Left Ventricular Dysfunction and Remodeling after Myocardial Infarction

Pathophysiological and therapeutical aspects

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Left Ventricular Dysfunction and Remodeling after Myocardial Infarction

Pathophysiological and therapeutical aspects

Linker ventrikel dysfunktie en remodelering na een myocard infarct

Pathofysiologische en therapeutische aspecten

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Nederlandse Hartstichting
vrienden van de hartstichting
Red het hart

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Aan: Mijn Familie

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Chapter 1

Introduction

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EPIDEMIOLOGY OF HEART FAILURE

Despite successful treatment of ischaemic heart disease in the western world cardiovascular disease is on the rise and is estimated to become the world's leading cause of death by the year 2020 (table 1.)(1-3). This is in part due to the improved treatment of infectious, parasitic and nutritional disease, together with a global change in life-style that allows populations to reach an age at which cardiovascular disease becomes manifest.(2)

Table 1. Estimated in rank order of disability-adjusted life years for the 10 leading causes of disease or injury worldwide from 1990 to 2020

Rank	1990	2020
1	Lower respiratory infections	Ischaemic heart disease
2	Diarrhoeal diseases	Unipolar major depression
3	Conditions arising during the perinatal period	Road traffic accidents
4	Unipolar major depression	Cerebrovascular disease
5	Ischaemic heart disease	Chronic obstructive pulmonary disease
6	Cerebrovascular disease	Lower respiratory infections
7	Tuberculosis	Tuberculosis
8	Measles	War
9	Road traffic accidents	Diarrhoeal diseases
10	Congenital anomalies	Human immunodeficiency virus

Data from Murray and Lopez[1,2]

These improvements are also shifting the cause and occurrence of congestive heart failure. In the western world a similar shift has already occurred. The main cause for congestive heart failure from 1900-1950 was rheumatic fever, however with the advent of antibiotic treatment and valve replacement surgery; the main cause became hypertension (1950-1980). With an improved treatment of hypertension as well as the improved survival of myocardial infarction, the importance of ischaemic heart disease infarction (with myocardial infarction as its most severe manifestation) increased and has become the most prominent cause of heart failure in the western world.(4) This rise in ischaemic heart disease is now being mimicked by the developing countries, and although etiology may vary per region worldwide heart failure is on the increase, with ischaemic related heart failure fast becoming the most prevalent cause. The increased life-expectancy combined with the baby boom after the second world war will only add to the growth of absolute number of people suffering from congestive heart failure.(5)

The increased number of heart failure patients is reflected in the increase in both prevalence (ranging from 1-2% of the total population) as well as incidence. Both rise with increasing age, approximately doubling with each decade of ageing above the age of 45 years.(6) However these figures do not include the prevalence of asymptomatic left ventricular dysfunction. The

asymptomatic numbers vary, according to criteria set, effectively doubling to tripling the number of patients when compared to the symptomatic number of patients.(7, 8) If treatment of patients by appropriate pharmacological intervention is started when they are asymptomatic, the progression to symptomatic left ventricular dysfunction and its adverse effects can be delayed or even prevented.(9, 10) Alternatively, if these numbers are not adequately addressed and treated they will accumulate and add to the growth of the existing number of symptomatic patients. The trend remains likely to grow even further, with predictions for the year 2010 foreseeing a 70% growth compared to 1985 in incidence in the Netherlands.(11)

From the first moment patients are diagnosed with heart failure their outlook is dismal. Mean survival of the first year once diagnosed is 60-70%, dropping to 35% at 5 years.(12-14) For the duration of the disease the patients are frequently admitted to hospital in need of medical attention, often for prolonged periods and requiring intensive medical treatment.(15, 16) Especially in the elderly patient re-admittance can be high, up to one-third may be re-admitted within 1 year of discharge.(17)

The cost for heart failure consumes about 1-2% of the national healthcare budget. The majority of these expenses are made during the patients stay in hospital; estimates vary form 60% to nearly 80% of the total cost of heart failure.(18, 19) The healthcare costs of these patients are also determined by the severity of the disease, for instance failure New York Heart Association class IV costs between 8 to 30 times more than New York Heart Association class II.(20) This increases the importance of preventing asymptomatic patients from progressing to symptomatic higher classes.

PATHOPHYSIOLOGY OF LEFT VENTRICULAR DYSFUNCTION AFTER MYOCARDIAL INFARCTION

There is no universally agreed upon definition of heart failure. Heart failure is a syndrome that develops as a consequence of cardiac disease. The syndrome is further recognized by a myriad of clinical signs and symptoms. Definitions that, have tried to capture this syndrome, resulted in for example: "the pathophysiological state in which an abnormality of cardiac function is responsible for the failure of the heart to pump blood at a rate commensurate with the requirements of the metabolizing tissues and/or to be able to do so only from an elevated filling pressure; Braunwald" or "a state in which the heart fails to maintain an adequate circulation for the needs of the body despite a satisfactory filling pressure; Wood".

As briefly mentioned before the etiology of congestive heart failure can be diverse. For instance rheumatic fever through it's degenerative effect on the aortic and mitral valve can impose a volume load on the myocardium. Longterm hypertension or aortic stenosis on the other hand will subject the myocardium to a pressure load. While these causes fundamentally differ from myocardial infarction, which mechanism is the production of myocardial damage, the resulting adaptive response follows a common pathway.

To better understand the pathophysiology of heart failure and the consequences for the hemodynamic regulation a short overview of the normal physiological situation is essential. The physiology of hemodyamic regulation is made up of a number of components (fig.1). Fiber shortening is regulated by the contractility of the myocardium, the preload (which influences the number of cross-bridges between actin and myosin) as well as the afterload or more precisely the wall stress that has to be generated. Preload or the loading conditions of the ventricle determine the left ventricular size, as well as influence the force development via the Frank-Starling mechanism. Left ventricular size is also a determinant of afterload as diameter or left ventricular size together with wall thickness determines, in accordance with the law of Laplace, the wall stress. The combination of left ventricular size and fiber shortening regulates the stroke volume. Together with the frequency of contractions (or heart rate) stroke volume makes up cardiac output. The cardiac output together with the resistance of the vasculature determine the perfusion pressure in the vasculature, as well as distributing the flow among the different organs.

When the demand of the body made upon the functional myocardium exceeds its capacity (either through damage or loading of the myocardium; see fig.1); the fall in cardiac output will result in a simultaneous lowering of the blood pressure. This will activate compensatory mechanisms: the hemodynamic defense reaction. Within a matter of seconds an activation of the sympathetic as well as a decrease in parasympathetic activity will occur.

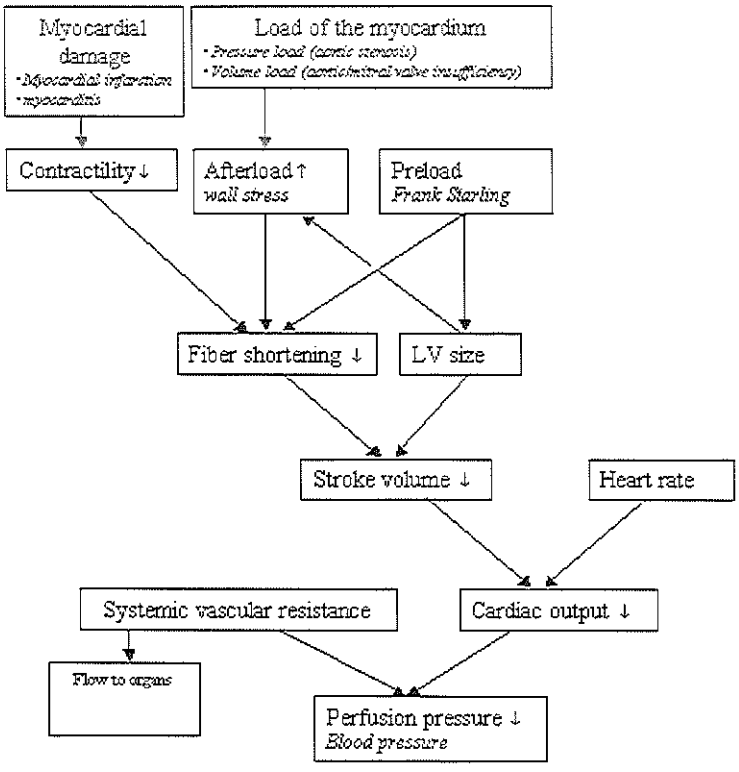

Figure 1. Physiologic regulation of perfusion pressure; LV: Left Ventricle

The heightening of the sympathetic activation will increase heart rate, contractility and vascular tone (fig. 2). A fall in the internal pressure of the kidney will result in the increased release of renin from the juxtaglomerular apparatus, thereby leading to activation of the renin angiotesin aldosteron system with its potent vasoconstrictor effects. The increase in vascular tone is not limited to the arterioles, the veins constrict as well, increasing the venous return of blood to the heart and thereby optimizing ventricular filling. These actions will increase cardiac output in order to maintain perfusion pressure and preserve the flow (through redistribution) to essential organs.

If the situation lasts up to hours or days; the sympathetic activation with help from the renin angiotesin aldosteron system and vasopressin will result in Na^+ and fluid retention to increase the intra-vascular volume allowing for optimal filling. Apart from increasing the circulating volume, these factors in their own right contribute directly to the increase in vasomotor tone.

When the activation persists for weeks or longer the activated compensatory mechanisms will result in a hypertrophic response of the myocardium. This hypertrophic response is further

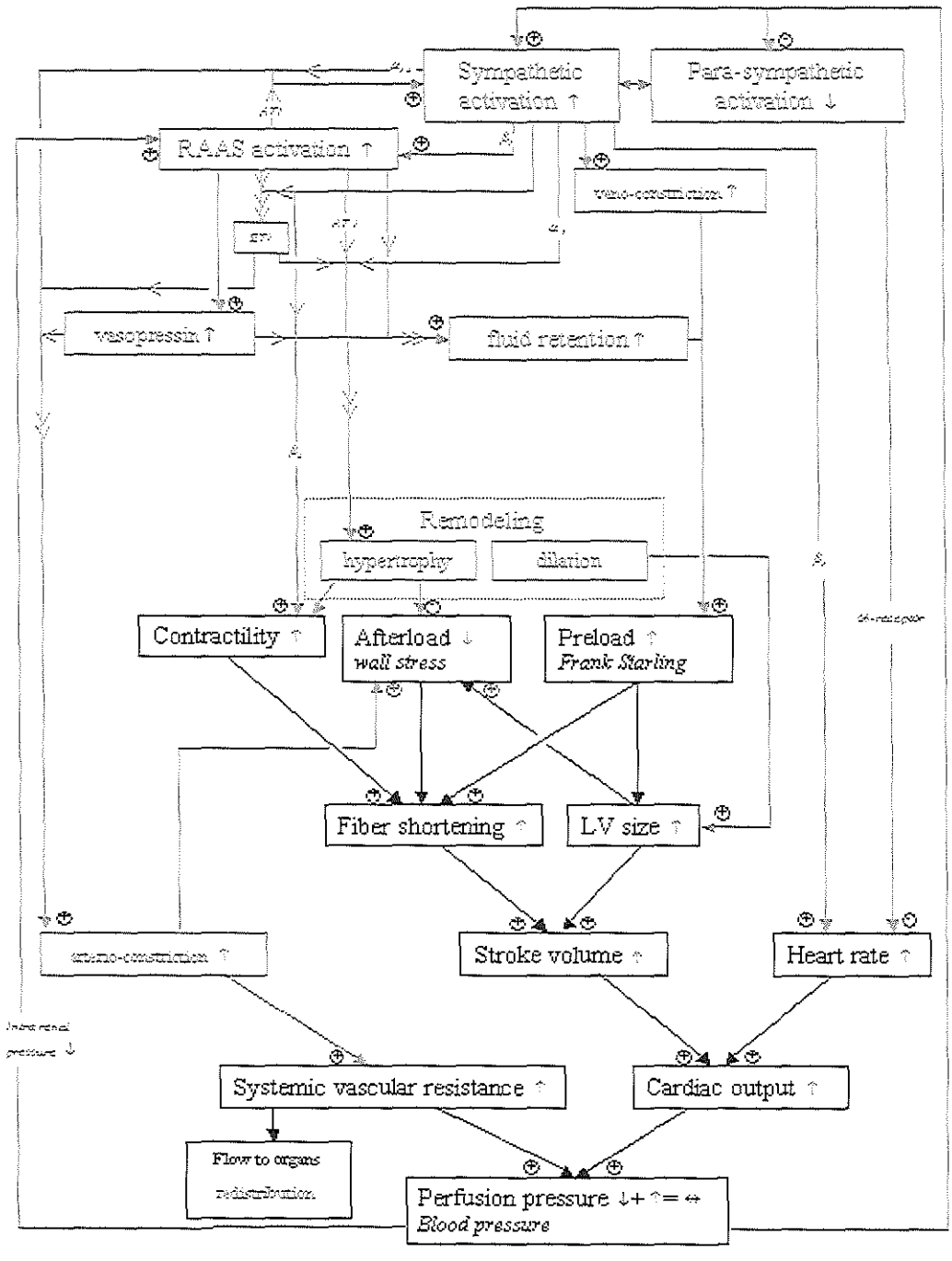

Figure 2. An overview of the hemodynamic defense reaction and its consequences; LV: Left Ventricle, β: β-adrenergic, α-: α-adrenergic, AT1 : angiotensin type 1 receptor,ET-1: endothelin- type 1 receptor, m : muscarinic receptor

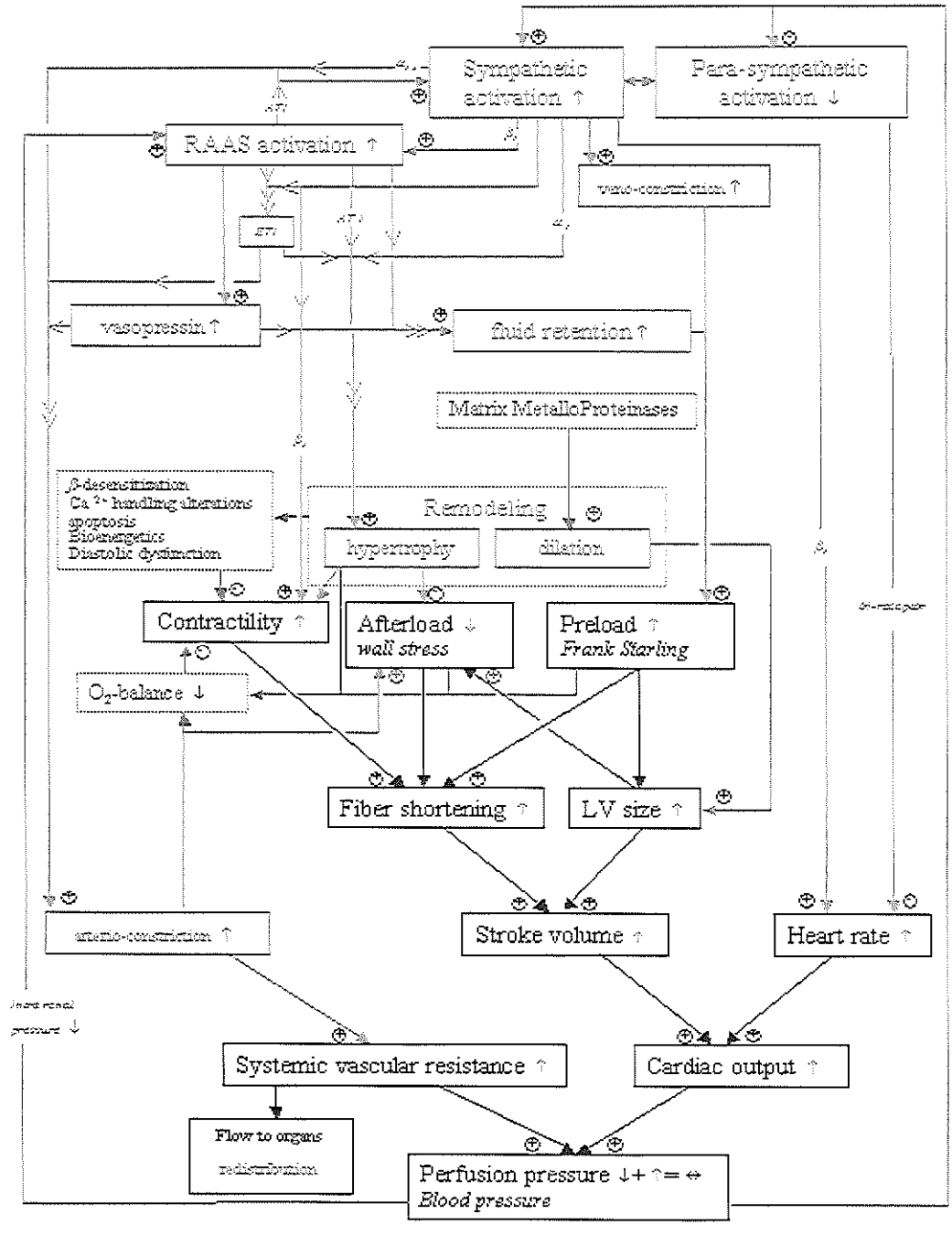

Figure 3. An overview of the effects of prolonged exposure to the hemodynamic defense reaction and its consequences; LV: Left Ventricle, *β*: *β*-adrenergic, *α*-: *α* adrenergic, AT1 : angiotensin type 1 receptor,ET-1: endothelin- type 1 receptor, m : muscarinic receptor

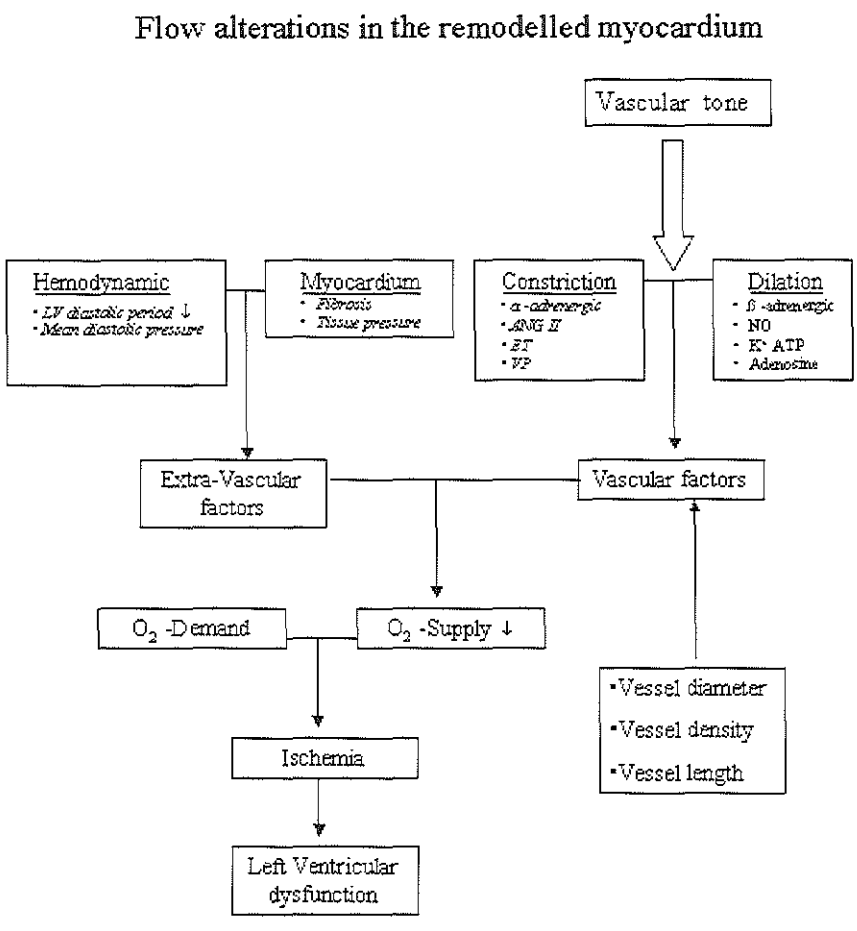

Figure 4. An overview of the flow alterations in the remodelled myocardium; LV: Left Ventricle, *β*-: *β*-adrenergic, *α*-: *α*-adrenergic, ANG: angiotensin, ET: endothelin, vp : vasopressin

strengthened by the activation of angiotensin receptors and endothelin among others. The hypertrophy of the myocardium will reduce the wall stress, which is the result of dilation of the ventricle, simultaneously reducing the workload of the individual myocytes.

However even when the hemodynamic situation has stabilized, but neurohumoral activation remains present, deterioration of the performance of the ventricle continues. Administration of neurohumoral blockers such as angiotensin converting enzyme inhibitors and/or *β*-adrenergic receptor-blockers(21) have been proven to reduce the rate of this deterioration. Comparing of pure vasodilators with angiotensin converting enzyme-inhibition showed that improved survival with angiotensin converting enzyme-inhibition must be attributed to a direct effect rather than an

unloading of the ventricle.(22, 23) It is now agreed upon that this neurohumoral activation, which acts to maintain blood pressure, also has harmful side effects.

As seen in fig.3 the progressive dilation of the ventricle in the remodeling process is attributable to the maintained neurohumoral activation, as well as the inflammatory response reaction following a myocardial infarction. Induction of Matrix-Metalloproteinases released from neutrophils will facilitate the dilation of the ventricle. The action of Matrix-Metalloproteinases will loosen the tight collagen network at the site of the infarction. Allowing elongation and side-to-side slippage of the myocytes to occur,(24, 25), resulting in a reduced number of myocytes at the site of the infarction,(26, 27) within the first hours of infarction. This dilation progresses further with the dilation of the non-infarcted area, stopping when the distending forces are countered via tensile strength (collagen depositions). As a consequence of this dilation a significant increase in the wall stress of the ventricle is achieved. With the progression of the ventricular dilation; inefficiency of the contraction and expulsion of blood is produced; the mitral valve orifice is widened and becomes insufficient.

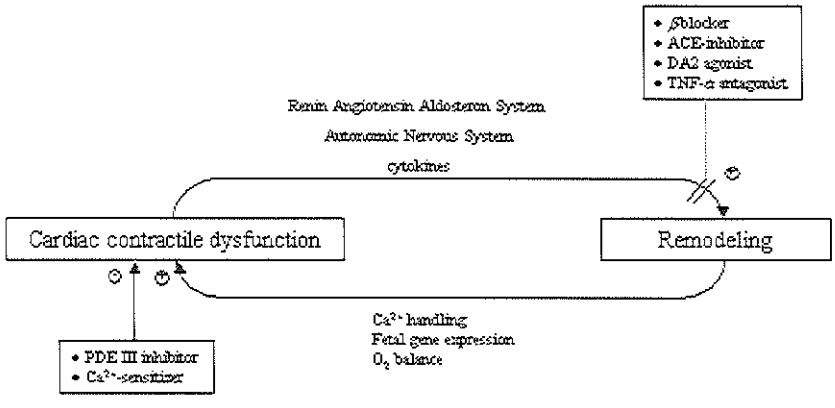

Figure 5. An overview of the active balance between hemodynamic defense reaction and cardiac remodeling with *ß: - ß adrenergic., ACE: angiotensin converting enzym, DA2: dopamine type 2, TNF: tumor necrosis factor, PDE: phosphodiesterase.*

The sympathetic activation in the form of norepinephrine release can stimulate the hypertrophic response both directly via the a_1-adrenergic receptor,(28) and indirectly via the $ß_1$-adrenergic receptor instigating the release of renin, which in turn promotes via angiotensin II the presynaptic release of norepinephrine and blocks its uptake thereby increasing its availability.(29) Both angiotensin II and norepinephrine are capable of enhancing endothelin-1 release, which is also a stimulus for hypertrophy.(30) Besides synthesis of collagen, myofibroblasts produce angiotensin I,

angiotensin II, aldosteron and receptors for angiotensin II, Transforming Growth Factor-ß1 and endothelin-1(31, 32) strengthening the hypertrophic stimulus.

The hypertrophic remodeling of the myocardium, while decreasing wall stress and energy demand per contractile element, will increase the total amount of energy required. This requirement is hindered by an increased intercapillary distance (or reduced capillary density) and elongation of the penetrating arterioles. The increased heart rate will shorten diastolic time essential for the perfusion of the myocardium, while the elevated filling pressures (mean diastolic left ventricle pressure or left atrial pressure) and tissue pressure will reduce the effective perfusion pressure of the subendocardium.

The increased total energy requirements of the myocardium will have to be met via vasodilation of the coronary vascular bed. However this is countered by the elevated plasma levels of vasoconstrictors such as catecholamines, angiotensin II, endothelin and vasopressine which are all present in the hemodynamic defense reaction. Although an increased vasoconstrictor tone may hinder the vasodilation of the coronary vasculature so too could a loss of vasodilating influences. For example a loss of nitric oxide availability could aggravate left ventricular dysfunction due to the peripheral vasoconstriction-induced increase in left ventricular afterload, coronary vasoconstriction, and increased myocardial O_2 consumption (33-36) Possibly, other vasodilator mechanisms may be less effective (K^+_{ATP}, prostacyclin and ß-adrenergic vasodilation). The energy starvation of the myocardium (in part due to a slowing of the phosphocreatinine shuttle) as seen in end-stage heart failure, may aggravate the situation. If the dysbalance between supply and demand of oxygen becomes too severe ischemia and even necrosis can ensue (fig.4).

The cardiac performance may also be threatened by the risk of cellular dropout as a result of apoptosis (programmed cell death) of cardiomyocytes. Reports suggest this phenomenon to be induced via hypoxia, stretch, angiotensin II, norepinephrine, cytokines (Tumor Necrosis Factor-β)(33), as well as being responsible for the majority of cell loss in the myocardium. Besides the possibility to lose cells, they may also become less responsive to stimulation. For instance β-adrenergic receptors desensitization as well as reduced levels of sarcoplasmatic reticulum Ca^{2+} pump (SERCA) and alterations in the MHC component.

Diastolic dysfunction is characterized (apart from stiffening of the ventricle) by a reduced rate of ventricular relaxation.(34) The reduced rate of relaxation can be the result of excessive deposition of collagen, contributing to the reduced performance as it hinders the ventricle to relax sufficiently. The synthesis of collagen 1 and 3 by myo-fibroblasts is modulated by a variety of factors: angiotensin II related mechanical deformation, fibroblasts growth factor; platelet derived growth factor, atrial natriuretic peptide and nitric oxide release. Aldosteron in its own right stimulates the transcription of collagen type I and III mRNA, and has been reported to be up to 17 times higher in

the myocardium compared to plasma values. (35). Activated macrophages (via γ-interferon) express angiotensin converting Enzyme thus providing a local source of angiotensin II, which is independent of the plasma angiotensin II, at the same time mediating the formation of fibrosis in the myocardium. The scar tissue that has formed on the site of the infarct will remain dynamic, vascularized, metabolically active and even capable of contraction. This contraction may contribute to the abnormal ventricular stiffness adding to diastolic dysfunction and hinder cardiac performance.(36)

When it comes to the contraction and relaxation of the cardiomyocytes the amount of intracellular calcium is of integral importance. Alterations in the intracellular calcium-cycling, especially reduced clearance, therefore can be of major importance in the diastolic dysfunction of the myocardium. The most prominent mechanism to reduce the amount of Ca^{2+} within the cell is by pumping the intracellular Ca^{2+} into the sarcoplasmatic reticulum via the sarcoplasmatic reticulum Ca^{2+} pump (SERCA). Another way of reducing the amount of Ca^{2+} within the cell is by expulsion into the sarcolemma via the Na^{+}/Ca^{2+} exchanger. During heart failure the amount of Na^{+}/Ca^{2+} exchanger is increased (37, 38) possible forming a compensatory mechanism to counter the reduced Ca^{2+} clearing due to decreased SERCA activity. However a price must be paid for this aid in myocardial relaxation as sarcoplasmatic reticulum calcium stores may become depleted especially during elevated heart rates, which can give rise to a blunted force-frequency response. (39) Decreased contractility does not have to be solely attributable to an altered calcium cycling, it may well be that alterations within the contractile apparatus are fundamental in the decreased performance. For instance, troponin isoforms, which can be seen to be induced in heart failure (40), have been implicated in an altered Ca^{2+} sensitivity. (41)

In summary, regardless of its etiology, congestive heart failure evokes similar response in the body and myocardium. It is this adaptive response or remodeling that is initially adaptive, but prolonged activation will only contribute to the deterioration of cardiac function it does so through various systems among them a disturbed O_2-balance and expression of fetal gene program. This deterioration typifies the natural history of heart failure. Therefore the interruption of this cycle through the administration of neurohumoral blockers such as angiotensin converting enzyme inhibitors and β-blockers have been shown to effectively prolong the life-expectancy of the individuals suffering from congestive heart failure. Furthermore Bristow has proposed that any therapeutic agent that would diminish the myocardial dysfunction without producing any adverse biological effects may improve the natural history of congestive heart failure (fig.5). A logical conclusion would be that a combination of an agent that improves the cardiac contractility and an agent that reduces the cardiac remodeling may meet these criteria.

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AIMS OF THE THESIS

Congestive heart failure is the only major cardiovascular syndrome of which the incidence and prevalence are currently increasing in the western world. This increase is in part due to the successful treatment of acute myocardial infarction in conjunction with an ageing population, and as a result myocardial infarction has replaced hypertension as the major cause of heart failure. Between 1950-1980 the treatment of congestive heart failure was solely based on the "hemodynamic model", which led to inotropic stimulation with sympathomimetics and/or unloading of the heart with vasodilators or inodilators in order to restore cardiac output. Between 1980-2000 the concept of congestive heart failure shifted and now encompasses the "neurohormonal model" in which the progressive remodeling with impaired cardiac chamber performance is considered to be a central feature. Consequently producing a change in the initiation of treatment, from decompensated state of ventricular dysfunction to one of compensation in order to prevent deterioration of myocardial function and thereby improving survival. Therefore, heart failure patients are now treated with blockers of neurohormones such as Angiotensin Converting Enzyme inhibitors and β-adrenergic receptor blockers.

Blunting of sympathetic activity via dopamine receptor stimulation

Several large scale clinical trials have shown that clinical deterioration of patients with heart failure is influenced by the sympathetic activation. Dopaminergic activation of the Dopamine 2 receptor limits the sympathetic activation and could prove beneficial in preventing deterioration of the remaining myocardium function. Activation of the Dopamine 1 receptor located on arterial smooth muscle cells in the kidney and mesenteric vascular bed and in some species in coronary and cerebral vessels,(1-4) causes vasorelaxation. Dopamine 1-receptors are also located on renal tubular cells and juxtaglomerular cells where they promote natriuresis and diuresis, respectively. These actions may prove effective in periods of exacerbation of congestive heart failure, when the need arises to decrease the afterload and congestion. The standard therapeutical option for unloading of the ventricle is still mainly performed via vasodilators and diuretics, that can elicit a baroreflex mediated activation of the sympathetic nervous system. We therefore examined in chapter 2 the pharmacologic actions of the dopamine agonist Z1046 under resting and exercise conditions.

Inotropic treatment of left ventricular dysfunction

Heart failure patients; especially end-stage; are repeatedly admitted to hospital requiring inotropic support. Inotropic support administered for short duration is well tolerated, while longterm support in clinical trials has generally led to disappointing results, as in a large number of studies improvements in cardiovascular function and quality of life have been overshadowed by an excess of sudden death.(5) Nevertheless, the depressed cardiac pump function remains a major concern in a significant proportion of patients, requiring treatment in order to prevent end organ failure because of lack of supply of nutrients. A major drawback of the traditional inotropic agents such as sympathomimetics and phosphodiesterase-III inhibitors is that they exert their actions through a cAMP-mediated enhancement of Ca^{2+}-transients which increases not only myocardial O_2-demand,(6) but also the risk of ventricular arrhythmias.

In contrast, Ca^{2+}-sensitizing agents augment myocardial contractility without increasing Ca^{2+}-transients and therefore will produce no or only minimal increments in myocardial O_2-consumption.(6, 7) It has been shown(8) that acute administration of the thiadiazinone derivative EMD 57033, a Ca^{2+}-sensitizing agent with negligible phosphodiesterase-III inhibiting properties,(9-11) improved myocardial contractility and mechanoenergetics without compromising left ventricular diastolic function in conscious dogs with pacing-induced congestive heart failure. Potentially adverse effects of Ca^{2+}-sensitizing agents on left ventricular diastolic function has been a major concern with this class of agents,(12, 13) but is mainly based on *in-vitro* studies (see(13)), as in several *in-vivo* studies employing animals with normal, stunned or failing hearts no adverse effects on diastolic function were demonstrated.(8, 14-17) The observation that an improvement in systolic function can be obtained without increasing energy expenditure and the risk of arrhythmias may therefore improve and change the natural history of heart failure. Chapter 3 therefore focuses on the pharmacologic actions of the calcium sensitizer EMD 57033 and compare these to those of pimobendan.

Role of renin angiotensin system in left ventricular remodeling

The progressive cardiac remodeling as seen in heart failure is seen as a central feature of heart failure. Angiotensin converting enzyme inhibitors prevent cardiac remodeling (ie, ventricular hypertrophy and chamber dilatation) after myocardial infarction. This occurs, in part, independent of their blood pressure–lowering effect, suggesting interference with tissue angiotensin II. However, angiotensin converting enzyme inhibitors do not always lower cardiac angiotensin II because of their pharmacokinetic properties (short half-life and lack of tissue penetration), angiotensin.converting enzyme upregulation during prolonged angiotensin converting enzyme inhibition, or alternative converting enzymes, such as chymase. Furthermore, previous angiotensin

II measurements in infarcted hearts have not taken into account the fact that cardiac angiotensin II is partly derived from the circulation. Plasma angiotensin II accumulates in the heart through angiotensin II type 1 receptor–mediated endocytosis, and changes in angiotensin II type 1 receptor density after myocardial infarction may influence this process.

angiotensin II type 1 receptor antagonists have opened new avenues in the investigation of the importance of cardiac angiotensin II after myocardial infarction. In general, these drugs increase rather than decrease angiotensin II levels, and this may lead to activation of unoccupied growth-inhibitory angiotensin II type 2 receptors. Currently, the effect of angiotensin II type 1 receptor antagonism on the angiotensin II content of the infarcted heart is unknown. Furthermore, it is still controversial whether angiotensin II type 1 receptor antagonism prevents the development of cardiac hypertrophy and increases survival after myocardial infarction.

Therefore, in chapter 4 we investigated changes in cardiac angiotensin II content and origin (local synthesis versus uptake from plasma) after myocaradial infarction in pigs, to compare the effects of angiotensin converting enzyme inhibition and angiotensin II type 1 receptor antagonism on these changes, and to evaluate whether the effects of both renin-angiotensin system blockers on cardiac angiotensin content are related to their effects on postinfarct remodeling.

Is myocardial blood flow perturbed in the remodelled left ventricle

Myocardial infarction; through hypertrophy as well as increases in heart rate and systemic vascular resistance; will increase myocardial O_2-demand. This increase in O_2-demand must result in an increase in myocardial blood flow. Reports in *in-vivo* studies in rats and pigs indicate a reduction in myocardial blood flow reserve of up to 35% in the surviving post-infarct left ventricle myocardium, three to eight weeks after infarction. However, the role of myocardial blood flow abnormalities in the progression of left ventricular dysfunction following infarction to overt heart failure is incompletely understood. Since hemodynamic and neurohumoral abnormalities associated with left ventricular dysfunction are exacerbated during exercise, myocardial blood flow increments could be impaired during increased O_2-demand produced by exercise, thereby limiting myocardial O_2-availability. Consequently, we investigated in chapter 5 the responses of myocardial blood flow and myocardial metabolism to treadmill exercise in normal swine and swine with a 3-week-old myocardial infarction to determine whether impaired myocardial blood flow responses limit myocardial O_2-availability and result in myocardial ischaemia. Since this is the first study of the responses to exercise in swine with a myocardial infarction, we also determined the exercise-responses of left ventricular function, systemic and pulmonary hemodynamics, and neurohormones.

Contribution of loss of nitric oxide availability to hemodynamic alterations in vasomotor tone following myocardial infarction

Heart failure is accompanied by increased production of neurohormones such as norepinephrine, angiotensin II and endothelin,(18, 19) which serves to maintain arterial pressure by partially restoring cardiac output and increasing peripheral vascular resistance, in order to maintain perfusion of essential tissues like brain and heart at the expense of renal, pancreatic and intestinal blood flow. Several studies have indicated that also endothelial dysfunction, in particular a decreased production of nitric oxide, contributes to an increased peripheral resistance in congestive heart failure.(20-24) A loss of nitric oxide availability could aggravate left ventricular dysfunction due to the peripheral vasoconstriction-induced increase in left ventricular afterload, coronary vasoconstriction, and increased myocardial O_2 consumption.(25-28) In support of this concept, a decreased nitric oxide production coincides with the transition of left ventricular dysfunction to overt congestive heart failure in models of pacing-induced heart failure.(24, 27)

However, as stated before, the most common cause for heart failure is myocardial infarction. Studies on the role of nitric oxide in regulation of vasomotor tone in myocardial infarction-induced left ventricular dysfunction in rats indicate that between 4 and 16 weeks after infarction, acetylcholine- or ADP-induced nitric oxide-mediated relaxation of systemic conductance arteries (29-33) and resistance vessels (34) is reduced, although this is not an unequivocal finding.(35, 36) In contrast, basal nitric oxide production is maintained in systemic conductance arteries.(31, 35) and in resistance vessels in various regional vascular beds *in-vivo*.(34, 36-38) These findings could be interpreted to suggest that early after infarction, a reduced nitric oxide-mediated vasodilator capacity could contribute to a blunted vasodilator response, and hence tissue hypoperfusion, during exercise,(39) at a time when basal nitric oxide production may still be intact. Recently, we reported that in resting and exercising normal swine inhibition of nitric oxide-synthase with N^{ω}-nitro-L-arginine decreased systemic and pulmonary vascular conductance, and flow to kidney, pancreas and part of the gut, whereas flow to skeletal muscle was unaffected.(25) A similar pattern was found in exercising swine with a 3-week old myocardial infarction,(40)*(see chapter 5)* suggesting that in this model of moderate left ventricular dysfunction nitric oxide availability is already impaired, in particular during exercise. Consequently, in chapter 6 we tested the hypothesis that loss of nitric oxide-mediated vasodilation contributes to abnormalities in vascular tone control in regional systemic, pulmonary and coronary beds in exercising swine with left ventricular dysfunction after infarction. Since our results indicated that the contribution of nitric oxide to exercise-induced vasodilation was maintained, and because inducible nitric oxide synthase may be upregulated after infarction,(41, 42) we further tested whether upregulation of inducible nitric oxide synthase compensated for the potential loss of endogenous nitric oxide synthase activity.

Autonomic balance in exercising swine with a recent myocardial infarction.

Heart failure is accompanied by a hemodynamic defense reaction consisting of salt and water retention, vasoconstriction and cardiac stimulation.(43) This hemodynamic defense reaction serves to partially restore cardiac output and to increase peripheral vascular resistance in order to maintain arterial pressure, and consequently perfusion of essential tissues like brain and heart. An integral part of the hemodynamic defense reaction involves alterations in the balance of autonomic activity,(44) i.e. an increase in sympathetic activity(45-47) and a decrease in parasympathetic activity.(48-50) Increased sympathetic activity contributes to the poor prognosis of heart failure as indicated by the correlation between elevation of plasma norepinephrine and mortality,(51, 52) and by the beneficial effects of β-adrenergic receptor blockers on mortality in patients with heart failure.(53-55) Conversely, a decreased parasympathetic activity may therefore potentially aggravat the catecholamine-induced fatal arythmia's by lowering the treshold for ventricular fibrillation.(56)

Most experimental studies on heart failure secondary to myocardial infarction have been performed in rats,(57) In contrast to large mammals such as humans and swine.(58) Rats already have a higher degree of sympathetic tone under basal resting conditions,(59) which makes interpretations of changes in autonomic balance during heart failure difficult. In chapter 3- 5 we observed that a 2-3 week old myocardial infarction in swine resulted in moderate left ventricular dysfunction characterized by a lower $LVdP/dt_{max}$, stroke volume and a slightly higher heart rate under awake resting conditions,(40)*(see chapter 5)* whereas resting catecholamine levels were still normal.(40, 60) *(chapters 5 and 4, respectively)* Animals displayed exaggerated catecholamine responses to treadmill exercise,(40) *(see chapter 5)* whereas the increases in heart rate and global left ventricular contractility were maintained and only slightly blunted at higher levels of exercise, respectively.(40) *(see chapter 5)* These findings could be interpreted to suggest that an attenuated cardiac β-adrenergic receptor responsiveness was compensated for by an increase in sympathetic drive during exercise at this stage of heart failure,(61) or that the exaggerated increase in sympathetic activity acted to compensate for a blunted further withdrawal of vagal tone during exercise. In chapter 5 we also found that systemic and coronary vasodilator responses to exercise were blunted 2-3 weeks after myocardial infarction,(40) *(see chapter 5)* which could be due to increased α-adrenergic vasoconstriction and/or diminished β-adrenergic vasodilation. Consequently, chapter 7 focusses on alterations in autonomic control of heart rate and cardiac pump function, as well as autonomic control of systemic and coronary vasomotion in swine with a 2-3 week old myocardial infarction at rest and during treadmill exercise.

Contribution of angiotensin II type I receptor blockade to the vasomotor tone.

Left ventricular dysfunction as induced via a myocardial infarction will lead to elevated plasma levels of angiotensin II, a potent vasoconstrictor and growth promotor. We already determined in chapter 4 that an elevated uptake of angiotensin II occurs via the angiotensin Type 1 receptor suggesting an increased amount of angiotensin Type 1 receptors in the myocardium. Furthermore, chapter 5 showed that coronary vascular tone was increased in the early stage of left ventricular dysfunction. This could suggest a role of angiotensin II in the increased coronary vascular tone after a myocardial infarction. We therefore studied in chapter 8 the hemodynamic effects during rest and exercise of the angiotensin Type 1 receptor blocker irbesartan to determine the possible contribution in the increased coronary vascular tone seen after a myocardial infarction.

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Chapter 2

Systemic, pulmonary and coronary haemodynamic actions of the novel dopamine receptor agonist Z1046

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ABSTRACT

1 In view of potential therapeutic application of specific dopamine receptor agonists in the treatment of hypertension and left ventricular dysfunction, we investigated the cardiovascular actions of the novel mixed D1/D2 dopamine receptor agonist Z1046 in awake swine at rest and during treadmill exercise.

2 Thirteen swine were chronically instrumented under sterile conditions for measurement of systemic, pulmonary, and coronary hemodynamics. Regional blood flows were determined with the radioactive microsphere technique.

Z1046 (1, 10, 100 μg/kg i.v.) produced dose-dependent reductions in central aortic blood pressure (up to 22±3%, $P \leq 0.05$) in awake resting swine which was accompanied by only minimal reflex activation of the sympathetic nervous system. The hypotensive response was principally the result of peripheral vasodilatation (systemic vascular resistance decreased up to 35±4%, $P \leq 0.05$), which was located in the cerebral, coronary, renal, mesenteric, adrenal, splenic and skeletal muscular vascular beds (vascular resistance decreased up to 30-40% after the highest dose in these beds). Only in the cerebral and mesenteric bed was the vasodilatation strong enough to overcome the decrease in blood pressure and result in an increased blood flow; the vasodilatation in the coronary bed was most likely due to metabolic regulation as neither coronary blood flow nor myocardial oxygen demand were changed significantly by Z1046. The systemic vasodilatation that was caused by the highest i.v. dose (100 μg/kg) was accompanied by transient and minor, reflex-mediated, increases in heart rate (15±5%, $P \leq 0.05$) and cardiac output (15±5%, $P \leq 0.05$) whereas after 10 μg/kg i.v. a slight decrease in cardiac output also contributed to the hypotension. Z1046 had no effect on pulmonary vascular resistance.

4 The systemic vasodilator response to Z1046 (100μg/kg i.v.) were sustained during treadmill exercise (1-4km/hr which produced heart rates of up to 233±10 beats/min), but with increasing treadmill speed attenuation of the exercise-induced increase in heart rate (-11±3%, $P \leq 0.05$) and hence cardiac output (-10±3%, $P \leq 0.05$), (as stroke volume was not altered by Z1046) contributed significantly to the lower aortic blood pressure (-20±3%, $P \leq 0.05$). Z1046 had no effect on pulmonary vascular resistance during exercise.

5 Oral administration (0.5, 1.5 mg/kg produced a fall in central aortic blood pressure (up to 15±3%, $P \leq 0.05$), which developed gradually during the first 90 min and lasted up to 4 hours after administration, again with negligible changes in heart rate and $LVdP/dt_{max}$.

6 Neither non-selective α- and β-adrenoceptor blockade, nor selective α-adrenoceptor blockade altered the vasodilator actions of Z1046, but non-selective α- and β-adrenoceptor blockade

abolished the cardiac responses to dopamine receptor stimulation, suggesting that its cardiac actions were principally caused by D_2-receptor mediated inhibition of catecholamine release, whereas the vasodilator response was likely the result of vascular D_1-receptor stimulation.

7 In conclusion, the novel dopamine receptor agonist Z1046 is an effective blood pressure lowering agent that elicits minimal reflex activation of sympathetic nervous system in awake resting swine. Systemic vasodilatation was not affected by combined α- and β- adrenoceptor blockade, which is consistent with a predominantly D_1 receptor dependent vasodilator mechanism. The hypotensive effect is maintained during treadmill exercise during which systemic vasodilatation and a lower cardiac output both contribute to the blood pressure lowering actions of Z1046. The cardiovascular profile of this orally active compound warrants further investigation of this class of drugs in experimental and clinical hypertension.

