PHARMACOLOGICAL MODULATION OF EARLY POSTINFARCTION REMODELING

Farmacologische Modulatie van Vroege Postinfarct Remodeling

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Thesis, Erasmus University, Rotterdam. With summary in Dutch

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PHARMACOLOGICAL MODULATION OF EARLY POSTINFARCTION REMODELING

Farmacologische modulatie van vroege postinfarct remodeling

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Aan allen die me lief zijn

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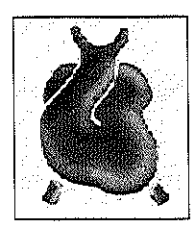
CHAPTER 1

General introduction



"The people who bind themselves to systems are those who are unable to encompass the whole truth and try to catch it by a tail; a system is like the tail of truth, but the truth is like a lizard; it leaves its tail in your fingers and runs away knowing full well that it will grow a new one in a twinkling"

-Ivan Turgenev-

1.1. Introduction

Cardiovascular disease remains one of the most important causes of morbidity and mortality in Western society, especially as the average age of the population increases [1]. Heart failure is clinical syndrome which may develop as a consequence of both peripheral and cardiac disorders (hypertension, aortic stenosis, cardiomyopathy, myocardial infarction), and is recognized clinically by a constellation of various signs and symptoms produced by complex circulatory and neurohormonal responses to cardiac dysfunction. Heart failure as a consequence of myocardial infarction is rapidly becoming one of the most prevalent cardiovascular disorders and the incidence of heart failure is expected to continue to increase for some time to come. The prognosis of heart failure is poor [2] and the economic impact of heart failure on health services is considerable because of the long-term pharmacological treatment and frequent hospitalizations associated with the syndrome [3].

Our conceptualization of heart failure is constantly evolving. Once considered a simple problem of left ventricular pump dysfunction, heart failure has now come to be understood as a highly complex clinical syndrome that is manifested by many extracardiac features, including neuroendocrine activation. The hemodynamic hypothesis has largely been abandoned, and embracement of the neurohormonal hypothesis has resulted in extraordinary changes in the clinical management of patients with chronic heart failure. A more contempory working hypothesis is that heart failure is a progressive disorder of neurohormonal activation and *left ventricular remodeling*, usually resulting from an index event, that culminates in a clinical syndrome characterized by impaired cardiac function and circulatory congestion (Fig 1).

Index Event ⟶	Structural Remodeling and Progression of disease ⟶	Clinical Syndrome of Heart Failure
Acute myocardial infarction	Myocyte hypertrophy	Salt and water retention
Gene mutation	Fibrosis, chamber dilation	Congestion, edema
Acute inflammation	Apoptosis	Low cardiac output
Onset of hypertension	Cell necrosis	Diastolic dysfunction
Valvular heart disease	Neuroendocrine activation	Increasing symptoms
	Cytokine release	
	Increased wall stress	
	Chamber dysfunction	

Fig.1 Working hypothesis of heart failure *(Francis GS, 2001)*

New therapies are now evolving that are designed to modulate *cardiac remodeling* by inhibition of neuroendocrine as well as cytokine activation and matrix metalloproteïnases, whereas drugs designed to heighten cardiac contractility have proven to be unhelpful in long-term management of heart failure. The modern treatment of chronic heart failure is now largely based on the neurohormonal hypothesis, which states that neuroendocrine activation and associated structural remodeling is important in the progression of heart failure and that inhibition of neurohormones is likely to have long-term benefit with regard to morbidity and mortality.

1.2. Pathophysiology of postinfarction induced heart failure

The consequence of a sustained segmental interruption of coronary blood flow to the myocardium, such as coronary artery occlusion is myocardial infarction. Loss of contractile tissue after myocardial infarction can severly affect pump function of the left ventricle, mainly depending on the magnitude of myocardial necrosis [4]. Large infarcts (>40%) lead to severe cardiac dysfunction, resulting in sudden cardiac death or chronic heart failure. In contrast, smaller infarctions may be initially associated with only minor alterations of hemodynamic parameters because of compensatory mechanisms. Acute myocardial infarction evokes activation of several mechanisms to

compensate for acute loss of function. Three major neurohormonal systems are activated to preserve tissue perfusion:

1. Sympathetic nervous system activity
2. Renin-angiotensin-aldosterone system
3. Arginine-vasopressin

Immediately activated mechanisms are sustained by neurohormonal activation, which is followed by a *structural response* of the heart. All structural alterations in cardiac architecture, at organ as well as at tissue and cellular level, are referred to as myocardial **'remodeling'**. Although cardiac remodeling occurs as a structural response to the increased hemodynamic load on the surviving myocardium, it may finally contribute to the development of heart failure. Several stages of postinfarction remodeling have been described. The *early* compensatory phase is followed by a more stable compensated phase which is characterized by a (partly) restored cardiac output but with decreased left ventricular function and ejection fraction. When symptoms of heart failure become overt, a decompensation phase develops driven by the same mechanisms as the compensation phase, but with a very poor prognosis. In Fig.3 an overview of major peripheral and cardiac mechanisms involved in the pathophysiology of heart failure is given.

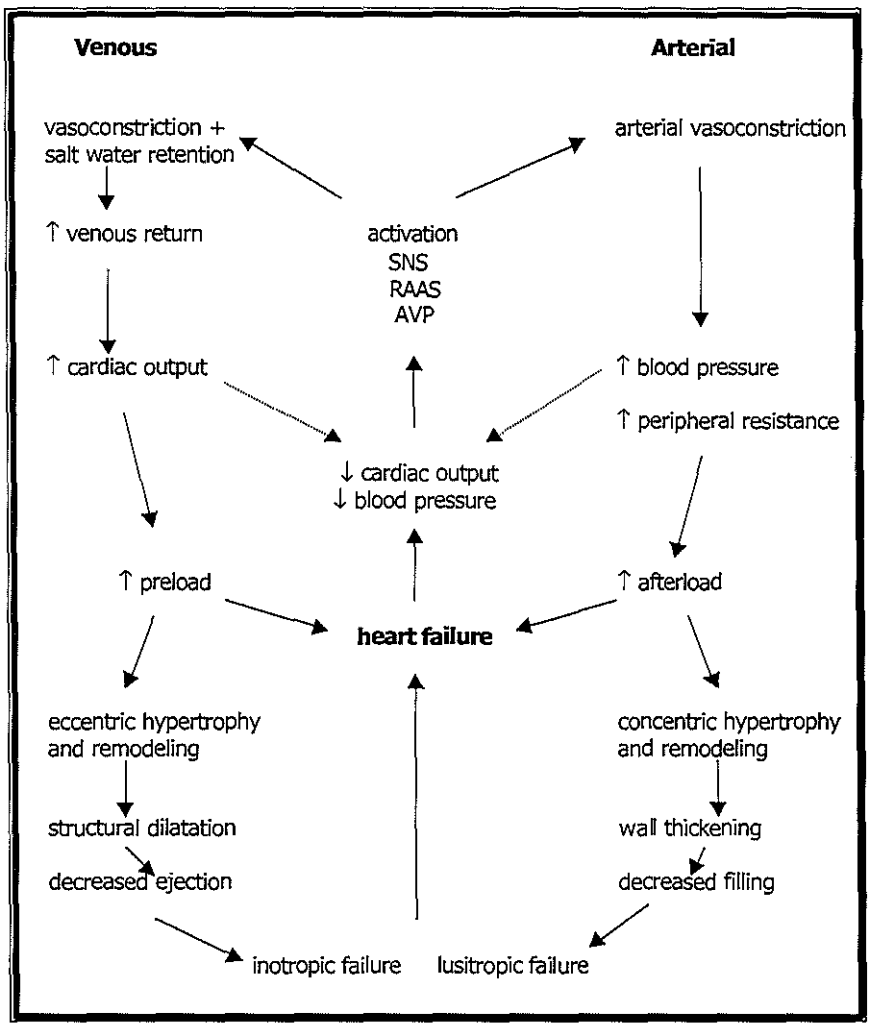


Fig.3 Peripheral and cardiac adaptation as a compensatory response to left ventricular dysfunction.

The different aspects of neuroendocrine activation and postinfarction remodeling as well as their possible role in the transition from postinfarction left ventricular dysfunction towards heart failure will be discussed more in detail in the following part of the thesis.

1.3. Neurohormonal activation

Several neurohormones are activated after myocardial infarction, the magnitude and time course of which are closely related to infarct size and degree of LV dysfunction [5]. The neurohormonal hypothesis postulates that neurohormones are initially adaptive by maintaining cardiac output and blood pressure, but later the same compensatory responses become pathological and contribute to adverse remodeling, progressive ventricular failure, and ultimately to the syndrome of heart failure [6].

1.3.1. Sympathetic nervous system

The first compensatory mechanism involved in heart failure is increased activity of the sympathetic nervous system, which act to maintain blood pressure when cardiac performance is initially depressed. This is achieved by stimulation of myocardial ß-adrenoceptors (inotropic stimulation) and of vascular α-adrenoceptors which increase venous return and arterial resistance. Although 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 [7].

Norepinephrine is an important but rather insensitive marker of mortality. Patients dying from progressive myocardial pump function failure have shown to display rapid progressively increasing norepinephrine levels [8]. Increased norepinephrine levels in heart failure are caused by increased norepinephrine spillover and decreased uptake. Adverse actions of high plasma catecholamines levels include trophic and toxic effects on cardiac myocytes [9], downregulation of β_1-adrenoceptors, increased renin secretion and myocardial as well as vascular hypertrophy [10,11]. Cytotoxicity of catecholamines may also be related to oxidative-related damage since catecholamines are metabolized partly through oxidation. In addition, sympathetic stimulation after myocardial infarction causes myocardial oxygen demand to rise, precipitating further ischemia, and the proarryhtmic effects of catecholamines may predispose to fatal arrythmias [9].

Sympathetic stimulation increases myocardial energy consumption and can promote ischemia especially at the level of subendocardial cells of a dilated ventricle. Increased energy consumption at the cellular level is induced since more actin-myosin cross-bridging between myofibrils has to occur during each cardiac cycle. The higher level of cross-bridging activity is achieved by an increase in calcium delivery to the myofibrils, which is pumped back either by the sarcoplasmatic reticulum or into the extracellular space by sarcolemma calcium pumps and sodium-calcium exchange [12]. Furthermore, sympathetic stimulation increases intracellular concentrations of cAMP and calcium which could promote myocyte deterioration and apoptosis.

1.3.2. Renin-angiotensin-aldosterone system

Sympathetic-dependent vasoconstriction induces a reduction in the capacity of the kidney to excrete excess sodium [13]. During the initial stages of heart failure, symptoms of sodium retention only occur in cases of excessive intake of sodium. As the disease progresses, sodium retention remains constant through the stimulation of the renin-angiotensin-aldosterone system, this being the second major compensatory mechanism. The release of renin increases indirectly with reduction in renal artery pressure, and directly by sympathetic tone via β_1-adrenoceptors. The combined stimulation of sympathetic and renin-angiotensin-aldosterone systems acts to maintain blood pressure through vasoconstriction and plasma volume expansion. Thus, sodium retention appears to be a key condition in heart failure. Plasma hormonal profiles have been studied extensively during heart failure. Initially the levels of plasma catecholamines are elevated during mild heart failure, with plasma renin activity increasing at later and more severe stages of the disease (Studies of Left Ventricular Dysfunction; SOLVD study) [14]. Consequences of such a renin-angiotensin stimulation are first, increased cardiac loading conditions (vasoconstriction), and second, cellular actions of angiotensin II through activation of phospholipase C, especially stimulation of cell growth and of collagen synthesis by fibroblasts. Furthermore, physiological actions of angiotensin II include inotropic and chronotropic effects on the myocardium [15], and effects on cardiovascular growth.

Aldosterone production is stimulated by angiotensin II and enhances sodium re-absorption by the distal tubule of the kidney. Some experimental work demonstrated that administration of high doses of aldosterone to rats and guinea pigs induces perivascular and interstitial collagen synthesis [16]. Such effects may promote the replacement of contractile fibers by fibrous tissue and further alter cardiac function: ventricle enlargement, arrythmias and ischemia by increasing the distance between capillaries and contractile sites of myocytes.

1.3.3. Arginine-vasopressin

The role of vasopressin system in the development of heart failure as well as the therapeutic possibilities of vassopressin antagonists are less investigated. Arginine-vasopressin is secreted by the posterior pituitary gland and is an antidiuretic hormone, and its two principal hemodynamic effects, vasoconstriction and fluid retention, are mediated via the V_{1a} and V_2 receptors, respectively [17,18]. Vasopressin is involved in regulation of circulating volume and osmolarity and has a suppressive effect on renin release by renal juxtaglomerulary cells [19]. Its secretion is stimulated by β-adrenergic activation and angiotensin II. Vasopressin has direct vasoconstrictive effects and indirect facilitating effects on sympathetic nervous system in an animal model [20]. Through activation of specific vasopressin V_2- receptors, vasopressin has some vasodilating action as well [21]. Overall, vasoconstrictive action prevails. Currently, there are only a limited number of studies examining the use of vasopressin antagonists as therapy for heart failure. Selective inhibition of the V_1 and V_2 receptor led to immediate improvement in hemodynamic parameters [22] and increased diuresis [23].

1.4. Postinfarction remodeling

When neurohormonal activation is not capable to restore hemodynamics after acute myocardial infarction, cardiac loading conditions remain increased evolving structural adaptation of the heart itself. This complex of structural changes is termed *remodeling* [24]. The cardiac remodeling process tends to reconstitute myocardial

tissue mass and to restore normal cardiovascular hemodynamics after large myocardial infarction [25]. Postinfarction remodeling has been divided into an *early* compensatory phase and *late* decompensation phase. Whereas in the *early phase* irreversibly damaged tissue is replaced by scar tissue and compensatory changes (myocyte hypertrophy) occur in the non-infarcted myocardium, *late* phase involves a continued remodeling process not accompanied by improved cardiac function and even contributing to progression of left ventricular dysfunction.

Initially, cardiac remodeling can be viewed as a *compensatory* mechanism (normalizing wall stress), which provides a functional advantage. However, at some point, these regenerative responses may become deleterious and contribute to heart failure [26,27]. It has become increasingly recognized that besides infarct size, the quality of the remaining viable tissue is a key determinant in ultimate patient prognosis. The acute loss of myocardium after myocardial infarction results in an abrupt increase in loading conditions that induces a unique pattern of remodeling involving the infarcted border zone and remote non-infarcted myocardium. Myocyte necrosis and the resultant increase in load trigger a cascade of biochemical intracellular signaling processes that initiates and subsequently modulates reparative changes, which include dilation, hypertrophy, and the formation of a discrete collagen scar. Ventricular remodeling may continue until the distending forces are counterbalanced by the tensile strength of the collagen network. This balance is determined by the size, location, and transmurality of the infarct, the extent of myocardial stunning, the patency of the infarct-related artery, and local tropic factors [24,28]. The failure to normalize increased wall stresses results in progressive dilation, recruitment of border zone myocardium into the scar, and deterioration in contractile function. Three major components of the myocardium following myocardial infarction are involved in the remodeling process: myocytes, extracellular matrix, and capillary microcirculation that services the contractile unit assembly. Consideration of all 3 components provides important insights into remodeling process and a rationale for future therapeutic strategies.

1.4.1. Infarcted myocardium

During the first hours after myocardial infarction, a reparative process is initiated to rebuild infarcted myocardium and maintain structural integrity of the ventricle. The repair process of the heart after myocardial infarction is considered a *wound healing* response, since it resembles the response of parenchymatous tissue to ischemic injury. In the infarcted area, myocyte necrosis and an acute inflammatory response at the border of the necrotic myocytes take place, followed by inflammation, fibroblast- and vascular cell proliferation, extracellular matrix deposition and resorption of necrotic tissue. Inflammatory response to cell death after myocardial infarction becomes evident by cellular infiltration (neutrophils followed by lymphocytes and plasma cells) progressing from the border zone into the more central areas of the infarct [29]. Other signs of inflammation are present, such as interstitial oedema and vascular congestion.

More recently, it was suggested that myocyte programmed cell death or apoptosis was the major initial, as early as 2 hours after infarct induction, form of myocyte loss produced by coronary artery occlusion in rats, whereas necrotic cell death followed apoptosis and contributed to the evolution of the infarct size [30]. Apoptosis was largely limited to hypoxic regions during acute infarction. Also in human infarcts extensive apoptosis occurred before neutrophilic infiltration was apparent. During the *healing phase* of the infarction, apoptosis has been reported to play an important role in the disappearance of infiltrated leucocytes [31].

Changes in collagen metabolism in the infarct segment have been subject of detailed investigation in the rat infarction model. Early after infarction degradation of the collagenous network surrounding cardiomyocytes and capillaries in the infarct zone occurs [32]. Collagen fibers are broken down by the activation of collagenases and related enzymes. The breakdown of cardiac structure can lead to slippage of surviving myocytes and, in addition, to myocyte loss. This process may cause disproportionate dilation and thinning of the infarct, also called *'infarct expansion'* [33]. Infarct expansion is a risk factor for the development of ventricular aneurysm or even rupture and is associated with adverse effects on ventricular function and prognosis [34]. Scar formation is the net result of a delicate balance between collagen

degradation and synthesis [35]. In infarcted rats, the expression of type I collagen gene is markedly enhanced in the infarcted zone on day 3 and persists for 90 days [32,36]. Fibrillar collagen deposition was observed by day 7 [35]. Collagen degradation exceeds synthesis during the very early phase of infarction. Enhanced collagen degradation after myocardial infarction involves a family of matrix metalloproteinases which mediate degradation of fibrillar collagen [37]. In turn, the activity of metalloproteinases is controlled by a family of tissue inhibitors of metalloproteinases. Thus, the balance between collagenases and tissue inhibitors of metalloproteinases ultimately determines the amount of collagenolysis in infarcted tissue [32].

Myofibroblasts have been shown to be the predominant cell responsible for collagen formation at sites of repair in the rat heart [32,36]. These myofibroblasts were shown to remain in mature scar tissue [38]. Increases of both types I and III procollagen mRNA levels have been observed within a few days after infarct induction, followed by collagen deposition [32]. The amount of other constituents of the extracellular matrix, such as fibronectin and tenascin, also changes after the induction of myocardial infarction [39,40].

1.4.2. Non-infarcted myocardium

At first sight the changes in the non-infarcted myocardium are not as dramatic as the changes in the infarct. Changes in the non-infarcted myocardium, however, affect both ventricles and affect all constituents of the myocardium, including the cardiomyocytes, vasculature as well as the extracellular matrix.

Myocyte hypertrophy

Compensation for the loss of viable tissue after infarction entails myocyte hypertrophy in the non-infarcted myocardium [41]. Cardiomyocyte hypertrophy occurs within several days after infarction and cardiomyocytes may increase their volume up to 112% in humans [42]. In cardiomyocytes, DNA synthesis is a limited process and involves less than 1% of the DNA synthetizing cells in rats [43,44]. Hypertrophy early after infarction may be seen as an useful adjustement to loss of contractile tissue, by increasing the number of sarcomeres and by restoring wall stress in the infarcted

ventricle [41]. In infarcted rats, side-to-side slippage of ventricular myocytes occurs in the first days after infarction and contributes to left ventricular enlargement [42]. After the acute phase, eccentric hypertrophy of myocytes further expands the left ventricle [45]. Besides eccentric hypertrophy, concentric growth of myocytes occurs in the non-infarcted part of the injured left ventricle. Reactive hypertrophy can be considered as an adequate response, though not always sufficient to normalize cardiac load. Besides through hypertrophy, myocardial mass may also increase through an increase in cell number. For a long time the prevailing perception has been that cardiomyocytes are terminally differentiated cells and lose their ability to proliferate. Myocytes lose the ability to divide shortly after birth [46], although myocyte polyploidy through nuclear division has been reported in hypertrophied hearts [47,48] and even the possibility of myocyte hyperplasia [42].

Cardiomyocyte apoptosis is also an important process which has been described in the non-infarcted myocardium, and may contribute to the remodeling process after infarction [49], and to the induction or progression of cardiac failure after infarction [50]. Indeed, a significant increase in myocyte apoptosis could be observed in the human failing heart [51]. Not only cell size and number, but also the cellular phenotype may change. Cardiac hypertrophy secondary to infarction is associated with altered gene expression, especially genes of the fetal phenotype [52]. For example, a transition of the expression of the normally expressed fast contractile protein α-myosin heavy chain to the slower β-myosin heavy chain results in a decreased capacity to develop contractile speed [52,53]. Such a change can be viewed to be adaptive because of a lower energy and oxygen consumption of the cardiac muscle.

Extracellular matrix

The extracellular matrix forms the structural backbone of the heart and is composed of a complex of macromolecules, which can be divided in at least five major components: collagens, basement membranes, elastic fibers, proteoglycans and structural glycoproteins. The collagen network is major constituent for the preservation of myocardial architecture and chamber geometry and plays an integral role in global ventricular remodeling [54]. Interstitial collagens I and III, which are

predominantly produced by fibroblasts, form the major components of the extracellular matrix [55]. An early accumulation of fibrillar collagen in the non-infarcted region has repeatedly been observed following infarction [43,56]. This increase in collagen seems not to be directly related to myocyte necrosis, but resembles the pattern of reactive fibrosis, characterized by thickening of the perimysium and occupation of intermuscular spaces by collagen fibers [32]. The elevation of collagen content in the non-infarcted septum is preceded by a 4 to 5-fold increased production of type I and III procollagen mRNA until 21 days post-infarction [32]. The stimulus for collagen deposition is not exactly kown, but potential candidates are angiotensin II, aldosterone, growth factors like transforming growth factor and catecholamines [35].

