-treated (open bars, n=10) and L-propionylcarnitine-treated (hatched bars, n=9) pigs at baseline (BL), after 30 and 60 min of ischemia (I), and 120 min of reperfusion (R). * P<0.05 vs baseline, only presented for 30 min and 60 min of ischemia; + changes vs baseline in the L-propionylcarnitine-treated animals are significantly different from the changes vs baseline in the saline-treated animals; • changes vs 60 min of ischemia in the L-propionylcarnitine-treated animals are significantly different from the changes vs 60 min of ischemia in the saline-treated animals.*

In the L-propionylcarnitine-treated animals there were similar decreases in mean arterial blood pressure cardiac output, stroke volume and left ventricular dP/dt_{max} (Fig. 1). Left ventricular end diastolic pressure initially increased from 8 ± 1 mmHg to 12 ± 1 mmHg but, at variance with the saline-treated pigs, decreased to 9 ± 1 mmHg in the last 30 min of LADCA flow reduction. In contrast to the decrease in cardiac output in the saline-treated animals, cardiac output of the L-propionylcarnitine-treated animals did not change during reperfusion. From the changes in cardiac output and mean arterial pressure it can be calculated that systemic vascular resistance did not change during reperfusion (Fig. 1).

Regional blood flows. After 60 min of ischemia the decreases in cardiac output of the saline-treated animals were not equally distributed, since blood flow to the kidneys (-12%), the iliopsoas muscle (-39%), the skin (-29%), the small intestine (-22%) and the brains (-18%) had not decreased to the same extent (Table 1). L-propionylcarnitine did not modify these ischemia-induced changes. Reperfusion did not lead to recovery of regional blood flows in the saline-treated animals. In the L-propionylcarnitine-treated animals blood flow to the skin and small intestine increased ($P<0.05$ *vs* saline-treated animals), but blood flow to the brains, skeletal muscle and the kidneys did not change.

Regional myocardial perfusion. The microsphere data revealed that inflation of the balloon had caused similar decreases in transmural myocardial blood flow in the saline-treated (from 79 ± 6 ml/min/100g to 19 ± 8 ml/min/100g, 76%) and the L-propionylcarnitine-treated animals (from 84 ± 7 ml/min/100g to 16 ± 7 ml/min/100g, 81%, Fig. 2) during the first 30 min of ischemia. There were no further changes in either group during the following 30 min of LADCA stenosis. After two hours of reperfusion transmural myocardial blood flow of the LADCA-perfused area had returned to 40 ± 5 ml/min/100g (53% of baseline) in the saline-treated animals. In the L-propionylcarnitine-treated animals, however, post-ischemic flow after two hours of reperfusion (69 ± 7 ml/min/100g, 82% of baseline) was significantly higher than in the saline-treated animals. The major factor contributing to the differences in post-ischemic flow was the increase in coronary vascular resistance from 1.3 ± 0.1 mmHg/(ml/min/100g) to 1.6 ± 0.3 mmHg/(ml/min/100g) ($P<0.05$) in the saline-treated animals, as such an increase in resistance was not seen in the L-propionylcarnitine-treated animals (1.1 ± 0.1 mmHg/(ml/min/100g) at baseline, and 1.0 ± 0.1 mmHg/ml/min/100g) after 2 hours of reperfusion).

In the saline-treated animals transmural blood flow to the non-ischemic area decreased during ischemia. This fall in perfusion was secondary to the fall in arterial blood pressure (coronary vascular resistance was 1.1 ± 0.1 mmHg/(ml/min/100g) at baseline and 1.0 ± 0.1 mmHg/(ml/min/100g) after 60 min of ischemia). During reperfusion coronary vascular resistance did not change (1.1 ± 0.1 mmHg/(ml/min/100g)). In the L-propionylcarnitine-treated animals blood flow at the end of reperfusion was not different from that at baseline (Fig. 2). In these animals coronary vascular resistance decreased during the experiment from 0.9 ± 0.1 mmHg/(ml/min/100g) at baseline, 0.8 ± 0.1 mmHg/(ml/min/100g) after 60 min of ischemia to 0.7 mmHg/(ml/min/100g) at the end of reperfusion.

Table 1 *Effect of L-propionylcarnitine on regional blood flows in open-chest anesthetized pigs*

		Baseline	min of ischemia		120 min of reperfusion
			30	60	
Kidneys	S	197 ± 21	207 ± 22	173 ± 16	118 ± 17
	P	199 ± 15	165 ± 17	152 ± 15*	147 ± 13•
Muscle	S	2.8 ± 0.3	2.2 ± 0.1*	1.7 ± 0.1*	1.6 ± 0.2
	P	3.1 ± 0.4	2.5 ± 0.3*	2.2 ± 0.2*	2.6 ± 0.7
Skin	S	0.78 ± 0.17	0.73 ± 0.15	0.55 ± 0.13	0.58 ± 0.11
	P	0.84 ± 0.25	0.78 ± 0.16	0.86 ± 0.18	1.5 ± 0.32+
Small intestine	S	36 ± 4	30 ± 3*	28 ± 3*	25 ± 3
	P	32 ± 1	29 ± 3	29 ± 1	36 ± 3+
Total brain	S	28 ± 3	25 ± 2	23 ± 1*	21 ± 2
	P	28 ± 1	26 ± 2	27 ± 1	27 ± 3

*Flow data (ml/min/100g) are presented as mean ± SE; P = L-propionylcarnitine-treated animals (n = 9); S = saline-treated animals (n = 10); * P<0.05 vs baseline, only presented for 30 and 60 min of ischemia; + L-propionylcarnitine-induced changes versus baseline are significant different from those in the saline-treated animals; • L-propionylcarnitine-induced changes versus 60 minutes of ischemia are significantly different from those in the saline-treated animals*

Myocardial oxygen extraction was almost maximal at baseline, since oxygen saturation of the coronary venous blood of the LADCA-perfused myocardium was 25 ± 5% and 24 ± 2% in the saline-treated and the L-propionylcarnitine-treated animals, respectively. After 2 hours of reperfusion oxygen extraction was still similar but less, as the coronary venous oxygen saturation was 64 ± 5% and 61 ± 8% for the saline- and the L-propionylcarnitine-treated animals, respectively. Hence myocardial oxygen consumption, defined as the product of coronary blood flow and the difference in the arteriovenous oxygen content, remained directly related to coronary blood flow and was therefore higher in the L-propionylcarnitine-treated animals at the end of reperfusion.

Regional myocardial wall function. Reduction of coronary blood flow caused thinning of the segment perfused by the LADCA, as EDT decreased from 12.7 ± 0.4 mm to 11.2 ± 0.4 mm in the saline-treated animals and from 12.0 ± 0.4 mm to 11.1 ± 0.4 mm in the L-propionylcarnitine-treated animals and complete loss of systolic thickening (baseline values 29 ± 2% and 31 ± 4% for the saline- and the L-propionylcarnitine-treated animals, respectively). After two hours of reperfusion EDT had increased to 14.2 ± 0.9 mm and 13.7 ± 0.6 mm in the saline- and the L-propionylcarnitine-treated animals, respectively, but there was no sign of return of systolic wall thickening. Post-systolic wall thickening was less then 2% at baseline, but 8 ± 1% and 6 ± 1% in the saline- and 11 ± 2% and 8 ± 2% in the L-propionylcarnitine-treated animals, after 60 min of ischemia and 2 hours of reperfusion, respectively.

High energy phosphates. Except for a 15% decrease ($P<0.05$ *vs* baseline) in the adenine nucleotide pool of the L-propionylcarnitine-treated animals at the end of reperfusion, there were no other changes in ATP, CP, energy charge, adenine nucleotide and creatine pools of the non-ischemic segment of either group (Figs. 3 and 4). ATP, CP, energy charge and the sum of the adenine nucleotides decreased in the ischemic segment of the myocardium perfused by the LADCA similarly in both groups during flow reduction. The sum of CP and creatine was, however, not affected (Figs. 3 and 4). Reperfusion did not lead to an improvement of the ATP levels of either group, but there was a considerable improvement in CP and the energy charge, with the increase in myocardial CP levels being most pronounced in the saline-treated animals (Figs. 3 and 4). In fact, CP recovered in the saline-treated animals, whereas it only returned to 55% of baseline in the L-propionylcarnitine-treated animals. The adenine nucleotide pool did not change in the saline-treated animals and even deteriorated further in the L-propionylcarnitine-treated animals (Fig. 4). The sum of CP and creatine remained constant in the saline-treated pigs but decreased to 47% of baseline ($P<0.05$ *vs* baseline) in the L-propionylcarnitine-treated animals.

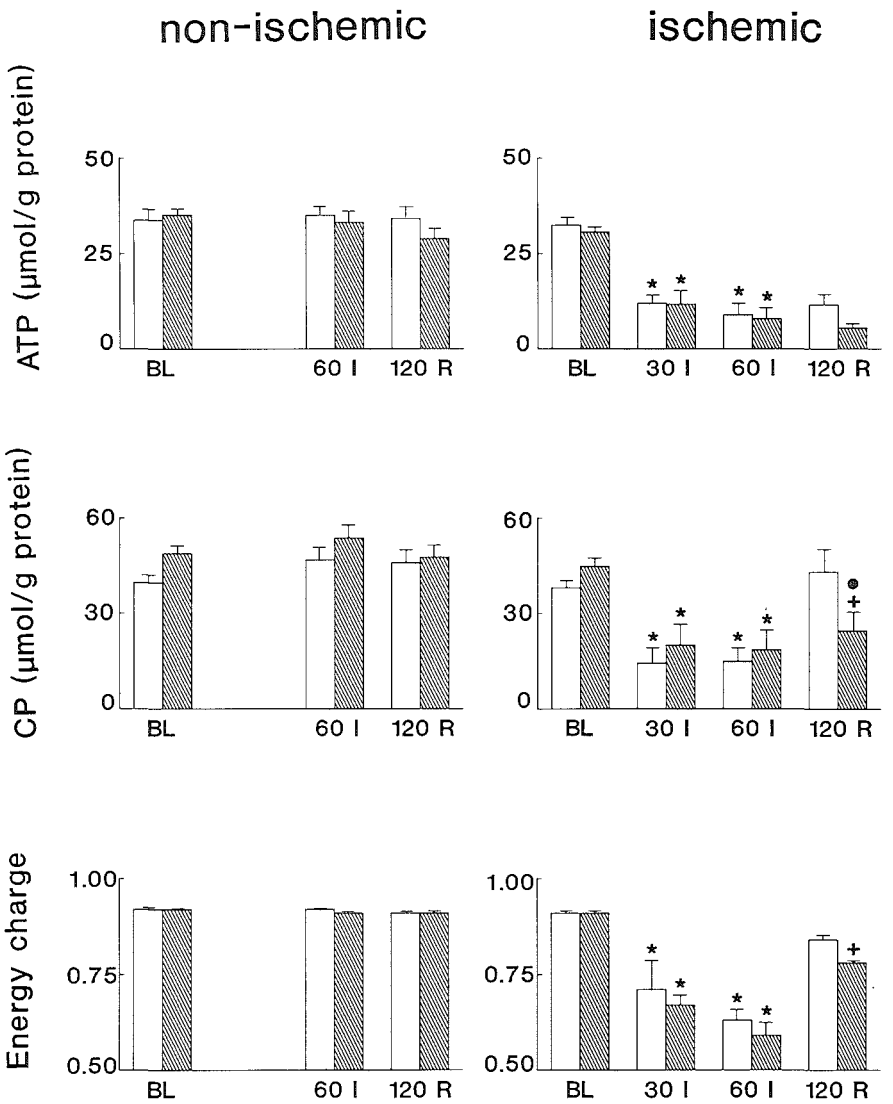

Figure 3 *ATP and CP (µmol/g protein) and energy charge ((ATP + ½ADP) / (ATP + ADP + AMP)) of the ischemic and non-ischemic myocardium of saline-treated animals (open bars, n=10) and L-propionylcarnitine-treated (hatched bars, n=9) at baseline (BL), at 30 (only for the ischemic segment) and 60 min of ischemia (I) and after 120 min of reperfusion (R). * P<0.05 vs baseline, only presented for 30 min and 60 min of ischemia; + changes vs baseline in the L-propionylcarnitine-treated animals are significantly different from the changes vs baseline in the saline-treated animals; • changes vs 60 min of ischemia in the L-propionylcarnitine-treated animals are significantly different from the changes vs 60 min of ischemia in the saline-treated animals.*

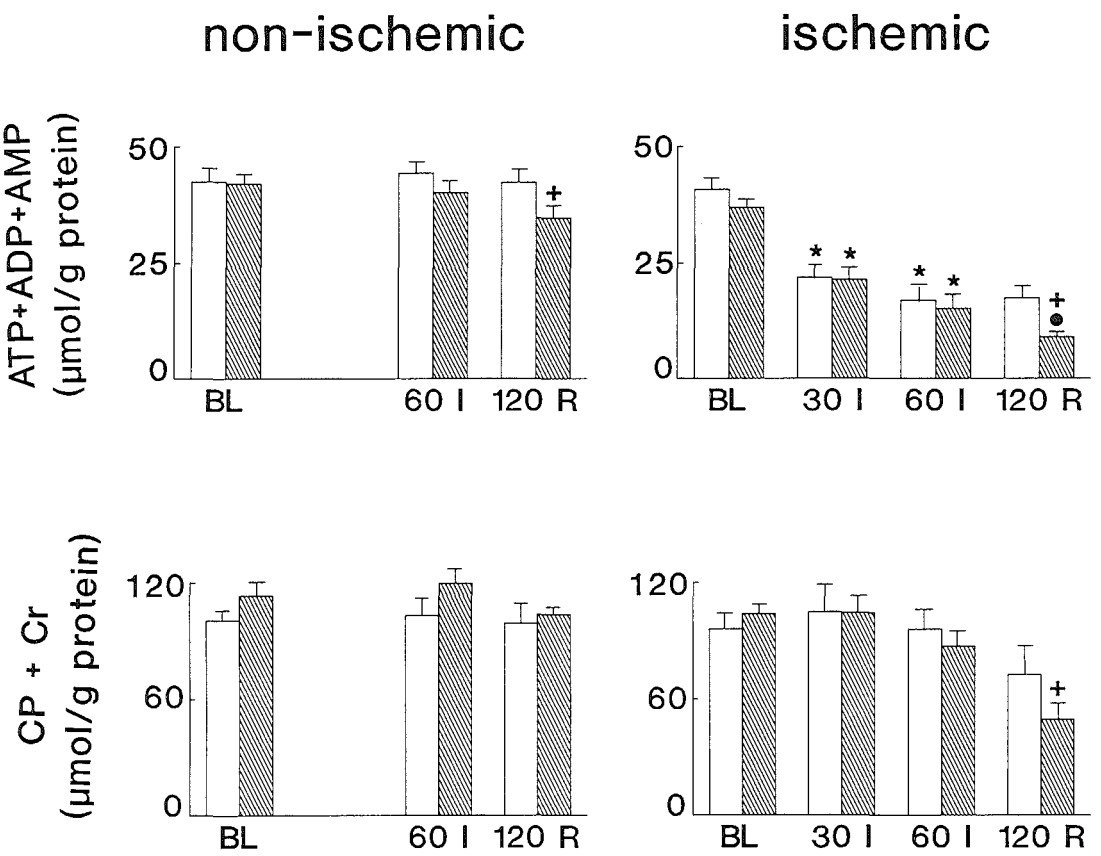

Figure 4 *Total adenine nucleotide (ATP + ADP + AMP) and creatine (CP + creatine) pools of the non-ischemic and ischemic myocardium in saline-treated (open bars, n=10) and L-propionylcarnitine-treated (hatched bars, n=9) pigs at baseline (BL), at 30 (only for the ischemic segment) and 60 min of ischemia (I) and after 120 min of reperfusion ((R). * P<0.05 vs baseline, only presented for 30 min and 60 min of ischemia; + changes vs baseline in the L-propionylcarnitine-treated animals are significantly different from the changes vs baseline in the saline-treated animals; • changes vs 60 min of ischemia in the L-propionylcarnitine-treated animals are significantly different from the changes vs 60 min of ischemia in the saline-treated animals.*

Ca^{2+} uptake and phospholamban phosphorylation. Ca^{2+} uptake and *in vitro* phosphorylation of phospholamban could only be determined at the end of reperfusion because of the considerable amount of myocardium (5 g), needed for the isolation of a sufficient amount of SR membrane vesicles. The rate of Ca^{2+} uptake of the non-ischemic segment of the L-propionylcarnitine-treated animals was 30% higher ($P < 0.05$) than that of the saline-treated animals, but that of the ischemic-reperfused segments was similar for both groups (Table 2). *In vitro* phosphorylation of phospholamban of both the non-ischemic and the ischemic-reperfused myocardium was less (by 40% and 50%, respectively) for the L-propionylcarnitine-treated than for the saline-treated animals (Table 2).

Table 2 *Effect of L-propionylcarnitine on Ca^{2+}uptake and in vitro ^{32}Pincorporation into phospholamban of sarcoplasmic reticulum of ischemic-reperfused myocardium in open-chest anesthetized pigs*

		Ca^{2+}-uptake (nmol/min/mg)	^{32}P incorporation (pmol/mg)
Non-ischemic myocardium	S	857 ± 102	1563 ± 150
	P	1164 ± 78	972 ± 35
Ischemic-reperfused myocardium	S	572 ± 190	1258 ± 234
	P	643 ± 151	621 ± 110

S = saline-treated, (n = 7); P = L-propionylcarnitine-treated, (n = 9); Data are presented as mean ± SE

Free carnitine and short-chain fatty acylcarnitine levels in plasma and myocardium. After a bolus of 50 mg/kg of L-propionylcarnitine the short-chain fatty acylcarnitine level in plasma increased 350-fold from 1.20 ± 0.49 μM to 682 ± 65 μM (Fig. 5). During ischemia and reperfusion the level of short chain fatty acylcarnitine gradually decreased to 67 ± 6 μM after 2 hours of reperfusion, which concentration was still 56-fold above baseline. Also free carnitine level increased above baseline (9-fold), immediately after the bolus infusion of L-propionylcarnitine and remained high (65 ± 10 μM) up to 2 hours of reperfusion.

At the end of reperfusion the myocardial level of free carnitine had decreased to about 60% of baseline value in both the saline- and the L-propionylcarnitine treated animals (Table 3). The levels of short chain fatty acyl carnitine were not significantly changed by neither ischemia nor subsequent reperfusion nor by L-propionylcarnitine treatment.

Table 3 *Effect of L-propionylcarnitine on myocardial tissue levels of free carnitine and short-chain fatty acylcarnitine in open-chest anesthetized pigs*

		Free carnitine			Short chain fatty acylcarnitine		
		Before ischemia					
Non-ischemic myocardium	S	4.9	±	0.7	1.8	±	0.4
	P	6.0	±	0.7	2.6	±	0.3
		After 120 min of reperfusion					
Non-ischemic myocardium	S	4.2	±	0.6	1.0	±	0.4
	P	5.9	±	0.6	1.9	±	0.3
Ischemic-reperfused myocardium	S	1.9	±	0.6*	1.1	±	0.3
	P	3.0	±	0.4*	1.3	±	0.5

*S = saline-treated animals (n = 3); P = L-propionylcarnitine-treated animals (n = 4); Data are in μmol/g protein; Data are presented as mean ± SE; * P<0.05 vs baseline*

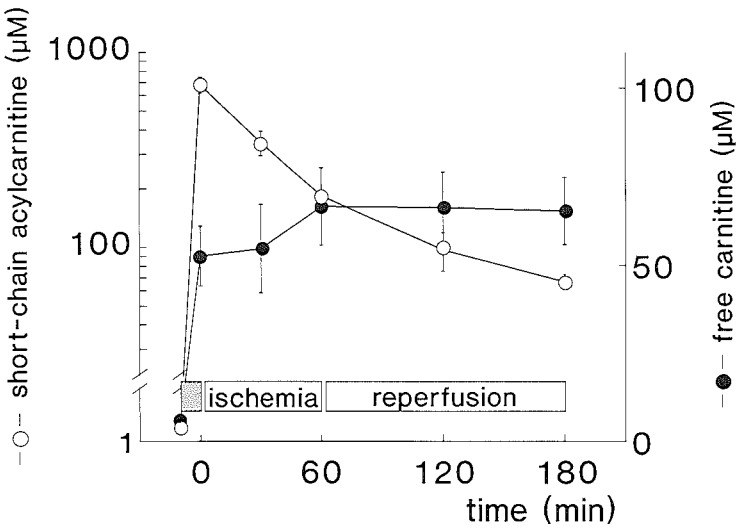

Figure 5 *Plasma levels of free carnitine and short chain acylcarnitine levels in L-propionylcarnitine-treated pigs at baseline, immediately after the L-propionylcarnitine infusion (shaded time period), after 30 and 60 min of ischemia and after 60 and 120 min of reperfusion.*

Discussion

The most important finding of the present study was that in spite of the similar arterial perfusion pressure, post-ischemic blood flow to the myocardium perfused by the LADCA was higher in the L-propionylcarnitine-treated than in the saline-treated animals. It has been well established that reperfusion after a prolonged period of myocardial ischemia does not always result in a complete return of blood flow since the vasculature of the jeopardized myocardium can become obstructed by extravascular compression or by intravascular obstructions (the "no-reflow"-phenomenon, 12, 13). It might therefore be hypothesized that L-propionylcarnitine-treatment attenuated this no-reflow phenomenon. It has been suggested that L-propionylcarnitine has a stabilizing action on plasmamembrane, during ischemia and associated acidosis of the Langendorff-perfused rat heart (11). Not only the sarcolemma of the cardiomyocytes, but also the vascular endothelium may be protected by the compound. Hinsberg and Scheffer (10) demonstrated in fura-2-loaded human endothelial cells in culture that L-propionylcarnitine decreased the resting cytoplasmic Ca^{2+} concentration, which also indicates a direct effect on the sarcolemma. Vascular endothelium has been recognized as an important functional unit involved with regulation of vascular smooth muscle tonus (8). This L-propionylcarnitine, by interacting directly with the surface membrane, could have protected the endothelial cell, attenuating the damage leading to the no-reflow phenomenon. L-propionylcarnitine, like L-carnitine (26) might also prevent cell damage by attenuating harmful accumulation of long-chain acylcoA. Furthermore, since the Ca^{2+} pump activity in SR plays a pivotal role in the development of cytosolic Ca^{2+} overload leading to reperfusion damage (14, 15, 22, 28), a higher rate of Ca^{2+} uptake may be beneficial. In the L-propionylcarnitine-treated animals, a higher Ca^{2+} uptake was observed in the non-ischemic segment but not in the ischemic-reperfused segment. The depression of the *in vitro* cyclic AMP-dependent phosphorylation of the regulatory subunit of the sarcoplasmic reticular Ca^{2+} pump by both ischemia and L-propionylcarnitine treatment may have consequences for the sensitivity of the myocardium to $ß_1$-adrenergic stimulation. The design of the present study does not provide confirmation, however.