INTRODUCTION

In the early seventies it was shown that dopamine produced vasodilatation by a mechanism independent from beta adrenoceptors (1). Since then, two functionally separate dopamine (D-) receptor subtypes have been identified. D_1-receptors are located on arterial smooth muscle cells in the kidney and mesenteric vascular bed and in some species in coronary and cerebral vessels (2-5), where their stimulation causes vasorelaxation. D_1-receptors are also located on renal tubular cells and juxtaglomerular cells where they promote natriuresis and diuresis, respectively. D_2-receptors are situated on sympathetic nerve endings (inhibition of norepininephrine release), in the zona glomerulosa cells of the adrenal cortex (inhibition of angiotensine II stimulated aldosterone release) and in sympathetic ganglia (inhibition of ganglionic transmission). Experimental and clinical evidence is accumulating that specific dopamine agonists may be of use in the treatment of hypertension and heart failure (1, 5-7). In particular, combined D_1-/ D_2-receptor agonists could be of benefit in hypertension as they are likely to minimize baroreflex mediated activation of the sympathetic nervous system.

Z1046 is an experimental dopamine receptor agonist which compared to dopamine, exerts equipotent D_1-receptor and 30 times more potent D_2-receptor agonistic activity in rabbit splenic artery and isolated ear preparations, respectively (8). Z1046 does not exhibit significant β_1 and β_2-agonistic properties in the guinea pig atrium and trachea, respectively. However, the drug may have weak α_1-antagonistic properties (rabbit aorta), while its α_2-agonistic activity in the guinea pig atrium may be similar to that of dopamine (8). In the present study we investigated the systemic-, pulmonary- and coronary haemodynamic responses to i.v. and oral administration of Z1046 in awake resting swine. In subsets of animals we also studied the responses to Z1046 after selective α_2-adrenoceptor blockade or combined non-selective α- and β-adrenoceptor blockade in order to determine whether adrenoceptor stimulation directly or indirectly via pre-synaptic inhibition of catecholamine release, contributes to the haemodynamic effects of Z1046. Finally, in view of observations that D_2-receptor mediated inhibition of catecholamine release may constitute a major component of Z1046's actions (8, 9) we also studied its haemodynamic actions during sympathetic stimulation produced by graded treadmill exercise.

METHODS

Thirteen swine were used in the present study. All experiments were performed in accordance with the "Guiding Principles in the Care and Use of Laboratory Animals" as approved by the Council of the American Physiological Society and after approval of the Animal Care Committee of the

Erasmus University Rotterdam. Adaptation of animals to the laboratory conditions started approximately 1 week prior to the day of surgery and continued until 1 week post-surgically.

Surgical procedures

After an overnight fast, crossbred Landrace x Yorkshire pigs of either sex (20-28kg) were sedated with ketamine (30 mg/kg i.v.) (Aescoket, Aesculaap BV, Boxtel, The Netherlands), intubated and mechanically ventilated with a mixture of oxygen and nitrous oxide (1:2) to which 0.2-2% (v/v) isoflurance was added. Anaesthesia was maintained withmidazolam(1 mg/kg/h, i.v.) and fentanyl(1 μg/kg/h, i.v.). Under sterile conditions, the chest was opened via the fourth left intercostal space and an 8French (F) fluid-filled polyvinylchloride (PVC) catheter was inserted into the aortic arch for measurement of blood pressure and was secured with a purse string suture. After the pericardium was opened, an electromagnetic flow probe (Skalar, Delft, The Netherlands) was positioned around the ascending aorta for measurement of aortic blood flow (cardiac output). A high fidelity pressure transducer (Konigsberg Instruments Inc., Pasadena, CA, U.S.A.) was inserted into the left ventricle via the apical dimple for recording of left ventricular pressure and its first derivative (LVdP/dt; obtained via electrical differentiation). An 8F PVC catheter was also inserted into the left ventricle for calibration of the Konigsberg transducer signal. Similar catheters were introduced into the left atrium for measurement of pressure and injection of radioactive microspheres to determine regional blood flows (see below) and in the pulmonary artery for measurement of pulmonary artery pressure and administration of drugs. A Doppler flow probe (f_0=20 MHZ) was placed around the proximal part of the left anterior descending coronary artery to measure local coronary blood flow. Electrical wires and catheters were tunnelled subcutaneously to the back, the chest was closed and the animals were allowed to recover. All electrical wires and catheters were protected with a vest.

Post-surgical period

During the first week after surgery animals received amoxicillin, 25mg/kg i.v. (Clamoxil, Beecham Farma B.V., Amstelveen, The Netherlands) and gentamycin, 5 mg/kg i.v. (Alfasan, Woerden, The Netherlands) each day. Every day catheters were flushed with physiological saline containing 2,000 IU/ml heparin.

Regional myocardial blood flow measurements

For the determination of regional blood flows, a batch of 1-2 x 10^6 carbonized plastic microspheres [15 ±1 μm (SD) in diameter] labelled with either ^{46}Sc, ^{95}Nb, ^{103}Ru, ^{113}Sn or ^{141}Ce was injected into the left atrium. Starting 5 seconds before and continuing until 60 seconds after completion of the injection, a reference sample was withdrawn from the aorta at a rate of 10 ml/min. After all experimental protocols had been completed, animals were killed with an overdose of sodium pentobarbital and various organs (heart, adrenals, liver, spleen, stomach, small, intestine, brains

and kidneys) and tissues (abdominal skin, various skeletal muscle groups) were excised, weighed and put into vials. The heart was divided into atria, right and left ventricle and fixed on formaldehyde (3.7% v/v). Two days later approximately 10g of myocardium of the left ventricular free wall was divided into three layers of equal thickness: subepicardium, mesocardium and subendocardium. The radioactivity was counted and the amount of blood flow to the various tissue (Q_{tis}) calculated as:

$Q_{tis}=(I_{tis}/I_{art}) \times Q_{art}$

where I_{tis} and I_{art} are the radioactivity (c.p.m.) in a particular tissue and the arterial blood sample, respectively, while Q_{art} is the rate of withdrawal of the blood sample. Blood flows were expressed as ml/min per 100 gram of tissue. Full details of the procedures and the calculation of flow data using this technique have been reported earlier (5, 10).

Experimental protocols

Studies were performed 1-5 weeks after surgery with animals either resting unrestrained and quietly in a cage or with animals exercising on a motor driven treadmill. The average weight of the thirteen animals increased from 28.5 $\pm$ 1.4 kg to 35.4 $\pm$ 1.0 kg over this time period.

Haemodynamic responses to increasing doses of intravenous Z1046 under resting conditions.

Systemic, pulmonary and coronary haemodynamic responses to three doses of Z1046 were studied in resting swine. After baseline haemodynamic measurements had been obtained, animals received i.v. injections, administered over a period of 1 min, of either 10 ml saline (vehicle, n=7), 1 μg/kg (n=9), 10 μg/kg (n=8), or 100 μg/kg (n=9) of Z1046 dissolved in 10 ml saline. Following administration of saline or Z1046 the animals were studied during a 90 min period. Doses were administered in random order and each animal received only one dose daily. Consecutive experiments in the same animals were performed at a minimum interval of 48 hours. Because of the limited number of radioisotopes that are available, regional blood flow measurements were made before and 5 minutes after administration of Z1046 in a dose of 10 μg/kg and before and 5 and 30 min after administration of Z1046 in a dose of 100µg/kg.

Haemodynamic responses to intravenous Z1046 during treadmill exercise.

In nine swine we studied the effects of Z1046 (100 μg/kg i.v.) during graded treadmill exercise. With swine resting on the treadmill (both lying and standing) measurements were made of systemic, pulmonary and coronary haemodynamics. Then, animals underwent a 3-stage exercise protocol (2, 3 and 4 km/hr), with each level lasting 2-3 min in duration. Measurements were made during the last 30 seconds of each level of exercise when haemodynamics had reached a stable level. Following completion of the exercise protocol, animals were allowed to rest. Sixty minutes later, when all variables had returned to baseline, animals received either saline (10 ml i.v.) or

Z1046 (100 μg/kg, in 10 ml saline i.v.). Five min later resting measurements were obtained and the exercise protocol was repeated.

Haemodynamic responses to orally administered Z1046.

In seven swine we studied the haemodynamic responses to orally administered Z1046. After baseline measurements had been recorded swine received either placebo (empty gel caps) (n=6) or Z1046 in a dose of 0.5 mg/kg (n=7) or 1.5 mg/kg (n=7) p.o. and were studied over a four hour period. Protocols were performed in random order at a minimum interval of 48 hours.

Modulation of adrenergic activity by Z1046.

In three resting swine we studied the effects of Z1046 (10 and 100 µg/kg i.v.) in the presence of combined α-and β-adrenoceptor blockade, using 1.0 mg/kg of the non-selective α-adrenoceptor blocker phentolamine followed by 1.0 mg/kg/hr i.v. (11) and 0.5 mg/kg of the non-selective β-adrenoceptor blocker propranolol followed by 0.5 mg/kg/hr i.v. {Duncker, 1987 #2}. Ten minutes after administration of the blockers, when haemodynamics had reached a new steady state, Z1046 was administered in a dose of either 10 or (on a different day) 100 μg/kg i.v. and haemodynamic measurements were made during the following 90 minutes.

In seven resting swine we studied the effects of Z1046 (100 μg/kg i.v.) in the presence of the α_2-adrenoceptor antagonist yohimbine. Yohimbine was administered in a dose of 30 μg/kg i.v. followed by a continuous infusion of 3 μg/kg/min i.v. After ten minutes, when haemodynamics had reached a steady state, Z1046 was administered and haemodynamic variables were recorded during the following 90 minutes.

Haemodynamic responses to repeated administration of an identical dose of intravenous Z1046.

On a different day we studied the systemic, pulmonary and coronary haemodynamic responses to two consecutive doses of 10 μg/kg Z1046 i.v. in 7 resting swine. The two doses of Z1046 were administered in 10 ml saline over 1 min separated by a 90-min interval.

Data acquisition and analysis

Data were recorded and digitized using an eight channel data-acquisition program ATCODAS (Dataq Instruments, Inc., Akron Ohio, USA)) and stored on a computer for later post-acquisition analysis with a program written in MatLab (The Mathworks Inc., Mass, USA). Systemic vascular resistance was calculated as the ratio of mean aortic pressure and cardiac output, pulmonary vascular resistance as the ratio of (mean pulmonary artery pressure - mean left atrial pressure) and cardiac output and regional vascular resistances as the ratio of mean aortic pressure and local blood flows.

Statistical analysis was performed using analysis of variance for repeated measures. When a significant effect was observed post-hoc testing was done using Wilcoxon signed rank test or

paired t-test. A *P*-value of less than or equal to 0.05 was considered statistically significant (two-tailed). All data are presented as mean ±s.e. mean.

Drugs

Z1046 ((S)-6-[[6-[[2-(2-Methoxyphenoxy)ethyl]amino]hexyl]propylamino]-5,6,7,8-tetrahydro-1,2-naphtalenediol dihydrochloride (courtesy of Dr F. Marchini, Zambon Group S.p.A., Italy), yohimbine and propranolol were dissolved in warm saline (30^0 C) to produce concentrations of 30 μg/kg/10 ml and 0.5 mg/kg/10 ml, respectively. Phentolamine was dissolved in water containing glucose (35mg/ml) and further diluted in saline to produce a final concentration of 1.0 mg/kg/10 ml. Fresh drug solutions were prepared on the day of each experiment.

RESULTS

Haemodynamic responses to increasing doses of intravenous Z1046 in awake resting swine

Saline

Administration of saline did not lead to significant changes in any of the systemic, pulmonary or coronary haemodynamic variables, demonstrating good cardiovascular stability over the 90 min observation period (Figure 1, Tables 1 and 2).

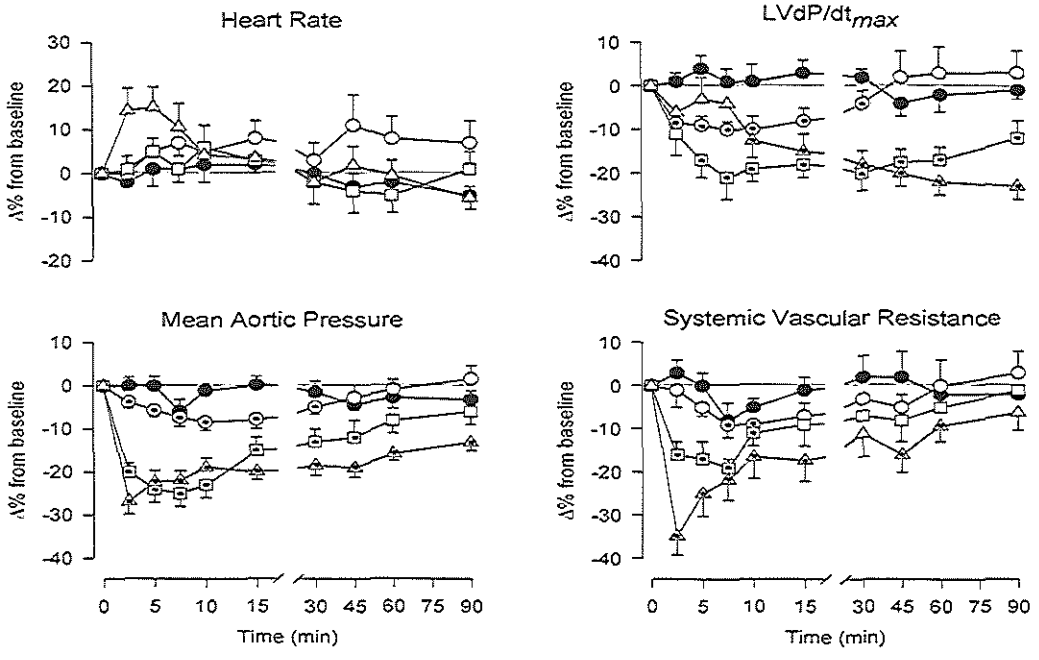

Figure 1. Systemic haemodynamic effects of Z1046 i.v. in awake resting swine. The four treatment groups are saline (control n=7, solid circle), and three doses of Z1046: 1μg/kg (n=9, open circles), 10μg/kg (n=9, open squares), and 100μg/kg (n=10, open triangles). Data are presented as mean±s.e. mean and reflect percent changes from pre-drug baseline. Solid dot inside a symbol indicates a significant change ($P \leq 0.05$) from baseline (0 min).

Table 1 Systemic and pulmonary haemodynamics after intravenous administration of Z1046 in awake resting pigs

	Z1046 ($\mu g\ kg^{-1}$)	*Time after administration (min)*									
		Baseline	*2.5*	*5*	*7.5*	*10*	*15*	*30*	*45*	*60*	*90*
CO	0	4.2±0.3	4.1±0.3	4.2±0.3	4.3±0.3	4.4±0.3	4.2±0.3	4.1±0.3	4.0±0.3	4.2±0.3	4.2±0.3
($l\ min^{-1}$)	1	3.7±0.2	3.6±0.3	3.6±0.2	3.7±0.3	3.7±0.2	3.6±0.2	3.6±0.2	3.7±0.3	3.7±0.3	3.7±0.2
	10	4.0±0.2	3.9±0.2	3.7±0.2	3.6±0.2*	3.5±0.2*	3.7±0.2	3.8±0.2	3.9±0.2	3.9±0.2	3.9±0.1
	100	4.2±0.3	4.8±0.3*	4.4±0.3*	4.2±0.2	4.2±0.3	4.2±0.2	3.9±0.2	4.1±0.2	3.9±0.1	3.9±0.1
SAP	0	122±4	122±5	122±6	115±4	120±5	122±5	119±5	117±3	117±4	117±3
(mmHg)	1	131±8	125±8*	122±8*	122±9*	120±9*	122±8*	125±9	129±10	130±10	133±9
	10	124±4	106±4*	101±4*	101±4*	103±3*	110±3*	111±4*	111±5*	115±4*	117±5
	100	127±4	106±4*	111±5*	110±5*	109±5*	107±4*	106±4*	103±5*	107±4*	108±4*
MAP	0	100±3	100±5	100±5	94±4	99±5	100±4	99±4	96±3	97±3	97±3
(mmHg)	1	106±6	102±6*	100±6*	99±7*	97±6*	98±6*	101±6*	104±7	106±7	108±6
	10	101±3	81±3*	77±4*	75±3*	78±2*	86±3*	88±3*	89±3*	93±3	95±4
	100	104±3	76±3*	81±3*	81±3*	84±3*	83±3*	85±3*	84±4*	88±3*	90±3*
DAP	0	77±6	76±7	78±6	70±4	75±6	78±5	77±5	72±5	74±4	74±4
(mmHg)	1	81±5	78±5	77±5*	75±6*	73±6*	74±6*	78±5	79±6	82±6	83±6
	10	78±4	59±3*	56±4*	55±3*	60±2*	65±3*	67±3	67±3	70±4	73±5
	100	80±4	53±4*	58±4*	58±3*	62±3*	62±3*	64±4*	65±4*	68±3*	71±3*
SVR	0	24.5±1.5	25.1±1.3	24.7±1.8	22.6±1.5	23.4±1.6	24.1±1.3	25.3±3.2	25.0±1.9	24.1±2.1	24.0±2.1
($mmHg\ min^{-1}$)	1	28.5±2.2	28.4±3.0	27.2±2.5	25.7±2.0*	25.8±2.1*	26.4±2.0*	27.7±2.4	27.1±2.5	28.4±2.9	29.2±2.5
	10	25.4±1.4	21.3±1.6*	21.1±1.9*	20.5±1.0*	23.0±1.5*	23.8±2.5	23.6±2.2	23.3±2.0	24.1±1.8	25.2±1.7
	100	25.4±1.7	16.3±1.4*	18.8±1.5*	19.4±0.9*	21.0±1.8*	20.5±1.0*	22.1±1.3	20.9±0.9*	22.6±1.0*	23.5±1.4
MPAP	0	17±2	17±5	19±1	17±1	18±2	19±2	19±2	17±2	17±1	16±1
(mmHg)	1	18±2	18±1	18±1	18±2	18±2	18±1	19±2	20±3	18±2	20±2
	10	20±2	20±2	18±1	17±1	17±2	18±2	16±1	15±1	16±1	18±1
	100	17±1	22±1*	21±2	19±2	17±1	17±1	16±2	17±2	17±2	17±1
LAP	0	4±1	4±1	5±2	4±2	5±2	4±2	6±2	4±2	5±2	3±2
(mmHg)	1	6±1	7±1	6±1	6±1	6±1	6±1	6±1	8±2	7±2	8±1
	10	5±1	4±1	3±1	3±1	5±1	5±2	4±1	4±1	4±1	5±1
	100	5±1	5±2	4±1	5±1	5±2	3±1	4±1	3±1	5±2	4±1
PVR	0	3.4±0.5	3.2±0.5	3.2±0.4	3.2±0.5	3.1±0.5	3.7±0.6	3.6±0.6	3.5±0.4	3.1±0.3	3.1±0.3
($mmHg\ min^{-1}$)	1	3.2±0.5	3.3±0.6	3.6±0.7	3.6±0.6	3.5±0.6	3.4±0.5	3.6±0.5	3.4±0.5	3.4±0.4	3.5±0.5
	10	3.5±0.5	4.1±0.7	4.0±0.7	3.7±0.6	3.2±0.9	4.0±0.5	4.0±0.9	3.8±0.9	3.6±0.8	4.1±0.4
	100	2.8±0.2	3.4±0.1	3.1±0.2	2.9±0.2	3.0±0.1	3.1±0.1	3.0±0.4	3.2±0.4	3.1±0.4	3.4±0.5

CO = cardiac output; SAP, MAP, DAP = systolic, mean and diastolic aortic blood pressure, respectively; SVR = systemic vascular resistance; MPAP = mean pulmonary artery pressure; MLAP = mean left atrial pressure PVR = pulmonary vascular resistance. Data are mean ± s.e.mean; control group contained 7 animals; the 1, 10 and 100 $\mu g\ kg^{-1}$ groups contained 9, 9 and 10 pigs, respectively; $^*P \leq 0.05$ vs baseline.

Table 2 Left ventricular and coronary haemodynamics after intravenous administration of Z1046 in awake resting pigs

	Z1046 (μg kg^{-1})	Time after administration (min)									
		Baseline	*2.5*	*5*	*7.5*	*10*	*15*	*30*	*45*	*60*	*90*
HR	0	124±3	121±4	125±5	124±5	127±7	126±3	123±4	119±6	121±5	118±3
(beats min^{-1})	1	115±7	116±8	122±8	124±9	122±9	124±8	118±8	126±8	124±8	123±8
	10	128±6	129±6	134±7	129±8	135±6	131±6	125±6	122±5	121±6	129±6
	100	122±4	139±7*	141±7*	135±8	127±7	127±6	120±7	124±6	121±4	115±4
LVSP	0	123±6	124±7	125±8	120±6	122±7	123±6	123±5	118±5	121±4	121±5
(mmHg)	1	130±11	124±10*	122±10*	121±11*	120±10*	123±10*	124±11*	124±12*	128±11	130±10
	10	122±3	112±4*	109±7*	102±4*	101±6*	112±5*	111±5*	115±6	117±4	118±6
	100	126±4	109±3*	115±5*	109±4*	109±3*	108±4*	108±4*	109±5*	108±4*	110±4*
RPP	0	14200±800	14200±1000	14900±1000	14200±900	14500±1200	14400±700	14200±800	13200±1100	13500±700	13300±600
(mmHg beats min^{-1})	1	14100±400	13400±600	14100±600	14100±600	13700±900	14000±700	13400±600	13600±400	14300±900	14400±800
	10	14800±900	13600±800	13900±900	12600±1300*	13300±1300	14100±1000	13100±700*	13300±500*	13200±1000	14400±900
	100	14900±600	14600±800	15700±1200	14400±1000	13600±900	13300±700*	12600±800*	13500±700*	12900±400*	12500±500*
LVEDP	0	8±1	10±2	9±2	7±2	8±2	9±2	10±2	7±1	8±2	6±2
(mmHg)	1	8±2	8±2	7±2	6±2	7±2	7±2	6±3	7±2	8±2	11±2
	10	8±2	6±1	5±2	5±2	5±2	6±2	5±2	5±2	6±2	7±2
	100	8±1	8±2	8±2	5±2	6±2	6±2	7±1	7±2	6±2	7±1
LVdP/dt$_{max}$	0	3360±180	3400±210	3490±210	3420±230	3410±220	3480±250	3430±190	3230±200	3290±190	3320±170
(mmHg s^{-1})	1	3120±240	2850±180*	2810±190*	2800±180*	2780±170*	2850±200*	3010±260*	3140±190	3200±270	3160±170
	10	3030±150	2720±190	2490±140*	2380±150*	2450±160*	2490±170*	2410±150*	2480±140*	2500±120*	2660±120*
	100	3320±180	3030±170	3170±210	3140±200	2880±160*	2790±160*	2720±160*	2710±190*	2590±160*	2560±140*
LVdP/dt$_{min}$	0	−2730±190	−2700±200	−2700±230	−2520±160	−2670±220	−2660±210	−2570±220	−2510±210	−2550±210	−2650±220
(mmHg s^{-1})	1	−2950±270	−2800±230*	−2690±200*	−2640±220*	−2590±220*	−2710±260*	−2810±250	−2820±180	−2900±220	−3030±360
	10	−2620±180	−2230±160*	−2010±190*	−1960±170*	−2060±160*	−2170±170*	−2220±180*	−2300±180*	−2330±170*	−2400±180
	100	−2590±150	−2030±140*	−2220±150*	−2120±170*	−2090±140*	−2130±150*	−2130±160*	−2120±170*	−2070±150*	−2190±150*
SV	0	33.9±2.4	33.7±2.1	33.6±2.3	34.5±2.4	34.8±2.8	33.7±2.3	33.7±3.0	33.4±2.5	35.1±3.2	35.5±2.6
(ml)	1	30.8±2.7	30.1±2.7	28.9±2.8	29.2±3.1	28.8±2.7	27.7±2.1	30.6±2.6	29.1±2.4	28.3±2.0	29.0±2.2
	10	32.0±1.9	30.6±2.0	27.5±1.8*	27.5±1.2*	25.5±1.0*	28.3±1.2*	29.2±1.2	31.2±1.4	31.4±1.2	30.2±1.8
	100	35.1±2.2	34.1±1.7	31.8±2.0*	31.9±2.0*	32.5±1.7	33.0±1.5	33.0±1.6	33.2±1.9	32.2±1.5	34.1±1.5
CBF	0	33±4	34±4	35±4	33±4	34±5	34±4	33±4	33±4	32±4	33±4
(ml min^{-1})	1	26±2	26±2	26±2	26±2	25±2	26±2	26±3	27±3	27±3	28±3
	10	28±3	30±4	27±3	28±4	27±3	28±3	28±3	28±4	28±3	30±3
	100	30±3	34±4	33±4	31±3	29±3	28±3	29±3	29±3	27±4	28±3
CVR	0	3.0±0.3	3.1±0.3	3.0±0.4	3.0±0.3	3.0±0.3	3.1±0.4	3.1±0.3	3.1±0.4	3.2±0.4	3.0±0.3
(mmHg min ml^{-1})	1	4.1±0.3	4.0±0.3	4.0±0.3	3.9±0.3	3.9±0.3	3.8±0.3	4.0±0.4	4.0±0.4	4.0±0.4	4.0±0.4
	10	3.9±0.5	3.0±0.4*	3.1±0.5*	3.0±0.4*	3.1±0.4*	3.3±0.4	3.5±0.5	3.7±0.6	3.8±0.5	3.4±0.4
	100	3.7±0.5	2.5±0.5*	2.7±0.5*	2.6±0.3*	3.2±0.6*	3.1±0.5*	3.1±0.4*	3.1±0.4*	3.5±0.6	3.4±0.5

HR = heart rate; LVSP = left ventricular peak systolic pressure; RPP = double product(HRxLVSP); LVEDP = LV end-diastolic pressure; LVdP/dt$_{max}$ = maximum rate of rise of LV pressure; LVdP/dt$_{min}$ = maximum rate of fall of LV pressure; SV = stroke volume; CBF = left anterior descending coronary artery blood flow; CVR = coronary vascular resistance. Data are mean ± s.e.mean; control group contained 7 animals; the 1, 10 and 100 μ kg^{-1} groups contained 9, 9 and 10 swine, respectively; *$P \leq 0.05$ vs baseline.

Z1046

Systemic and pulmonary haemodynamics.

Z1046 in doses of 1, 10 and 100 μg/kg produced dose-dependent decreases in mean aortic blood pressure which reached their peak values of 9±3%, 21±6 % and 22±3 %, respectively, within the first 10 min after administration (Figure 1; Table 1). Thereafter, aortic pressure gradually recovered over the remainder of the 90 min period; the recovery was complete after 1 μg/kg and 10 μg/kg but not after 100 μg/kg. Similar decreases were observed in systolic and diastolic aortic pressure, so that arterial pulse pressure did not change. The Z1046-induced fall in blood pressure was caused by systemic vasodilatation as cardiac output (and its two determinants heart rate and stroke volume) was virtually unchanged. Only after administration of 10 μg/kg did cardiac output and stroke volume decrease slightly, whereas after 100 μg/kg cardiac output and heart rate transiently increased which was likely due to reflex-mediated sympathetic activation (see below). Mean pulmonary artery and left atrial pressures were not affected except for a transient increase in pulmonary pressure during the first 2.5 minutes following administration of 100μg/kg, which parallelled the increase in cardiac output, consequently pulmonary vascular resistance was not significantly altered by any dose of Z1046 (Tables 1 and 2). $LVdP/dt_{max}$ and $LVdP/dt_{min}$ decreased dose-dependently by up to 23±3 % and 21±3%, respectively, after 100 μg/kg (both $P \leq 0.05$) which may in part have been related to the decreases in diastolic aortic pressure and left ventricular systolic pressure, respectively.

Coronary and myocardial haemodynamics.

Coronary artery blood flow measured with the Doppler flow probe was not affected by any of the doses of Z1046, although coronary flow tended to increase during the first 7.5 min after administration of the highest dose, and tended to decrease thereafter, in parallel with the changes in myocardial oxygen demand, reflected by heart rate or the "double product" (heart rate x left ventricular systolic blood pressure)(Table 2). Consequently, the decreases in computed coronary vascular resistance that occurred with the highest two doses were the result of autoregulation to compensate for the decrease in mean aortic blood pressure. Z1046 had no effect on the distribution of myocardial blood flow across the left ventricular wall, measured with radioactive microspheres, 5 min after administration of 10 μg/kg or 5 and 30 min after administration of 100 μg/kg (Table 3).

Distribution of regional blood flows and vascular resistances).

Z1046 in doses of 10 and 100 μg/kg i.v. produced vasodilatation in the brain (up to 35±4% decrease in vascular resistance 5 min after administration of 100 μg/kg), small intestine (40±7%), kidneys (30±6%), adrenals (31±6%), spleen (33±7%) and skeletal muscle (43±14%)(Table 4). Only in the brain and small intestine was the vasodilatation sufficiently large to overcome the

decrease in aortic blood pressure so that blood flow to these organs increased. Neither dose of Z1046 produced significant changes in vascular resistance in the stomach, liver or skin.

Haemodynamic responses to intravenous Z1046 during treadmill exercise

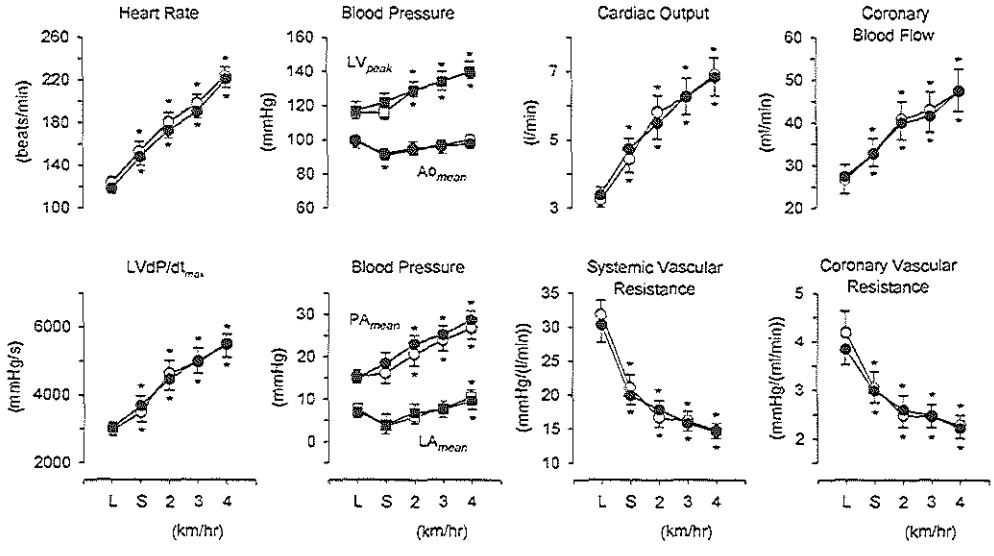

Figure 2. Systemic haemodynamic variables during two consecutive periods of graded treadmill exercise in 9 swine. LV_{peak} = Left ventricular peak systolic pressure, Ao_{mean} = mean aortic pressure, PA_{mean} = mean pulmonary artery pressure, LA_{mean} = mean left atrial pressure. L = lying, S = standing. Animals were studied during control conditions (open circles) and following administration of saline (10 ml, solid circles). Data are presented as mean±s.e. mean, *$P \leq 0.05$ versus Lying down, •$P \leq 0.05$ Saline vs Control.

Saline. Exercise produced increases in heart rate from 124±5 beats/min at rest to 224±8 beats/min at the highest level of exercise (4 km/hr), left ventricular systolic pressure from 116±4 to 140±4 mmHg, $LVdP/dt_{max}$ from 2960±170 to 5510±280 mmHg/s and cardiac output from 3.2±0.2 to 6.9±0.5 l/min (Figure 2). While mean aortic pressure increased only slightly from 91±3 (quietly standing) to 100±3 mmHg at the highest level of exercise, mean pulmonary artery pressure almost doubled from 15±1 to 27±3 mmHg. These responses reflect the intense systemic vasodilatation versus the unaltered pulmonary vascular resistance. Coronary blood flow increased from 27±3 ml/min at rest to 48±5 ml/min during the highest level of exercise. Two consecutive exercise periods separated by 60 min of rest, resulted in highly reproducible systemic, pulmonary and coronary haemodynamic responses.

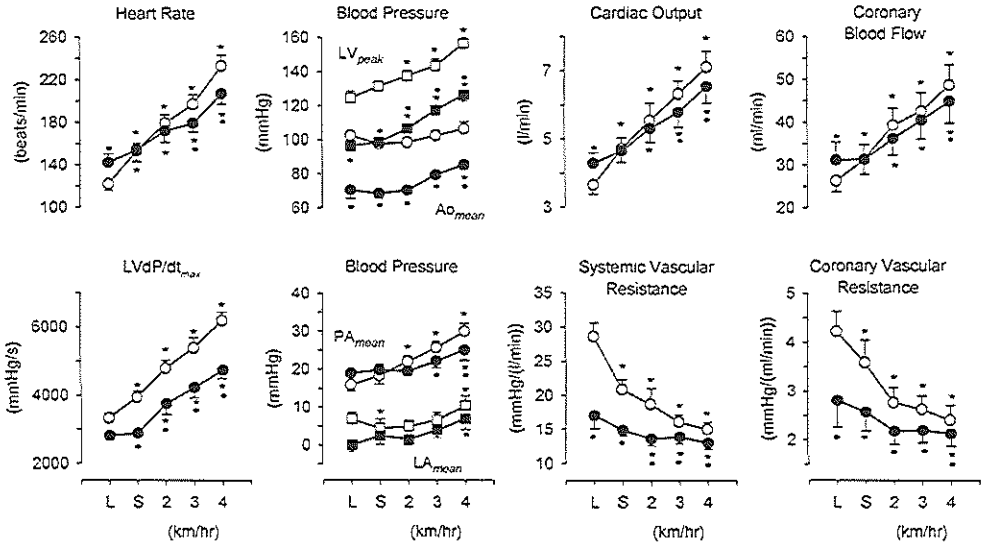

Figure 3. Systemic haemodynamic effects of Z1046 i.v. in 9 swine during graded treadmill exercise. LV_{peak} = Left ventricular peak systolic pressure, Ao_{mean} = mean aortic pressure, PA_{mean} = mean pulmonary artery pressure, LA_{mean} = mean left atrial pressure, L = lying down, S = standing. Animals were studied during control conditions (open circles) and in the presence of Z1046 (100μg/kg, solid circles). Data are presented as mean±s.e. mean. *P<0.05 versus Lying down, •P<0.05 Z1046 vs Control

Table 3 Myocardial blood flow and vascular resistance responses to intravenous administration of Z1046 in awake resting pigs

	Z1046 (μg kg^{-1})	*Baseline*	*5 min*	*30 min*
Blood flows (ml min^{-1} 100 g^{-1})				
Right atrium	10	30±3	38±5	–
	100	33±5	46±10	35±8
Left atrium	10	39±6	68±25	–
	100	47±7	81±27	60±15
Right ventricle	10	88±11	114±18	–
	100	108±14	125±25	104±16
LVmean	10	141±20	161±21	–
	100	161±18	173±31	152±25
LVepi	10	116±18	142±20	–
	100	137±17	150±27	132±23
LVmeso	10	155±22	174±22	–
	100	178±21	190±36	167±29
LVendo	10	167±21	178±23	–
	100	183±21	191±34	174±26
endo/epi	10	1.49±0.09	1.28±0.06	–
	100	1.35±0.08	1.28±0.10	1.35±0.05
Vascular resistances (mmHg min ml^{-1} 100g)				
Right atrium	10	3.48±0.39	2.62±0.54*	–
	100	3.48±0.42	2.15±0.39*	3.12±0.62
Left atrium	10	3.39±0.94	1.85±0.34*	–
	100	2.32±0.29	1.31±0.24*	1.83±0.43
Right ventricle	10	1.22±0.12	0.87±0.15*	–
	100	1.01±0.10	0.71±0.09*	0.90±0.12
LVmean	10	0.81±0.13	0.58±0.08*	–
	100	0.65±0.05	0.50±0.06*	0.61±0.07
LVepi	10	1.00±0.17	0.67±0.10*	–
	100	0.78±0.07	0.57±0.06*	0.71±0.09
LVmeso	10	0.74±0.13	0.53±0.07*	–
	100	0.59±0.04	0.46±0.06*	0.56±0.07
LVendo	10	0.67±0.11	0.52±0.07	–
	100	0.58±0.05	0.46±0.06*	0.53±0.05

LV = left ventricle; mean = mean transmural, epi = subepicardium, meso = midmyocardium, endo = subendocardium. Data are mean±s.e.mean; n = 8; *$P \leq 0.05$ vs baseline.

Table 4 Regional blood flow and vascular resistance response to intravenous administration of Z1046 in awake resting pigs

	Z1046 ($\mu g\ kg^{-1}$)	*Baseline*	*5 min*	*30 min*
Blood flows (ml min^{-1} 100 g^{-1})				
Brain	10	81±4	92±10	–
	100	87±5	98±5*	82±4
Kidneys	10	321±30	368±31	–
	100	339±26	381±26	350±27
Adrenals	10	114±8	134±11	–
	100	129±12	143±17	158±23
Small intestine	10	116±18	136±23	–
	100	120±18	147±27*	95±13
Stomach	10	76±14	85±17	–
	100	85±18	67±11	56±6
Liver	10	23±6	19±10	–
	100	20±7	13±5	21±10
Spleen	10	155±25	162±25	–
	100	160±22	202±26	171±19
Skeletal muscle	10	6.7±1.3	9.8±2.3	–
	100	9.7±2.5	12.1±1.9	6.7±1.5
Skin	10	7.2±1.4	7.1±0.8	–
	100	7.2±0.9	8.6±1.5	7.5±1.1
Vascular resistances (mmHg min ml^{-1} 100 g)				
Brain	10	1.29±0.09	0.94±0.09*	–
	100	1.24±0.08	0.81±0.06*	1.03±0.07
Kidneys	10	0.35±0.04	0.23±0.03*	–
	100	0.32±0.03	0.21±0.02*	0.25±0.03*
Adrenals	10	0.93±0.07	0.63±0.06*	–
	100	0.86±0.08	0.61±0.10*	0.64±0.14*
Small intestine	10	1.06±0.16	0.76±0.15	–
	100	1.07±0.17	0.67±0.12*	1.01±0.16
Stomach	10	1.84±0.40	1.27±0.27	–
	100	1.68±0.30	1.43±0.28	1.67±0.26
Liver	10	9.2±3.2	18.3±5.3	–
	100	10.8±2.7	10.5±2.2	11.5±2.8
Spleen	10	0.83±0.16	0.62±0.13*	–
	100	0.82±0.17	0.46±0.10*	0.53±0.07
Skeletal muscle	10	18.6±3.4	11.6±2.4*	–
	100	15.5±3.8	7.2±1.1*	15.0±2.5
Skin	10	18.5±3.3	12.5±1.6	–
	100	15.9±1.4	19.1±10.4	12.5±1.5

Data are mean±s.e.mean; $n=9$; *$P \leq 0.05$ vs baseline.

Z1046.

Z1046 in a dose of 100 μg/kg i.v. produced markedly different haemodynamic responses during treadmill exercise than during resting conditions (Figure 3). At rest the fall in aortic pressure was the result of systemic vasodilatation (35% decrease in systemic vascular resistance) despite a reflex-mediated increase in heart rate and cardiac output (both 15%), whereas during exercise both vascular resistance (15%) and cardiac output (10%) decreased. The decrease in cardiac output produced by Z1046 during exercise resulted from a lower heart rate as stroke volume was maintained. The latter occurred despite an elevated preload (LV end-diastolic pressure) and a reduction in afterload (LV systolic pressure) suggesting a lower contractile state in the presence of Z1046, which could be explained by D_2-receptor mediated inhibition of catecholamine release from sympathetic nerve endings. This is also suggested by the lower $LVdP/dt_{max}$ data although underestimation of contractility may have occurred because of a lower aortic blood pressure. The effects of Z1046 on coronary blood flow during exercise paralleled its effects on heart rate and the double product, but only at the highest level of exercise was coronary blood flow lower than during

control exercise. In view of the fall in aortic pressure after Z1046 the coronary vascular resistance was lower at rest and during exercise compared to control conditions.

Haemodynamic responses to oral Z1046

Placebo.

Administration of placebo did not result in significant changes in heart rate, mean aortic pressure (Figure 4), or any of the other systemic haemodynamic variables (not shown) over the 4 hour observation period.

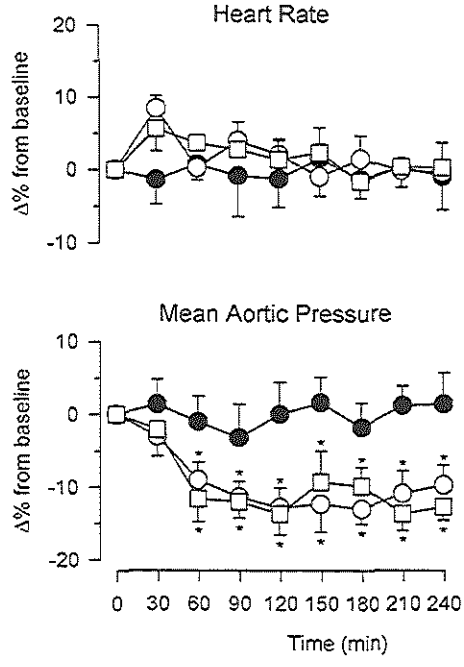

Figure 4. Systemic haemodynamic effects of Z1046 p.o. in awake resting swine. The three treatment groups are placebo (control n=6, solid circle), and two doses of Z1046: 0.5 mg/kg (n=7, open circles) and 1.5mg/kg (n=7, open squares). Data (mean±s.e. mean) are presented as percentage changes from pre-drug baseline. *$P \leq 0.05$ vs baseline (0 min).

Z1046.

Oral administration of Z1046 in doses of 0.5 mg/kg and 1.5 mg/kg resulted in similar decreases in mean aortic blood pressure, which developed gradually over the first 90 min, reached a peak at 120 min (up to 15 ±2% and 15±3% after 0.5 and 1.5 mg/kg, respectively) and remained virtually unchanged during the remainder of the four hour observation period (Figure 4). The decrease in mean aortic pressure was produced by a decrease in systemic vascular resistance (up to 11±3% and 14±4% after 0.5 and 1.5 mg/kg, respectively) whereas the slight decrease in cardiac output was not significant (not shown). $LVdP/dt_{max}$ decreased by up to 15% with either dose, while left ventricular end-diastolic pressure did not change (not shown).

Modulation of adrenergic activity by Z1046

Non-selective α- and ß-adrenoceptor blockade.

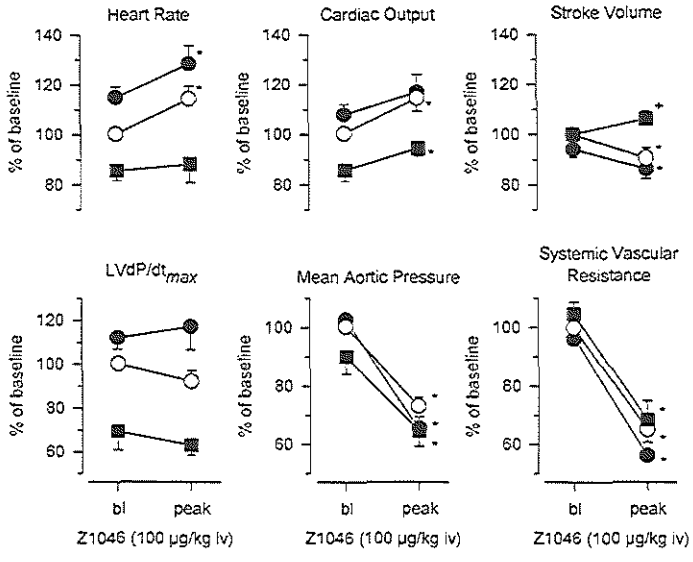

Figure 5. Systemic haemodynamic peak effects of Z1046 i.v. in awake resting swine. Z1046 was administered in a dose of 100 μg/kg under control conditions (n=10, open circles), in the presence of selective α_2-adrenoceptor blockade (n=7, solid circles), and in the presence of combined α- and β-adrenoceptor blockade (n=3, solid squares). Data are presented as mean±s.e. mean and as percent of predrug (i.e. pre-adrenoceptor blockade) baseline. *$P \leq 0.05$ vs baseline, +$p \leq 0.05$ vs change in the non-blocked animals.

Non-selective α- and β-adrenoceptor blockade in three swine resulted in decreases in heart rate and $LVdP/dt_{max}$ by approximately 20% and 35%, respectively, and a small increase in left ventricular end-diastolic pressure; mean aortic pressure fell by 15% due to the 18% decrease in cardiac output (Figure 5). Coronary blood flow decreased parallel to the decrease in double product. Z1046 in a dose of 100 μg/kg i.v. produced similar decreases in aortic pressure and systemic vascular resistance compared to animals with unblocked adrenoceptors, but with no change in heart rate. Thus, combined non-selective α- and β-adrenoceptor blockade did not modify the vasodilator activity of Z1046, but prevented the mild reflex-tachycardia produced by the high dose of Z1046.

When Z1046 in a dose of 10 μg/kg i.v. was administered in the presence of combined adrenoceptor blockade it produced considerably smaller (17 mmHg) decreases in aortic pressure compared to animals with unblocked adrenoceptors (26 mmHg, Figure 6). The decrease in pressure was produced solely by a decrease in systemic vascular resistance whereas the decreases in cardiac output and $LVdP/dt_{max}$, that was observed when adrenoceptors were unblocked, were absent in the presence of non-selective α- and β-adrenoceptor blockade. These findings suggest that the cardiac effects of Z1046 but not the systemic vasodilator response were the result of inhibition of sympathetic activity.

Selective α_2-adrenoceptor blockade.

Pretreatment with the selective α_2-adrenoceptor antagonist yohimbine (30 μg/kg and 3 μg/kg/min, i.v.) resulted in slightly higher levels of heart rate, $LVdP/dt_{max}$ and the double product (Figure 5), which was likely the result of increased norepinephrine release produced by presynaptic α_2-adrenoceptor blockade. However, yohimbine did not alter the haemodynamic response to Z1046

(100 μg/kg i.v.), indicating that Z1046 did not inhibit catecholamine release via presynaptic α_2-adrenoceptor stimulation and that Z1046 was devoid of postsynaptic α_2-adrenoceptor-mediated vasoconstrictor actions in awake swine.