Changes in collagen content might be considered, again, as beneficial as well as maladaptive. On the one hand, collagen deposition probably can serve an adaptive role in the initial response to overload, where increased wall stiffness can reduce dilation and, as a consequence of the law of Laplace, lessen wall tension in the overloaded heart [57]. On the other hand, it has been shown that even small increases in the amount of collagen can cause adverse effects on diastolic relaxation and result in stiffness [58]. Indeed, deposition of collagen in the non-infarcted myocardium has been related to an increased myocardial stiffness and impaired diastolic heart function [59].

Microcirculation

As the heart progressively enlarges, coronary blood flow is limited by microvascular abnormalities and the high intramyocardial wall tension caused by the altered geometry of the now dilated and hypertrophied ventricle. Vascular adaptation of the vascular beds which are perfused by the remaining patent coronary arteries after myocardial infarction is required. The amount of contractile tissue which the patent coronary arteries feed has increased due to compensatory hypertrophy, and this myocardium has to operate at increased wall stress [45]. Therefore, a greater amount of nutrients and oxygen is needed by the spared part of the heart. Increased oxygen delivery to hypertrophied viable tissue can be achieved by increasing resting coronary blood flow through dilatation of resistance arteries [60]. If peak oxygen

demand of the hypertrophied viable myocardium exceeds the supply by the fully dilated vascular bed, ischemic stress will threaten function and viability of myocytes [61]. In addition to this potentially inadequate oxygen supply by the coronary arteries, oxygen availibility may also be hampered at a cellular level by increased pathway length for oxygen to diffuse from interstitial capillaries to mitochondria within the cardiomyocyte [41].

In the non-infarcted myocardium, a decrease of the total capillary density and capillary to myocyte ratio has been reported 30 days after coronary artery occlusion in the rat [62]. In the majority of studies, a deficit in the microvascular network was shown after myocardial infarction. Early investigations demonstrated that the capillarity is decreased in the hypertrophied myocardium whereas intercapillary distance increased due to a rise in cardiomyocyte diameter [63]. Similar findings have been obtained in many subsequent experimental studies, in which the capillary supply to the surviving hypertrophic cells was decreased in the *early* and *late* phases after infarction, as expressed in morphometric parameters like capillary density, capillary luminal volume, capillary surface density and elevated diffusion distances [64,65]. From these studies, it was concluded that capillary density in the hypertrophied non-infarcted part decreased by up to 22% such that diffusion distances for oxygen were increased by 16%. The response of the coronary vasculature within the surviving myocardium could generate a relative energy starvation that, in turn, may lead to local ischemia and tissue damage [47] and may account for the impaired performance of infarcted hearts.

In the remodeled myocardium, vessel density is the result of angiogenesis and myocyte hypertrophy. In the non-infarcted myocardium of rat infarcted hearts, capillary angiogenesis is inadequate in maintaining normal tissue capillarization. Decreased capillary density is especially pronounced in the non-infarcted tissue near the infarct region [66]. Small studies using autopsy material from human myocardium suggest that both capillary and arteriolar density are maintained [67], and even tend to be increased in infarction induced hypertrophy. This may suggest that angiogenesis in the vascular beds of the hypertrophied myocardium must have occurred. However, it remains unclear if besides infarction induced remodeling, additional ischemia due

to coronary artery atherosclerosis of the arteries supplying non-infarcted tissue increases the angiogenic response.

Biochemical alterations

Although reactive hypertrophy after myocardial infarction has the potential to restore the amount of contractile myocardium, the increased volume per cell is parallelled by a number of biochemical alterations in the myocytes that may be involved in the ultimate hemodynamic deterioration towards cardiac failure. 1) Mean oxygen diffusion distance is increased due to reduced cardiomyocyte surface to intercellular volume ratio, which is further aggravated by interstitial tissue volume and decreased capillary density [45,68]. This seems to be most pronounced in the area bordering the infarct zone. Cellular hypoxia may be detrimental, by limiting ATP production by mitochondrial oxidative phosphorylation in already functionally stressed myocytes. In addition, cellular hypoxia might trigger apoptosis, which would further reduce the numer of viable myocytes [69]. Cellular loss would then demand further hypertrophy of remaining myocytes, resulting in a vicious circle of events leading to heart failure. 2) Imbalance between ATP generating and ATP consuming processes is indicated by a decrease in ratio of mitochondria to myofibril number. Indeed, in the non-infarcted hypertrophied myocardium, energy reserve (intracellular levels of high-energy phosphates) [70] and mitochondrial oxygen consumption rate are decreased [71]. Remodeled hearts display enhanced anaerobic metabolism, which was suggested by increased lactate hydrogenase activity and impaired creatine kinase activity in the residual contractile tissue bordering the infarct [72]. 3) In hypertrophied myocytes there seems to be an impaired calcium handling. Indeed, a significant reduction of ATP-dependent calcium uptake activity of sarcoplasmic reticulum membrane fractions has been observed in the non-infarcted myocardium bordering the infarcted area [73]. Furthermore, calcium sensitivity of myofibrils from hypertrophied myocardium has been reported to be reduced [73]. 4) In the non-infarcted myocardium of rats, a decreased ATPase activity of contractile apparatus has been reported resulting from a switch to a slower V_3 myosin isoform [74]. This chemical conversion can be regarded as an adaptive process that limits the excessive increase in oxygen consumption that might be expected with a significant increase in total muscle mass. 5) There seems

to be an altered response to sympathetic stimulation in the non-infarcted myocardium due to a down-regulation of β-adrenoceptor number and reduced intracellular transmission of the signal [75,76].

Thus, increased diffusion distance for oxygen may be the initiating stimulus for the aforementioned biochemical changes, since it can result from an increase of myocyte volume. Relative oxygen deficit of the myocyte and impaired oxidative phosphorylation capacity could be related to decreased potential of generating high-energy phosphates and result in a number of changes affecting the contractile apparatus of the cardiomyocyte, such as lower ATPase activity of myofibrils and other energy-requiring processes.

1.5. Pharmacological intervention on postinfarction remodeling

In the 1970s, it was believed that vasodilator drugs worked primarily by reducing high systemic vascular resistance and thus by "unloading" the failing left ventricle. Undoubtedly, the picture is far more complex than this . It is clear that the concept of chronic afterload reduction of the failing heart with pure arteriolar vasodilator is now challenged and may no longer be tenable. This concept is now largely relegated to the treatment of acute or decompensated heart failure. The chronic use of therapies which increase ejection fraction by directly stimulating cardiac contractility (dobutamine, milrinone) or by decreasing impedance to left ventricular ejection through relaxation of peripheral blood vessels may relieve symptoms in the short term, but do not necessarily produce clinical benefits in the long term and may even increase mortality [3,77]. However, favourable experience with long-term vasodilator therapy in heart failure has been observed with the combination of isosorbide dinitrate and hydralazine in the V-HeFT II Trial [78]. Initially combined because of their complementary dilating actions on periheral bloodvessels, recent evidence suggests that hydralazine and isosorbide dinitrate may act principally at a biochemical level rather than as conventional vasodilators. Pharmacological treatments may also improve ejection fraction by affecting the process of cardiac remodeling. This approach utilized by neurohormonal blockade with ACE-inhibitors or β-adrenergic receptor blockers [3] has been shown to improve left ventricular

performance and patient survival when used on a chronic basis for the treatment of heart failure. In addition, diuretics with aldosterone blocking properties such as spironolactone have been shown to improve survival in patients with class III and IV heart failure, adding further support for the neurohormonal hypothesis [79].

The above observations suggest that the most effective means of decreasing risk of hospitalization and death in patients with heart failure is to adress the primary mechanisms that contribute to disease progression rather than to stimulate the contractility of the failing left ventricle. The current working hypothesis in the treatment of heart failure is represented in Fig.2.

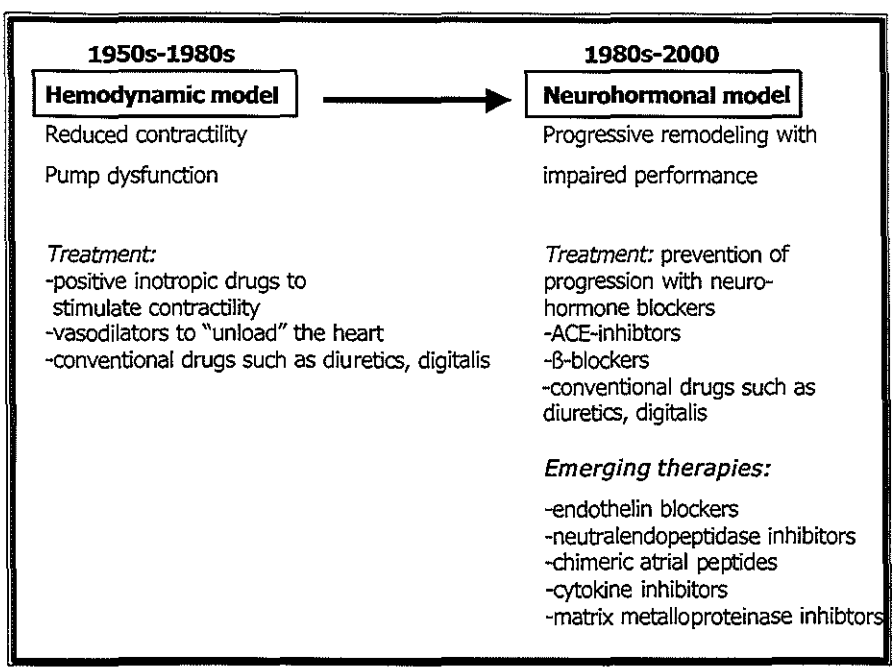


Fig.2 Heart failure: a changing paradigm *(Francis GS, 2001).*

1.5.1. Angiotensin converting enzyme (ACE)-inhibitors

ACE-inhibtors have emerged as the cornerstone of heart failure therapy. The efficacy of ACE-inhibitors in attenuating left ventricular dilation after infarction was first demonstrated in the rat, and this effect on remodeling was associated with

improved survival. Observations on the effects of ACE-inhibition in infarcted rats [80,81] have not only stressed the importance of cardiac remodeling in determing the clinical outcome, but also triggered numerous follow-up studies addressing the role of the renin-angiotensin-aldosterone system in the control of remodeling. The effects of captopril, furosemide, and placebo were studied in patients with asymptomatic left ventricular dysfunction 1 week after myocardial infarction [82]. Captopril treatment resulted in a significant reduction in left ventricular end-systolic volume index, with increases in stroke volume index and ejection fraction, whereas treatment with furosemide and placebo was associated with significant increases in echocardiographic left ventricular volumes.

The mechanism of improvement with ACE-inhibition is related in part to peripheral vasodilation, ventricular unloading, and the attenuation of ventricular dilation. There may be additional beneficial effects on the coronary circulation and intrinsic plasminogen-activating system [83]. Although coronary hemodynamic data have suggested a balanced effect of ACE-inhibitors on the coronary circulation, one study in patients with heart failure indicated that such treatment may worsen ischemia, because of the hypotension that compromises myocardial perfusion [84]. Importantly, ACE-inhibition may have a direct effect on myocardial tissue [85] preventing the inappropiate growth and hypertrophy stimulated by angiotensin II and other growth factors. Besides effects on hypertrophy, observations on increased interstitial collagen deposition and the inhibitory effect of ACE-inhibitor thereon resulted in the hypothesis that the renin-angiotensin-aldosterone system might be involved in the control of collagen synthesis in the cardiac interstitium [86].

Previous experimental studies [81,87] have clearly demonstrated that *early* treatment with captopril in chronically infarcted rats resulted in prevention of hypertrophy which was associated with deterioration of *in vivo* haemodynamics. On the other hand in the rat myocardial infarction model, improved heart function was found with delayed captopril treatment [81]. Furthermore, clinical trials evaluating *early* intervention with ACE-inhibitors in patients with acute myocardial infarction have not yielded uniform results. Whereas decreased mortality has been reported from some trials [71,88,89], others did not find improved survival [90-93].

1.5.2. Angiotensin receptor blockers

An alternative approach to inhibiting the actions of angiotensin II in patients with heart failure is the use of drugs that block the angiotensin II receptor. Whether angiotensin receptor blockers are equivalent or superior to ACE-inhibitors is still unclear, but several clinical trials are now underway. Several angiotensin II receptor antagonists have been approved for the treatment of hypertension (losartan, valsartan, irbesartan, candasartan and eprosartan), but none has been approved for use in heart failure. The actions of angiotensin II depend upon the presence of relevant angiotensin receptors. Angiotensin II receptors in the heart consist of at least two major subtypes [94] and have been characterized by using potent, highly selective antagonists of the angiotensin type 1 and 2 receptor (AT_1-R and AT_2-R). In the non-infarcted myocardium an increase in AT_1-R number and AT_1-R mRNA have been reported as well as an upregulation of AT_2-R and AT_2-R mRNA in whole ventricular tissue [95,96]. Activation of the AT_1-R lead to the majority of known functions of the renin-angiotensin-aldosterone system, like vasoconstriction, renal salt and water retention, the drinking response, inotropy, chronotropy, and cell growth [97]. The function of AT_2-R is less clear, but it has a possible role in growth and development, differentation and blood pressure regulation. Early AT_1-R blockade in rats, significantly reduced interstitial collagen following myocardial infarction, without affecting cell proliferation. In contrast, early AT_2-R blockade in rats abolished the increased cell proliferation [98], but completely lacked an effect on collagen deposition [99,100]. More importantly, using the same experimental setup *late* but not *early* captopril treatment restored cardiac function following myocardial infarction. Starting the treatment with ACE-inhibitors, 7 or 14 days after infarction, improves cardiac function and the 1 year survival rate of infarcted rats [80]. AT_2- but not AT_1-R blockade had similar effects [100]. Since this improvement coincided with effects on cell proliferation and not with effects on collagen, this implies that proliferative phenomena, rather than interstitial collagen, determine cardiac function following myocardial infarction. Moreover, since AT_2-R blockade does not affect aldosterone, the role for angiotensin II seems to dominate. Intervention on the renin-angiotensin-aldosterone system with AT_1-antagonists also inhibits the hypertrophic response.

Although, again, afterload reduction may contribute to this effect, studies with AT-receptor antagonists indicate a specific AT_1-receptor mediated hypertrophic response in cardiomyocytes [97]. The ELITE-study, which compared captopril with losartan, showed a trend towards a reduction in mortality of AT_1-antagonist treated patients [101]. ELITE II, an ongoing trial designed to examine mortality, may further extend our understanding of the mechanisms underlying postinfarction remodeling, including the relative actions played by the kinins [102].

1.5.3. β-adrenergic receptor blockers

Benefit of β-blockade therapy as a secondary preventive therapy for myocardial infarction has been demonstrated by data from large number of trials, which showed a significant mortality reduction [103,104]. Clinical trials of several different β-blockers have shown that these drugs can produce hemodynamic and symptomatic improvement in chronic heart failure due to non-ischemic and ischemic cardiomyopathy [105,106]. β-blockers have been shown to establish neuroendocrine, anti-arrythmic and anti-ischemic effects [107]. Non-selective agents without intrinsic sympathomimetic activity appeared to have the maximal potential benefit [108]. The mechanisms whereby β-adrenergic blockers improve left ventricular performance and reduce mortality are still not entirely clear. Early studies suggested that upregulation of reduced density of myocardial β-adrenergic receptors was a prime mechanism of action of β-blockers [109], but this has not been demonstrated with the newer β-adrenergic blockers, such as carvedilol [110]. A large multicentre heart failure trial has indicated that the addition of carvedilol to conventional therapy is associated with a decrease in mortality [107].

Non-selective β-blockers such as carvedilol may attenuate remodeling, an effect associated with a significant reduction in subsequent adverse cardiac events [111]. Whether β-blocking agents provide a benefit additional to ACE-inhibitor treatment in heart failure remains unknown. Although the rationale for combination treatment is strong when extrapolating from clinical trials with β-blockers after myocardial infarction, definitive data are lacking. The mortality benefit from β-blockade in heart failure patients is due to a reduction in both progressive heart

failure and sudden death. Thus, in patients with heart failure, combination neurohormonal blockade may be optimal, although occasionally limited by hypotension.

1.6. Outline of the thesis and hypothesis

The response to acute myocardial infarction can be divided into different phases:

1. Acute myocardial infarction induces a sudden decrease of cardiac output related to the size of infarcted area.
2. *Compensatory phase*: A decreased cardiac output evokes activation of compensatory mechanisms, such as the sympathetic nervous system and renin-angiotensin-aldosterone sytem. If these mechanisms cannot bring cardiac output back to acceptable levels, structural changes in the heart called hypertrophy and remodeling will follow.
3. *Compensated phase*: A relatively stabile phase with (partly) restored cardiac output but with decreased left ventricular function and ejection fraction has been established; a stage compensated heart failure, in which patients are often not recognized.
4. *Decompensation phase*: When symptoms of heart failure become overt, decompensation develops driven by the same mechanisms as in the previous phases, but with a very poor prognosis.

Therapy during the different phases require different strategies. During phase 1 the objective of therapy is to limit infarct size by establishing reperfusion as quick as possible. Then, theoretically, phase 2 requires stimulation, phase 3 stabilization and support, and phase 4 inhibition of the same ongoing processes. Although still patients at stage 4 are clinically the most important ones, inclination towards earlier intervention is growing. The problem that may arise, however, is that earlier treatment, especially at stage 2 is aimed at the opposite direction as the later therapy. This problem is substantiated by the results of the 2 CONSENSUS trials, where delayed ACE-inhibitor (enalapril) treatment had clearly beneficial effects

(CONSENSUS I), whereas similar treatment during early phases had detrimental effects (CONSENSUS II)[91]. Pharmacological research has mainly focused on *stage 4* when congestive heart failure is diagnosed and prognosis becomes very poor. Except for treatment with ACE-inhibitors, prognosis has not benefited substantially from all these research. During this stage, cardiac hypertrophy and remodeling have become a major target for therapy since they are associated with improved prognosis [112]. When treatment should be started earlier, that is with ventricular dysfunction but before signs of heart failure, it implies interference with cardiac hypertrophy and remodeling, which during the *early phase* are regarded a pathophysiological response to compensate for the loss of contractile tissue. This indicates that treatment should therefore be selected carefully [81,91]. Thus, although the importance of treatment during the **compensatory stage** is now well acknowledged, a strategy is not established yet.

Hypothesis

We hypothesize that treatment during the *early* compensatory stage should be directed to preservation of the total amount of contractile tissue, with optimal perfusion.

The former implicates that reactive hypertrophy should not be inhibited, whereas perfusion can be enhanced by increased vascularization, or optimizing the use of existing vascularization by prolonged diastolic time or improved cardiac relaxation. In addition, interference with collagen should be minimized in order to preserve sufficient tensile strength and prevent left ventricular dilation.

Experimental model

Over the years, several animal models for heart failure have been developed mostly based upon conditions that lead to heart failure in man. In the present thesis, ligation of the left anterior descending coronary artery in rats, resulting in transmural infarction of the LV free wall, was used to study the structural and functional

consequences of a large myocadial infarction. The rat heart failure model has been shown to be a well established model of cardiac failure, which is associated with neurohormonal activation and *remodeling* of the cardiac structure [29,113].

To test our hypothesis in this rat heart failure model, the following sets of experiments were performed:

- In **chapter 2**, the effects of *early* treatment with a low-dose aspirin and methylprednisolone have been used to study the intereference with collagen deposition and its relation to left ventricular diastolic function.
- In **chapter 3**, the effects of *early* sympatholytic treatment with moxonidine are evaluated on postinfarction induced sympathetic activation and its consequences for ventricular hypertrophy and collagen deposition.
- In **chapter 4**, the effects of *early* moxonidine therapy on capillary density in relation to sensitivity to ischemia are investigated.
- In **chapter 5,** the effects of *early* treatment with vasopressin V_{1A} and V_2 antagonists on *in vivo* hemodynamics and postinfarction remodeling have been studied.
- In **chapter 6**, we compared the effects of *early* therapeutic intervention with aspirin, methylprednisolone, moxonidine and captopril on *in vivo* and *intrinsic* heart rate in relation to capillary growth in the non-infarcted myocardium.