L-propionylcarnitine could also have produced a direct coronary vasodilatory effect. The data obtained immediately after administration of the drug do not support this idea but at the end of the experiment, the higher blood flows to the non-ischemic myocardium and some organs may have been due to a direct vasodilatory action of L-propionylcarnitine. We must keep in mind, however, that the higher myocardial work in the L-propionylcarnitine-treated animals resulted in metabolic autoregulation secondary to the higher myocardial oxygen demand. In order to determine if a coronary vasodilatory action could be demonstrated after a prolonged period of time, we also followed two identically instrumented animals for a period of 3 hours, without inducing ischemia. These additional experiments provided no evidence that L-propionylcarnitine exerted a vasodilatory effect.

The higher post-ischemic blood flow in the L-propionylcarnitine-treated animals was not accompanied by a return of systolic contractile function during early reperfusion. The explanation for this can be threefold. Firstly, the myocardial tissue is irreversibly injured. Although we have demonstrated that even four weeks after 60 min of total coronary artery occlusion recovery of contractile function was absent (25, 33), others found that 30-40% of the myocardium at risk was still viable (9). In the present study we reduced coronary blood flow to 20% of baseline during the 60 min of ischemia and a significant fraction of the affected myocardium must therefore have been viable. Secondly, the low level of ATP of the post-ischemic myocardium prevented contractile function. However, it has repeatedly been demonstrated that enhanced recovery of function occurs while the low ATP-levels are not affected (1, 5, 32). Thirdly, Krause et al. have suggested that the inability of the stunned myocardium to function normally is due to a reduction of the activity of the Ca^{2+} pump (15). In the present study the Ca^{2+} pump activity of the jeopardized myocardium was still considerably lower than that of the non-ischemic segment. It is most likely that the stunning of the myocardium was so severe that it also became unresponsive to any stimulation (3). We can therefore not refute the suggestion by Liedtke et al. (21) that in their study L-propionylcarnitine enhanced recovery of regional myocardial function by a positive inotropic action.

After reperfusion, the total adenine and creatine pools were lower in the L-propionylcarnitine-treated group. A more marked leakage of creatine and adenosine, possibly due to the higher coronary blood flow, may have contributed to the impaired resynthesis of high energy phosphates, but an increased utilization of ATP and CP to meet the higher energy requirements can also not be excluded. Moreover, the energy charge, the accepted marker of the cellular phosphorylation potential, tended to recover similarly in both groups.

Recently, post-systolic wall thickening, determined at the end of ischemia (30) or the reduction of the Ca^{2+} uptake rate of sarcoplasmic reticulum, measured during early reperfusion (25, 28, 33) have been shown to be related to the viability of myocardial ischemic tissue. These parameters do not suggest that in the present study the long-term outcome in the L-propionylcarnitine-treated animals will be different from that in the saline-treated animals, in spite of a lower level of CP after the two hour reperfusion period.

In our study we observed a concomitant increase of plasma free carnitine following administration of L-propionylcarnitine. This is not due to impurity of the substance, but may be the consequence of a rapid enzymatic hydrolysis of L-propionylcarnitine or a rapid exchange of the short-chain acylcarnitine with intracellular carnitine. Liedtke et al. (21) showed in the open-chest pig model that in non-ischemic myocardium L-propionylcarnitine lowered cardiac free carnitine concentration, which result supports the idea of transmembrane counterflux of carnitine derivatives. However, no depletion of cardiac free carnitine due to L-propionylcarnitine administration is observed in the present study. Apparently, the cardiac free carnitine concentration is rapidly restored by intracellular hydrolysis of L-propionylcarnitine. The results indicate that ischemia followed by reperfusion indeed depletes the intracellular carnitine pool which, however,

was not prevented by L-propionylcarnitine.

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Chapter 7

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

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

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

Introduction

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

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

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

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Materials and methods

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

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

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

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

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

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

Results

Intravenous infusions of nicorandil in anaesthetized animals

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

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

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

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

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

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

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

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

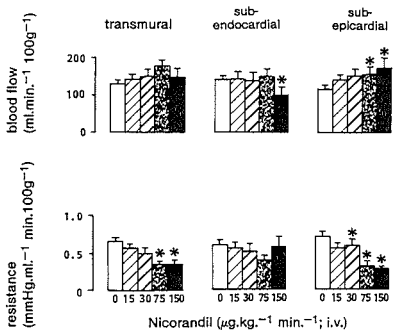

Fig. 1. Left ventricular blood flow and coronary vascular resistance after consecutive 10 min intravenous infusions of nicorandil in 11 anaesthetized pigs. Although transmural blood flow did not change, there was a redistribution in favour of the epicardium. All data have been presented as means ± SEM.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

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

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

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

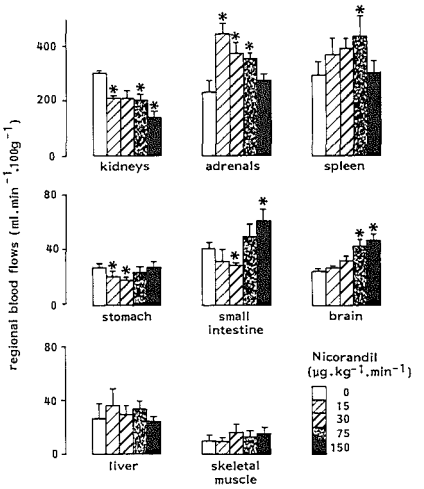

Fig. 2. Regional blood flows after consecutive 10 min intravenous infusions of nicorandil in 12 anaesthetized pigs. All data have been presented as means ± SEM. * $P < 0.05$ vs. pre-nicorandil

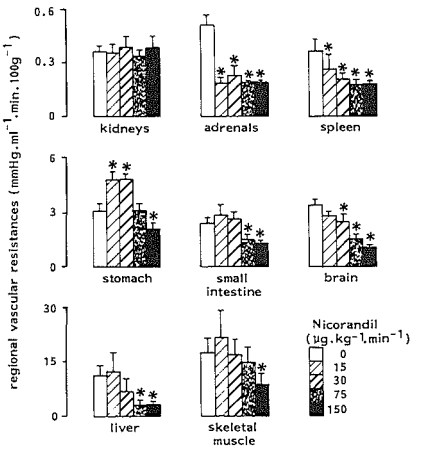

Fig. 3. Regional vascular resistances after consecutive 10 min intravenous infusions of nicorandil in 12 anaesthetized pigs. All data have been presented as means ± SEM. * $P < 0.05$ vs. pre-nicorandil

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

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

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

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

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

Intracoronary infusions of nicorandil in anaesthetized animals

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

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

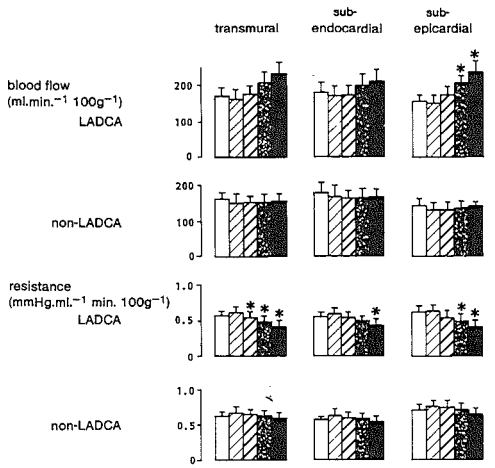

Fig. 4. Myocardial blood flow and coronary vascular resistance after intracoronary infusions of nicorandil into the left anterior descending coronary artery (LADCA) of 5 anaesthetized pigs. No changes were observed in the area adjacent to the LADCA perfused myocardium. The intracoronary infusion rates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

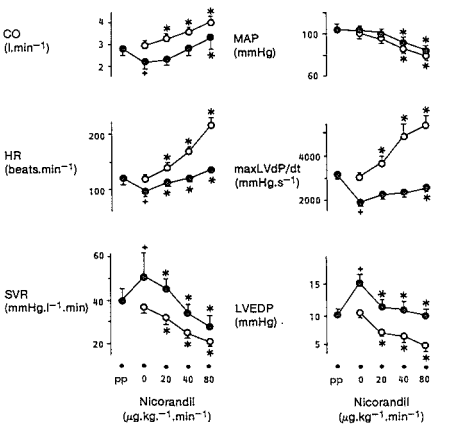

Fig. 5. The effects of continuous 10 min intravenous infusions of nicorandil without (○; $n = 6$) and after (●; $n = 4$) beta-adrenoceptor blockade in conscious pigs. All data have been presented as mean ± SEM. * $P < 0.05$ vs. pre-nicorandil (○). + $P < 0.05$ vs. pre-propranolol (*PP*)

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

Intravenous infusions of nicorandil in conscious pigs

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

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

Discussion

Systemic haemodynamics

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

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

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

Regional haemodynamics

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

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

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

Myocardial oxygen consumption and haemodynamics

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

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

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Chapter 8

Nicorandil and cardiovascular performance in anaesthetized pigs with a concentric coronary artery stenosis

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Summary. The present investigation compares the systemic and regional haemodynamics in nicorandil-treated and solvent-treated pigs with a concentric stenosis around the left anterior descending coronary artery. The stenosis per se led to a decrease in mean arterial blood pressure, cardiac output, stroke volume, maximum rate of rise in left ventricular pressure and transmural (more marked in the endocardium than in the epicardium) blood flow to and myocardial wall motion in the post-stenotic segment. Infusions of nicorandil (15 and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$, intravenously) decreased arterial blood pressure, cardiac output and the maximum rate of rise in left ventricular blood pressure. There was a tendency for epicardial blood flow in the non-stenotic segment to increase but blood flow to the ischaemic myocardium (epicardium as well as endocardium) was further compromised. Using the postsystolic wall thickening as an index for the viability of the myocardium and the Bretschneider formula for myocardial oxygen demand and the calculated myocardial oxygen consumption, we found that nicorandil further compromised the oxygen balance but did not jeopardize the viability of the myocardium. Regionally, nicorandil decreased renal blood flows but enhanced blood flows to the brains and adrenals. It is concluded that nicorandil lacks beneficial effects on the ischaemic myocardium in pigs with a concentric coronary artery stenosis. Apparently, the potential adverse effect (decrease in coronary perfusion pressure) of nicorandil outweighs its potential salutary effects (coronary vasodilatation and decrease in myocardial oxygen consumption due to peripheral vasodilatation).

Key words: Myocardial ischaemia – Nicorandil – Regional blood flows – Regional myocardial wall thickening – Systemic haemodynamics

Introduction

Nicorandil is a new vasodilator agent which is structurally related to the nitrates but also possesses a nicotinamide moiety. Some characteristics of nitrates – dilatation of large "conductance" coronary arteries and reduction of venous return to the heart – have been confirmed in man as well as in a number of different animal species; however, in this respect the drug is less potent than nitroglycerine (Taira et al. 1979; Sakai et al. 1980; Thorman et al. 1983; Taira 1987). Nicorandil increases potassium conductance which may largely be responsible for the cardiac effects of the drug (Taira et al. 1979; Yanagisawa et al. 1979; Yanagisawa and Taira 1980). However, mainly due to its vasodilating action, the drug is employed in the treatment of ischaemic heart disease. This idea is supported by the observation that nicorandil increases collateral blood flow to the ischaemic myocardium in dogs with a ligated coronary artery (Lamping and Gross 1984a, b). In addition, nicorandil has been shown to possess a perfusion-unrelated metabolic component which may also contribute to its anti-ischaemic actions (Pieper and Gross 1987).

The use of vasodilator drugs carries the risk of promoting ischaemic events by indirectly reducing perfusion of the post-stenotic myocardium due to hypotension (Gross and Warltier 1981). This risk is particularly great in patients with concentric stenoses. In the present investigation we therefore induced myocardial ischaemia by a concentric narrowing of a coronary artery in pigs (a species without a collateral circulation) and studied the effects of nicorandil on myocardial perfusion and function. Although the effects of nicorandil on regional systemic perfusion have been studied in animals with an intact coronary circulation (Verdouw et al. 1987a) these are not yet known for animals with myocardial ischaemia. A disadvantage of the acute stenosis is that the change in perfusion does not provide information about long term recovery of myocardial function (Van der Giessen et al. 1988). However, recent studies (Brown et al. 1987; Takayama et al. 1988) have shown that the post-systolic wall thickening is a useful marker for the ultimate recovery of function.

Methods

General. Cross-bred Landrace × Yorkshire pigs ($n = 21$, 24–30 kg) were sedated with 120 mg, i.m. azaperone (Janssen Pharmaceutica, Beerse, Belgium) and, subsequently, anaesthetized with 150 mg, i.v. metomidate (Janssen Pharmaceutica, Beerse, Belgium). After intubation the animals were artificially ventilated with a mixture of 30% oxygen and 70% nitrous oxide. Anaesthesia was maintained with 160 $mg \cdot kg^{-1}$, i.v. α-chloralose (Merck, Darmstadt, FRG) followed by an infusion of 5 $mg \cdot kg^{-1} \cdot h^{-1}$ pentobarbitone sodium (Sanofi, Paris, France) via a catheter placed in the superior vena cava by way of a jugular vein. Respiratory rate and tidal volume were regulated to control arterial blood gas values (ABL-3, Radiometer, Copenhagen,

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Denmark) to 7.40 ± 0.01 (pH), 41 ± 5 mm Hg (pCO_2), 160 ± 4 mm Hg (pO_2) and 95 ± 2% (O_2 saturation), respectively. Haemaccel (Behringwerke, Marburg, FRG) was infused to correct blood loss. Left ventricular and central aortic pressures were monitored with catheters tipped with a micromanometer (Honeywell-Philips, Best, The Netherlands). After the heart was exposed using a midsternal split, electromagnetic flow probes (Skalar, Delft, The Netherlands) were placed around the ascending aorta and the left anterior descending coronary artery (LADCA). The vein accompanying the LADCA was cannulated with a polyethylene catheter for the withdrawal of blood samples for determination of coronary venous blood gases. The left atrial appendage was catheterized for the injection of radioactive microspheres. Finally, a hydraulic occluder (RE Jones, Silver Spring, MD, USA) was placed around the LADCA distal to the flow probe and connected to a 1 ml syringe (Hamilton Bonaduz, Bonaduz, Switzerland) which was driven by a micrometer.

Regional myocardial function. Regional myocardial function was estimated from myocardial wall thickness recordings obtained with the aid of a 5 MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, PA, USA) sutured onto a part of the epicardial surface perfused by the LADCA (Verdouw et al. 1985). From the wall thickness at end-diastole (EDT) and end-systole (EST), percentage systolic wall thickening (SWT) was calculated as: SWT (%) = 100 × (EST − EDT)/EDT. Postsystolic wall thickening (PSWT) was calculated as: PSWT (%) = 100 × (maxT − EST)/EDT, in which maxT is maximum diastolic wall thickness (Brown et al. 1987; Takayama et al. 1988).

Regional blood flow. Regional blood flow measurements were obtained using the radioactive microsphere technique (^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce; 15 ± 1 (SD) μm diameter, NEN Company, Dreieich, FRG). After completion of the experiment, the ischaemic myocardium was identified by intra-arterial injection of methylene blue (P4125, Sigma Chemical Company, St. Louis, MO, USA) while the LADCA was completely occluded at the site of the inflatable balloon. The heart, the brains, the adrenals, the kidneys and the iliopsoal muscle were removed and treated as described before to obtain regional perfusion data (see Saxena et al. 1980; Duncker et al. 1986). Regional cerebral blood flow was determined by dissecting the brain in left and right hemisphere, diencephalon, cerebellum and brain stem and placing them in separate vials. Regional conductances were calculated by the ratio of the mean arterial blood pressure and the regional blood flows.

Experimental protocols. After systemic haemodynamics had been stable for at least 30 min following the surgical procedures, baseline values of systemic haemodynamics, regional myocardial function and arterial and coronary venous blood gases were obtained while a batch of microspheres was injected. LADCA flow was then reduced gradually by slowly inflating the balloon until regional systolic wall thickening had decreased to less than 35% of its baseline value. If necessary, minor adjustments in the degree of flow reduction were performed during the first 5 min of ischaemia but, thereafter, no further manipulations were allowed. Five animals had an episode of ventricular fibrillation during the first 15 min of ischaemia and were excluded from further study. When, however, this arrhythmia occurred during drug or solvent treatment the animals were promptly defibrillated with DC-countershock. After 15 min of ischaemia in 9 animals an infusion of nicorandil at a rate of 15 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was started which was increased to 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$ after 30 min. The 7 other animals received the same amount of solvent. The measurements of systemic and regional haemodynamics and myocardial performance were repeated before and at the end of each infusion period. No antiarrhythmic drugs were administered during the course of the experiments.

Drugs. Apart from the anaesthetics during the surgical procedures, only nicorandil (Rhône-Poulenc, Amstelveen, The Netherlands) or solvent (see below) was used in this study. Nicorandil was dissolved in 1.5 ml ethyl alcohol and 0.5 ml polyethylene glycol and, subsequently, final volumes were reached by adding isotonic saline. This solvent has no cardiovascular effects in the pig (Duncker et al. 1986).

Data presentation and statistical analysis. All data have been presented as arithmetic means ± SEM. The significance of the changes produced by LADCA flow reduction in the animals was evaluated by Duncan's new multiple range test once an analysis of variance had revealed that the samples represented different populations. The significance of the nicorandil-induced changes was determined by comparing these changes with those observed in the solvent-treated animals at comparable points of time using an unpaired Student's *t*-test. *P*-Values less than 0.05 were considered statistically significant.

Results

Ventricular arrhythmias

Five animals had an episode of ventricular fibrillation in the first 15 min of ischaemia and were excluded from further study. In both groups the incidence of ventricular fibrillation after 15 min of ischaemia was similar (four animals in each group), and they were all defibrillated successfully within 15 s after the onset of this arrhythmia. Except for the short period of ventricular fibrillation, this arrhythmia had no effect on systemic haemodynamics, as each animal recovered immediately and completely. The incidence of other ventricular dysrhythmias was too low to affect systemic haemodynamics at the time the measurements were taken.

Systemic haemodynamics

Figure 1 shows that after 15 min the reduction of LADCA blood flow in both the nicorandil-treated animals and the solvent-treated animals had caused decreases in mean arterial blood pressure (by 15% and by 14%, respectively), cardiac output (by 30% and by 26%, respectively), stroke volume (by 22% and by 21%, respectively) and max$LVdP/dt$ (by 24% and by 25%, respectively) an increase in LVEDP (by 4 and by 3 mm Hg, respectively), and no change in heart rate. After infusions of nicorandil (15 and 30 $\mu g \cdot kg^{-1} \cdot min^{-1}$) heart rate increased by 14% ($P > 0.05$) at the highest dose over the value obtained after 15 min of ischaemia whereas during the comparable time interval in the solvent-treated animals heart rate decreased with 7%. During nicorandil infusions mean arterial blood pressure decreased by 37% at the highest dose which is significantly different

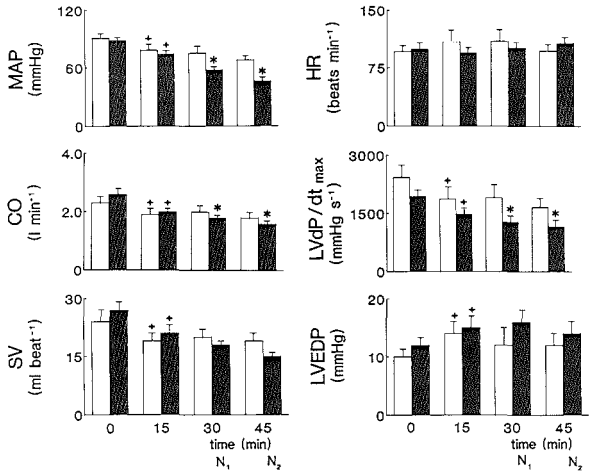

Fig. 1. Comparison of the effects on systemic haemodynamic variables in nicorandil-treated (■) and solvent-treated (□) pigs with a partial stenosis of the LADCA; *HR* heart rate; *MAP* mean arterial blood pressure; *maxLVdP/dt* maximal rate of rise in left ventricular pressure; *CO* cardiac output; *SV* stroke volume; *LVEDP* left ventricular end-diastolic pressure). $^{+}P < 0.05$ vs. baseline, only presented for 15 min of ischaemia; * nicorandil-induced changes are significantly different from the changes in the solvent-treated animals. Stenosis was applied after baseline measurements were taken at $t = 0$ min. Infusion of nicorandil, at the lower infusion rate (15 $\mu g \cdot kg^{-1} \cdot min^{-1}$, N_1), or solvent was started at $t = 15$ min and doubled at $t = 30$ min after collection of the data with the lower infusion rate. The last set of data (N_2) were recorded after 15 min infusion at the higher rate. Date are means ± SEM

from the 13% decrease in the solvent-treated animals. The fall in mean arterial blood pressure was not only due to a further decrease in cardiac output but also to vasodilatation as systemic vascular resistance decreased with 28% at the highest infusion rate (not shown), which was significantly different from the 7% decrease in the solvent-treated animals. Nicorandil also caused a further decrease in stroke volume and max LV*dP/dt* (by 19% and by 29%, respectively, of the value obtained after 15 min of ischaemia) whereas in the solvent-treated animals stroke volume did not change and maxLV*dP/dt* decreased only by 11% ($P < 0.05$). Nicorandil had no effect on left ventricular end diastolic pressure (Fig. 1).

Regional myocardial perfusion

The microsphere data revealed that 15 min after inflation of the balloon around the LADCA, in the nicorandil-treated and the solvent-treated animals transmural blood flow to the myocardium perfused by the LADCA was reduced to values of 31% and 39% of baseline, respectively. Since blood flow to the subendocardial layers was more severely affected than that to the subepicardial layers, the ratio of the normalized subendocardial to subepicardial blood flow (endo/epi ratio) was reduced in both groups. Administration of nicorandil further decreased the blood flow to the ischaemic myocardium. This decrease was similar for both myocardial layers and was significantly different from the changes observed in the solvent-treated animals (Fig. 2).