Haemodynamic responses to repeated administration of an identical dose of intravenous Z1046

Ninety minutes following administration of Z1046 (10 μg/kg i.v.), at a time when all haemodynamic variables had returned to baseline except for $LVdP/dt_{max}$ which was still slightly (14±4%, $P \leq 0.05$) below baseline levels, a second dose of 10 μg/kg resulted in a markedly smaller decrease of aortic pressure (9±2 mmHg versus 23±6 mmHg, $P \leq 0.05$). This attenuated hypotensive effect was not due to mitigation of the systemic vasodilator response but was the result of a maintained stroke volume and hence cardiac output following the second administration of 10 μg/kg (Figure 6).

Although the baseline conditions were different, the responses to Z1046 in the presence of α- and β-adrenergic blockade resembled the second 10 μg/kg administration of Z1046 (Figure 6). This could suggest that the altered response to the second repeated administration of Z1046 (10 μg/kg) may actually have been the result of persisting inhibition of catecholamine release.

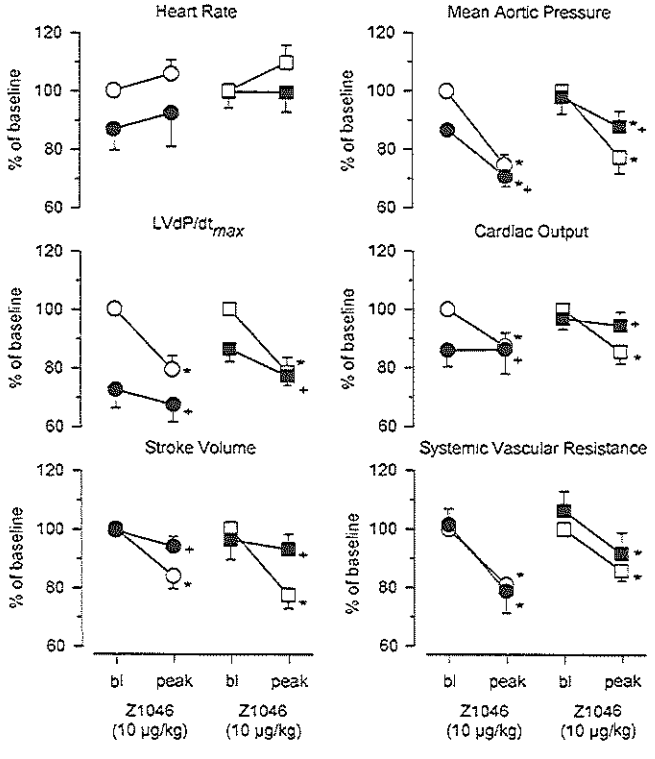

Figure 6. Systemic haemodynamic peak effects of Z1046 i.v. in awake resting swine. Z1046 was administered in a dose of 10 μg/kg under control conditions (n=9, open circles) and in the presence of combined α- and β-adrenoceptor blockade (n=3, solid circles) or during two consecutive administrations of 10 μg/kg under control conditions (n=7). The second dose (solid squares) was administered 90 min after the first dose (open squares). Data (mean±s.e. mean) are presented as percentage of predrug baseline (i.e. pre-adrenoceptor blockade or pre-first dose). *$P \leq 0.05$ vs baseline, +$P \leq 0.05$ vs change in the non-blocked animals or vs change produced by first dose.

DISCUSSION

The most important findings of the present study were that (*i*) i.v. or oral administration of the non-selective dopamine receptor agonist Z1046 produced dose-dependent reductions in central aortic blood pressure in awake resting swine which was accompanied by only minimal reflex activation of the sympathetic nervous system; (*ii*) the hypotensive response was principally the result of peripheral vasodilatation, in particular of the cerebral, coronary, renal, mesenteric, adrenal, splenic and skeletal muscular vascular beds; (*iii*) the vasodilatation that was caused by the highest i.v. dose (100 μg/kg) was accompanied by transient and minor, reflex-mediated, increases in heart rate and cardiac output, whereas after an i.v. dose of 10 μg/kg a slight decrease in cardiac output also contributed to the hypotension; (*i.v.*) the systemic vasodilator response to dopamine receptor stimulation was sustained during treadmill exercise, but with increasing intensity of exercise attenuation of the increase in cardiac output contributed significantly to the fall in pressure (*v*) Non-selective α- and β-adrenoceptor blockade did not alter the vasodilator actions of Z1046, but abolished the cardiac responses to dopamine receptor stimulation, suggesting that its cardiac actions were principally caused by D_2-receptor mediated inhibition of catecholamine release, whereas the vasodilator response was likely the result of vascular D_1-receptor stimulation.

Haemodynamic effects of D-receptor stimulation in awake resting swine.

Z1046 in doses of 1, 10 and 100 μg/kg i.v. produced dose-dependent decreases in systemic vascular resistance of 10%, 20% and 35%, respectively. The response of cardiac output to Z1046 differed with each dose. Thus, while 1 μg/kg had no effect, 10 μg/kg produced a decrease and 100 μg/kg produced a transient increase in cardiac output. The decrease in cardiac output produced by 10 μg/kg was due to a decrease in stroke volume with no change in heart rate. A decrease in stroke volume in the face of a decrease in left ventricular systolic pressure indicates a negative inotropic effect, which is supported by the moderate decrease in $LVdP/dt_{max}$. Interpretation of changes in $LVdP/dt_{max}$ as changes in contractility in the presence of changes in aortic blood pressure is complicated by the dependency of $LVdP/dt_{max}$ on diastolic aortic pressure. Nonetheless, $LVdP/dt_{max}$ was still depressed 90 min after administration of 10 μg/kg at a time when diastolic aortic pressure had returned to baseline levels. Furthermore, when 10 μg/kg was administered after adrenoceptor blockade, no decrease in $LVdP/dt_{max}$ occurred despite a significant decrease in aortic pressure. In awake resting swine, non-selective β-adrenoceptor blockade produced by propranolol results in decreases in $LVdP/dt_{max}$ of 25%, indicating that even under quiet resting conditions there is some degree of sympathetic activity. The observation that Z1046 decreased contractility in the present study is therefore consistent with inhibition of norepinephrine release from the sympathetic nerve endings produced by presynaptic D_2-like receptor stimulation. Z1046 in a dose of 100 µg/kg

produced a dramatic decrease in mean aortic pressure which was entirely due to a decrease in systemic vascular resistance. The minimal effect of the highest dose on cardiac output was somewhat surprising. If 10 μg/kg produced a decrease in cardiac output via D_2-like receptor mediated inhibition of sympathetic activity to the heart (which is suggested by the mild decreases in cardiac output and $LVdP/dt_{max}$), it would be expected that a higher dose would produce an even greater D_2-receptor mediated depression of cardiac output. However, the peripheral vasodilatation produced by the high dose likely resulted in reflex-mediated activation of sympathetic nervous system and withdrawal of cardiac vagal tone thereby counteracting the direct effects of D_2-like receptor stimulation on sympathetic activity. This hypothesis is supported by the observation that 100 µg/kg resulted in transient increases in heart rate and cardiac output, which were absent when animals were pretreated with combined α- and β-adrenoceptor blockade. It is important to note that in our awake swine model Z1046 produced severe hypotension with considerably less reflex-tachycardia than produced by pure vasodilators such as the dihydropyridine calcium channel blockers (12) or K^+_{ATP} channel activators like nicorandil (13) and bimakalim (10) that we have studied in the same model (Figure 7). The dopamine receptor agonist epinine, which also has some β_2-adrenergic agonistic properties in this model, produces slightly greater increases in heart rate at a given reduction in mean aortic blood pressure (Figure 7)(14).

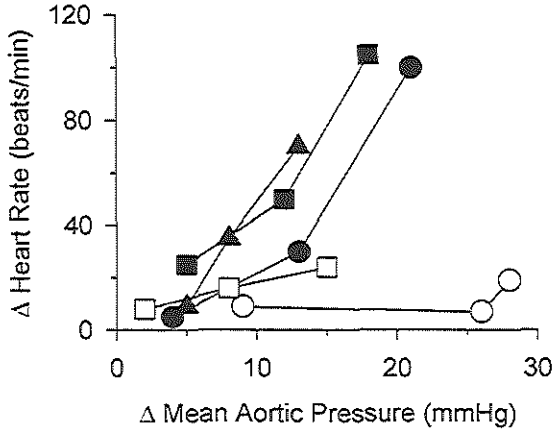

Figure 7. Comparison of the reflex-tachycardia in response to systemic hypotension produced by Z1046 (open circles, present study), the dopamine receptor agonist epinine (open squares), the calcium channel blocker nisoldipine (solid circles), and the K^+_{ATP} channel activators nicorandil (solid squares) and bimakalim (solid triangles) in awake resting swine. Data are presented as absolute changes in heart rate vs absolute changes in aortic pressure. For the sake of clarity we have only shown mean data. Note that Z1046 lowered aortic blood pressure with minimal reflex-tachycardia.

Z1046 has previously been reported to exhibit weak α_1-adrenoceptor antagonistic properties in rabbit aorta and α_2-agonistic properties in guinea pig atria (8). In the present study, the systemic vasodilator response to Z1046 was not altered by combined α- and β-adrenoceptor, suggesting that vasodilatation by Z1046 did not involve α- or β-adrenoceptors. However, balanced α_1-antagonistic and α_2-agonistic activities could have obscured an effect of non-selective α-adrenoceptor blockade with phentolamine. That this is unlikely is suggested by the observation that selective the α_2-adrenoceptor blocker yohimbine had no effect on the systemic dilation produced by Z1046. The

vasodilatation produced by Z1046 was located in the brain, small intestine, kidneys, adrenals, spleen and skeletal muscle, with regional vascular resistances decreasing by 30-40%. Vasodilatation in the brain, kidneys, small intestine and adrenals was most likely the result of stimulation of D_1-like receptors which are abundant in these vascular beds. In support of this hypothesis, we have previously observed in the identical swine model that dopamine (in the absence or presence of non-selective α- and β-adrenoceptor blockade) or the selective D_1-like receptor agonist fenoldopam produced vasodilatation in these beds (5). Conversely, pretreatment with the selective D_1-like receptor antagonist SCH23390 abolished the renal vasodilatation produced by Z1046 administered in a dose of 30 μg/kg i.v. (15).

We previously observed that non-selective α- and β-adrenoceptor blockade abolished the vasodilator response to dopamine in the spleen while the vasodilatation in the kidneys and small intestine persisted (5), suggesting that the vasodilator response to Z1046 in the spleen could be due to D_2-like mediated withdrawal of α-adrenergic tone. It is possible that the splenic D_2-like vasodilatation is species specific for swine, since vasodilatation produced by Z1046 in the isolated rabbit splenic artery is antagonized by the D_1-like receptor antagonist SCH23390 (8). That the skeletal muscle vasodilatation is most likely due to D_2 -like receptor stimulation is suggested by a previous study in anaesthetized dogs, in which Z1046 (30 μg/kg i.v.) caused a 47% decrease (which was blocked by the selective D_2 -like receptor antagonist domperidone) in resistance of the femoral bed, representing mainly skeletal muscle resistance. In contrast, in anaesthetized swine we recently failed to observe vasodilatation in skeletal muscle tissue in response to Z1046 administered in doses of 10 and 100 μg/kg (Duncker *et al.*, unpublished observations). Similarly, we previously reported that dopamine either in the absence or presence of α- and β-adrenoceptor blockade failed to elicit a vasodilator response in skeletal muscle of anaesthetized swine (5). In pentobarbital anaesthetized dogs sympathetic activity is higher than in pentobarbital anaesthetized swine, due to pentobarbital-induced vagal withdrawal which is prominent in dogs (16). This would allow D_2-like receptor stimulation to produce vasodilatation via inhibition of α-adrenergic constriction in dogs. In awake swine sympathetic activity is higher than in anaesthetized animals (5), which may be due to the inhibitory effects of barbiturates on sympathetic outflow (17).

In the present study Z1046 had no effect on coronary artery blood flow or its distribution across the left ventricular myocardial wall. Since the double product was also minimally affected it is most likely that the decrease in coronary vascular resistance was principally the result of autoregulation. D_1-like receptor stimulation has been reported to produce coronary vasodilatation in anaesthetized dogs (3, 18, 19) although one study in dogs was negative (20). In swine, we failed to observe D_1-like mediated coronary vasodilatation after intracoronary infusions of fenoldopam or i.v. infusions of dopamine in the presence of adrenergic blockade (5). Those findings suggest that the presence of

D_1-like receptors in the coronary circulation could be species dependent. In human isolated coronary arteries precontracted with norepinephrine, fenoldopam failed to elicit a vasodilator response in 6 out of 7 patients (21), suggesting that human conduit coronary arteries do not possess a significant amount of D_1-like receptors.

Haemodynamic effects of D-receptor stimulation during treadmill exercise.

In the present study we observed that Z1046 (100 μg/kg i.v.) produced markedly different haemodynamic responses during treadmill exercise than during resting conditions. Thus, at rest the fall in aortic pressure was the result of systemic vasodilatation (35% decrease in systemic vascular resistance) despite a mild reflex tachycardia and increase in cardiac output, whereas during exercise hypotension was due to both a 15% decrease in vascular resistance and a 10% decrease in cardiac output. The progressive inhibition of increases in heart rate, $LVdP/dt_{max}$ and cardiac output at higher levels of sympathetic activity can be explained by the D_2-mediated inhibition of catecholamine release from sympathetic nerve endings. In support of our observations, Girbes (22) reported that the D_1/D_2 dopamine receptor agonist pro-drug ibopamine, which is converted into epinine, blunted the increases in circulating norepinephrine levels in man produced by bicycle exercise. Their finding that ibopamine did not blunt the exercise-induced tachycardia could have been due to the direct β_1- / β_2-adrenoceptor stimulation by the compound (23), a property which Z1046 appears to lack (8).

In conclusion the novel dopamine receptor agonist Z1046 is an effective blood pressure lowering agent that elicits minimal reflex activation of sympathetic nervous system in awake resting swine. Systemic vasodilatation was not affected by combined α- and β- adrenoceptor blockade, which is consistent with a predominantly D_1 receptor dependent vasodilator mechanism. The hypotensive effect is maintained during treadmill exercise during which systemic vasodilatation and a lower cardiac output both contribute to the blood pressure lowering actions of Z1046. The cardiovascular profile of this orally active compound warrants further investigation of this class of drugs in experimental and clinical hypertension.

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Chapter 3

Beneficial effects of the Ca^{2+} sensitizer EMD 57033 in exercising pigs with infarction-induced chronic left ventricular dysfunction

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ABSTRACT

1 It is unknown how cardiac stimulation by Ca^{2+} sensitisation modulates the cardiovascular response to exercise when left ventricular (LV) function is chronically depressed following a myocardial infarction. We therefore investigated the effects of EMD 57033 at rest and during exercise and compared these to those of the mixed Ca^{2+}-sensitizer/ phosphodiesterase III inhibitor pimobendan.

2 Pigs were chronically instrumented for measurement of cardiovascular performance. At the time of instrumentation, infarction was produced by coronary artery ligation (MI, *n=12*). Studies in MI were performed in the awake state, 2-3 weeks after infarction.

3 MI were characterized by a lower resting cardiac output (18%), stroke volume (30%) and $LVdP/dt_{max}$ (18%), and a doubling of LV end-diastolic pressure, compared to normal pigs (N, *n=13*).

4 In 11 resting MI, intravenous EMD 57033 (0.2-0.8 mg kg^{-1} min^{-1}) increased $LVdP/dt_{max}$ (57±5%) and stroke volume (26±6%) with no effect on heart rate, LV filling pressure, and myocardial O_2-consumption, similar to N.

5 In MI, the effects of EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v.) on stroke volume and $LVdP/dt_{max}$ were maintained during treadmill exercise up to 85% of maximal heart rate, while heart rate was lower compared to control exercise (all $P<0.05$). In contrast, the effects of EMD57033 gradually waned in N at increasing intensity of exercise.

6 Compared to N, the cardiostimulatory effects of pimobendan (20 μg kg^{-1} min^{-1}, i.v.) were blunted in MI both at rest and during exercise compared to N.

7 In conclusion, the positi.v.e inotropic actions of the Ca^{2+} sensitizer EMD 57033 are unmitigated in resting and exercising MI compared to N, while those of the mixed Ca^{2+}-sensitizer/ phosphodiesterase III inhibitor pimobendan are blunted

INTRODUCTION

Clinical trials exploring the usefulness of inotropic support in patients with chronic heart failure (CHF) have generally led to disappointing results, as in a large number of studies improvements in cardiovascular function and quality of life have been overshadowed by an excess of sudden death (1). Nevertheless, the depressed cardiac pump function remains a major concern in a significant proportion of patients, requiring treatment in order to prevent end organ failure because of lack of supply of nutrients. A major drawback of the traditional inotropic agents such as sympathomimetics and phosphodiesterase-III (PDE-III) inhibitors is that they exert their actions through a cAMP-mediated enhancement of Ca^{2+}-transients which increases not only myocardial O_2-demand (2) but also the risk of ventricular arrhythmias. In contrast, Ca^{2+}-sensitizing agents augment myocardial contractility without increasing Ca^{2+}-transients and therefore with no or only minimal increments in myocardial O_2-consumption (2, 3). In a recent study, (4) showed that acute administration of the thiadiazinone derivative EMD 57033, a Ca^{2+}-sensitizing agent with negligible PDE-III inhibiting properties (5, 6), improved myocardial contractility and mechanoenergetics without compromising left ventricular diastolic function in conscious dogs with pacing-induced CHF. An adverse effect of Ca^{2+}-sensitizing agents on left ventricular diastolic function has been a major concern with this class of agents (7, 8), but is mainly based on *in vitro* studies (see (8), as in several *in vivo* studies employing animals with normal, stunned or failing hearts no adverse effects on diastolic function were demonstrated (4, 9-11). The observation that an improvement in systolic function can be obtained without increases in energy expenditure and without increasing the risk of arrhythmias may therefore open avenues for inotropic treatment of CHF.

Evidence is emerging that physical activity may be beneficial in patients suffering from CHF (12, 13). If, and how, the responses to exercise are modulated when such patients are treated with Ca^{2+}-sensitizing agents is currently unknown. This appears to be relevant as we have earlier shown that in normal exercising pigs the EMD 57033-induced increase in myocardial contractility is partially offset by the increased β-adrenergic activity (9). This blunting effect of exercise on the inotropic actions of EMD 57033 was not present after β-adrenergic receptor blockade suggesting that EMD 57033 might be useful in patients with LV dysfunction who are still capable of performing moderate exercise despite a loss of cardiac β-adrenergic responsiveness (14). We therefore investigated the cardiovascular effects of intravenous (i.v.) EMD 57033 in exercising pigs with chronic LV dysfunction caused by myocardial infarction. A suitable dose was chosen based on the haemodynamic response to three cumulative doses administered to these animals at rest. Furthermore, in order to establish whether a combination of Ca^{2+} sensitization and PDE-III inhibition is advantageous over Ca^{2+} sensitization alone, we compared the effects of EMD 57033 to

those of pimobendan (15-17). Because in an earlier study (9) we have described the effects of EMD 57033 in normal exercising pigs, we have included in some figures the responses of these normal pigs to establish whether responses of the drugs are different for pigs with infarction and normal pigs. Four new normal animals have been added to exclude confounding factors such as time.

METHODS

Experiments were performed in pigs (Land race x Yorkshire) of either sex with approval of the Animal Care Committee of the Erasmus University Rotterdam and in accordance with the 'Guiding Principles in the Care and Use of Laboratory Animals' of the Council of the American Physiological Society. Adaptation of animals to the laboratory and experimental conditions started 1 week prior to instrumentation and was continued post-operatively.

Surgery

Full details of anaesthesia, surgical procedures and instrumentation have been described earlier (9, 18). After a thoracotomy under sterile conditions, midazolam/fentanyl anaesthetised pigs (23±1 kg) were instrumented with fluid-filled polyvinylchloride 8Fr catheters for measurement of blood pressure in the aortic arch, pulmonary artery and left atrium and for blood sampling. An electromagnetic flow probe (Skalar) was positioned around the ascending aorta, and a Doppler flow probe (Triton) was placed around the proximal part of the left anterior descending coronary artery (LAD), while a microtipped pressure transducer (Konigsberg Instruments) was inserted into the left ventricle via the apex for recording of left ventricular (LV) pressure and LVdP/dt. Myocardial infarction was produced by permanent ligation of the proximal part of the left circumflex coronary artery, which causes infarction of approximately 20% of the total LV mass (19, 20). In three of these animals a catheter was inserted into the anterior interventricular vein for coronary venous sampling. Three of the 17 animals that were subjected to infarction died within 1 hour after occlusion due to recurrent ventricular fibrillation. After electrical wires and catheters had been tunnelled subcutaneously to the back, the chest was closed and the animals were allowed to recover. All electrical wires and catheters were protected with an elastic vest.

Post-surgical care

During the first 48 hours after surgery, animals received daily intravenous injections of buprenorphine (0.3 mg) as treatment for pain, and during the first week also of amoxicillin (25 mg kg^{-1}) and gentamycin (5 mg kg^{-1}) to prevent infection. Catheters were flushed daily with physiological saline containing heparin (2,000 IU ml^{-1}). Two animals with a myocardial infarction

died during the first night after surgery, and experimental protocols were therefore performed in 12 pigs.

Experimental protocols

The effects of consecutive 10-min i.v. infusions of EMD 57033 (0.2, 0.4 and 0.8 mg kg^{-1} min^{-1}, n=12) or vehicle (0.5, 1.0 and 2.0 ml min^{-1} propylene glycol, n=10) were studied approximately 3 weeks after instrumentation with the animals resting in a cage. Experiments were performed in random order and EMD 57033 and solvent infusions were separated by at least 24 hours.

On different days, the effects of EMD 57033 (n=6) and pimobendan (n=6) on the responses to graded treadmill exercise were studied. After resting measurements (lying and standing on the treadmill) were made, the animals underwent a 4-stage exercise protocol (1, 2, 3 and 4 km h^{-1}), with each level lasting 2-3 min (9). Measurements were made during the last 30 s of each level of exercise when haemodynamics had reached a stable level. Following completion of the exercise protocol, the animals were allowed to rest for 60 min, in which period all variables returned to baseline levels (9). The animals then received either EMD 57033 (0.4 mg kg^{-1} min^{-1}, i.v.) or pimobendan (20 μg^{-1} kg min^{-1}, i.v.) or no treatment. The EMD 57033 dose was chosen because it had no effect on heart rate in MI, and the effect on $LVdP/dt_{max}$ was unmitigated by propranolol in normal resting pigs (9), indicating negligible PDE-III inhibitory properties of this dose. In addition, it proved that at this dose, the vehicle propylene glycol did not contribute to the effects of the drug (see Table 1). The pimobendan dose (20 μg kg^{-1} min^{-1}, i.v.) was selected because it is equipotent to EMD 57033 (0.4 mg kg^{-1} h^{-1}, i.v.) with respect to their effects on $LVdP/dt_{max}$ in awake resting normal pigs (9, 16).

In order to evaluate whether the responses to EMD 57033 were altered in animals with myocardial infarction (MI) compared to normal pigs (N), we also present the responses to EMD 57033 in 13 N. The results of 9 of these animals have been described before (9), but data of 4 additional animals were included in the present study to exclude time as a confounding factor.

Data acquisition and analysis

Data were recorded and digitized on-line using an eight channel data-acquisition program CODAS (Dataq Instruments) and stored for analysis with a program written in MatLab (The Mathworks), as previously described (9, 10).

Statistical analysis

Analysis of the resting protocols was performed using three-way (group [MI vs N], treatment [EMD 57033 vs vehicle] and dose), two-way and one-way analysis of variance, followed by Dunnett's test, paired t-test or unpaired t-test, as appropriate. Statistical analysis of the exercise data was performed using two-way (treatment [control run vs intervention run], and exercise level) analysis of variance for repeated measures, followed by paired t-test (treatment) or one-way

analysis of variance for repeated measures and Dunnett's test (exercise level). Significance (two-tailed) was accepted for $P \leq 0.05$. All data are mean ± standard error of the mean.

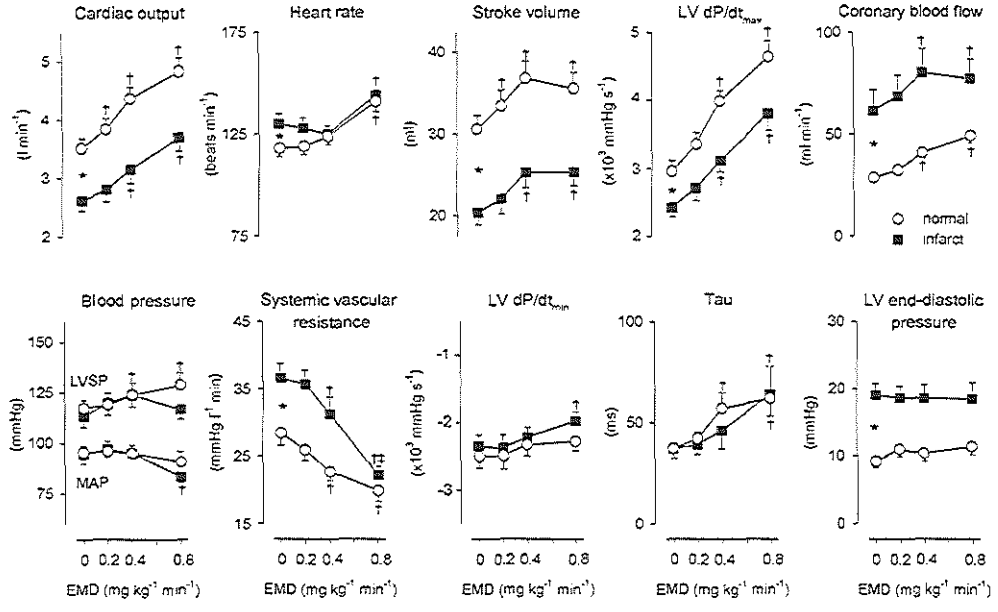

Figure 1 Systemic haemodynamic and cardiac effects of cumulative 10-min intravenous infusions of EMD 57033 in resting MI (n=12) and N (n=13); Tau=time constant of decay of left ventricular (LV) pressure. Data are mean±s.e.mean; *$P \leq 0.05$ baseline MI vs baseline N, †$P \leq 0.05$ vs baseline (0 mg kg^{-1} min^{-1}), ‡EMD 57033-induced change from baseline in MI is significantly different ($P \leq 0.05$) from EMD 57033-induced change from baseline in N.

Baseline characteristics of MI

MI were characterized by a lower cardiac output (18%), stroke volume (30%) and myocardial contractility ($LVdP/dt_{max}$, 18%), a higher heart rate (15%), a doubling of the LV end-diastolic pressure and an elevated systemic vascular resistance (SVR, 28%) compared to N (Figure. 1). Mean arterial pressure (MAP) was normal in MI because the increases in SVR balanced the decrease in cardiac output. Mean pulmonary artery pressure (MPAP) had doubled, due to a quadrupling of mean left atrial pressure and a doubling of pulmonary vascular resistance (Figure 2).

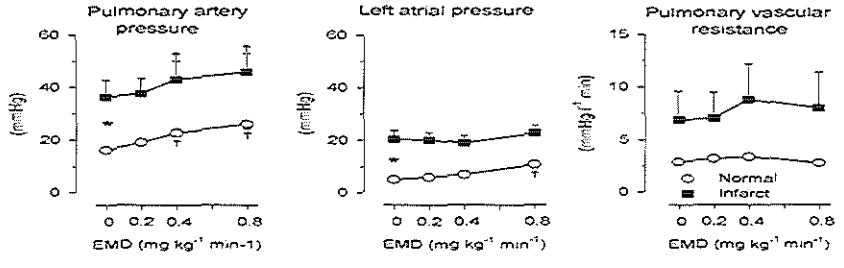

Figure 2 Pulmonary haemodynamic effects of cumulative 10-min intravenous infusions of EMD 57033 in resting MI (n=5) and N (n=7). For further details see Figure 1.

Table 1 Systemic, pulmonary and coronary haemodynamic effects of propylene glycol in 10 resting swine with a myocardial infarction.

		Propylene glycol (ml min^{-1})		
	Baseline	*0.5*	*1.0*	*2.0*
Systemic haemodynamics				
CO (l min^{-1})	2.8 ± 0.2	2.9 ± 0.3	2.9 ± 0.3	3.0 ± 0.3
HR (beats min^{-1})	134 ± 5	134 ± 6	140 ± 7	139 ± 7
SV (ml)	22 ± 2	22 ± 2	21 ± 2	22 ± 2
LVdP/dt$_{max}$ (mmHg s^{-1})	2630 ± 220	2670 ± 250	2650 ± 230	2660 ± 250
LVdP/dt$_{min}$ (mmHg s^{-1})	-2480 ± 170	-2590 ± 190	-2510 ± 190	-2520 ± 190
LVEDP (mmHg)	19 ± 2	21 ± 2	20 ± 2	22 ± 2*
Tau (ms)	37 ± 4	38 ± 3	39 ± 3	39 ± 4
LVSP (mmHg)	110 ± 3	113 ± 3	111 ± 2	113 ± 3
MAP (mmHg)	90 ± 3	93 ± 3	92 ± 2	95 ± 3
SVR (mmHg min l^{-1})	34.6 ± 3.1	35.6 ± 2.8	36.2 ± 4.5	35.5 ± 3.5
Pulmonary haemodynamics				
MPAP [a] (mmHg)	35 ± 7	37 ± 6	39 ± 6	41 ± 8*
MLAP [a] (mmHg)	17 ± 3	18 ± 3	19 ± 3	20 ± 3
PVR [b] (mmHg min l^{-1})	7.6 ± 2.7	7.7 ± 2.8	8.7 ± 3.7	9.8 ± 4.2
Coronary haemodynamics				
CBF (ml min^{-1})	63 ± 12	68 ± 12	70 ± 12*	71 ± 12*
CVR (mmHg min ml^{-1})	2.0 ± 0.3	1.8 ± 0.2	1.6 ± 0.2	1.7 ± 0.2
LVW (mmHg l min^{-1})	310 ± 36	322 ± 41	313 ± 37	335 ± 44*

CO, cardiac output; HR, heart rate; SV, stroke volume; LVdP/dt$_{max}$, maximum rate of rise of left ventricular pressure; LVdP/dt$_{min}$, maximum rate of fall of left ventricular pressure; LVEDP, left ventricular end-diastolic pressure; Tau, time constant of decay of LV pressure; LVSP, left ventricular peak systolic pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; PVR, pulmonary vascular resistance; CBF, coronary blood flow, CVR, coronary vascular resistance; LVW, LV work (LVSPxCO). Data are mean ± s.e. mean, *n*, 10 ([a]n=7, [b]n=6); *$P \leq 0.05$ vs baseline.

Blood flow in the non-infarcted myocardium increased up to during EMD 57033 (24±5%, Figure 1), but approximately 50% of this effect must be ascribed to the solvent (Table 1). Myocardial O_2-consumption, although measured in only 3 pigs, increased less than 15% during EMD 57033 infusion despite a 59±15% increase in LV work (Figure 3), reflecting an increased LV mechanical efficiency (LV work/myocardial O_2-consumption). The contribution of the vehicle to the latter proved to be of minor importance (Table 1).

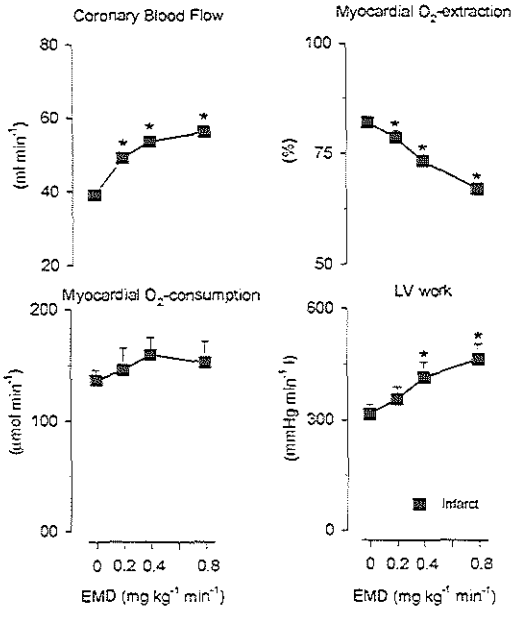

Figure 3 Effect of cumulative 10-min intravenous infusions of EMD 57033 on myocardial O_2-consumption and left ventricular (LV) work in resting MI (n=3). *$P \leq 0.05$ vs baseline (0 mg kg^{-1} min^{-1}).

Responses to exercise

During exercise at 4 km h^{-1}, heart rate of untreated MI increased to 235±8 beats/min which was 66±10% above its resting (standing) heart rate and approximately 85% of the maximum heart rate in the pig (21). Heart rate and cardiac output increased sharply at 1 km h^{-1} and more gradually thereafter (Table 2). Stroke volume remained unchanged, implying that tachycardia was responsible for the exercise-induced increase in cardiac output. MAP also remained unchanged as peripheral vasodilation balanced the increase in cardiac output. $LVdP/dt_{max}$ increased up to 50±11% ($P<0.05$), while $LVdP/dt_{min}$ became more negative (14±5%, $P<0.05$) and τ did not change (44±11 ms at rest and 39±10 ms at 4 km h^{-1}) suggesting that exercise did not adversely affect LV relaxation. Figure 4 also shows that the slope of the relations between cardiac output and $LVdP/dt_{max}$ versus heart rate (i.e. exercise load) was less steep in MI than in N (both $P<0.05$). MPAP and left atrial pressure increased, with a trend towards vasodilation in the pulmonary vascular bed ($P=0.09$, Table 2).

Effects of EMD 57033. In MI, the responses of cardiac output and $LVdP/dt_{max}$ to exercise remained unaltered in the presence of EMD 57033, i.e. the EMD 57033-induced increases in the resting values of cardiac output, stroke volume and $LVdP/dt_{max}$ were maintained during exercise (Table 2 and Figure 4). The EMD 57033-induced increase in $LVdP/dt_{max}$ in MI at all levels of exercise differed from N in which $LVdP/dt_{max}$ values became similar at higher workloads, independent of the presence of EMD 57033. $LVdP/dt_{min}$ (Table 2) and τ (42±9 ms at 4 km h^{-1}) were not affected by EMD 57033 during exercise. Similar to the control run, MAP was not affected by exercise in the presence of EMD 57033. The response to exercise of the pulmonary circulation of MI was, similar to N (not shown), not modified by EMD 57033 (Table 2).

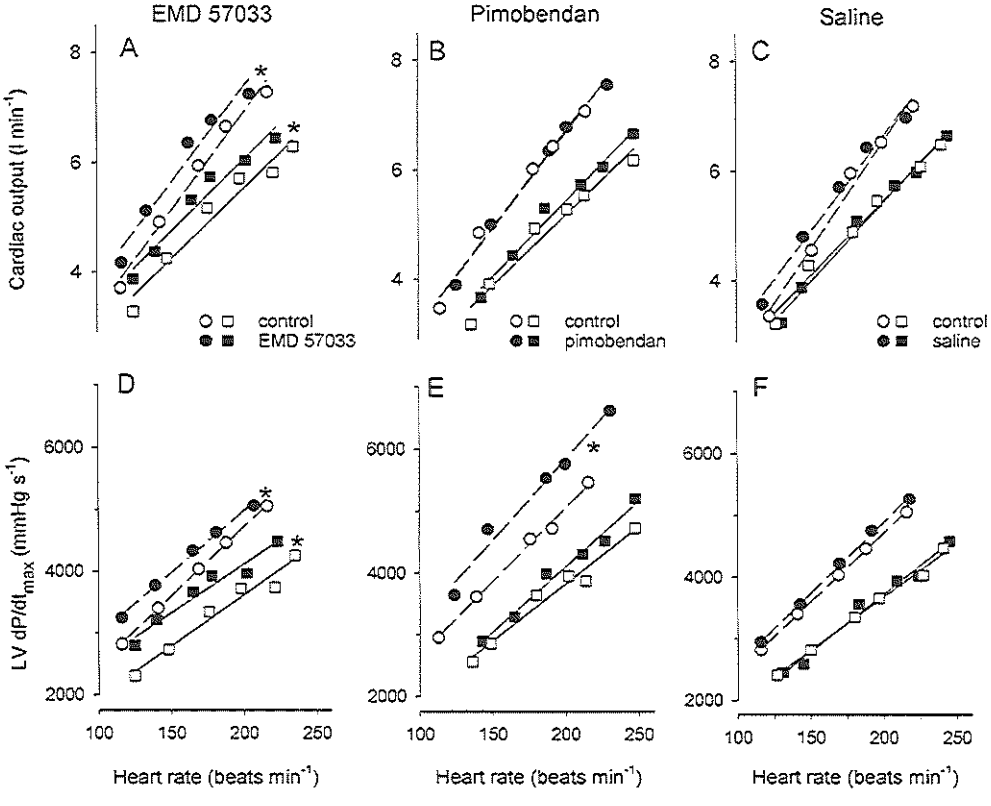

Figure 4 Responses of cardiac output and $LVdP/dt_{max}$ to exercise in the absence and presence of EMD 57033 (0.4 mg kg^{-1} min^{-1}) or pimobendan (20 μg kg^{-1} min^{-1}) in MI (n=6; squares) and N (n=8; circles). Data have been plotted as a function of heart rate. The reproducibility of two consecutive control exercise periods is presented in panels C and F. *$P \leq 0.05$ vs corresponding control conditions (first exercise period).

Tabel 2 Haemodynamic responses to EMD 57033 in swine with a myocardial infarction during graded treadmill exercise.

		Rest		*Exercise (km h^{-1})*			
	Treatment	*Lying*	*Standing*	*1*	*2*	*3*	*4*
SYSTEMIC							
CO	Control	3.3 ± 0.2	4.2 ± 0.1*	5.2 ± 0.1†	5.7 ± 0.2†	5.8 ± 0.2†	6.3 ± 0.3†
(l min^{-1})	EMD 57033	3.9 ± 0.1‡	4.4 ± 0.2	5.3 ± 0.2†	5.7 ± 0.2†	6.0 ± 0.2†	6.5 ± 0.3†
HR	Control	125 ± 7	148 ± 8*	176 ± 7†	198 ± 8†	221 ± 8†	235 ± 8†
(beats min^{-1})	EMD 57033	125 ± 6	140 ± 6*	165 ± 10†	178 ± 8†‡	202 ± 9†‡	223 ± 11†‡
SV	Control	26 ± 1	28 ± 2*	30 ± 2	29 ± 2	27 ± 2	27 ± 1†
(ml)	EMD 57033	31 ± 2‡	32 ± 3‡	33 ± 2‡	32 ± 1‡	30 ± 1‡	29 ± 1‡
LV dP/dt_{max}	Control	2310 ± 150	2730 ± 340	3340 ± 440†	3720 ± 500†	3740 ± 460†	4260 ± 470†
(mmHg s^{-1})	EMD 57033	2800 ± 230‡	3210 ± 350‡	3670 ± 480†	3930 ± 470†	3970 ± 490†‡	4490 ± 580†
LV dP/dt_{min}	Control	-2560 ± 340	-2660 ± 370	-2880 ± 460	-2880 ± 490	-2910 ± 500	-3300 ± 570
(mmHg s^{-1})	EMD 57033	-2640 ± 410	-2520 ± 410	-2590 ± 440	-2700 ± 430	-2710 ± 460	-3180 ± 500
LVSP	Control	115 ± 6	116 ± 8	119 ± 7	121 ± 7	122 ± 8	128 ± 9
(mmHg)	EMD 57033	116 ± 6	118 ± 6	122 ± 6	123 ± 7	125 ± 8†	131 ± 10†
MAP	Control	101 ± 4	96 ± 7	94 ± 6	95 ± 6	95 ± 6	97 ± 6
(mmHg)	EMD 57033	97 ± 5	91 ± 5	88 ± 6	88 ± 5	90 ± 6	92 ± 6‡
SVR	Control	31.5 ± 2.6	22.7 ± 2.1*	18.1 ± 1.0†	16.7 ± 1.1†	16.4 ± 1.0†	15.6 ± 1.3†
(mmHg min l^{-1})	EMD 57033	24.6 ± 1.7‡	21.7 ± 2.7	16.1 ± 1.2†‡	15.4 ± 0.8†‡	14.9 ± 0.8†‡	14.4 ± 1.0†‡
Pulmonary haemodynamics							
MPAP	Control	27 ± 5	28 ± 4	32 ± 4†	36 ± 4†	37 ± 4†	37 ± 3†
(mmHg)	EMD 57033	27 ± 3	28 ± 4	34 ± 5†	36 ± 4†	39 ± 4†	39 ± 3†
MLAP	Control	14 ± 3	12 ± 2	14 ± 2	15 ± 2	17 ± 3†	18 ± 2†
(mmHg)	EMD 57033	13 ± 1	11 ± 2	14 ± 3	16 ± 2	19 ± 2†	21 ± 3†
PVR	Control	3.9 ± 0.7	4.1 ± 0.4	3.6 ± 0.5	3.6 ± 0.5	3.5 ± 0.4	3.1 ± 0.4
(mmHg min l^{-1})	EMD 57033	3.7 ± 0.6	3.7 ± 0.3	3.9 ± 0.5	3.5 ± 0.5	3.4 ± 0.5	2.9 ± 0.3

CO, cardiac output; HR, heart rate; SV, stroke volume; $LVdP/dt_{max}$, maximum rate of rise of left ventricular pressure; LV dP/dt_{min}, maximum rate of fall of left ventricular pressure; LVSP, left ventricular peak systolic pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; PVR, pulmonary vascular resistance. Data are mean ± s.e. mean; n=6; *$P \leq 0.05$ standing versus lying; †$P \leq 0.05$ exercise versus standing; ‡ $P \leq 0.05$ EMD 57033 versus corresponding control.

Tabel 3 Haemodynamic responses to pimobendan in swine with a myocardial infarction during graded treadmill exercise.

		Rest		*Exercise (km h^{-1})*			
	Treatment	*Lying*	*Standing*	*1*	*2*	*3*	*4*
Systemic haemodynamics							
CO	Control	3.2 ± 0.3	4.0 ± 0.2*	5.0 ± 0.2†	5.3 ± 0.3†	5.6 ± 0.3†	6.2 ± 0.3†
(l min^{-1})	Pimobendan	3.7 ± 0.2‡	4.5 ± 0.3*	5.4 ± 0.3†‡	5.8 ± 0.3†‡	6.1 ± 0.3†‡	6.7 ± 0.3†‡
HR	Control	136 ± 7	149 ± 6	180 ± 6†	202 ± 9†	214 ± 8†	248 ± 5†
(beats min^{-1})	Pimobendan	143 ± 6‡	165 ± 9‡	187 ± 7†	212 ± 10†‡	227 ± 9†	248 ± 4†
SV	Control	24 ± 2	27 ± 2*	28 ± 2	26 ± 1	26 ± 1	25 ± 1†
(ml)	Pimobendan	26 ± 2‡	27 ± 2	29 ± 2	27 ± 2	27 ± 2	27 ± 1
LV dP/dt_{max}	Control	2560 ± 210	2860 ± 260	3640 ± 310†	3960 ± 430†	3870 ± 410†	4730 ± 590†
(mmHg s^{-1})	Pimobendan	2890 ± 240‡	3290 ± 280	3990 ± 400	4310 ± 510†	4520 ± 550†	5210 ± 730†
LV dP/dt_{min}	Control	-2540 ± 350	-2520 ± 360	-2680 ± 330	-2690 ± 350	-2710 ± 360	-3160 ± 540
(mmHg s^{-1})	Pimobendan	-2580 ± 400	-2450 ± 320	-2700 ± 420	-2730 ± 420†	-2690 ± 380	-3010 ± 510†
LVSP	Control	113 ± 5	109 ± 6	118 ± 4†	119 ± 4†	117 ± 4†	124 ± 6†
(mmHg)	Pimobendan	112 ± 5	111 ± 4	120 ± 6†	121 ± 5†	120 ± 4†	125 ± 8†
MAP	Control	97 ± 4	87 ± 5*	88 ± 3	88 ± 4	88 ± 4	93 ± 5
(mmHg)	Pimobendan	93 ± 5	86 ± 4	89 ± 6	88 ± 5	86 ± 4	88 ± 5
SVR	Control	31.8 ± 2.2	22.4 ± 1.5*	18.0 ± 0.8†	16.8 ± 0.8†	16.1 ± 0.7†	15.2 ± 0.7†
(mmHg min l^{-1})	Pimobendan	26.0 ± 1.8‡	20.1 ± 1.8*	17.0 ± 1.0†	15.5 ± 0.8†	14.3 ± 0.7†‡	13.3 ± 0.8†‡
Pulmonary haemodynamics							
MPAP	Control	29 ± 5	28 ± 5	34 ± 5†	38 ± 5†	40 ± 5†	39 ± 3†
(mmHg)	Pimobendan	31 ± 5	33 ± 5	37 ± 5	42 ± 5†‡	42 ± 5†	39 ± 3†
MLAP	Control	15 ± 3	10 ± 3	12 ± 3	14 ± 2	17 ± 2†	18 ± 3†
(mmHg)	Pimobendan	14 ± 3	13 ± 3	15 ± 3‡	17 ± 3‡	17 ± 3	18 ± 4†
PVR	Control	4.9 ± 0.9	4.0 ± 0.4	4.5 ± 0.6	4.7 ± 0.6	4.4 ± 0.6	3.4 ± 0.3
(mmHg min l^{-1})	Pimobendan	4.6 ± 0.8	4.6 ± 0.6	4.4 ± 0.7	4.4 ± 0.7	4.3 ± 0.8	3.2 ± 0.3

CO, cardiac output; HR, heart rate; SV, stroke volume; $LVdP/dt_{max}$, maximum rate of rise of left ventricular pressure; LV dP/dt_{min}, maximum rate of fall of left ventricular pressure; LVSP, left ventricular peak systolic pressure; MAP, mean arterial pressure; SVR, systemic vascular resistance; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure; PVR, pulmonary vascular resistance. Data are mean ± s.e. mean; n=6; *$P \leq 0.05$ standing versus lying; †$P \leq 0.05$ exercise versus standing; ‡ $P \leq 0.05$ Pimobendan versus corresponding control.