1.7. References

1. Berry C, Murdoch DR, McMurray JJ. Economics of chronic heart failure. *Eur J Heart Fail.* 2001;3:283-91.
2. Ho KK, Pinsky JL, Kannel WB, Levy D. The epidemiology of heart failure: the Framingham Study. *J Am Coll Cardiol.* 1993;22:6A-13A.
3. Packer M. New concepts in the pathophysiology of heart failure: beneficial and deleterious interaction of endogenous haemodynamic and neurohormonal mechanisms. *J Intern Med.* 1996;239:327-33.
4. Mathey D, Biefield W, Hanrath P, Effert S. Attempt to quantitate relation between cardiac function and infarct size in acute myocardial infarction. *Br Heart J.* 1974;36:271-9.
5. McAlpine HM, Morton JJ, Leckie B, Rumley A, Gllen G, Dargie HJ. Neuroendocrine activation after acute myocardial infarction. *Br Heart J.* 1988;60:117-24.
6. Packer M. Pathophysiology of chronic heart failure. *Lancet* 1992;340:88-92.
7. Francis GS. Neurohumoral activation and progression of heart failure: hypothetical and clinical considerations. *J Cardiovasc Pharmacol.* 1998;32:S16-21.
8. Francis GS, Boosalis PJ. Mechanism of death in patients with congestive cardiac failure: the change in plasma norepinephrine and its relation to sudden death. *Cardioscience.* 1990;1:29-32.
9. Rupp H, Dhalla KS, Dhalla NS. Mechanisms of cardiac cell damage due to catecholamines: significance of drugs regulating central sympathetic outflow. *J Cardiovasc Pharmacol.* 1994;24:S16-24.
10. Francis GS, McDonald KM, Cohn JN. Neurohumoral activation in preclinical heart failure. Remodeling and the potential for intervention. *Circulation.* 1993;87:IV90-6.
11. Ceconi C, Curello S, Ferrari R. Catecholamines: the cardiovascular and neuroendocrine system. *Eur Heart J* 1998;19:F2-6.
12. Theroux P. A symposium: sodium-hydrogen exchange inhibition. Introduction. *Am J Cardiol.* 1999;83:1G-2G.
13. Volpe M, Tritto C, DeLuca N, Rubattu S, Rao MA, Lamenza F, Mirante A, Enea I, Rendina V, Mele AF, et al. Abnormalities of sodium handling and of cardiovascular adaptations during high salt diet in patients with mild heart failure. *Circulation.* 1993;88:1620-7.
14. Effect of enalapril on mortality and the development of heart failure in asymptomatic patients with reduced left ventricular ejection fractions. The SOLVD Investigattors. *N Engl J Med.* 1992;327:685-91.
15. Lindpaintner K, Ganten D. The cardiac renin-angiotensin system. An appraisal of present experimental and clinical evidence. *Circ Res.* 1991;68:905-21.
16. Weber KT, Villarreal D. Role of aldosterone in congestive heart failure. *Postgrad Med.* 1993;93:203-7, 211-2, 216-8 passim.

17. Burrell LM, Phillips PA, Stephenson JM, Risvanis J, Johnston CI. Vasopressin and a nonpeptide antidiuretic hormone receptor antagonist (OPC-31260). *Blood Press.* 1994;3:137-41.
18. Thibonnier M, Conarty DM, Preston JA, Wilkins PL, Berti-Mattera LN, Mattera R. Molecular pharmacology of human vasopressin receptors. *Adv Exp Med Biol.* 1998;449:251-76.
19. Ostrowski NL, Lolait SJ, Bradley DJ, O'Carroll AM, Brownstein MJ, Young WS, 3rd. Distribution of V1a and V2 vasopressin receptor messenger ribonucleic acids in rat liver, kidney, pituitary and brain. *Endocrinology.* 1992;131:533-5.
20. Stone CK, Liang CS, Imai N, Sakamoto S, Sladek CD, Hood WB, Jr. Short-term hemodynamic effects of vasopressin V1-receptor inhibition in chronic right-sided congestive heart failure. *Circulation.* 1988;78:1251-9.
21. Hirsch AT, Dzau VJ, Majzoub JA, Creager MA. Vasopressin-mediated forearm vasodilation in normal humans. Evidence for a vascular vasopressin V2 receptor. *J Clin Invest* 1989;84:418-26.
22. Creager MA, Faxon DP, Cutler SS, Kohlmann O, Ryan TJ, Gavras H. Contribution of vasopressin to vasoconstriction in patients with congestive heart failure: comparison with the renin-angiotensin system and the sympathetic nervous system. *J Am Coll Cardiol.* 1986;7:758-65.
23. Wong LL, Verbalis JG. Vasopressin V2 receptor antagonists. *Cardiovasc Res.* 2001;51:391-402.
24. Pfeffer MA, Braunwald E. Ventricular remodeling after myocardial infarction. Experimental observations and clinical implications. *Circulation.* 1990;81:1161-72.
25. Ginzton LE, Conant R, Rodrigues DM, Laks MM. Functional significance of hypertrophy of the noninfarcted myocardium after myocardial infarction in humans. *Circulation.* 1989;80:816-22.
26. Pfeffer JM, Pfeffer MA, Fletcher PJ, Braunwald E. Progressive ventricular remodeling in rat with myocardial infarction. *Am J Physiol.* 1991;260:H1406-14.
27. Anversa P, Li P, Zhang X, Olivetti G, Capasso JM. Ischaemic myocardial injury and ventricular remodelling. *Cardiovasc Res.* 1993;27:145-57.
28. Warren SE, Royal HD, Markis JE, Grossman W, McKay RG. Time course of left ventricular dilation after myocardial infarction: influence of infarct-related artery and success of coronary thrombolysis. *J Am Coll Cardiol.* 1988;11:12-9.
29. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. *Am J Pathol.* 1978;90:57-70.
30. Bialik S, Geenen DL, Sasson IE, Cheng R, Horner JW, Evans SM, Lord EM, Koch CJ, Kitsis RN. Myocyte apoptosis during acute myocardial infarction in the mouse localizes to hypoxic regions but occurs independently of p53. *J Clin Invest* 1997;100:1363-72.
31. Takemura G, Ohno M, Hayakawa Y, Misao J, Kanoh M, Ohno A, Uno Y, Minatoguchi S, Fujiwara T, Fujiwara H. Role of apoptosis in the disappearance of infiltrated and proliferated interstitial cells after myocardial infarction. *Circ Res.* 1998;82:1130-8.
32. Cleutjens JP, Verluyten MJ, Smiths JF, Daemen MJ. Collagen remodeling after myocardial infarction in the rat heart. *Am J Pathol.* 1995;147:325-38.

33. Weisman HF, Bush DE, Mannisi JA, Bulkley BH. Global cardiac remodeling after acute myocardial infarction: a study in the rat model. *J Am Coll Cardiol.* 1985;5:1355-62.
34. Vannan MA, Taylor DJ. Ventricular remodelling after myocardial infarction. *Br Heart J.* 1992;68:257-9.
35. Weber KT. Monitoring tissue repair and fibrosis from a distance. *Circulation.* 1997;96:2488-92.
36. Sun Y, Ramires FJ, Zhou G, Ganjam VK, Weber KT. Fibrous tissue and angiotensin II. *J Mol Cell Cardiol.* 1997;29:2001-12.
37. Tyagi SC, Ratajska A, Weber KT. Myocardial matrix metalloproteinase(s): localization and activation. *Mol Cell Biochem.* 1993;126:49-59.
38. Willems IE, Havenith MG, De Mey JG, Daemen MJ. The alpha-smooth muscle actin-positive cells in healing human myocardial scars. *Am J Pathol.* 1994;145:868-75.
39. Willems IE, Arends JW, Daemen MJ. Tenascin and fibronectin expression in healing human myocardial scars. *J Pathol.* 1996;179:321-5.
40. Ulrich MM, Janssen AM, Daemen MJ, Rappaport L, Samuel JL, Contard F, Smits JF, Cleutjens JP. Increased expression of fibronectin isoforms after myocardial infarction in rats. *J Mol Cell Cardiol.* 1997;29:2533-43.
41. Anversa P, Loud AV, Levicky V, Guideri G. Left ventricular failure induced by myocardial infarction. II. Tissue morphometry. *Am. J. Physiol.* 1985;248:H883-9.
42. Olivetti G, Melissari M, Balbi T, Quaini F, Cigola E, Sonnenblick EH, Anversa P. Myocyte cellular hypertrophy is responsible for ventricular remodelling in the hypertrophied heart of middle aged individuals in the absence of cardiac failure. *Cardiovasc Res.* 1994;28:1199-208.
43. van Krimpen C, Smits JF, Cleutjens JP, Debets JJ, Schoemaker RG, Struyker Boudier HA, Bosman FT, Daemen MJ. DNA synthesis in the non-infarcted cardiac interstitium after left coronary artery ligation in the rat: effects of captopril. *J. Mol. Cell. Cardiol.* 1991;23:1245-53.
44. Capasso JM, Bruno S, Cheng W, Li P, Rodgers R, Darzynkiewicz Z, Anversa P. Ventricular loading is coupled with DNA synthesis in adult cardiac myocytes after acute and chronic myocardial infarction in rats. *Circ Res.* 1992;71:1379-89.
45. Olivetti G, Capasso JM, Meggs LG, Sonnenblick EH, Anversa P. Cellular basis of chronic ventricular remodeling after myocardial infarction in rats. *Circ Res.* 1991;68:856-69.
46. Ueno H, Perryman MB, Roberts R, Schneider MD. Differentiation of cardiac myocytes after mitogen withdrawal exhibits three sequential states of the ventricular growth response. *J Cell Biol.* 1988;107:1911-8.
47. Anversa P, Palackal T, Sonnenblick EH, Olivetti G, Meggs LG, Capasso JM. Myocyte cell loss and myocyte cellular hyperplasia in the hypertrophied aging rat heart. *Circ Res.* 1990;67:871-85.
48. Liu Y, Cigola E, Cheng W, Kajstura J, Olivetti G, Hintze TH, Anversa P. Myocyte nuclear mitotic division and programmed myocyte cell death characterize the cardiac myopathy induced by rapid ventricular pacing in dogs. *Lab Invest* 1995;73:771-87.

49. Kajstura J, Cheng W, Reiss K, Clark WA, Sonnenblick EH, Krajewski S, Reed JC, Olivetti G, Anversa P. Apoptotic and necrotic myocyte cell deaths are independent contributing variables of infarct size in rats. *Lab Invest* 1996;74:86-107.
50. Sabbah HN, Sharov VG, Goldstein S. Programmed cell death in the progression of heart failure. *Ann Med.* 1998;30:33-8.
51. Olivetti G, Abbi R, Quaini F, Kajstura J, Cheng W, Nitahara JA, Quaini E, Di Loreto C, Beltrami CA, Krajewski S, Reed JC, Anversa P. Apoptosis in the failing human heart. *N Engl J Med.* 1997;336:1131-41.
52. Lompre AM, Mercadier JJ, Schwartz K. Changes in gene expression during cardiac growth. *Int Rev Cytol.* 1991;124:137-86.
53. Kajstura J, Zhang X, Reiss K, Szoke E, Li P, Lagrasta C, Cheng W, Darzynkiewicz Z, Olivetti G, Anversa P. Myocyte cellular hyperplasia and myocyte cellular hypertrophy contribute to chronic ventricular remodeling in coronary artery narrowing-induced cardiomyopathy in rats. *Circ Res.* 1994;74:383-400.
54. Weber KT, Sun Y, Tyagi SC, Cleutjens JP. Collagen network of the myocardium: function, structural remodeling and regulatory mechanisms. *J Mol Cell Cardiol.* 1994;26:279-92.
55. Bishop JE, Laurent GJ. Collagen turnover and its regulation in the normal and hypertrophying heart. *Eur Heart J.* 1995;16:38-44.
56. Ju H, Zhao S, Jassal DS, Dixon IM. Effect of AT1 receptor blockade on cardiac collagen remodeling after myocardial infarction. *Cardiovasc Res.* 1997;35:223-32.
57. Katz AM. Heart failure in 2001: a prophecy. *Am J Cardiol.* 1992;70:126C-131C.
58. Covell JW. Factors influencing diastolic function. Possible role of the extracellular matrix. *Circulation.* 1990;81:III155-8.
59. Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OH. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. *Circulation.* 1995;91:161-70.
60. Chilian WM, Eastham CL, Marcus ML. Microvascular distribution of coronary vascular resistance in beating left ventricle. *Am J Physiol.* 1986;251:H779-88.
61. Hearse DJ. Ischemia, reperfusion, and the determinants of tissue injury. *Cardiovasc Drugs Ther.* 1990;4:767-76.
62. Xie Z, Gao M, Batra S, Koyama T. The capillarity of left ventricular tissue of rats subjected to coronary artery occlusion. *Cardiovasc Res.* 1997;33:671-6.
63. Turek Z, Grandtner M, Kubat K, Ringnalda BE, Kreuzer F. Arterial blood gases, muscle fiber diameter and intercapillary distance in cardiac hypertrophy of rats with an old myocardial infarction. *Pflugers Arch.* 1978;376:209-15.
64. Anversa P, Beghi C, Kikkawa Y, Olivetti G. Myocardial infarction in rats. Infarct size, myocyte hypertrophy, and capillary growth. *Circ Res.* 1986;58:26-37.
65. Hudlicka O, Brown MD, Walter H, Weiss JB, Bate A. Factors involved in capillary growth in the heart. *Mol Cell Biochem.* 1995;147:57-68.

66. Sladek T, Sladkova J, Kolar F, Papousek F, Cicutti N, Korecky B, Rakusan K. The effect of AT1 receptor antagonist on chronic cardiac response to coronary artery ligation in rats. *Cardiovasc Res.* 1996;31:568-76.

67. Yarom R, Zirkin H, Stammler G, Rose AG. Human coronary microvessels in diabetes and ischaemia. Morphometric study of autopsy material. *J Pathol.* 1992;166:265-70.

68. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. *Circulation.* 2000;101:2981-8.

69. Cheng W, Li B, Kajstura J, Li P, Wolin MS, Sonnenblick EH, Hintze TH, Olivetti G, Anversa P. Stretch-induced programmed myocyte cell death. *J Clin Invest* 1995;96:2247-59.

70. Neubauer S, Horn M, Naumann A, Tian R, Hu K, Laser M, Friedrich J, Gaudron P, Schnackerz K, Ingwall JS, et al. Impairment of energy metabolism in intact residual myocardium of rat hearts with chronic myocardial infarction. *J Clin Invest* 1995;95:1092-100.

71. Sanbe A, Tanonaka K, Kobayasi R, Takeo S. Effects of long-term therapy with ACE inhibitors, captopril, enalapril and trandolapril, on myocardial energy metabolism in rats with heart failure following myocardial infarction. *J Mol Cell Cardiol.* 1995;27:2209-22.

72. Laser A, Ingwall JS, Tian R, Reis I, Hu K, Gaudron P, Erti G, Neubauer S. Regional biochemical remodeling in non-infarcted tissue of rat heart post-myocardial infarction. *J Mol Cell Cardiol.* 1996;28:1531-8.

73. Cheung JY, Musch TI, Misawa H, Semanchick A, Elensky M, Yelamarty RV, Moore RL. Impaired cardiac function in rats with healed myocardial infarction: cellular vs. myocardial mechanisms. *Am J Physiol.* 1994;266:C29-36.

74. Geenen DL, Malhotra A, Scheuer J. Regional variation in rat cardiac myosin isoenzymes and ATPase activity after infarction. *Am J Physiol.* 1989;256:H745-50.

75. Warner AL, Bellah KL, Raya TE, Roeske WR, Goldman S. Effects of beta-adrenergic blockade on papillary muscle function and the beta-adrenergic receptor system in noninfarcted myocardium in compensated ischemic left ventricular dysfunction. *Circulation.* 1992;86:1584-95.

76. Yousef ZR, Redwood SR, Marber MS. Postinfarction left ventricular remodeling: a pathophysiological and therapeutic review. *Cardiovasc Drugs Ther.* 2000;14:243-52.

77. Cowie MR, Mosterd A, Wood DA, Deckers JW, Poole-Wilson PA, Sutton GC, Grobbee DE. The epidemiology of heart failure. *Eur. Heart J.* 1997;18:208-25.

78. Cohn JN. Lessons from V-HeFT: questions for V-HeFT II and the future therapy of heart failure. *Herz.* 1991;16:267-71.

79. Pitt B, Zannad F, Remme WJ, Cody R, Castaigne A, Perez A, Palensky J, Wittes J. The effect of spironolactone on morbidity and mortality in patients with severe heart failure. Randomized Aldactone Evaluation Study Investigators. *N Engl J Med.* 1999;341:709-17.

80. Pfeffer JM, Pfeffer MA, Braunwald E. Hemodynamic benefits and prolonged survival with long-term captopril therapy in rats with myocardial infarction and heart failure. *Circulation.* 1987;75:I149-55.

81. Schoemaker RG, Debets JJ, Struyker-Boudier HA, Smits JF. Delayed but not immediate captopril therapy improves cardiac function in conscious rats, following myocardial infarction. *J. Mol. Cell. Cardiol.* 1991;23:187-97.

82. Sharpe N, Murphy J, Smith H, Hannan S. Treatment of patients with symptomless left ventricular dysfunction after myocardial infarction. *Lancet* 1988;1:255-9.

83. Magrini F, Shimizu M, Roberts N, Fouad FM, Tarazi RC, Zanchetti A. Converting-enzyme inhibition and coronary blood flow. *Circulation.* 1987;75:I168-74.

84. Cleland JG, Henderson E, McLenachan J, Findlay IN, Dargie HJ. Effect of captopril, an angiotensin-converting enzyme inhibitor, in patients with angina pectoris and heart failure. *J Am Coll Cardiol.* 1991;17:733-9.

85. Weber KT. Extracellular matrix remodeling in heart failure: a role for de novo angiotensin II generation. *Circulation.* 1997;96:4065-82.

86. van Krimpen C, Schoemaker RG, Cleutjens JP, Smits JF, Struyker-Boudier HA, Bosman FT, Daemen MJ. Angiotensin I converting enzyme inhibitors and cardiac remodeling. *Basic Res. Cardiol.* 1991;86:149-55.

87. Gay RG. Early and late effects of captopril treatment after large myocardial infarction in rats. *J Am Coll Cardiol.* 1990;16:967-77.

88. Ambrosioni E, Borghi C, Magnani B. The effect of the angiotensin-converting-enzyme inhibitor zofenopril on mortality and morbidity after anterior myocardial infarction. The Survival of Myocardial Infarction Long-Term Evaluation (SMILE) Study Investigators [see comments]. *N Engl J Med.* 1995;332:80-5.

89. GISSI-3 I. Six-month effects of early treatment with lisinopril and transdermal glyceryl trinitrate singly and together withdrawn six weeks after acute myocardial infarction: the GISSI-3 trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico. *J Am Coll Cardiol.* 1996;27:337-344.

90. Sharpe N, Smith H, Murphy J, Greaves S, Hart H, Gamble G. Early prevention of left ventricular dysfunction after myocardial infarction with angiotensin-converting-enzyme inhibition [published erratum appears in Lancet 1991 May 11;337(8750):1174]. *Lancet* 1991;337:872-6.

91. Swedberg K, Held P, Kjekshus J, Rasmussen K, Ryden L, Wedel H. Effects of the early administration of enalapril on mortality in patients with acute myocardial infarction. Results of the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS II) [see comments]. *N Engl J Med.* 1992;327:678-84.

92. Ray SG, Pye M, Oldroyd KG, Christie J, Connelly DT, Northridge DB, Ford I, Morton JJ, Dargie HJ, Cobbe SM. Early treatment with captopril after acute myocardial infarction. *Br Heart J* 1993;69:215-22.

93. CCS-1 I. Oral captopril versus placebo among 13,634 patients with suspected acute myocardial infarction: interim report from the Chinese Cardiac Study (CCS-1). *Lancet* 1995;345:686-687.

94. Timmermans PB, Wong PC, Chiu AT, Herblin WF, Benfield P, Carini DJ, Lee RJ, Wexler RR, Saye JA, Smith RD. Angiotensin II receptors and angiotensin II receptor antagonists. *Pharmacol Rev.* 1993;45:205-51.

95. Meggs LG, Coupet J, Huang H, Cheng W, Li P, Capasso JM, Homcy CJ, Anversa P. Regulation of angiotensin II receptors on ventricular myocytes after myocardial infarction in rats. *Circ Res.* 1993;72:1149-62.

96. Nio Y, Matsubara H, Murasawa S, Kanasaki M, Inada M. Regulation of gene transcription of angiotensin II receptor subtypes in myocardial infarction. *J Clin Invest* 1995;95:46-54.

97. Baker KM, Booz GW, Dostal DE. Cardiac actions of angiotensin II: Role of an intracardiac renin-angiotensin system. *Annu Rev Physiol.* 1992;54:227-41.

98. Unger T, Culman J, Gohlke P. Angiotensin II receptor blockade and end-organ protection: pharmacological rationale and evidence. *J Hypertens Suppl.* 1998;16:S3-9.

99. Smits JF, van Krimpen C, Schoemaker RG, Cleutjens JP, Daemen MJ. Angiotensin II receptor blockade after myocardial infarction in rats: effects on hemodynamics, myocardial DNA synthesis, and interstitial collagen content. *J Cardiovasc Pharmacol.* 1992;20:772-8.

100. Kuizinga MC, Smits JF, Arends JW, Daemen M. AT2 receptor blockade reduces cardiac interstitial cell DNA synthesis and cardiac function after rat myocardial infarction. *J Mol Cell Cardiol.* 1998;30:425-34.

101. Pitt B, Poole-Wilson P, Segal R, Martinez FA, Dickstein K, Camm AJ, Konstam MA, Riegger G, Klinger GH, Neaton J, Sharma D, Thiyagarajan B. Effects of losartan versus captopril on mortality in patients with symptomatic heart failure: rationale, design, and baseline characteristics of patients in the Losartan Heart Failure Survival Study--ELITE II. *J Card Fail.* 1999;5:146-54.

102. Gainer JV, Morrow JD, Loveland A, King DJ, Brown NJ. Effect of bradykinin-receptor blockade on the response to angiotensin-converting-enzyme inhibitor in normotensive and hypertensive subjects. *N Engl J Med.* 1998;339:1285-92.

103. Yusuf S, Peto R, Lewis J, Collins R, Sleight P. Beta blockade during and after myocardial infarction: an overview of the randomized trials. *Prog Cardiovasc Dis.* 1985;27:335-71.

104. Chadda K, Goldstein S, Byington R, Curb JD. Effect of propranolol after acute myocardial infarction in patients with congestive heart failure. *Circulation.* 1986;73:503-10.

105. Olsen SL, Gilbert EM, Renlund DG, Taylor DO, Yanowitz FD, Bristow MR. Carvedilol improves left ventricular function and symptoms in chronic heart failure: a double-blind randomized study. *J Am Coll Cardiol.* 1995;25:1225-31.