In the myocardium adjacent to that nourished by the narrowed LADCA (non-ischaemic myocardium) the changes in blood flow at the corresponding time (i.e. 15 min after LADCA flow reduction) in both groups were not marked; only the reduction (by 21 ± 7%) in the subendocardial layers was statistically significant, compared to the 4% increase in the solvent-treated animals (Fig. 2). As opposed to the control group, in the treated group the subepicardial fraction tended to increase during the drug infusions. Because nicorandil did not affect transmural blood flow, the nicorandil-induced decrease in endo-epi blood flow

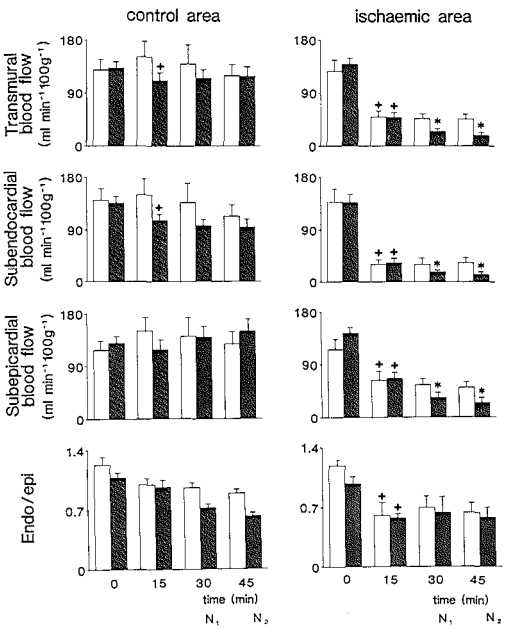

Fig. 2. Comparison of the effects on regional myocardial blood flows and the normalized subendocardial/subepicardial (endo/epi) blood flow ratio in nicorandil-treated (■) and solvent-treated (□) pigs with a partial stenosis of the LADCA. For further details see Fig. 1

ratio was more a consequence of the luxury subepicardial blood flow rather than a deficit of the subendocardial blood flow (Fig. 2).

In view of the decrease in mean arterial blood pressure following nicorandil infusions after 15 min of ischaemia, it

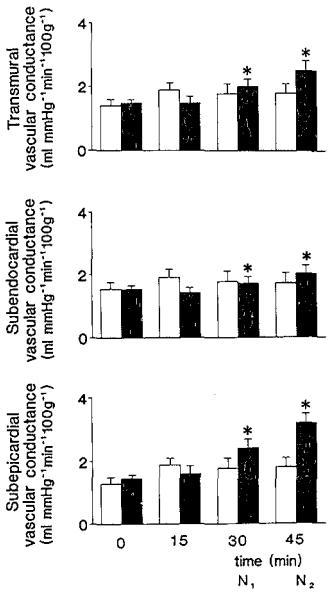

Fig. 3. Comparison of the effects on vascular conductance in the non-ischaemic segment in nicorandil-treated (■) and solvent-treated pigs (□) with a partial stenosis of the LADCA. For further details see Fig. 1

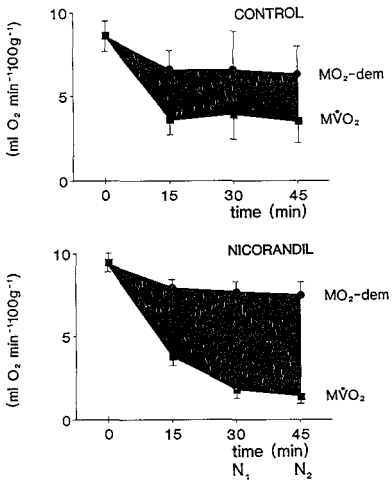

Fig. 4. Myocardial oxygen demand (MO_2-dem; ●) and myocardial oxygen consumption ($M\dot{V}O_2$; ■), resulting in the myocardial oxygen deficit (*shaded area*) in the solvent-treated and the nicorandil-treated pigs with a partial stenosis of the LADCA at baseline and 15, 30 and 45 min of ischaemia. For further details see Fig. 1

is obvious that coronary vasodilatation, especially of the subepicardium, is responsible for the maintenance of the transmural blood flow of the non-ischaemic myocardium. Indeed, while there was no change in the solvent-treated animals conductance of the subepicardial layers doubled after nicorandil. The vascular conductance in the subendocardial layers increased by 41% compared to a 10% decrease in the solvent-treated animals (Fig. 3).

Figure 4 shows that the difference in myocardial oxygen demand (MO_2-dem) and consumption ($M\dot{V}O_2$) (i.e. myocardial deficit), calculated according to the Bretschneider formula (Hoeft et al. 1984), tended to increase in the nicorandil-treated animals as a result of the decreasing myocardial oxygen consumption ($P < 0.05$).

Regional myocardial wall function

Reduction of the coronary blood flow in both groups caused a 23–33% decrease in the systolic wall thickening in the segment perfused by the LADCA, which did not change in the solvent-treated animals and also remained unaffected by nicorandil. At 15 min of ischaemia, post-systolic wall thickening in the solvent and in the nicorandil-treated pigs was $14 \pm 3\%$ and $17 \pm 2\%$ ($P > 0.05$), respectively, which did not change in either group during the remainder of the ischaemic period.

Regional blood flow and conductances in some other organs

In all the nicorandil-treated animals, but in only four of the solvent-treated pigs cerebral, renal, adrenal and skeletal muscle blood flows were measured. In both groups the ischaemia-induced decrease in cardiac output was not homogeneously distributed as perfusion of some organs and tissues decreased (kidneys by 20% in the treated and by 15% in the solvent-treated animals, adrenals by 15% in the treated and by 18% in the solvent-treated animals, skeletal muscle by 23% in the treated and by 27% in the solvent-treated animals), whereas cerebral blood flow and perfusion of the non-ischaemic left ventricular myocardium (see above) were not significantly affected (Table 1).

The nicorandil-induced decrease in cardiac output was relatively minor (though significantly different from the decrease during the comparable time interval in the solvent-treated animals), but compared to the solvent-treated animals renal blood flow decreased ($P < 0.05$) and skeletal muscle flows did not change and adrenal and cerebral blood flows increased significantly (Table 1). Consequently, dose-dependent vasodilatation occurred during infusions of nicorandil in the aforementioned organs and tissues, except the kidneys (Table 1). With respect to the brain it is intriguing that the different regions responded differently to the drug (Table 2) as the increase in vascular conductance in the brain stem (by $264 \pm 58\%$) was larger than in the diencephalon (by $194 \pm 41\%$), cerebellum (by $151 \pm 27\%$) and the right (by $129 \pm 26\%$) and left cerebral hemispheres (by $123 \pm 22\%$), while in the solvent-treated pigs there was no change in conductance of any part of the brain.

Discussion

Systemic haemodynamics

The changes in systemic haemodynamic parameters following the reduction in myocardial blood flow in pigs have been extensively documented and those reported here are

Table 1. Comparison of the effects on regional blood flows and conductances in nicorandil-treated (N, $n = 9$) and solvent-treated (S, $n = 4$) open-chest anaesthetized pigs with a coronary artery stenosis

		Baseline values	LADCA stenosis	Nicorandil ($\mu g \cdot kg^{-1} \cdot min^{-1}$)	
				15	30
Flow ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)					
Kidneys	S	274 ± 40	232 ± 34 +	212 ± 30	210 ± 36
	N	303 ± 19	242 ± 13 +	192 ± 24	123 ± 17*
Adrenals	S	131 ± 46	108 ± 32	85 ± 9	118 ± 10
	N	116 ± 28	98 ± 19	194 ± 32*	189 ± 24*
Total brain	S	28 ± 4	27 ± 3	25 ± 4	25 ± 3
	N	28 ± 2	24 ± 1	29 ± 3*	36 ± 4*
Skeletal muscle	S	3.3 ± 0.5	2.4 ± 0.2 +	2.3 ± 0.2	2.4 ± 0.4
	N	3.0 ± 0.2	2.3 ± 0.2 +	2.3 ± 0.2	2.2 ± 0.1
Conductance ($ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 100\ g^{-1}$)					
Kidneys	S	3.22 ± 0.44	3.10 ± 0.42	2.79 ± 0.35	2.80 ± 0.42
	N	3.49 ± 0.34	3.30 ± 0.23	3.42 ± 0.46	2.63 ± 0.35
Adrenals	S	1.60 ± 0.65	1.45 ± 0.44	1.12 ± 0.11	1.57 ± 0.07
	N	1.39 ± 0.40	1.38 ± 0.35	3.48 ± 0.60*	4.14 ± 0.56*
Total brain	S	0.34 ± 0.07	0.36 ± 0.05	0.34 ± 0.06	0.34 ± 0.05
	N	0.32 ± 0.03	0.32 ± 0.02	0.53 ± 0.06*	0.79 ± 0.10*
Skeletal muscle	S	0.037 ± 0.006	0.031 ± 0.004	0.030 ± 0.004	0.032 ± 0.007
	N	0.034 ± 0.002	0.032 ± 0.003	0.041 ± 0.004*	0.048 ± 0.003*

+ $P < 0.05$ versus baseline, only presented for 15 min of LADCA-stenosis
* Nicorandil-induced changes versus 15 min of LADCA stenosis are significantly different from the changes in the solvent-treated animals

Table 2. Comparison of the effects on regional cerebral blood flows and conductances in nicorandil-treated (N, $n = 9$) and solvent-treated (S, $n = 4$) open-chest anaesthetized pigs with a coronary artery stenosis

		Baseline values	LADCA stenosis	Nicorandil ($\mu g \cdot kg^{-1} \cdot min^{-1}$)	
				15	30
Flow ($ml \cdot min^{-1} \cdot 100\ g^{-1}$)					
Hemispheres					
Right	S	28 ± 4	28 ± 3	25 ± 3	25 ± 3
	N	28 ± 2	24 ± 1	28 ± 2*	33 ± 4*
Left	S	28 ± 4	28 ± 3	25 ± 3	25 ± 3
	N	28 ± 2	24 ± 1	28 ± 2*	33 ± 4*
Diencephalon	S	25 ± 3	27 ± 3	25 ± 4	25 ± 3
	N	26 ± 2	20 ± 2	28 ± 3*	36 ± 5*
Cerebellum	S	31 ± 5	30 ± 3	29 ± 4	29 ± 3
	N	33 ± 4	27 ± 2	35 ± 4*	43 ± 8*
Brain stem	S	24 ± 4	25 ± 3	22 ± 4	22 ± 2
	N	28 ± 4	24 ± 2	36 ± 4*	53 ± 8*
Conductance ($ml \cdot min^{-1} \cdot mm\ Hg^{-1} \cdot 100\ g^{-1}$)					
Hemispheres					
Right	S	0.34 ± 0.04	0.37 ± 0.06	0.34 ± 0.05	0.34 ± 0.05
	N	0.32 ± 0.03	0.32 ± 0.02	0.51 ± 0.06*	0.73 ± 0.09*
Left	S	0.32 ± 0.06	0.36 ± 0.05	0.33 ± 0.06	0.35 ± 0.05
	N	0.31 ± 0.03	0.32 ± 0.02	0.50 ± 0.05*	0.72 ± 0.08*
Diencephalon	S	0.30 ± 0.06	0.32 ± 0.04	0.31 ± 0.05	0.31 ± 0.04
	N	0.29 ± 0.03	0.27 ± 0.02	0.50 ± 0.07*	0.79 ± 0.13*
Cerebellum	S	0.38 ± 0.08	0.40 ± 0.05	0.38 ± 0.06	0.39 ± 0.06
	N	0.38 ± 0.05	0.37 ± 0.03	0.62 ± 0.09*	0.93 ± 0.15*
Brain stem	S	0.29 ± 0.06	0.34 ± 0.05	0.29 ± 0.06	0.30 ± 0.04
	N	0.32 ± 0.05	0.33 ± 0.04	0.66 ± 0.11*	1.15 ± 0.17*

* Nicorandil-induced changes versus 15 min of LADCA stenosis are significantly different from the changes in the solvent-treatment animals

consistent with existing data (Schamhardt et al. 1981; Verdouw et al. 1985; Sassen et al. 1988). Nicorandil modified several parameters, most important of which was the decrease in mean arterial blood pressure. Similarly, in accordance with earlier observations (Preuss et al. 1985; Lamping et al. 1984a, b; Verdouw et al. 1987a), nicorandil dilated systemic arterial beds. In the nicorandil-treated animals diastolic arterial blood pressure, an important determinant of max$LV dP/dt$, and max$LV dP/dt$ decreased significantly compared to the solvent-treated animals. The decrease in systemic vascular resistance is similar to that reported for non-ischaemic animals, but the absence of any effect on left ventricular filling pressure is at variance with earlier observations (Verdouw et al. 1987a) and cannot be readily explained.

Regional left ventricular blood flow

As expected, the reduction in blood flow in the LADCA led to a more severe decrease in the subendocardium than in the subepicardium. In the solvent-treated animals both systemic and regional haemodynamic variables remained relatively stable during the time interval corresponding to that of the nicorandil infusions. Furthermore, negative chronotropy following either a specific bradycardic agent (Schamhardt et al. 1981) or β-adrenoceptor antagonism (Verdouw et al. 1985; Sassen et al. 1988) or an increase in mean arterial blood pressure (Schamhardt et al. 1979; Lekven and Kiil 1975) improves myocardial perfusion with subendocardial layers benefiting the most.

The present study demonstrates that nicorandil decreased the endo/epi blood flow ratio in the non-ischaemic segment. It must be emphasized, however, that this decrease in the endo/epi ratio was not due to an adverse effect in the subendocardial layers but more to a preferential vasodilatation in the subepicardial layers. In the ischaemic myocardial segment nicorandil did not improve myocardial wall motion and caused a clear reduction in the transmural blood flow that was equally distributed over the subepicardial and subendocardial layers. Therefore, in the present porcine experimental model (concentric stenosis and no collateral circulation), which may be a representation of a substantial group of, in particular, young patients with a sudden onset of symptoms of coronary artery disease (Schwartz et al. 1978; Kober et al. 1978), nicorandil failed to have a beneficial effect on the ischaemic myocardium. This is consistent with the observations made with pinacidil, another compound that increases potassium conductance (Sakamoto et al. 1987). However, in patients with an eccentric stenosis nicorandil may improve transmural perfusion as the drug has been shown to be capable of dilating the large coronary arteries (Suryapranata et al. 1988).

The adverse effect of nicorandil on the perfusion of the ischaemic segment seems to be related to the nicorandil-induced decrease in mean arterial blood pressure. The primary goal of vasodilators is to lower the oxygen demand of the myocardium and possibly to improve perfusion by an increase in collateral blood flow. The potential unfavorable effect of such a drug is the reduction in myocardial blood flow due to decreased perfusion pressure. The latter effect is especially likely to be observed under conditions of maximal vasodilatation. In this model of concentric stenosis of the LADCA in a species with poor native collaterals, the disadvantages of nicorandil outweighed its advantages as myocardial oxygen demand was not affected by the drug. It has been well documented, however, that the oxygen balance can be positively affected in this model (Schamhardt et al. 1979, 1980; Verdouw et al. 1986, 1987b).

Despite the increase in oxygen deficit there was no indication that during the ischaemic period the viability of the myocardial tissue was affected, as post-systolic wall thickening, a marker for potential of recovery (Brown et al. 1987; Takayama et al. 1988) was similar in both groups.

Regional blood flows and conductances

In a number of studies it has been emphasized that measurement of a central haemodynamic parameter such as cardiac output may be of limited value in describing the cardiovascular effects of pharmacological agents. In myocardial ischaemia and, particularly, in heart failure the effects on perfusion of some peripheral organs and tissues may be more informative (Drexler et al. 1985, 1986). The present study shows that the ischaemia-induced decrease in cardiac output caused a decrease in renal and skeletal muscle blood flow but had no effect on cerebral and adrenal blood flow. The decrease in renal and skeletal muscle flow was due to the fall in perfusion pressure as the regional conductances were not significantly affected. The present data suggest that in spite of a slight additional decrease in cardiac output, nicorandil distributed flow in favour of the adrenals and brain, due to a marked vasodilatory action. These findings are consistent with those described for the drug in non-ischaemic animals (Verdouw et al. 1987a).

In conclusion, our experiments show that nicorandil does not have any beneficial effect on myocardial perfusion and performance in pigs with a concentric stenosis around the LADCA.

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Chapter 9

Haemodynamic profile of the potassium channel activator EMD 52692 in anaesthetized pigs

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Haemodynamic profile of the potassium channel activator EMD 52692 in anaesthetized pigs

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1 The systemic and regional haemodynamic effects of the potassium channel activator EMD 52692 or its solvent were investigated after intravenous and after intracoronary administration in anaesthetized pigs.

2 Consecutive intravenous 10 min infusions of EMD 52692 (0.15, 0.30, 0.60, 1.20 $\mu g\,kg^{-1}\,min^{-1}$; $n = 7$) dose-dependently decreased mean arterial blood pressure by up to 50%. This was entirely due to peripheral vasodilatation, since cardiac output did not change. Heart rate increased by up to 50%, while left ventricular end diastolic pressure decreased dose-dependently from 6 ± 1 mmHg to 3 ± 1 mmHg ($P < 0.05$), and stroke volume decreased from 30 ± 2 ml to 21 ± 2 ml ($P < 0.05$). Left ventricular dP/dt_{max} was not affected.

3 Although cardiac output did not change, EMD 52692 caused a redistribution of blood flow from the arteriovenous anastomoses to the capillary channels. Blood flow to the adrenals, small intestine, stomach, bladder, spleen and brain increased while renal blood flow decreased and blood flow to several muscle groups and skin were not altered. Vascular conductance was increased dose-dependently in all organs, except for the kidneys, where after the initial increase, vascular conductance returned to baseline with the highest dose. Particularly striking were the effects on the vascular of the brain. With the highest dose of EMD 52692 blood flow more than doubled, while vascular conductance increased four fold.

4 Transmural myocardial blood flow increased slightly, which was entirely due to an increase in subepicardial blood flow. Myocardial O_2-consumption and segment length shortening were not significantly affected.

5 After consecutive 10 min intracoronary infusions (0.0095, 0.019, 0.0375 and 0.075 $\mu g\,kg^{-1}\,min^{-1}$; $n = 7$) into the left anterior descending coronary artery (LADCA), mean arterial blood pressure was maintained with the two lowest doses, but decreased by up to 15% with the higher doses, whereas heart rate increased by up to 24%. Blood flow to the LADCA-perfused myocardium doubled with the highest dose, the subepicardium benefitting the most. Coronary venous O_2-saturation increased dose-dependently from $23 \pm 2\%$ to $60 \pm 4\%$, while myocardial O_2-consumption of the LADCA-perfused myocardium was not affected by the drug.

6 It is concluded that EMD 52692 is a potent vasodilator, with particularly pronounced effects on vasculature of the brain. Its selectivity for vascular smooth muscle cells exceeds that for the myocytes, since with doses that much higher than those of potential clinical no negative inotropic effects were observed. The compound primarily dilates arteries but some venodilatation may also occur.

Introduction

Diverse pharmacological treatments can have equal hypotensive effects in essential hypertension, despite apparently distinct modes of action (e.g. Ca^{2+} antagonism, (ACE)-inhibition). Recently, a new class of vasodilators, the potassium channel activators, has been introduced. These drugs act on the vascular smoooth muscle cells, causing hyperpolarization of the membranes, which reduces the availability of Ca^{2+} and thereby attenuates contraction (Hamilton *et al.*, 1986). Furthermore, the cells become less sensitive to depolarizing stimuli (Clapham & Wilson, 1987; Cook, 1988; 1989). The vasodilator and cardiac effects of drugs like cromakalim (Hamilton *et al.*, 1986; Weir & Weston, 1988; Cook & Hof, 1988) and pinacidil (Bray *et al.*, 1987) and also the cardiac effects of nicorandil (Weir & Weston, 1988) are mediated by opening of adenosine 5'-triphosphate (ATP)-dependent potassium channels. These potassium channels have been shown to be important in mediating the hypoxic dilatation of coronary arteries (Daut *et al.*, 1990).

Cromakalim, at doses causing similar decreases in arterial blood pressure as nifedipine, exhibits a vasodilator profile which is clearly different from that of the calcium antagonists (Buckingham *et al.*, 1986). The aim of the present study was to investigate the systemic haemodynamics and the distribution of cardiac output (radioactive microsphere technique) in response to intravenous infusions of EMD 52692 in the anaesthetized pig. EMD 52692 (Figure 1), a novel benzopyran derivative, structurally identical to SR 44866 (Findlay *et al.*, 1989), has been shown to hyperpolarize smooth muscle cells, which has been attributed to opening of ATP-dependent potassium channel activation (Findlay *et al.*, 1989; De Peyer *et al.*, 1989; Gericke *et al.*, 1989). In order to distinguish direct from indirect (secondary to systemic haemodynamic changes)

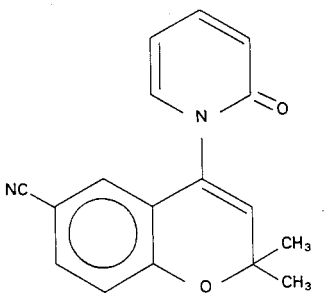

Figure 1 Chemical structure of EMD 52692.

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effects, we also studied the effects of EMD 52692 on myocardial blood flow after intracoronary infusion of the compound.

Methods

General

After an overnight fast, cross-bred Landrace × Yorkshire pigs of either sex (22–28 kg, $n = 29$) were sedated with 120 mg azaperone (Stresnil) i.m., anaesthetized with 150 mg metomidate (Hypnodil) i.v., intubated and connected to a ventilator for intermittent positive pressure ventilation with a mixture of O_2 and N_2O (1:2, v/v). Respiratory rate and tidal volume were set to keep arterial blood gases within the normal range: $7.41 < pH < 7.44$; $42\,mmHg < Pco_2 < 45\,mmHg$ and $130\,mmHg < Po_2 < 170\,mmHg$. 7F catheters were placed in the superior caval vein for administration of $160\,mg\,kg^{-1}$ α-chloralose followed by an infusion of a low dose of sodium pentobarbitone ($5\,mg\,kg^{-1}\,h^{-1}$); for administration of the muscle relaxant pancuronium bromide (4 mg) prior to thoracotomy; and for administration of haemaccel (Behringwerke A.G., Marburg, F.R.G.) to replace blood loss. Catheters were also positioned in the descending aorta for withdrawal of blood samples and measurement of central aortic blood pressure. A micromanometer-tipped catheter (7F Millar), inserted via the left carotid artery, was used to measure left ventricular pressure and its first derivative (LV dP/dt). After thoractomy, an electromagnetic flow probe (Skalar, Delft, The Netherlands) was placed around the ascending aorta, while the great cardiac vein was cannulated for collection of blood in which the haemoglobin concentration and O_2-saturation were determined (OSM2, Radiometer, Copenhagen, Denmark). In 15 of the animals the proximal left anterior descending coronary artery was dissected free for placement of an electromagnetic flow probe around the vessel, while a cannula (outer diameter 0.8 mm) was inserted for intracoronary infusions of EMD 52692.