Effects of Pimobendan. The exercise-induced increases in cardiac output in MI during pimobendan were similar to those during EMD 57033 (Table 3). However, in contrast to EMD 57033, the cardiac output – heart rate relation was not affected by pimobendan (Figure 4), implying that the pimobendan-induced increase in cardiac output was solely caused by tachycardia. Similarly, the relation between $LVdP/dt_{max}$ and heart rate was virtually identical in untreated and pimobendan-treated MI, although at the highest exercise level, and at similar heart rates, $LVdP/dt_{max}$ tended to be higher in the pimobendan-treated than in the untreated animals (Figure 4). In contrast, for a given heart rate in N, $LVdP/dt_{max}$ was consistently

higher during pimobendan. Similar to EMD 57033, the pimobendan-induced systemic vasodilatation balanced the increase in cardiac output, thereby leaving MAP unchanged. The response of the pulmonary circulation to exercise in MI was not modified by pimobendan in MI (Table 3) or N (data not shown).

Reproducibility of exercise responses. When in MI the control run was repeated after the 60 min resting period all cardiovascular parameters (Figure 4), showed the same good reproducibility as N (9).

DISCUSSION

The main findings of the present study in chronically instrumented pigs with a 3-week old myocardial infarction are that (*i*) The positi.v.e inotropic action of EMD 57033 was virtually identical to that in N with minimal increments in myocardial O_2-consumption and only at the highest dose a minimal increase in heart rate and LV relaxation time. (*ii*) The positive inotropic actions of EMD 57033 in exercising MI were similar to those in N. In contrast, the positive inotropic and chronotropic actions of pimobendan were blunted in exercising MI compared to N.

In vivo models of heart failure

Most large animal studies produce CHF by rapid ventricular pacing, although clinically the most common cause of CHF is myocardial infarction (22). We therefore elected to perform studies in a model in which LV dysfunction was produced by permanent coronary ligation. This model shows at 3-6 weeks a milder degree of LV dysfunction and neurohumoral acti.v.ation compared to the pacing model. Thus, in pacing-induced CHF resting levels of catecholamines are markedly elevated within 6 weeks after onset of pacing (23), whereas we found near normal resting catecholamine and angiotensin II levels up to 6 weeks after MI (24), although catecholamine levels are increased during treadmill exercise (unpublished observations from our laboratory) and β-adrenergic receptor responsi.v.eness is blunted (25). Moreover, the LV dilates and hypertrophies

after infarction (24), whereas in pacing-induced CHF LV_{weight} fails to increase despite severe dilation resembling end-stage dilated cardiomyopathy (22).

Ca^{2+}-sensitizers in heart failure

Studies investigating the cardiovascular actions of Ca^{2+}-sensitizing agents have been hampered by the additional PDE-III inhibiting properties of most of these agents. In awake normal pigs and dogs (16, 26, 27), pimobendan increases cardiac output, $LVdP/dt_{max}$ and reduces LV filling pressure. The increase in cardiac output is predominantly heart rate-mediated (16, 26, 27), while the chronotropic and inotropic actions are markedly attenuated by beta-blockade (16), indicating significant PDE-III inhibition. This explains why the chronotropic and inotropic actions of pimobendan are blunted in pigs and dogs with CHF (17, 26), although they are better preserved than those of the pure PDE-III inhibitor amrinone (27). In humans with LV dysfunction or overt CHF pimobendan results in short-term improvements of the haemodynamic status, but also increases exercise capacity and quality of life after long-term treatment (28, 29).

Levosimendan increases $LVdP/dt_{max}$ and cardiac output in awake dogs (30), but the increases in heart rate and the amenability of the cardiostimulatory actions to autonomic blockade suggest a major action via PDE-III inhibition (30). In patients with LV dysfunction, levosimendan caused a heart rate-mediated increase in cardiac output (31), whereas in patients with severe CHF the dose-dependent increase in cardiac output was primarily due to an increase in stroke volume, with heart rate only contributing at higher doses (32). Similarly, in dogs with pacing-induced CHF, the chronotropic response was absent, while the inotropic response was blunted (11, 33). Also (34) reported in dogs a marked loss of the inotropic response to levosimendan in pacing-induced CHF, which paralleled the loss of response to isoproterenol. Because levosimendan also increases Ca^{2+}-transients dose-dependently in isolated rat hearts, these studies indicate that levosimendan exerts considerable PDE-III inhibition.

MCI-154, another mixed Ca^{2+}-sensitizer/PDE-III inhibitor, also improves LV contractility and cardiac output in dogs with pacing-induced severe CHF (35) and in patients with LV dysfunction (36). Interestingly the inotropic effects of MCI-154 were very similar to those in normal dogs (35), while in the patients with LV dysfunction in a dose that produced a similar increase in cardiac output as dobutamine, MCI-154 had no effect on heart rate and myocardial O_2-consumption (35, 36), whereas dobutamine increased O_2-consumption by 40% (36). Importantly, none of the aforementioned mixed agents affected diastolic function adversely and often improved diastolic performance (11, 17, 26-28, 31, 33, 35, 36), which is likely related to PDE-III inhibition.

The principal mode of action EMD 57033 appears to be Ca^{2+} sensitization, both in vitro (5, 6, 37) and in vivo (38, 39), although the drug possesses some minor PDE-III inhibitory effects in vitro (40). Indeed, the inotropic responses to EMD 57033 were not affected by β-adrenoceptor blockade

(9, 10), which explains why in the present study the effects of EMD 57033 in MI were virtually identical to those in N, despite a reduced β-adrenergic responsiveness (25). (41)) reported that EMD 57033 (0.6 mg kg^{-1} min^{-1}, i.v.) increased $LVdP/dt_{max}$ and cardiac output, which was accompanied by decreases in heart rate and LV end-diastolic pressure in dogs with pacing-induced CHF. (4) also reported that EMD 57033 in a dose of 0.4 mg kg^{-1} min^{-1} i.v. produced similar inotropic effects in normal and failing dogs, whereas the increase in LV contractility by 0.8 mg kg^{-1} min^{-1} i.v. was blunted in failing compared to normal dogs, most likely due to modest PDE-III inhibition at this dose (9).

The negligible PDE-III inhibitory effects might raise concern that EMD 57033 exerts detrimental effects on diastolic function (7). In pigs with a 3-week old myocardial infarction, end-diastolic and end-systolic LV diameter and LV end-diastolic pressure are increased (24). However, early diastolic relaxation parameters ($LVdP/dt_{min}$ and τ) in MI were not different from N, possibly because the elevated heart rate masked subtle relaxation abnormalities in MI. EMD 57033 did not alter LV end-diastolic pressure of MI (whereas it increased during infusion of the vehicle), and only increased $LVdP/dt_{min}$ and τ at 0.8 mg kg^{-1} min^{-1} in resting swine. In addition, 0.4 mg kg^{-1} min^{-1} had no effect on τ either at rest or during exercise in MI, indicating that EMD 57033 at a dose that produces an increase in LV pump function had no adverse effect on this index of early relaxation or LV filling. The contrasting observation of a lack of significant diastolic abnormalities in in-vivo studies (4) and the impairment of diastolic function in isolated myocardial muscle strips (42) could be explained by increased elastic restoring forces in the in-vivo heart, due to ejection to a lower end-systolic volumes, which counteract any potential untoward effects of EMD 57033 on diastolic function (4, 43). This mechanism is only relevant in ejecting but not in isolated isometrically contracting tissues and may therefore explain why in vitro but not in-vivo studies report an impairment of diastolic function with EMD 57033.

Ca^{2+}-sensitizers and exercise responses in LV dysfunction

In normal pigs (9) and dogs (27), pimobendan enhances the exercise-induced increases in $LVdP/dt_{max}$. This facilitation of exercise-induced response of contractility is a typical feature of PDE-III inhibition and is most effective when β-adrenergic stimulation (and hence cAMP production) is high, such as during exercise (27, 44). Indeed the facilitation by pimobendan of exercise-induced inotropic stimulation was virtually abolished when normal pigs were pretreated with propranolol to minimize β -adrenoceptor-mediated cAMP production (9).

The positive inotropic actions of EMD 57033 were blunted during exercise in N, which may be related to the exercise-induced increase in cAMP which reduces myofilament Ca^{2+} sensitivity. Indeed, when cAMP production was minimized by propranolol the positive inotropic effects of

EMD57033 in N were maintained at all levels of exercise (9). These findings explain why in the present study the positive inotropic effects of EMD 57033 were maintained during exercise in MI, whereas those of pimobendan were blunted compared to N. Our findings are supported by the observations in dogs with pacing-induced CHF (27), although in that study the 50% loss in inotropy with pimobendan was still less than the 80% loss observed with the pure PDE-III inhibitor amrinone. The findings are best explained by the loss of β-adrenergic responsiveness that occurs not only in pacing-induced CHF (45) also in our MI model (25). Interestingly, (28) reported beneficial haemodynamic effects of pimobendan in patients with CHF, both at rest and during exercise. Thus, whereas in these patients pimobendan had no effect on heart rate, stroke volume was markedly increased and pulmonary capillary wedge pressure was decreased both at rest and during exercise. Moreover, the effects at rest were similar to the effects during exercise, much like the effect of EMD 57033 in the present study. These findings suggest that the contribution of PDE-III inhibition to the positive chronotropic and inotropic actions of mixed Ca^{2+}-sensitizers/PDE-III inhibitors is progressively lost with increasing severity of LV dysfunction and concomitant loss of β-adrenergic responsiveness, whereas the Ca^{2+}-sensitizing effects are preserved. This also explains why in our MI the effects of EMD 57033 were fully maintained during exercise. Exercise in the presence of EMD 57033 resulted in slightly lower heart rates, whereas pimobendan resulted in higher heart rates compared to control exercise. This implies that exercise at a given workload will be accompanied by a lower myocardial O_2-demand during EMD 57033 compared to control exercise and particularly compared to pimobendan.

In conclusion, the positive inotropic actions of EMD 57033 were maintained in MI compared to N both at rest and during exercise, whereas the positive inotropic actions of pimobendan were blunted. Finally, for a drug to be attractive for daily use oral route of administration is desired. It is therefore of interest that oral administration of EMD 82571, a prodrug of EMD 57033, which is converted to EMD 57033 upon absorption increased myocardial contractility in 4 N (45% increase in $LVdP/dt_{max}$) and 6 MI (30% increase in $LVdP/dt_{max}$) up to 8 hours after administration (unpublished data from our laboratory).

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Chapter 4

ACE Inhibition and AT_1 Receptor Blockade Prevent Cardiac Remodeling in Pigs After Myocardial Infarction.Role of Tissue Angiotensin II.

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ABSTRACT

Background-The mechanisms behind the beneficial effects of renin-angiotensin (Ang) system blockade following myocardial infarction (MI) are not fully elucidated, but may include interference with tissue Ang II.

Methods and Results-Forty-nine pigs underwent coronary artery ligation or sham operation and were studied up to six weeks. To determine coronary Ang I-II conversion and to distinguish plasma-derived Ang II from locally synthesized Ang II, ^{125}I-labeled and endogenous Ang I and II were measured in plasma, infarcted and non-infarcted left ventricle (LV) during ^{125}I-Ang I infusion. AT_1 receptor-mediated uptake of circulating ^{125}I-Ang II was increased at 1 and 3 weeks in non-infarcted LV, and this uptake was the main cause of the transient elevation in Ang II levels in the non-infarcted LV at 1 week. Ang II levels and AT_1 receptor-mediated uptake of circulating Ang II were reduced in the infarct area at all time points. Coronary Ang I-II conversion was unaffected by MI. Captopril and the AT_1 receptor antagonist eprosartan attenuated post-infarct remodeling, although both drugs increased cardiac Ang II production. Captopril blocked coronary conversion by >80% and normalized Ang II uptake in the non-infarcted LV. Eprosartan did not affect coronary conversion and blocked cardiac Ang II uptake by >90%.

Conclusions-Both circulating and locally generated Ang II contribute to remodeling after MI. The rise in tissue Ang II production during ACE inhibition and AT_1 receptor blockade suggests that the antihypertrophic effects of these drugs do not only result from diminished AT_1 receptor stimulation, but also from increased stimulation of growth-inhibitory AT_2 receptors.

INTRODUCTION

ACE inhibitors prevent cardiac remodeling (i.e., ventricular hypertrophy and chamber dilatation) following myocardial infarction (MI). This occurs, in part, independent of their blood pressure-lowering effect,(1) suggesting interference with tissue angiotensin (Ang) II. However, ACE inhibitors do not always lower cardiac Ang II,(2-4) due to *(i)* their pharmacokinetic properties (short half life, lack of tissue penetration),](3, 4) *(ii)* ACE upregulation during prolonged ACE inhibition,(5) or *(iii)* alternative converting enzymes such as chymase.(6) Furthermore, previous Ang II measurements in infarcted hearts(4, 7, 8) have not taken into account that cardiac Ang II is partly derived from the circulation. Plasma Ang II accumulates in the heart through AT_1 receptor-mediated endocytosis,(2, 9) and changes in AT_1 receptor density following MI(10, 11) may influence this process.

AT_1 receptor antagonists have opened new avenues to investigate the importance of cardiac Ang II after MI. In general, these drugs increase rather than decrease Ang II levels,(12) and this may lead to activation of unoccupied, growth-inhibitory(13) AT_2 receptors. Currently, the effect of AT_1 receptor antagonism on the Ang II content of the infarcted heart is unknown. Furthermore, it is still controversial whether AT_1 receptor antagonism prevents the development of cardiac hypertrophy and increases survival following MI.(14, 15)

Therefore, the aim of the present study was to investigate changes in cardiac Ang II content and origin (local synthesis versus uptake from plasma) following MI in pigs, to compare the effects of ACE inhibition and AT_1 receptor antagonism on these changes, and to evaluate whether the effects of both RAS blockers on cardiac angiotensin content are related to their effects on post-infarct remodeling.

METHODS

Animals

Experiments were performed in accordance with the "Guiding Principles in the Care and Use of Laboratory Animals" of the Council of the American Physiological Society. Forty-nine 2-3 month-old Yorkshire x Landrace pigs of either sex entered the study. Adaptation of animals to laboratory conditions started 1 week prior to surgery.

Surgical Procedure

Animals were sedated with ketamine (30 mg/kg, im), anesthetized with thiopental (10 mg/kg, i.v.), intubated and ventilated with a mixture of O_2 and N_2O to which 0.2-1.0% (v/v) isoflurane was added.(16) Anesthesia was maintained with midazolam (2 mg/kg + 1 mg/kg/hour, i.v.) and fentanyl

(10 μg/kg/hour, iv). The chest was opened via a left intercostal space and a fluid-filled polyvinylchloride catheter was inserted into the aortic arch for hemodynamic monitoring and blood sampling.(16) Subsequently, the pericardium was opened, the proximal left circumflex coronary artery (LCXCA) dissected out, and a suture placed around the LCXCA. In 35 animals the LCXCA was permanently ligated, while in 14 animals (sham) the suture was removed. The pericardium was closed and the aortic catheter tunneled subcutaneously to the back. The chest was closed and animals were allowed to recover. Animals received analgesia (0.3 mg buprenorphine, im) for 2 days, and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin, i.v.) for 5 days.(16)

Experimental Groups

Animals were followed for 1, 3 or 6 weeks. Of the 3-week MI animals, 6 received captopril (25 mg b.i.d. orally), and 7 received the AT_1 receptor antagonist eprosartan (400 mg b.i.d. orally; a gift of Dr. P.K. Weck, SmithKlineBeecham, Collegeville, PA). This dose of eprosartan blocks Ang II-induced pressor responses by >95% (n=3). Treatment started 12-24 hours following LCXCA ligation and was continued for 3 weeks.

Echocardiography

At the end of the follow-up period, animals were sedated with ketamine, and 2-D echocardiographic recordings of the left ventricular (LV) short axis at midpapillary level were obtained (Sonos 5500, Hewlett-Packard) and stored for off-line analysis. LV end-diastolic (EDA) and end-systolic (ESA) cross-sectional areas were determined and ejection fraction (EF) was calculated as (EDA-ESA)/EDA x 100%.

Infusion of ^{125}I-Angiotensin I

Following echocardiography, pigs were anesthetized and prepared for hemodynamic monitoring, administration of ^{125}I-Ang I, and blood and tissue sampling.(9, 17) After collecting baseline measurements, animals were subjected to a 60-minute infusion of ^{125}I-Ang I ($\approx 4 \times 10^6$ cpm/minute) into the LV cavity. ^{125}I-Ang I and II reach steady-state levels in plasma and cardiac tissue within 10 and 60 min, respectively.(9)

Blood and Tissue Sampling

During follow-up, arterial blood samples were collected in the morning from awake animals, for measurement of norepinephrine (NE), epinephrine (E), atrial natriuretic peptide (ANP), N-terminal ANP (N-ANP), Ang I and Ang II.(2, 18, 19) During ^{125}I-Ang I infusion, arterial and coronary venous blood samples were collected from anesthetized animals for measurement of endogenous and ^{125}I-labeled Ang I and II.(2, 9) With the ^{125}I-Ang I infusion still running, the heart was stopped by fibrillation, left and right ventricle were separated and weighed, and 0.5-1 gram samples were

rapidly obtained from non-infarcted anterior LV wall, lateral LV wall (containing the infarct area and borderzone after MI), interventricular septum (IVS), and right ventricular (RV) wall. Samples were immediately frozen in liquid nitrogen, and stored at -70°C.

Biochemical Measurements

NE, E, ANP and n-ANP were measured as described before.(18, 19) Endogenous and ^{125}I-labeled Ang I and II were measured, after SepPak extraction and HPLC separation, by gammacounting and radiommunoassay, respectively.(2, 9)

Data Analysis

Fractional conversion and degradation of ^{125}I-Ang I in the coronary vascular bed, i.e., the percentage of arterially delivered ^{125}I-Ang I that is converted to ^{125}I-Ang II or degraded to other angiotensin metabolites during coronary passage, were calculated as described previously.(20) To quantify cardiac Ang I and II synthesis, tissue levels of Ang I and II were corrected for uptake from plasma using the steady-state plasma and tissue levels of ^{125}I-Ang I and II.(2) Cardiac tissue ^{125}I-Ang I was undetectable in nearly all animals. To prevent underestimation of the contribution of plasma Ang I to tissue Ang I, in-situ synthesized tissue Ang I was quantified under the assumption that tissue ^{125}I-Ang I equals 5% of the steady-state plasma levels of ^{125}I-Ang I.(2) Differences between sham and MI animals, and differences between treated and untreated animals were tested by two-way ANOVA or MANOVA, followed by Student's t-test. Statistical significance was accepted for $P<0.05$. Data are expressed as mean±SEM.

RESULTS

Mortality

All sham animals survived the follow-up period, but two sham animals died during ^{125}I-Ang I infusion due to technical failure. Eight MI animals died within 12 hours after induction of MI and 2 during eprosartan treatment on days 7 and 15.

Hemodynamic and Neurohumoral Characteristics

One day after surgery, mean arterial pressure (MAP) was lower and heart rate was higher in MI pigs compared to sham pigs (Figure 1). MAP partially recovered in the MI group during the first week, but remained below sham levels during follow-up. Plasma NE and E levels were similar in MI and sham animals, while plasma ANP and N-ANP were higher in MI animals during the entire follow-up period. Captopril and eprosartan did not affect any of the hemodynamic or neurohumoral parameters (data not shown).

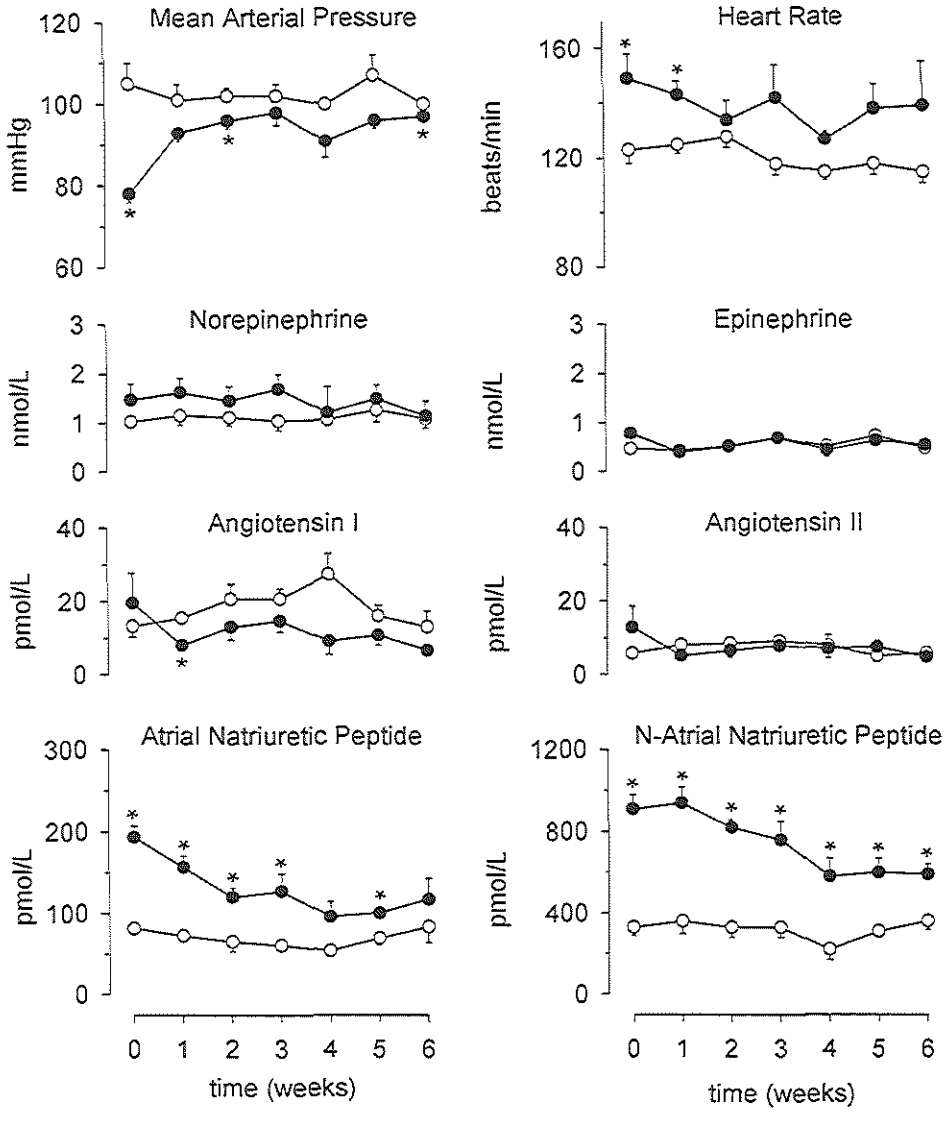

Figure 1. Mean arterial blood pressure, heart rate, and arterial plasma levels of neurohormones in sham (open circles) and MI (closed circles) animals. N-atrial natriuretic peptide indicates N-terminal atrial natriuretic peptide. * $P<0.05$ vs sham.

Remodeling

LV EDA and ESA were increased at 1 week after MI, so that EF was lower in MI animals (Table 1). EF recovered during the follow-up period, although LV EDA and ESA remained increased compared to sham hearts. Captopril and eprosartan blunted the increases in LV dimension, thereby slightly increasing EF.

Table 1. Hemodynamics and morphology

Parameter	1 Week		3 Weeks				6 Weeks	
	SHAM (n=4)	MI (n=4)	SHAM (n=5)	MI (n=6)	MI+Captopril (n=6)	MI+Eprosartan (n=5)	SHAM (n=3)	MI (n=3)
MAP, (mm Hg)	95±4	90±3	93±4	96±6	85±4	89±2	94±6	100±3
Heart rate, (bpm)	115±5	124±11	101±6	103±8	103±5	113±8	118±9	130±8
Cardiac output, (L/min)	2.2±0.1	1.9±0.2	3.0±0.2	2.7±0.1	2.8±0.3	2.2±0.3	2.8±0.2	3.5±0.2
LV dP/dt_{max} (mmHg/s)	2343±214	2221±301	2053±138	1706±78	1872±162	1690±127	1940±104	2053±99
LVEDP, (mm Hg)	7±2	10±2	8±2	14±2*	11±1	11±3	7±1	9±2
PAP, (mm Hg)	21±4	31±1	16±3	28±2*	25±4	24±3	23±5	31±3
EDA, (cm^2)	8.1±0.1	13.6±0.3*	9.7±1.4	12.8±1.5	11.8±1.2	11.6±0.7	10.0±0.3	15.8±4.2
ESA, (cm^2)	4.1±0.5	10.4±0.6*	4.6±0.8	8.6±1.6*	6.9±1.0	7.0±1.3	5.7±0.8	9.7±2.8
EF, (%)	50±6	24±3*	53±4	33±9*	42±3*	42±8	43±6	40±4
BW, (kg)	23.7±0.5	23.5±1.2	30.0±1.4	28.7±1.1	28.3±1.4	28.4±0.6	36.5±1.5	40.1±1.5
LVW, (g)	75.1±1.4	90.6±1.1*	82.7±6.2	102.2±5.2*	83.2±3.2†	82.5±4.4†	102.1±1.0	131.3±12.2*
RVW, (g)	30.7±2.7	32.5±2.8	27.3±1.0	40.1±3.1*	31.9±1.5*†	30.3±2.7†	27.8±2.5	52.9±4.7*
LVW/BW, (g/kg)	3.17±0.11	3.90±0.22*	2.75±0.16	3.57±0.14*	3.17±0.25†	2.91±0.16†	2.80±0.09	3.32±0.42
RVW/BW, (g/kg)	1.30±0.10	1.39±0.12	0.91±0.04	1.39±0.06*	1.14±0.06*†	1.06±0.08†	0.77±0.10	1.34±0.16*

Values are mean ±SEM. MAP indicates mean arterial pressure, LVEDP, LV end-diastolic pressure; PAP, pulmonary artery pressure,BW, bodyweight; LVW, LV weight; and RVW, RV weight.* $P<0.05$ vs. SHAM, † $P<0.05$ vs. untreated MI.

Table 2. Plasma angiotensin levels, angiotensin II/I ratios, and coronary angiotensin I metabolism.

Parameter	1 Week		3 Weeks				6 Weeks	
	Sham (n=4)	MI (n=4)	Sham (n=5)	MI (n=6)	MI+Captopril (n=6)	MI+Eprosartan (n=5)	Sham (n=3)	MI (n=4)
Aorta								
Ang I (pmol/L)	4.5±1.7	8.4±4.3	4.9±1.6	2.9±1.0	24.7±8.5*†	19.0±4.7*†	2.1±0.3	3.0±1.0
Ang II (pmol/L)	1.4±0.1	6.6±2.9	3.6±1.2	1.9±0.5	4.2±1.6	16.5±3.4*†	1.1±0.1	2.1±0.7
Ang II/I ratio	0.54±0.22	0.82±0.08	0.62±0.08	0.73±0.04	0.16±0.05*†	0.92±0.09	0.50±0.12	0.49±0.20
^{125}I-Ang I (10^3 cpm/L)	546±76	501±52	420±28	545±106	620±63	563±73	369±28	459±50
^{125}I-Ang II (10^3 cpm/L)	263±19	255±33	232±46	286±62	100±15*†	385±15	186±12	352±81
^{125}I-Ang II/I ratio	0.50±0.05	0.51±0.05	0.56±0.12	0.57±0.14	0.17±0.03*†	0.73±0.09	0.51±0.01	0.74±0.13
Coronary vein								
Ang I (pmol/L)	2.6±0.8	7.1±3.2	4.7±1.1	2.5±1.0	21.8±7.6*†	15.8±3.9*†	1.9±0.3	3.1±0.8
Ang II (pmol/L)	1.1±0.2	4.8±2.0	2.8±0.8	1.4±0.3	4.5±1.4	18.0±3.2*†	1.3±0.2	1.9±0.6
Ang II/I ratio	0.58±0.22	0.63±0.08	0.49±0.03	0.66±0.10	0.18±0.06*†	0.96±0.16	0.65±0.25	0.59±0.13
^{125}I-Ang I (10^3 cpm/L)	257±35	323±35	203±31	267±74	455±50	225±28	193±25	250±26
^{125}I-Ang II (10^3 cpm/L)	277±23	268±38	223±43	289±52	97±11*†	346±16	223±33	349±58
^{125}I-Ang II/I ratio	1.12±0.08	0.82±0.05	1.14±0.16	1.22±0.20	0.21±0.04*†	1.67±0.26	1.17±0.17	1.21±0.17
Coronary ^{125}I-Ang I metabolism								
fractional conversion (%)	24±1	17±1	20±4	25±2	4±1*†	26±4	26±4	24±1
fractional degradation (%)	29±2	20±4	31±6	31±4	23±1	31±5	22±4	22±2

Values are mean ± SEM. * P<0.05 vs. SHAM; † P<0.05 vs. untreated MI.

After MI, the surviving myocardium hypertrophied, as reflected by the increased LV weight (W) and RVW and LVW and RVW/body weight (BW) ratios (Table 1). Captopril and eprosartan attenuated LV and RV hypertrophy.

Hemodynamics during Anesthesia

Hemodynamics did not differ between MI and sham animals, except LV end-diastolic pressure (LVEDP) and pulmonary arterial pressure (PAP), which were higher (P<0.05) in MI pigs at 3 weeks (Table 1). Neither captopril nor eprosartan affected hemodynamics.

Angiotensin Levels in Plasma

In the awake state, plasma Ang I and II (Figure 1) were somewhat higher than under anesthesia (Table 2). However, the changes produced by MI and renin-angiotensin system (RAS) blockade in the awake state parallelled those under anesthesia. The Ang I and II levels in arterial and coronary venous plasma in MI animals were marginally higher (P=NS) than those in corresponding sham animals at 1 week. At 3 and 6 weeks, the levels were similar in both groups. Captopril increased plasma Ang I 5-10 fold, but did not alter plasma Ang II in MI animals. The plasma Ang II/I ratio decreased by approximately 80% during captopril treatment, indicating effective ACE inhibition.

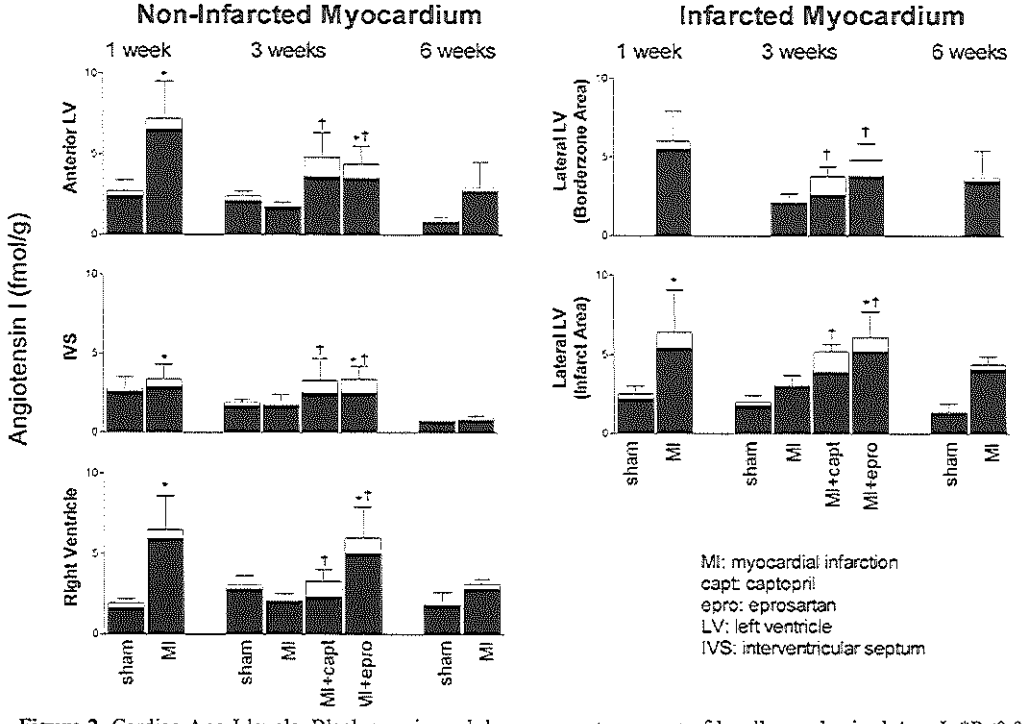

Figure 2. Cardiac Ang I levels. Black area in each bar represents amount of locally synthezised Ang I. *P<0.05 vs sham; †P<0.05 vs untreated MI.

Eprosartan increased plasma Ang I and II in MI animals 5-10 fold, without altering the plasma Ang II/I ratio. Neither the steady-state ^{125}I-Ang I and II levels nor the ^{125}I-Ang II/I ratios in arterial and

coronary venous plasma differed between sham and MI animals at any time point. Captopril reduced the ^{125}I-Ang II levels and the ^{125}I-Ang II/I ratio at both sampling sites, while eprosartan did not alter the steady-state plasma ^{125}I-Ang I and II levels. Angiotensin I Metabolism in the Coronary Vascular Bed

Coronary ^{125}I-Ang I-II conversion and ^{125}I-Ang I degradation in sham animals (Table 2) were not different from that reported in non-instrumented normal pigs.(21) MI did not alter Ang I conversion or degradation at any time point. Captopril inhibited conversion, but did not affect degradation. Eprosartan affected neither conversion nor degradation.

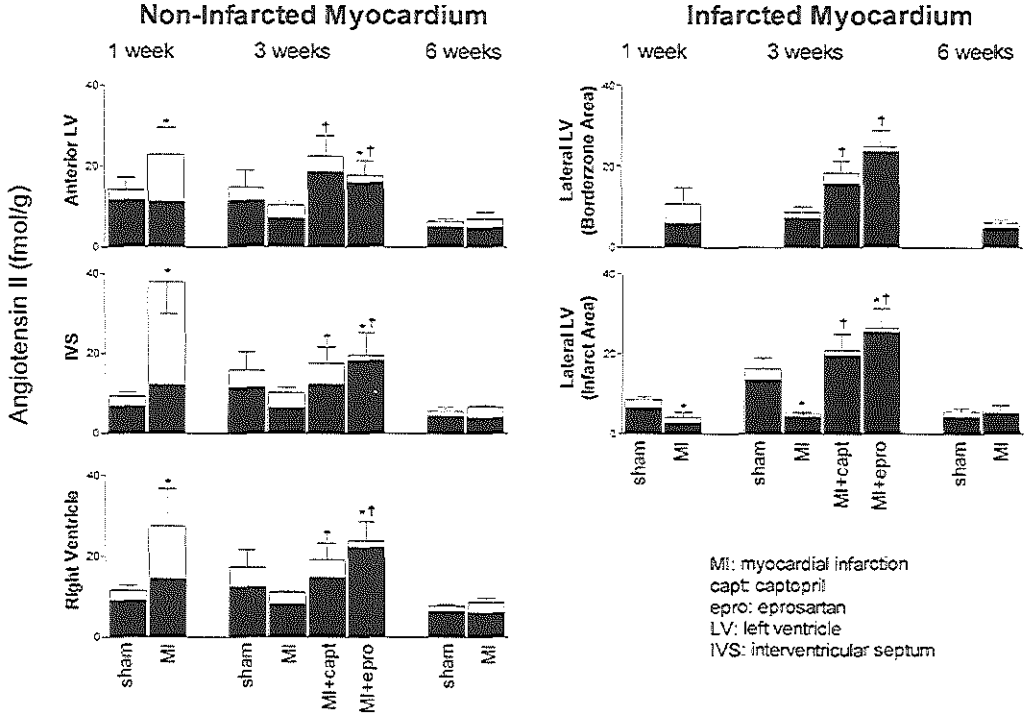

Figure 3. Cardiac Ang II levels. Black area in each bar represents amount of locally synthezised Ang II. *P<0.05 vs sham; †P<0.05 vs untreated MI

Angiotensin Levels in Tissue

Ang I and II levels in the non-infarcted myocardium were higher in MI animals than in sham animals at 1 week but not at 3 and 6 weeks (Figures 2 and 3). In sham animals, Ang II levels were similar at all myocardial sites, while in MI animals Ang II levels were lowest in the center of the infarct region and intermediate in the borderzone. Both captopril and eprosartan increased Ang I and II 2-3 fold at all tissue sites in MI animals (Figures 2 and 3), without altering the tissue Ang II/I ratios significantly (data not shown).

^{125}I-Ang I was undetectable in all regions, indicating that virtually all tissue Ang I had been produced in situ (Figure 2). ^{125}I-Ang II accumulated in cardiac tissue, reaching steady-state levels in the non-infarcted myocardium that were comparable to or higher than those in arterial plasma (Figure 4). Its levels in the infarcted and borderzone lateral LV wall were approximately 20-30% and 60-70% of those in arterial plasma. Blockade of cardiac ^{125}I-Ang II accumulation by eprosartan (Figure 4) indicates that this process is AT_1 receptor-mediated. There were no changes in ^{125}I-Ang II accumulation in the RV wall and the infarcted and borderzone lateral LV wall during the follow-up period. In sham animals ^{125}I-Ang II accumulation in the non-infarcted anterior LV wall and the IVS was increased at 1 week, but not at 3 and 6 weeks, as compared to normal, non-instrumented animals,(9) while it was elevated in MI animals at 1 week and 3 weeks but not at 6 weeks. Captopril abolished the increased ^{125}I-Ang II accumulation in the non-infarcted myocardium of MI animals at 3 weeks. Using the tissue ^{125}I-Ang II levels to correct for uptake of circulating Ang II, it appeared that the increase in cardiac Ang II levels in MI animals at 1 week was mainly due to uptake from plasma, whereas the increases in cardiac Ang II levels in captopril- and eprosartan-treated animals were due to Ang II generated in situ from locally synthesized Ang I (Figure 3).

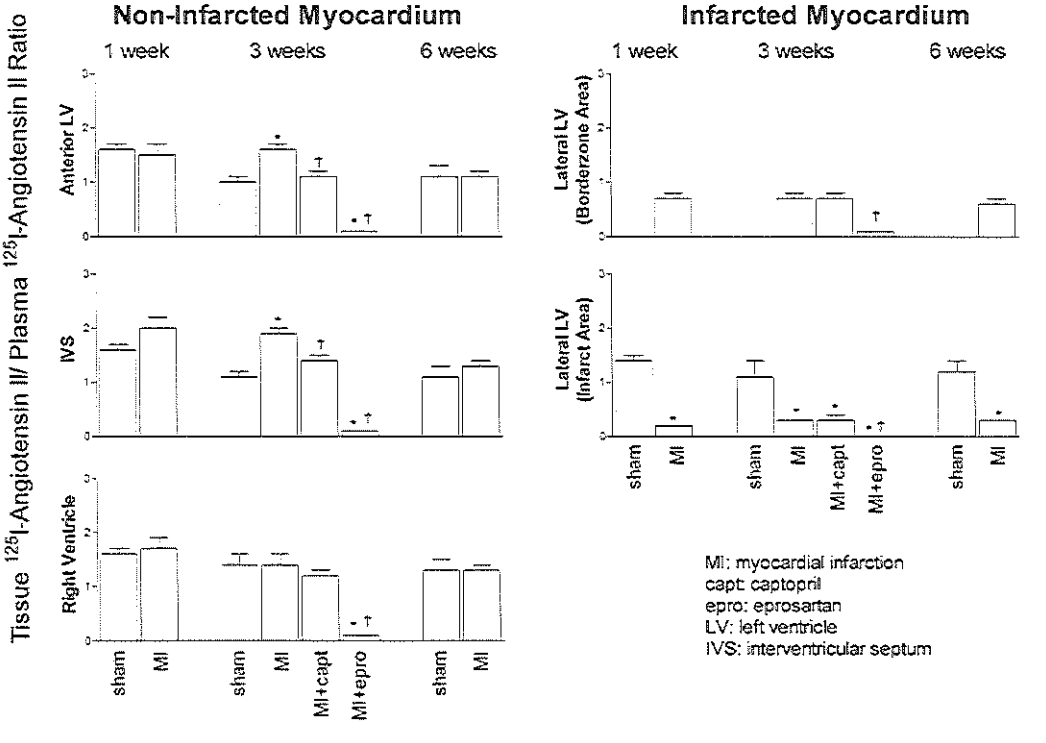

Figure 4. Tissue/plasma concentration ratios of ^{125}I-Ang II. *P<0.05 vs sham; †P<0.05 vs untreated MI.

DISCUSSION

This study demonstrates that, following MI, the Ang II levels in the non-infarcted left ventricle are transiently increased due to enhanced AT_1 receptor-mediated uptake of circulating Ang II. Captopril and eprosartan normalized or blocked this uptake and attenuated post-infarct remodeling, but increased local cardiac Ang II production. These results suggest that the antihypertrophic effects of RAS blockade results not only from diminished AT_1 receptor stimulation but also from increased stimulation of growth-inhibitory AT_2 receptors.

LCXCA ligation caused left and right ventricular hypertrophy within 1-3 weeks. MI was accompanied by a transient but severe decrease in MAP, which, via stimulation of renal renin release, may have caused the modest (non-significant) rise in plasma angiotensin levels in the first week. The hemodynamic and neurohumoral profile of the MI animals indicates that permanent LCXCA occlusion, which results in 15-25% infarction of the porcine LV, was associated with mild-to-moderate LV dysfunction, requiring minimal sympathetic activation to maintain cardiovascular homeostasis. This contrasts with an atrial pacing model in pigs, which led to severe heart failure and significant neurohumoral activation within 3 weeks.(22) Interestingly, in the latter model, LV AT_1 receptor density was decreased, while after MI LV AT_1 receptors are usually upregulated.(10, 23) The present findings are in agreement with such upregulation, since the amount of plasma-derived ^{125}I-Ang II sequestered by the non-infarcted LV myocardium via AT_1 receptor-mediated internalization at 1 week and 3 weeks after MI was twice as high as that in a previous study in normal pigs.(9) However, similar increases were observed in sham animals at 1 week after surgery, suggesting that this procedure, possibly through the induction of a fibrogenic response,(23) is partly responsible for the increase in AT_1 receptor density. It must be realized that ^{125}I-Ang II uptake in the heart is mediated exclusively via AT_1 receptors, since AT_2 receptors do not internalize Ang II.(9, 24) Furthermore, ^{125}I-Ang II formation from ^{125}I-Ang I that has diffused into the interstitial space does not contribute significantly to the steady-state tissue levels of ^{125}I-Ang II.(9, 21)

The increase in AT_1 receptor-mediated uptake of circulating Ang II is responsible for the transient rise in Ang II levels in the non-infarcted LV myocardium, suggesting that circulating Ang II is among the factors initiating the development of LV hypertrophy, even when plasma Ang II is only marginally increased. This observation extends previous studies demonstrating that elevation of circulating Ang II, either through infusion of Ang II or through renovascular hypertension, induces biventricular hypertrophy within 2 weeks.(25) Increased ^{125}I-Ang II levels in the non-infarcted LV myocardium were no longer observed at 6 weeks, nor were they present in captopril-treated animals at 3 weeks. This, in combination with the notion that eprosartan fully blocked the uptake of circulating ^{125}I-Ang II at all myocardial locations, suggests that RAS blockade prevents the

development of LV hypertrophy, at least in part, by attenuating or blocking the AT_1 receptor-mediated uptake of circulating Ang II.

In addition to their effects on cardiac ^{125}I-Ang II uptake, both captopril and eprosartan increased plasma and cardiac Ang I. Since cardiac renin is derived from the circulation, both under normal circumstances and after MI,(26-28) these increases most likely reflect the rise in renal renin release that normally accompanies RAS blockade. It is currently unknown whether enzymes other than renin (e.g., cathepsin D[29]) contribute to Ang I generation in infarcted hearts. Similarly, the origin of cardiac angiotensinogen post-MI has not been fully elucidated, although recent data suggest that most angiotensinogen in infarcted hearts, (27) like in normal hearts, (26) is plasma-derived.

In the eprosartan-treated pigs, plasma and cardiac Ang II rose in parallel with Ang I, while in the captopril-treated pigs Ang II did not change in the circulation but increased in the heart. Consequently, the Ang II/I ratio, a measure of ACE activity, was decreased in plasma but not in the heart following captopril treatment.

The rise in cardiac Ang II synthesis with captopril may have several causes. First, captopril may not have entered the heart in sufficient quantities. This seems unlikely, since in an earlier study we observed a clear reduction in the cardiac Ang II/I ratio following 3 days of treatment with the same dose of captopril.(2) It is also unlikely in view of the favorable cardiac effects of captopril in humans following MI.(1) Second, cardiac Ang II synthesis might occur at intracellular sites(21) that cannot be reached by ACE inhibitors. Third, the initial captopril-induced blockade of cardiac tissue Ang I-II conversion(2) may have been compensated for during long-term treatment with this drug, either through an upregulation of ACE(5) or because alternative converting enzymes such as chymase(6) have come into play. Our findings do not provide evidence for changes in cardiac or coronary ACE activity, since neither the cardiac Ang II/I ratio nor coronary Ang I-II conversion were altered in MI animals. Although MI-induced ACE upregulation has been described,(23, 29) transgenic rats overexpressing cardiac ACE 40-fold have normal cardiac Ang II levels.(30) Moreover, we failed to observe ACE gene I/D polymorphism-related differences in vascular Ang I-II conversion despite profound effects of this polymorphism on plasma and tissue ACE levels.(31) Thus, elevated cardiac ACE levels, if present in MI pigs, do not necessarily result in elevated Ang II levels or Ang II/I ratios, but may explain why captopril decreases the cardiac Ang II/I ratio less effectively. Furthermore, chymase is present in the porcine heart,(32) and its concentration increases following MI.(33)

Does the rise in cardiac Ang II production during RAS blockade have a physiological function? If caused by chymase, one must realize that chymase is present in the cytosol of mast cells and in the extracellular matrix,(6) whereas ACE is located on the cell membrane, in close proximity to AT_1

receptors.(23, 34) Consequently, Ang II generated by chymase may couple less efficiently to AT_1 receptors than Ang II generated by ACE.(35)

AT_2 receptor antagonism abolishes the beneficial effects of AT_1 receptor blockade in MI rats.(36) This raises the possibility that the rise in Ang II in the present study results in stimulation of growth-inhibitory AT_2 receptors.(13, 24) The AT_2 receptor density is increased in infarcted and failing hearts,(10, 11) and because the net effect of Ang II depends on the AT_1/AT_2 receptor ratio,(13) it is indeed conceivable that growth inhibition occurs, not only during AT_1 receptor antagonism, but also during ACE inhibition, since the latter prevents the rise in AT_1 receptor density following MI (this study), and is, possibly, accompanied by chymase-dependent Ang I-II conversion at sites distant from AT_1 receptors.(34, 35)

Enhanced AT_2 receptor stimulation, together with diminished AT_1 receptor stimulation, might also explain why captopril and eprosartan prevented RV hypertrophy in MI animals. Both drugs minimally affected the increase in PAP that, in combination with the elevated RV Ang II levels at one week after MI, may have contributed to this hypertrophy.