106. Bristow MR. beta-adrenergic receptor blockade in chronic heart failure. *Circulation.* 2000;101:558-69.

107. Packer M. Beta-adrenergic blockade in chronic heart failure: principles, progress, and practice. *Prog Cardiovasc Dis.* 1998;41:39-52.

108. Fowler MB. Beta-blockers in heart failure. Do they improve the quality as well as the quantity of life? *Eur. Heart J.* 1998;19:P17-25.

109. Heilbrunn SM, Shah P, Bristow MR, Valantine HA, Ginsburg R, Fowler MB. Increased beta-receptor density and improved hemodynamic response to catecholamine stimulation during long-term metoprolol therapy in heart failure from dilated cardiomyopathy. *Circulation.* 1989;79:483-90.

110. Gilbert EM, Abraham WT, Olsen S, Hattler B, White M, Mealy P, Larrabee P, Bristow MR. Comparative hemodynamic, left ventricular functional, and antiadrenergic effects of chronic treatment with metoprolol versus carvedilol in the failing heart. *Circulation.* 1996;94:2817-25.

111. Basu S, Senior R, Raval U, van der Does R, Bruckner T, Lahiri A. Beneficial effects of intravenous and oral carvedilol treatment in acute myocardial infarction. A placebo-controlled, randomized trial. *Circulation.* 1997;96:183-91.

112. Nauman D, Greenberg B. Studies of Left Ventricular Dysfunction (SOLVD). *Am J Geriatr Cardiol.* 1993;2:28-36.

113. Schoemaker RG, Urquhart J, Debets JJ, Struyker Boudier HA, Smits JF. Acute hemodynamic effects of coronary artery ligation in conscious rats. *Basic Res Cardiol.* 1990;85:9-20.

CHAPTER 2

Altered cardiac collagen and associated changes in diastolic function of infarcted rat hearts

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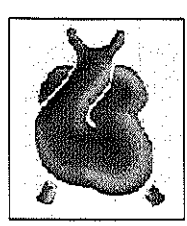

ALTERED CARDIAC COLLAGEN AND ASSOCIATED CHANGES IN DIASTOLIC FUNCTION OF INFARCTED RAT HEARTS

Abstract

Anti-inflammatory drugs have been shown to modulate collagen deposition during myocardial infarction (MI) induced remodeling. Chronic effects of methylprednisolone (5 mg/kg/day) and low-dose aspirin (25 mg/kg/day) on cardiac collagen and left ventricular diastolic function were studied in rat hearts, 21 days after MI. Left ventricular function was assessed at baseline and after β-adrenergic stimulation with isoproterenol in isolated perfused hearts, using an intraventricular balloon. After diastolic arrest, left ventricular pressure-volume curves were obtained. Left ventricular dilation was defined as the corresponding left ventricular volume at 20 mmHg left ventricular diastolic pressure. In histological sections, perivascular and interstitial collagen content were quantified morphometrically as the Sirius Red positive area in the non-infarcted interventricular septum. Impaired baseline left ventricular function of MI-hearts was improved by methylprednisolone but not by low-dose aspirin. Isoprotenerol significantly enhanced systolic function in all hearts, whereas it augmented the decrease in left ventricular diastolic pressure only in methylprednisolone treated MI-hearts. The rightward shift of the pressure-volume curve after MI was aggravated by methylprednisolone but not with low-dose aspirin treament. Low-dose aspirin reduced perivascular but not interstitial collagen whereas methylprednisolone decreased both perivascular and interstitial collagen. Our findings indicate that MI-induced collagen deposition in the spared myocardium can be affected by chronic therapy with low-dose aspirin or methylprednisolone. The effects on interstitial collagen seemed reflected in an altered left ventricular diastolic function.

2.1. Introduction

Large myocardial infarction (MI) is known to induce alterations in the collagen matrix of both the infarcted and non-infarcted region of the heart. In the course of post-MI remodeling, infarcted tissue is replaced by scar tissue with a high collagen content while in the non-infarcted tissue collagen content is increased as well [1,2]. Deposition of collagen in the non-infarcted myocardium has been related to an increased myocardial stiffness and impaired diastolic heart function [3-5]. In addition, the structural remodeling of the myocardial collagen matrix appears to contribute considerably to left ventricular (LV) dilation [6] and progression of LV dysfunction [7,8].

In a previous study, we have shown in a rat MI-model that chronic administration of aspirin, in a non anti-inflammatory dose, prevented collagen accumulation in the spared myocardium in the first two weeks after MI [9]. This

observation was associated with improved *in vivo* hemodynamics and absence of further increased LV cavity dimensions [10]. On the other hand, high-dose aspirin, providing plasma levels associated with anti-inflammatory actions in humans was shown to be able to inhibit both the synthesis [11] and degradation of collagen in other rat tissues [12]. Furthermore, anti-inflammatory therapy after MI may lead to a too large reduction of the tensile strength of the LV collagen network resulting in aggravation of chamber dilation, as has been reported with non-steroid anti-inflammatory drugs (NSAIDs) as well as with steroids [13-15]. Moreover, chronic treatment with steroids and NSAIDs have been shown to retard collagen deposition in scar tissue resulting in infarct thinning [16,17]. Thus, although post-MI cardiac fibrosis may contribute to impaired cardiac function it is not clear whether the infarcted heart would benefit from anti-fibrotic treatment.

Therefore, in the present study the effects of chronical methylprednisolone and low-dose aspirin on interstitial and perivascular collagen deposition in 3 week old infarcted rat hearts were investigated, and related to effects on LV diastolic function.

2.2. Methods

2.2.1. Animals

Male Wistar rats (Harlan, Zeist, The Netherlands) weighing 270-300 g were housed in groups of 2 or 3 on a 12-hour light-dark cycle with standard rat chow and water available *ad libitum*. The animals were subjected to sham-surgery or coronary artery ligation. All experiments were carried out after approval of the University ethics committee for the use of experimental animals and conform with the *Guide for Care and Use of Laboratoy Animals.*

2.2.2. Myocardial infarction

Under pentobarbital anesthesia (60 mg/kg i.p.), MI was induced by ligation of the left anterior descending coronary artery [18]. Briefly, after intubation of the trachea an incision was made in the skin overlying the fourth intercostal space, with the overlying muscles separated and kept aside. The animals were put on positive pressure ventilation (frequency 65-70/min, tidal volume 3 ml) and the thoracic cavity

was opened by cutting the intercostal muscles. The heart was carefully pushed to the left and 6-0 silk suture was looped under the left descending coronary artery near the origin of the pulmonary artery. After returning the heart to its normal position, the suture was tied. Intercostal space was closed by pulling the ribs with 3-0 silk, the muscles were returned to their normal position and the skin incision was sutured. Sham-operated animals underwent the same surgical procedure, without the actual coronary artery ligation. Proper occlusion of the coronary artery resulted in an extensive transmural infarction comprising a major part of the LV free wall, with small variations in size [19]. Infarct size was determined by planimetry at mid-ventricular levels in transverse slices [20] as the percentage of LV circumference [9].

2.2.3. Experimental protocols

Infarcted rats were randomized to receive saline, aspirin or methylprednisolone. Aspirin (25 mg/kg; lysine-acetylsalicylic, Aspégic®, Lorex B.V., Maarssen, The Netherlands) was dissolved in saline and administered as daily i.p. injections of 1 ml/kg, starting 2 days before surgery until the end of the experiment at 21 days after surgery. Methylprednisolone (5 mg/kg; methylprednisolone sodiumsuccinate, Methypresol®, Pharmachemie B.V., Haarlem, The Netherlands) was given as daily i.p. injections starting at the end of the acute inflammatory phase [18], from 7 days to 21 days after surgery.

Untreated control rats were receiving once daily saline injections of 1ml/kg i.p. from 2 days before until 21 days after surgery (control for asprin treatment) or from 7 days to 21 days after surgery (control for methylprednisolone treatment). Because of no differences between controls, data were pooled.

2.2.4. Left ventricular function

At the end of the protocol, the hearts were rapidly excized under pentobarbital anesthesia and mounted for perfusion with an oxygenated Krebs-Henseleit buffer (composition in mM: NaCl 125, KCl 4.7, $CaCl_2$ 1.35, $NaHCO_3$ 20, NaH_2PO_4 0.4, D-glucose 10; pH=7.4; 37°C) at a constant pressure of 85 mmHg, using the Langendorff technique. Hearts were paced at 350 beats/min (4V, 2ms). LV function was measured as isovolumetric developed pressure against a fluid-filled latex balloon

placed in the left ventricle, and connected via a fluid-filled catheter to a miniature low-volume displacement pressure transducer. The LV end-diastolic pressure was set to 10 mmHg by adjusting the balloon volume. Although this value for LV end-diastolic pressure is above the physiological values of sham-hearts, this was performed to be able to obtain any β-agonist mediated decreases of the LV diastolic pressure. Coronary flow was measured by a flow probe (Transonic Systems, Ithaca, NY, USA) placed in the tubing just before the ostia of the coronary arteries.

After a stabilization period of 15 min, variables were measured at baseline. In order to determine maximal LV performance during β-agonist stimulation, responses to increasing doses of isoprotenerol (L-isoprotenerol hydrochloride, Sigma Chemicals, St. Louis, USA), ranging from 10^{-9} to 10^{-5} M, were determined. For each dose, 100 µl of isoprotenerol solution, dissolved in saline, was injected into the perfusing medium just before entering the coronary arteries. During the administration of isoprotenerol pacing was set to 450 beats/min in order to minimize arrythmias. After a re-stabilization period, hearts were arrested in diastole with a 0.5 ml injection of a 1 M potassium chloride into the perfusing buffer. At 10 to 20 different LV ballon volumes, diastolic pressures in the range of 0 to 40 mmHg were obtained. For each heart, values were fitted into: $pressure=c.e^{k.volume}+a$. As a measure for LV dilation the corresponding volume at 20 mmHg LV pressure, V_{20}, was calculated.

2.2.5. Ventricular hypertrophy

After completion of the functional measurements, hearts were removed from the Langendorff preparation and weighed after exclusion of the atria and large vessels. Ventricular hypertrophy was defined as the ratio of ventricular weight and body weight.

2.2.6. Collagen content

Briefly, ventricles were cut into 4 transversal slices from apex to base and fixated with 3.6% phosphate-buffered formaldehyde for at least 24 h. After fixation, the slices were dehydrated and paraffin-embedded. Deparaffinized 5 µm thick sections were incubated for 5 min with 0.2% (wt/vol) aqueous phosphomolybdic acid and subsequently incubated for 45 min with 0.1% Sirius Red F3BA (C.I. 35780,

Polysciences Inc., Northampton, U.K.) in saturated aqueous picric acid, washed for 2 min with 0.01 M HCl, dehydrated and mounted with Entellan (Merck, Darmstadt, Germany).

Collagen content was quantified by morphometry. In the interventricular septum, remote from the infarcted area, interstitial collagen was determined as the Sirius Red positive area in 40 high power fields per heart [9,21]. Areas that enclosed signs of replacement fibrosis or bloodvessels, were excluded from analysis. Perivascular collagen was measured around 12 resistance arteries (lumen diameter <150 μm) per heart, in the non-infarcted interventricular septum and viable left ventricular free wall. Perivascular collagen area was corrected for luminal area of the vessel [22].

2.2.7. Data analysis

All data are presented as means ± s.e.m. Data of infarcted rats were only included if the infarction comprized the major part of the LV free wall, since small infarctions are found to be hemodynamically fully compensated [23,24]. Estimation of infarct size by macroscopic appearance has proven to be a reliable method to recognize too small infarctions (< 20%) [19]. Differences between groups were analyzed (SigmaStat™, Jandel Scientific, Erkrath, Germany) using one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc *t*-tests for multiple group comparisons [25]. Responses to increasing doses isoprotenerol in the different experimental groups were analyzed using two-way ANOVA for repeated measurements. Differences were considered statistically significant if $P<0.05$.

2.3. Results

2.3.1. Ventricular hypertrophy

Three weeks after MI, infarct size as well as body and ventricular weight were measured in the different experimental groups (Table 1). Compensatory hypertrophy in MI-hearts, defined by the ventricular weight body weight ratio, was indicated by a 14 % rise of ventricular mass despite replacement of the major part of the LV free wall by lighter scar tissue. Treatment with low-dose aspirin did not affect cardiac

hypertrophy when compared to hearts from untreated MI-rats. Both body and ventricular weight of methylprednisolone treated MI-rats were significantly reduced when compared to untreated MI-rats. However, this resulted in an unchanged ventricular weight body weight ratio. No differences in infarct size were observed between the different groups.

Table 1. Infarct size, body and ventricular weight measured in the different experimental groups 21 days after surgery

	Sham	MI	MI+MP	MI+ASP
n	10	12	7	6
Infarct size (%)	-	43±2	41±3	44±3
Body weight (g)	346±8	347±8	300±10 [bc]	354±11
Ventricular weight (mg)	1061±52	1211±54 [b]	1059±64 [c]	1261±61 [b]
Ventricular weight/body weight (mg/g)	3.1±0.1	3.5±0.1 [b]	3.5±0.1 [b]	3.6±0.2 [b]

[a] Data are presented as means ± s.e.m. MI: myocardial infarction; MP: methylpred-nisolone (5 mg/kg/day); ASP: aspirin (25 mg/kg/day). [b]: $P<0.05$ vs Sham; [c]: $P<0.05$ vs MI.

2.3.2. Cardiac collagen

Photomicrographs of picrosirius red stained sections of interventricular septum containing interstitial and perivascular collagen for the different experimental groups are shown in Fig.1. Post-MI remodeling is associated with an increase in both interstitial and perivascular collagen. Whereas methylprednisolone treatment reduced interstitial as well as perivascular collagen deposition, low-dose aspirin only affected perivascular collagen. These observations were substantiated by the actual measurements as shown in Fig.2.

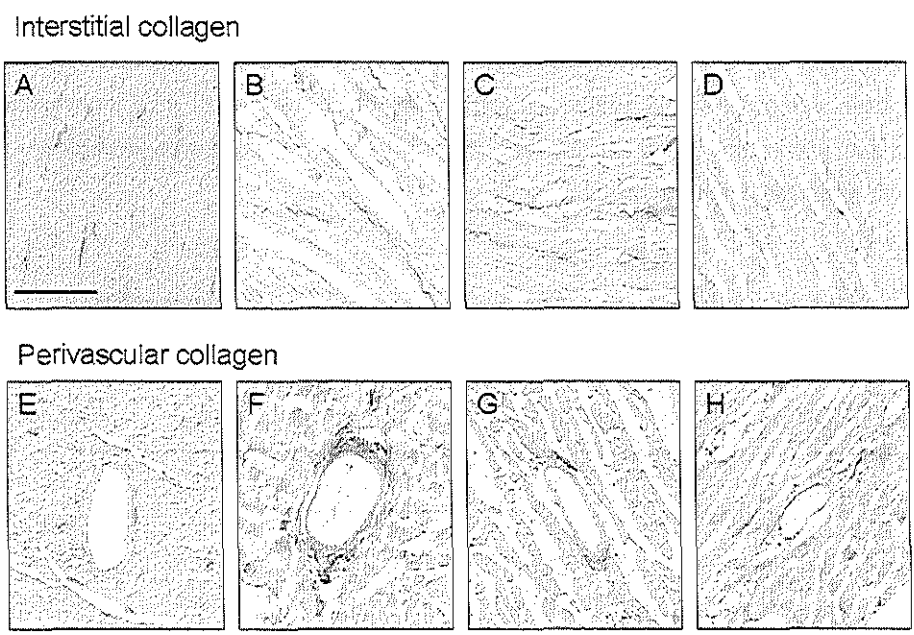

Fig. 1. Picrosirius red stained sections of interventricular septum showing interstitial (A/B/C/D) and perivascular collagen (E/F/G/H) 3 weeks after surgery. A/E: sham heart, B/F: untreated MI-heart, C/G: aspirin treated MI-heart, D/H: methylprednisolone treated MI-heart. The bar in photomicrograph A indicates 100μm, and accounts for all micrographs.

Methylprednisolone, however, reduced both interstitial and perivascular collagen when compared to untreated MI-hearts. Moreover, methylprednisolone treatment did not significantly reduce scar collagen (34±4% in methylprednisolone treated MI-hearts versus 43±5% in untreated MI-hearts).

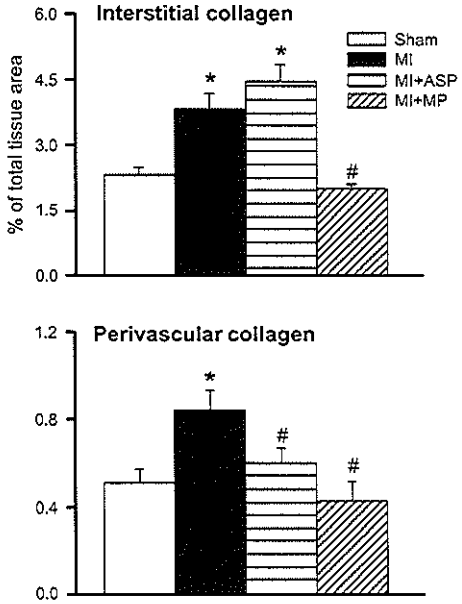

Fig. 2. Effects of treatment on interstitial *(upper panel)* and perivascular collagen *(lower panel)*. Interstitial collagen is expressed as percentage of total tissue area while perivascular collagen as collagen to lumen ratio of resistance arteries. MI: myocardial infarction; ASP: low-dose aspirin (25 mg/kg/day); MP: methylprednisolone (5 mg/kg/day). *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

2.3.3. Left ventricular function

Variables of LV function as well as cardiac perfusion measured at baseline in isolated perfused rat hearts are summarized in Table 2.

Table 2. Characterization of the experimental groups

	Sham	MI	MI+MP	MI+ASP
n	10	12	7	6
LVSP (mmHg)	89±6	58±6 [b]	78±7	67±6
LVEDP (mmHg)	14±3	11±3	9±3	10±1
$+dP/dt_{max}$ (mmHg/s)	3180±307	1731±197 [b]	2752±270 [c]	2049±230 [b]
$-dP/dt_{max}$ (mmHg/s)	2197±224	1246±177 [b]	1714±155	1462±195
Coronary flow (ml/min)	10.9±1.0	10.3±1.0	7.7±0.9	12.2±0.7
Cardiac perfusion (ml/min.g)	10.3±0.7	8.7±1.0	7.3±0.6	9.7±0.6

[a] Baseline values obtained from isolated perfused rat hearts paced at 350 beats/min. Data are presented as means ± s.e.m. MI: myocardial infarction; MP: methylprednisolone (5 mg/kg/day); ASP: aspirin (25 mg/kg/day); LVSP: left ventricular systolic pressure; LVEDP: left ventricular end-diastolic pressure; $+dP/dt_{max}$ and $-dP/dt_{max}$: maximum velocity of pressure rise and decline; [b] : $P<0.05$ vs Sham; [c]: $P\leq0.05$ vs MI.

In Fig.3, effects of increasing doses isoprotenerol on LV function are shown. LV systolic dysfunction in untreated MI-hearts was evidenced from a decreased baseline LV systolic pressure and peak velocity of LV contraction $+dP/dt_{max}$. Methylprednisolone treated MI-hearts showed an improved LV systolic function as manifested by an increased baseline $+dP/dt_{max}$, whereas maximal values obtained after isoprotenerol infusion were not different from hearts of untreated MI-rats. Cardiac perfusion at baseline was not changed in untreated MI-hearts when compared to sham-hearts. This was neither altered by treatment with aspirin or methylprednisolone. Cardiac perfusion in methylprednisolone treated MI-hearts was significantly lower at maximal stimulation with 10^{-5} M isoprotenerol (12.1±0.8 ml/min.g) when compared to untreated MI-hearts (16.0±1.1 ml/min.g). In low-dose aspirin treated MI-hearts, LV function nor cardiac perfusion were significantly different from untreated MI-hearts at baseline and after isoprotenerol.

Diastolic dysfunction in MI-hearts was substantiated by a significantly reduced baseline $-dP/dt_{max}$ which was not altered by low-dose aspirin or methylprednisolone. In methylprednisolone treated MI-hearts, isoprotenerol significantly augmented the decrease in LV end-diastolic pressure when compared to sham-hearts and untreated MI-hearts. The LV end-diastolic pressure in low-dose aspirin treated MI-hearts did not differ from untreated MI-hearts at any timepoint.