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 either ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce. Fifteen 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. At the end of each experiment in which the animals received intracoronary infusions of drug or solvent (see below), the area perfused by the left anterior descending coronary artery was identified by intracoronary injection of patent blue violet (Sigma, St. Louis, MO, U.S.A.). All the animals were killed with an overdose of sodium pentobarbitone. From the i.v. treated animals (see below) various organs (adrenals, bladder, stomach, small intestine, brains and kidneys) and tissues (abdominal skin, different muscle groups) were excised, weighed and put into vials. In the animals treated with intracoronary infusions only the heart was excised. The hearts were fixed in formaldehyde (10% v/v) and 48 h later divided into atria and right and left ventricle. The myocardium of the left ventricle was divided into three layers of equal thickness: subepicardium, mesocardium and subendocardium. The kidneys were divided into medulla and three cortical layers of equal thickness. The cerebral hemispheres, cerebellum, diencephalon and brain stem were separated in order to obtain regional blood flow data from the different parts of the brain.

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

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

where I_{tis} and I_{art} are, respectively, the radioactivity (c.p.m.) in a particular tissue and that of the arterial blood sample, while Q_{art} is the rate of withdrawal of the blood sample. Although the lungs receive microspheres via both peripheral arteriovenous anastomoses and bronchial arteries, the contribution via the latter route appears to be only about 1% (Baile *et al.*, 1982). Thus, the values for the lung blood flow can be used as an index of the arteriovenous anastomotic flow (i.e. nonnutrient part of the cardiac output). The nutrient part of the cardiac output was calculated by subtracted 'lung flow' from the cardiac output. All regional vascular conductances were calculated as the ratio between Q_{tis} and the mean arterial blood pressure. Full details of the procedures and the calculation of flow data using this technique have been described earlier (Saxena *et al.*, 1980).

Myocardial oxygen consumption and contractile function

Myocardial O_2-consumption (MVO_2) was calculated as the product of coronary blood flow and the difference in the O_2 contents of the arterial and coronary venous blood.

Regional myocardial segment length shortening was measured by sonomicrometry (Triton Technology Inc., San Diego, CA, U.S.A.) by a pair of ultrasonic crystals implanted approximately 10–15 mm apart in the subepicardial layers (during intracoronary infusions) or the midmyocardial layers (during the intravenous infusions), oriented parallel to the short axis (Freeman *et al.*, 1985). In order to differentiate the effect of EMD 52692 on the different layers of the myocardium, another pair of crystals was implanted in the subendocardial layers of the animals treated with EMD 52692 directly into the left anterior descending coronary artery. At the end of each experiment the position of the subendocardial crystals was confirmed. From the tracings, 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

Four consecutive 10 min intravenous infusions of EMD 52692 (0.15, 0.3, 0.6 and $1.2\,\mu g\,kg^{-1}\,min^{-1}$; total cumulative doses of 1.5, 4.5, 10.5 and $22.5\,\mu g\,kg^{-1}$; $n = 7$) and equal volumes of the solvent ($n = 7$) or four consecutive 10 min intracoronary infusions of EMD 52692 (0.0095, 0.019, 0.0375 and $0.075\,\mu g\,kg^{-1}\,min^{-1}$; total cumulative doses of 0.10, 0.29, 0.66 and $1.41\,\mu g\,kg^{-1}$; $n = 7$) or equal volumes of the solvent ($n = 8$) were administered. Systemic haemodynamics, regional myocardial function and the distribution of coronary blood flow were determined in all four series of experiments, but the distribution of cardiac output was only determined during the intravenous infusions of EMD 52692 or its solvent.

Determination of EMD 52692 plasma concentrations

At the end of each infusion step blood samples (5 ml) were collected in heparinized tubes for the determination of plasma levels of EMD 52692. The blood samples were centrifuged at 1500 *g* at 0°C for 10 min and the plasma (2–3 ml) was stored at −80°C until further analysis. Known amounts of hexadeuterated EMD 52692 were added as internal standard to the plasma samples. EMD 52692 and internal standard were extracted with isopropyl ether. The extracts were evaporated to dryness under nitrogen, taken up in 20 to 60 μl of methanol, and aliquots of 1 μl analysed by gas chromatography/mass fragmentography (Hewlett Packard GC 5890 II and autoinjector HP 7673).

Drugs

For the i.v. infusions, EMD 52692 (4-(1,2-dihydro-2-oxo-1-pyridyl)-2,2-dimethyl-2*H*-1- benzopyran-6-carbonitrile; courtesy Dr P. Schelling, Merck Darmstadt, F.R.G.) was dissolved in 4% (v/v) polyethylene glycol for the two highest (0.60 and $1.20\,\mu g\,kg^{-1}\,min^{-1}$) doses and subsequently further diluted

with NaCl (0.9%) to give the two lower (0.15 and 0.30 $\mu g\,kg^{-1}\,min^{-1}$) doses. The infusion rate was 1 ml min^{-1} for the lowest and the third, and 2 ml min^{-1} for the second and the highest dose. for the intracoronary infusions the EMD 52692 was dissolved in 1% (v/v) polyethylene glycol and the required doses were reached by adjusting the infusion rate (from 0.25 ml min^{-1} to 2 ml min^{-1}).

Statistical evaluation

All data are presented as the arithmetic mean ± s.e.mean. The significance of the effects of the solvent or EMD 52692 on the different variables was evaluated by Duncan's new-multiple range-test once an analysis of variance (randomized block design) had revealed that the samples represented different populations. Statistical significance was defined as $P < 0.05$ (two-tailed).

Results

Intravenous infusions of EMD 52692

Plasma concentrations of EMD 52692 As the infusion rates (0.15, 0.30, 0.75 and 1.5 $\mu g\,kg^{-1}\,min^{-1}$) of EMD 52692 were increased, the arterial plasma concentrations reached levels of 5.9 ± 0.5, 14.6 ± 1.1, 30.8 ± 1.1 and 62.5 ± 2.3 $\mu g\,l^{-1}$, respectively.

Systemic haemodynamics No significant changes occurred during infusion of the solvent in any of the systemic haemodynamic variables (Table 1). EMD 52692 lowered the arterial blood pressure dose-dependently (by 51% with the highest dose). This fall in arterial blood pressure was caused by systemic vasodilatation (fall in systemic vascular resistance in 52%) as cardiac output was not affected. Cardiac output was maintained but stroke volume decreased (by up to 30%, $P < 0.05$), most likely secondary to the fall in left ventricular filling pressure (from 6 ± 1 mmHg to 3 ± 1 mmHg, $P < 0.05$). Heart rate increased dose-dependently from 103 ± 7 beats min^{-1} to 151 ± 13 beats min^{-1} ($P < 0.05$) with the highest dose. LV$dP/dt_{max}$ was not affected.

Left ventricular blood flow and performance The solvent had no effect on the transmural blood flow and its distribution over the subendocardial and the subepicardial layers and on the coronary vascular conductance (Figure 2). Infusions of the highest dose of EMD 52692 had no significant effect on transmural left ventricular blood flow, but with the two highest doses there was a redistribution in favour of the subepicardium, as the flow to the subepicardial layers increased dose-dependently from 154 ± 14 ml min^{-1} 100 g^{-1} to 257 ± 18 ml min^{-1} 100 g^{-1} ($P < 0.05$), and flow to the subendocardium did not change. In view of the decrease in arterial perfusion pressure it can be calculated that vasodilatation occurred in all layers of the myocardium (increase in vascular conductance). The increase in the subependicardium (by up to 268 ± 41%) was more pronounced than the increase (by up to 65 ± 16%) in the subendocardium (Figure 2).

In the solvent-treated animals there was a minor narrowing of the difference in the O_2 contents of the arterial and coronary venous blood (5%, $P < 0.05$), reflected by the small

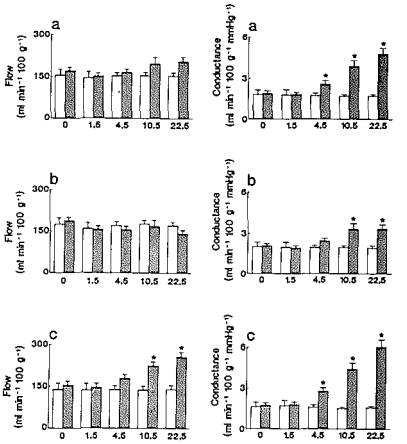

Figure 2 Effect of cumulative 10 min infusions (total dose 0, 1.5, 4.5, 10.5 and 22.5 $\mu g\,kg^{-1}$) of EMD 52692 (hatched columns, $n = 7$) and equal volumes of its solvent (open columns, $n = 7$) on transmural left ventricular (a), subendocardial (b) and subepicardial (c) blood flows and conductances in anaesthetized pigs. Data are presented as mean and the bars show s.e.mean. * Indicates that the drug-induced changes from baseline are significantly different ($P < 0.05$) from the solvent-induced changes from baseline.

Table 1 Systemic haemodynamics after continuous intravenous 10 min infusions of EMD 52692 or its solvent in anaesthetized pigs

		EMD 52692 ($\mu g\,kg^{-1}$) or equal volumes of its solvent				
		0	*1.5*	*4.5*	*10.5*	*22.5*
MAP	Solvent	89 ± 5	88 ± 4	88 ± 4	90 ± 4	88 ± 4
	EMD 52692	90 ± 3	82 ± 2*	65 ± 2*	51 ± 4*	44 ± 4*
APP	Solvent	43 ± 2	46 ± 2	45 ± 2	46 ± 2	45 ± 2
	EMD 52692	42 ± 3	39 ± 3	39 ± 3	42 ± 3	44 ± 4
CO	Solvent	2.8 ± 0.2	2.8 ± 0.2	2.8 ± 0.2	2.7 ± 0.2	2.7 ± 0.2
	EMD 52692	3.0 ± 0.2	3.1 ± 0.2	3.0 ± 0.2	3.0 ± 0.3	3.1 ± 0.2
SVR	Solvent	34 ± 4	33 ± 4	33 ± 3	35 ± 3	34 ± 3
	EMD 52692	31 ± 3	28 ± 2	22 ± 2*	18 ± 2*	15 ± 2*
HR	Solvent	99 ± 6	96 ± 6	99 ± 8	98 ± 6	99 ± 6
	EMD 52692	103 ± 7	111 ± 8*	129 ± 7*	141 ± 12*	151 ± 13*
LV$dP/dt_{max}$	Solvent	2670 ± 170	2610 ± 130	2650 ± 130	2540 ± 150	2530 ± 140
	EMD 52692	2710 ± 120	2630 ± 120	2880 ± 130	2730 ± 280	2800 ± 300
LVEDP	Solvent	6 ± 1	6 ± 1	6 ± 1	6 ± 1	7 ± 1
	EMD 52692	6 ± 1	5 ± 1*	4 ± 1*	3 ± 1*	3 ± 1*
SV	Solvent	28 ± 1	29 ± 1	29 ± 2	27 ± 1	27 ± 1
	EMD 52692	30 ± 2	28 ± 2*	23 ± 2*	22 ± 2*	21 ± 2*

Data are mean ± s.e.mean; $n = 7$ for both groups. MAP = mean arterial blood pressure (mmHg); APP = arterial pulse pressure (mmHg); CO = cardiac output (l min^{-1}); SVR = systemic vascular resistance (mmHg l min^{-1}); HR = heart rate (beats min^{-1}); LV$dP/dt_{max}$ = maximal rate of rise of the left ventricular pressure (mmHg s^{-1}); LVEPD = left ventricular end diastolic pressure (mmHg); SV = stroke volume (ml).

* Changes versus baseline in the EMD 52692-treated animals are significantly ($P < 0.05$) different from those in the solvent-treated animals.

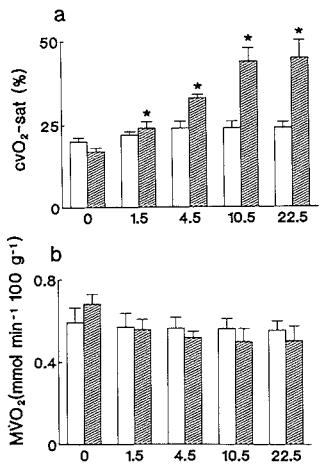

Figure 3 Effect of cumulative 10 min infusions of EMD 52692 (hatched columns, total dose 0, 1.5, 4.5, 10.5 and 22.5 $\mu g\,kg^{-1}$, $n = 7$) or equal volumes of its solvent (open columns, $n = 7$) on (a) coronary venous O_2-saturation (cvO_2-sat) and (b) left ventricular O_2-consumption (MVO_2). Data are presented as mean and the bars show s.e.mean. * Indicates that the drug-induced changes from baseline are significantly different ($P < 0.05$) from the solvent-induced changes from baseline.

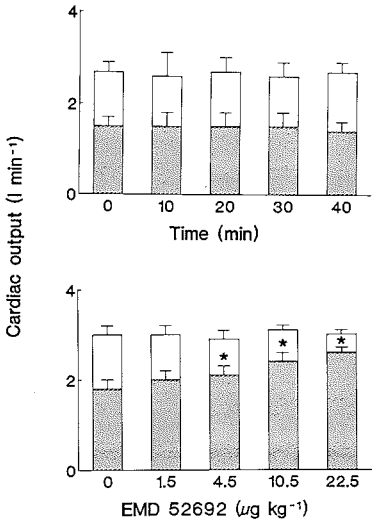

Figure 4 Fractionation of cardiac output in arteriovenous anastomotic (open columns) and capillary (hatched columns) blood flow during cumulative 10 min infusions of EMD 52692 (total dose 0, 1.5, 4.5, 10.5 and 22.5 $\mu g\,kg^{-1}$, $n = 7$) or equal volumes of its solvent ($n = 7$) anaesthetized pigs. Data are presented as mean and the bars show s.e.mean. * Indicates that the drug-induced changes from baseline are significantly different ($P < 0.05$) from the solvent-induced changes from baseline.

increase (from $20 \pm 1\%$ to $24 \pm 2\%$) in the O_2-saturation in the great cardiac vein (Figure 3). With EMD 52692, the decrease (35% after the highest infusion rate) in O_2-extraction was considerably large as the coronary venous O_2-saturation almost tripled (from $17 \pm 1\%$ to $45 \pm 5\%$ with the highest dose, $P < 0.05$). Myocardial O_2-consumption was not affected by the solvent (591 ± 73, 570 ± 66, 563 ± 57, 562 ± 49 and 554 ± 46 at baseline and after 10, 20, 30 and 40 min, respectively) or by the drug (686 ± 49, 558 ± 49, 519 ± 29, 499 ± 62 and 505 ± 71 $\mu mol\,min^{-1}\,100\,g^{-1}$ at baseline and after 1.5, 4.5, 10.5 and 22.5 $\mu g\,kg^{-1}$, respectively).

Myocardial segment length shortening, determined from the crystals placed in the mid-myocardium, did not change either

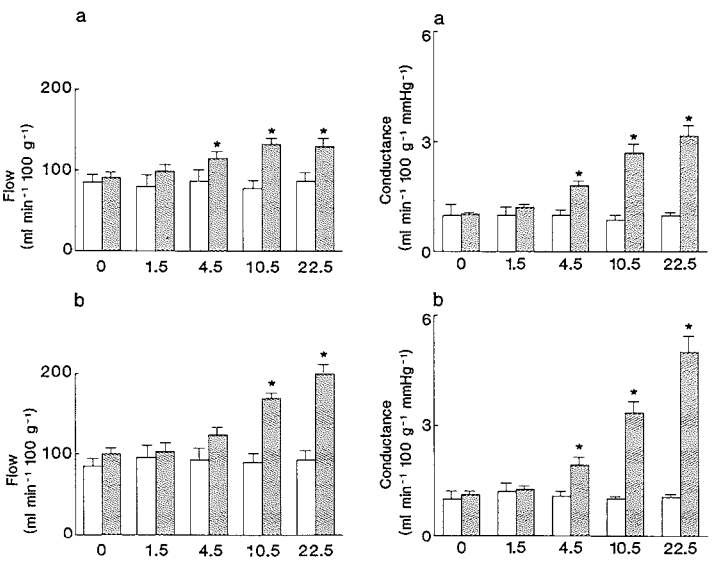

Figure 5 Effect of cumulative 10 min infusions (total dose 0, 1.5, 4.5, 10.5 and 22.5 $\mu g\,kg^{-1}$) of EMD 52692 (hatched columns, $n = 7$) and equal volumes of its solvent (open columns, $n = 7$) on atrial (a) and right ventricular blood flow (b) and conductance in anaesthetized pigs. Data are presented as mean and the bars show s.e.mean. * Indicates that the drug-induced changes from baseline are significantly different ($P < 0.05$) from the solvent-induced changes from baseline.

during infusion of the solvent (19 ± 1%, 18 ± 1%, 19 ± 1%, 19 ± 1% and 20 ± 2% at baseline and after 10, 20, 30 and 40 min, respectively) or during infusion of EMD 52692 (20 ± 1%, 19 ± 1%, 18 ± 2% and 18 ± 1% at baseline, and after cumulative infusions of 0.15, 0.30, 0.60 and 1.2 $\mu g\,kg^{-1}\,min^{-1}$ of EMD 52692, respectively).

Distribution of cardiac output

Fractionation of cardiac output into arteriovenous anastomotic and capillary flows In anaesthetized animals a large fraction of the cardiac output is shunted through arteriovenous anastomoses (Kaihara *et al.*, 1968; Van Woerkens *et al.*, 1990) and without affecting total cardiac output, antihypertensive drugs may affect this non-nutritional flow differently (Hof & Hof, 1989). Figure 4 illustrates that, under baseline conditions, in both groups approximately 40% of the cardiac output was shunted through arteriovenous anastomoses. This fraction did not change during infusion of the solvent. Although EMD 52692 did not affect cardiac output, there was a redistribution in favour of the capillary flow, which increased to 85% after the highest dose.

Regional blood flows Blood flow to the atria and the right ventricle increased dose-dependently after the EMD 52692 infusions (Figure 5). With respect to the decrease in perfusion pressure it can be calculated that the increments in vascular conductance were even more pronounced (Figure 5). EMD 52692 caused pronounced increases in total cerebral blood flow (32 ± 3, 34 ± 2, 43 ± 3, 66 ± 7 and 69 ± 7 $ml\,min^{-1}\,100\,g^{-1}$ at baseline and after 1.5, 4.5, 10.5 and 22.5 $\mu g\,kg^{-1}$, respectively). Although flow to all parts of the brain increased (Figure 6), the increase was more pronounced in the brain stem (216 ± 24%) and diencephalon (178 ± 40%) than in the cerebellum (113 ± 14%) and the hemispheres (100 ± 10%). The increases in regional conductances were even more impressive than the increases in flow (Figure 6).

Total renal blood flow remained constant with the two lowest doses, but decreased by 43 ± 7% after infusion of the highest dose (Table 2). Further analysis revealed that this was predominantly due to a dose-dependent decrease in glomerular blood flow of the outer cortex, as the glomerular blood flow of the inner cortex decreased only slightly and flow to the medulla even increased (Table 2). The decreases in flow were secondary to the fall in arterial blood pressure, as total renal conductance increased significantly by as much as

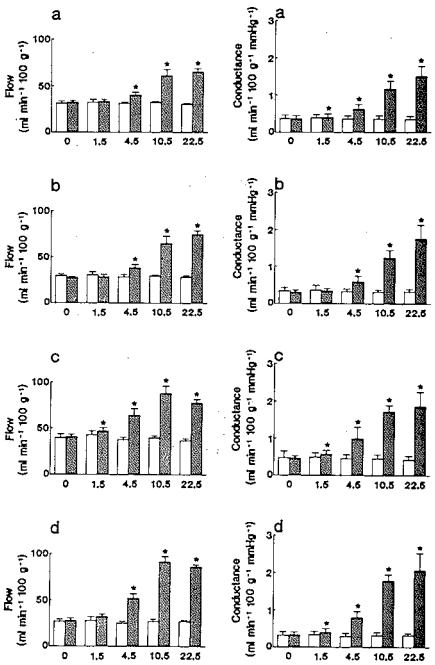

Figure 6 Effect of cumulative 10 min infusions of EMD 52692 (hatched columns, total dose 0, 1.5, 4.5, 10.5 and 22.5 $\mu g\,kg^{-1}$, $n = 7$) or equal volumes of its solvent (open columns; $n = 7$) on blood flow and conductance of the cerebral hemispheres (a), diencephalon (b), cerebellum (c) and brain stem (d) in anaesthetized pigs. Data are presented as mean and the bars show s.e.mean. * Indicates that the drug-induced changes from baseline are significantly different ($P < 0.05$) from the solvent-induced changes from baseline.

110 ± 50% after the third dose. With the highest dose renal vascular conductance started to return to baseline values. The increases in glomerular vascular conductance of the inner

Table 2 Effect of continuous intravenous 10 min infusions of EMD 52692 or its solvent on the renal blood flow and conductance in anaesthetized pigs

		EMD 52692 ($\mu g\,kg^{-1}$) or equal volumes of its solvent				
Blood flow ($ml\,min^{-1}\,100\,g^{-1}$)		*0*	*1.5*	*4.5*	*10.5*	*22.5*
Kidneys	Solvent	252 ± 22	267 ± 35	253 ± 24	249 ± 24	236 ± 15
	EMD 52692	247 ± 19	225 ± 14	228 ± 16	191 ± 11*	137 ± 17*
Glomeruli of the inner cortex	Solvent	210 ± 18	210 ± 19	202 ± 16	190 ± 13	183 ± 11
	EMD 52692	163 ± 10	161 ± 10	178 ± 9	155 ± 9	115 ± 13
Glomeruli of the outer cortex	Solvent	393 ± 25	407 ± 32	405 ± 32	412 ± 33	387 ± 26
	EMD 52692	386 ± 31	333 ± 19	316 ± 25*	258 ± 18*	179 ± 23*
Medulla	Solvent	58 ± 7	59 ± 6	53 ± 5	46 ± 5	46 ± 5
	EMD 52692	42 ± 5	44 ± 5	55 ± 7*	58 ± 5*	55 ± 6*
Conductance ($ml\,min^{-1}\,100\,g^{-1}\,mmHg^{-1}$)						
Kidneys	Solvent	2.89 ± 0.31	3.14 ± 0.25	2.95 ± 0.34	2.79 ± 0.31	2.67 ± 0.17
	EMD 52692	2.78 ± 0.23	2.74 ± 0.17	3.35 ± 0.33*	3.88 ± 0.33*	3.19 ± 0.34
Glomeruli of the inner cortex	Solvent	2.40 ± 0.25	2.54 ± 0.22	2.33 ± 0.22	2.12 ± 0.18	2.08 ± 0.14
	EMD 52692	1.84 ± 0.13	1.96 ± 0.12	2.79 ± 0.18*	3.18 ± 0.35*	2.73 ± 0.36*
Glomeruli of the outer cortex	Solvent	4.43 ± 0.34	4.89 ± 0.32	4.62 ± 0.41	4.62 ± 0.42	4.37 ± 0.20
	EMD 52692	4.32 ± 0.35	4.05 ± 0.19	4.95 ± 0.48	5.22 ± 0.43	4.14 ± 0.45
Medulla Solvent	Solvent	0.67 ± 0.08	0.68 ± 0.07	0.62 ± 0.07	0.51 ± 0.06	0.53 ± 0.06
	EMD 52692	0.48 ± 0.05	0.53 ± 0.06	0.84 ± 0.09*	1.16 ± 0.11*	1.27 ± 0.11*

Data are mean ± s.e.mean; $n = 7$ for both groups. * Changes versus baseline in the EMD 52692-treated animals are significantly ($P < 0.05$) different from those in the solvent-treated animals.