Interestingly, the lowest ^{125}I-Ang II accumulation and local Ang II production were observed in the infarct area. The latter finding could indicate that in this area, possibly secondary to the reduced blood flow, renin uptake was diminished in parallel with the diminished ^{125}I-Ang II uptake. Alternatively, and perhaps more likely in view of the unaltered local Ang I production in this area, the low ^{125}I-Ang II and Ang II levels might be the consequence of increased local Ang II degradation.

In summary, MI results in a transient upregulation of AT_1 receptors in spared, non-infarcted myocardium which will cause enhanced sequestration of plasma Ang II even in the absence of changes in the circulating RAS. RAS inhibitors prevent the rise in plasma Ang II sequestration, either by interfering with myocardial AT_1 receptor upregulation or by blocking these receptors. Furthermore, these inhibitors increase tissue Ang II production, which through stimulation of cardiac AT_2 receptors may minimize post-infarct remodeling.

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Chapter 5

Minimal impairment of myocardial blood flow responses to exercise in the remodeled left ventricle early after myocardial infarction, despite significant hemodynamic and neurohumoral alterations.

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ABSTRACT

Objectives: Previous studies have demonstrated a decreased flow reserve in the surviving hypertrophied left ventricle (LV) early after myocardial infarction. Exacerbation of hemodynamic abnormalities and neurohumoral activation during exercise could exhaust flow reserve and thereby impair myocardial blood flow and O_2-availability. Consequently, we studied hemodynamic, neurohumoral and regional myocardial perfusion and metabolic responses to exercise in pigs with LV hypertrophic remodeling 3 weeks after a myocardial infarction produced by permanent left circumflex coronary artery ligation.

Methods and Results: Chronically instrumented pigs were exercised on a treadmill up to 85% of maximum heart rate. Pigs with a myocardial infarction (MI) had a lower cardiac output (21%), stroke volume (28%), $LVdP/dt_{max}$ (18%), systemic (22%) and pulmonary (20%) vascular conductance, and increased left atrial (225%) and pulmonary artery (75%) pressures, compared to normal pigs. In MI, the exercise-induced increases in cardiac pump function, and systemic and pulmonary vasodilation were blunted compared to normals. Consequently, perfusion of visceral organs became impaired during strenuous exercise, but cerebral and skeletal muscle blood flows were maintained. Exercise-induced increases in norepinephrine and endothelin levels were exacerbated and while relative sympathetic drive was maintained, cardiac responsiveness to norepinephrine was attenuated. Despite lower capillary densities in the hypertrophied non-infarcted LV and relative subendocardial hypoperfusion during exercise, which necessitated a slight increase in O_2-extraction, there was no metabolic evidence of overt myocardial ischemia during exercise as indicated by the arterio-coronary venous pH difference.

Conclusions: LV dysfunction and neurohumoral activation were present in pigs with a 3-week-old infarction, particularly during exercise. However, although myocardial perfusion and O_2-supply were slightly impaired, myocardial ischemia did not occur even during exercise up to 85% of maximum heart rate, suggesting that perfusion abnormalities do not contribute to LV dysfunction early after infarction.

INTRODUCTION

Heart failure is currently the only major cardiovascular disorder of which the incidence is increasing. Myocardial infarction is becoming an increasingly important risk factor for heart failure due to the reduction in acute infarction-associated mortality. The role of myocardial blood flow (MBF) abnormalities in the progression of left ventricular (LV) dysfunction following infarction to overt heart failure is incompletely understood, but *in vivo* studies in rats (1, 2) and pigs (3) indicate a reduction in MBF reserve of up to 35% in the surviving post-infarct LV myocardium, three to eight weeks after infarction. Since hemodynamic and neurohumoral abnormalities associated with LV dysfunction are exacerbated during exercise, MBF increments could be impaired during increased O_2-demand produced by exercise, thereby limiting myocardial O_2-availability. Consequently, we investigated the responses of MBF and myocardial metabolism to treadmill exercise in normal pigs (N) and pigs with a 3-week-old myocardial infarction (MI) to determine whether impaired MBF responses limit myocardial O_2-availability and result in myocardial ischemia. Since this is the first study of the responses to exercise in pigs with a myocardial infarction, we also determined the exercise-responses of LV function, systemic and pulmonary hemodynamics, and neurohormones.

METHODS

Animals

Studies were performed in accordance with the *Guide for the Care and Use of Laboratory Animals* (NIH publication 86-23, revised 1985), and with approval of the Animal Care Committee of the Erasmus University Rotterdam. Thirty-three 2-to-3-months-old Yorkshire X Landrace pigs (23±1 kg at the time of surgery) of either sex entered the study; 17 were designated to the MI group and 16 to the N group. Daily adaptation of animals to laboratory conditions started 1 week before surgery.

Surgery

Pigs were sedated (ketamine, 20 mg/kg IM), anesthetized (thiopental, 10 mg/kg i.v.), intubated and ventilated with O_2 and N_2O to which 0.2%-1% (vol/vol) isoflurane was added (4-7). Anesthesia was maintained with midazolam (2 mg/kg followed by 1 mg/kg per hour IV) and fentanyl (10 μg/kg per hour IV). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid filled polyvinylchloride catheter was inserted into the aortic arch for mean aortic pressure measurement (Combitrans® pressure transducers, Braun) and blood sampling for determination of blood gases (Acid-Base Laboratory Model 505, Radiometer), O_2-saturation and

hemoglobin concentration (OSM2, Radiometer), and computation of O_2-content, O_2-supply, and O_2-consumption (VO_2) (5, 6). An electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of aortic blood flow. A microtipped pressure-transducer ($P_{4.5}$, Konigsberg Instruments) was inserted into the LV via the apex. Polyvinylchloride catheters were inserted into the LV to calibrate the Konigsberg transducer LV pressure signal (5, 6), and into the left atrium to measure pressure and inject radioactive microspheres to determine regional blood flows (7). Catheters were inserted into the pulmonary artery to measure pressure, administer drugs and collect mixed venous samples (5, 6). An angio-catheter was inserted into the anterior interventricular vein for blood sampling, while a Doppler flow probe (2.0-3.0 mm, Crystal Biotech) was placed around the left anterior descending coronary artery (5, 6). The proximal left circumflex coronary artery (LCx) was permanently occluded in 17 MI (4, 8), which were monitored for one hour and if needed internally defibrillated (10-30 Joules). Two MI died due to recurrent fibrillation. Catheters were tunnelled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine IM) for 2 days and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin i.v.) for 5 days (5, 6). Four MI died during the first night after surgery.

Experimental protocols

Central and regional systemic, pulmonary and coronary hemodynamic and neurohumoral responses to exercise were studied in 16 N (31±1 kg) and 11 MI (29±1 kg), at 19±2 and 20±1 days post-surgery, respectively. After baseline measurements (lying, 0_L, and standing, 0_S) were obtained, a five stage treadmill exercise protocol was begun (1-5 km/h in N and 1-4 km/h in MI); data were collected during the last 30 s of each 3 min exercise stage [5,6].

Regional blood flows

In 11 N and 10 MI, regional blood flows were determined using the radioactive microsphere technique (4, 6). In N, radioactive microspheres were injected at rest (lying) and during exercise at 3 and 5 km/h. In MI, microspheres were injected at rest (lying) and during exercise at 3 and 4 km/h.To study the progression of LV dysfunction over time, hemodynamic responses to treadmill exercise were additionally studied in N at 10±1 and 32±2 days, and in 9 MI at 9±1 and 32±1 days after surgery.

Coronary morphometry

In 6 N and 7 MI, hearts were fixed with 3% buffered formaldehyde via perfusion fixation and the anterior LV wall at mid-papillary level was dissected for morphometric analysis, dehydrated and embedded in paraffin. A minimum of 4 sections (4 μm thickness) were obtained, mounted on poly-L-Lysine coated glass slides, rehydrated and stained with hematoxylene/eosin (routine staining), resorcin/fuchsin to stain the elastic layers of arterioles, or lectine to stain capillary walls. Using a

microscopy image analysis system (Impak C, Clemex Vision Image analysis system, Clemex Technologies Inc.) slides were analyzed at 4X and 40X magnification for arteriolar and capillary densities, respectively.

Data analysis

Digital recording and off-line analysis of hemodynamics and regional blood flows have been described previously (5-7). We have previously shown that epicardial coronary artery Doppler blood flow measurements show an excellent correlation with myocardial tissue blood flow measurements with the radioactive microsphere technique (6, 9). Consequently, Doppler flow data were normalized per gram of myocardium using the flow data obtained with the radioactive microsphere technique, as previously described (9). In arterial blood samples, catecholamines, renin, angiotensin II, atrial natriuretic peptide (ANP), N-terminal ANP (N-ANP), and endothelin were determined (7). Statistical analysis was performed using two-way (exercise level and infarction) analysis of variance (ANOVA) for repeated measures, followed by Dunnett's test (exercise effect) or unpaired *t*-testing (MI vs N). Analysis of co-variance (ANCOVA) for repeated measures was used to test for statistically significant differences in relations between hemodynamic or neurohumoral variables and body or myocardial VO_2 (with VO_2 as a co-variate) and between hemodynamic variables and norepinephrine (with norepinephrine as a co-variate) in MI versus N. Significance was accepted when $P<0.05$. Data are mean±SEM.

RESULTS

Hemodynamics and O_2-transport and O_2-utilization

Systemic hemodynamics

Under resting conditions, MI were characterized by a lower cardiac output (20%), stroke volume (28%), and $LVdP/dt_{max}$ (18%), compared to N, while left atrial pressure was approximately two-fold higher (all $P<0.05$) and heart rate tended to be higher (12%, $P=0.07$; Fig. 1). Mean aortic pressure was maintained in MI due to a 22% decrease in systemic vascular conductance. In MI, the exercise-induced responses of cardiac output, $LVdP/dt_{max}$, LV systolic pressure and systemic vascular conductance were blunted compared to N.

Pulmonary hemodynamics

In resting MI, mean pulmonary artery pressure was elevated (75%), due to a higher left atrial pressure and a lower pulmonary conductance (Fig. 1). In both MI and N, mean pulmonary artery pressure increased during exercise, but in contrast to N, pulmonary vascular conductance did not increase in MI.

Systemic O_2-transport and O_2-utilisation

Arterial PO_2 was slightly lower in MI (98±3 mmHg at rest and 93±6 mmHg at 4 km/h) than in N (107±1 and 104±3 mmHg, respectively), but this did not result in arterial hemoglobin desaturation (Fig. 2). In MI, mixed venous SO_2 was lower than in N, both at rest and during exercise, reflecting an increased O_2-extraction which compensated for the lower cardiac output. Consequently, body VO_2 index (i.e. body VO_2 per kg body weight) was maintained in MI. There were no differences in arterial or mixed venous PCO_2 or pH between MI and N (not shown).

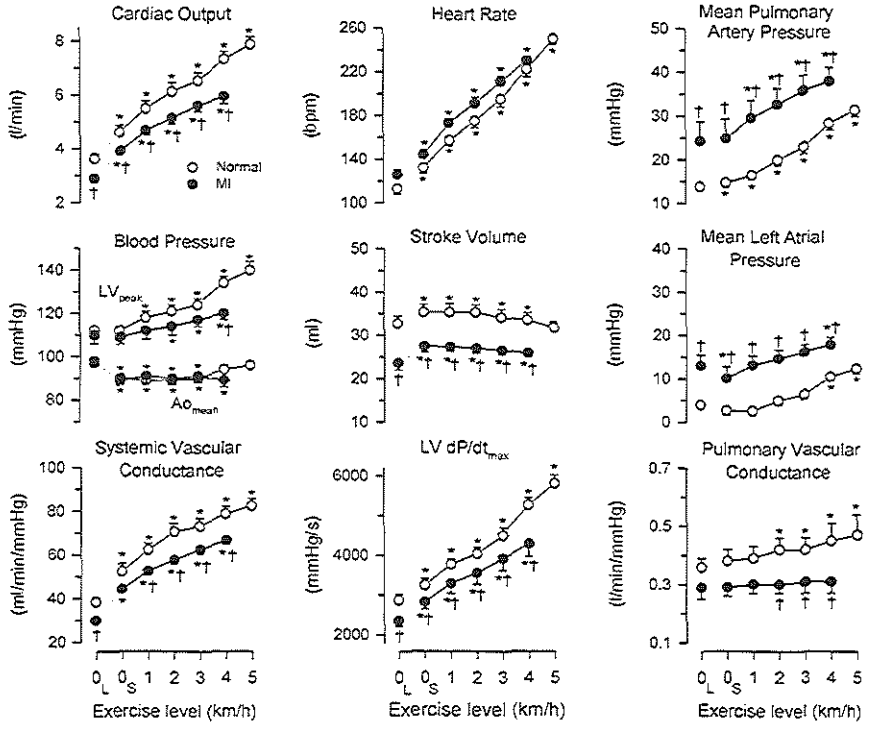

Figure. 1. Cardiovascular function in exercising MI ~3 weeks after myocardial infarction. 0_L=lying, 0_S=standing. Data are mean±SEM; *P<0.05 vs 0_L, †P<0.05 MI vs Normal at corresponding exercise level.

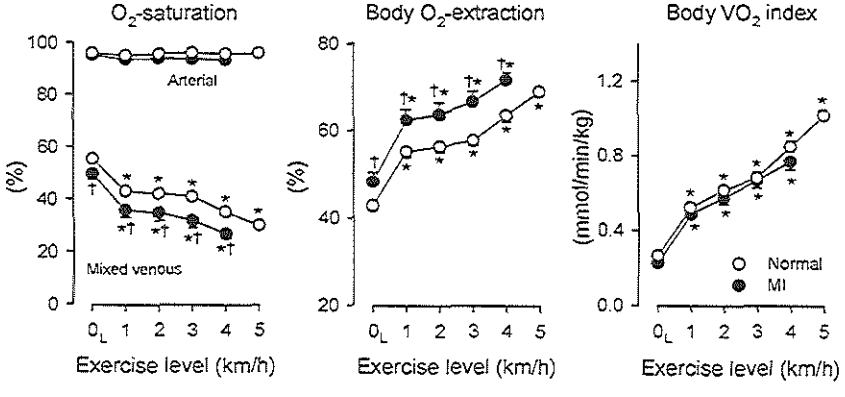

Figure. 2. Whole body O_2-balance in exercising MI ~3 weeks after myocardial infarction. VO_2=O_2-consumption. Data are mean±SEM; *P<0.05 vs 0_L, †P<0.05 MI vs Normal at corresponding exercise level.

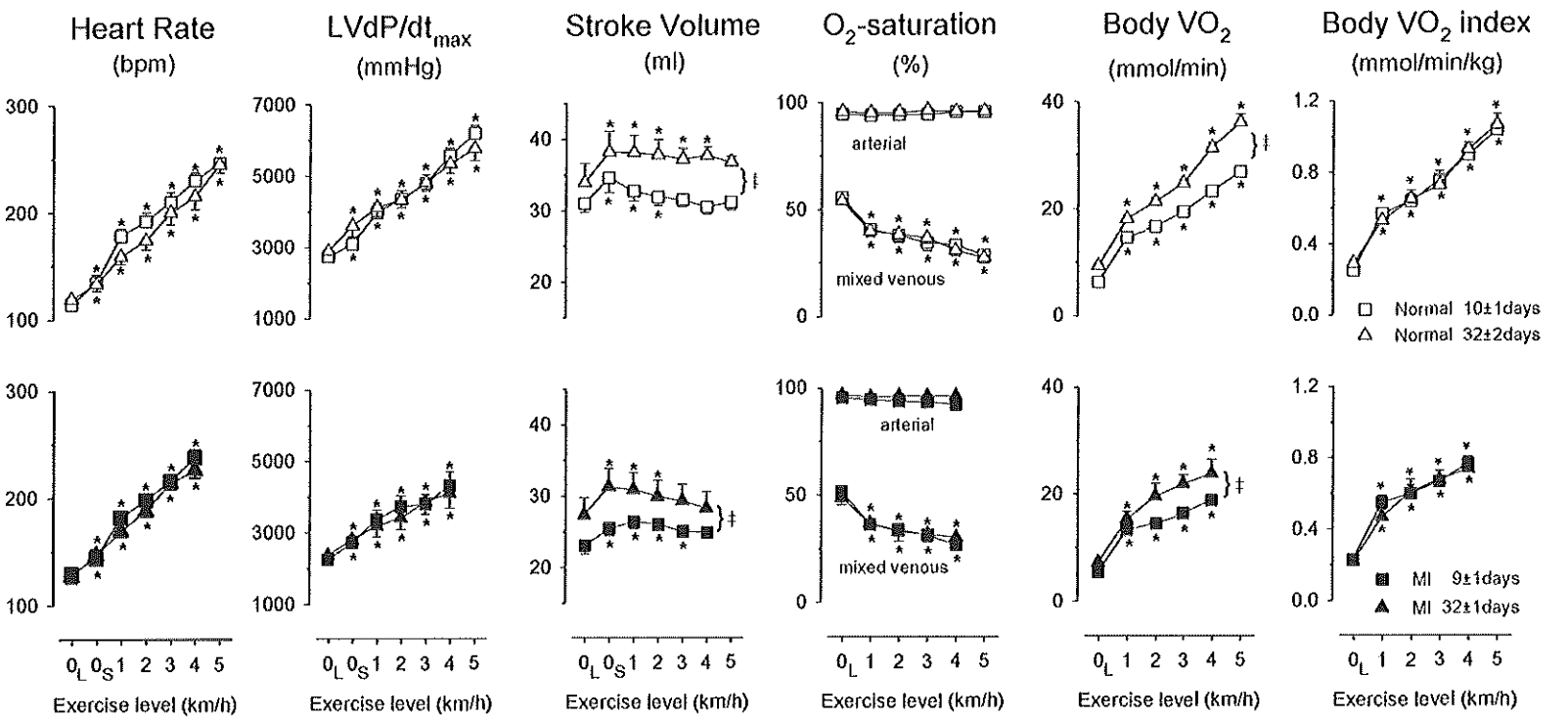

Figure 3.Time course of LV pump function and whole body O_2-balance in exercising MI -10 days and -30 days after myocardial infarction. VO_2=O_2-consumption. Data are mean±SEM; *P<0.05 vs 0_L, ‡P<0.05 -30 days vs -10 days (significant effect of time by two-way ANOVA).

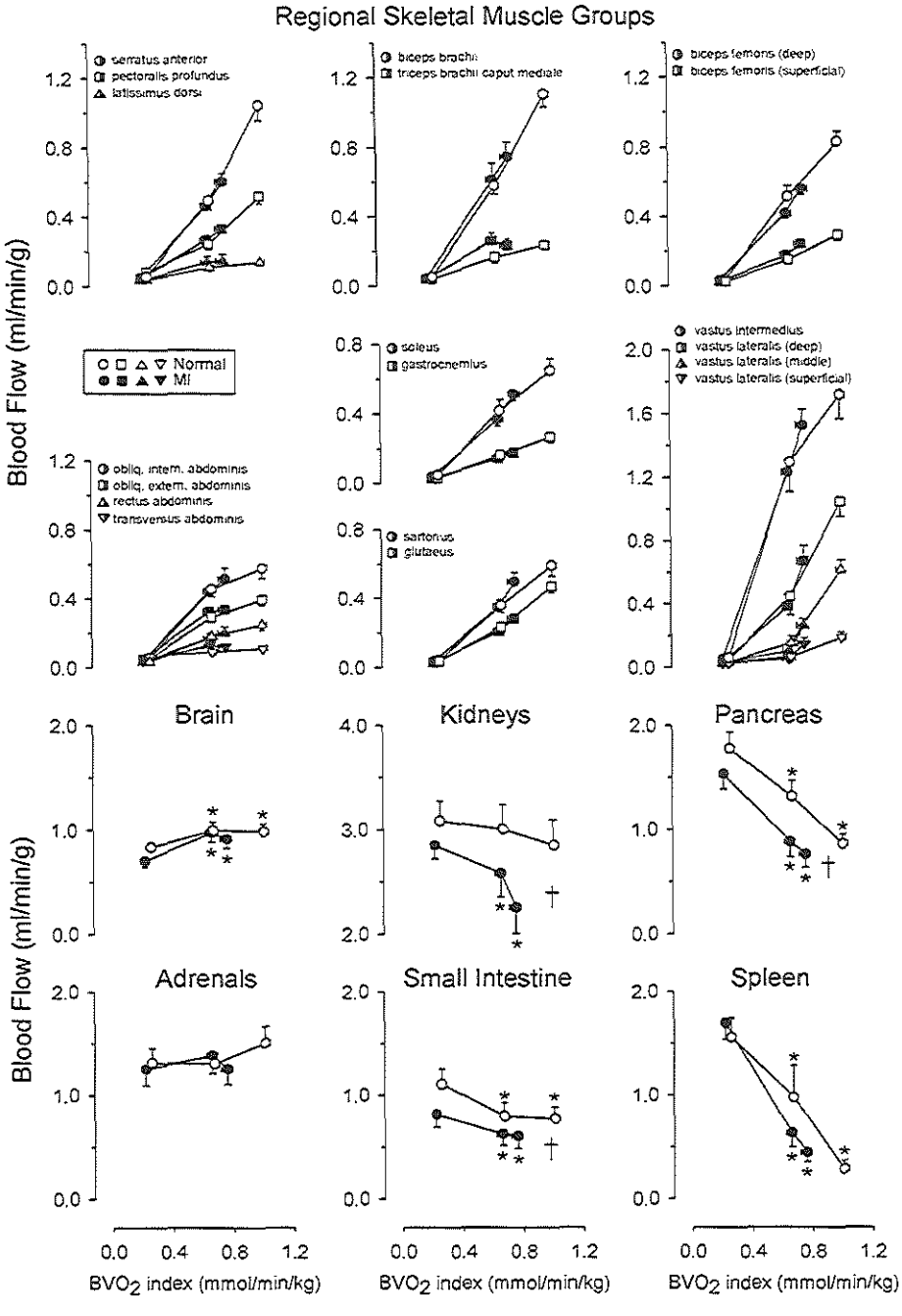

Figure. 4. Regional blood flows in exercising MI ~3 weeks after myocardial infarction. Data shown for N represent 0_L, 3 and 5 km/h; data shown for MI represent 0_L, 3 and 4 km/h. VO_2=O_2-consumption. Data are mean±SEM; *P<0.05 vs 0_L, †P<0.05 MI vs Normal (significant effect of infarction by ANCOVA). For the sake of clarity, symbols indicating a significant exercise-induced increase in skeletal muscle blood flow (which occurred at all exercise levels in all skeletal muscle groups for both MI and N) have been omitted.

Time course of hemodynamics and O_2-transport and O_2-utilization.

The hemodynamic responses to exercise between ~10 and ~32 days after surgery did not change in MI and N (Fig. 3). Only stroke volume and BVO_2 increased over this period, which was likely due to body growth (from 25±1 to 33±1 kg in MI and from 26±1 to 33±1 kg in N), because stroke volume index (not shown) and BVO_2 index were unchanged. These findings indicate that the degree of LV dysfunction and the circulatory adaptations in MI were stable over this time period.

Regional blood flows

In MI, resting blood flows and exercise-responses of blood flow to the brain, adrenals, spleen and various skeletal muscle groups were similar to those in N (Fig. 4), but the relations between body VO_2 and blood flows to kidneys, small intestine, and pancreas were shifted down towards lower blood flows ($P<0.05$ by ANCOVA).

Neurohormones

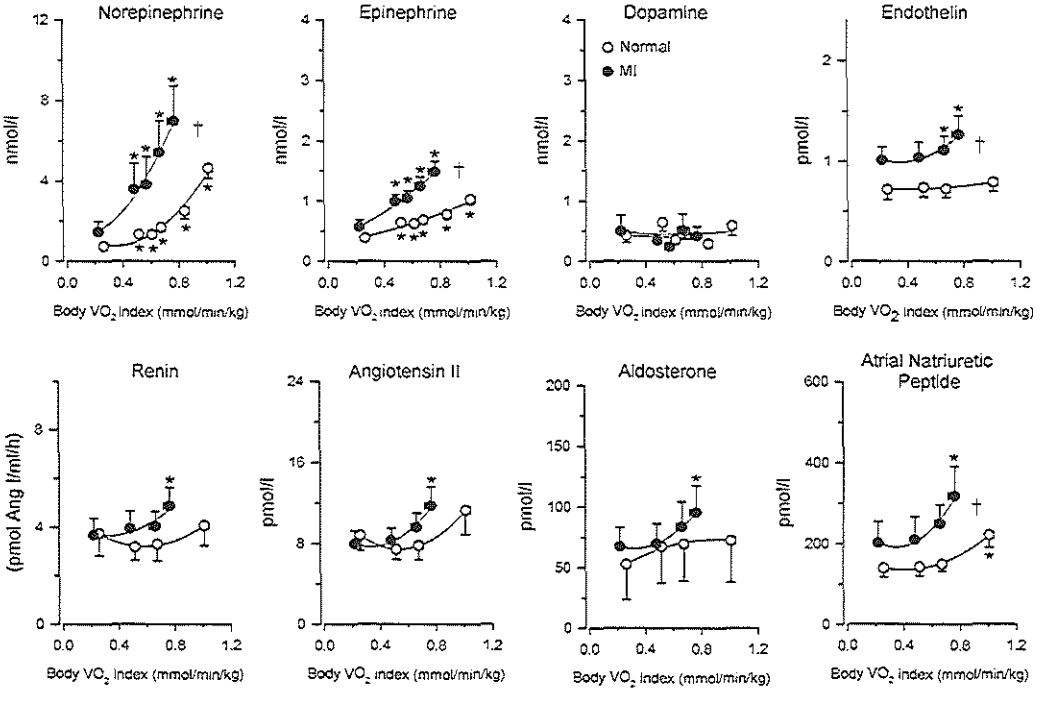

Figure 5..Neurohumoral responses in exercising MI ~3 weeks after myocardial infarction. In the norepinephrine, epinephrine and dopamine panels, data shown for N represent 0_L, 1, 2, 3, 4 and 5 km/h and data for MI represent 0_L, 1, 2, 3 and 4 km/h. In all other panels, data shown for N represent 0_L, 1, 3 and 5 km/h and data for MI represent 0_L, 1, 3 and 4 km/h. Mean data points were fitted with second order curves. VO_2=O_2-consumption. Data are mean±SEM; *$P<0.05$ vs 0_L, †$P<0.05$ MI vs Normal (by ANCOVA).

In MI, circulating levels of renin, angiotensin II and aldosterone were not different from N under resting conditions, while norepinephrine, epinephrine and ANP tended (all $P>0.10$) to be higher. Endothelin levels (Fig. 5) and N-ANP levels (1.04±0.19 vs 0.71±0.10 nmol/l, $P<0.05$, one-tailed), however, were significantly elevated in MI. Linear regression analysis of pooled individual data of both MI and N as well as of individual data within the MI group showed that in resting pigs endothelin levels correlated with pulmonary artery pressure ($r^2=0.49$ and $r^2=0.75$, respectively, both $P<0.01$) and pulmonary vascular conductance ($r^2=0.58$ and $r^2=0.66$, respectively, both $P<0.01$).

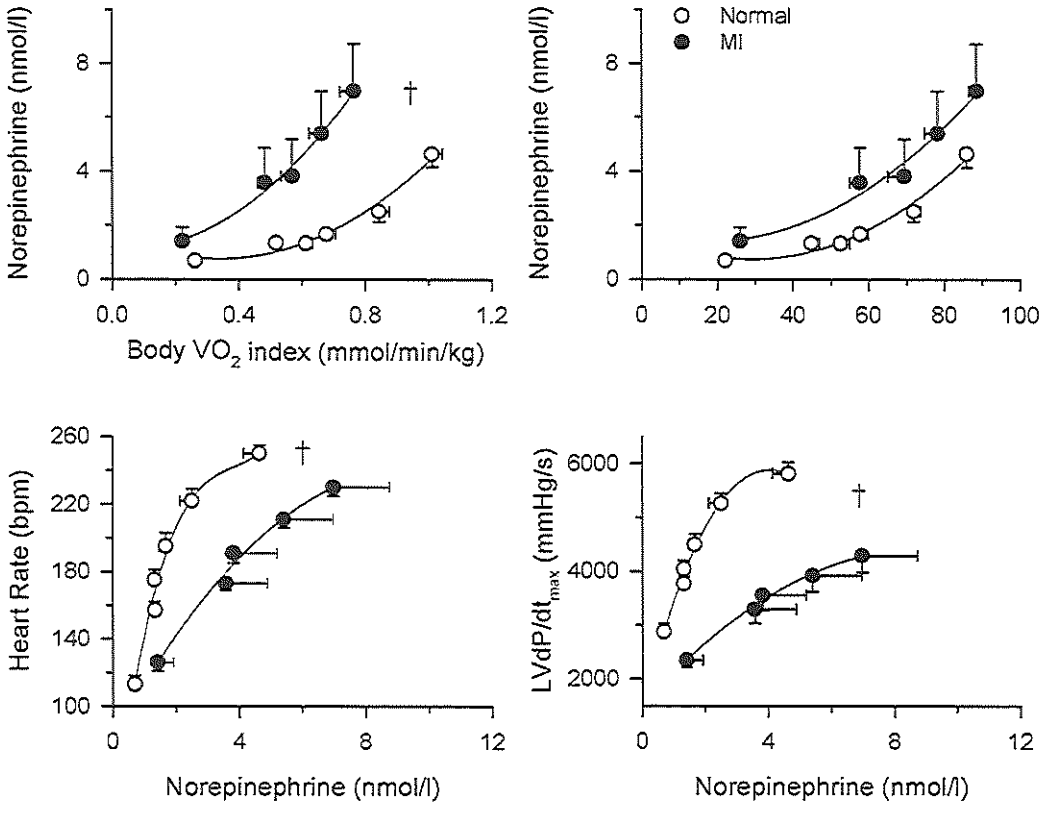

Figure. 6. Sympathetic responsiveness of the body and cardiac responsiveness to exercise-induced increases in norepinephrine in MI ~3 weeks after infarction. In the top right panel body VO_2 was expressed as a percentage of the estimated maximum body VO_2. In each pig, heart rate at the highest level of exercise was normalised as a percentage of 295 beats/min, which is the maximum heart rate for normal young pigs (6). Since heart rate and body VO_2 as a percentage of maximum correlate closely during exercise, maximum body VO_2 was computed and all body VO_2 values were normalised as a percentage of the estimated maximum body VO_2. In MI, we previously observed that heart rates during exercise at 4 km/h in the presence of atropine did not increase above 260 beats/min. Consequently, we normalised heart rate at 4 km/h to the estimated maximum heart rate of 260 beats/min, and normalised body VO_2 accordingly. Data shown for N represent 0_L, 1, 2, 3, 4 and 5 km/h and for MI represent 0_L, 1, 2, 3 and 4 km/h. Mean data points were curve fitted with second order or third order (normal animals in the lower panels) curves. Data are mean±SEM. Symbols indicating significant differences versus resting (0_L)

In MI, exercise resulted in exaggerated increases in circulating levels of norepinephrine, epinephrine, and ANP (all $P<0.05$ vs rest, and $P<0.05$ vs N), while endothelin, angiotensin II and aldosterone increased in MI (all $P<0.05$ vs rest at 4 km/h) but not in N. However, the exercise-induced increases in aldosterone, angiotensin II and the trend towards increased renin ($P=0.11$) in MI were not different from the responses in N.

Fig. 6 illustrates that the norepinephrine response to exercise, plotted as a function of percent of estimated maximum BVO_2, was similar in MI and N, indicating a preserved relative sympathetic drive. In contrast, cardiac responsiveness to circulating norepinephrine levels was blunted in MI compared to N, as higher levels of norepinephrine were required to produce the same level of heart rate and global LV contractility.

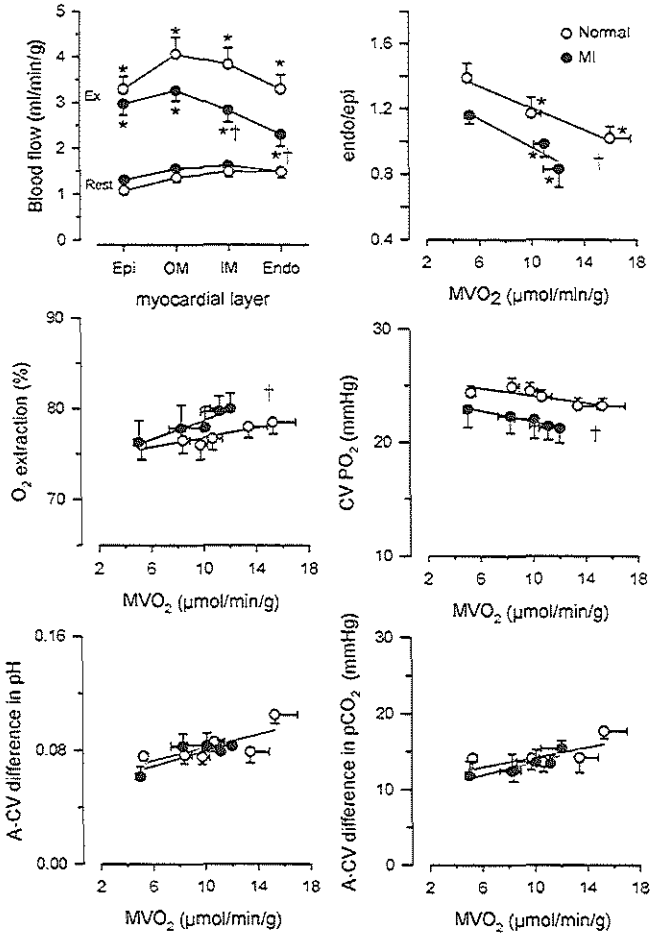

Figure 7. Myocardial blood flow and O_2-balance in the left ventricular anterior wall of N and MI ~3 weeks after myocardial infarction. Epi=subepicardial, OM=outer mid; IM=inner mid; Endo=subendocardial. MVO_2=myocardial O_2-consumption; A-CV = arterio-coronary-venous. In the top left panel data myocardial blood flow data are shown for resting (Rest, lying) conditions, and during maximum exercise (Ex, 5 km/h in N and 4 km/h in MI). Data are mean±SEM; $^*P<0.05$ vs 0_L, $^\dagger P<0.05$ MI vs Normal (by ANCOVA).

Cardiac anatomical data

Normalized right ventricular (RV) weight (RV to body weight ratio) was 47% higher in MI (1.62±0.20 g/kg) than in N (1.10±0.06 g/kg, $P<0.05$). Stepwise multivariate linear regression analysis showed that the degree of RV hypertrophy corresponded best with resting pulmonary artery pressure ($r^2=0.81$), with no additional contribution of resting levels of endothelin, or of any other neurohormone. LV to body weight ratio was 3.63±0.15 g/kg in MI and 3.23±0.08 g/kg in N, representing a 12% increase ($P<0.05$). The degree of LV hypertrophy did not correspond with any of the hemodynamic determinants (e.g. left atrial pressure, $r^2=0.15$) or resting levels of endothelin ($r^2=0.10$) or norepinephrine ($r^2=0.04$), but this may have been, at least in part, the result of the opposing effects of a large infarct on loss of viable tissue versus elevated loading conditions on the surviving myocardium.

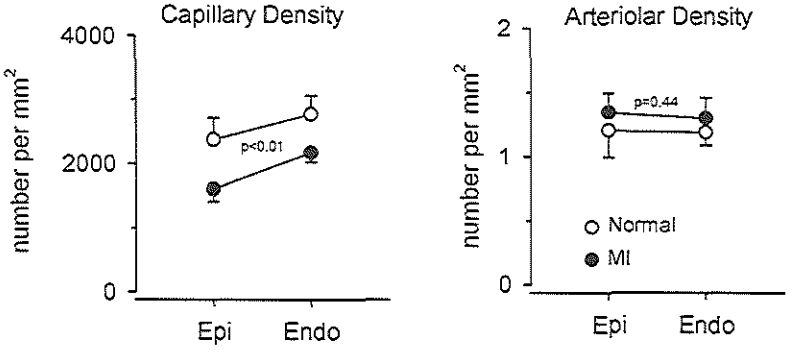

Figure 8. Capillary and arteriolar densities in the LV anterior wall in MI. Epi=subepicardial layer, Endo=subendocardial layer. Data are mean±SEM.

Coronary circulation

In MI, the relations between myocardial O_2-consumption and myocardial O_2-extraction (ratio of O_2-consumption and O_2-supply) and between myocardial O_2-consumption and coronary venous PO_2 in the LV anterior free wall were shifted up- and downward, respectively, compared to those in N (Fig. 7), indicating that arterial O_2-supply was slightly impeded in MI. The impediment of blood flow occurred principally in the inner half of the LV wall, but was only small and was not associated with a widening of the arterio–coronary venous pH difference, suggesting that ischemia did not occur.

In the LV anterior free wall, arteriolar densities were similar in MI and N, while capillary densities were lower in MI (Fig. 8).

DISCUSSION

This is the first study to investigate the integrated responses of LV pump function, neurohormones, (regional) systemic, pulmonary and coronary hemodynamics to graded treadmill exercise in a large animal model of LV dysfunction produced by myocardial infarction. The major findings in this study are that: (*i*) the exercise-induced increases in cardiac pump function and pulmonary and systemic vasodilation are blunted in MI, which is associated with impaired perfusion of splanchnic organs during strenuous exercise, but maintained cerebral and skeletal muscle blood flows. (*ii*) Exercise produces exaggerated increases in circulating norepinephrine, epinephrine, ANP and endothelin levels in MI, but the relative sympathetic drive is still maintained. In contrast, cardiac responsiveness to exercise-induced increases in norepinephrine is blunted. (*iii*) Myocardial capillary density is decreased and the subendocardium is hypoperfused in the surviving hypertrophied LV myocardium 3 weeks after MI, which necessitates a small increase in O_2-extraction during exercise but does not appear to cause overt myocardial ischemia. These findings suggest that perfusion abnormalities do not contribute significantly to the LV dysfunction that occurs in the first weeks following myocardial infarction.

Characteristics of LV dysfunction after myocardial infarction

LV dysfunction

LV dysfunction was produced by permanent ligation of the left circumflex coronary artery, which results in a circumscript transmural infarction of the lateral LV wall, comprising 20-25% of the total LV (10). LV dysfunction in awake resting MI was characterised by 20-30% decreases in cardiac output, stroke volume and $LVdP/dt_{max}$ and a tripling of LV filling pressure (13±3 mmHg). Previously we observed that during the first week after infarction significant LV dilation occurs, but that between 1 and 6 weeks after infarction no further LV dilation occurs (8). The present study shows that the difference between $LVdP/dt_{max}$, cardiac output and stroke volume in N and MI remained constant between ~10 and ~32 days, indicating that the degree of LV dysfunction and the circulatory adaptations in MI were stable during this observation period. In contrast, the global biventricular cardiomyopathy produced by 3-4 weeks of rapid ventricular pacing in dogs or pigs produces symptomatic and progressive heart failure (which is often reversible upon cessation of pacing, particularly during the initial stages of the process) that mimics severe end-stage human heart failure, reflected by 35-70% reductions in cardiac output and stroke volume (11-13), $LVdP/dt_{max}$ (12, 14), LV fractional shortening (12, 13), and dramatic elevations in LV filling pressures (up to 30 mmHg (12-14)).

During exercise, cardiac output, LV systolic pressure and $LVdP/dt_{max}$ increased almost in parallel in MI and N until 4 km/h, when curves began to diverge; 4 km/h was also the maximally attainable exercise level for most MI. In dogs with pacing-induced heart failure, exercise-induced increases in LV systolic pressure and $LVdP/dt_{max}$ are even more reduced (up to 70%), reflecting the more severe degree of dysfunction (14).

Neurohumoral activation.

Neurohumoral activation in resting MI was characterized by a trend towards elevated plasma levels of catecholamines but normal levels of the circulating renin-angiotensin-aldosterone system. The latter may have been due to the 50% increments in ANP and endothelin, which can suppress renin and aldosterone release (15). In a recent study we observed that over a six week follow-up period in resting MI, ANP doubled within 24 hours, recovered to 50% above normal values within 2 weeks and remained stable between 2 and 6 weeks after infarction, while renin and norepinephrine levels remained normal (8). In contrast, the more severe hemodynamic abnormalities observed in pacing-induced heart failure are accompanied by marked neurohumoral activation including four-to-twenty-fold elevations in plasma levels of catecholamines, renin, aldosterone and endothelin (13, 16).

In contrast to the discrete neurohumoral activation in resting MI, exercise resulted in exaggerated increases in catecholamines and ANP and increases in endothelin, angiotensin II, and aldosterone. Patients with increasing severity of heart failure display a progressive blunting of the relative sympathetic drive (17). While in the present study resting circulating levels of norepinephrine were still normal and the relative sympathetic drive in response to exercise was preserved in MI, the cardiac responsiveness to exercise (both heart rate and $LVdP/dt_{max}$) was already blunted 3 weeks after infarction. A selective increase in cardiac sympathetic drive, which precedes generalized activation in heart failure patients (18), may have contributed to the blunted cardiac responsivess to exercise-induced increases of norepinephrine, via β-adrenoceptor desensitization and/or downregulation.

Systemic circulation: O_2-transport and regional blood flows.

\Regional blood flows were slightly lower in visceral organs, under resting conditions and particularly during exercise, most likely due to the elevated catecholamine and endothelin levels. In contrast to the decreased skeletal muscle perfusion observed in rats (19, 20), skeletal muscle blood flow was maintained in MI, both at rest and during exercise. Nitric oxide is particularly important in maintaining blood flow during exercise in slow oxidative and fast-oxidative but not in fast glycolytic muscle fibers (21), and because nitric oxide production is blunted in heart failure we hypothesized that a possible loss of nitric oxide would impair blood flow particularly in those muscle groups that contain the highest number of oxidative fibers (i.e. the deep red muscle) (22).

However, inspection of Fig. 4 shows that flows were maintained in all skeletal muscle groups, suggesting that either nitric oxide production is unperturbed at this stage of post-infarction LV dysfunction or that other vasodilators compensated. These results also suggest that skeletal muscle perfusion abnormalities do not contribute to the impaired exercise capacity early after MI, which supports rehabilitation starting early after infarction, when skeletal muscle perfusion and function are still normal.

MI were still capable of maintaining a normal body O_2-consumption, even during exercise, by increasing O_2-extraction as compensation for the lower cardiac output. In contrast, in pacing-induced heart failure cardiac output can decrease up to 70% (13), which simply cannot be compensated by an increased O_2-extraction, resulting in limited O_2-availability even under resting conditions.

Pulmonary circulation.

Pulmonary artery pressure had almost doubled at ~3 weeks after infarction, which was associated with a 47% increase in RV weight. The increase in pulmonary artery pressure resulted from an increased left atrial pressure together with, at strenuous exercise-levels, a decrease in pulmonary conductance. The mechanism for the increased pulmonary tone cannot be determined from the present study, but could be due to loss of NO production. In addition, a role for endothelin is suggested by the inverse relation between endothelin concentrations and pulmonary conductance in resting pigs, which is in agreement with recent experimental and clinical heart failure studies (23). The present study also shows that ~3 weeks following infarction, exercise-induced pulmonary vasodilation is virtually absent in MI, possibly due to an exercise-induced increase in endothelin levels.

Coronary circulation

Marked decreases in myocardial perfusion occur in pacing-induced severe heart failure in pigs (24) and dogs (14, 25), especially in the more vulnerable subendocardial layers. Although one study in dogs indicated that the lower myocardial blood flow is principally the result of a lower myocardial VO_2 (14), studies in pigs suggest that the impaired perfusion is responsible for the deterioration of LV function because the ultrastructural changes resemble those that are associated with ischemia (i.e. interstitial edema and disruption of collagen fibers in the subendocardium) (26, 27). In animals models of compensated pressure – overload – induced severe LV hypertrophy, selective underperfusion of the subendocardium can produce myocardial ischemia during exercise and result in post-exercise myocardial stunning (28, 29). Repetitive exercise-induced stunning may lead to progressive functional and structural ischemic changes contributing to the deterioration of LV function over time (28, 29).

The contribution of perfusion abnormalities in the remote surviving myocardium after a myocardial infarction to the LV dysfunction remains unclear. Studies in rats (1, 2) demonstrated a 25-40% reduction in coronary flow reserve in the surviving myocardium at four (1) and eight (2) weeks after myocardial infarction. In addition, maximum subendocardial blood flow was blunted by 40% in anesthetized pigs with heart failure 3 weeks after a myocardial infarction (3). We hypothesized that the decreased flow reserve could limit the increase in myocardial blood flow to the hypertrophied myocardium during exercise when hemodynamic abnormalities and neurohumoral activation are exacerbated, thereby impairing myocardial O_2-supply. LV filling pressures were elevated in MI (to a similar level as in the study of Zhang et al. (3)), while endothelin and norepinephrine levels were increased, particularly during exercise.