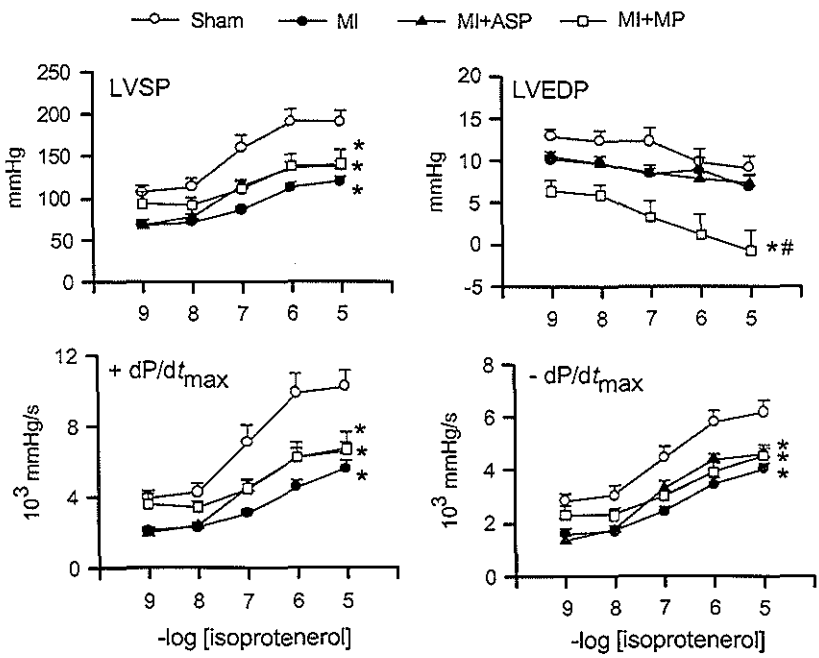

Fig. 3. Left ventricular functional variables measured in isolated perfused treated and untreated rat hearts after increasing concentrations of isoprotenerol. Effects of treatment on left ventricular systolic (LVSP) and end-diastolic pressure (LVEDP) are shown in the upper left and right panel while the effects on peak velocity of contraction ($+dP/dt_{max}$) and relaxation ($-dP/dt_{max}$) are presented in the lower left and right panel. MI: myocardial infarction; ASP: low-dose aspirin (25 mg/kg/day); MP: methylprednisolone (5 mg/kg/day). *: curve significantly different from sham values $P<0.05$; #: curve significantly different from MI values $P<0.05$.

2.3.4. Diastolic pressure-volume curves

Post MI-remodeling of the left ventricle resulted into a rightward shift of the diastolic pressure-volume (PV) curve (Fig.4.). Furthermore, the diastolic PV curves of MI-hearts (n=13) were less steep compared to sham-hearts (n=25), as indicated by significantly reduced k-values of the exponential PV relationship: 76±8 (10^{-4}) versus

118±7 (10^{-4}) in hearts from sham-operated rats. Low-dose aspirin treated MI-hearts (n=9) manifested no alterations in the diastolic PV curves when compared to untreated MI-hearts, whereas methylprednisolone (n=6) resulted in a further rightward shift of the diastolic PV relationship. LV dilation of MI-hearts, as indicated by an increased LV volume at 20 mmHg pressure (V_{20}= 0.850±0.037 in sham and 1.268±0.066 ml/kg in non-treated infarcted hearts) was not affected by aspirin (V_{20}= 1.250±0.107 ml/kg) but significantly aggravated by methylprednisolone (V_{20}= 1.496±0.079 ml/kg). The steepness k of the diastolic PV relationship remained unchanged in both low-dose aspirin (58±8 (10^{-4})) and methylprednisolone (96±13 (10^{-4})) treated MI-hearts when compared to untreated MI-hearts.

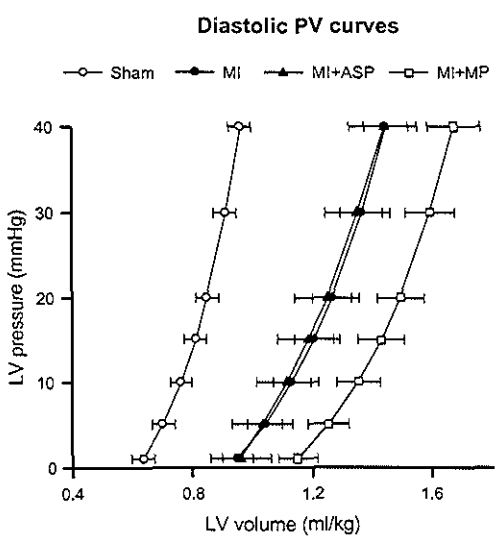

Fig. 4. Diastolic pressure-volume (PV) relationships obtained from treated and untreated rat hearts arrested in diastole. MI: myocardial infarction; ASP: low-dose aspirin (25 mg/kg/day); MP: methylprednisolone (5 mg/kg/day).

2.4. Discussion

The present study was carried out to investigate whether treatment with aspirin or methylprednisolone would attenuate cardiac collagen and alter LV diastolic function in the 3 week old post-MI remodeled rat heart. In a previous study, we have shown that a daily dose of 25 mg/kg/day (low-dose) aspirin inhibits thromboxane but not prostaglandin synthesis as the rat dose equivalent to chronic low-dose aspirin

treatment in patients [9]. This low-dose of aspirin prevented interstitial and perivascular fibrosis in the spared myocardium, while leaving wound healing and reactive hypertrophy relatively unaffected during the first two weeks of post-MI remodeling. Besides a possible delay in the development, aspirin treatment showed no effect on *in vitro* LV dysfunction. However, 3 weeks of this low-dose aspirin treatment is associated with improved *in vivo* hemodynamics in conscious rats [10].

The effects of low-dose aspirin were compared with the effects of methylprednisolone treament which has been shown to aggravate post-MI LV dilation [15]. In order to avoid interference with the initial wound healing, methylprednisolone treatment was started 1 week after MI, at the end of the acute inflammatory phase [18].

2.4.1. Collagen and diastolic pressure-volume relationship

In the present study, LV dilation in MI-hearts was indicated by a rightward shift of the diastolic PV relationship and substantiated by an increased volume at 20 mmHg. Post-MI remodeling induced accumulation of both interstitial and perivascular collagen in the non-infarcted interventricular septum. Although LV stiffness is strongly determined by collagen concentration and degree of fibrosis [3], the steepness of the diastolic PV relationship in MI-hearts decreased. This phenomenon may be explained by an effect of changed LV geometry on the steepness of the PV relationship. Indeed, early after MI the PV curve is shifted to the left with increased stiffness, while during the process of remodeling a rightward shift of the PV relation and decreased LV stiffness were observed [8].

Methylprednisolone was shown to retard the rate of MI healing, cause scar thinning and induce cavity dilation in MI-rats [15]. To limit the effects on healing and scar formation treatment with methylprednisolone was started one week after MI. As was previously shown for aspirin, where at 2 weeks post-MI infarct collagen content was already normalized [9], methylprednisolone did not significantly reduce scar collagen content. In agreement with the studies of Mannisi, chronic treatment with methylprednisolone in MI-rats resulted in a further rightward shift of the diastolic PV curves, indicating aggravation of LV dilation whereas low-dose aspirin did not affect the diastolic PV relationship in MI-hearts. In previous studies, deleterious effects of

methylprednisolone treatment in terms of LV dilation have been explained by its interference with replacement fibrosis in the infarcted area [14,15] in addition to its effects on collagen in the non-infarcted myocardium. It could also be possible that methylprednisolone did aggravate the initial process of breakdown of pre-existing collagen fibers correlating with post-MI infarction [6]. Aggravation of LV dilation in MI-hearts treated with methylprednisolone is associated with both decreased interstitial and perivascular collagen. On the other hand, therapy with low-dose aspirin did not aggravate LV dilation and only reduced perivascular collagen. This observation suggests that a too strong prevention of interstitial collagen build-up in the non-infarcted myocardium may promote LV dilation. In addition, prevention of collagen deposition by methylprednisolone or low-dose aspirin was not associated with alterations in diastolic stiffness.

The changes in the diastolic PV relationship related to collagen may also be explained by an effect on the physical properties of the collagen network in MI-hearts rather than the absolute amount of collagen. In the present study, only total collagen content was measured. Changes in collagen type, degree of cross-linking, fiber organization and fiber thickness could also alter the mechanical properties of the myocardium as reported by previous investigators [26].

2.4.2. Left ventricular function

LV systolic dysfunction 3 weeks after MI was evidenced from a decreased baseline LV systolic pressure and $+dP/dt_{max}$. Depressed LV function was not attributed to differences in cardiac perfusion, since this was not different between sham- and MI-hearts at any timepoint. As expected, isoprotenerol infusion augmented contractile function and relaxation in all experimental groups.

Treatment with methylprednisolone improved baseline $+dP/dt_{max}$, but maximal values with isoprotenerol were not different from untreated MI-hearts. Glucocorticosteroids are known to be able to increase β-receptor number. A higher β-receptor number / responsiveness at similar endogenous stimulation would explain a higher baseline contractility. Moreover, methylprednisolone was shown to accelerate the recovery of the decrease in β-adrenergic responsiveness caused by successive administrations isoprotenerol in rat hearts [27]. Interesting in this regard is the

significant lower perfusion of methylprednisolone treated MI-hearts, seen at the highest dose of isoprotenerol. Treatment with low-dose aspirin did not change LV function or myocardial perfusion compared to untreated MI-hearts.

LV diastolic dysfunction in MI-hearts was substantiated by a significantly reduced baseline $-dP/dt_{max}$ which was not altered by low-dose aspirin or methylprednisolone. However, β-stimulation with isoprotenerol induced a significant decrease in diastolic pressure in methylprednisolone, but not in low-dose aspirin or untreated MI-hearts. A possible explanation for the enhanced active relaxation observed in methylprednisolone treated hearts could be related to its effects on interstitial collagen network. Moreover, besides a reduction of total collagen amount, methylprednisolone has been reported to have a pronounced effect on the tensile strengh of the collagen network [28], which could have contributed to the altered active relaxation in the isolated MI-heart. This could also explain the aggravated LV dilation seen in methylprednisolone treated MI-hearts. Although generally regarded as detrimental, if the rightward shift of the PV-curve at similar slope would be responsible for the improved active relaxation with β-stimulation, even some benefit may be considered. Alternatively, the above described interactions between methylprednisolone and the β-adrenergic pathway could as well be involved in the improved diastolic function. Nevertheless, an enhanced relaxation normally would be associated with an increased perfusion. Therefore it remains difficult to explain why an augmented relaxation seen after maximal isoprotenerol was associated with a decreased cardiac perfusion in the methylprednisolone treated hearts.

2.4.3. Conclusions

The present findings indicate that pharmacological interference with the deposition of collagen in the non-infarcted myocardium may result in an altered LV diastolic function. Changes in interstitial rather than perivascular collagen seem to be important in the regulation of passive as well as active relaxation of the isolated heart. Whether improving LV relaxation could beneficially influence cardiac function remains to be elucidated.

2.5. References

1. van Krimpen C, Smits JF, Cleutjens JP, Debets JJ, Schoemaker RG, Struyker Boudier HA, Bosman FT, Daemen MJ. DNA synthesis in the non-infarcted cardiac interstitium after left coronary artery ligation in the rat: effects of captopril. *J Mol Cell Cardiol.* 1991;23:1245-53.
2. Volders PG, Willems IE, Cleutjens JP, Arends JW, Havenith MG, Daemen MJ. Interstitial collagen is increased in the non-infarcted human myocardium after myocardial infarction. *J Mol Cell Cardiol.* 1993;25:1317-23.
3. Jalil JE, Doering CW, Janicki JS, Pick R, Shroff SG, Weber KT. Fibrillar collagen and myocardial stiffness in the intact hypertrophied rat left ventricle. *Circ Res.* 1989;64:1041-50.
4. Litwin SE, Litwin CM, Raya TE, Warner AL, Goldman S. Contractility and stiffness of noninfarcted myocardium after coronary ligation in rats. Effects of chronic angiotensin converting enzyme inhibition. *Circulation.* 1991;83:1028-37.
5. Conrad CH, Brooks WW, Hayes JA, Sen S, Robinson KG, Bing OH. Myocardial fibrosis and stiffness with hypertrophy and heart failure in the spontaneously hypertensive rat. *Circulation.* 1995;91:161-70.
6. Whittaker P, Boughner DR, Kloner RA. Role of collagen in acute myocardial infarct expansion. *Circulation.* 1991;84:2123-34.
7. Fletcher PJ, Pfeffer JM, Pfeffer MA, Braunwald E. Left ventricular diastolic pressure-volume relations in rats with healed myocardial infarction. Effects on systolic function. *Circ Res.* 1981;49:618-26.
8. Raya TE, Gay RG, Lancaster L, Aguirre M, Moffett C, Goldman S. Serial changes in left ventricular relaxation and chamber stiffness after large myocardial infarction in rats. *Circulation.* 1988;77:1424-31.
9. Kalkman EA, van Suylen RJ, van Dijk JP, Saxena PR, Schoemaker RG. Chronic aspirin treatment affects collagen deposition in non-infarcted myocardium during remodeling after coronary artery ligation in the rat. *J Mol Cell Cardiol.* 1995;27:2483-94.
10. Schoemaker RG, Saxena PR, Kalkman EA. Low-dose aspirin improves in vivo hemodynamics in conscious, chronically infarcted rats. *Cardiovasc Res.* 1998;37:108-14.
11. Solheim LF, Ronningen H, Barth E, Langeland N. Effects of acetylsalicylic acid and naproxen on the mechanical and biochemical properties of intact skin in rats. *Scand J Plast Reconstr Surg.* 1986;20:161-3.
12. Solheim LF, Ronningen H, Langeland N. Effects of acetylsalicylic acid and naproxen on the synthesis and mineralization of collagen in the rat femur. *Arch Orthop Trauma Surg.* 1986;105:1-4.
13. Brown EJ, Jr., Kloner RA, Schoen FJ, Hammerman H, Hale S, Braunwald E. Scar thinning due to ibuprofen administration after experimental myocardial infarction. *Am J Cardiol.* 1983;51:877-83.

14. Hammerman H, Kloner RA, Hale S, Schoen FJ, Braunwald E. Dose-dependent effects of short-term methylprednisolone on myocardial infarct extent, scar formation, and ventricular function. *Circulation.* 1983;68:446-52.
15. Mannisi JA, Weisman HF, Bush DE, Dudeck P, Healy B. Steroid administration after myocardial infarction promotes early infarct expansion. A study in the rat. *J Clin Invest* 1987;79:1431-9.
16. Hammerman H, Kloner RA, Schoen FJ, Brown EJ, Jr., Hale S, Braunwald E. Indomethacin-induced scar thinning after experimental myocardial infarction. *Circulation.* 1983;67:1290-5.
17. Jugdutt BI, Basualdo CA. Myocardial infarct expansion during indomethacin or ibuprofen therapy for symptomatic post infarction pericarditis. Influence of other pharmacologic agents during early remodelling. *Can J Cardiol.* 1989;5:211-21.
18. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. *Am J Pathol.* 1978;90:57-70.
19. Kalkman EA, Bilgin YM, van Haren P, van Suylen RJ, Saxena PR, Schoemaker RG. Determinants of coronary reserve in rats subjected to coronary artery ligation or aortic banding. *Cardiovasc Res.* 1996;32:1088-95.
20. Nelissen-Vrancken HJ, Kuizinga MC, Daemen MJ, Smits JF. Early captopril treatment inhibits DNA synthesis in endothelial cells and normalization of maximal coronary flow in infarcted rat hearts. *Cardiovasc Res.* 1998;40:156-64.
21. Whittaker P, Kloner RA, Boughner DR, Pickering JG. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. *Basic Res Cardiol.* 1994;89:397-410.
22. Brilla CG, Janicki JS, Weber KT. Impaired diastolic function and coronary reserve in genetic hypertension. Role of interstitial fibrosis and medial thickening of intramyocardial coronary arteries. *Circ Res.* 1991;69:107-15.
23. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ Res.* 1979;44:503-12.
24. Schoemaker RG, Debets JJ, Struyker-Boudier HA, Smits JF. Delayed but not immediate captopril therapy improves cardiac function in conscious rats, following myocardial infarction. *J Mol Cell Cardiol.* 1991;23:187-97.
25. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. *Circ Res.* 1980;47:1-9.
26. McCormick RJ, Musch TI, Bergman BC, Thomas DP. Regional differences in LV collagen accumulation and mature cross- linking after myocardial infarction in rats. *Am J Physiol.* 1994;266:H354-9.
27. Sugiyama S, Hayashi K, Hanaki Y, Taki K, Ozawa T. Effect of methylprednisolone on recovery of beta-adrenergic desensitization in rat hearts. *Arzneimittelforschung.* 1991;41:439-43.
28. Vivaldi MT, Eyre DR, Kloner RA, Schoen FJ. Effects of methylprednisolone on collagen biosynthesis in healing acute myocardial infarction. *Am J Cardiol.* 1987;60:424-5.

CHAPTER 3

Chronic administration of moxonidine suppresses sympathetic activation in a rat heart failure model

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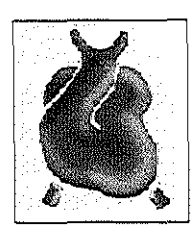

CHRONIC ADMINISTRATION OF MOXONIDINE SUPPRESSES SYMPATHETIC ACTIVATION IN A RAT HEART FAILURE MODEL

Abstract

Excessive sympathetic activity contributes to cardiovascular abnormalities which negatively affect the prognosis of heart failure. The present study evaluated the effects of moxonidine, an imidazoline I_1 receptor agonist, on sympathetic activation and myocardial remodeling in a rat heart failure model. Rats were subjected to coronary artery ligation, and treated with moxonidine, 3 or 6 mg/kg/day, from 1 to 21 days after myocardial infarction. After 21 days, heart rate and blood pressure were measured in conscious, chronically instrumented rats. Plasma catecholamine levels were determined by high-performance liquid chromatography. Effects on post-myocardial infarction remodeling were evaluated from the ventricular weight body weight ratio and interstitial collagen deposition, measured morphometrically in the interventricular septum remote from the infarcted area. Moxonidine dose-dependently decreased myocardial infarction induced tachycardia but did not affect myocardial infarction reduced blood pressure. Plasma noradrenaline levels which were elevated after myocardial infarction, decreased below sham-values with 6 mg/kg/day moxonidine. Ventricular weight body weight ratio as well as interstitial collagen were significantly elevated in myocardial infarcted rats, and restored to sham values with 6 mg/kg/day moxonidine. These data suggest that moxonidine suppresses myocardial infarction induced sympathetic activation in a dose-dependent way as indicated by reduced heart rate and plasma noradrenaline levels. Furthermore, post-myocardial infarction remodeling may be attenuated at a higher dose-range of moxonidine as shown by normalisation of ventricular weight body weight ratio and interstitial collagen.

3.1. Introduction

Myocardial infarction is one of the major causes of heart failure. Although activation of neurohormonal compensatory mechanisms following myocardial infarction initially may appear beneficial and adaptive to preserve blood pressure and cardiac output, excessive and prolonged sympathetic stimulation negatively affects the prognosis of heart failure [1,2]. Adverse actions of high plasma catecholamine levels include trophic and toxic effects on cardiac myocytes [3], downregulation of β_1-adrenoceptors, increased renin secretion and myocardial as well as vascular hypertrophy [4,5].

Recently, imidazoline I_1 receptors in the rostral ventrolateral medulla oblongata have been recognized as a new target for centrally-acting sympatholytic drugs such as moxonidine and rilmenidine [6,7]. Moxonidine is used as a centrally active antihypertensive drug which reduces sympathetic outflow and circulating levels of

catecholamines [8]. While its antihypertensive action is well-characterized in experimental and clinical studies [9,10], the potential efficacy of moxonidine in heart failure needs to be established.

The present study was carried out to investigate the effects of chronic moxonidine therapy on myocardial infarction induced sympathetic activation and its consequences for cardiac remodeling in chronically, infarcted rats. Myocardial infarction in rats has proven to be a clinically relevant model for the consequences of myocardial infarction leading to heart failure [11,12]. Twenty-one days after myocardial infarction, heart rate and blood pressure were measured in conscious, chronically instrumented rats. Plasma catecholamine levels were measured in collected arterial blood samples. Regarding the effects of moxonidine on post-myocardial infarction remodeling, cardiac hypertrophy as well as interstitial collagen in the interventricular septum were determined.

3.2. Materials and methods

3.2.1. Animals

Male Wistar rats (Harlan, Zeist, The Netherlands) weighing 270-300 g were housed in groups of 2 or 3 on a 12 h light-dark cycle with standard rat chow and water available *ad libitum*. The experimental protocol was approved by the University ethics committee for the use of experimental animals and conformed with the *Guide for Care and Use of Laboratoy Animals*.

3.2.2. Myocardial infarction

Rats were subjected to sham surgery or coronary artery ligation. Under pentobarbital anesthesia (60 mg/kg i.p.), myocardial infarction was induced by ligation of the left anterior descending coronary artery [13]. Briefly, after intubation of the trachea an incision was made in the skin overlying the fourth intercostal space, with the overlying muscles separated and kept aside. The animals were put on positive pressure ventilation (frequency 65-70/min, tidal volume 3 ml) and the thoracic cavity was opened by cutting the intercostal muscles. The heart was carefully pushed to the left and 6-0 silk suture was looped under the left descending

coronary artery near the origin of the pulmonary artery. After returning the heart to its normal position, the suture was tied. Intercostal space was closed by pulling the ribs with 3-0 silk, the muscles were returned to their normal position and the skin incision was sutured. Sham-operated animals underwent the same surgical procedure, without the actual coronary artery ligation. Proper occlusion of the coronary artery resulted in an extensive transmural infarction comprizing a major part of the left ventricular free wall, with small variations in size [14].

3.2.3. Treatment

Infarcted rats were randomized to receive subcutaneous implantation of osmotic minipumps (Alzet® 2001, ALZA Pharmaceuticals, Palo Alto, CA) filled with moxonidine. Minipumps were replaced each week under ether anesthesia. Sham-rats and non-treated myocardial infarcted rats underwent the same anesthesia and surgical procedure without the actual implantation of the minipumps. Moxonidine was dissolved in a 1 ml saline buffer with 20 µl HAc and adjusted to pH=6-6.5 with NaOH to provide a final daily dose of 3 (low-dose) or 6 mg/kg (high-dose). Administration of moxonidine was started 24 h following myocardial infarction and continued until the end of the experiment at 21 days after surgery.