Table 3 Effect of continuous intravenous 10 min infusions of EMD 52692 or its solvent on regional blood flow and conductance in anaesthetized pigs

Blood flow ($ml\,min^{-1}\,100\,g^{-1}$)		*EMD 52692 ($\mu g\,kg^{-1}$) or equal volumes of its solvent*				
		0	*1.5*	*4.5*	*10.5*	*22.5*
Spleen	Solvent	271 ± 46	317 ± 52	273 ± 43	254 ± 55	267 ± 56
	EMD 52692	255 ± 23	311 ± 31	453 ± 36	375 ± 42	272 ± 31
Adrenals	Solvent	131 ± 16	139 ± 14	142 ± 7	144 ± 12	149 ± 22
	EMD 52692	180 ± 27	220 ± 23	332 ± 20*	291 ± 19*	231 ± 14*
Stomach	Solvent	21 ± 1	20 ± 1	23 ± 2	21 ± 2	20 ± 2
	EMD 52692	21 ± 2	20 ± 1	19 ± 2	21 ± 3	29 ± 5
Small Intestine	Solvent	38 ± 3	37 ± 2	36 ± 2	39 ± 2	36 ± 2
	EMD 52692	44 ± 5	46 ± 8	51 ± 9	62 ± 9	77 ± 7*
Skin	Solvent	1.9 ± 0.5	1.9 ± 0.9	1.5 ± 0.4	2.0 ± 0.7	2.3 ± 1.0
	EMD 52692	2.4 ± 0.4	2.7 ± 0.5	2.2 ± 0.5	2.6 ± 0.4	2.5 ± 0.3
Bladder	Solvent	3.5 ± 0.3	4.3 ± 0.4	4.0 ± 0.4	3.9 ± 0.3	3.9 ± 0.2
	EMD 52692	5.0 ± 0.5	4.9 ± 0.5	4.5 ± 0.6	4.5 ± 0.5	7.6 ± 0.9
Conductance ($ml\,min^{-1}\,100\,g^{-1}\,mmHg^{-1}$)						
Spleen	Solvent	3.13 ± 0.60	3.70 ± 0.60	3.16 ± 0.51	2.84 ± 0.65	3.05 ± 0.64
	EMD 52692	2.83 ± 0.23	3.79 ± 0.35	7.00 ± 0.38*	7.30 ± 0.54*	6.18 ± 0.39*
Adrenals	Solvent	1.53 ± 0.22	1.72 ± 0.14	1.64 ± 0.11	1.60 ± 0.14	1.67 ± 0.19
	EMD 52692	2.04 ± 0.32	2.70 ± 0.30	5.16 ± 0.36*	5.83 ± 0.42*	5.45 ± 0.44*
Stomach	Solvent	0.24 ± 0.02	0.25 ± 0.02	0.26 ± 0.02	0.23 ± 0.02	0.23 ± 0.03
	EMD 52692	0.24 ± 0.03	0.25 ± 0.02	0.31 ± 0.03	0.42 ± 0.06*	0.67 ± 0.11*
Small Intestine	Solvent	0.44 ± 0.05	0.44 ± 0.03	0.42 ± 0.030	0.44 ± 0.03	0.42 ± 0.03
	EMD 52692	0.49 ± 0.06	0.56 ± 0.09	0.81 ± 0.017	1.23 ± 0.16*	1.79 ± 0.18*
Skin	Solvent	0.022 ± 0.007	0.022 ± 0.012	0.018 ± 0.005	0.023 ± 0.008	0.028 ± 0.013
	EMD 52692	0.027 ± 0.005	0.033 ± 0.007	0.036 ± 0.009	0.051 ± 0.007*	0.058 ± 0.006*
Bladder	Solvent	0.041 ± 0.004	0.066 ± 0.006	0.046 ± 0.002	0.044 ± 0.004	0.044 ± 0.003
	EMD 52692	0.056 ± 0.006	0.059 ± 0.006	0.072 ± 0.013	0.090 ± 0.011*	0.178 ± 0.022*

Data are mean ± s.e.mean; $n = 7$ for both groups. Data are in $ml\,min^{-1}\,100\,g^{-1}\,mmHg^{-1}$: * changes versus baseline in the EMD 52692-treated animals are significantly different ($P < 0.05$) from those in the solvent-treated animals.

cortex and the vascular conductance of the medulla were not observed in the outer cortex (Table 2).

Flow to a number of other organs was well maintained (Table 3). Increases were observed in flow to the small intestine (by 81 ± 19% with the highest dose, $P < 0.05$) and the adrenals (by up to 38 ± 15%, $P < 0.05$). Since the fall in arterial perfusion pressure was larger than the decrease in flow, vascular conductance was increased in all these organs (Table 3).

The blood flow to eight different muscle groups was measured, of which only flow to the M. sternocleidomastoideus increased from 5.6 ± 0.7 $ml\,min^{-1}\ 100\,g^{-1}$ at baseline to 12.4 ± 2.7 $ml\,min^{-1}$ ($P < 0.05$), with the highest dose. Flow to all the other muscles (M. iliopsoas, pars costalis diaphragmatis, M. pectoralis, M. erector spinae, M. gluteus, M. masseter and the M. rectus abdominis) remained constant (baseline values between 2 and 6 $ml\,min^{-1}\ 100^{-1}$). Vasodilatation occurred in all muscle groups as vascular conductance had increased 2 to 5 fold after infusion of the highest dose.

Intracoronary infusions of EMD 52692

Systemic haemodynamics No arrhythmias were observed during infusions of either the solvent or EMD 52692. Infusion of the solvent had no effect on systemic haemodynamics (Table 4). During infusion of the two lowest doses of EMD 52692 the only change was a 5% increase in heart rate. With the higher infusion rates there was a further increase in heart rate (by up to 22 ± 4%) and a 16 ± 3% decrease in mean arterial blood pressure, due to peripheral vasodilatation (decrease in systemic vascular resistance of 16 ± 4%). Cardiac output and left ventricular filling pressure did not change, while LV dP/dt_{max} showed an increase (24 ± 8%, $P < 0.05$) after infusion of the highest dose.

Table 4 Systemic haemodynamics after continuous intracoronary 10 min infusions of EMD 52692 or its solvent in anaesthetized pigs

		EMD 52692 ($\mu g\,kg^{-1}$) or equal volumes of its solvent				
		0	*0.10*	*0.29*	*0.66*	*1.41*
HR	Solvent	111 ± 6	111 ± 6	111 ± 5	113 ± 5	114 ± 6
	EMD 52692	116 ± 5	117 ± 5	123 ± 6*	130 ± 8*	144 ± 10*
MAP	Solvent	87 ± 4	85 ± 3	84 ± 3	85 ± 4	84 ± 5
	EMD 52692	93 ± 4	94 ± 4	89 ± 5	86 ± 4*	79 ± 5*
CO	Solvent	2.6 ± 0.3	2.6 ± 0.2	2.6 ± 0.3	2.6 ± 0.3	2.5 ± 0.3
	EMD 52692	2.3 ± 0.1	2.4 ± 0.2	2.3 ± 0.2	2.3 ± 0.1	2.3 ± 0.1
SVR	Solvent	35 ± 3	35 ± 3	34 ± 3	35 ± 3	35 ± 3
	EMD 52692	42 ± 3	41 ± 3	39 ± 3	38 ± 2*	35 ± 1*
LV$dP/dt_{max}$	Solvent	2490 ± 290	2460 ± 280	2460 ± 290	2610 ± 370	2580 ± 370
	EMD 52692	2270 ± 160	2330 ± 190	2500 ± 250	2680 ± 300	2830 ± 320*
LVEDP	Solvent	7 ± 1	6 ± 1	6 ± 1	6 ± 1	6 ± 1
	EMD 52692	7 ± 1	7 ± 1	7 ± 1	7 ± 1	6 ± 1

Data are mean ± s.e.mean; $n = 8$ for the solvent-treated and $n = 7$ for the EMD 52692-treated animals. HR = heart rate ($beats\,min^{-1}$); MAP = mean arterial blood pressure (mmHg); CO = cardiac output ($l\,min^{-1}$); SVR = systemic vascular resistance ($mmHg\,min\,l^{-1}$); LV$dP/dt_{max}$ = maximal rate of rise of ventricular blood pressure ($mmHg\,s^{-1}$); LVEDP = left ventricular end-diastolic blood pressure (mmHg). * Changes versus baseline in the EMD 52692-treated are significantly different ($P < 0.05$) from those in the solvent-treated animals.

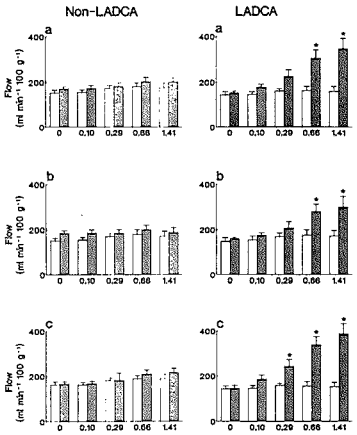

Figure 7 Transmural (a), subendocardial (b) and subepicardial (c) blood flow after 10 min cumulative intracoronary infusions of EMD 52692 (hatched columns, total doses 0, 0.10, 0.29, 0.66 and 1.41 $\mu g\,kg^{-1}$, $n = 7$) or its solvent (open columns, $n = 8$) directly into the left anterior descending coronary artery (LADCA). Non-LADCA = area not perfused by the LADCA (posterior wall of the left ventricle). Data are presented as mean and the bars show s.e.mean. * Indicates that the EMD 52692-induced changes from baseline are significantly different ($P < 0.05$) from solvent-induced changes from baseline.

Myocardial performance Intracoronary infusions of the solvent had no effect on myocardial perfusion (Figure 7). A representative example of the effects of EMD 52692 is presented in Figure 8, which clearly shows the EMD 52692-induced increases in coronary blood flow. EMD 52692 dose-dependently increased transmural blood flow of the myocardium perfused by the left anterior descending coronary artery by up to 128% (Figure 7). Although all layers benefited from the increase in transmural flow, the increments in the subepicardium ($242 \pm 55\,ml\,min^{-1}\ 100\,g^{-1}$) were considerably larger than in the subendocardium ($140 \pm 46\,ml\,min^{-1}\ 100\,g^{-1}$). There were no significant changes in myocardial blood flow (Figure 7) or vascular conductance (not shown) of the posterior wall of the left ventricle (the myocardium not perfused by the left anterior descending coronary artery).

The solvent had no effect on the myocardial O_2-consumption (499 ± 39, 486 ± 41, 593 ± 29, 529 ± 52, $523 \pm 59\,\mu mol\,min^{-1}\ 100\,g^{-1}$ at baseline and after 10, 20, 30 and 40 min of solvent infusion, respectively) or on regional subendo- and subepicardial segment length shortening (Table 5). Subendocardial segment length shortening decreased from $18.2 \pm 1.6\%$ to $16.8 \pm 1.0\%$ with the highest dose of EMD 52692 ($P < 0.05$). Subepicardial segment length shortening tended to increase, but the increments did not reach levels of significance (Table 5).

Myocardial O_2-consumption of the LADCA-perfused myocardium was not affected by the drug (542 ± 43, 522 ± 26, 560 ± 33, 618 ± 17 and $521 \pm 46\,\mu mol\,min^{-1}\ 100\,g^{-1}$ at baseline and after 0.095, 0.29, 0.66 and 1.41 $\mu g\,kg^{-1}$, respectively).

Discussion

In the present study the plasma levels of EMD 52692 ranged from 5.1 to 62 $\mu g\,l^{-1}$. This range compares well with the effective *in vitro* concentrations described by De Peyer and colleagues (1989). In this last study the EC_{50} of EMD 52692 to hyperpolarize the membrane of the vascular smooth muscle cells of porcine coronary arteries was $0.6–1 \times 10^{-7}$ M, which corresponds to a concentration of 16–26 $\mu g\,l^{-1}$.

EMD 52692 is a potent systemic vasodilator. Its potency exceeds that of nicorandil, tested in the same model by a factor of 100 (Verdouw *et al.*, 1987). Furthermore EMD 52692 was shown to be 5 and 50 times more potent in anaesthetized

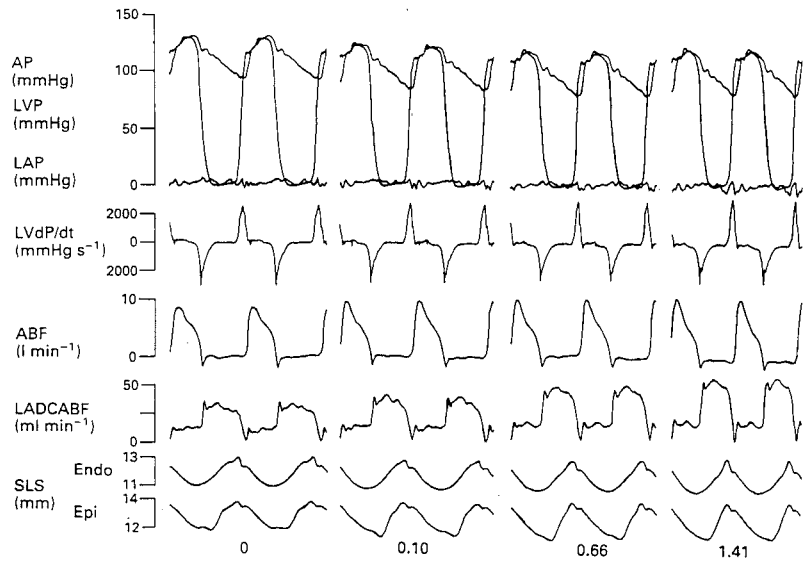

Figure 8 Representative tracing of the effects of intracoronary (left anterior descending coronary artery) infusions of EMD 52692 on systemic haemodynamics and regional myocardial blood flow and function in an anaesthetized pig. AP, LVP and LAP are central aortic, left ventricular and left atrial blood pressure, respectively; LV$dP/dt$ = first derivative of LVP; ABF = ascending aortic blood flow; LADCABF = left anterior descending coronary artery blood flow; SLS(Endo) = subendocardial segment length shortening; SLS(Epi) = subepicardial segment length shortening. The infusion rates of EMD 52692 (total dose, $\mu g\,kg^{-1}$) are given at the bottom of the figure.

Table 5 Effect of continuous intracoronary 10 min infusions of EMD 52692 or its solvent on subendocardial and subepicardial segment length shortening in anaesthetized pigs

		EMD 52692 ($\mu g\,kg^{-1}$) or equal volumes of its solvent				
		0	*0.10*	*0.29*	*0.66*	*1.41*
Subendocardium	Solvent	17.3 ± 1.4	17.7 ± 1.5	16.8 ± 1.5	17.2 ± 1.7	17.5 ± 1.5
	EMD 52692	18.2 ± 1.6	18.3 ± 1.2	17.8 ± 1.8	17.7 ± 1.2	16.8 ± 1.0*
Subepicardium	Solvent	15.1 ± 1.1	14.9 ± 1.4	14.6 ± 1.4	15.1 ± 1.6	15.0 ± 1.3
	EMD 52692	16.7 ± 1.7	17.1 ± 1.7	17.4 ± 1.4	17.9 ± 1.4	17.6 ± 1.4

Data are mean ± s.e.mean; $n = 8$ for the solvent-treated animals and $n = 7$ for the EMD 52692-treated animals. Data are expressed as %. * Changes versus baseline in the EMD 52692-treated animals are significantly different from those in the solvent-treated animals.

dogs than cromakalim and pinacidil, respectively (Schliep *et al.*, 1989), two other compounds of which the pharmacological actions have been ascribed to potassium channel opening (Hamilton *et al.*, 1986; Bray *et al.*, 1987; Weir & Weston, 1988). The potency of EMD 52692 to dilate the systemic vascular bed, also compares favorably with that of the most potent representatives of the dihydropyridine calcium antagonists such as nisoldipine (Duncker *et al.*, 1986) and elgodipine (Sassen *et al.*, 1990).

Contraction of vascular smooth muscle cells in capacitance vessels depends on a rise in intracellular free calcium, mediated by activation of receptor-operated rather than voltage-operated events (Bolton, 1979). Webb and colleagues (1989) found that cromakalim produced dose-dependent dilatation of forearm resistance vessels, but did not dilate dorsal hand veins preconstricted with noradrenaline and concluded that this potassium channel activator was arterioselective. However, we observed at similar increments in heart rate (e.g. compare the second infusion rate of the intravenous and the highest infusion rate of the intracoronary experiments), that left ventricular end-diastolic pressure decreased after the intravenous, but not after the intracoronary infusions. The dose-dependent decrease in left ventricular filling pressure during the intravenous infusions can therefore not solely be attributed to the tachycardia and EMD 52692 most likely caused some venodilatation during the intravenous infusions.

The increase in heart rate, which has also been demonstrated for cromakalim (Buckingham *et al.*, 1986), was most likely a reflex-mediated response, and is a common feature of acute administration of vasodilators. Despite the tachycardia, cardiac output did not increase. We have shown that in the same model vasodilators such as nisoldipine (Duncker *et al.*, 1986) and nicorandil (Verdouw *et al.*, 1987) elicited similar responses. In conscious pigs these compounds caused marked increases in heart rate, left ventricular dP/dt_{max} and cardiac output (Verdouw *et al.*, 1987; Duncker *et al.*, 1988), indicating that the anaesthesia may have attenuated the reflex-mediated responses.

Potassium channel activators have potentially negative inotropic properties (Yanagisawa *et al.*, 1988), although the selectivity for vascular smooth muscle outweighs that for the myocardium (Cohen & Colberg, 1986; Longman *et al.*, 1988; Gotanda *et al.*, 1988). SR 44866, a compound which is structurally identical to EMD 52692 has been shown to have inhibitory effects on the electrical and mechanical activity of cardiac muscle (Findlay *et al.*, 1989), but the required dose is much higher for cardiac muscle than for smooth muscle. We did not see any changes in $LVdP/dt_{max}$, a frequently used measure of myocardial contractility. This index is, however, also influenced by changes in heart rate and pre- and afterload, all of which were affected considerably by EMD 52692. However, the two lowest intracoronary infusion rates (where heart rate, pre- and afterload were only slightly affected, with no changes in segment length shortening) demonstrated that at comparable intravenous infusions EMD 52692 exhibits no negative inotropic action. With the highest intracoronary dose subendocardial layers segment length shortening decreased significantly, while subepicardial segment length shortening was not affected. At present these results are difficult to interpret. However, it is unlikely that these findings would have clinical implications since the dose used ($0.075\,\mu g\,kg^{-1}\,min^{-1}$, into the coronary artery) exceeds the doses that can be used in the clinical situation.

In anaesthetized animals a substantial fraction of the cardiac output is shunted through arteriovenous anastomoses (Kaihara *et al.*, 1968; Saxena & Verdouw, 1985), which is reflected by the large entrapment of the microspheres in the lungs after induction of anaesthesia (Van Woerkens *et al.*, 1990). Figure 4 shows that although cardiac output did not change, the nutritional fraction increased with increasing doses of EMD 52692. This increase was most prominent in the brain, adrenals and small intestine, but absent in skeletal muscle. This study therefore confirms earlier investigations that potassium channel activators at equihypotensive dosages have a different vasodilator profile than for instance the calcium antagonists (Duncker *et al.*, 1986; Buckingham *et al.*, 1986; Sassen *et al.*, 1990). When comparing the vasodilator profile of EMD 52692 with two compounds that were tested in the same model, nicorandil (Verdouw *et al.*, 1987) and elgodipine (Sassen *et al.*, 1990), a calcium antagonist very similar to nisoldipine (Duncker *et al.*, 1986), it appears that EMD 52692 closely resembles nicorandil; similar increases in adrenal, cerebral, left ventricular and intestinal blood flow occurred while no effect on skeletal muscle blood flow at comparable increases in systemic vascular conductances was observed. Furthermore, it can be concluded that EMD 52692 dilates cerebral and adrenal vascular beds much more potently than elgodipine, but that the increase in vascular conductance of skeletal muscle is less affected by the potassium channel activator than by the calcium antagonist. Of interest is that the initial vasodilatation in the kidneys, as observed with elgodipine and EMD 52692 was followed by a decrease with the highest dose although systemic vascular conductance still increased. These vascular responses in the kidneys differed markedly in the inner and outer cortex and the medulla, as a result of which blood flow was redistributed away from the outer to the inner layers. A similar redistribution of renal flow during hypotention has been obtained by other investigators (see Carrière, 1975) and may thus be a perfusion pressure-dependent response rather than a direct drug action. The reduction in cortical glomerular blood flow might serve to enhance fluid retention to counterbalance the severe hypotension (Wright & Briggs, 1979).

Only a few studies have described the vasodilator profiles of other potassium channel activators (Arrigoni-Martelli *et al.*, 1980; Buckingham *et al.*, 1986; Cook & Hof, 1988). Cook & Hof (1988) studied the effects of cromakalim on regional blood flows in anaesthetized rabbits, and shbat doses inducing a blood pressure drop of up to 20% that renal blood flow decreased, skeletal muscle flow was maintained and blood flow to the stomach and small intestine were enhanced. These responses correspond well with our experiments. At variance with our findings, cerebral blood flow did not change significantly in their study. Arrigoni-Martelli and colleagues (1980) showed that pinacidil also did not affect blood flow through the femoral artery (representing muscle blood flow).

Myocardial O_2-consumption did not change after both the intravenous and the intracoronary infusions. In both experi-

ments the increase in O_2-demand caused by the increase in heart rate was fully compensated for by the decrease in arterial blood pressure. The increase in flow has therefore no metabolic origin and indicates that the coronary blood flow response is the result of a direct action of the drug on the coronary vascular bed. The finding that with the intravenous infusions the increase in flow was almost exclusively confined to the subepicardial layers is not surprising in view of the tachycardia and hypotension (Domenech & Goich, 1976); this has also been demonstrated for other vasodilators (Verdouw *et al.*, 1987). Chronic treatment often leads to a reduction of the reflex tachycardia (Kiowski *et al.*, 1983). If the coronary vasodilator action were to be sustained with chronic treatment, the lower heart rate would most likely not only lower myocardial O_2-demand but also shift coronary blood flow in favour of the subendocardial layers.