Three weeks after infarction, myocardial blood flow per gram of tissue in the LV anterior wall of resting MI, was similar to that in resting N, confirming previous studies in rats and pigs (1-3). In contrast, during the initial 14 days after infarction, an increase in basal flow per gram of myocardium in the remote surviving part of the LV has been reported (23), which most likely reflects the increased myocardial metabolic demands at a time when LV dilation is present (8) but hypertrophy is still in progress. Interestingly, we observed a small trend towards higher blood flows in the outer two layers (P=0.09), suggesting that despite hypertrophy of the surviving myocardium (8), metabolic demand per gram of myocardium in the outer, but not the inner, layers was still slightly elevated, 3 weeks after infarction. The increase in LV to body weight ratio increased by only 12%, suggesting that LV hypertrophy in response to the volume-overload (in contrast to the 47% increase in RV to body weight secondary to the 75% increase in pulmonary artery pressure) was only modest. However, the LV to body weight ratio likely underestimates the degree of hypertrophy in the surviving portion of the LV myocardium, due to the loss of 20-25% of viable LV myocardium and replacement by scar tissue. Consequently, based on previous observations from our laboratory, the degree of hypertrophy of the surviving portion of the LV was more likely in the range of 25-30% (4).

During exercise, myocardial blood flow increased but was distributed away from the subendocardium towards the subepicardium in MI compared to N. The impediment of subendocardial blood flow and hence O_2-supply in MI, necessitated a small increase in O_2-extraction which resulted in a lower coronary venous PO_2. This effect was only small, although lower myocardial capillary density may have prevented a greater increase in O_2-extraction and a further lowering of coronary venous PO_2 in MI compared to N. On the other hand, the arterio-coronary venous pH difference was similar to N, suggesting that anaerobic metabolism was absent and that myocardial blood flow and O_2-consumption were still matched up to exercise levels at 85% of maximum heart rate. These findings indicate that despite increases in extravascular forces

(elevated LV filling pressures), increased circulating levels of catecholamines and endothelin and possibly loss of NO production, there is sufficient coronary vasodilator reserve to maintain myocardial blood flow commensurate with metabolic needs.

Clinical relevance

The present study indicates that 3 weeks after myocardial infarction, LV dysfunction is clearly present evidenced by hemodynamic abnormalities and neurohumoral activation at rest and particularly during exercise, but that myocardial blood flows are only slightly impaired leaving myocardial O_2-consumption unaffected. The lack of overt anaerobic metabolism even during heavy exercise suggests that myocardial perfusion abnormalities within the surviving myocardium do not contribute to LV dysfunction in the first few weeks after infarction. This also suggests that patients with a recent myocardial infarction can safely perform light to moderate exercise (<75% of maximum heart rate) without an increased risk of encountering myocardial ischemia, provided there is no significant coronary artery disease within the non-infarcted myocardial regions, and supports increasing efforts to initiate rehabilitation early after myocardial infarction as a therapeutic adjuvant to prevent deconditioning and progression of LV dysfunction (30).

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Chapter 6

Nitric oxide production is maintained in exercising swine with chronic left ventricular dysfunction

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ABSTRACT

Left ventricular (LV) dysfunction caused by myocardial infarction is accompanied by endothelial dysfunction, most notably a loss of nitric oxide (NO) availability. We tested the hypothesis that endothelial dysfunction contributes to impaired tissue perfusion during increased metabolic demands as produced by exercise, and determined the contribution of NO to regulation of regional systemic, pulmonary and coronary vasomotor tone in exercising swine with LV dysfunction produced by a 2-3-week old myocardial infarction (MI). LV dysfunction resulted in blunted systemic and coronary vasodilator responses to ATP, whereas the responses to nitroprusside were maintained. Exercise resulted in blunted systemic and pulmonary vasodilator responses in MI that resembled the vasodilator responses in normal swine (N) following blockade of NO synthase with N^{ω}-nitro-L-arginine (NLA, 20 mg/kg i.v.). However, NLA resulted in similar decreases in systemic (43±3% in N and 49±4% in MI), pulmonary (45±5% in N and 49±4% in MI) and coronary (28±4% in N and 35±3% in MI) vascular conductances in N and MI under resting conditions, while similar effects were also observed during treadmill exercise. Selective inhibition of iNOS with aminoguanidine (20 mg/kg i.v.) had no effect on vascular tone in MI. These findings indicate that while agonist-induced vasodilation is already blunted early after myocardial infarction, the contribution of endothelial NO synthase derived NO to regulation of vascular tone under basal conditions and during exercise is maintained.

INTRODUCTION

Heart failure is accompanied by increased production of neurohormones such as norepinephrine, angiotensin II and endothelin (2, 3), which serves to maintain arterial pressure by partially restoring cardiac output and increasing peripheral vascular resistance, in order to maintain perfusion of essential tissues like brain and heart at the expense of renal, pancreatic and intestinal blood flow. Several studies have indicated that also endothelial dysfunction, in particular a decreased production of nitric oxide (NO), contributes to an increased peripheral resistance in congestive heart failure (4-8). A loss of NO availability could aggravate left ventricular (LV) dysfunction due to the peripheral vasoconstriction-induced increase in LV afterload, coronary vasoconstriction, and increased myocardial O_2 consumption (6, 9-11). In support of this concept, a decreased NO production coincides with the transition of left ventricular dysfunction to overt congestive heart failure in models of pacing-induced heart failure (6, 10).

Clinically, the most common cause for heart failure is myocardial infarction. Studies on the role of NO in regulation of vasomotor tone in myocardial infarction-induced LV dysfunction in rats indicate that between 4 and 16 weeks after infarction, acetylcholine- or ADP-induced NO-mediated relaxation of systemic conductance arteries (12-16) and resistance vessels (17) is reduced, although this is not an unequivocal finding (18, 19). In contrast, basal NO production is maintained in systemic conductance arteries (12, 18), and in resistance vessels in various regional vascular beds *in vivo* (17, 19-21). These findings could be interpreted to suggest that early after infarction, a reduced NO-mediated vasodilator capacity could contribute to a blunted vasodilator response, and hence tissue hypoperfusion, during exercise (22), at a time when basal NO production may still be intact. Recently, we reported that in resting and exercising normal swine inhibition of NO-synthase with N^{ω}-nitro-L-arginine (NLA) decreased systemic and pulmonary vascular conductance, and flow to kidney, pancreas and part of the gut, whereas flow to skeletal muscle was unaffected (11). A similar pattern was found in exercising swine with a 3-week old myocardial infarction (23), suggesting that in this model of moderate LV dysfunction NO availability is already impaired, in particular during exercise. Consequently, we tested in the present study the hypothesis that loss of NO-mediated vasodilation contributes to abnormalities in vascular tone control in regional systemic, pulmonary and coronary beds in exercising swine with LV dysfunction after infarction. Since our results indicated that the contribution of NO to exercise-induced vasodilation was maintained, and because inducible NO synthase (iNOS) may be upregulated after infarction (24), we further tested whether upregulation of iNOS compensated for the potential loss of eNOS activity.

MATERIALS AND METHODS

Studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 86-23, revised 1985), and with approval of the Animal Care Committee of the Erasmus University Rotterdam. Thirty-six 2 to 3-month old Yorkshire X Landrace swine (22±1 kg at the time of surgery) of either sex entered the study; 22 swine were designated to the myocardial infarction group (MI) and 14 to the normal group (N). Results from 11 of the 14 N have been previously reported (11). Daily adaptation of animals to laboratory conditions started 1 week before surgery.

Surgical Procedures

Swine were sedated (ketamine, 20 mg/kg im), anesthetized (thiopental, 10 mg/kg iv), intubated and ventilated with O_2 and N_2O to which 0.2%-1% (vol/vol) isoflurane was added (1, 25-27). Anesthesia was maintained with midazolam (2mg/kg followed by 1 $mg \cdot kg^{-1} \cdot h^{-1}$ iv, for two hours) and fentanyl (5-10 $\mu g \cdot kg^{-1} \cdot h^{-1}$ iv). Under sterile conditions, the chest was opened via the fourth left intercostal space and a fluid filled polyvinylchloride catheter was inserted into the aortic arch for aortic blood pressure measurement (Combitrans® pressure transducers, Braun) and blood sampling for determination of blood gases (Acid-Base Laboratory Model 505, Radiometer), O_2-saturation and hemoglobin concentration (OSM2, Radiometer), and computation of O_2-content, O_2-supply, and O_2-consumption (26, 27). An electromagnetic flow probe (14-15 mm, Skalar) was positioned around the ascending aorta for measurement of cardiac output. A microtipped pressure-transducer (P4.5, Konigsberg Instruments) was inserted into the LV via the apex. Polyvinylchloride catheters were inserted into the LV to calibrate the Konigsberg transducer LV pressure signal (26, 27), and into the left atrium to measure pressure and inject radioactive microspheres to determine regional blood flows (25). Catheters were inserted into the pulmonary artery to measure pressure, administer drugs and collect mixed venous samples, while an angio-catheter was inserted into the anterior interventricular vein for blood sampling, and a Doppler flow probe (2.0-3.0 mm, Crystal Biotech) was placed around the left anterior descending coronary artery (26, 27). The proximal left circumflex coronary artery (LCx) was permanently occluded in 22 MI (1), which were monitored for one hour and if needed internally defibrillated (10-30 Joules). Six MI died due to recurrent fibrillation. Catheters were tunneled to the back and animals were allowed to recover, receiving analgesia (0.3 mg buprenorphine IM) for 2 days and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin iv) for 5 days (26, 27). Four MI died overnight during the first week after surgery.

Experimental Protocols

Agonist-induced vasodilation. The hemodynamic responses to the vasodilator ATP (50-200 $\mu g \cdot kg^{-1} \cdot min^{-1}$, iv) were determined in 5 resting MI and compared to 6 N. ATP was employed instead of acetylcholine, because acetylcholine is less effective as an endothelium-dependent vasodilator in the porcine coronary circulation. We have previously shown in N (11) that vasodilation produced by these doses of ATP is virtually abolished by pretreatment with NLA (20 mg/kg i.v.), indicating that at these doses ATP mediates vasodilation principally via NO. To study the responsiveness of the vascular beds to NO, we determined in the same animals the hemodynamic responses to the endothelium-independent NO-donor sodium nitroprusside (SNP, 0.5-5 $\mu g \cdot kg^{-1} \cdot min^{-1}$, i.v.).

Exercise. Central and regional systemic, pulmonary and coronary hemodynamic responses to exercise were studied in 14 N (29±1 kg) and 12 MI (27±1 kg), approximately 2-3 weeks after surgery. After baseline measurements (lying, 0_L, and standing, 0_S) were obtained, a treadmill exercise protocol was begun (1-4 km/h); data were collected during the last 30 s of each 3 min exercise stage (26, 27). After completion of the exercise protocol the swine were allowed to rest for 90 min, then NLA (20 mg/kg iv) was administered and the exercise protocol repeated (15).

On another day (separated by at least 4 days from the NLA protocol and performed in random order with the NLA protocol) the reproducibility of the responses to exercise were studied. Animals underwent a second control exercise trial 90 min after the first trial, as previously described (11, 26, 27).

On a different day (separated by at least 4 days from the NLA protocol), the exercise protocol was repeated in 4 MI, but before the second exercise protocol the iNOS inhibitor aminoguanidine (10-50-fold selectivity iNOS/eNOS (28, 29)) was administered in a dose of 20 mg/kg iv (infused in 15 min starting 30 min prior to exercise), which is in the dose range (10-30 mg/kg) that has been reported to produce a high degree of iNOS inhibition in rats (30), rabbits (31) and swine (32).

Regional Blood Flows

In 7 N and 7 MI, regional blood flows were determined on a separate day using the radioactive microsphere technique (11, 25). In N, radioactive microspheres were injected at rest (lying, 0_L) and during exercise at 5 km/h, while in MI, microspheres were injected at rest (lying) and during exercise at 4 km/h (maximum treadmill speed for most MI (23)).

Data Analysis

Digital recording and off-line analysis of hemodynamics and regional blood flow have been described previously (25-27). Statistical analysis was performed using two-way (exercise level and infarction) analysis of variance (ANOVA) for repeated measures, followed by Dunnett's test (exercise effect) or unpaired t-test (MI vs N). Analysis of co-variance (ANCOVA) for repeated measures was used to detect statistically significant differences of relations between hemodynamic

variables and body or myocardial VO_2 (with VO_2 as a co-variate) in MI versus N. Significance was accepted when $P<0.05$. Data are mean±SEM.

RESULTS

Cardiac anatomical data

Despite the loss of viable LV myocardial tissue due to infarction, the LV weight to body weight ratio was 15% higher than in normal swine (3.69±0.16 g/kg vs 3.18±0.17 g/kg, $P<0.05$). Right ventricular weight to body weight ratio was also higher in MI compared to N (1.71±0.23 g/kg vs 1.14±0.13 g/kg, $P<0.05$), which correlated well with the elevated pulmonary artery pressure in MI ($r^2=0.63$; $P<0.05$).

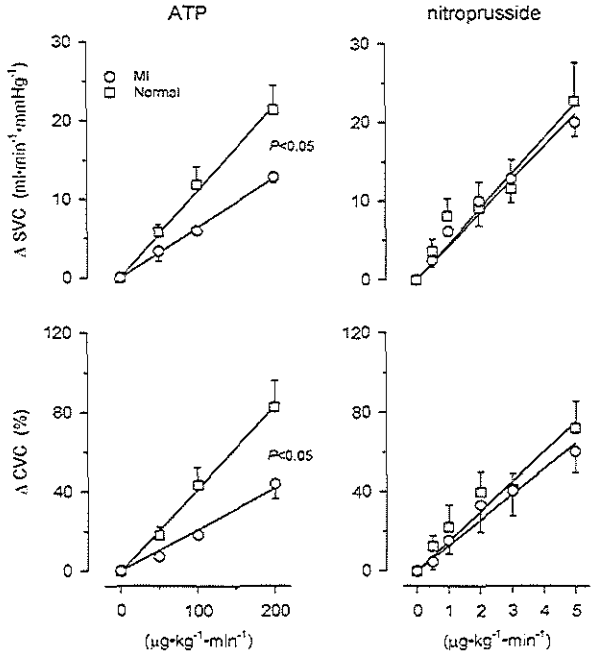

Figure 1. Increases in systemic vascular conductance (SVC = cardiac output / mean arterial pressure) and coronary vascular conductance (CVC = coronary blood flow / [mean arterial pressure – mean left atrial pressure]) vascular conductances in MI and N. The changes in CVC have been presented as % changes because of differences in baseline flow (see Table 1) due to the presence of LV hypertrophy in MI (1). Note that ATP-induced vasodilation was blunted ($P<0.05$ by ANOVA) whereas nitroprusside-induced vasodilation was maintained. Data are mean±SEM.

Agonist-induced vasodilation

The NO-dependent vasodilator ATP caused dose-dependent dilation of the systemic and coronary circulation (Fig. 1), but did not result in significant pulmonary vasodilation (not shown). At each dose of ATP, dilation was less in MI than in N, indicating either a reduced NO production, an increased NO degradation or reduced vascular smooth muscle responsiveness to NO after MI. The vasodilator response of systemic and coronary vascular beds to the NO donor SNP were comparable in MI and N, suggesting that the blunted vasodilator response to ATP was not due to a reduced bioavailability or vascular responsiveness to NO.

Role of NO at rest and during exercise

Table 1: *Effects of NO synthase inhibition on hemodynamics in MI and N*

Group		Treatment	Rest		Exercise Level (km/h)			
			Lying	Standing	1	2	3	4
Systemic hemodynamics								
HR	N	Control	118 ± 5	137±5*	169±6*	186±7*	204±6*	233±5*
(bpm)		NLA	94 ± 5†	120±4*†	136±4*†	148±5*†	165±6*†	192±7*†
	MI	Control	134 ± 4‡	145±5*	178±4*	195±5*	212±6*	230±6*
		NLA	103 ± 6†	124±6*†	143±4*†	155±4*†	172±5*†	196±6*†
CO	N	Control	3.79 ± 0.20	5.10±0.29*	6.09±0.30*	6.60±0.30*	7.05±0.29*	7.63±0.33*
(L/min)		NLA	2.78 ± 0.13†	4.11±0.24*†	4.86±0.28*†	5.22±0.29*†	5.84±0.32*†	6.60±0.31*†
	MI	Control	3.09 ± 0.21‡	3.85±0.25*‡	4.66±0.23*‡	5.05±0.24*‡	5.33±0.28*‡	5.79±0.36*‡
		NLA	2.09 ± 0.11†‡	2.84±0.16*†‡	3.43±0.17*†‡	3.70±0.19*†‡	3.93±0.29*†‡	4.39±0.35*†‡
SV	N	Control	32 ± 1	37±2*	36±1*	36±1*	35±1*	33±1
(ml)		NLA	30 ± 1	34±2*	36±2*	35±1*	36±2*	35±2*
	MI	Control	23 ± 2‡	26±2*‡	26±1*‡	26±1*‡	25±1*‡	25±1*‡
		NLA	20 ± 1†‡	22±	24±1*†‡	24±1*†‡	23±2*†‡	22±2*†‡
MAP	N	Control	99 ± 2	91±2*	90±1*	89±1*	91±1*	90±1*
(mmHg)		NLA	131 ± 3†	122±2*†	126±3*	127±2*†	126±2*†	124±2*†
	MI	Control	91 ± 3‡	80±3*‡	82±3*‡	83±3*‡	84±3*‡	83±3*‡
		NLA	125 ± 5†	110±5*†	115±5*†	115±6*†	117±7*†	115±7*†
LVSP	N	Control	115 ± 4	117±2	121±3	123±2*	127±3*	133±3*
(mmHg)		NLA	142 ± 6†	142±4†	144±4†	145±4†	147±3†	150±4†
	MI	Control	103 ± 4‡	101±4*‡	107±4*‡	108±4*‡	110±4*‡	111±4*‡
		NLA	131 ± 5†‡	121±6*†‡	122±6*†‡	122±4*†‡	123±6*†‡	125±6*†‡
$LVdP/dt_{max}$	N	Control	2970 ± 150	3470±180*	4220±240*	4480±230*	4930±270*	5500±230*
(mmHg/s)		NLA	2290 ± 90†	2900±120*†	3480±160*†	3820±200*†	4510±250*†	5510±360*
	MI	Control	2360 ± 160‡	2660±160*‡	3140±220*‡	3430±240*‡	3600±250*‡	3890±250*‡
		NLA	2140 ± 110	2630±140*	3080±110*	3280±200*	3540±160*‡	4130±270*‡
Coronary hemodynamics								
CBF	N	Control	44 ± 4	62±6*	72±6*	76±6*	85±8*	93±11*
(ml/min)		NLA	40 ± 3	57±5*	70±6*	±7*	81±	±9*
	MI	Control	65 ± 3‡	76±5*‡	92±6*‡	102±8*‡	110±9*‡	121±9*‡
		NLA	59 ± 3†‡	70±5*†‡	78±5*†‡	86±8*†‡	94±6*†‡	108±8*†‡
CVC	N	Control	0.46 0.04	0.69±0.06*	0.82±0.07*	0.88±0.08*	0.98±0.09*	1.12±0.14*
($ml \cdot min^{-1} \cdot mmHg^{-}$)		NLA	0.32 ± 0.03†	0.48±0.04*†	0.57±0.05*†	0.60±0.05*†	0.67±0.06*†	0.75±0.07*
	MI	Control	0.81 ± 0.05‡	1.04±0.08*‡	1.29±0.08*‡	1.45±0.10*‡	1.60±0.13*‡	1.84±0.15*‡
		NLA	0.52 ± 0.03†‡	0.66±0.04*†‡	0.74±0.04*†‡	0.82±0.05*†‡	0.88±0.06*†‡	1.04±0.08*†‡
Pulmonary hemodynamics								
MPAP	N	Control	14 ± 1	14±1	18±2*	21±2*	23±2*	27±2*
(mmHg)		NLA	20 ± 1†	24±2*†	28±2*†	30±2*†	33±2*†	37±2*†
	MI	Control	26 ± 5‡	26±5‡	30±5*‡	33±5*‡	36±5*‡	38±4*‡
		NLA	34 ± 5†‡	37±5†‡	42±5*†‡	44±5*†‡	48±4*†‡	51±4*†‡
MLAP	N	Control	4 ± 1	2±1	2±2	3±2	6±2	9±2*
(mmHg)		NLA	8 ± 1	4±1*	4±1*	6±1	7±1	10±1*
	MI	Control	12 ± 3‡	9±2‡	11±2‡	13±1‡	15±1‡	16±1‡
		NLA	15 ± 1‡	10±1‡	13±1‡	13±1‡	15±1‡	15±1‡

HR, heart rate; CO, cardiac output; SV, stroke volume; MAP, mean arterial pressure; LV, left ventricular; LVSP, left ventricular systolic pressure; $LVdP/dt_{max}$, maximum rate of rise in LV pressure; CBF, coronary blood flow; CVC, coronary vascular conductance (CVC = CBF/(MAP-MLAP)) were computed); MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure. NLA: N^{ω}-nitro-L-arginine 20 mg/kg iv.

Data are mean ± SEM; N, n=14; MI, n=12; *$P \leq 0.05$ vs. Rest (Lying); †$P \leq 0.05$ vs. corresponding Control; ‡$P \leq 0.05$ MI vs. N.

peripheral vasoconstriction in both MI and N, as indicated by the decrease in systemic vascular conductance (SVC, Fig. 2). The systemic vasoconstriction markedly increased aortic blood pressure despite concomitant decrease in cardiac output, which was due to a decrease in heart rate, probably mediated by the baroreceptor reflex (Table 1). The decrease in cardiac output was accompanied by an increase in body O_2 extraction, so that whole body O_2 consumption was maintained, resulting in a decrease in mixed venous PO_2 (Fig. 2). These responses to NLA were similar in MI and N, indicating that the contribution of NO to the regulation of vasomotor tone in the systemic vascular bed was maintained 2-3 weeks after infarction.

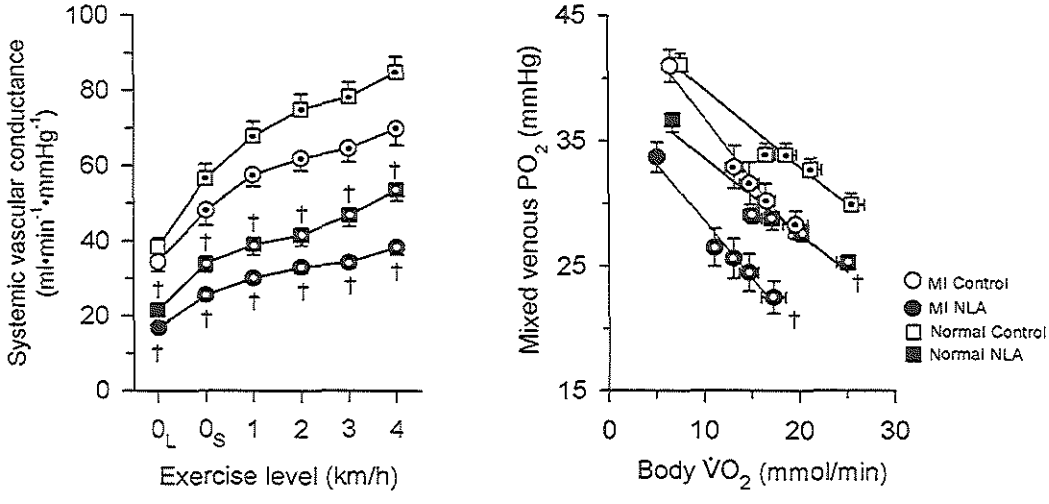

Figure 2. Effect of inhibition of NO synthase by NLA (20 mg/kg iv) on systemic vascular conductance at rest (0_L, lying; 0_S, standing) and during treadmill exercise (1-4 km/h; left panel), and on the relation between mixed venous PO_2 at rest (lying) and during treadmill exercise (1-4 km/h; right panel) in MI and N. Data are mean±SEM. The dot inside a symbol denotes $P<0.05$ vs. 0_L; $^{\dagger}P<0.05$ NLA vs. corresponding Control; there were no significant differences in the responses to NLA between MI and N.

In N, NLA blunted the exercise-induced increase in SVC (ΔSVC from 0_L to 4 km/h) from 46±2 during control exercise to 32±2 ml•min^{-1}•mmHg^{-1} during exercise in the presence of NLA ($P<0.05$, Fig. 2). In MI, the exercise-induced increase in SVC (35±3 ml•min^{-1}•mmHg^{-1}, $P<0.05$ vs control run in N) resembled that in N after NLA (P=NS). This should not be interpreted to suggest that a loss of NO production contributed to the blunted exercise-induced increase of SVC in MI, as NLA blunted the exercise-induced increase in SVC in MI (21±2 ml•min^{-1}•mmHg^{-1}) to the same extent as in N. Similarly, the effects of NLA on whole body O_2 extraction and mixed venous PO_2 were

virtually identical in N and MI (Fig. 2), indicating that the contribution of NO to the exercise-induced vasodilation of the systemic vascular bed was unperturbed 2-3 weeks after infarction.

Regional systemic vascular beds. NLA did not affect skeletal muscle blood flow in either N or MI, either at rest or during exercise (Fig. 3). The exercise-induced decrease in blood flow to various abdominal organs was more pronounced in MI than N. However, the NLA-induced decrease in flow to these organs was not different between MI and N both at rest and during exercise.

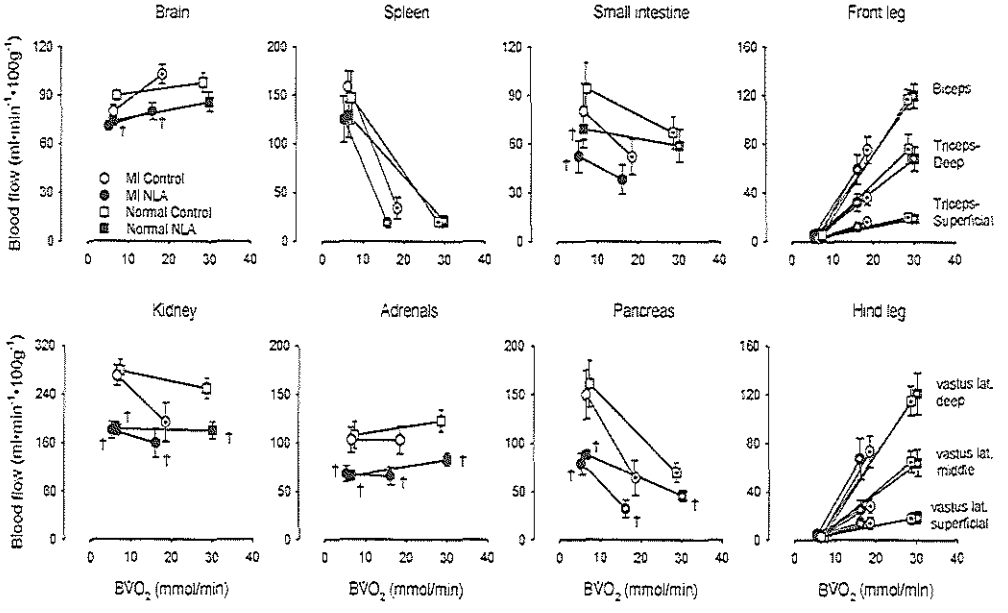

Figure 3. Effect of inhibition of NO synthase by NLA (20 mg/kg iv) on regional blood flows at rest (lying) and during treadmill exercise at 4 km/h (MI) or at 5 km/h (N). Regional blood flows are plotted as a function of whole body O_2 consumption (VO_2) to facilitate comparison of the exercise data obtained in MI (4 km/h) and N (5 km/h). Data are mean±SEM. The dot inside a symbol denotes $P<0.05$ vs. 0_L; $^{\dagger}P<0.05$ NLA vs. corresponding Control. There were no differences in the responses to NLA between MI and normal swine either at rest or during exercise.

Coronary circulation. NLA decreased coronary vascular conductance (CVC) at rest and during exercise, to a similar extent in MI and N (Table 1). The decrease in CVC was not simply the result of myogenic vasoconstriction secondary to the increase in aortic blood pressure, as it limited myocardial O_2 supply thereby necessitating an increase in myocardial O_2 extraction and resulting in a decrease of coronary venous PO_2 (Fig. 4). Importantly, the effects of NLA on CVC, O_2 extraction and coronary venous PO_2 were not different in MI and N, either at rest or during exercise, indicating that the contribution of NO to the regulation of vasomotor tone in the coronary resistance vessels was maintained in MI both at rest and during exercise.

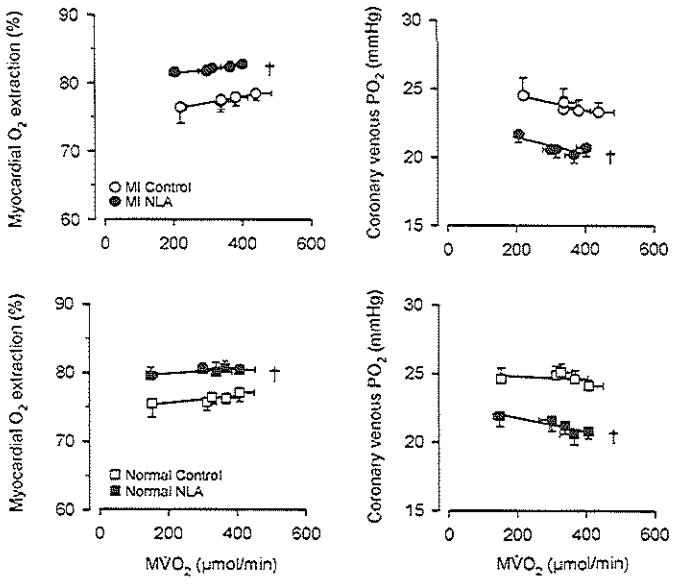

Figure 4. Effect of inhibition of NO synthase by NLA (20 mg/kg iv) on myocardial O_2 extraction and coronary venous PO_2 at rest (lying) and during treadmill exercise (1-4 km/h) in MI and N. Data are mean±SEM. The dot inside a symbol denotes $P<0.05$ vs. 0_L; †$P<0.05$ NLA vs. Control; there were no significant differences in the responses to NLA between MI and N either at rest or during exercise.

Pulmonary circulation. NLA produced similar increases in MPAP and decreases in PVC in MI and N under resting conditions (Table 1, Fig. 5). In N, NLA blunted the exercise-induced increase in PVC (ΔPVC from 0_L to 4 km/h) from 119±40 ml•min^{-1}•mmHg^{-1} during control exercise ($P<0.05$) to 53±33 ml•min^{-1}•mmHg^{-1} during exercise in the presence of NLA (Fig. 5). In MI, the exercise-induced increase in PVC (48±22 ml•min^{-1}•mmHg^{-1}) resembled that in N after NLA, suggesting that a loss of NO production contributed to the blunted exercise-induced in PVC in MI. However, the effects of NLA on PVC during exercise were similar in MI and N. These findings indicate that the contribution of NO to basal tone and the exercise-induced vasodilation of the pulmonary resistance vessels is unperturbed 2-3 weeks after infarction.

Contribution of iNOS

Since NLA in the dose used inhibits both eNOS and iNOS, we used the specific iNOS inhibitor aminoguanidine to determine whether increased levels of iNOS-derived NO acted to compensate for a loss of eNOS-derived NO. Aminoguanidine did not have any effect on systemic and coronary vascular conductance or on whole body and myocardial O_2-extraction either at rest or during exercise (Table 2), indicating that NO production by iNOS influenced neither basal tone nor exercise-induced vasodilation 2-3 weeks after infarction.

Reproducibility of the responses to exercise

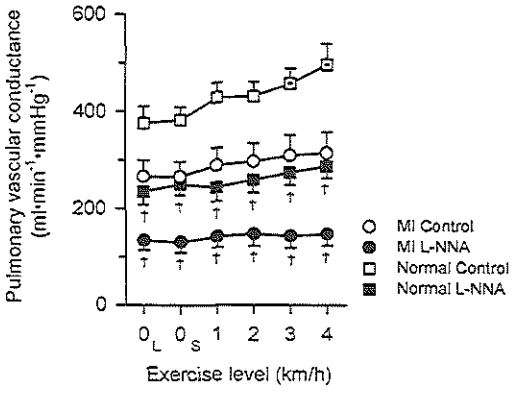

Figure 5. Effect of inhibition of NO synthase by NLA (20 mg/kg iv) on pulmonary vascular conductance (cardiac output / [mean pulmonary artery pressure - mean left atrial pressure]) at rest (0_L, lying; 0_S, standing) and during treadmill exercise (1-4 km/h) in MI and normal swine. Data are mean±SEM. The dot inside a symbol denotes $P<0.05$ vs. 0_L; †$P<0.05$ NLA vs. Control; there were no significant differences in the responses to NLA between MI and N.

Ninety minutes after the first control exercise period, at a time when all hemodynamic variables had returned to baseline resting values, a second period of exercise resulted in virtually identical hemodynamic responses to exercise (Table 3).

Table 2: *Effects of inducible NO synthase inhibition on hemodynamics in MI*

	Treatment	Rest	Exercise Level (km/h)			
		Lying	1	2	3	4
HR	Control	118 ± 4	164 ± 5*	181 ± 6*	201 ± 7*	236 ± 4*
(bpm)	AG	123 ± 6	171 ± 3*	184 ± 4*	198 ± 4*	228 ± 7*
MAP	Control	88 ± 1	79 ± 6*	80 ± 6*	83 ± 6*	84 ± 5*
(mmHg)	AG	90 ± 8	82 ± 6*	84 ± 6*	83 ± 6*	84 ± 6*
SVC	Control	43 ± 8	66 ± 7	69 ± 7*	72 ± 7*	78 ± 7*
($ml \cdot min^{-1} \cdot mmHg^{-1}$)	AG	37 ± 1	67 ± 8	69 ± 7*	74 ± 7*	79 ± 6*
BEO_2	Control	44 ± 1	52 ± 2	54 ± 3	60 ± 2	64 ± 2
(%)	AG	43 ± 1	54 ± 2†	57 ± 3†	60 ± 3	66 ± 2
CVC	Control	0.83 ± 0.07	1.26 ± 0.05*	1.36 ± 0.07*	1.51 ± 0.05*	1.88 ± 0.15*
($ml \cdot min^{-1} \cdot mmHg^{-1}$)	AG	0.93 ± 0.05	1.37 ± 0.08*	1.48 ± 0.06*	1.62 ± 0.04*	1.89 ± 0.12*
MEO_2	Control	75 ± 2	76 ± 2	75 ± 2	78 ± 1	78 ± 1
(%)	AG	74 ± 3	74 ± 2	75 ± 1	75 ± 1	77 ± 1

HR, heart rate; MAP, mean arterial pressure; SVC, systemic vascular conductance; BEO_2, Body O_2 extraction; CVC, coronary vascular conductance (CVC = CBF/(MAP-MLAP)) ; MEO_2, Myocardial O_2 extraction.
AG, aminoguanidine 20 mg/kg iv
Data are mean ± SEM; n=4; *$P \leq 0.05$ vs. Rest (Lying).

DISCUSSION

Table 3: *Reproducibility of systemic hemodynamic responses to exercise in MI and N*

	Gro	Treatment	Rest		Exercise Level (km/h)			
			Lying	Standing	1	2	3	4
Systemic								
HR	N	Control I	119±4	140±4*	166±5*	184±5*	208±6*	232±6*
(bpm)		Control II	118±4	144±6*	163±5*	175±5*	201±6*	223±7*
	MI	Control I	125±4	143±5*	172±7*	192±7*	222±6*	237±6*
		Control II	124±5	142±4*	172±6*	196±8*	214±6*	235±7*
CO	N	Control I	3.85±0.24	5.10±0.32*	5.69±0.29*	6.41±0.33	7.04±0.33	7.82±0.38
(L/min)		Control II	3.96±0.26	5.23±0.28*	5.95±0.28*	6.44±0.31	7.05±0.32	7.72±0.36
	MI	Control I	3.12±0.18‡	4.13±0.23*‡	4.83±0.30*‡	5.33±0.31*‡	5.84±0.41*‡	6.15±0.43*‡
		Control II	3.06±0.21‡	3.76±0.17*‡	4.79±0.29*‡	5.30±0.30*‡	5.75±0.39*‡	6.16±0.46*‡
MAP	N	Control I	98±2	91±2*	90±2*	92±2*	92±2*	93±2*
(mmHg)		Control II	98±2	90±2*	91±3*	90±2*	90±2*	92±2*
	MI	Control I	98±4	88±3*	92±4*	91±3*	93±3*	92±3*
		Control II	97±4	90 4*	91±4*	93±4	93±4	94±4
$LVdP/dt_{max}$	N	Control I	3030±140	3630±200*	4090±200*	4590±220*	5100±230*	5740±230*
(mmHg/s)		Control II	3050±160	3690±200*	4120±200*	4290±	4790±190*	5360±210*
	MI	Control I	2470±130‡	2890±160*‡	3350±120*‡	3610±170*‡	4050±190*‡	4310±250*‡
		Control II	2430±130‡	2740 160*‡	3340±180*‡	3770±230*‡	3890±190*‡	4260±250*‡
Coronary								
CBF	N	Control I	49±4	60±7*	69±7*	82±8*	87±9*	94±10*
(ml/min)		Control II	50±5	63±7*	73±7*	77±7*	86±8*	93±10*
	MI	Control I	63±7	77±8*	89±10*	99±13*	113±15*	119±16*
		Control II	59±6	75±8*	86±9*	100±11*	107±13*	118±14*
Pulmonary								
MPAP	N	Control I	15±1	13±1	18±1*	20±1*	25±2*	29±1*
(mmHg)		Control II	16±1	17±1	20±1*	21±1*	25±1*	29±1*
	MI	Control I	24±5	24±4‡	30±5*‡	33±4*‡	38±4*‡	40±4*‡
		Control II	24±4	25±4	30±5*‡	34±5*‡	36±4*‡	41±4*‡
	N		4±	-0 1	2±1	3±1	6±1	10±1*
(mmHg)		Control II	4±1	1±1	2±1	3±1	5±2	9±1*
	MI	Control I	10±3	7±2‡	11±2‡	13±	16±2*‡	17±2*‡
		Control II	12±2‡	9±2‡	11±2‡	13±2‡	15±2*‡	16±1*‡

HR, heart rate; CO, cardiac output; MAP, mean arterial pressure; LV, left ventricular; $LVdP/dt_{max}$, maximum rate of rise in LV pressure; CBF, coronary blood flow; MPAP, mean pulmonary artery pressure; MLAP, mean left atrial pressure.
Data are mean ± SEM; N, n=14; MI, n=12.

*$P \leq 0.05$ vs. Rest (Lying); †$P \leq 0.05$ vs. Corresponding Control I; ‡$P \leq 0.05$ MI vs. N.

The major findings of the present study are that in swine with LV dysfunction due to a 2-3 week old myocardial infarction: (*i*) agonist-induced receptor-mediated NO production was blunted compared to normal swine, whereas the vasodilator response to the endothelium-independent but NO-mediated vasodilation by nitroprusside was maintained. (*ii*) Inhibition of NO production by N^{ω}-nitro-L-arginine resulted in similar increments in vascular tone compared to normal swine in the (regional) systemic, pulmonary and coronary beds under basal resting conditions as well as during exercise up to 85% of maximum heart rate. (*iii*) Inhibition of NO production by inducible

NOS had no effect on vascular tone in the systemic, pulmonary and coronary beds either at rest or during exercise.

Endothelial dysfunction and heart failure

Agonist-induced vasodilation.

Advanced heart failure is associated with endothelial dysfunction, in particular a reduced biological availability of NO. Thus, clinical studies have shown that chronic heart failure is accompanied by blunted vasodilator responses to endothelium-dependent receptor mediated vasodilators (particularly acetylcholine) in the microcirculation of the LV myocardium (33), leg (8, 34), and forearm (35-38). In the canine model of pacing-induced end-stage congestive heart failure, attenuated vasodilator responses of resistance vessels to acetylcholine *in vivo* have also been observed in the microvasculature of the hindleg circulation (17, 39) and the coronary circulation (6). Also, in rats with myocardial infarction-induced LV dysfunction, vasodilator responses in the microcirculation of the hindlimb to acetylcholine were blunted 10 weeks after infarction (17), although this was not confirmed in another study at 1, 2, 3, 5 and 13 weeks after infarction in rats (6). An explanation for the discrepancy between the two studies is not readily found but may be related to the use of blood (17), a scavenger of NO, versus buffered solution (6) for perfusion of the hindlimb. In the present study we also observed reduced vasodilator responses in the systemic and coronary microvasculature to ATP, in doses which we have previously shown to be completely abolished by pretreatment with NLA (11). These findings of a blunted ATP-induced vasodilation are in agreement with the hypothesis that agonist-induced NO synthase mediated NO production is blunted 2-3 weeks after myocardial infarction, but could also be explained by a decreased biological availability of NO or a reduced vascular smooth muscle responsiveness to NO. Thus, while several clinical studies reported that the responses to nitroglycerine or nitroprusside were maintained in patients with chronic heart failure (8, 35, 36, 40), other studies reported a blunted vasodilator response to these NO donors (34, 37, 38). The severity of heart failure may underly these equivocal findings as Bank et al. (41) observed blunted vasodilator responses to methacholine in the forearm of patients with mild or severe heart failure, whereas nitroprusside-induced vasodilation was blunted only in patients with severe heart failure. In agreement with this notion, the vasodilation by nitroglycerine or nitroprusside was maintained in LV dysfunction produced by myocardial infarction (17, 20), whereas vasodilation by nitroglycerine was blunted in the model of pacing-induced end-stage heart failure (6). The reduction in vasodilator response to NO donors could result from a decreased vascular smooth muscle responsiveness to NO or from a reduced half life of NO due to increased levels of NO scavenging substances such as O_2^-, which may be increased in chronic severe heart failure in part due to an increased production of TNFα (42).

The preserved nitroprusside-induced vasodilation in the present study indicates that NO degradation and vascular smooth muscle responsiveness to NO were normal in MI. Therefore, the blunted vasodilator response to ATP is in agreement with the hypothesis that endothelial NO production in response to ATP was attenuated. However, it could be argued that in MI, accelerated ATP breakdown contributed to the attenuated vasodilation that was observed during ATP infusion in MI compared to N. Indeed one cannot exclude that following administration of endothelium-dependent vasodilators such as acetylcholine, substance P, bradykinin and ADP (endogenous compounds that usually employed to assess endothelial function in heart failure), increased breakdown of these compounds contributes to the blunted vasodilator response. In previous experiments we observed in normal animals that the vasodilation in response to intravenous infusion of ATP in doses of 50-200 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was abolished by NLA both in the systemic and coronary vascular beds, indicating that ATP produces vasodilation exclusively via NO synthase mediated NO production (11). Part of this vasodilation could be due to the production of ADP and adenosine (43). ADP has been shown to produce endothelium-dependent NO mediated vasodilation via activation of the purinergic P_{2Y} receptor, which is at least equipotent to ATP (44, 45). Adenosine which is also equipotent to ATP (43) produces vasodilation that is primarily endothelium-dependent at lower doses, while principally endothelium-independent at higher doses (46, 47). This may explain our previous findings in normal swine that the vasodilation produced by ATP in doses 300-500 $\mu g \cdot kg^{-1} \cdot min^{-1}$ was only partly attenuated by nitro-L-arginine (11). The finding that the vasodilator response to nitroprusside was maintained suggests that vascular smooth muscle responsiveness is unaltered and hence that the endothelium-independent component of adenosine-induced vasodilation would likely be unperturbed. Thus, if ATP infusion in MI would have resulted in higher adenosine levels this would have lead to a greater degree of endothelium-independent vasodilation, which could then actually have contributed to an underestimation of the degree of endothelial dysfunction. Although we cannot entirely exclude that ATP breakdown to ADP and adenosine was enhanced in MI, the finding that the vasodilator response to ATP infusion was blunted in MI compared to N is in agreement with the hypothesis that agonist-induced (ATP, ADP and adenosine) endothelium-dependent NO mediated vasodilation is blunted in swine with LV dysfunction 2-3 weeks after infarction.

Basal NO biological availability. A loss of NO-mediated vasodilation in heart failure could contribute to reduced tissue perfusion. In addition, the peripheral vasoconstriction can increase the workload of the heart, which together with an increased coronary vasomotor tone may lead to myocardial ischemia thereby aggravating LV dysfunction, and enhancing the progression of LV dysfunction to heart failure. This is supported by studies in dogs with pacing-induced dilated cardiomyopathy, in which the loss of basal NO production in the LV myocardium coincides with

the progression from LV dysfunction to overt heart failure (6, 10). In contrast, we found no evidence of a reduced vasodilator influence of endogenous NO, represented by the similar decreases in (regional) systemic, pulmonary and coronary conductances produced by NLA (blocking both eNOS and iNOS) in resting swine, 2-3 weeks after myocardial infarction. Similar observations were made in rats in which up to 13 weeks after a large myocardial infarction (40% of the left ventricle) constrictor responses to the NO synthase inhibitor N^{ω}-nitro-monomethyl-L-arginine (LNMMA) were not different from those in normal rats in the total systemic bed (21) and resistance vessels in the renal, cutaneous, mesenteric, cerebral and hindlimb microcirculation (17, 19, 20). Only in the coronary bed was vasoconstriction by NLA less at 8 weeks after infarction compared to normal rats (20). In humans with chronic heart failure, studies on the contribution of NO to basal microvascular tone in the forearm, leg, or total systemic bed have yielded equivocal results with reponses varying from decreased (4, 8, 38), maintained (48), to increased (35, 49) vascular tone following NO synthase inhibition. It is possible that a maintained or increased production of NO that was observed in some studies was the result of increased expression of iNOS (24, 50), as part of a generalized inflammatory response in end-stage heart failure, that occurred in the presence of either a decreased (24, 51) or increased (52, 53) expression of eNOS. Interestingly, iNOS upregulation has been suggested to be of greater importance in idiopathic dilated cardiomyopathy than in ischemic cardiomyopathy (54), although this could not be confirmed by others (52, 55, 56). An explanation for the equivocal findings in clinical studies could lie in differences in severity and etiology of heart failure. However, most clinical studies used patient populations that consisted of mixed etiology and a range of severities and findings are therefore difficult to reconcile.