3.2.4. Heart rate and mean arterial blood pressure

At day 19 after coronary artery ligation, rats were re-anesthetized and a catheter (PE-10 heat-sealed to PE-50) was inserted in the abdominal aorta through the femoral artery to measure mean arterial blood pressure. The heparinized saline filled catheter was tunnelled under the skin, exteriorized at the back of the neck and closed with a metal plug. The animals were housed separately and allowed to recover for another 2 days before measurements. On the experimental day, the arterial catheter was connected to a pressure transducer (Viggo-spectramed, DT-XX, Bilthoven, The Netherlands) and signal was fed into a 68B09-based microprocessor and compatible computer, sampling at 500 Hz. After one h stabilization, baseline values of mean arterial blood pressure and heart rate were obtained. Heart rate was measured as the frequency of the pulsatile pressure signal.

3.2.5. Catecholamines

After the registration of heart rate and mean arterial blood pressure was completed, a 1 ml arterial blood sample was collected in a syringe containing 10 µl EDTA (0.1 M) and put on ice. After centrifugation (4000 rpm for 1 min), plasma was collected in prechilled tubes filled with 1.2 mg glutathione. Plasma was stored at -80°C until assay. High-performance liquid chromatography was used to measure plasma concentrations of noradrenaline, adrenaline and dopamine, as described in detail by Boomsma et al. [15].

3.2.6. Cardiac hypertrophy

When the functional measurements were completed, rats were deeply anesthetized with pentobarbital. Then, the hearts were excised, blotted dry and weighed after removal of the atria and large vessels. Cardiac hypertrophy was defined as the ratio of ventricular weight and body weight.

3.2.7. Interstitial collagen

Hearts were cut into 4 transversal slices from apex to base and fixated by perfusion with 3.6% phosphate-buffered formaldehyde for at least 24 h. After fixation, the slices were dehydrated and paraffin-embedded. Deparaffinized 5 µm thick sections were incubated for 5 min with 0.2% (wt/vol) aqueous phosphomolybdic acid and subsequently incubated for 45 min with 0.1% Sirius Red F3BA (C.I. 35780, Polysciences Inc., Northampton, U.K.) in saturated aqueous picric acid, washed for 2 min with 0.01 M HCl, dehydrated and mounted with Entellan (Merck, Darmstadt, Germany). In the interventricular septum, remote from the infarcted area, interstitial collagen was determined as the Sirius Red positive area in 40 high power fields per heart [16,17]. Areas that enclosed signs of replacement fibrosis or bloodvessels, were excluded from analysis.

3.2.8. Data analysis

All data are presented as means ± S.E.M. Data of infarcted rats were only included if the infarction comprized the major part of the left ventricular free wall,

since small infarctions are found to be hemodynamically fully compensated [12,18]. Estimation of infarct size by macroscopic appearance has proven to be a reliable method to recognize too small infarctions (<20 %) [14]. Differences between groups were analyzed (SigmaStat™, Jandel Scientific, Erkrath, Germany) using one-way analysis of variance (ANOVA) followed by Bonferroni's post-hoc *t*-tests for multiple group comparisons [19]. Differences were considered statistically significant if $P<0.05$.

3.3. Results

Results comprise data from sham-rats (n=14), untreated (n=12), low- (n=7) and high-dose (n=7) moxonidine treated myocardial infarcted rats. Overall mortality following myocardial infarction was 38% and did not depend on the treatment used since death mainly occurred within the first 24 h after coronary artery ligation. No other than surgery related death were observed during the treatment period.

3.3.1. Heart rate and mean arterial blood pressure

As shown in Fig. 1, chronically infarcted rats showed a significantly increased heart rate (*left panel*) compared to sham-rats, which was associated with a decreased mean arterial blood pressure (*right panel*). Chronic administration of low-dose moxonidine resulted in a normalization of myocardial infarction induced tachycardia to sham-values whereas high-dose moxonidine decreased heart rate even significantly below sham-values. The observed decrease of mean arterial blood pressure in myocardial infarcted rats remained unaffected by moxonidine treatment.

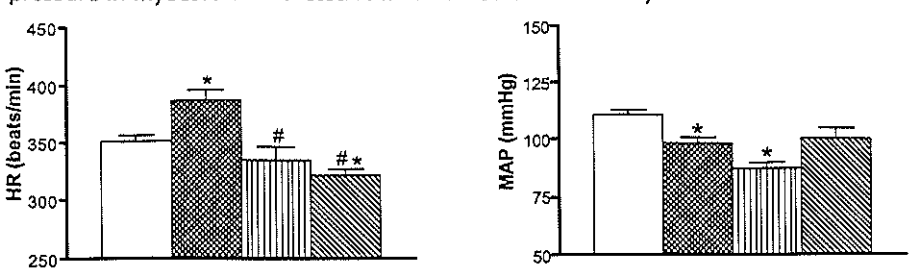

Fig. 1. Heart rate (*left panel*) and mean arterial pressure (*right panel*) measured in conscious rats with chronic MI. HR: heart rate; MAP: mean arterial pressure; MI: myocardial infarction; MOX_L: low-dose moxonidine (3 mg/kg/day); MOX_H: high-dose moxonidine (6 mg/kg/day). *: $P<0.01$ vs Sham; #: $P<0.01$ vs MI.

3.3.2. Catecholamines

Plasma catecholamine levels in the different experimental groups are shown in Fig. 2. Plasma noradrenaline levels were significantly elevated in untreated myocardial infarcted rats when compared to sham-rats. High-dose moxonidine reduced noradrenaline levels to about 50% the values of sham-rats. Plasma adrenaline and dopamine levels were not different between the experimental groups.

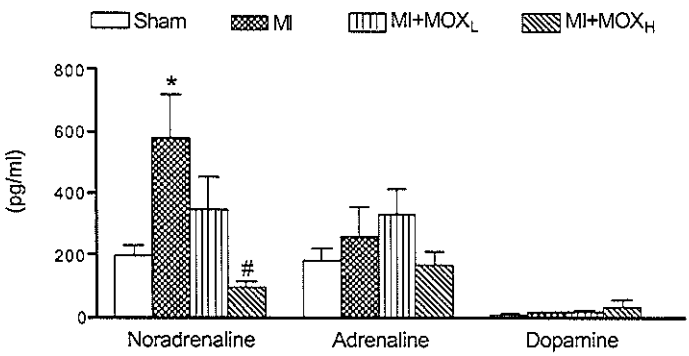

Fig. 2. Plasma concentrations of catecholamines measured in arterial blood samples obtained from resting, conscious rats. MI: myocardial infarction; MOX_L: low-dose moxonidine (3 mg/kg/day); MOX_H: high-dose moxonidine (6 mg/kg/day). *: $P<0.01$ vs Sham; #: $P<0.01$ vs MI.

3.3.3. Cardiac hypertrophy

Body weight before surgery as well as ventricular weight and body weight after 21 days for the different experimental groups are summarized in Table 1. Although started at similar body weight, sham-rats gained more weight (37±7 g) as compared to myocardial infarcted rats (23±9 g). Low-dose moxonidine did not affect this (21±9 g) but rats treated with high-dose moxonidine did not gain weight during the experimental period (1±6 g). Although not accounting for all high-dose moxonidine treated infarcted rats, overall appearance of rats treated with high-dose moxonidine was less healthy as judged from their general behaviour and condition of the fur[20,21]. Untreated myocardial infarcted hearts weighed significantly more than sham-operated control hearts despite replacement of the major part of the left ventricular free wall by lighter scar tissue.

Table 1. Body weight and ventricular weight of rats in the different experimental groups

	Sham	MI	MI+MOX_L	MI+MOX_H
n	14	12	7	7
Body weight at day 0 (g)	296±5	301±4	279±6	297±5
Body weight at day 21 (g)	333±7	320±10	301±5	299±9 [a]
Ventricular weight at day 21 (mg)	1174±37	1543±75 [a]	1410±104	1076±24 [b]

Data are presented as means ± s.e.m. MI: myocardial infarction; MOX_L: low-dose moxonidine (3 mg /kg/day); MOX_H: high-dose moxonidine (6 mg/kg/day). [a]: $P<0.01$ vs Sham; [b]: $P<0.01$ vs MI.

Ventricular weight body weight ratio was increased after myocardial infarction and significantly reduced to sham-values in myocardial infarcted rats treated with high-dose moxonidine (Fig. 3, upper panel). Low-dose moxonidine induced no changes in ventricular weight body weight ratio compared to untreated myocardial infarcted rats.

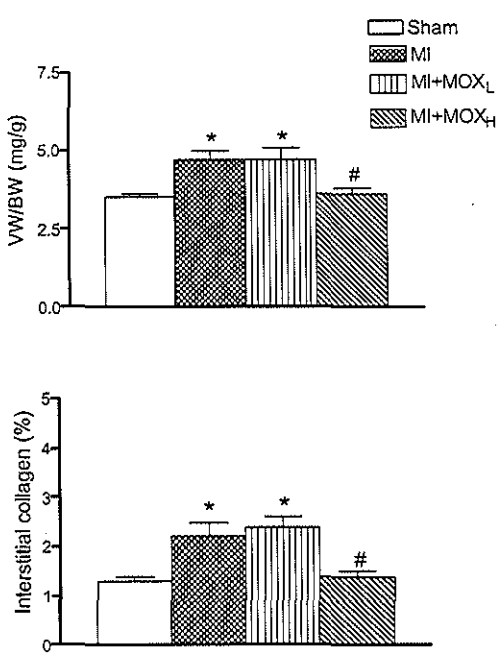

Fig. 3. Effects of moxonidine on post-MI remodeling. Cardiac hypertrophy indicated as the heart weight body weight ratio *(upper panel)* and interstitial collagen expressed as percentage of total tissue area *(lower panel)*, are presented for the different experimental groups. VW/BW: ventricular weight body weight ratio; MI: myocardial infarction; MOX_L: low-dose moxonidine (3 mg/kg/day); MOX_H: high-dose moxonidine (6 mg/kg/day). *: $P<0.01$ vs Sham; #: $P<0.01$ vs MI.

3.3.4. Interstitial collagen

Photomicrographs of picrosirius red stained sections of interventricular septum containing interstitial collagen are shown in Fig.4.

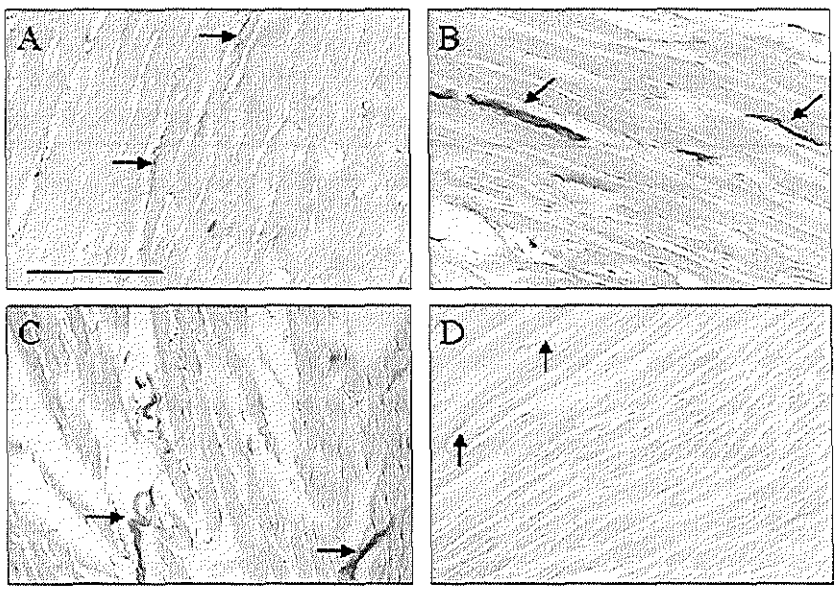

Fig. 4. Picrosirius red stained sections of interventricular septum showing interstitial collagen (see arrows) 3 weeks after surgery. A: sham heart, B: untreated MI-heart, C: low-dose moxonidine (3 mg/kg/day) treated MI-heart, D: high-dose moxonidine (6 mg/kg/day) treated MI-heart. The bar in photomicrograph A indicates 100µm, and accounts for all micrographs.

Myocardial infarction induced remodeling was associated with a significant increase in interstitial collagen content of the interventricular septum compared to sham-values. Whereas interstitial collagen in low-dose moxonidine treated myocardial infarcted hearts did not differ from untreated myocardial infarcted hearts, treatment with high-dose moxonidine significantly reduced interstitial collagen compared to the values of untreated myocardial infarcted hearts.

3.4. Discussion

3.4.1. General

In recent years, both experimental studies and clinical trials have provided insight into the potential of drugs, which directly or indirectly attenuate the activity of the sympathetic nervous system such as angiotensin-converting enzyme inhibitors [22,23] and β-adrenoceptor anatagonists [24,25] to prevent progression of heart failure. The development of newer sympatholytic drugs such as moxonidine may offer an interesting alternative to peripheral β- and α-adrenoceptor blockade for the treatment of heart failure. Moxonidine has been introduced into clinical practice for the treatment of hypertension [26] and shows less adverse side effects compared to first-generation centrally-acting antihypertensive drugs such as clonidine [27]. In the light of these observations, the effects of chronic moxonidine therapy on sympathetic activation and post-myocardial infarction remodeling were studied in a well-established rat heart failure model [11,12,28].

3.4.2. Sympathetic activation

Chronically infarcted rats manifested signs of sympathetic activation, i.e. a significantly increased heart rate accompanied by significantly elevated plasma noradrenaline levels. Accordingly, an augmented sympathetic activity associated with hemodynamic signs of heart failure, i.e. a decreased blood pressure and cardiac output, has been reported in one of our previous studies with myocardial infarcted rats [29]. The observed tachycardia in myocardial infarcted rats could be explained by enhanced sympathetic activity and elevated plasma noradrenaline levels, since heart rate *in vivo* is strongly regulated by both sympathetic nerve activity and catecholamine concentrations [30]. Interestingly, if these myocardial infarcted hearts were isolated and perfused, heart rate is still higher than in sham-operated rats, suggesting changes in intrinsic activation of the sinus node [29].

In keeping with a central reduction in sympathetic tone, myocardial infarcted rats treated with moxonidine showed a dose-dependent decrease in heart rate and plasma noradrenaline levels. A similar reduction in heart rate and plasma noradrenaline concentrations was observed in patients with severe congestive heart failure, receiving moxonidine orally for 2 to 12 weeks [31]. In the present study,

moxonidine therapy did not affect mean arterial blood pressure in myocardial infarcted rats. A blood pressure lowering effect of moxonidine was not likely to occur in normotensive rats. On the other hand, in spontaneously hypertensive rats long-term administration of moxonidine was shown to exhibit a dose-dependent and long-lasting reduction in blood pressure usually accompanied by a reduction in heart rate [6]. This may be a chronic rather than an acute effect of moxonidine, since in acute experiments (data not shown) a reduced heart rate was associated with an increase in blood pressure mediated by vascular α_2-adrenoceptors [32].

From an energetic point of view, the lower heart rate induced by moxonidine would be beneficial to the failing heart. Infarcted hearts display a lower mechanical efficiency at rest, which is amplified by tachycardia, thereby negatively affecting the relation between myocardial oxygen demand and supply [33]. A reduction in hearte rate is, independent of its origin, associated with increased capillary growth, which would greatly improve oxygenation of the heart [34]. Moreover, a decreased heart rate may lead to longer diastolic filling phase of the heart and improve diastolic function in heart failure. Since cardiac output and stroke volume are significantly reduced in severe heart failure, the lower heart rate with moxonidine could only be beneficial if stroke volume would be correspondingly increased to maintain cardiac output. Accordingly, as shown by Motz *et al.* (1998), a 10% decrease in heart rate was associated with a 20% increase in stroke volume in heart failure patients treated with oral moxonidine for 3 months.

3.4.3. Cardiac remodeling

Cardiac remodeling after myocardial infarction is strongly determined by cardiac loading conditions and neuroendocrine activation [35]. In the rat heart failure model, large myocardial infarction evokes alterations in shape and size of the injured left ventricle and compensatory hypertrophy of the non-infarcted myocardium [11]. In the present study, myocardial infarcted rats were characterized by an increased ventricular weight body weight ratio at 21 days after surgery. Since body weight was not changed in these rats, compensatory cardiac hypertrophy is indicated by a rise of ventricular mass despite replacement of the major part of the left ventricular wall by lighter scar tissue. Myocardial infarcted rats treated with high-dose moxonidine had

not gained weight 21 days after myocardial infarction. One explanation could be a decreased tolerance of moxonidine at higher doses since the general aspect of these rats was less healthy than other myocardial infarcted rats. Another explanation could be a reduced food intake. This suggestion is supported by data from spontaneously hypertensive rats chronically treated with 8 mg/kg/day moxonidine which also manifested loss of body weight [6].

In hypertensive patients [36] as well as rats [37], moxonidine was shown to be highly effective in reversing cardiac hypertrophy and protecting myocardial structure, an effect which coincided with decreased plasma noradrenaline and renin concentrations. In the present study, prevention of cardiac hypertrophy was seen in myocardial infarcted rats treated with high-dose moxonidine, whereas low-dose moxonidine had no effect on cardiac hypertrophy compared to untreated myocardial infarcted rats. The pronounced effect of high-dose moxonidine on cardiac hypertrophy is in accordance with a strong suppression of sympathetic activity i.e. lower heart rate and plasma noradrenaline levels when compared to the lower dose.

Although *regression* of hypertrophy with angiotensin-converting enzyme inhibitors and β-adrenoceptor antagonists [22,38,39] has been recognized to improve heart function and prognosis in heart failure patients [40] as well as in myocardial infarcted rats, *prevention* of early hypertrophy after myocardial infarction may be regarded as compensatory and should be interfered with care. Previous experimental studies [12,41] have clearly demonstrated that early treatment with the captopril in chronically infarcted rats resulted in prevention of hypertrophy which was associated with deterioration of *in vivo* hemodynamics. On the other hand in the rat myocardial infarction model, improved heart function was found with delayed captopril treatment [12]. Furthermore, clinical trials evaluating early intervention with angiotensin converting enzyme inhibitors in patients with acute myocardial infarction have not yielded uniform results. Whereas decreased mortality has been reported from some trials [42-44], others did not find improved survival [45-48]. Therefore, our results suggest that the high-dose moxonidine in the present study may be too high for an optimal chronic therapy .

Another event associated with post-myocardial infarction remodeling is the accumulation of interstitial [49] and perivascular collagen [17,50]. Collagen accumulation in

non-infarcted tissue has been shown to increase ventricular stiffness in myocardial infarcted rats [51]. In the present study, a significant accumulation of interstitial collagen in the non-infarcted area was observed in hearts from untreated myocardial infarcted rats. High-dose moxonidine, which prevented cardiac hypertrophy, restored interstitial collagen content to sham-values, whereas low-dose moxonidine did not alter collagen content nor hypertrophy compared to untreated myocardial infarcted rats. In spontaneously hypertensive rats, moxonidine has been reported to normalise myocardial fibrosis and capillarization to physiological levels [37]. On the other hand, a too large reduction of tensile strength of the collagen network could result in aggravation of chamber dilation, as has been reported with non-steroidal anti-inflammatory drugs and steroids [52,53].

Based on the findings of the present study, we conclude that moxonidine (3 mg/kg/day) effectively suppresses sympathetic activation after myocardial infarction without effects on cardiac hypertrophy and fibrosis. Moxonidine at 6 mg/kg/day, however, attenuated sympathetic activity even significantly below sham values, indicating the loss of a major hemodynamic regulatory system, which coincided with prevention of cardiac hypertrophy and fibrosis.

3.4.4. Clinical implications

Therapeutic management of heart failure should be focused on the reduction of sympathetic activation and protection of cardiac function. Research into moxonidine has contributed to the renewal of interest in centrally-acting sympatholytics and their possible benefit in the syndrome of heart failure. Preliminary clinical observations so far, suggest that the efficacy of moxonidine in the treatment of heart failure is comparable to currently used drugs such as angiotensin-converting enzyme inhibitors or β-adrenoceptor anatagonists. Chronic heart failure patients treated with oral moxonidine during a 3 month period, showed decreased plasma noradrenaline levels with an improved hemodynamic profile [31,54]. Furthermore, the adverse side effects which have been reported with earlier central sympatholytics, seem to be less severe.

According to the clinical observations, the findings of the present study show that moxonidine therapy effectively suppresses myocardial infarction induced

sympathetic activation in conscious, chronically infarcted rats as indicated by reduced heart rate and plasma noradrenaline levels. Furthermore, post-myocardial infarction cardiac remodeling, which also is an important prognostic factor in heart failure, could also be attenuated by moxonidine. However, the dose of moxonidine in the mangement of heart failure needs to be chosen carefully. Therapy with a high-dose of moxonidine may lead to an unphysiologically low sympathetic tone and prevent compensatory hypertrophy, which may negatively affect prognosis of heart failure. Whether the beneficial effects of moxonidine will be associated with an improved clinical outcome in heart failure patients, needs to be further investigated.