In conclusion, EMD 52692 is a potent arterial vasodilator with particularly pronounced effects on the cerebral vasculature. At doses exceeding the clinically relevant range, negative inotropic actions were not observed. The cardiovascular profile suggests that the drug may be useful in the treatment of hypertension and, in view of the lack of negative inotropic actions, of heart failure. The pronounced coronary, cerebral and mesenteric vasodilatation warrants investigations with this compound in syndromes of myocardial, cerebral and intestinal ischaemia.

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Chapter 10

Bisoprolol improves perfusion of ischaemic myocardium in anaesthetized pigs.

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Bisoprolol improves perfusion of ischaemic myocardium in anaesthetized pigs

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1 The ability of the cardioselective β-adrenoceptor antagonist bisoprolol ((±)-1-[4-(2-isopropoxyethoxymethyl)-phenoxy]-3-isopropyl-amino-2-propanol hemifumarate, EMD 33512) to suppress isoprenaline-induced increases in heart rate and maximal rate of rise in left ventricular pressure ($LVdP/dt_{max}$) was studied in 6 anaesthetized pigs given 4 cumulative doses (16, 64, 256 and 1024 $\mu g\,kg^{-1}$). Bisoprolol was about 2 times more effective in suppressing isoprenaline-induced increases in $LVdP/dt_{max}$ than those in heart rate.

2 In 8 animals which had a partial stenosis of the left anterior descending coronary artery (LADCA), the effects of 3 consecutive doses (50, 200 and 750 $\mu g\,kg^{-1}$) of bisoprolol were studied on systemic haemodynamics, regional myocardial perfusion and function. The effects of the drug were compared with those obtained in a group of 9 animals with LADCA stenosis which did not receive any treatment.

3 The lowest dose of bisoprolol (50 $\mu g\,kg^{-1}$) increased perfusion of the ischaemic myocardium (which had been reduced from $123 \pm 20\,ml\,min^{-1}\,100\,g^{-1}$ to $42 \pm 11\,ml\,min^{-1}\,100\,g^{-1}$) by $21 \pm 10\,ml\,min^{-1}\,100\,g^{-1}$ ($P < 0.05$). In particular the subendocardial layers, which were most severely affected by the stenosis (a decrease from $128 \pm 19\,ml\,min^{-1}\,100\,g^{-1}$ to $20 \pm 6\,ml\,min^{-1}\,100\,g^{-1}$) benefited from the administration of the drug (an increase of $30 \pm 10\,ml\,min^{-1}\,100\,g^{-1}$). Perfusion of the subepicardium was not significantly affected. With the higher dose only a minor additional improvement in perfusion of the ischaemic myocardium was observed.

4 The negative chronotropic response is the most likely factor leading to the improvement in perfusion.

5 Myocardial wall thickening, which decreased from 41 ± 2% to 9 ± 4% ($P < 0.05$) due to the hypoperfusion, did not improve after administration of the drug. This lack of improvement may possibly be due to the duration of ischaemia before and the magnitude of the flow deficit after bisoprolol administration.

6 Between 15 and 60 min of ischaemia, 5 of the 9 untreated animals had an episode of ventricular fibrillation compared with only 1 of the 8 animals treated with bisoprolol, in spite of an initially larger flow reduction in the treated animals. The more homogeneous flow distribution after bisoprolol might account for the lower incidence of arrhythmias in this group.

7 It was demonstrated that bisoprolol improves perfusion of ischaemic myocardium in anaesthetized pigs even at doses (50 $\mu g\,kg^{-1}$) that only moderately antagonize isoprenaline-induced cardiostimulatory effects.

Introduction

In spite of the increasing number of drugs of different mechanisms available, β-adrenoceptor antagonists still remain the drugs of choice for the treatment of myocardial ischaemia. Since recent studies have shown that this class of drugs may also be useful in preventing sudden cardiac death after acute myocardial infarction, the area of application of β-adrenoceptor antagonists has widened (Norwegian Multicenter Study Group, 1981; Hjalmarson *et al.*, 1981). One such new drug is bisoprolol

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((±)-1-[4-(2-isopropoxyethoxymethyl)-phenoxy]-3-isopropylamino-2-propanol hemifumarate, EMD 33512) which is devoid of intrinsic sympathomimetic activity (Harting *et al.*, 1986) and exhibits selectivity for β_1-adrenoceptors in several studies performed in anaesthetized dogs, guinea-pigs and cats (Schliep & Harting, 1984; Schliep *et al.*, 1986). In conscious pigs, bisoprolol antagonized isoprenaline-induced increases in the maximum rate of rise in left ventricular pressure ($LVdP/dt_{max}$) more effectively than those in heart rate. In the same model, propranolol was more potent than bisoprolol but inhibited isoprenaline-induced changes in these two parameters equally (Duncker *et al.*, 1987).

The cardiovascular profile of bisoprolol is well documented and it resembles other β-adrenoceptor antagonists (Harting *et al.*, 1986; Verdouw *et al.*, 1987a,b). Although bisoprolol reduces ST-segment elevation during short-lasting intermittent occlusions of the left anterior descending coronary artery (LADCA) in anaesthetized dogs (Harting *et al.*, 1986), information about its effects on blood flow in and function of the ischaemic myocardium is not available. In this study we describe the effects of three consecutively administered doses of bisoprolol on myocardial perfusion and performance in anaesthetized open-chest pigs with a graded coronary artery stenosis. In order to discover whether anaesthesia affected the β-adrenoceptor antagonist potency of bisoprolol we also studied the drug's ability to inhibit isoprenaline-induced changes in heart rate and myocardial contractility.

Methods

General

Studies were performed on Yorkshire pigs (21–35 kg) after an overnight fast. After sedation with 120 mg azaperone i.m. (Heykant *et al.*, 1971), the animals were anaesthetized with 150 mg metomidate i.v. (Dimigen & Reetz, 1970) and subsequently intubated for artificial ventilation with a mixture of oxygen and nitrous oxide (1:2). Respiratory rate and tidal volume were adjusted to control arterial blood gas values (ABL-3, Radiometer, Copenhagen, Denmark). In the present study arterial blood pH, P_{CO_2} and P_{O_2} were 7.44 ± 0.01, 41 ± 2 mmHg and 160 ± 5 mmHg, respectively.

Catheters were placed in the superior vena cava via the jugular vein for the administration of 100 mg kg^{-1} α-chloralose (Merck, Darmstadt, F.R.G.) followed by an infusion of 5 mg kg^{-1} h^{-1} pentobarbitone sodium (Sanofi, Paris, France). When necessary, sodium bicarbonate (8.4%) and Haemaccel (Behringwerke, Marburg, F.R.G.) were infused to correct base deficit and blood loss. Left ventricular and central aortic pressures were monitored with microtipped catheters (Honeywell-Philips, Best, the Netherlands).

After the heart had been exposed by a midsternal split, electromagnetic flow probes (Skalar, Delft, the Netherlands) were placed around the ascending aorta and the LADCA. The vein accompanying the LADCA was cannulated with a polyethylene catheter for the withdrawal of blood samples for determination of coronary venous blood gases. The left atrial appendage was catheterized for the injection of radioactive microspheres. Finally, a commercially available inflatable balloon (R.E. Jones, Silver Spring, Maryland, U.S.A.) was placed around the LADCA distal to the flow probe and connected to a 1 ml syringe (Hamilton Bonaduz, Bonaduz, Switzerland) which was driven by a micrometer.

Regional myocardial function

Regional myocardial function was estimated from myocardial wall thickness recordings obtained with the aid of a 5 MHz ultrasonic transducer (Krautkramer-Branson, Lewistown, Pa, U.S.A.) sutured onto a part of the epicardial surface perfused by the LADCA (Verdouw *et al.*, 1981). From the wall thickness at end-diastole (EDT) and end-systole (EST), percentage systolic wall thickening (SWT) was calculated as: $SWT\ (\%) = 100 \times (EST - EDT)/EDT$.

Regional myocardial blood flows

Just before the injection of about 2×10^6 microspheres (15 ± 1 (s.d.) μm diameter) (NEN Company, Dreieich, F.R.G.), labelled with either ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce, the withdrawal of an arterial reference sample was started at a flow rate of 10 ml min^{-1} and continued for a period of about 1 min after injection of the microspheres. After completion of the experiment, the ischaemic myocardium was identified by intra-arterial injection of methylene blue (P4125, Sigma Chemical Company, St Louis, MO, U.S.A.) while the LADCA was completely occluded at the site of the inflatable balloon. Then the animal was killed and the excised heart was fixed in 4% formalin for at least 24 h. Epicardial fat and large vessels were removed. The atria and the right ventricle were separated from the left ventricle, including the intraventricular septum. Radioactivity in the tissues was counted to obtain blood flow data as described previously (see Saxena & Verdouw, 1984).

Experimental protocols

In the first group of six animals the responses of heart rate and $LVdP/dt_{max}$ to isoprenaline (0.025,

0.05, 0.1, 0.2, 0.4 and 0.8 $\mu g\,kg^{-1}$) were evaluated following four doses (16, 64, 256 and 1024 $\mu g\,kg^{-1}$) of bisoprolol. Each of the doses of bisoprolol was administered over a period of 2 min at 60 min intervals. Dose-ratios of isoprenaline after each dose of bisoprolol (cumulative doses 16, 80, 336 and 1360 $\mu g\ kg^{-1}$) were calculated for increases in heart rate of 40 beats min^{-1} and in $LVdP/dt_{max}$ of 2000 $mmHg\,s^{-1}$, which is approximately 50% of the maximal response for each of the two parameters.

In the second group of 21 animals, baseline values were obtained for systemic haemodynamics, regional myocardial function and arterial and coronary venous blood gases, while a batch of microspheres was injected after systemic haemodynamic parameters had been stable for at least 30 min. Subsequently, the flow in the LADCA was reduced gradually by a slow inflation of the balloon until regional systolic wall thickening had decreased to approximately 20% of baseline. The presence of ischaemia was also confirmed by the reduction of LADCA blood flow and the appearance of myocardial proton release. In this model the latter is an accurate reflection of myocardial lactate production (Schamhardt *et al.*, 1981a). When necessary, minor adjustments in the degree of flow reduction were performed during the first 5 min of ischaemia, but thereafter, the degree of stenosis was kept constant. After post-ischaemic (15 min) measurements the animals were divided into two groups. Nine animals did not receive any treatment and served for evaluation of the stability of the preparation. In these animals all measurements were repeated after 30, 45 and 60 min of flow reduction. Eight other animals received 3 consecutive doses of bisoprolol (50, 200 and 750 $\mu g\,kg^{-1}$), administered over a period of two min at 15 min intervals. In these animals all measurements were repeated 10–12 min after the administration of each dose of bisoprolol. Because of technical failure, in one animal regional blood flows could not be determined after the highest two doses.

Since the magnitude of the systolic wall thickening depends on the location of the transducer on the myocardium (Verdouw *et al.*, 1980), we did not determine the regional function of the adjacent control segment, but evaluated the effects of bisoprolol on systolic wall thickening of the LADCA perfused area in a separate group of 10 animals with an intact coronary circulation.

Four animals had an episode of ventricular fibrillation in the first 15 min of ischaemia and were excluded from further study. When, however, ventricular fibrillation occurred after 15 min of ischaemia, the animals were included in the study until precipitation of this arrhythmia. Antiarrhythmic drugs were not administered during the course of these experiments.

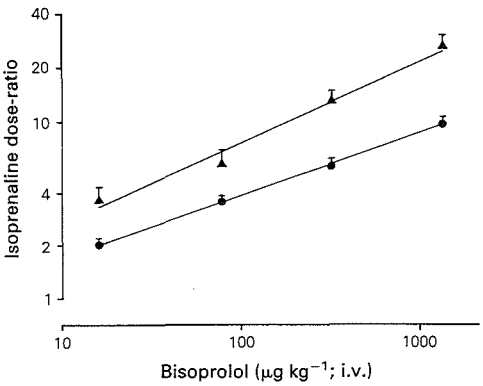

Figure 1 Effect of bisoprolol on isoprenaline-induced increases in heart rate (●) and left ventricular (LV) dP/dt_{max} (▲) in 6 anaesthetized pigs. Note that bisoprolol was about 2 times more effective in antagonizing $LVdP/dt_{max}$ changes than heart rate changes induced by isoprenaline. Data have been presented as means with vertical lines showing s.e.mean.

Drugs

Except for the anaesthetic drugs only (−)-isoprenaline sulphate (Pharmacy Department, Erasmus University, Rotterdam) and bisoprolol hemifumarate (Merck, Darmstadt, F.R.G.) were used. Both drugs were dissolved in isotonic saline and doses refer to the respective salts.

Data presentation and statistical analysis

All data are presented as mean ± s.e.mean. The significance of the changes produced by the reductions in LADCA flow in the untreated and bisoprolol-treated animals was evaluated by Duncan's new multiple range test once an analysis of variance had revealed that the samples represented different populations (Steel & Torrie, 1980). The significance of the bisoprolol-induced changes was determined by comparing these changes with those observed in the untreated animals at comparable points of time by use of Student's unpaired *t*-test.

Results

Antagonism of cardiac responses to isoprenaline

Modifications of isoprenaline-induced changes in heart rate and $LVdP/dt_{max}$ by bisoprolol are presented in Figure 1. It is clear that bisoprolol was approximately twice as effective in antagonizing the

effects of isoprenaline on $LVdP/dt_{max}$ compared with those on heart rate.

Ventricular arrhythmias between 15 and 60 min after LADCA stenosis

In 5 of the 9 animals in the untreated group the protocol was not completed because of ventricular fibrillation (in 2 animals between 30 and 45 min and in 3 animals between 45 and 60 min). The incidence of this fatal arrhythmia was considerably lower in the animals which received bisoprolol since ventricular fibrillation occurred in only 1 of the 8 treated animals after administration of the second dose between 30 and 45 min.

Myocardial proton release

Within two minutes of LADCA blood flow reduction, the coronary arterio-venous difference in pH increased from 0.05 ± 0.01 to 0.28 ± 0.04 in the untreated animals and from 0.06 ± 0.01 to 0.27 ± 0.03 in the animals which later received bisoprolol. These data did not change significantly during the first 15 min but started to decline gradually thereafter. This pattern has been well described and excluded myocardial proton release as a variable to evaluate the stability of the preparation (Verdouw *et al.*, 1978). Additionally, the rate of decline in myocardial proton release also depends on factors unrelated to the severity of ischaemia. Therefore, this variable is not suitable for the evaluation of the effects of pharmacological interventions (Verdouw *et al.*, 1978) and was not further studied during the course of experiments.

Regional myocardial blood flow

During the first 15 min of ischaemia, perfusion of the myocardium supplied by the LADCA in the untreated animals decreased from $132 \pm 14\,ml\,min^{-1}\,100\,g^{-1}$ to $71 \pm 14\,ml\,min^{-1}\,100\,g^{-1}$ (Figure 2). This decrease was not equally distributed over all myocardial layers since the deficit in perfusion of the subendocardial layers (65%) was more severe than that in the subepicardial layers (25%). Up to 45 min of flow reduction, perfusion values did not change significantly; the data obtained at 60 min showed a tendency for the ischaemic area to deteriorate further. As a result of the small number of observations ($n = 4$), we could not determine if there was an additional significant decrease in perfusion compared with that observed after 15 min of ischaemia. Perfusion of the control segment tended to increase during the first 30 min of ischaemia, but a significant increase could only be demonstrated for

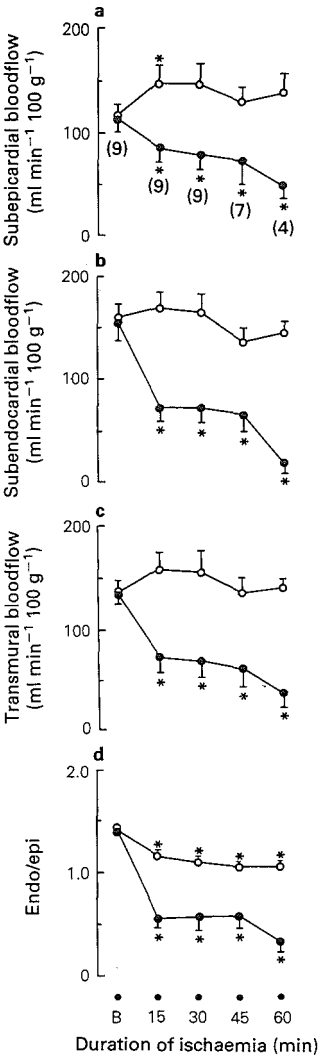

Figure 2 Myocardial perfusion in 9 open-chest anaesthetized pigs with a graded stenosis in the left anterior descending coronary artery. (a) Subepicardial blood flow, (b) subendocardial blood flow and (c) transmural blood flow. (d) Endo/epi = ratio of normalized subendocardial and subepicardial blood flows. (○) Non-ischaemic segment, (●) ischaemic segment. The numbers in parentheses indicate the number of observations. *$P < 0.05$ vs baseline (B). Data have been presented as means with vertical lines showing s.e.mean.

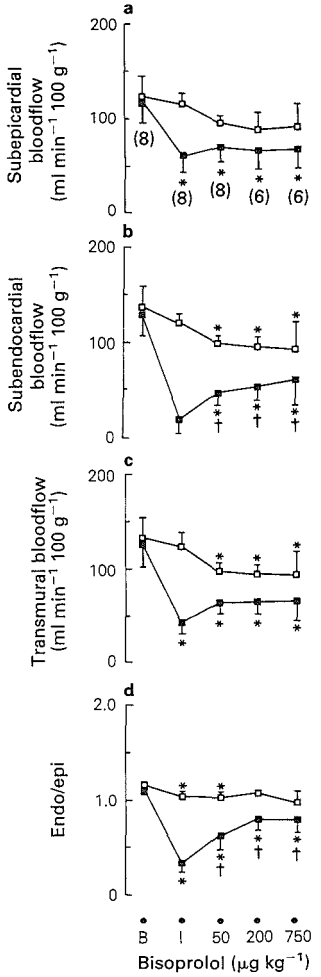

Figure 3 Effect of bisoprolol on myocardial perfusion in 8 open-chest anaesthetized pigs with a graded stenosis in the left anterior descending coronary artery. (a) Subepicardial blood flow, (b) subendocardial blood flow and (c) transmural blood flow. (d) Endo/epi = ratio of normalized subendocardial and subepicardial blood flows. The three doses of bisoprolol were administered over a period of 2 min at 15 min intervals. The first dose was given after 15 min of ischaemia (I); (□) non-ischaemic segment; (■) ischaemic segment. The numbers in parentheses indicate the number of observations. * $P < 0.05$ vs baseline (B). † $P < 0.05$ vs I. Data have been presented as means with vertical lines showing s.e.mean.

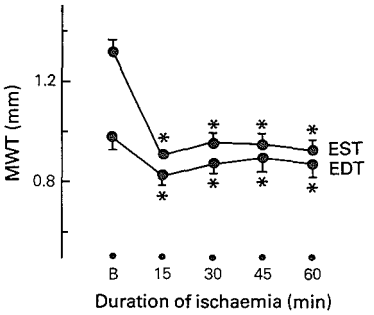

Figure 4 Myocardial wall thickness (MWT) of the ischaemic segment in 9 open-chest anaesthetized pigs with a graded stenosis in the left anterior descending coronary artery. EDT and EST are the wall thickness at end-diastole and end-systole, respectively. * $P < 0.05$ vs baseline (B). Data have been presented as means with vertical lines showing s.e.mean.

the subepicardial layers after 15 min of ischaemia. Although the perfusion of the individual layers was not grossly affected, there was a slight decrease in the ratio of the normalized subendocardial to subepicardial blood flow (endo/epi ratio).

In animals which were later given bisoprolol, the initial reduction in LADCA flow was larger (−65%) than in the untreated animals (−45%): the perfusion of myocardium supplied by the LADCA was reduced from $123 \pm 20\,ml\,min^{-1}\,100\,g^{-1}$ to $42 \pm 11\,ml\,min^{-1}\,100\,g^{-1}$ before administration of bisoprolol (Figure 3). The subendocardium was more severely affected than the subepicardium (−81% vs −50%, respectively). Administration of $50\,\mu g\,kg^{-1}$ bisoprolol improved the perfusion of the ischaemic segment by $21 \pm 10\,ml\,min^{-1}\,100\,g^{-1}$ ($P < 0.05$). This improvement was limited, however, to the subendocardial layers as perfusion of the subepicardial layers was not significantly modified (Figure 3). With the higher doses of bisoprolol, there were only minor additional changes. It has to be noted, however, that there was no indication of further deterioration in the ischaemic segment; this is in contrast to that in the control group at 60 min of flow reduction.

Since perfusion of the non-ischaemic myocardium decreased slightly after administration of bisoprolol, in contrast with the control group, the normalized flow ratio of the ischaemic and non-ischaemic myocardium was calculated in order to gain a better insight into the flow deficit of the ischaemic segment after bisoprolol administration. Table 1 clearly demonstrates that bisoprolol distinctly levelled out the perfusion differences between epicardium and endocardium while these remained unchanged in the control group.

Table 1 Flow deficit in ischaemic myocardium, expressed as the ratio of the normalized flows to the ischaemic and non-ischaemic myocardium, in untreated (U) and bisoprolol-treated (B) pigs before (baseline) and after partial stenosis of LADCA

			Time after ischaemia (min)			
		Baseline	*15*	*30*	*45*	*60*
			Dose ($\mu g\,kg^{-1}$)			
	U		0 ($n = 9$)	0 ($n = 9$)	0 ($n = 7$)	0 ($n = 4$)
	B		0 ($n = 8$)	50 ($n = 8$)	250 ($n = 7$)	750 ($n = 7$)
Transmural	U	0.98 ± 0.02	0.46 ± 0.10*	0.51 ± 0.12*	0.46 ± 0.12*	0.27 ± 0.10*
	B	0.96 ± 0.03	0.34 ± 0.08*	0.64 ± 0.11*†	0.71 ± 0.11*†	0.74 ± 0.11*†
Subendocardial	U	0.99 ± 0.05	0.33 ± 0.08*	0.39 ± 0.12*	0.39 ± 0.13*	0.14 ± 0.07*
	B	0.96 ± 0.05	0.19 ± 0.07*	0.52 ± 0.16*†	0.60 ± 0.13*†	0.66 ± 0.13*†
Subepicardial	U	0.97 ± 0.04	0.62 ± 0.12*	0.60 ± 0.12*	0.59 ± 0.14*	0.38 ± 0.12*
	B	1.03 ± 0.03	0.49 ± 0.09*	0.72 ± 0.09*†	0.78 ± 0.09*†	0.80 ± 0.10*†

LADCA, Left anterior descending coronary artery. * $P < 0.05$ vs baseline; † $P < 0.05$ vs changes in untreated group. Data have been presented as means ± s.e.mean.