The inflammatory reaction that occurs after myocardial infarction not only causes an increase in reactive oxygen species (42), but could also upregulate iNOS (24, 50), particularly in the myocardium (57). Consequently, in the present study iNOS-mediated NO production could have increased and thereby have masked a possible reduction in eNOS activity. Since NLA, in the concentration used, blocks all three isoforms of NOS (29), we performed additional experiments in which we blocked iNOS with aminoguanidine in a dose of 20 mg/kg, iv. This dose was based on studies in rabbits, in which a dose of 10 mg/kg aminoguanidine reduced cGMP levels and blocked iNOS activity by over 80% (31), and in rats, in which a dose of 15 mg/kg i.v. prevented and reversed endotoxic shock induced systemic hypotension (30). More recently, a study was published that reported that 30 mg/kg, administered subcutaneously, *completely* abolished the induction of iNOS activation and cardioprotective by mono-phosphoryl lipid A in swine during a 24 hour period (32). Hence, the dose of aminoguanidine of 20 mg/kg given intravenously 10 min prior to the exercise trial will produce effective blockade of iNOS. The observation that iNOS blockade had no

effect on either basal systemic, pulmonary or coronary vascular tone is in agreement with the hypothesis that NO production via iNOS does not contribute significantly to vascular tone in MI, and, consequently, that basal endothelial NO production is maintained early after MI despite the presence of LV dysfunction.

It could also be argued that endothelial NO production was already maximal so that ATP could not result in any further stimulation of NO production. However, this is unlikely because ATP did produce, albeit blunted, vasodilation in MI. Finally, ATP may activate eNOS through a different mechanism than shear stress; the latter is present in blood vessels in vivo, even under resting conditions. Indeed, ATP-induced activation of eNOS is mediated through a calcium-calmodulin dependent pathway (58) whereas shear stress activates eNOS through Akt-mediated phosphorylation (59), resulting in calcium-independent activation of eNOS. Hence, it is possible that perturbations in the calcium homeostasis of endothelial cells contributed to the selective impairment of ATP-induced vasodilation in swine with MI.

NO availability during treadmill exercise

A loss of endothelial NO-mediated vasodilator influence in heart failure could contribute to impaired tissue perfusion, when myocardial O_2 demand is increased such as during exercise. The exercise-induced vasodilation in the systemic and pulmonary beds of MI during control was similar to the vasodilation observed in N during exercise in the presence of NLA, which might suggest that a loss of NO production contributed to the blunted systemic and pulmonary vasodilation in MI. However, the effects of NLA were similar in MI and normal swine, both at rest and during exercise. This observation implies that while agonist-induced receptor-mediated NO production was reduced, the exercise-induced NO production was maintained. Several possibilities might explain the difference between agonist- and exercise induced NO production. Firstly, Traverse et al. (60) have shown that the amount of NO, produced after stimulation with an agonist is larger than the amount of NO produced with moderate exercise (60% increase in heart rate). Hence, the maximal capacity of NO production may be reduced, whereas the capacity of eNOS is sufficient to maintain basal and exercise-induced NO production. This explanation is unlikely since agonist-induced dilation is already affected at the lowest dose of ATP administered (which probably releases less NO than strenuous exercise), and higher doses of ATP still produce more dilation. Secondly, as stated above, ATP may activate eNOS through a different mechanism than shear stress (58, 59), so that calcium homeostasis of endothelial cells is selectively affected by MI.

Similar to the systemic bed as a whole, we also observed similar responses to NLA in regional vascular beds in MI and N. In patients with chronic heart failure (ejection fraction 22%, NYHA class II-III; (4)) inhibition of NO production by LNMMA had no effect on forearm blood flow either at rest or during exercise. In contrast, LNMMA decreased forearm blood flow and vascular

conductance in normal subjects both at rest and during handgrip exercise. Interpretation of that study is difficult, however, because forearm blood flow was measured with venous occlusion plethysmography which requires interruption of exercise, and the measurements reflect blood flow during the early recovery from exercise. Indeed, Radegran and Saltin (61) have recently shown that inhibition of NO hastens the recovery of blood flow following exercise but does not reduce skeletal muscle blood flow during exercise. In rats with heart failure produced by a 6-week old myocardial infarction, inhibition of NO production elicited smaller decreases in slow oxidative (i.e. red) skeletal muscle blood flow and vascular conductance, as compared to normal rats. In contrast, in the present study NLA had no effect on skeletal muscle blood flow in either N or MI, while NLA-induced decrease in skeletal muscle vascular conductance was similar in MI and N in both red and white muscle groups.

Possible causes for the increased vascular tone after MI.

The present findings indicate that a reduction in NO production does not contribute to the increased tone of the systemic, pulmonary and coronary resistance vessels at rest and the reduced dilatory response in these beds to exercise in 2-3 weeks after MI. We previously found that early after infarction the neurohumoral status has changed, so that circulating levels of endothelin, norepinephrine, epinephrine and angiotensin II increased more during exercise in MI than in N (24), which may have contributed to the decreased dilator response to exercise. Also, other vasodilator systems, such as prostacyclin exert a tonic vasodilator influence in pigs (62). Since prostacyclin has been shown to contribute to shear stress-induced vasodilation (63) and its production from endogenously administered arachidonic acid is reduced in the large coronary arteries of dogs with pacing-induced heart failure (64), a reduced prostacyclin production may also have contributed to the increased peripheral resistance at rest and during exercise.

Conclusion

Although agonist-induced vasodilation is already blunted, basal and exercise-induced production of NO is maintained in swine with moderate left ventricular dysfunction 2-3 weeks after myocardial infarction.

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Chapter 7

Autonomic imbalance in exercising swine with a recent myocardial infarction

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INTRODUCTION

Heart failure is accompanied by a hemodynamic defense reaction consisting of salt and water retention, vasoconstriction and cardiac stimulation. This hemodynamic defense reaction serves to partially restore cardiac output and to increase peripheral vascular resistance in order to maintain arterial pressure, and consequently perfusion of essential tissues like brain and heart. An integral part of the hemodynamic defense reaction involves an alteration of the balance in autonomic activity,(1) i.e. an increase in sympathetic activity(2-4) and a decrease in parasympathetic activity.(5-7) Increased sympathetic activity contributes to the poor prognosis of heart failure as indicated by the correlation between elevation of plasma norepinephrine and mortality,(8, 9) and by the beneficial effects of β-blockers on mortality in patients with heart failure.(10-13) Conversely, a decreased parasympathetic activity after is a predictor of cardiovascular mortality,(14) likely due to a lowering of ventricular fibrillation treshold.(15) Patients with end-stage heart failure are characterized by increased resting heart rates and catecholamine levels and reduced vagal tone. (6, 16, 17) In contrast, the exercise-induced increments in heart rate are markedly attenuated,(16, 17) despite exaggerated sympathetic activation during exercise,(16-20) which is due to a blunted β-adrenoceptor responsiveness,(17, 21) in conjunction with a reduced reserve to withdraw parasympathetic tone during exercise owing to the already reduced parasympathetic tone under resting conditions.(6, 17, 22)

Currently, the main cause of heart failure is ischemic heart disease, in particular the loss of viable contractile cardiac tissue as a result of myocardial infarction (MI). Recently, we observed that a 2-3 week old MI in swine resulted in moderate left ventricular dysfunction characterized by a lower global left ventricular (LV) contractility (as indicated by $LVdP/dt_{max}$), stroke volume and a slightly higher heart rate under awake resting conditions.(23) However, resting catecholamine levels were normal compared to normal swine.(23, 24) During treadmill exercise, an exaggerated increase in catecholamine levels occurred, compared to normal swine, while the increases in heart rate were maintained and the increases in global LV contractility were blunted only at higher levels of exercise.(23) These findings suggest that early after myocardial infarction, an attenuated cardiac β-adrenergic receptor responsiveness can still be compensated for by an increase in sympathetic drive during exercise at this stage of heart failure.(19) Alternatively, the exaggerated increase in sympathetic activity may act to compensate for a blunted (further) withdrawal of vagal tone during exercise. In addition, we observed that particularly systemic, but also coronary, vasodilator responses to exercise were blunted 2-3 weeks after myocardial infarction,(23) which could be due to increased α-adrenergic vasoconstriction and/or diminished ß-adrenergic vasodilation. In the

present study we investigated the alterations in autonomic control of heart rate and cardiac pump function, as well as autonomic control of systemic and coronary vasomotion in swine with a 2-3 week old myocardial infarction at rest and during treadmill exercise.

MATERIALS AND METHODS

Studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 86-23, revised 1985), and with prior approval of the Animal Care Committee of the Erasmus University Rotterdam. Thirty-crossbred Landrace x Yorkshire swine (2-3 months old; 22±1 kg at the time of surgery; 14 normal swine and 16 swine with a myocardial infarction (MI)) entered the study. Full details of the experimental procedures have been published previously.(25, 26) Results from 11 of the 14 normal swine have been previously reported previously.(25, 26)

Surgical procedures

Swine were sedated (ketamine 20-30 mg/kg, im), anesthetised (thiopental 10-15 mg/kg, i.v.), intubated and ventilated with a mixture of O_2 and N_2O (1:2) to which 0.2-1.0% (v/v) isoflurane was added.(24-26) Anesthesia was maintained with midazolam (2 mg/kg + 1 mg/kg/h, i.v.;) and fentanyl (10 μg/kg/h, i.v.;). Under sterile conditions, the chest was opened via the fourth left intercostal space and a polyvinylchloride catheter was inserted into the aortic arch, for the measurement of mean aortic pressure (Combitrans® pressure transducers, Braun) and blood sampling to determine PO_2, PCO_2 and pH (Acid-Base Laboratory model 505), O_2-saturation and hemoglobin concentration (OSM2, Radiometer), for the determination of O_2-consumption (VO_2).(25, 26) After the pericardium was opened, an electromagnetic flow probe (14-15 mm inner diameter, Skalar) was positioned around the ascending aorta for measurement of cardiac output (CO). A high fidelity pressure transducer (Konigsberg Instruments) was inserted into the left ventricle (LV) via the apex. A fluid filled catheter also for calibration of the Konigsberg pressure transducer signal.(25, 26) Fluid filled catheters were also implanted in the left atrium for pressure measurements and in the pulmonary artery for pressure measurements, infusion of drugs and collection of mixed venous blood samples. A small angiocatheter was inserted into the anterior interventricular vein for coronary venous blood sampling. A Doppler flow probe (2.0-3.0 mm, Crystal Biotech) was placed around the proximal left anterior descending coronary artery (LAD).(25) In both groups the proximal part of the left coronary circumflex artery (LCx) was exposed, but only in MI was the LCx permanently occluded with a silk suture.(23, 24) For the following hour animals were carefully monitored and in case of fibrillation internally defibrillated (10-50 Joules); two swine died due to recurrent fibrillation. Electrical wires and catheters were tunneled subcutaneously to the back, the chest was closed and animals were allowed to recover.

Animals received analgesia (0.3 mg buprenorphine im, for 2 days) and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin, iv, for 5 days). Catheters were flushed daily with physiologic saline containing 2,000 IU/ml heparin.(25) Three MI swine died overnight during the first week after surgery.

Experimental protocols

Studies were performed 2-4 weeks after surgery in 14 normal swine and in 11 MI swine, with animals exercising on a motor driven treadmill.(25, 26) After hemodynamic measurements (lying and standing), blood samples (lying), rectal temperature (standing) was obtained, swine were subjected to a four-stage treadmill exercise protocol (1-4 km/h). Hemodynamic variables were continuously recorded and blood samples collected during the last 60 seconds of each 3 min exercise stage, at a time when hemodynamics had reached a steady state. Following the last stage, swine were allowed to rest for 90 minutes, after which animals received either vehicle (physiologic saline) or non-selective blockade of muscarinic receptors (atropine, 30 μg/kg/min i.v., 14 normal and 8 MI swine), β-adrenoceptors (propranolol, 0.5 mg/kg iv, 14 normal and 8 MI swine), the α-adrenoceptors (phentolamine, 1 mg/kg i.v., 8 normal and 9 MI swine) and underwent a second exercise trial.(25, 26) Animals that had received atropine underwent a third exercise trial 90 min later, in the presence of atropine (30 μg/kg/min i.v.) and propranolol (0.5 mg/kg i.v.).(25, 26) Animals that had received propranolol underwent a third exercise trial 90 min later, in the presence of propranolol (0.25 mg/kg i.v.) and phentolamine (1 mg/kg i.v.).(25, 26) All experiments were performed on different days in random order. All drugs were freshly prepared each day.

Data analysis

Digital recording and off-line analysis of hemodynamic data and computation of myocardial and body VO_2 have been described in detail elsewhere.(25, 27) Statistical analysis was performed using three-way, two-way and one-way analysis of variance for repeated measures followed by post-hoc testing with Dunnett's test or t-test, as appropriate. Analysis of co-variance for repeated measures was used to analyse changes in whole body and myocardial oxygen balance. Statistical significance was accepted at $P < 0.05$. Data are mean±SEM.

RESULTS

Effects of MI on exercise response

Under resting conditions MI swine were characterized by a lower cardiac output (21%), stroke volume (20%), $LVdP/dt_{max}$ (28%), and a three-fold higher left atrial pressure, but a maintained heart rate (Fig. 1). Mean arterial pressure was also maintained due to a lower systemic vascular conductance (20%). Exercise resulted in blunted increments of $LVdP/dt_{max}$, cardiac output and

systemic vascular conductance, while the increase in heart rate was maintained. Catecholamines were not significantly elevated in MI swine under resting conditions, but showed an exaggerated increase during exercise (Fig. 1).

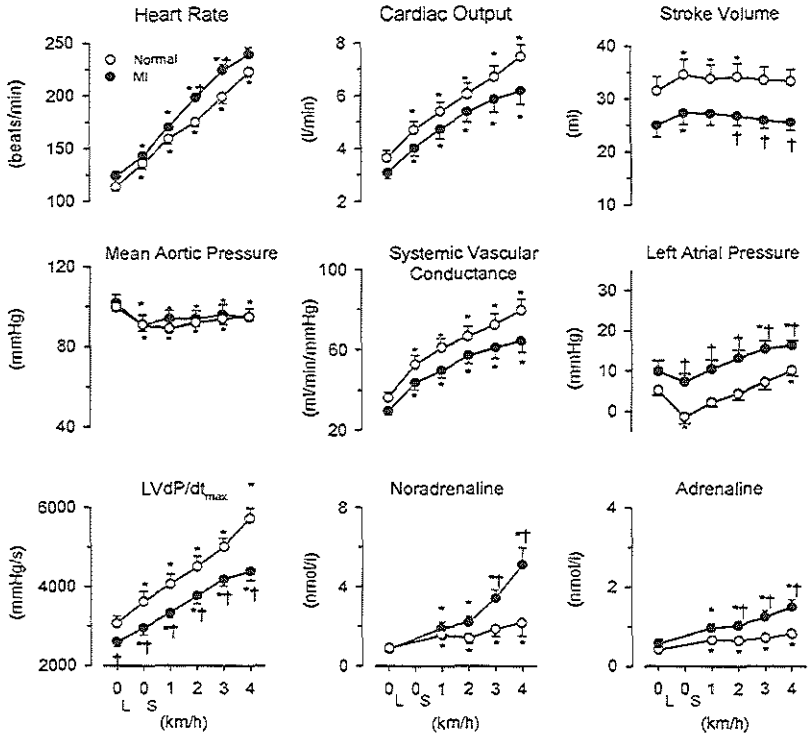

Figure 1. Responses to treadmill exercise of left ventricular pump function, systemic hemodynamics and catecholamine in normal swine (open circles) and MI swine (solid circles). 0_L = 0 km/h, lying; 0_S = 0 km/h, standing. **P*<0.05 vs. corresponding 0_L; †*P*<0.05 vs corresponding measurement in normal swine.
Data are mean±SEM.

Autonomic control of cardiac function.

ß-adrenergic and muscarinic receptor mediated control. Infusion of propranolol produced a decrease in heart rate and cardiac output at rest and particularly during exercise in both normal and MI swine, without significantly affecting stroke volume in either group (Fig. 2). The decrease in heart rate and cardiac output were slightly larger, whereas the propranolol-induced decrease in $LVdP/dt_{max}$ was blunted, in MI compared to normal swine (both $P<0.05$ MI vs normal). Propranolol increased left atrial pressure ($P<0.05$) and tended to decrease mean aortic pressure in MI swine, but this failed to reach levels of statistical significance ($P>0.10$).

Atropine increased heart rate and to a lesser extent cardiac output (due to a concomitant decrease in stroke volume) in normal swine at rest but also during exercise (Fig. 2). These atropine-induced changes were blunted in MI compared to normal swine particularly at higher levels of exercise.

Atropine increased $LVdP/dt_{max}$ in normal swine but had no effect on $LVdP/dt_{max}$ in MI swine ($P<0.05$ vs normal). In both groups of animals, atropine tended to increase left atrial pressure and mean aortic pressure, but this failed to reach levels of statistical significance.

In the presence of atropine, the propranolol-induced decreases in heart rate and $LVdP/dt_{max}$ and the increase in stroke volume were blunted in MI compared to normal swine (Fig. 1). Conversely, in the presence of propranolol, the addition of atropine had little effect on hemodynamics except for an increase in mean aortic pressure and heart rate and a decrease in stroke volume, responses that were similar in MI and normal swine. These findings indicate that most of the atropine-induced effects were mediated through enhanced β-adrenoceptor activation. Interestingly during combined M-and β-blockade heart rate still increased during exercise in MI swine.

α-Adrenergic and β-adrenergic receptor mediated control. Phentolamine produced similar decreases in blood pressure and increases in heart rate in normal and MI swine, whereas the increases in $LVdP/dt_{max}$ were blunted in MI swine ($P<0.05$; Fig. 3). The effects of phentolamine on heart rate and LVdP/dtmax were mediated via increased β-adrenoceptor stimulation, since these effects were absent when phentolamine was administered in the presence of propranolol. Phentolamine produced slightly greater increases in cardiac output in MI swine, as stroke volume was better maintained (in the absence of propranolol) or slightly increased (in the presence of propranolol) after phentolamine in MI compared to normal animals. Since left atrial pressure decreased more in response to phentolamine in MI swine (particularly in the absence of propranolol), it is possible that a decrease in systolic wall stress and hence left ventricular afterload may have contributed to the maintained stroke volume in MI swine.

Autonomic control of systemic vascular tone.

β-adrenergic and muscarinic receptor mediated control. Propranolol resulted in fairly similar decreases in systemic vascular conductance (Fig. 2) and mixed venous O_2 saturation (Fig. 4) in MI compared to normal swine (both in the absence and presence of atropine), although up to 1 km/h a trend was observed towards smaller decreases in systemic vascular conductance by propranolol in MI compared to normal swine.

Atropine increased systemic vascular conductance ($P<0.05$ in MI swine), which was associated with a trend towards a small increase in mixed venous O_2 saturation; these effects were slightly greater in MI than in normal swine. The actions of atropine appeared to be due to an increased ß-adrenoceptor vasodilator influence, because concomitant β-adrenoceptor blockade negated atropine's effects on systemic vascular conductance and mixed venous O_2 saturation in both normal and MI animals (Figs. 2 and 4).

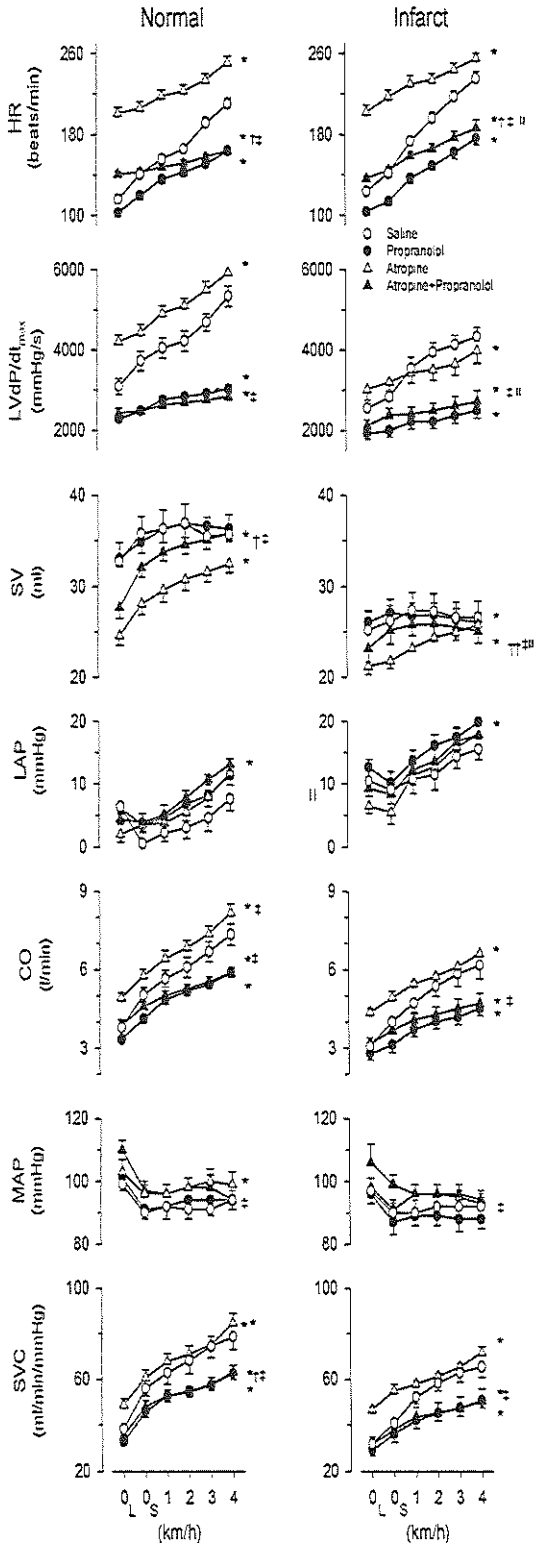

Figure 2. Effects of ß-adrenoceptor blockade (propranolol 0.5 mg/kg iv) and/or muscarinic receptor blockade (atropine 30 µg/kg/min iv) on the responses of left ventricular pump function and systemic hemodynamic to treadmill exercise in normal swine (left panels) and MI swine (right panels). 0_L = 0 km/h, lying; 0_S = 0 km/h, standing. *P<0.05 vs. corresponding Saline; †P<0.05 Atropine+Propranolol vs corresponding Propranolol; ‡P<0.05 Atropine+Propranolol vs corresponding Atropine; ¶response vs corresponding Saline was significantly different (P<0.05) in MI from normal swine; §response to Atropine+Propranolol vs corresponding Propranolol was significantly different (P<0.05) in MI from normal swine; ǂresponse to Atropine+Propranolol vs corresponding Atropine was significantly different (P<0.05) in MI from normal swine. Data are mean±SEM.

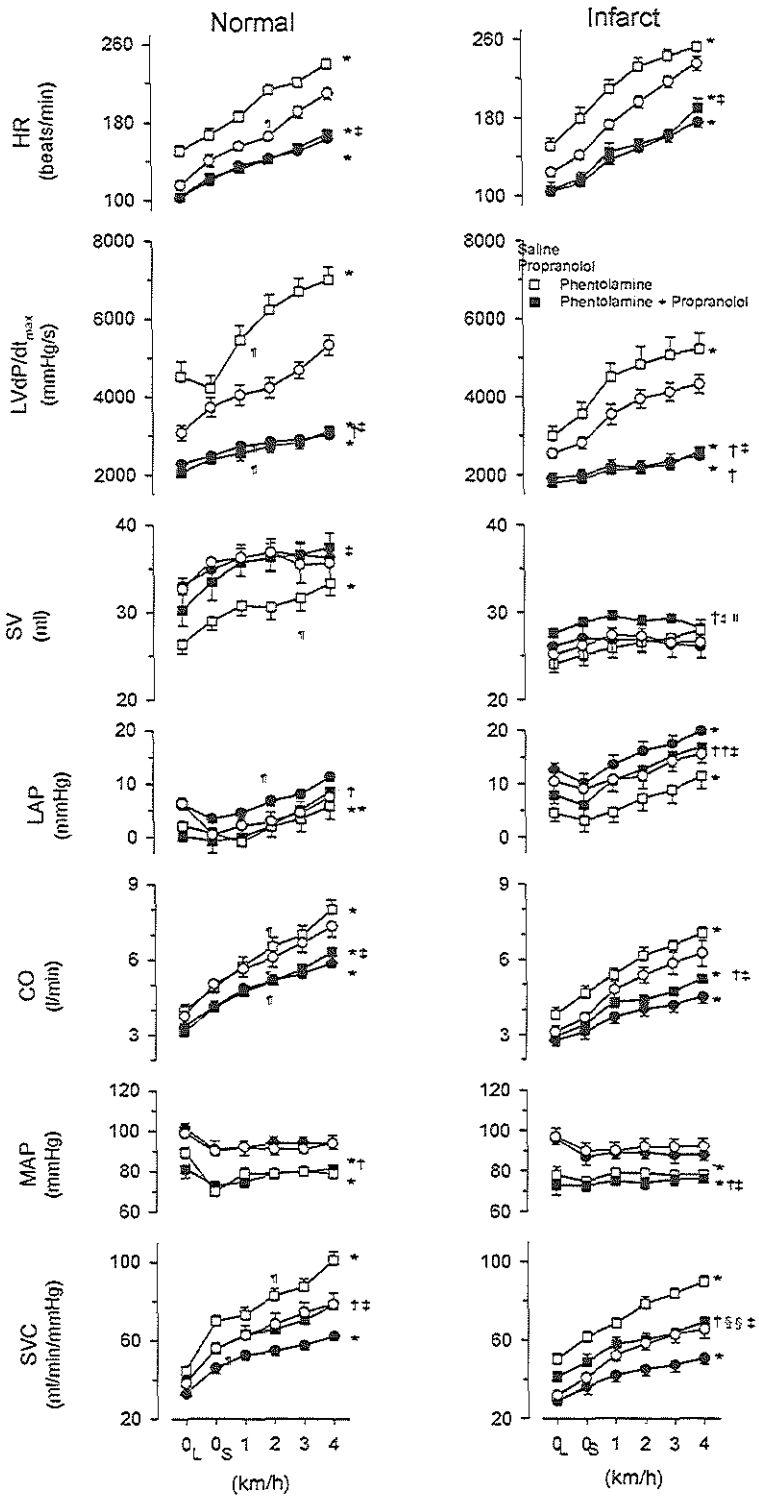

Figure 3. Effects of ß-adrenoceptor blockade (propranolol 0.5 mg/kg iv) and/or α-adrenoceptor blockade (phentolamine 1 mg/kg iv) on the responses of left ventricular pump function and systemic hemodynamic to treadmill exercise in normal swine (left panels) and MI swine (right panels). 0_L = 0 km/h, lying; 0_S = 0 km/h, standing. *P<0.05 vs. corresponding Saline; †P<0.05 Phentolamine+Propranolol vs corresponding Propranolol; ‡P<0.05 Phentolamine+Propranolol vs corresponding Phentolamine; ¶response vs corresponding Saline was significantly different (P<0.05) in MI from normal swine; §response to Phentolamine+Propranolol vs corresponding Propranolol was significantly different (P<0.05) in MI from normal swine; ‡response to Phentolamine+Propranolol vs corresponding Phentolamine was significantly different (P<0.05) in MI from normal swine. Data are mean±SEM.

α-Adrenergic and ß-adrenergic receptor mediated control. The effect of phentolamine on systemic vascular conductance was slightly more pronounced in MI than in normal swine both at rest and during exercise ($P<0.05$; Fig. 3). This effect was maintained in the presence of propranolol, indicating an increased α-adrenergic vasoconstrictor influence on the systemic vasculature. An increased α-adrenergic vasoconstriction in MI swine during exercise is also suggested by the greater phentolamine-induced counter-clockwise rotations of the relation between body VO_2 and mixed venous O_2 saturation, in MI compared to normal swine ($P<0.05$ both in the absence and presence of propranolol; Fig. 4).

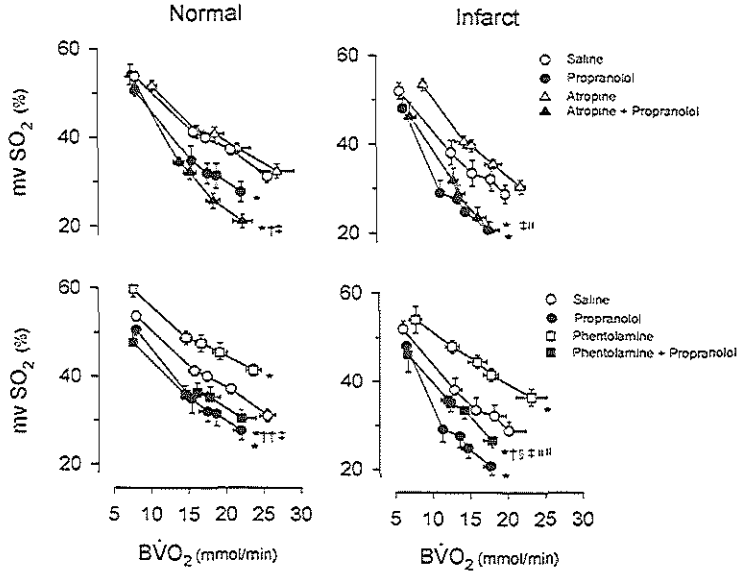

Figure 4. Effect of muscarinic receptor blockade (atropine, 30 μg/kg/min, i.v.), α-adrenoceptor blockade (phentolamine, 1 mg/kg iv) and or ß-adrenoceptor blockade (propranolol, 0.5 mg/kg i.v.) on the response of mixed venous O_2 saturation (mv SO_2) plotted as a function of body O_2 consumption (BVO_2) in normal and MI swine. *$P<0.05$ vs. corresponding Saline; †$P<0.05$ Atropine+Propranolol vs corresponding Propranolol or Phentolamine+Propranolol vs corresponding Propranolol; ‡$P<0.05$ Atropine+Propranolol vs corresponding Atropine or Phentolamine+Propranolol vs corresponding Phentolamine; ¶response vs corresponding Saline was significantly different ($P<0.05$) in MI from normal swine; §response to Atropine+Propranolol or Phentolamine+Propranolol vs corresponding Propranolol was significantly different ($P<0.05$) in MI from normal swine; ‡response to Atropine+Propranolol or Phentolamine+Propranolol vs corresponding Atropine or Phentolamine, respectively, was significantly different ($P<0.05$) in MI from normal swine. Data are mean±SEM.

Autonomic control of coronary vascular tone.

ß-adrenergic and muscarinic receptor mediated control. In both normal and MI swine, propranolol progressively impaired myocardial O_2 delivery during exercise, necessitating an increase in myocardial O_2 extraction resulting in a decreased coronary venous PO_2, indicative of ß-adrenergic feed-forward coronary vasodilation during exercise. The ß-adrenergic feed-forward coronary

vasodilation was of a similar magnitude in MI and normal swine. Atropine produced coronary vasodilation as indicated by the increased coronary venous PO_2 (Fig. 5). This vasodilation was principally mediated by an increased β-adrenergic coronary vasodilation, because atropine plus propranolol resulted in a similar relation between MVO_2 and $CVPO_2$ as propranolol alone, in both groups of animals.

α-Adrenergic and β-adrenergic receptor mediated control. Phentolamine had no effect on coronary resistance vessel tone in either MI or normal swine as indicated by the unchanged relation between MVO_2 and coronary venous PO_2, either in the absence or presence of β-adrenergic blockade (Fig. 5).

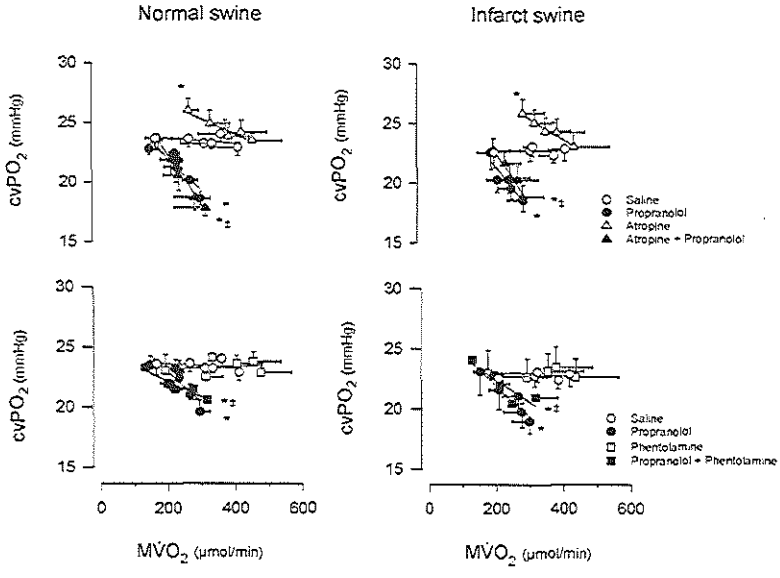

Figure 5. Effect of muscarinic receptor blockade (atropine, 30 μg/kg/min, i.v.), α-adrenoceptor blockade (phentolamine, 1 mg/kg iv) and or β-adrenoceptor blockade (propranolol, 0.5 mg/kg i.v.) on the response of coronary venous O_2 tension ($cvPO_2$) plotted as a function of myocardial O_2 consumption (MVO_2) in normal and MI swine. *P<0.05 vs. corresponding Saline; $^{\dagger}P$<0.05 Atropine+Propranolol vs corresponding Propranolol or Phentolamine+Propranolol vs corresponding Propranolol; $^{\ddagger}P$<0.05 Atropine+Propranolol vs corresponding Atropine or Phentolamine+Propranolol vs corresponding Phentolamine; ¶response vs corresponding Saline significantly different (P<0.05) in MI from normal swine; §response to Atropine+Propranolol or Phentolamine+Propranolol vs corresponding Propranolol significantly different (P<0.05) in MI from normal swine; ‡response to Atropine+Propranolol or Phentolamine+Propranolol vs corresponding Atropine or Phentolamine significantly different (P<0.05) in MI from normal swine. Data are mean±SEM.

DISCUSSION

The major findings of this study are that in swine with a 2-3 week old myocardial infarction: (*i*) parasympathetic influence on the heart is decreased during exercise; (*ii*) circulating catecholamine levels are elevated resulting in an increased β-adrenergic influence on heart rate and α-adrenergic influence on the systemic vasculature; (*iii*) β-adrenergic influence on global left ventricular contractility is decreased reflecting a blunted left ventricular ß-adrenergic responsiveness; (*iv*) β-adrenergic vasodilator influences in both the coronary and systemic vascular bed were not altered, which, in conjunction with the increased catecholamine levels, suggests that systemic and coronary vascular β-adrenergic responsiveness is decreased.

Parasympathetic control of cardiovascular function in swine with a 2-3 week old myocardial infarction

Patients with advanced severe heart failure are characterized by a shift in the sympathovagal balance, with an increased sympathetic activity(17, 19) and a blunted parasympathetic activity that is reflected in reduced heart rate variability and reduced baroreceptor reflex sensitivity.(5-7, 17) In patients that suffer a myocardial infarction, parasympathetic tone is reduced, already on the day of the infarction.(28, 29) Normalization of parasympathetic activity may either occur over a period varying from several days(28, 29) to months,(30-33) or not at all in patients who subsequently go on to develop heart failure.(34) Indeed the degree of LV dysfunction is correlated with the extent of parasympathetic withdrawal,(7) which may in part explain why parasympathetic activity following a myocardial infarction depends on the size and location of the infarcted region.(35, 36) Kruger et al(37) observed in rats that a maximal dose of atropine (0.5 mg/kg i.v.) produced smaller increases in heart rate compared to sham animals at 3 and 28 days, but not at 56 days after myocardial infarction of the LAD region. In the present study, we observed that a maximal dose of atropine produced a similar increase in heart rate in swine with a 2-3 week old MI, compared to normal swine, suggesting a normal parasympathetic activity. In contrast, the increase in $LVdP/dt_{max}$ that was observed in resting normal swine (1180±230 mmHg/s, $P<0.05$) was virtually absent in resting MI swine (370±210 mmHg/s, P=ns; $P<0.05$ vs normal swine). The cardiovascular effects of parasympathetic activity have been proposed to be mediated, at least in part, via presynaptic modulation of sympathetic activity.(38) The blunted effect of atropine on $LVdP/dt_{max}$ could thus be due to a selective loss of muscarinic receptor mediated presynaptic inhibition of neuronal catecholamine release in the LV myocardium, so that in MI swine atropine no longer exerted an effect on LV catecholamine release in MI swine. This is supported by the observation that in the presence of β-adrenoceptor blockade, the effect of atropine on $LVdP/dt_{max}$ was abolished in both normal and MI swine. At this stage of LV dysfunction the loss of parasympathetic

inhibition of sympathetic activity appears to have occurred selectively in the LV myocardium, because atropine increased heart rate to a similar extent in normal and MI swine and because circulating catecholes were not significantly elevated at rest in MI compared to normal swine. It could also be speculated that the observation that an increase in cardiac sympathetic drive precedes the increase in general sympathetic drive(39) is due to an early loss parasympathetic inhibition of sympathetic activity in the LV myocardium.

The present study is the first to study in animals with a MI the withdrawal of parasympathetic tone during incremental levels of treadmill exercise. In contrast to the effects under resting conditions, the atropine-induced increase in heart rate during exercise (particularly at higher exercise levels) was blunted in MI compared to normal swine, while the increase in $LVdP/dt_{max}$ was abolished. These results indicate that the withdrawal of parasympathetic tone during exercise was significantly more pronounced in MI compared to normal swine. Importantly, these findings suggest that at a time when parasympathetic tone under basal resting conditions is normal, an exaggerated withdrawal of parasympathetic activity can be present during exercise. The cardiovascular effects of parasympathetic activity have been proposed to be mediated, at least in part, via presynaptic modulation of sympathetic activity.(38) Consequently, it is likely that in the present study the greater degree of withdrawal of parasympathetic tone during exercise contributed to the exaggerated increase in sympathetic activity during exercise, as reflected in the β-adrenoceptor-mediated increases in circulating catecholamines. The observation that in the presence of propranolol the effects of atropine were no longer different between MI and normal swine also supports the concept that the more pronounced withdrawal of parasympathetic tone during exercise contributed to the exaggerated exercise-induced increase in sympathetic activity.

In resting dogs, parasympathetic activity exerts a vasodilator influence on coronary resistance vessels that is mediated via nitric oxide.(40) Conversely, in resting swine parasympathetic activity exerts a vasoconstrictor effect on the coronary resistance vessels (which wanes during exercise) that is mediated via inhibition of β-adrenergic vasodilation.(25) In contrast to the loss of the parasympathetic inhibitory influence on β-adrenoceptor mediated inotropy, we observed that the inhibitory influence of the parasympathetic system on the coronary circulation appeared to be maintained in MI compared to normal swine. Similarly, in the systemic vascular bed of resting swine, atropine also resulted in significant vasodilation, with the increase being slightly greater in MI than in normal swine. In both groups of animals, the atropine-induced increase in systemic vascular conductance was mediated through an increased β-adrenergic vasodilator influence. These findings indicate that at this stage of LV dysfunction parasympathetic control of β-adrenoceptor mediated systemic and coronary vasodilation is unimpaired.

Advanced heart failure is characterized by increased plasma norepinephrine levels under resting conditions,(2, 18) that result principally from increased sympathetic nerve activity although impaired reuptake may also contribute.(41) Prolonged exposure to elevated norepinephrine levels results in desensitization and downregulation of the adrenergic receptors.(21, 42, 43) During exercise, an exaggerated increase in catecholamine levels occurs at similar absolute levels of exercise,(20) which is aimed at maintaining chronotropic and inotropic responses to exercise.(44) Recently, we observed exaggerated increases in catecholamine levels during treadmill exercise in swine with LV dysfunction produced by a 2-3 week old MI,(23) at a time when catecholamine levels were not significantly elevated under resting conditions.(23, 24) The exaggerated exercise-induced increases in catecholamine levels may serve to maintain the chronotropic and inotropic responses to acute exercise. This concept is supported by the observation that β-adrenoceptor blockade produced a more pronounced decrease in the chronotropic response during exercise in MI than in normal swine (Fig. 2), which can be attributed to the higher levels of norepinephrine (Fig. 1). In contrast, β-adrenoceptor blockade in MI swine resulted in a blunted decrease in global LV contractility compared to normal swine, in particular during exercise. The latter findings are consistent with a reduced left ventricular myocardial β-adrenoceptor responsiveness, at a time when chronotropic responses are only slightly blunted.(23)

β-adrenergic receptor activation contributes to the systemic vasodilation(26) and coronary vasodilation(25) that occurs during exercise. The β-adrenergic coronary vasodilation results in an increase in myocardial oxygen delivery that is commensurate with the increase in oxygen consumption, so that myocardial oxygen extraction and hence coronary venous O_2 tension remain constant during exercise.(25) Previous studies in pacing-induced severe heart failure in dogs have indicated that β-adrenergic vasodilation of pulmonary(45) and systemic arteries(46) is attenuated. More recently, Gaballa et al(47) suggested that also MI-induced LV dysfunction was associated with a blunted vasodilator response of aortic rings to isoproterenol of rats, although the decrease in systemic vascular resistance to isoproterenol was increased in MI compared to normal rats.(47) In the present study, the β-adrenergic vasodilator influence on the systemic circulation (Figs. 2 and 4) as well as that on the coronary circulation (Fig. 5) appeared to be unchanged in MI swine compared to normal animals. However, in view of the increased levels of norepinephrine and epinephrine these findings suggest a diminished β-adrenergic responsiveness of the systemic and coronary resistance vessels. From the present study, we cannot determine which β-adrenergic receptor subtype is affected. However, in view of its preferential distribution in the resistance vessels, it is

likely that the principal subtype involved is the β_2-adrenoceptor. This is also supported by previous observations that in ischaemic cardiomyopathy (in contrast to idiopathic dilated cardiomyopathy), both β-adrenoceptors are downregulated.(48)

α-Adrenergic activation limits the exercise-induced increase in systemic vascular conductance in swine.(26) In the present study, phentolamine produced a slightly greater increase in systemic vascular conductance and, consequently, a slightly greater increase in mixed venous O_2 saturation. These results indicate an increased influence of α-adrenergic vasoconstrictor tone, likely as a result of the increased circulating catecholamine levels. Systemic vascular α-adrenoceptor responsiveness has been reported to be enhanced in pacing-induced severe heart failure in dogs.(49) In contrast in MI-induced heart failure in the rat vascular α-adrenoceptor responsiveness has been reported to be unchanged(50, 51) or slightly attenuated.(52) Although these disparate results could be due to either species differences and / or pathogenesis of heart failure, a study in humans with heart failure due to either dilated cardiomyopathy or ischemic heart disease, found that α-adrenoceptor responsiveness was unchanged.(53) The weight of evidence suggests that systemic vascular α-adrenoceptor responsiveness is minimally affected in LV dysfunction produced by a myocardial infarction.

In the dog, α-adrenoceptor activation limits the exercise-induced increase in coronary blood flow, thereby necessitating an increase in myocardial O_2 extraction, which leads to a decrease in coronary venous PO_2.(54) In contrast, in swine α-adrenoceptors do not contribute to regulation of coronary blood flow during exercise.(25) In the present study we found that administration of phentolamine had also no effect on coronary venous PO_2 of MI swine. These findings indicate that even in the presence of exaggerated increments in catecholamine levels in MI swine during exercise, α-adrenoceptors do not contribute to regulation of tone in coronary resistance vessels.

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Chapter 8

Cardiac AT_1 receptor upregulation does not contribute to an increased coronary vasomotor tone in swine with a myocardial infarction

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INTRODUCTION

Heart failure is currently the only major cardiovascular disorder of which both incidence and prevalence is on the increase. This is the result of a reduced mortality associated with acute myocardial infarction, as well as an overall improvement in healthcare culminating in an aging of the population. The most prominent cause of heart failure is ischemic induced. Directly following a myocardial infarction there is set in motion a variety of mechanisms in an attempt to maintain stroke volume. Apart from an increase in sympathetic tone there is a structural remodeling of the remaining myocardium consisting of dilatation and hypertrophy. However LV remodeling has been shown to be a key factor in the transition of asymptomatic dysfunction towards overt heart failure. A major role in the remodeling process is played by Angiotensin II, which also exerts a considerable vasoconstriction itself. Previously we confirmed a Angiotensin II in remodeling of the left ventricle of swine with a myocardial infarction.(1) These animals showed functional up-regulation of Cardiac AT_1 receptor binding, which action could be attenuated due to either an ACE inhibitor or administration of an AT_1 blocker. Earlier data from our laboratory demonstrated that in exercising swine with a 3-week old myocardial infarction myocardial O_2 balance was perturbed, necessitating an increase in O_2 extraction. This coincided with a rise in an increase in plasma Angiotensin versus normal (N).(2) This led us to hypothesize that AT_1 receptor up-regulation might contribute to the increased tone in the coronary resistance vessels. To test our hypothesis we exercised chronically instrumented swine with a myocardial infarction on a motor driven treadmill to study the coronary vascular and myocardial O_2 balance response to the AT_1 receptor antagonist irbesartan.

MATERIALS AND METHODS

Studies were performed in accordance with the Guide for the Care and Use of Laboratory Animals (NIH publication 86-23, revised 1985), and with prior approval of the Animal Care Committee of the Erasmus University Rotterdam. Thirty two Crossbred Landrace x Yorkshire swine (2-3 months old; 22±1 kg at the time of surgery; 9 normal swine (N) and 12 swine with a myocardial infarction (MI)) were included. Adaptation of animals to the laboratory conditions started 1 week prior to the day of surgery and continued until 10 days post-operatively.