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3.5. References

1. Packer M. Pathophysiology of chronic heart failure. *Lancet* 1992;340:88-92.
2. Francis GS. Neurohumoral activation and progression of heart failure: hypothetical and clinical considerations. *J. Cardiovasc. Pharmacol.* 1998;32:S16-21.
3. Rupp H, Dhalla KS, Dhalla NS. Mechanisms of cardiac cell damage due to catecholamines: significance of drugs regulating central sympathetic outflow. *J. Cardiovasc. Pharmacol.* 1994;24:S16-24.
4. Francis GS, McDonald KM, Cohn JN. Neurohumoral activation in preclinical heart failure. Remodeling and the potential for intervention. *Circulation.* 1993;87:IV90-6.
5. Ceconi C, Curello S, Ferrari R. Catecholamines: the cardiovascular and neuroendocrine system. *Eur. Heart J.* 1998;19:F2-6.
6. Ziegler D, Haxhiu MA, Kaan EC, Papp JG, Ernsberger P. Pharmacology of moxonidine, an I1-imidazoline receptor agonist. *J. Cardiovasc. Pharmacol.* 1996;27:S26-37.
7. van Zwieten PA. Central imidazoline (I1) receptors as targets of centrally acting antihypertensives: moxonidine and rilmenidine. *J. Hypertens.* 1997;15:117-25.
8. Nurminen ML, Culman J, Haass M, Chung O, Unger T. Effect of moxonidine on blood pressure and sympathetic tone in conscious spontaneously hypertensive rats. *Eur. J. Pharmacol.* 1998;362:61-7.
9. Luft FC, Mann JF. New classes of antihypertensive drugs and new findings with established agents. *Curr. Opin. Nephrol. Hypertens.* 1992;1:91-9.
10. Kuppers HE, Jager BA, Luszick JH, Grave MA, Hughes PR, Kaan EC. Placebo-controlled comparison of the efficacy and tolerability of once- daily moxonidine and enalapril in mild-to-moderate essential hypertension. *J. Hypertens.* 1997;15:93-7.
11. Anversa P, Loud AV, Levicky V, Guideri G. Left ventricular failure induced by myocardial infarction. I. Myocyte hypertrophy. *Am. J. Physiol.* 1985;248:H876-82.
12. Schoemaker RG, Debets JJ, Struyker-Boudier HA, Smits JF. Delayed but not immediate captopril therapy improves cardiac function in conscious rats, following myocardial infarction. *J. Mol. Cell. Cardiol.* 1991;23:187-97.
13. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. *Am. J. Pathol.* 1978;90:57-70.
14. Kalkman EA, Bilgin YM, van Haren P, van Suylen RJ, Saxena PR, Schoemaker RG. Determinants of coronary reserve in rats subjected to coronary artery ligation or aortic banding. *Cardiovasc. Res.* 1996;32:1088-95.
15. Boomsma F, Alberts G, van Eijk L, Man in 't Veld AJ, Schalekamp MA. Optimal collection and storage conditions for catecholamine measurements in human plasma and urine. *Clin. Chem.* 1993;39:2503-8.

16. Whittaker P, Kloner RA, Boughner DR, Pickering JG. Quantitative assessment of myocardial collagen with picrosirius red staining and circularly polarized light. *Basic Res. Cardiol.* 1994;89:397-410.
17. Kalkman EA, van Suylen RJ, van Dijk JP, Saxena PR, Schoemaker RG. Chronic aspirin treatment affects collagen deposition in non-infarcted myocardium during remodeling after coronary artery ligation in the rat. *J. Mol. Cell. Cardiol.* 1995;27:2483-94.
18. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ. Res.* 1979;44:503-12.
19. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. *Circ. Res.* 1980;47:1-9.
20. Schoemaker RG, Kalkman EA, Smits JF. 'Quality of life' after therapy in rats with myocardial infarction: dissociation between hemodynamic and behavioral improvement. *Eur J Pharmacol.* 1996;298:17-25.
21. Schoemaker RG, Smits JF. Behavioral changes following chronic myocardial infarction in rats. *Physiol Behav.* 1994;56:585-9.
22. Pfeffer MA, Stevenson LW. Beta-adrenergic blockers and survival in heart failure. *N. Engl. J. Med.* 1996;334:1396-7.
23. Remme WJ. Effect of ACE inhibition on neurohormones. *Eur. Heart J.* 1998;19:J16-23.
24. Investigators TS. Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N. Engl. J. Med.* 1991;325:293-302.
25. Gu XH, Kompa AR, Summers RJ. Regulation of beta-adrenoceptors in a rat model of cardiac failure: effect of perindopril. *J. Cardiovasc. Pharmacol.* 1998;32:66-74.
26. Haxhiu MA, Dreshaj I, Schafer SG, Ernsberger P. Selective antihypertensive action of moxonidine is mediated mainly by I1-imidazoline receptors in the rostral ventrolateral medulla. *J. Cardiovasc. Pharmacol.* 1994;24:S1-8.
27. Planitz V. Crossover comparison of moxonidine and clonidine in mild to moderate hypertension. *Eur. J. Clin. Pharmacol.* 1984;27:147-52.
28. Anversa P, Loud AV, Levicky V, Guideri G. Left ventricular failure induced by myocardial infarction. II. Tissue morphometry. *Am. J. Physiol.* 1985;248:H883-9.
29. Schoemaker RG, Saxena PR, Kalkman EA. Low-dose aspirin improves in vivo hemodynamics in conscious, chronically infarcted rats. *Cardiovasc. Res.* 1998;37:108-14.
30. Daly PA, Sole MJ. Myocardial catecholamines and the pathophysiology of heart failure. *Circulation.* 1990;82:I35-43.
31. Motz W, Scheler S, Mox B. The potential clinical application of moxonidine in congestive heart failure. *Rev. Contemp. Pharmacother.* 1998;9:473-79.
32. Ernsberger P. The I_1-imidazoline receptor agonist moxonidine: molecular, cellular and organismic actions. *Rev. Contemp. Pharmacother.* 1998;9:441-62.
33. Stewart JT, Simpson IA, Smith RE, Callicott C, Gray HH, Camm AJ. Left ventricular energetics: heat production by the human heart. *Cardiovasc. Res.* 1993;27:1024-32.

34. Hudlicka O, Brown MD, Walter H, Weiss JB, Bate A. Factors involved in capillary growth in the heart. *Mol. Cell. Biochem.* 1995;147:57-68.

35. Levy D, Garrison RJ, Savage DD, Kannel WB, Castelli WP. Prognostic implications of echocardiographically determined left ventricular mass in the Framingham Heart Study. *N. Engl. J. Med.* 1990;322:1561-6.

36. Ollivier JP, Christen MO. I1-imidazoline-receptor agonists in the treatment of hypertension: an appraisal of clinical experience. *J. Cardiovasc. Pharmacol.* 1994;24:S39-48.

37. Mall G, Greber D, Gharenhbaghi H, Wiest G, Amann K, Mattfeldt T. Effects of nifedipine and moxonidine on cardiac structure in spontaneously hypertensive rats (SHR)--stereological studies on myocytes, capillaries, arteries, and cardiac interstitium. *Basic Res. Cardiol.* 1991;86:33-44.

38. Fowler MB. Beta-blockers in heart failure. Do they improve the quality as well as the quantity of life? *Eur. Heart J.* 1998;19:P17-25.

39. Pfeffer JM, Pfeffer MA, Braunwald E. Hemodynamic benefits and prolonged survival with long-term captopril therapy in rats with myocardial infarction and heart failure. *Circulation.* 1987;75:I149-55.

40. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ, Jr., Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. *N. Engl. J. Med.* 1992;327:669-77.

41. Gay RG. Early and late effects of captopril treatment after large myocardial infarction in rats. *J Am Coll Cardiol.* 1990;16:967-77.

42. Ambrosioni E, Borghi C, Magnani B. The effect of the angiotensin-converting-enzyme inhibitor zofenopril on mortality and morbidity after anterior myocardial infarction. The Survival of Myocardial Infarction Long-Term Evaluation (SMILE) Study Investigators [see comments]. *N Engl J Med.* 1995;332:80-5.

43. Sanbe A, Tanonaka K, Kobayasi R, Takeo S. Effects of long-term therapy with ACE inhibitors, captopril, enalapril and trandolapril, on myocardial energy metabolism in rats with heart failure following myocardial infarction. *J Mol Cell Cardiol.* 1995;27:2209-22.

44. GISSI-3 I. Six-month effects of early treatment with lisinopril and transdermal glyceryl trinitrate singly and together withdrawn six weeks after acute myocardial infarction: the GISSI-3 trial. Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto Miocardico. *J Am Coll Cardiol.* 1996;27:337-344.

45. Sharpe N, Smith H, Murphy J, Greaves S, Hart H, Gamble G. Early prevention of left ventricular dysfunction after myocardial infarction with angiotensin-converting-enzyme inhibition [published erratum appears in Lancet 1991 May 11;337(8750):1174]. *Lancet* 1991;337:872-6.

46. Swedberg K, Held P, Kjekshus J, Rasmussen K, Ryden L, Wedel H. Effects of the early administration of enalapril on mortality in patients with acute myocardial infarction. Results of

the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS II) [see comments]. *N Engl J Med.* 1992;327:678-84.

47. Ray SG, Pye M, Oldroyd KG, Christie J, Connelly DT, Northridge DB, Ford I, Morton JJ, Dargie HJ, Cobbe SM. Early treatment with captopril after acute myocardial infarction. *Br Heart J* 1993;69:215-22.
48. CCS-1 I. Oral captopril versus placebo among 13,634 patients with suspected acute myocardial infarction: interim report from the Chinese Cardiac Study (CCS-1). *Lancet* 1995;345:686-687.
49. van Krimpen C, Smits JF, Cleutjens JP, Debets JJ, Schoemaker RG, Struyker Boudier HA, Bosman FT, Daemen MJ. DNA synthesis in the non-infarcted cardiac interstitium after left coronary artery ligation in the rat: effects of captopril. *J. Mol. Cell. Cardiol.* 1991;23:1245-53.
50. Sun Y, Cleutjens JP, Diaz-Arias AA, Weber KT. Cardiac angiotensin converting enzyme and myocardial fibrosis in the rat. *Cardiovasc. Res.* 1994;28:1423-32.
51. Raya TE, Gay RG, Lancaster L, Aguirre M, Moffett C, Goldman S. Serial changes in left ventricular relaxation and chamber stiffness after large myocardial infarction in rats. *Circulation.* 1988;77:1424-31.
52. Brown EJ, Jr., Kloner RA, Schoen FJ, Hammerman H, Hale S, Braunwald E. Scar thinning due to ibuprofen administration after experimental myocardial infarction. *Am. J. Cardiol.* 1983;51:877-83.
53. Mannisi JA, Weisman HF, Bush DE, Dudeck P, Healy B. Steroid administration after myocardial infarction promotes early infarct expansion. A study in the rat. *J. Clin. Invest.* 1987;79:1431-9.
54. Swedberg K, Waagstein F, Dickstein K, Wiltse C, McNay J. Moxonidine, a centrally acting sympathetic inhibitor, causes sustained reduction of plasma norepinephrine in heart failure patients. *J. Am. Coll. Cardiol.* 1997;29:220A.

CHAPTER 4

Restored capillary density in the spared myocardium of infarcted rats improves ischemic tolerance

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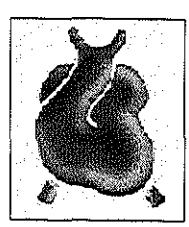

RESTORED CAPILLARY DENSITY IN THE SPARED MYOCARDIUM OF INFARCTED RATS IMPROVES ISCHEMIC TOLERANCE

Abstract

Myocardial infarction (MI) induced hypertrophy is associated with a decreased capillary density, which may negatively affect ischemic tolerance of the spared myocardium. The present study investigated the effects of moxonidine, a centrally-acting sympatholytic, on left ventricular (LV) hypertrophy and capillary density in relation to sensitivity to ischemia in infarcted hearts. Infarcted rats were randomized to recieve 3 or 6 mg/kg/day moxonidine from 1 to 21 days after MI. LV hypertrophy after MI was indicated by increased ventricular to body weight ratio, and was significantly inhibited by moxonidine. Histological analysis revealed that MI-induced concentric hypertrophy of the spared myocardium, as indicated by almost double cross-sectional area of Gomori stained myocytes, was completely prevented by 6 mg/kg/day moxonidine. This effect was accompanied by a a restored number of Lectin stained capillaries per tissue area. However, capillary to myocyte ratio was similar in all groups. LV dysfunction after MI, measured in isolated perfused hearts, was confirmed by decreased LV systolic pressure and $+(dP/dt)_{max}$ and was not affected by moxonidine. Low flow ischemia, induced by lowering perfusion pressure from 85 mmHg to 15 mmHg for 30 min resulted in a further reduction of cardiac perfusion compared to sham-rats, which was normalized with 6 mg/kg/day moxonidine. Ischemic sensitivity in MI-hearts, as reflected by increased maximal coronary flow during reperfusion, was reduced with moxonidine. This was further supported by substantially lower purines and lactate concentrations in the coronary effluent during ischemia. These results indicate that moxonidine induced prevention of hypertrophy, may preserve capillary density without affecting capillary number, thereby improving ischemic tolerance of the spared myocardium.

4.1. Introduction

Myocardial infarction (MI) induces compensatory hypertrophy and remodeling of the spared myocardium [1]. Inadequate structural adaptation of the vascular bed to the hypertrophy of the surrounding myocytes may play a role in the progression from left ventricular (LV) dysfunction into heart failure [2]. Indeed, vascular growth is inadequate to maintain normal capillary density of hypertrophied myocardium as shown in infarcted rat hearts [3]. Moreover, a lower capillary density as a consequence of myocyte hypertrophy may limit oxygen supply and lead to ischemia during periods of increased coronary flow demand

Therapeutic strategies, which focus on the prevention of MI-induced hypertrophy, have been shown to improve cardiac perfusion in both experimental and clinical studies [4-6]. Chronic angiotensin-converting enzyme (ACE) inhibition was shown to reduce cardiac hypertrophy, and improve myocardial capillary density in

chronically infarcted rats [7]. Similar findings were described in coronary ligated rats treated with the AT_1 receptor antagonist losartan [8]. Furthermore, treatment with captopril was shown to induce regression of hypertrophy thereby restoring maximal cardiac perfusion and leading to a better preservation of aerobic ATP production during ischemia [9]. Besides ACE-inhibitors or β-adrenoceptor antagonists, prevention of hypertrophy after MI can also be achieved by treatment with centrally-acting sympatholytic drugs such as moxonidine [10,11].

Recently, we showed that moxonidine effectively suppressed MI-induced sympathetic activation and prevented LV hypertrophy in 3 weeks old infarcted rat hearts [12]. Moreover, this study showed that other aspects of cardiac remodeling, such as interstitial fibrosis, were prevented by moxonidine treatment as well. Accordingly, in hypertensive rats moxonidine was shown to improve coronary microcirculation, as a consequence of decreased hypertrophy and fibrosis [10]. However, whether these effects are related to preservation of adequate oxygen supply in the infarcted hypertrophied heart, is still unkown. Therefore, the aim of the present study was to investigate whether the effects of moxonidine on myocardial hypertrophy and capillary density are associated with a decreased sensitivity to ischemia.

4.2. Materials and Methods

4.2.1. Animals

Male Wistar rats (Harlan, Zeist, The Netherlands) weighing 270-300 g were housed in groups of 2 or 3 on a 12 h light-dark cycle with standard rat chow and water available *ad libitum*. The experimental protocol was approved by the University ethics committee for the use of experimental animals and conformed with the *Guide for Care and Use of Laboratoy Animals.*

4.2.2. Myocardial infarction

Rats were subjected to sham-surgery or coronary artery ligation. Under pentobarbital anesthesia (60 mg/kg i.p.), MI was induced by ligation of the left anterior descending coronary artery [13]. Briefly, after intubation of the trachea an incision was made in the skin overlying the fourth intercostal space, with the

overlying muscles separated and kept aside. The animals were put on positive pressure ventilation (frequency 65-70/ min, tidal volume 3 ml) and the thoracic cavity was opened by cutting the intercostal muscles. The heart was carefully pushed to the left and 6-0 silk suture was looped under the left descending coronary artery near the origin of the pulmonary artery. After returning the heart to its normal position, the suture was tied. Intercostal space was closed by pulling the ribs with 3-0 silk, the muscles were returned to their normal position and the skin incision was sutured. Sham-operated animals underwent the same surgical procedure, without the actual coronary artery ligation. Proper occlusion of the coronary artery resulted in an extensive transmural infarction comprising a major part of the LV free wall, with small variations in size [9]. Infarct size was determined by planimetry at mid-ventricular levels in transverse slices as the percentage of LV circumference.

4.2.3. Moxonidine treatment

Moxonidine was a generous gift from Solvay Pharma (Hannover, Germany). Infarcted rats were randomized for treatment with moxonidine at two different doses. For that, moxonidine was dissolved in saline (pH= 6-6.5) to provide a final daily dose of 3 (low-dose) or 6 mg/kg (high-dose) using subcutaneously implanted osmotic minipumps (Alzet® 2001, ALZA Pharmaceuticals, CA, USA). Administration of moxonidine was started 24 h following MI and continued until the end of the experiment at 21 days after surgery. Minipumps were replaced each week under ether anesthesia. Sham-rats and untreated MI-rats underwent the same anesthesia and surgical procedure without the actual implantation of the minipumps.

4.2.4. Isolated heart perfusion

At the end of the protocol, the hearts were rapidly excized under pentobarbital anesthesia and mounted for perfusion with an oxygenated Krebs-Henseleit buffer (composition in mM: NaCl 125, KCl 4.7, $CaCl_2$ 1.35, $NaHCO_3$ 20, NaH_2PO_4 0.4, D-glucose 10; pH=7.4; 37°C) at a constant pressure of 85 mmHg, using the Langendorff technique. After 15 min of stabilization, hearts were paced at 350 beats/min (4V, 2ms). LV function was measured as the isovolumetric developed pressure against a fluid-filled latex balloon placed in the left ventricle, connected to a

miniature low-volume displacement pressure transducer. LV end-diastolic pressure was set to 5 mmHg by adjusting the balloon volume. Coronary flow was measured by a flow probe (Transonic Systems, Ithaca, NY, USA) placed in the tubing just before the ostia of the coronary arteries. Cardiac perfusion was defined as the coronary flow corrected for ventricular weight.

4.2.5. Ischemia and reperfusion

To induce ischemia, perfusion pressure was lowered to 15 mmHg. At baseline and at the end of ischemia, coronary effluent was sampled on ice and stored at -80°C until assayed for purines and lactate concentrations. After 30 min of low-flow ischemia, perfusion pressure was reset to 85 mmHg. Reactive hyperemia was determined as the maximal coronary flow during reperfusion. When hemodynamics had stabilized again, at 25 min reperfusion, LV function was recorded and compared to baseline values before ischemia to indicate functional recovery. To examine whether maximal coronary flow during reperfusion indeed represent a functional response to the ischemic period rather than changes in absolute maximal coronary flow, the latter is obtained at the end of the experiments by bolus (0.1 ml) injection of a 10^{-2} M sodium nitroprusside solution (Dijkzigt University Hospital's pharmacy, Rotterdam, The Netherlands).

4.2.6. Purines and lactate

Release of purines into the coronary effluent was used to investigate loss of ATP catabolites from the heart [14,15]. Cardiac loss of ATP catabolites during ischemia correlates well with myocardial ATP breakdown, as measured with [^{31}P]- nuclear magnetic resonance [16]. Concentration of purines was determined as described in detail by Smolenski et al. [17]. Briefly, the ATP catabolites uric acid, adenosine, inosine, xanthine and hypoxanthine were determined by high-performance liquid chromatography on a C_{18}-μBondapak column (Millipore Waters Co., Milford, Mass, USA). Coronary effluent (100μl) was injected directly into the system, eluted with a 15% (v/v) solution of acetonitrile in 150mM potassium dihydrogen orthophosphate,

containing 150mM potassium chloride adjusted to pH 6.0 with potassium hydroxide. Peaks were monitored by absorption at 254 nm or 280 nm for uric acid.

The release of lactate into the coronary effluent was used as an indicator of anaerobic glycolysis in the cardiomyocyte. Lactate concentration in coronary effluent was determined as described in detail by Marbach & Weil (reagents: Sigma Diagnostics, Deisenhofen, Germany). Briefly, lactic acid was converted by lactate oxidase to pyruvate and H_2O_2. In the presence of the formed H_2O_2, peroxidase catalysed the oxidative condensation of chromogen precursors to produce a coloured dye with an absorption maximum at 540 nm. Lactate concentration could be determined, being directly proportional to the increase of absorption at 540 nm.

4.2.7. Ventricular hypertrophy

After completion of the functional measurements, hearts were removed from the Langendorff preparation and weighed after exclusion of the atria and large vessels. Ventricular hypertrophy was defined as the ratio of ventricular weight to body weight. Ventricles were cut into 4 transversal slices from apex to base and fixated with 3.6% phosphate-buffered formaldehyde for at least 24 h. After fixation, the slices were dehydrated and paraffin-embedded. Deparaffinized 5 µm thick sections were stained with a Gomori's silver staining [18] in order to visualize individual myocytes of the viable LV wall. Concentric myocyte hypertrophy in the viable LV free wall, remote from the infarcted area, was measured as the cross-sectional area of transversally cut myocytes showing a nucleus. Myocyte density was calculated as the average number of myocytes per tissue area.

4.2.8. Capillary density

To visualize the capillaries in the myocardium, endothelial cells were stained with Lectin GSI (Sigma-Aldrich Chemie©, Zwijndrecht, The Netherlands), as previously described by Nelissen-Vrancken. Sections of 5 µm thickness were deparaffinized and rehydrated, and endogenous peroxidase was inhibited by methanol/H_2O_2 (0.3%) for 15 minutes. The sections were incubated overnight with the biotinylated Lectin GSI (1:100) at room temperature. Then, in a second step, the signal was intensified with an ABC-complex containing peroxidase labeled biotins

(1:100) (Lab vision, CA, USA). Finally, the sections were incubated with a Ni-Co amplified DAB solution to which a stable peroxide substrate buffer was added (Pierce©, CA, USA). Endothelial cells of capillaries and larger vessels are visualized in the myocardium as a brown precipitate. A background staining was not used in order to avoid interference with the Lectin staining. The number of capillaries were counted in the same region of the viable LV free wall in which myocyte area was determined. Capillary density in the viable LV wall was calculated as the number of capillaries per tissue area. Capillary to myocyte ratio was calculated as capillary density divided by myocyte density.