Regional myocardial function

The decrease in perfusion led to decreases in end-diastolic and, in particular, in end-systolic wall thickness. Consequently, absolute systolic wall thickening (EST − EDT) decreased from 3.5 ± 0.2 mm to 0.8 ± 0.2 mm and percentage systolic wall thickening (SWT) fell from 36 ± 3% to 10 ± 3% ($P < 0.05$) after the first 15 min of flow reduction in the untreated animals (Figure 4). There were no additional changes during the remainder of the experiment.

In animals that were later treated with bisoprolol the reduction in LADCA blood flow also caused an immediate decrease in absolute systolic wall thickening from 4.6 ± 0.2 mm to 0.8 ± 0.3 mm and in

Table 2 Systemic haemodynamics in untreated (U) and bisoprolol-treated pigs (B) before (baseline) and after partial stenosis of LADCA

			Time after ischaemia (min)			
		Baseline	*15*	*30*	*45*	*60*
			Dose ($\mu g\,kg^{-1}$)			
	U		0 ($n = 9$)	0 ($n = 9$)	0 ($n = 7$)	0 ($n = 4$)
	B		0 ($n = 8$)	50 ($n = 8$)	250 ($n = 7$)	750 ($n = 7$)
CO ($l\,min^{-1}$)	U	2.23 ± 0.16	1.97 ± 0.18	2.06 ± 0.17	1.94 ± 0.23	1.83 ± 0.03
	B	2.50 ± 0.34	2.04 ± 0.16*	1.84 ± 0.17*	1.84 ± 0.12*	1.67 ± 0.10*†
HR (beats min^{-1})	U	102 ± 4	123 ± 9*	127 ± 9*	116 ± 7	115 ± 10
	B	111 ± 10	113 ± 12	94 ± 8†	91 ± 7*†	84 ± 6*†
SV (ml)	U	22 ± 2	16 ± 1*	16 ± 1*	17 ± 1*	16 ± 1*
	B	22 ± 1	19 ± 1*	20 ± 1	21 ± 1	20 ± 1
MAP (mmHg)	U	91 ± 5	78 ± 6*	74 ± 6*	66 ± 5*	60 ± 2*
	B	79 ± 5	64 ± 3*	61 ± 3	59 ± 3*	57 ± 3*
$LVdP/dt_{max}$ ($mmHg\,s^{-1}$)	U	2360 ± 180	2070 ± 260*	2020 ± 280	1810 ± 280*	1800 ± 160*
	B	2640 ± 440	1860 ± 380*	1230 ± 190*†	1240 ± 210*†	1070 ± 150*†
LVEDP (mmHg)	U	9 ± 1	13 ± 2*	12 ± 2	11 ± 2	14 ± 3
	B	9 ± 1	15 ± 1*	15 ± 2*	15 ± 3*	15 ± 3*

LADCA, Left anterior descending coronary artery; CO, cardiac output; HR, heart rate; SV, stroke volume; MAP, mean arterial blood pressure; $LVdP/dt_{max}$, maximum rate of rise in left ventricular pressure; LVEDP, left ventricular end diastolic pressure. * $P < 0.05$ vs baseline; † $P < 0.05$ vs changes in untreated group. Data have been presented as means ± s.e.mean.

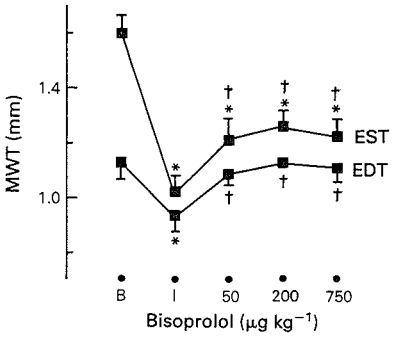

Figure 5 Effect of bisoprolol on myocardial wall thickness of the ischaemic segment in 8 open-chest anaesthetized pigs with a graded stenosis in the left anterior descending coronary artery. The three doses of bisoprolol were administered over a period of 2 min at 15 min intervals. The first dose was given after 15 min of ischaemia (I). EDT and EST are the wall thickness at end-diastole and end-systole, respectively. * $P < 0.05$ vs baseline (B). † $P < 0.05$ vs I. Data have been presented as means with vertical lines showing s.e.mean.

percentage systolic wall thickening from 41 ± 2% to 9 ± 4% ($P < 0.05$) (Figure 5). Although bisoprolol increased both EDT and EST, it failed to improve absolute as well as percentage systolic wall thickening.

As stated before (see experimental protocol) the effects of bisoprolol (cumulative doses: 16, 80, 336 and 1024 $\mu g\,kg^{-1}$) on the LADCA perfused myocardium were evaluated in a separate group of 10 animals with an intact coronary circulation. Systolic wall thickening values (%) before and after the four doses of bisoprolol were 48 ± 2, 52 ± 4, 45 ± 4, 44 ± 3 and 34 ± 3, respectively. Therefore, bisoprolol only slightly reduced systolic wall thickening and that too after the highest dose.

Systemic haemodynamics

The reduction in LADCA blood flow caused a number of immediate changes in systemic haemodynamic variables: decreases in cardiac output (10%), stroke volume (25%), mean arterial blood pressure (15%) and LV$dP/dt_{max}$ (12%), while both heart rate (20%) and left ventricular filling pressure (45%) increased (Table 2). These changes lasted for the entire 60 min period of observation in the untreated group. In the group treated with bisoprolol similar effects were noticed except that heart rate decreased (instead of a moderate increase) and LV$dP/dt_{max}$ decreased more prominently (Table 2).

Discussion

The reduction in LADCA flow produced by the stenosis led to immediate changes in regional myocardial blood flow and wall motion and in systemic haemodynamics. These effects remained stable during the first 45 min of ischaemia, but between 45 and 60 min, the untreated preparations tended to show signs of further deterioration (see Figure 2). However, because of the low number of observations at the end of the 60 min period (4 animals died because of ventricular fibrillation), statistical significance was difficult to ascertain. Therefore, it appears that systemic and regional haemodynamic variables and systolic myocardial wall thickening and perfusion are suitable for evaluating the effects of pharmacological agents during this period.

The present study demonstrated that bisoprolol improves perfusion of the ischaemic myocardium in anaesthetized open-chest pigs. This is, however, not surprising since a large number of other β-adrenoceptor antagonists also possess this property (Vatner *et al.*, 1977; Tomoike *et al.*, 1978; Berdeaux *et al.*, 1979; Buck *et al.*, 1979; 1981; Gross *et al.*, 1979; Saxena, 1983; Verdouw *et al.*, 1986). On the other hand, negative results have also been obtained in earlier studies (Becker *et al.*, 1975; Kloner *et al.*, 1976; Berdeaux *et al.*, 1977). It is interesting that bisoprolol increases blood flow to the ischaemic myocardial segment, in particular to the subendocardium, even at doses which only moderately inhibit isoprenaline-induced increases in heart rate and contractility (Duncker *et al.*, 1987; present results). Using other β-adrenoceptor antagonists or specific bradycardiac drugs, several investigators have shown that the endocardium, especially, benefits from a prolongation of the duration of diastole (Berdeaux *et al.*, 1979; Gross *et al.*, 1979; Buck *et al.*, 1981; Schamhardt *et al.*, 1981b; Saxena, 1983; Daemmgen *et al.*, 1985; Verdouw *et al.*, 1986; Guth *et al.*, 1987).

In contrast with the increase in blood flow, there was no improvement in segmental myocardial function. Several arguments can be put forward to explain this apparent discrepancy. Firstly, the improvement in flow may be too small to guarantee an improvement in contractile function. In the same preparation, we have shown that reduction of LADCA flow to 50% of baseline values causes an almost complete loss of systolic wall thickening (Verdouw *et al.*, 1980). Based on the data presented in Figure 3, some slight improvement might have been expected. Secondly, most β-adrenoceptor antagonists, and bisoprolol is no exception, exhibit negative inotropic properties which could, but do not necessarily, decrease systolic wall thickening of the normally perfused myocardium. We therefore

studied systolic wall thickening of the myocardium perfused by the LADCA in an additional group of animals with an intact coronary circulation. Since, in animals with an intact coronary circulation, doses lower than $1000\,\mu g\,kg^{-1}$ did not affect systolic wall thickening, it is unlikely that the drug-induced negative inotropic effect prevented an improvement in regional function. Moreover, a number of β-adrenoceptor antagonists have been shown to improve the regional contractile function of ischaemic myocardium despite their negative inotropic action on the normal myocardium (Tomoike *et al.*, 1978; Gross *et al.*, 1979, Buck *et al.*, 1981). A third reason may be the duration of ischaemia preceding the administration of bisoprolol: numerous studies in dogs and pigs have shown that even after complete restoration of blood flow subsequent to partial or complete occlusion of a coronary artery lasting up to 20 min, recovery of contractile function takes place only after a considerable delay (Heyndrickx *et al.*, 1975; Ramanathan *et al.*, 1978; van der Giessen *et al.*, 1986). During bisoprolol treatment recovery of perfusion is still incomplete, therefore, it is not surprising that regional contractile function of the ischaemic segment did not improve. It is of interest, however, that although regional contractile function did not concomitantly return to normal, both end-diastolic and end-systolic wall thickness improved slightly. This implies that 'bulging' of the ischaemic myocardium became less, which may be a first step towards the return of contractile function.

In the present experiments using a model with partially restricted coronary blood flow, the incidence of ventricular fibrillation was considerably lower in the bisoprolol-treated than in the untreated animals, despite a more severe reduction in blood flow during the first 15 min before drug administration in the bisoprolol group. In recent experiments using repeated, complete coronary occlusions, bisoprolol was shown to be ineffective in preventing ventricular fibrillation in pigs (Verdouw *et al.*, 1987b). This is not surprising because most β-adrenoceptor antagonists, including propranolol, pindolol and sotalol, fail to prevent ventricular fibrillation after ligation of a coronary artery in this species (Frank *et al.*, 1978; Bergey *et al.*, 1984; Benfey *et al.*, 1984; Muller *et al.*, 1986; Verdouw & Hartog, 1986). Metoprolol has been shown to be effective against ventricular fibrillation (Muller *et al.*, 1986), but the dose used ($20\,mg\,kg^{-1}$) is not clinically relevant as it far exceeded that required to obtain adequate β-adrenoceptor blockade, and it is therefore doubtful that the antifibrillatory action was related to β-adrenoceptor blockade. With complete vascular occlusion, the differences in perfusion of the normal and the acutely ischaemic myocardium, and in perfusion of the different layers of the ischaemic segment, are not affected by pharmacological agents since the porcine heart has only few native collaterals (Schaper & Wüsten, 1979; Millard, 1980. However, in the conditions used in the present study, where only a partially obstructed vascular supply was used, the flow differences between the various myocardial areas were attenuated (Table 1). This might have contributed to the reduction in the incidence of fatal ventricular arrhythmias in bisoprolol-treated animals.

In conclusion, bisoprolol appears to produce beneficial effects in an animal model of acute myocardial ischaemia. The finding that the drug improves the perfusion of ischaemic myocardium and prevents ventricular arrhythmias at doses that have relatively moderate effects on β-adrenoceptors, may be clinically advantageous.

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Chapter 11

General discussion

GENERAL DISCUSSION

Coronary vasoreactivity after an induction and after a post-induction diet

The endothelial cells play an important role in modulating the responsiveness for the underlying smooth muscle cells (Furchgott and Zawadski, 1980). In particular, the endothelium of large arteries exerts a protective action opposing the constrictor effects of platelet products (Vanhoutte and Houston, 1985; Cohen et al., 1983). Atherosclerosis not only causes vascular occlusion but also alters the vascular responses to a variety of neurohumoral stimuli. Patients with atherosclerosis are susceptible to the development of coronary vasospasm, particularly at sites of coronary stenosis (Maseri et al., 1978). A variety of vasoactive substances (e.g. serotonin, thromboxane, histamine, thrombin and adrenergic stimuli) are putative mediators of vasospasm (Vanhoutte and Houston, 1985).

Regression of atherosclerosis or regeneration of the endothelium does not necessarily imply return of the endothelium-dependent vasodilatory ability of an artery. This has been demonstrated in vitro for porcine vessels (Shimokawa et al., 1989). It has however also been demonstrated that dietary treatment of atherosclerosis in monkeys abolishes augmented vasoconstrictor responses to serotonin (Heistad et al., 1987a, 1987b), while vasodilator responses failed to improve.

In chapter 3 we have shown that fish oil causes regression of atherosclerosis of the abraded arteries of pigs but the function of the endothelium was not investigated. In a more recent series of experiments we tried to intensify the induction of atherosclerosis by adding bile acids to the diet and prolonging the induction period to 8 months. The post-induction period was 4 months and the effect of addition of a combination of 5% of lard fat and 5% (w/w) of fish oil was compared with lard fat addition (10%) alone. In these experiments no regression of coronary artery sclerosis was found. To investigate the endothelial function we performed angiograms after intracoronary infusions of continuous doses of histamine, 5-hydroxytryptamine, a thromboxane A_2 agonist and the Ca^{2+}-agonist Bay K 8644. For 5-hydroxytryptamine and Bay K 8644 the decrease in mean diameter of the abraded vessel of the post-induction pigs was lower than in the induction pigs, while after the thromboxane A_2 agonist there were no differences (Figure 1). For histamine only in the fish oil treated animals the vasoconstrictor response was reduced. Comparing the two post-induction groups, for histamine and Bay K 8644 the vasoconstrictor effect was less in the fish oil fed than in the lard fat fed animals.

Bay K 8644 (Ruybani et al., 1985) and serotonin (Shimokawa et al., 1987) cause release of EDRF. However for histamine there seems to be species-dependency since in rats histamine induces the release of EDRF, but in humans prostacyclin is also liberated (Baenziger et al., 1981). The 4 months of low-cholesterol feeding may have caused regeneration of the endothelium while not affecting the intimal thickening. The

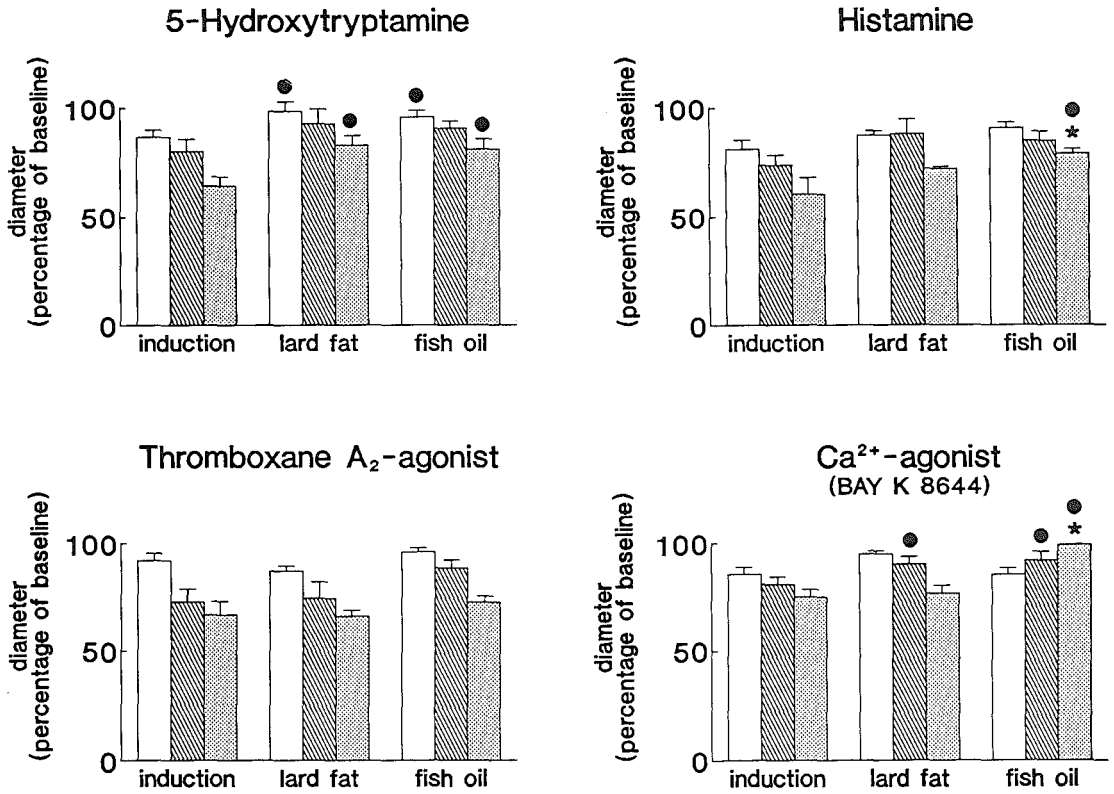

*Figure 1 Effect of continuous 5 min intracoronary infusions (□ , ▨ , ▩) of 5-hydroxytryptamine (2, 10, 100 μg/min), histamine (30, 75, 300 μg/min), a thromboxane A_2 agonist (1, 5, 10 μg/min) and the Ca^{2+}-agonistBay K 8644 (40, 100, 200 μ/min) on the coronary diameter (% of baseline) in pigs after a high-cholesterol induction diet or a low-cholesterol post-induction diet with lard fat or fish oil addition. • changes versus baseline are significantly different from those in the induction group; * changes versus baseline in the fish oil treated animals are significantly different from those in the lard fat fed animals.*

EDRF-release, impaired in the induction group, may therefore have reappeared in both the post-induction groups. At variance with the present study, Shimokawa et al. (1989) showed that the morphologically regenerated endothelium did not regenerate functionally. However, in the present study the function of the endothelium was investigated after a period of 4 months, whereas only after four weeks in the study by Shimokawa et al. (1989). The difference in vasoreactivity to histamine between both post-induction groups might be explained by an additional release of prostacyclin

elicited by histamine. It can then be theorized that the well known effects of n-3 fatty acids on the prostaglandin metabolism (see introduction) may have caused the discrepancy between the groups. The difference in reaction to Bay K 8644 can however not be readily explained. It should however be noted that n-3 fatty acids induce large changes in fatty acid composition of cellular plasma membranes, in which the Ca^{2+}-agonist/antagonist-receptors are embedded.

Further studies in which also in vivo the prostacyclin-induced vasodilation is abolished by prior infusion of indomethacin may elucidate whether endothelium-dependent vasodilation through EDRF is restored.

Antiischemic effects of nisoldipine, nicorandil and bisoprolol

The use of vasodilators in ischemia however, in some instances carry the risk of aggravating ischemia. Experimentally, when applying a fixed concentric stenosis in an animal model with no coronary collateral circulation, the autoregulatory process is exhausted and the perfusion of the postischemic myocardium therefore becomes entirely dependent on the perfusion pressure. In chapter 8 we investigated the effects of 3 doses of nicorandil on myocardial perfusion and function in pigs with such a fixed concentric stenosis causing a 70% flow reduction. In the case of a concentric stenosis in a patient the vessel wall is circumferentially affected and can actually not respond adequately to vasodilatory therapy (Lichtlen and Ebner, 1986). Nicorandil has been shown to be capable of dilating the epicardial coronary arteries in patients (Suryapranata et al., 1988). However, in the presently used model this effect was eliminated by the concentric configuration of the stenosis. A model utilizing an eccentric stenosis would therefore be more valuable to reveal an antiischemic effect of nicorandil. One can use an intracoronary balloon, which obstructs the coronary blood flow. The factor that the entire vessel wall can respond to vasoactive drugs on the other hand also does not mimic a clinical situation. The aforementioned limitations of the model highlighted the negative effects of nicorandil (decrease in coronary blood flow and no effect on myocardial contractile function). Moreover, at variance with the non-ischemic and conscious pigs (chapter 7), nicorandil failed to decrease preload in the pigs with myocardial ischemia (chapter 8).

We also investigated the antiischemic effects of a low dose of the Ca^{2+}-antagonist nisoldipine in pigs with an 80% reduction in coronary blood flow (chapter 4). Comparing the ischemic episode only, one can observe that, in the same model (concentric stenosis), nicorandil decreased and nisoldipine slightly increased myocardial perfusion. Heart rate, an important determinant of coronary blood flow was similar for both the nisoldipine- and nicorandil-treated animals. Furthermore, left ventricular enddiastolic pressure in both groups increased after applying the stenosis but did not change in either group during ischemia.

The observation that nisoldipine modestly increased coronary blood flow to the

ischemic myocardium is in itself not surprising as it has been shown that in both anaesthetized pigs (Gewirtz et al., 1984; Pantely et al., 1985) and in dogs (Heusch et al., 1987) with severe ischemia, vasodilator reserve is not completely exhausted. Gewirtz et al (1984) have shown that nifedipine increased flow to the ischemic segment in anaesthetized pigs with a severe fixed coronary artery obstruction as used in our study. Vatner et al (1988) studied the effect of a similar dose of nisoldipine on myocardial perfusion in conscious baboons, also a species with a very limited collateral circulation. These investigators showed that a nisoldipine infusion, which started 1 hour post-occlusion, more than doubled blood flow to the subepicardial ischemic segment (from 6 ml/min/100g to 15 ml/min/100g), whereas it increased by a much smaller percentage in the non-ischemic segment. From the above we may conclude that recruitment of coronary vasodilator reserve occurred in the ischemic segment, probably via both collateral and non-collateral vessels. From the design of the study we are not in the position to resolve the mechanism underlying this beneficial effect of nisoldipine. It must be emphasized however, that in anaesthetized preparations (Pantely et al, 1985) sympathetic tone is usually high and that in such preparations calcium antagonists like nisoldipine may exert a beneficial effect by a functional antagonism against α-adrenergic constrictor tone in the ischemic coronary vasculature (Heusch et al, 1987).

It has been demonstrated that Ca^{2+}-antagonists are capable of affecting the ratio of maximum to resting flow (coronary flow reserve, Merril et al., 1982; Dymek et al., 1984; Foult et al., 1986). We determined for a number of drugs the relationship between coronary perfusion pressure and maximal coronary blood flow (determined by administering 20 μg/kg adenosine into the left anterior descending coronary artery at different degrees of stenosis). The Ca^{2+}-antagonist nifedipine, structurally related with nisoldipine, dose-dependently decreased the slope of the flow-pressure line and caused a translocation of the x-intercept (Unpublished data; Figure 2). However, when

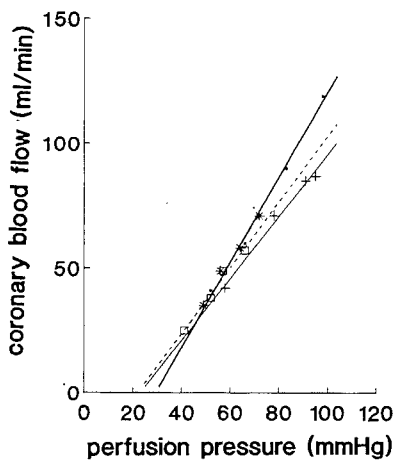

Figure 2 *Example of the maximum coronary flow-pressure lines following intracoronary adenosine at baseline (•), 0.5 (+), 1 (*) and 2 (□) μg/kg/min of nifedipine.*

extrapolating this line one can speculate that at a post-stenotic coronary pressure below 40 to 50 mmHg, maximal coronary blood flow after treatment is higher than before treatment. A lower preload can cause this translocation of the x-intercept (Aversano et al., 1984). However, as stated above, left ventricular end diastolic pressure was not affected by nisoldipine during ischemia. Although we did not measure post-stenotic pressure, we must assume that with the severe degree of stenosis the post-stenotic pressure was below 40-50 mmHg. Since flow had decreased below baseline values the autoregulatory process was exhausted. Yet, nisoldipine caused a significant rise in myocardial blood flow during ischemia. A decrease in the slope of the flow-pressure line in combination with a translocation of the x-intercept, resulting in a higher maximal flow at lower post-stenotic pressures, could possibly account for this phenomenon.