Surgical procedures

Swine were sedated (ketamine 20-30 mg/kg, im), anaesthetized (thiopental 10-15 mg/kg, i.v.), intubated and ventilated with a mixture of O_2 and nitrous oxide (1:2) to which 0.2-1.0% (v/v) isoflurane was added(1, 3, 4). Anesthesia was maintained with midazolam (2 mg/kg + 1 mg/kg/h,

i.v.;) and Fentanyl (10 μg/kg/h, i.v.;). Under sterile conditions, the chest was opened via the fourth left intercostal space and a polyvinylchloride catheter was inserted into the aortic arch, for the measurement of mean aortic pressure (MAP, Combitrans® pressure transducers, Braun) and blood sampling to determine PO_2, PCO_2 and pH (Acid-Base Laboratory model 505), O_2-saturation and hemoglobin concentration (OSM2, Radiometer), for the determination of O_2-consumption (4, 5). After the pericardium was opened, an electromagnetic flow probe (14-15 mm inner diameter, Skalar) was positioned around the ascending aorta for the measurement of cardiac output (CO). A high fidelity pressure transducer (Konigsberg Instruments) was inserted into the left ventricle (LV) via the apex. A fluid filled catheter was inserted into the LV for calibration of the Konigsberg pressure transducer signal (4, 5) and the withdrawal of arterial blood samples. A similar catheter was positioned in the left atrium for pressure measurements, while two catheters were inserted into the pulmonary artery for pressure measurements as well as infusion of drugs and collection of mixed venous blood samples. A small angiocatheter was inserted into the anterior interventricular vein for coronary venous blood sampling. A Doppler flow probe (2.0-3.0 mm, Crystal Biotech) was placed around the proximal left anterior descending coronary artery (LADCA) (4, 5). In both groups the proximal part of the Left Coronary Circumflex Artery (LCx) was exposed, but only in MI was the LCx permanently occluded with a silk suture(1). For the following hour the animal was carefully monitored and in case of fibrillation internally defibrillated (10-50 Joules); three swine died due to recurrent fibrillation. Electrical wires and catheters were tunnelled subcutaneously to the back, the chest was closed and the animals allowed to recover. Animals received analgesia (0.3 mg buprenorphine im, for 2 days) and antibiotic prophylaxis (25 mg/kg amoxicillin and 5 mg/kg gentamycin, i.v., for 5 days). Catheters were flushed daily with physiologic saline containing 2,000 IU/ml heparin(4, 5). Two MI died overnight during the first week after surgery.

Experimental protocols

Studies were performed between 2 and 4 weeks in 9 normal swine (N) and in 7 swine with a myocardial infarction (MI), with animals exercising on a motor driven treadmill. After hemodynamic measurements (lying and standing), blood samples (lying), rectal temperature (standing) were obtained, swine were subjected to a four-stage treadmill exercise protocol (1-4 km/h). Hemodynamic variables were continuously recorded and blood samples collected during the last 60 seconds of each 3 min exercise stage, at a time when hemodynamics had reached a steady state. Following the last stage, swine were allowed to rest for 90 minutes of rest, after which animals received either vehicle (physiologic saline) or selective AT_1 blocker (Irbesartan, 1mg/kg; iv) and underwent a second exercise trial. Experiments were performed in a random order seperated by a minumum of 24 hours. Solutions were prepared fresh each day.

Data analysis

Digital recording and off-line analysis of hemodynamic data have been described previously (4, 5). Statistical analysis was performed using three-way, two-way and one-way analysis of variance for repeated measures, as appropriate. Analysis of co-variance for repeated measure was used to analyse changes in the relation between hemodynamic variables and oxygen consumption. To determine statistically significance Post-hoc testing was performed using Dunnett's test or t-test. Significance (two-tailed) was accepted at $P \leq 0.05$. Data are mean±SEM.

RESULTS

Systemic effects of AT_1 blockade

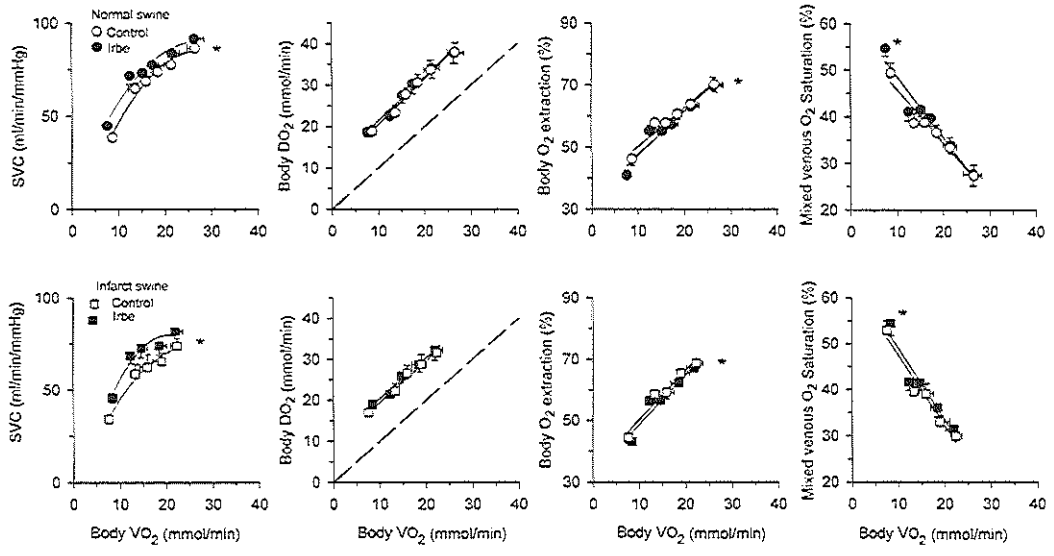

Figure 1. Effect of AT_1 receptor blockade (Irbesartan, 1mg/kg; iv) on the response of systemic vascular conductance, Body O_2 delivery, Body O_2 extraction and mixed venous O_2 saturation as a function of whole Body O_2-Consumption in N and MI.* $P<0.05$ vs. corresponding control. Data are mean±SEM

Administration of Irbesartan produced a significant decrease in MAP in both MI and N. Which elicited a reflectoir increase in HR, albeit not significant. As there was a small increase in SV, CO rose, which only reached statistical values under resting conditions. Consequently giving rise to the SVC. Throughout the entire exercise protocol the effect of the blockade remained stable. There were no discernible effects on the $LVdP/dt_{max}$ or the LAP

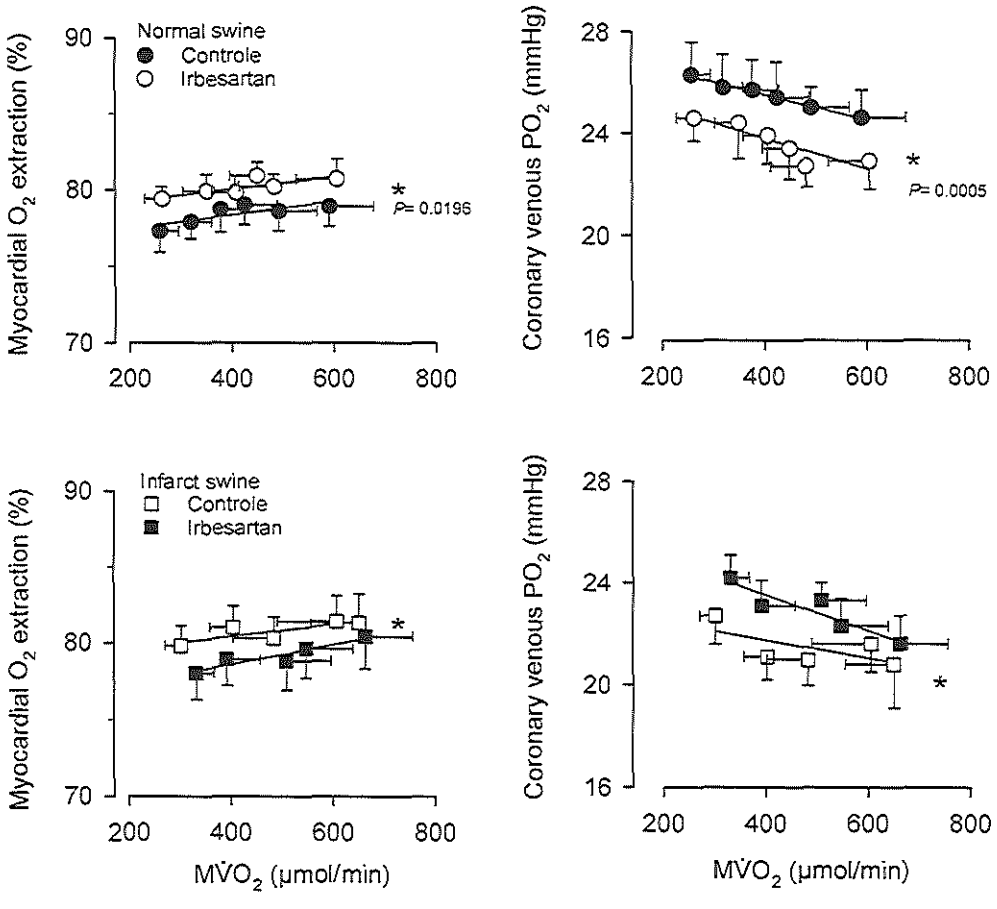

Figure 2. Effect of AT_1 receptor blockade (Irbesartan, 1mg/kg; iv) on the response of coronary venous O_2 tension and myocardial O_2 extraction as a function of Myocardial O_2-Consumption in N and MI.* $P<0.05$ vs. corresponding control. Data are mean±SEM

The systemic administration of Irbesartan resulted in both groups in a similar decrease in myocardial extraction. Leading to an increase in cv PO_2. While there was a small increase in extraction and subsequent decrease in cv PO_2, this effect followed the same response as under control situations. This is best illustrated as a parallel shift in, when the cv PO_2 and myocardial extraction are plotted against the $M\dot{V}O_2$.

DISCUSSION

The major findings of the present study are that in normal swine and swine with LV dysfunction due to a 2-3 week old myocardial infarction: (*i*) AT_1 receptors contribute to the resistance vessel

tone in the systemic and coronary bed. *(ii)* AT_1 mediated sympathetic stimulation is not involved in chronically instrumented swine with or without a MI. *(iii)* However upregulation of AT_1-receptors do not contribute to the increased vasomotor tone in swine with a myocardial infarction.

Angiotensin II is capable of producing a wide variety of actions through its receptors AT_1 and AT_2. Among these actions are the vasoconstrictive response, sympathetic stimulation and hypertrophic response ascribed to AT_1 and a cell protective, growth inhibitory and apoptosis inducing quality mediated by the AT_2. The presence of both AT_1 and AT_2 receptors in the myocardium, has been reported in a variety of species, including rats, rabbits, monkeys(6) and sheep.(7) Both endogenous Angiotensin II in rats(8), rabbits(9) as well as exogenous(10) Angiotensin II are capable of facilitating the exocytotic release of catecholamines from the presynaptic varicosities, which is mediated by the AT_1 receptor(11, 12). However administration of Irbesartan in our study failed to produce a significant decrease in HR and dP/dt_{max}. Other data from our laboratory confirm this observation. This study, performed in anaesthetized swine, failed to produce a significant rise in interstitial cardiac catecholamines, upon an intra-coronary infusion of exogenously Angiotensin II, despite a prominent increase in arterial plasma Angiotensin II.(13) These findings are in contrast with what has been found in dogs.(10) This contrast may be attributed to the higher dose of Angiotensin that was infused in these animals. However it has been proposed that a chronic infusion of Angiotensin II can produce a rise in catecholamines, were a short infusion failed to elicit this response.(11, 14) Besides its quality in facilitating a exocytotic reaction of the catecholamines Angiotensin II produces both in systemic and coronary vasculature a direct pressor effect.(15-18) The presence of both AT_1 and AT_2 receptors in the myocardium, has been reported in a variety of species, including rats, rabbits, monkeys(6) and sheep.(7) Evidence suggests that after a myocardial infarction an increase in the role by angiotensin is played. Our study is the first to prove the direct tonic effect off Angiotensin II (ANG II) on the coronary vasculature in swine after a myocardial infarction.

It has been well documented that among the alterations that occur after a myocardial infarction, there is an increased activation of the RAAS system and of its main component the octapeptide Angiotensin II. Earlier findings reported in rats, an upregulation in genetic transcription and protein expression,(19-21) as well as binding-profile.(22) A similar increase in AT_1 receptor gene transcription(23) has been described for humans. Previously we reported in our model that a 2-3 week old MI coincides with an increased uptake of Ang II mediated by AT_1 receptor.(1) However blockade of the AT_1 receptors in MI produced no significant increase in dilatory response compared to N animals, neither at rest nor during exercise. Indicating that the increased binding capacity is not located or directly linked with the vascular response of ANG II.

Studies performed in rats have reported an increase in AT_1 receptors, chiefly in the infarcted area or its border zone, localizing the upregulation within the myofybroblasts.(24) The upregulation of AT_1 receptors as seen in the hypertrophic remodeling response has been implicated with both inflammatory response following myocardial infarction(25, 26) and increase in stretch upon the cardiomyocytes.(27-29) Whereas with the inflammatory response the upregulation of the AT_1 receptors is predominantly located in the myofibroblasts. The increase in AT_1 gene transcription due to the increased stretch, as seen in the increased filling-pressures, can be found in the cardiomyocytes. While some inflammation was present in the sham-operated animals, there could be observed a transient increase in the ANG II concentration, a direct effect can be ascribed to the infarction, as the response was greater in the Infarct animals. Although a number of studies performed in humans have reported a decrease in AT_1 receptor density.(30-32) The studies where performed in these patients diagnosed with end-stage heart failure, often exposed for a extended period to the elevated plasma levels of ANG II. Prolonged exposure to ANG II, as often seen in older or more severe forms of myocardial infarction,(30) induces a downregulation of the AT_1 receptor.

While in our model the ANG II does not seem to play a major role this maybe enhanced when the situation progresses. It could conceivable become instrumental in this process as AT_2 receptor has been implicated in the apoptosis induction described in heart failure.(28, 33) This is especially interesting when several studies have reported an increase in AT_2 receptors(34).

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DISCUSSION AND SUMMARY

The conceptualization of the pathophysiology of heart failure is constantly evolving. Heart failure when studied in the 1940-60's was primarily considered to be a cardiorenal disorder marked by salt and water retention, and in part due to the renal blood flow abnormalities.(1) Treatment options were limited and medication to treat heart failure was constrained in the 1950' s-1960's to diuretics to decrease the water retention, and digitalis to improve total cardiac output.(2)

The cardiorenal model shifted to a cardiocirculatory model in the 1970's and 1980's(1) as hemodynamic measurements revealed a reduced cardiac output and increased resistance the prevailing view became that congestive heart failure was primarily a hemodynamic problem due to an ineffective pump. It was believed that through an unloading of the the heart with vasodilators (from the 1970's on(1, 3, 4)) and the inotropic stimulation with sympathomimetics or the combination through inodilators could be made to restore cardiac output. The onset of inotropic medication in the 1980's,(1) made it possible to increase cardiac performance. However, while an improvement in the condition of the patient could be made, this was overshadowed by their decreased survival.

When the comparison of pure vasodilators with Angiotensin Converting Enzyme-inhibition showed an improved survival for the Angiotensin Converting Enzyme-inhibition the hypothesis was put forward that the deteriorating function of the heart may be instigated by the sympathetic stimulation.(5, 6) This concept was supported by earlier observations, already in the 1970's that administration of β-adrenergic agents conveyed functional benefits in dilated cardiomyopathy.(7) The functionality of this treatment modality sparked renewed interest from the 1990's onward. As administration of β-adrenergic blockers proved to improve survival in patients with congestive heart failure.(8) The encouraging results made from other randomized clinically controlled studies have produced a paradigm shift(9, 10) revising not only the way we treat, but conceptually look at the syndrome that encompasses heart failure. The hypothesis that the deteriorating performance of the myocardium may be instigated by the sympathetic stimulation is now well accepted, even in the more severe stages of the heart failure, especially as improvements in more severe stages of heart failure can be seen with the effective blockade of neurohumoral response through Angiotensin Converting Enzyme inhibitors.(11, 12) The consensus is that regardless of the nature of the initiating factor for myocardial damage, increased activation of neurohormonal compensatory mechanisms will be the result. Although the activation of neurohormonal compensatory mechanisms may appear beneficial and adaptive to preserve blood pressure and cardiac output, excessive and prolonged sympathetic stimulation negatively affects the prognosis of heart failure.(13)

The paradigm shift from the hemodynamic to the neurohumoral concept has forced us to search for treatment modalities at a time when the hemodynamic situation is still compensated. The treatment of left ventricular dysfunction with neurohumoral blockers has been shown to be effective in delaying or even preventing the progression. The possibility remains that more maladaptations are already present early on after a myocardial infarction that may prove susceptible to interventions and prolong the survival as well as improve the quality of life.

In chapter 2 we studied the pharmacologic actions of i.v. and oral administration of the dopamine agonist Z1046 in awake chronically instrumented swine under resting and exercise conditions. This compound is an experimental dopamine receptor agonist, which compared to dopamine, exerts equipotent Dopamine 1-receptor and 30 times more potent Dopaminergic 2-receptor agonistic activity in rabbit splenic artery and isolated ear preparations respectively (14)
Consequently the dopamine receptor agonist Z1046 is an effective blood pressure lowering agent, that acts through a predominantly Dopamine 1-receptor dependent vasodilator mechanism. While it elicits minimal reflex activation of sympathetic nervous system in awake resting swine by activating Dopamine 2-receptor mediated inhibition of catecholamine release.(14, 15) Systemic vasodilatation was not affected by combined α- and β-adrenergic receptor blockade, which is consistent with a predominantly Dopamine 1-receptor dependent vasodilator mechanism. The hypotensive effect is maintained during treadmill exercise during which systemic vasodilatation and a lower cardiac output both contribute to the blood pressure lowering actions of Z1046. Administration could prove advantageous in a setting of left-ventricular dysfunction, where a decrease in sympathetic activation has been shown to increase life-expectancy. The blood pressure lowering qualities of Z1046, may be advantageous as it will lower afterload and thus decrease O_2–demand of the myocardium. The increased flow to the kidney will similarly decrease the activation of the renin angiotensin aldosteron system and therefore possible improve the progresssive remodelling of the ventricle

In chapter 3 we studied the pharmacologic actions of EMD 57033 compared to pimobendan in chronically instrumented swine with a myocardial infarction at rest and during exercise. The principal mode of action EMD 57033 appears to be Ca^{2+} sensitization, with only minimal Phophodiesterase-III inhibitory effects at the highest dose (0.8 mg kg^{-1} min^{-1} i.v.).(16)
The positive inotropic action of EMD 57033 in left ventricular dysfunction animals was virtually identical to that in normal swine with minimal increments in myocardial O_2-consumption and only at the highest dose a minimal increase in heart rate and left ventricular relaxation time. The increased elastic restoring forces in the *in-vivo* heart, due to ejection to a lower end-systolic

volumes may be the cause of the minimal increase in relaxation time, and thus can counteract any potential untoward effects of EMD 57033 on diastolic function.(17, 18)
The positive inotropic actions of EMD 57033 in exercising swine with left ventricular dysfunction were similar to those in normal swine, contrary to the blunted positive inotropic and chronotropic actions of pimobendan. This shows that the desensitization of β-adrenergic receptors which accompanies LV dysfunction together with the impaired ventricular performance can be positively influenced without increasing myocardial O_2-demand, thus providing an alternative inotropic support for congestive heart failure.

In chapter 4 we determined whether the changes in cardiac angiotensin II content and origin (local synthesis versus uptake from plasma) after myocardial infarction in swine, to compare the effects of angiotensin converting enzym inhibition and angiotensin type 1 receptor antagonism on these changes, and to further evaluate whether the effects of both renin angiotensin system blockers on cardiac angiotensin content are related to their effects on post-infarct remodelling. The performed study demonstrates that after myocardial infarction, the angiotensin II levels in the non-infarcted left ventricle are transiently increased because of enhanced angiotensin type 1 receptor–mediated uptake of circulating angiotensin II. Captopril and eprosartan normalized or blocked this uptake and attenuated postinfarct remodeling, but increased local cardiac angiotensin II production. These results suggest that the antihypertrophic effects of renin angiotensin system blockade result not only from diminished angiotensin type 1 receptor stimulation but also from increased stimulation of growth-inhibitory angiotensin type 2 receptors. The increased uptake could contributed to the coronary vascular tone which is increased after a myocardial infarction.

In chapter 5 we investigated the integrated responses of left ventricular pump function, neurohormones, and (regional) systemic, pulmonary and coronary hemodynamics to graded treadmill exercise in a large animal model of left ventricular dysfunction produced by myocardial infarction. The performed study showed that the exercise-induced increases in cardiac pump function and pulmonary and systemic vasodilation are blunted in myocardial infarction; the latter is associated with impaired perfusion of splanchnic organs during strenuous exercise, but maintained cerebral and skeletal muscle blood flows. Furthermore, exercise produced exaggerated increases in circulating norepinephrine, epinephrine, atrial natriuretic peptide and endothelin levels in myocardial infarction, while the relative sympathetic drive was maintained. However, cardiac responsiveness to exercise-induced increases in norepinephrine is blunted. Decreased myocardial capillary density contributed to the hypoperfusion of the subendocardium is in the surviving hypertrophied left ventricular myocardium 3 weeks after myocardial infarction, necessitating a

small increase in O_2 extraction during exercise, but does not appear to cause overt myocardial ischaemia. These findings suggest that perfusion abnormalities while present do not contribute significantly to the left ventricular dysfunction that occurs in the early stage of left ventricular remodeling.

In chapter 6 we tested the hypothesis that loss of nitric oxide-mediated vasodilation contributes to abnormalities in vascular tone control in regional systemic, pulmonary and coronary beds in exercising swine with left ventricular dysfunction after infarction as was observed in chapter 5. The study showed that in swine with left ventricular dysfunction agonist-induced receptor-mediated nitric oxide production was blunted compared to normal swine, whereas the vasodilator response to the endothelium-independent but nitric oxide-mediated vasodilation by nitroprusside was maintained. Inhibition of nitric oxide production by N^{ω}-nitro-L-arginine in both groups resulted in similar increments in vascular tone compared in the (regional) systemic, pulmonary and coronary beds under basal resting conditions as well as during exercise up to 85% of maximum heart rate. The possibility of a masking reduction in endogenous nitric oxide synthase activity of nitric oxide production via the induction of inducible nitric oxide synthase, early after a myocardial infarction was excluded. The present findings indicate that a reduction in nitric oxide production does not contribute to the increased tone of the systemic, pulmonary and coronary resistance vessels at rest and the reduced dilatory response in these beds to exercise in 2-3 weeks after myocardial infarction.

In chapter 7 we investigated whether the increase in angiotensin II mediated uptake in the left ventricle as seen in chapter 4 exerted an increased vasoconstrictor influence. angiotensin type 1 receptors contribute to the resistance vessel tone in the coronary bed. However there was no evidence for an increased angiotensin type 1 –mediated coronary vasoconstrictor influence in swine with a myocardial infarction.

In chapter 8 we studied the presence of alterations in the autonomic balance early after a myocardial infarction. The results showed that already early (~2weeks) after a myocardial infarction the parasympathetic influence on the heart is decreased. Elevated levels of circulating catecholamine result in an increased β-adrenergic influence on heart rate and α-adrenergic influence on the systemic vasculature, but a depressed influence on global left ventricular contractility, reflecting a blunted left ventricular β-adrenergic responsiveness. The maintained β-adrenergic vasodilator influences in both the coronary and systemic vascular, in conjunction with

the increased catecholamine levels, suggests that systemic and coronary vascular β-adrenergic responsiveness is decreased albeit less than the β-adrenergic responsiveness of the myocardium.

Conclusions:

The studies we have performed, have all contributed to improve our understanding of the early period of myocardial dysfunction following a myocardial infarction. Interestingly is that although in end-stage heart failure flow alterations are apparent and may contribute to the further progression, flow alterations are only minimally present in the early period and does not contribute to the deterioration. The decrease in left ventricular function is in part due to the loss of contractile tissue, but also the result of a decreased β-adrenergic responsiveness. Furthermore the administration of angiotensin converting enzym or angiotensin receptor blocker can prevent the cardiac remodeling that occurs early after a myocardial infarction. It is believed that this remodeling continous even when hemodynamic situation has stabilized and in so doing plays an important part in the deterioration from left ventricular dysfunction towards overt heart failure.

The hypertrophic response which forms such an integral part of the remodeling process and seems to be an adequate response, may form the very reason for the continuing deterioration. The question arises whether hypertrophy is fundamentally unwanted or will some measure of hypertrophy aid the function of the surviving myocardium? One of the key factors that helps drive this response and maintains this are the elevated plasma levels of catecholamines, which are even capable of directly deteriorating the ventricular function through apoptosis. This rise in catecholamines can be curbed by the administration of Dopamin 2 receptor agonist, and so may from an alternative method to counter the effects of the remodeling process. The therapy for heart failure has now focused on slowing or possible even halting the progression of remodeling by trying to the stimulus. However the stimulus for the hypertrophic response still remains present with this therapy and may even overcome the β-adrenergic blockade. Another therapy, potentially even more beneficial, may be to block the stimulus and simultaneously support the heart so the stimulus is minimized at the same time. This added effect may be performed by a calcium sensitizer which increases cardiac performance without a concommittant rise in myocardial O_2 demand.

It seems likely that the near future will be charactherized by an improved understanding of the process of the adaptation both genetic and proteiomic fields. And from this knowledge may stem an entirely new treatment modality. It creates the possibility that during surgical open chest revascularization by means of an injection muscle-derived stem cells can be placed within the infarcted area grafting them, where they may regain their function.(19) This treatment modality may also be performed via catheters, allowing for faster recovery. These therapies may for the first

time provide the possibility to not only reduce the ventricular dysfunction but reverse it as a whole. Patients who are at high risk of sudden death due to a history of myocardial infarction or reduced systolic left ventricular function stand to gain benefits from implantable cardiac defibrillators.(20) However trials concerning cardiac defibrillators in heart failure patients are ongoing. Already it has been shown that patients who develop secondary to their ischemic damage a conduction induced contraction delay stand to benefit from resynchronization of the ventricles.(21)

Nederlandse Samenvatting

De functie van het hart is het pompen van bloed door de kleine circulatie, alwaar in de longen deze van zuurstof wordt voorzien, alvorens het verder door de grote circulatie heen te pompen om uiteindelijk weer, via de veneuze vaten, bij het hart aan te komen. Dit doet het hart onafgebroken een heel mensenleven lang. Bovendien past het hart zijn pompsnelheid ook zonodig aan als er meer of minder bloed moet rondgepompt worden om aan de vraag van het lichaam te blijven voldoen, bijvoorbeeld tijdens inspanning. De grootste bedreiging dat het hart niet in staat is om aan de vraag te voldoen, wordt gevormd door een belemmerde doorbloeding van de hartspier zelf, vaak op basis van aderverkalking. Dit kan zo ernstig worden dat een deel van de hartspier te lang verstoken blijft van bloed en als gevolg afsterft. Deze situatie wanneer ongemoeid gelaten heeft een hoge sterftekans. De aanpassingen in de laatste jaren hebben de moderne geneeskunde in staat gesteld om steeds vaker mensen te laten overleven met een deels beschadigde hartspier. Het hart past zich aan de beschadiging aan door de overgebleven hartspier in dikte te laten toenemen om uiteindelijk toch aan de vraag naar zuurstofrijk bloed van het lichaam te kunnen voldoen.

Dit lijkt aanvankelijk succesvol, maar in de loop van de tijd zal de pompfunctie van het hart nog verder verslechteren zodat uiteindelijk het aanbod vanuit het hart zal falen in de vraag van het lichaam.

De beschrijving van het ziekteproces van deze belangrijke ziekte is over de decennia's onderhevig geweest aan en staat nog steeds onder, de invloed van verschuivende opvattingen over de pathofysiologie ervan. Toen hartfalen voor het eerst bestudeerd werd in de jaren '40-'60 werd het met name beschouwd als een cardiorenal ziektebeeld gekenmerkt door het vasthouden van zout en vocht, en ten dele door afwijkingen in de nierdoorbloeding.(1) Behandeling was beperkt en medicijnen voor hartfalen bestonden in de periode van 1950 tot 1960 uit diuretica, om het vastgehouden vocht af te drijven, en uit digitalis om het hartminuutvolume te verbeteren.(2)

Dit cardiorenale model veranderde in een cardiocirculatoir model in de jaren '70 tot '80(1) toen hemodynamische metingen aantoonde dat het hartminuutvolume was verminderd en de weerstand toegenomen. Vanaf dit moment werd hartfalen meer gezien als een hemodynamisch probleem, met als voornaamste reden een falende pomp. Hieruit vloeide voort dat geprobeerd werd door het ontlasten van het hart met vaatverwijders (verkrijgbaar vanaf 1970(1, 3, 4)) of door de pompkracht te verhogen door sympathomimetische medicijnen of het gecombineerd ontlasten van het hart en tegelijk de pompkracht te verhogen, het hartminuutvolume te normaliseren. Sinds 1980, toen inotrope therapie beschikbaar werd,(1) kon men het hartminuutvolume verhogen. Hoewel dit

leidde tot een verbeterde uithoudingsvermogen van de patiënt ging dit gepaard met een verkorte overleving van de patiënten.

Met de ontdekking dat na toediening van een angiotensine converterend enzyme een verbeterde overleving zichtbaar werd in vergelijking tot toediening van een pure vaatverwijder, werd de hypothese geopperd dat de verslechtering van de hartspierfunctie samenhing met de sympatische stimulatie.(5, 6) Dit concept werd ook ondersteund door eerdere observaties. Al in 1970 werd na toediening van β-adrenerge blokkade bij gedilateerde cardiomyopathy een functionele verbetering gezien.(7) Sinds de jaren negentig is er een hernieuwde interesse in deze vorm van behandeling gekomen. Toen werd bekend dat de toediening van β-adrenerge blokkers een verbeterde overleving liet zien in patiënten met hartfalen.(8) Tezamen met andere bemoedigende resultaten van gerandomiseerde klinische gecontroleerde studies werd een visieverandering in gang gezet(9, 10) welke niet alleen onze kijk op hartfalen beïnvloedt en daarmee ook de behandeling, maar ook onze kijk op het ziekteproces zelf is nu aangepast. De gedachte dat de verslechtering van de functie van het hart mogelijk wordt aangestuurd door de sympatische stimulatie is nu algemeen geaccepteerd, zelfs voor de ernstige stadia van hartfalen. Het bewijs hiervoor word geleverd door studies die in de gevorderde stadia van hartfalen verbetering lieten zien na effectieve blokkade van de neurohumorale respons door toediening van Angiotensine Converting Enzyme inhibitoren.(11, 12) Nu bestaat de consensus dat ongeacht de initiële schade die verantwoordelijk is voor de beschadiging van de hartspier, het lichaam zal reageren door de neurohumorale activatie te verhogen. In eerste instantie lijkt dit een adequate response te zijn, daar bloeddruk en hartminuutvolume gewaarborgd lijken, maar een langdurige periode van activatie heeft slechtere vooruitzicht voor hartfalen.(13)

De visie verandering maakt het noodzakelijk om te zoeken naar andere behandelopties en deze te starten terwijl er nog geen sprake is van hartfalen. De behandeling van linkerkamerdysfunktie, de voorbode van hartfalen, door middel van neurohumorale blokkade, is aangetoond effectief in het vertragen van de verslechtering of om deze geheel af te wenden. De mogelijkheid bestaat dat vroeg na een hartaanval er meerdere "aanpassingen" bestaan die wanneer ze beïnvloed worden, de overleving doen verlengen, mogelijk zelfs de kwaliteit van het leven positief beïnvloeden.

In hoofdstuk 2 bestudeerden we de farmacologische eigenschappen van de zowel i.v. als oraal toegediende dopamine agonist Z1046 in wakkere chronisch geïnstrumenteerde varkens tijdens rust en tijdens inspanning. Deze stof is een experimentele dopamine receptor agonist, die in vergelijking met dopamine, even krachtig de Dopamine 1-receptor en 30 maal zo sterk de Dopamine 2-receptor respectievelijk in konijnen darmarteriëen en in geïsoleerde oorpreparatenstimuleert.(14)

Met als gevolg dat de dopamine receptor agonist Z1046 een effectieve bloeddruk verlagende werking heeft, met als voornaamste werkingsmechanisme de Dopamine 1-receptor gemedieerde vaatverwijding. Dit effect wordt versterkt daar er slechts een geringe reflectoire activatie van het sympatische zenuwstelsel plaatsvindt in de wakkere rustende varkens. Dit laatste is te wijten aan de simultane activatie van de Dopamine 2-receptor die de afgifte van catecholamines tegengaat.(14, 15) De systemische vaatverwijding werd niet beïnvloed door een gecombineerde α- en β-adrenerge receptor blokkade., Dit is in overeenstemming met de voornamelijk Dopamine 1-receptor gemedieerde vaatverwijding. Het bloeddrukverlagende effect blijft gehandhaafd tijdens een gereguleerde periode van inspanning op een tredmolen, waarbij systemische vaatverwijding en een verlaagd hartminuutvolume bijdragen aan het bloeddruk verlagende effect van Z1046. Potentieel zou toediening van dit medicament een rol kunnen spelen in de behandeling van linkerkamerdysfunktie, waar al is aangetoond dat een verlaging van sympathische activatie een gunstig effect heeft op de overleving van patiënten. De bloeddruk verlagende effecten op zich zouden ook een gunstig effect kunnen hebben aangezien het verlagen van de nabelasting van de hartspier de zuurstofvraag van de hartspier doet afnemen. De toegenomen doorbloeding van de nieren zal ervoor zorgen dat de activatie van het renine angiotensine aldosteron systeem wordt verminderd en zal op deze manier de voortschrijdende remodelering van de hartspier staken.

In hoofdstuk 3 hebben we de farmacologische activiteit van EMD 57033 bestudeerd en deze vergeleken met die van pimobendan in het chronisch geïnstrumenteerd varken model na een hartinfarct in rust en tijdens inspanning. Het voornaamste werkingsmechanisme van EMD 57033 lijkt te berusten op een Ca^{2+}- sensitisatie, met slechts een beperkte Phophodiesterase-III inhibitoir effect bij de hoogste dosering (0.8 mg kg^{-1} min^{-1} i.v).(16)

Het positief inotrope effect van EMD 57033 in dieren met linkerkamerdysfunktie was bijna identiek aan normale dieren met slechts minimale stijging van de zuurstofbehoefte van de hartspier en enkel bij de hoogste dosering gepaard gaand met een minimale toename van de hartfrequentie en linkerventrikel relaxatietijd. De toename van de elastische herstellende krachten in het *in-vivo* hart, waarschijnlijk het gevolg van een verlaagd eind-systolisch volume die de relaxatietijd deed verkorten, zou de potentiële negatieve effecten van EMD 57033 op de diastolische funktie kunnen tegengaan.(17, 18)

De positief inotrope gevolgen van EMD 57033 bij inspannende varkens met linkerkamerdysfunktie zijn gelijk aan normale varkens. Dit is in tegenstelling tot de afgezwakte positief inotrope en chronotrope effecten van pimobendan. Dit laatste onderschrijft het fenomeen dat linkerkamerdysfunktie gepaard gaat met een ongevoeligheid van de β-adrenergic receptoren Deze dysfunktie is dus ten dele te beïnvloeden zonder een toename in de zuurstofvraag van de hartspier

te genereren, en kan op deze alternatieve manier een ondersteuning van de hartspierfunktie te bereiken met name tijdens hartfalen.

In hoofdstuk 4 hebben we bepaald of er veranderingen in cardiale angiotensine II gehalte en oorsprong (lokaal synthesis versus opname uit het plasma) na een hartinfarct in varkens optreden, en vergeleken of de toediening van angiotensine converterend enzyme inhibitie en die van angiotensine type 1 receptor antagonist een verandering teweegbracht in cardiale angiotensine II gehalte, en om te bepalen hoe beide mechanismen, met hun effect op de renine angiotensine systeem, ingrijpen op de post-infarcering remodelering van de hartspier. De studie toonde aan dat na een hartinfarct, de spiegels van angiotensine II in het niet-geïnfarceerde deel van het hart kortdurend verhoogd zijn omdat er een toegenomen angiotensine type 1 receptor–gemedieerde opname van circulerend angiotensine II bestaat. Captopril en eprosartan voorkwamen beide deze opname en verminderden de post-infarct remodelering, maar tegelijk versterkten ze de lokale (in het hart) aanmaak van angiotensine II. Deze resultaten suggereren dat het anti-hypertrophe effect van deze stoffen niet alleen berust op het blokkeren van het renine angiotensine systeem met als gevolg verminderde stimulatie van de angiotensine type 1 receptor maar ook de toegenomen stimulatie van de angiotensine type 2 receptoren en hun groei-remmend effect. De toegenomen opname via de angiotensine type 1 receptor kunnen bijdragen aan de coronaire vaatweerstand die toegenomen is na een hartinfarct.

In hoofdstuk 5 onderzochten we de samenhang tussen de linkerkamer pompfunktie, neurohormonen en de hemodynamiek van verscheidene vaatbedden, te weten het systemische, pulmonaire en coronaire en de reactie van deze parameters op verschillende niveau's van inspanning in een groot proefdier-model van linkerkamerdysfunktie secundair aan een hartinfarct. Deze studie liet zien dat de inspanningsgeïnduceerde stijging van de pompfunktie van het hart en pulmonaire en systemische vaatverwijding verminderd zijn na een hartinfarct; het gaat ook gepaard met verminderde doorbloeding van inwendige organen tijdens zware inspanning, terwijl de doorbloeding van de spieren en hersenen gehandhaafd blijft. Hiernaast leidde, bij dieren met een hartinfarct, de inspanning tot een versterkte afgifte van circulerend noradrenaline, adrenaline, atriaal natriuretisch peptide en endotheline, terwijl de relatieve sympatische activiteit gehandhaafd bleef. Daarentegen, is de gevoeligheid van het hart voor inspanningsgebonden toename van noradrenaline verminderd. Een afgenomen capillaire dichtheid heeft bijgedragen aan de verminderde doorbloeding van de binnenste lagen van de hartspier van de niet-geïnfarceerde en gehypertropheerde deel van het hart, drie weken na een hartinfarct. Maar ondanks een toename in de extractie van zuurstof tijdens inspanning leidde dit niet tot een zuurstoftekort voor de hartspier.

Deze bevindingen suggereren dat ondanks dat er vroeg na een hartinfarct aanwijzingen zijn voor doorbloedingsafwijkingen, deze niet in grote mate bijdrage aan de verslechterde linkerkamerfunktie.

In hoofdstuk 6 hebben we de hypothese getest of een verlies van stikstofmonoxide bijdraagt aan de verhoogde vaattonus van het systemische, pulmonaire en coronaire vaatbed bij inspannende varkens met linkerkamerdysfunktie na een hartinfarct zoals beschreven in hoofdstuk 5. Dit onderzoek liet zien dat, in varkens met linkerkamerdysfunktie, agonist geïnduceerde receptorgebonden productie van stikstofmonoxide verminderd was ten opzichte van normale dieren, daarentegen bleef de stikstofmonoxide gemedieerde vaatverwijdingsresponse voor endotheel onafhankelijke vaatverwijder nitroprusside gehandhaafd. De inhibitie van de stikstofmonoxide productie door N^{ω}-nitro-L-arginine leidde in beide groepen tot een vergelijkbare toename van de vaattonus in de verscheidene vaatbedden, systemisch, pulmonair en coronair, zowel in rust als tijdens inspanning, tot 85% van de maximale hartfrequentie. De mogelijkheid dat vroeg na een hartinfarct een verlaging in de endogene stikstofmonoxide synthase activiteit tot een verlaging van de stikstofmonoxide productie had geleid, maar dat deze was gemaskeerd door een toegenomen activiteit van de induceerbare vorm stikstofmonoxide synthase, werd uitgesloten. De bevindingen van deze studie ondersteunen de gedachte dat hoewel er een vermindering in de agonist geïnduceerde receptorgebonden productie van stikstofmonoxide bestaat deze niet leidt tot de toegenomen vaattonus van de verschillende vaatbedden in rust noch tot de verminderde inspanningsgebonden vaatverwijdingsresponse 2-3 weken na een hartinfarct.

In hoofdstuk 7 bestudeerden we of de toegenomen van de Angiotensine II gemedieerde opname in de linkerkamer, zoals beschreven in hoofdstuk 4, een toename van de vasoconstrictoire invloed op het coronaire vaatbed betekende. Het is bekend dat angiotensine type 1 receptoren een vasoconstritoire invloed uitoefenen met name ook in het coronaire vaatbed, maar in onze studie waren er geen aanwijzingen voor een toename van deze invloeden in varkens, vroeg na een hartinfarct.

In hoofdstuk 8 onderzochten we of er reeds vroeg na een hartinfarct aanwijzingen zijn voor veranderingen van de autonome balans. De resultaten lieten zien dat reeds vroeg (~2weken) na een hartinfarct de parasympatische invloed op het hart verminderd is. De toegenomen circulerende plasmaspiegels van catecholamine resulteerde in een toename van de β-adrenerge invloed op de hartfrequentie en de α-adrenerge invloed op de systemische vasculatuur, maar lieten een verlaagde invloed zien op de contractiliteit van de gehele hartspier, welke een afgenomen gevoeligheid

suggereert van de β-adrenerge receptoren van de linkerkamer. Zowel in de normale als in de geïnfarceerde dieren was het β-adrenerge gemedieerde vaatverwijdende effect identiek in zowel de coronaire als systemische vaatbedden, dit ondanks de gestegen plasma catecholamie spiegels in de laatste groep. Dit suggereert dat in beide vaatbedden de gevoeligheid voor β-adrenerge stimulatie is verminderd maar in een mindere mate dan dat van de hartspier zelf.

Conclusies:

De studies zoals uitgevoerd, hebben allemaal bijgedragen om onze begripsvorming te verbeteren van de vroege periode van hartspierdysfunktie na een hartinfarct. Opvallend is dat hoewel in het eindstadium van hartfalen, wanneer doorbloedingsafwijkingen opvallend zijn en kunnen bijdragen aan de verdere verslechtering van het hartfalen, deze afwijkingen alleen minimaal aanwezig zijn in de vroege periode na een hartinfarct en dragen niet bij aan de initiële verslechtering van de hartspierfunktie. De verslechtering van de linkerkamerfunktie is deels toe te schrijven aan het verlies van contractiel weefsel, maar ook aan de verminderde gevoeligheid van het hart voor β-adrenerge stimulatie. Hiernaast kan het remodeleringsproces na een hartinfarct voorkomen worden met het toedienen van angiotensine converterende enzyme of angiotensine receptor blokkers. Men gelooft dat de remodelering een continue proces is, welke zelfs actief is tijdens hemodynamisch stabiele situatie en zodoende een belangrijke rol vervult in de verslechtering van linkerkamerdysfunktie naar hartfalen.

De hypertrofieringsresponse die optreedt, maakt integraal deel uit van het remodelingsproces en hoewel dit ogenschijnlijk een adequate response is kan dit juist de reden zijn waarom verslechtering van het hart blijft doorgaan. Dit roept de vraag op of hypertrofie in principe ongewenst is of dat een bepaalde mate van hypertrofiering nog wel gewenst is omdat dit de funktie van het overlevend myocard verbetert. Een sleutelrol in de onderhouding van deze response wordt gespeeld door de verhoogde plasma catecholamine spiegels. Deze laatste kunnen zelfs direct verantwoordelijk zijn voor de verslechtering van de hartspierfunctie door celdoodinductie (apoptosis) te induceren. De stijging van de catecholamines kunnen afgeremd worden door de toediening van Dopamine 2 receptor agonist, en zou op die manier een alternatieve methode zijn om het remodeleringsproces tegen te gaan. De huidige therapie is nu gericht op het vertragen van het remodeleringsproces, of het zelfs te doen staken, door de stimulus te remmen. Een nadeel bij deze therapie is het feit dat de stimulus blijft bestaan en eventueel de kans krijgt om de blokkade te overwinnen. Een andere mogelijkheid berust daarop dat de stimulus geblokkeerd wordt met tegelijk het ondersteunen van de hartfunktie zodat de stimulus zelf ook tot een minimum beperkt blijft. Dit laatste zou bijvoorbeeld bereikt kunnen worden door het toedienen van een calcium sensitizer die het hartminuutvolume vergroot zonder de zuurstofvraag van de hartspier te verhogen.

De nabije toekomst zal waarschijnlijk gekenmerkt worden door een toegenomen begripsvorming van het ziekteproces. Dit komt met name door de komst van genetische en eiwitbepalingen met hun functionele nut. Uit deze nieuwe kennis kan dan weer een nieuwe therapie voortkomen. Het creëert bijvoorbeeld de mogelijkheid dat tijdens open hartchirurgie via een injectie stamcellen worden geplaatst in een voorheen afgestorven weefsel om dan weer een functionerend stuk hartspier te maken.(19) Deze optie kan zelfs gebeuren doormiddel van een catheter waardoor herstel wordt bespoedigd. Wanneer deze therapie succesvol blijkt te zijn, zal men voor het eerst in staat zijn om niet alleen de verslechtering te stoppen maar zelfs terug te draaien. Patiënten die een verhoogd risico hebben, op basis van hartinfarct of verminderde systolische linker kamer funktie, om te sterven aan plotse hartdood lijken baat te hebben bij het geïmplanteerd krijgen van een implanteerbare cardiac defibrillator.(20) Op dit moment lopen er studies naar het effect van deze implanteerbare defibrilatoren. Wel is al aangetoond dat patiënten die op basis van ischemie naast contractiele schade oplopen ook een verstoring van het contractie patroon krijgen door geleidingsstoornissen baat ondervinden bij het resynchroniseren van de beide hartkamers.(21)

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ABSTRACTS

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CURRICULUM VITAE

David Bjørn Haitsma was born on 10th October 1972, in Singapore. As part of his medical education he performed an extended twelve-month research period at the laboratory of Experimental Cardiology under the supervision of Prof. Dr. P.D. Verdouw. After graduating as a medical doctor in 1998 from the Erasmus University Rotterdam he started work as a trainee research assistant (AIO) at the same department and remained there until June 2002, resulting in this thesis. In June 2002 he started his clinical internship at the Albert Schweitzer Ziekenhuis, Dordwijk (supervisor Dr. A.C.M. van Vliet) as part of his training to become a cardiologist. He will finish this in the Thoraxcentre, Erasmus Medical Centre, Rotterdam in 2008 (supervisor Prof. Dr. M.L. Simoons).