The decrease in heart rate, resulting in a decrease in myocardial oxygen demand, but also in increased subepi- but more so subendocardial perfusion, is the origin of the antiischemic effects of ß-blockers. Bisoprolol also proved to exert these effects. Obviously, these beneficial effects can be acquired irrespective of the nature of the stenosis (concentric or eccentric).

Vasodilatory profile of elgodipine, EMD 52692 and nicorandil

In the chapters 5, 7 and 9 it has not only been shown that elgodipine (Ca^{2+}-antagonist), nicorandil (mixed nitrate and K^+ channel activator) and EMD 52692 (pure K^+ channel activator) are potent vasodilators, but due to their different modes of action there are also some large regional differences in the vasodilatory capacity of these compounds. This is clearly demonstrated in Figure 3, where the changes in regional vascular conductances are plotted against the changes in systemic vascular conductances. As expected there is a close resemblance between nicorandil and EMD 52692, which could be anticipated from the K^+ channel activating capacities of nicorandil (Weir and Weston, 1988). However, for some regional vascular beds the differences with the Ca^{2+}-antagonist elgodipine are striking. These differences were not apparent in the left ventricle. However, it must be kept in mind that the myocardial oxygen consumption decreased with elgodipine and nicorandil, but not with EMD 52692, and with the two former drugs the decrease in myocardial oxygen demand therefore opposed the drug-induced effect on coronary blood flow. Of interest is that the initial vasodilation in the kidneys, as observed with elgodipine and EMD 52692 was followed by a decrease with the highest dose although systemic vascular conductance still increased. The severe hypotension observed with these highest doses is most likely responsible for this effect. From Figure 3 it can also be concluded that, while EMD 52692 and nicorandil strikingly increase total cerebral conductance, elgodipine has almost no effect. This also demonstrates the heterogeneity of Ca^{2+}-antagonists with respect to their vasodilatory profile, since for instance nimodipine in the same model doubled cerebral conductance (Duncker et al., 1986).

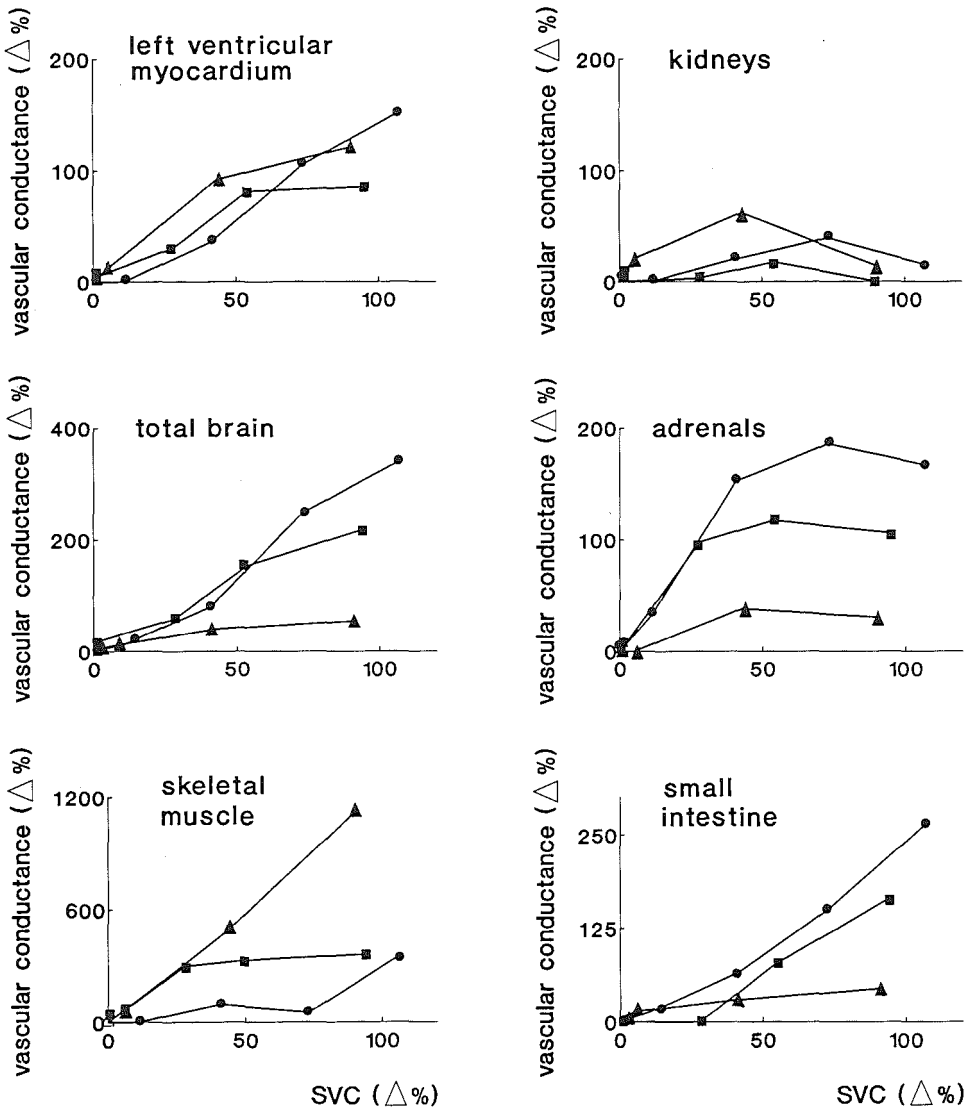

Figure 3 *The changes in regional vascular conductance in various organs are plotted against the changes in systemic vascular conductance (SVR) in nicorandil-treated (■,chapter 7), EMD 52692-treated (●,chapter 9) and elgodipine-treated (▲,chapter 5) pigs. For the sake of clarity the standard errors are not shown.*

Potential antiischemic effects of EMD 52692

A characteristic effect of direct-acting vasodilator drugs is to induce tachycardia via a reflex increase in sympathetic drive to the heart, which obviously is inopportune when applying the drug for antiischemic purposes. This also holds true for EMD 52692 and for cromakalim, a K^+ channel activator, structurally closely related to EMD 52692. The cromakalim-induced increases in heart rate can however be abolished by prior administration of the ß-adrenoceptor antagonist bopindolol (Berthold et al., 1981) suggesting this effect to be reflex-mediated rather than a direct action. Moreover, other studies with K^+ channel activators have shown that, due to resetting of the baroreceptors, this tachycardia abates with time (Buckingham et al., 1986). The potential for exerting antiischemic effects may however be present although some precautions must be considered. The resemblance of nicorandil and EMD 52692 suggests that possible antiischemic effects of EMD 52692 will not be disclosed when tested in the same model as we employed for nicorandil (no collaterals, concentric stenosis, chapter 8). Furthermore, another factor important in determining the antiischemic effect of nicorandil is the ability to decrease preload, which quality is not shared by EMD 52692. Nevertheless, during ischemia left ventricular end diastolic pressure was not affected by nicorandil (chapter 8), which is consistent with the observations by others (Lamping et al., 1984; Lamping and Gross, 1984).

The potential advantageous effects during ischemia greatly depend on the ability of the drug to dilate the large epicardial coronary arteries. To date, it has not been investigated whether K^+ channel activators hold this feature. In additional experiments with closed-chest anesthetized pigs (n=4) we infused EMD 52692 directly into the left anterior descending coronary artery and performed coronary angiograms. The diameter of the midportion of the coronary artery increased as much as 20% at doses similar as those used during the intracoronary infusions (chapter 9). Whether an increase in diameter is exclusively due to a direct effect of the drug on the epicardial vessels or the what extent flow-dependent vasodilation contributes could not be deduced from these experiments. Flow-dependent vasodilation is an endothelium-dependent process, which depends on the existance of intact endothelium and most likely is mediated by EDRF (Kaiser et al., 1985). Substantial flow-dependent coronary dilatation has also been shown in humans (Drexler et al., 1989), which was however markedly impaired in atherosclerotic coronary arteries of patients (Cox et al., 1989), most likely reflecting dysfunction of the endothelium. Infusion of EMD 52692 in animals in which myocardial blood flow is maximal and flow-dependent vasodilation is thus excluded, can reveal the true vasodilatory response. Holtz et al. (1983) applied a flow-limiting stenosis and showed that the increases in diameter of epicardial vessels by non-nitrate drugs like nifedipine, diltiazem and verapamil is predominantly generated by this phenomenon.

The above-mentioned experiments in the 4 additional animals do not provide a definite clue as to the ability of EMD 52692 to dilate the large epicardial coronary arteries. Moreover, in view of the small fraction (3-4%) of the cardiac output used for

myocardial perfusion, a 30 times higher dose (2.25 μg/kg/min) would be needed to observe this effect. Further studies in patients with coronary artery disease are needed to elucidate the clinical importance of the effect.

It has been shown that EMD 52692 improves blood flow to collateral-dependent myocardium (Maruyama et al, 1990). In pigs, collaterals can be formed by combining ischemia with regular exercise. In such a model, representing the patient with repetitive ischemic events in the medical history, the antiischemic actions of EMD 52692 might become apparent. Additionally, since K^+ channel activators have also displayed direct cardioprotective effects (Grover et al., 1988, Grover et al., 1990), whereas nicorandil is dependent on peripheral actions for its efficacy (Grover et al., 1990), it seems of interest to explore the antiischemic actions of EMD 52692 in future studies.

L-propionylcarnitine and ischemia

Besides the indirect effects of nisoldipine, this compound also exerts direct cellular effects (see Table 2, General Introduction, chapter 1). L-propionylcarnitine is a compound which does not seem to exert any systemic hemodynamic effect and most likely only has direct cell-salvaging effects (see Table 2, General Introduction, Chapter 1). Liedtke et al (1988) have demonstrated that pretreatment with L-propionylcarnitine caused reversal of myocardial stunning in a porcine model of 45 min of mild ischemia followed by 60 min of reperfusion. However, regarding the fact that ischemia was more severe in the untreated animals, evidenced by the presence of a greater systolic wall thickening during ischemia in the pretreated animals, this conclusion seems precarious. The results of our study on L-propionylcarnitine and myocardial ischemia (chapter 6) indicate that the compound exerts an advantageous effect on postischemic myocardial blood flow and we postulated that an attenuation of the no-reflow phenomenon by a direct cell-salvaging action might have been the basis for this observation. However, myocardial contractile function was not influenced. Probably, the ischemic episode was too lengthy and the degree of ischemia too severe to allow return of function. Experiments with a lesser degree of ischemia would constitute a model in which the potential beneficial effects of the drug could become manifest. For instance: in untreated pigs, 10 min of total occlusion followed by 30 min of reperfusion causes myocardial wall thickening to change from 31 ± 8 (mean ± sd) % at baseline to 14 ± 2 % at the end of reperfusion (unpublished results of our laboratory).

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Samenvatting

Coronair vaatlijden wordt veroorzaakt door de systemische aandoening atherosclerosis, en myocardischemie is het uiteindelijke gevolg. Therapeutisch kan de aandoening op zeer verschillende manieren worden benaderd. Men kan het atherosclerose proces aanpakken, via veranderingen in het dieet en levensstijl of door het toedienen van geneesmiddelen. In de praktijk echter presenteert de patient zich pas wanneer hij al lijdt aan een van de verschillende uitingsvormen van myocardischemie en zal dit dus bestreden moeten worden.

In dit proefschrift is bij de studie naar de behandeling van de verschillende aspecten van coronair vaatlijden voor het varken als proefdier gekozen. Zowel voor wat betreft de vorming van atherosclerose als voor de bestudering van myocardischemie is het varken een diermodel dat in vele fysiologische en pathofysiologische opzichten goed vergelijkbaar is met de mens.

Ca^{2+}-antagonisten zijn medicamenten waarvan dierexperimenteel onderzoek met konijnen en ratten heeft aangetoond dat ze in extreem hoge doseringen de vorming van atherosclerose kan doen verminderen. In hoofdstuk 2 zijn de potentiele anti-atherosclerotische effecten bestudeerd van de Ca^{2+}-antagonist diltiazem. In ons streven de humane situatie zo goed mogelijk te benaderen zijn wij zowel qua diermodel (hypercholesterolemisch varken met coronairvaten waarvan het endotheel mechanisch verwijderd is *versus* hypercholesterolemisch konijn) als qua dosering van het medicament (2 maal de maximale humane dosering *versus* tot 1000 maal de humane dosis) afgeweken van de in de literatuur gebruikelijke methodes en doseringen en konden geen gunstig effect van de stof aantonen.

Dat regressie van atherosclerose in proefdiermodellen mogelijk is, is reeds eerder aangetoond. Of een dietaire maatregel deze regressie kan versterken werd in hoofdstuk 3 bestudeerd. Twee doseringen makreelolie, toegevoegd aan een basisdieet, werden vergeleken met reuzelvet, toegevoegd aan het basisdieet. Makreelolie veroorzaakte regressie van de coronairsclerose (gemeten als intimaproliferatie), terwijl geen effect werd gevonden op de lipideninhoud van de lesies in de aorta. Het verschil in effect op eenzelfde proces in verschillende vaatbedden kan mogelijk verklaard worden door de verschillende meetmethodes die voor deze vaatbedden gebruikt werden. Zowel de cholesterolverlagende invloed, als de effecten op plaatjesaggregatie hebben waarschijnlijk bijgedragen aan het gunstige effect van de n-3 bevattende makreelolie. Het dosisafhankelijke effect, dat in deze studie werd gevonden kan daarmee echter niet verklaard worden. Onbekende factoren of andere, door ons in deze studie niet gemeten maar in de literatuur beschreven effecten van n-3 polyonverzadigde vetzuren, moeten ook hebben bijgedragen aan de gunstige invloed die visolie heeft op de regressie van atherosclerose in varkens.

In de hoofdstukken 4, 6, 8 en 10 is bij het genarcotiseerde varken myocardischemie geinduceerd door een concentrische stenose aan te brengen rond een coronair arterie (ramus anterior arteriae coronariae sinistrae). In hoofdstuk 4 laten we zien dat de Ca^{2+}-antagonist nisoldipine in staat is, al enigszins tijdens ischemie, maar

vooral na twee uur reperfusie de coronaire bloedstroom te verhogen. Waarschijnlijk speelde vasodilatiatie van het coronaire vaatbed een rol maar vermindering van het "no-reflow" fenomeen zou ook een rol hebben kunnen gespeeld. Een snellere refosforylering van ADP leidde tot een toename van creatine fosfaat, waardoor tijdens de ischemische episode bedreigde cellen konden worden gespaard. Bovendien zorgde nisoldipine voor een hogere activiteit van de Ca^{2+}-pomp van het sarcoplamatisch reticulum, wat mogelijk kan hebben bijgedragen tot een vermindering van de Ca^{2+} overload.

L-propionylcarnitine is een stof zonder duidelijke systemische hemodynamische effecten en oefent zijn mogelijk gunstige invloed tijdens ischemie uit door direct cellulaire effecten, zoals het herstellen van de carnitine pool, het wegvangen van de schadelijke lange keten vetzuren, die zich ophopen in de cel tijdens ischemie en het stimuleren van de vetzuuroxidatie. Wij bestudeerden de effecten van de stof (hoofdstuk 6) op de coronaire bloedstroom, energiehuishouding en contractiele functie van het myocard tijdens 1 uur ischemie gevolgd door 2 uur reperfusie. Het meest opvallend was de bevinding dat bij gelijke arteriele perfusiedruk de post-ischemische coronaire bloedstroom significant hoger was in de behandelde groep, vergeleken met de controlegroep. Wij hebben getracht dit te verklaren door aan te nemen dat er door de direkte cellulaire effecten van L-propionylcarnitine minder endotheelschade is opgetreden, wat aan de basis ligt van het "no-reflow" fenomeen. De toegenomen bloedstroom ging echter niet gepaard met herstel van functie in de behandelde groep, zodat mogelijk deze celbeschermende werking van L-propionylcarnitine niet op ging voor de myocyten.

In hoofdstuk 5 bestudeerden we de cardiovasculaire effecten van elgodipine, een nieuwe generatie Ca^{2+}-antagonist, behorend tot de groep van de fenyldihydropyridines. Bij intraveneuze toediening in het genarcotiseerde varken bleek de stof zeer potent te zijn voor wat betreft systemische en coronaire vaatverwijding, zonder dat dat gepaard ging met negatief inotrope of positief chronotrope eigenschappen. De stof lijkt daarom geschikt voor gebruik bij essentiele hypertensie, myocardischemie en mogelijk bij matig hartfalen.

Eenzelfde sterke daling van de perifere weerstand werd gevonden met nicorandil, een nitraat-achtige stof met K^+ kanaal openende eigenschappen (hoofdstuk 7). Deze bloeddrukdaling ging hier echter wel gepaard met een reflex-gemedieerde tachycardie.

Het evenwicht tussen de potentiele gunstige effecten (coronaire vaatverwijding, daling van de zuurstofconsumptie door daling van de perifere weerstand en daling van de preload) en de mogelijk nadelige effecten (daling van de coronaire perfusiedruk) tijdens ischemie werd bestudeerd in hoofdstuk 8. We toonden aan dat nicorandil in het gebruikte model (concentrische stenose, geen collateraalcirculatie) de bloedstroom naar de verschillende lagen van het ischemische myocard verminderde. Het is aangetoond dat nicorandil in staat is de collateraalflow te vergroten bij honden met een coronair ligatie. Bovendien veroorzaakt nicorandil bij patienten dilatatie van de epicardiale vaten. Deze twee eigenschappen maken de stof bij een bepaalde groep van patienten (eccentrische stenose met collateraalcirculatie) geschikt als geneesmiddel bij myocardischemie. Uit onze studie blijkt dat bij de jonge patient met een acuut ontstaan van klachten van myocardischemie, waar zich nog geen collateraalcirculatie heeft gevormd, de stof ook

nadelig kan werken.

EMD 52692 is in tegenstelling tot nicorandil een selectieve opener van de ATP-afhankelijke K^+ kanalen. K^+-kanaal openers veroorzaken hyperpolarisatie van de membranen van gladde spiercellen, waardoor de beschikbaarheid van Ca^{2+} in de cel, nodig voor contractie, daalt. EMD 52692 is een zeer potente vasodilator, met het meest duidelijk effect op de hersenvaten en de intestinale vaten (hoofdstuk 9). Ook de selectiviteit voor de vaatspiercellen was aanmerkelijk groter dan die voor de hartspiercellen, omdat met doses die veel hoger waren dan die van klinisch belang geen negatieve inotropie werd gevonden. De stof zou van belang kunnen zijn bij de therapie van essentiele hypertensie, matig hartfalen, ischemische aandoeningen van de hersenen, angine abdominale en mogelijk ook myocardischemie, hoewel de reflex-gemedieerde tachycardie, in combinatie met de sterke daling van de perfusiedruk, ischemie zou kunnen induceren.

Beta-adrenenoceptor antagonisten zijn stoffen die nog steeds van belang zijn in de behandeling van myocardischemie. Bisoprolol is een cardioselectieve beta-adrenoceptor antagonist en in hoofdtuk 10 beschrijven we de effecten van 3 doseringen van deze stof op de bloedstroom door en de functie van het myocard van genarcotiseerde varkens met een partiele stenose van een coronair arterie. Bisoprolol verbeterde de bloedstroomvan het ischemische myocard, meest waarschijnlijk door het negatief chronotrope effect van de stof, waardoor de diastolische perfusietijd verlengd werd. Dit ging echter niet gepaard met herstel van functie, mogelijk door de duur van de ischemische episode gecombineerd met de toch nog lage bloedstroom na bisoprolol. In de met bisoprolol behandelde groep varkens was ook het aantal dieren dat uitviel als gevolg van ventrikelfibrillatie significant lager. Dit effect zou mogelijk verklaard kunnen worden, doordat bisoprolol een meer homogene bloodstroomverdeling veroorzaakte.

Samengevat wordt in dit proefschrift aangetoond dat in varkens diltiazem geen anti-atherosclerotisch effect heeft en makreelolie de regressie van coronairsclerose bevordert. Verder zorgen zowel nisoldipine als L-propionylcarnitine voor een stijging van de post-ischemische bloedstroom van het myocard. Waarschijnlijk geschiedt dit door een vermindering van het "no-reflow" fenomeen, en in het geval van nisoldipine ook door directe coronaire vasodilatatie. In beide studies echter ging dit niet gepaard met herstel van de contractiele functie van het myocard. Nicorandil, een stof met zowel nitraat-achtige werking als effecten van een K^+ kanaal opener, de meer zuivere K^+-kanaal opener EMD 52692 en de Ca^{2+}-antagonist elgodipine zijn alle zeer potente vasodilatoren, wat ze geschikt maakt voor de behandeling van myocardischemie. De reflectoire tachycardie in combinatie met de sterke perfusiedrukdaling kan echter bij de stoffen nicorandil en EMD 52692 de myocardischemie onder bepaalde condities verergeren. Voor nicorandil hebben we dit inderdaad geobserveerd in een varkensmodel (concentrische stenose, geen collateraalcirculatie) wat model staat voor een bepaalde groep patienten (jonge patienten, acuut begin van ischemie). In hetzelfde model toonden we ook aan dat de cardioselectieve beta-adrenoceptor antagonist bisoprolol de bloedstroom naar het ischemische myocard bevorderde, meest waarschijnlijk dankzij een negatief chronotroop effect.

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Curriculum vitae

De schrijfster van dit proefschrift werd geboren op 19 april 1962 te Gouda. Na het behalen van het VWO diploma aan de Rijksscholengemeenschap Oud-Beijerland in 1980 werd met de studie Geneeskunde begonnen aan de Erasmus Universiteit Rotterdam. Tijdens de studie Geneeskunde werkte zij als student-assistent op de afdeling Experimentele Cardiologie onder leiding van Prof. Dr. P.D. Verdouw. Vanaf het behalen van het artsexamen in 1987 tot op heden is zij als wetenschappelijk onderzoeker werkzaam op dezelfde afdeling.

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