4.2.9. Data analysis

All data are presented as means ± S.E.M. Data of infarcted rats were only included if the infarction comprized the major part of the LV free wall, since small infarctions (<20%) are found to be hemodynamically fully compensated [5,19]. Estimation of infarct size by macroscopic appearance has proven to be a reliable method to recognize too small infarctions (<20 %) [9]. Differences between groups were analyzed (SigmaStat™, Jandel Scientific, Erkrath, Germany) using one-way analysis of variance (ANOVA) followed by Dunnett's and Bonferroni's post-hoc *t*-tests for multiple group comparisons [20]. A non-parametric data analysis (ANOVA on Ranks) was performed to analyze differences in purines and lactate levels. Differences were considered statistically significant if $P<0.05$.

4.3. Results

Overall mortality following MI was 38 % and did not depend on the treatment, since death mainly occurred within the first 24 h after coronary artery ligation. No other than surgery related death was observed during the treatment period with moxonidine. Results comprize data from sham-rats (n=14), untreated (n=12), low- (n=7) and high-dose (n=7) moxonidine treated MI-rats. In tables and figures low- and high-dose moxonidine will be abbreviated as mox_L and mox_H, respectively.

4.3.1. Cardiac remodeling

Three weeks after MI, body and ventricular weight as well as infarct size were measured for the different experimental groups (Table 1). The infarct size between untreated and moxonidine treated MI-hearts remained unchanged. Body weight of high-dose moxonidine treated rats was lower when compared to sham-rats. Ventricular weight of untreated MI-rats was significantly higher than sham-operated control hearts despite replacement of the major part of the left ventricular free wall by lighter scar tissue. Treatment with high-dose moxonidine normalized ventricular weight to sham-values.

Table 1. Infarct size, body and ventricular weight measured in the experimental groups 21 days after surgery.

n = 7-14	SHAM	MI	MI+mox$_L$	MI+mox$_H$
Infarct size (%)	-	45 ± 3	47 ± 5	44 ± 3
Body weight (g)	333 ± 7	320 ± 10	301 ± 5	299 ± 9 [a]
Ventricular weight (mg)	1174 ± 37	1543 ± 75 [a]	1408 ± 104	1076 ± 24 [b]

Data are presented as means±S.E.M. MI: myocardial infarction; mox$_L$: low-dose moxonidine (3 mg/kg/day); mox$_H$: high-dose moxonidine (6 mg/kg/day); [a] $P<0.05$ vs. SHAM; [b] $P<0.05$ vs.MI.

Moreover, ventricular weight to body weight ratio, which increased after MI, was significantly reduced to sham-values in high-dose moxonidine treated MI-rats (Figure 2, *lower panel*).

Photomicrographs of Gomori stained sections in the LV viable wall are shown in Figure 1. MI-induced hypertrophy, which was microscopically confirmed by an increased myocyte area and decreased myocyte density, was dose-dependently restored to sham-values with moxonidine.

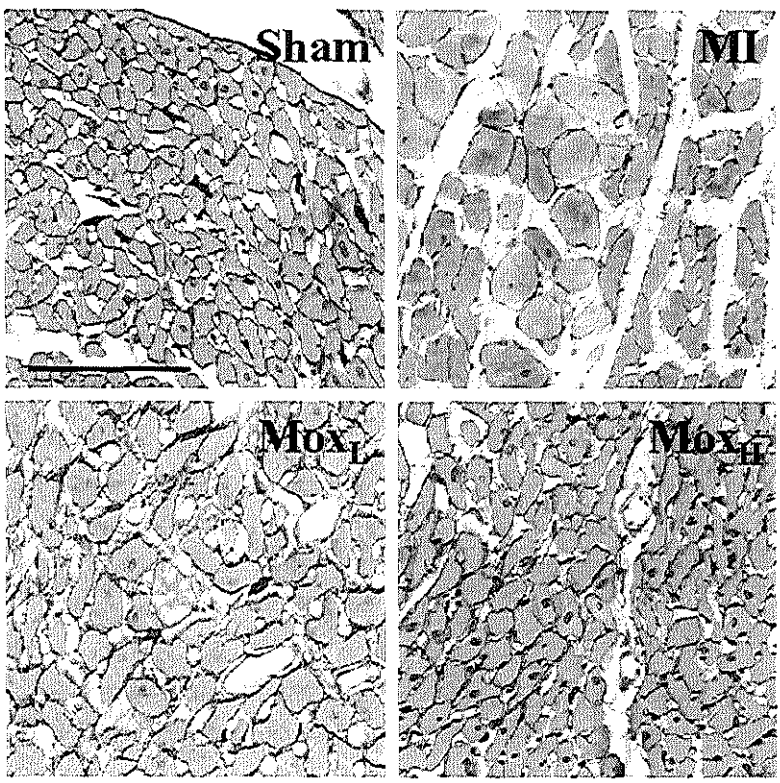

Fig. 1. Gomori stained sections of LV viable wall obtained from the different experimental groups, showing individual myocytes. The bar in left upper photomicrograph indicates 100 µm, and accounts for all micrographs.

These observations were substantiated by the actual measurements in Figure 2 (*upper and middle panel*).

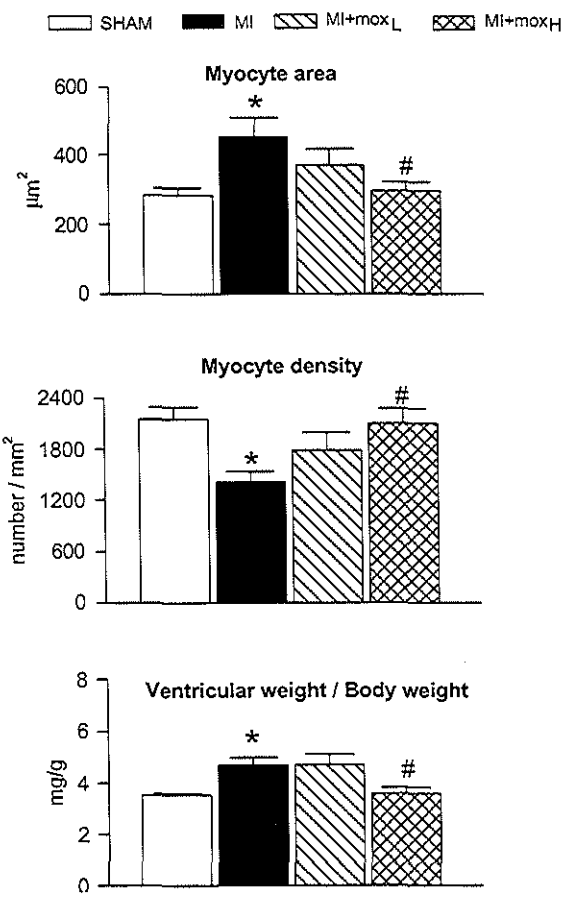

Fig. 2. Myocyte area *(upper panel)*, myocyte density *(middle panel)* and ventricular weight to body weight ratio *(lower panel)* are presented for the different experimental groups. MI: myocardial infarction; mox$_L$: low-dose moxonidine (3 mg/kg/day); mox$_H$: high-dose moxonidine (6 mg/kg/day). *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

Photomicrographs of Lectin stained sections in the LV viable wall showing capillaries are represented in Figure 3. The decreased capillary density observed in hypertrophied MI-hearts, could be restored to sham-values with high-dose moxonidine.

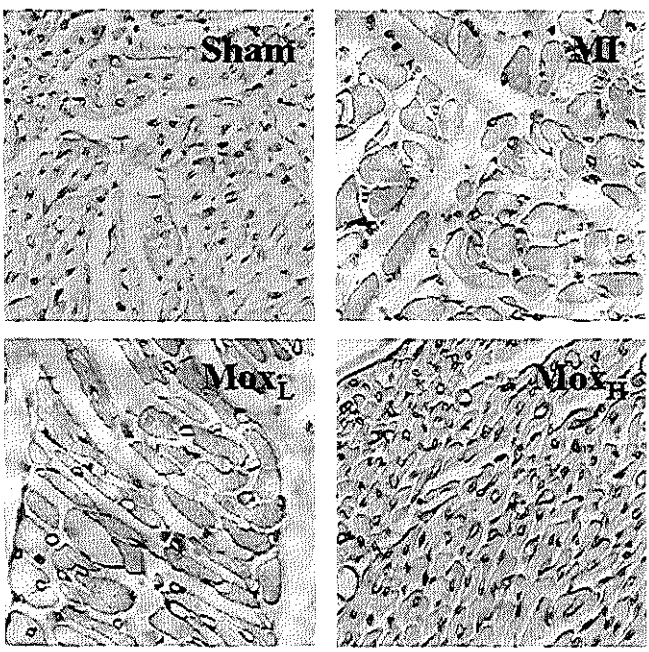

Fig. 3. Lectin stained sections of LV viable wall obtained from the different experimental groups, showing individual capillaries.

These observations were substantiated by the actual measurements in Figure 4.

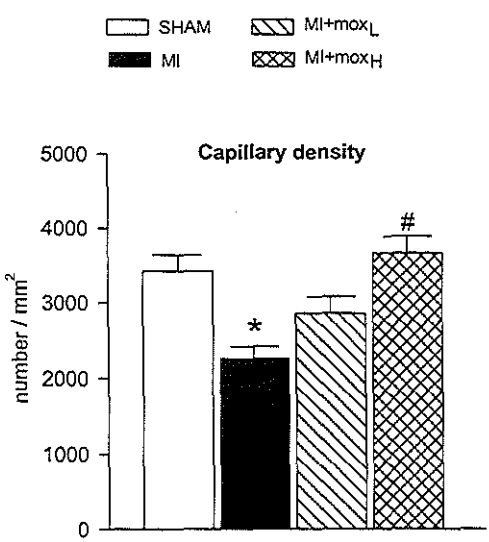

Fig. 4. Capillary density measured in untreated as well as moxonidine treated MI-hearts. MI: myocardial infarction; mox$_L$: low-dose moxonidine (3 mg/kg/day); mox$_H$: high-dose moxonidine (6 mg/kg/day). *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

The changes in capillary density could not be attributed to a change in actual capillary number, since capillary to myocyte ratio was similar in all groups (Sham:1.60±0.08; MI:1.70±0.20; mox_L:1.70±0.17; mox_H: 1.83±0.20).

4.3.2. In vitro LV function

In vitro heart rate, LV systolic and diastolic pressure as well as coronary flow, measured at baseline, before and after pacing, are summarized in Table 2. At baseline, untreated MI-hearts were characterized by decreased LV systolic pressure and tachycardia, which was not affected by moxonidine treatment. LV diastolic pressure and coronary flow remained unaffected between the different experimental groups.

Table 2. *In vitro* functional parameters measured at baseline before and after pacing

n = 7-14	SHAM	MI	MI+mox_L	MI+mox_H
Heart rate (beats/min)	208 ± 12	256 ± 11[a]	281 ± 18[a]	239 ± 17
paced	354 ± 1	353 ± 2	352 ± 1	355 ± 1
LV systolic pressure (mmHg)	77 ± 6	51 ± 7[a]	66 ± 10	44 ± 5[a]
paced	73 ± 6	45 ± 7[a]	68 ± 11	48 ± 7[a]
LV diastolic pressure (mmHg)	5 ± 1	6 ± 2	6 ± 1	4 ± 1
paced	4 ± 2	6 ± 1	5 ± 1	4 ± 1
Coronary flow (ml/min)	9.9 ± 0.9	9.6 ± 0.8	10.5 ± 1.8	10.2± 1.0
paced	12.9 ± 1.1	10.1 ± 0.9	11.8 ± 2.0	10.9± 1.3

Data are presented as means±S.E.M. MI: myocardial infarction; mox_L: low-dose moxonidine (3 mg/kg/day); mox_H: high-dose moxonidine (6 mg/kg/day); [a] $P<0.05$ vs. SHAM.

LV dysfunction in paced untreated MI-hearts was further confirmed by a significantly decreased peak velocity of contraction and relaxation at baseline as well as during ischemia and reperfusion (Figure 5, *middle* and *lower panel*). Moxonidine treatment did not affect peak velocity of contraction and relaxation. After 25 min reperfusion, LV function was fully recovered.

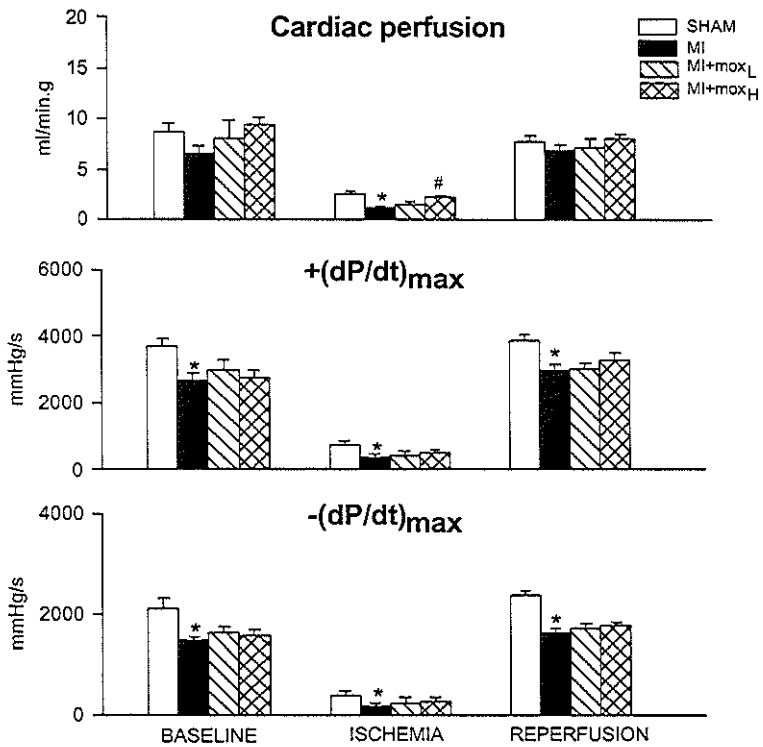

Fig. 5. Functional parameters measured in paced hearts at baseline, during ischemia and reperfusion. *Upper panel:* Cardiac perfusion. *Middle and lower panel:* Peak velocity of contraction and relaxation. MI: myocardial infarction; mox$_L$: low-dose moxonidine (3 mg/kg/day); mox$_H$: high-dose moxonidine (6 mg/kg/day). *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

4.3.3. Ischemic sensitivity

Whereas cardiac perfusion (coronary flow corrected for ventricular weight) at baseline and during the reperfusion period was similar for all groups, ischemia depressed cardiac perfusion in all hearts but most pronounced in untreated MI-hearts (Figure 5, *upper panel*). Furthermore, improved cardiac perfusion during ischemia was observed in high-dose moxonidine treated MI-hearts. At reperfusion, coronary flow increased to a maximum within 5 min and returned to baseline after 20 min.

Reactive hyperemia, as reflected by maximal coronary flow during reperfusion, was significantly increased in MI-hearts and decreased with moxonidine treatment (Figure 6, *left panel*). Maximal *absolute* values for coronary flow, which were obtained after a bolus injection with sodium nitroprusside, were similar in all groups (Figure 6, *right panel*).

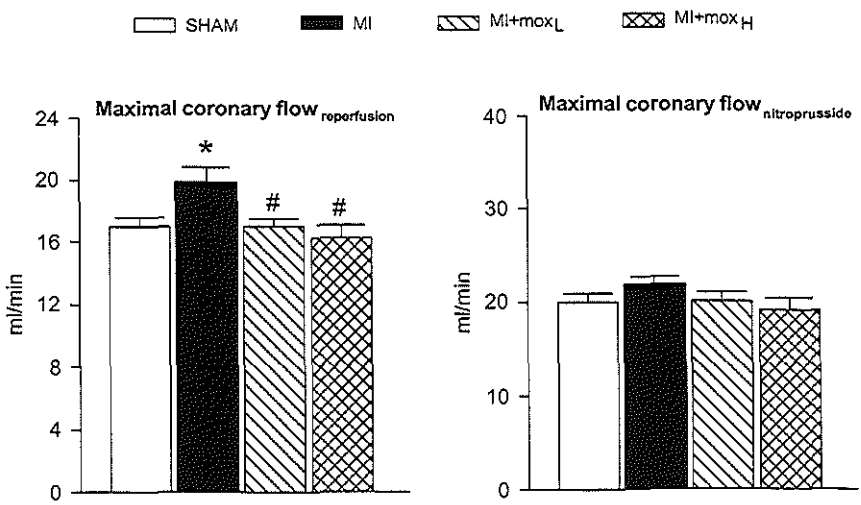

Fig. 6. Maximal coronary flow during reperfusion and after bolus injection of sodium nitroprusside in sham-, and untreated as well as moxonidine treated MI-hearts. MI: myocardial infarction; mox$_L$: low-dose moxonidine (3 mg/kg/day); mox$_H$: high-dose moxonidine (6 mg/kg/day). *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

Baseline values for purines and lactate concentrations were not different for the experimental groups. Low-flow ischemia, which resulted in an approximately 90% decrease of coronary flow when compared to baseline (*Ischemic flow:* Sham:1.1±0.1; MI:1.1±0.1; mox$_L$:1.4±0.2; mox$_H$:1.5±0.1 ml/min), was associated with on average 14 times higher purines and 20 times higher lactate concentrations in the coronary effluent. The values of purines and lactate concentrations measured in the coronary effluent at the end of ischemia are shown in Figure 7. No differences for ischemic purines and lactate concentrations were observed between sham-hearts and untreated MI-hearts. In accordance with the observed decrease in reactive

hyperemia, high-dose moxonidine treated MI-hearts were characterized by substantially lower ischemic purines (Figure 7, *left panel*) and lactate (Figure 7, *right panel*) concentrations.

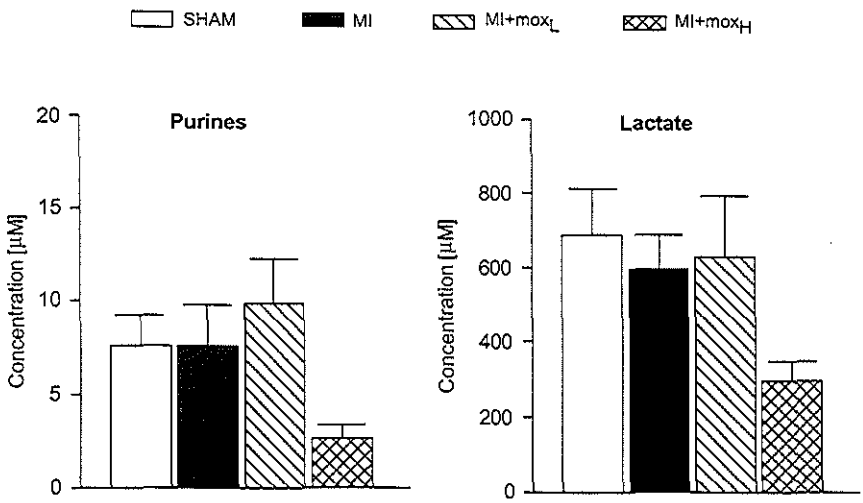

Fig. 7. Concentration of purines *(left panel)* and lactate *(right panel)* in the coronary effluent measured at the end of ischemia. MI: myocardial infarction; mox$_L$: low-dose moxonidine (3 mg/kg/day); mox$_H$: high-dose moxonidine (6 mg/kg/day).

4.4. Discussion

4.4.1. Cardiac remodeling

Post-MI remodeling is associated with alterations in shape and size of the injured left ventricle and compensatory hypertrophy of the non-infarcted myocardium [3,11]. In the present study, ventricular hypertrophy in MI-hearts was macroscopically shown by an increased ventricular weight to body weight ratio. Since body weight was not changed in MI-rats, compensatory hypertrophy is indicated by a rise of ventricular mass despite replacement of the major part of the LV free wall by lighter scar tissue [12]. Concentric rather than eccentric hypertrophy has been shown to be related with increased ischemic sensitivity [5]. Furthermore, in a previous study we have shown that a decreased maximal myocardial perfusion is limited to the area

with the most pronounced cardiomyocyte hypertrophy, the spared part of the LV free wall [21]. In this area, concentric hypertrophy following MI was microscopically confirmed by an almost double myocyte cross-sectional area and decreased myocyte density. Whereas, in the present study, cardiac perfusion at baseline and during the reperfusion period was similar for all groups, ischemia depressed cardiac perfusion in all hearts but most pronounced in untreated MI-hearts. Furthermore, improved cardiac perfusion during ischemia was observed in high-dose moxonidine treated MI-hearts. The impaired ischemic cardiac perfusion in untreated MI-hearts may be explained by a disproportional degree of hypertrophy relative to vascular adaptation. Improved cardiac perfusion observed with high-dose moxonidine is mainly due to a lower ventricular weight, since absolute coronary flow was not changed. Indeed, complete prevention of ventricular hypertrophy was only seen with high-dose moxonidine, which restored myocyte area and ventricular weight to body weight ratio. These findings correspond with other clinical and experimental studies, in which moxonidine was shown to be highly effective in reversing cardiac hypertrophy [12] and protecting myocardial structure [10,11].

In hypertensive rats, cardiac hypertrophy has been found to be associated with an inadequate compensation of the capillary bed brought about by a relative decrease in capillary numerical density with no change in capillary size [22]. Similarly in the present study, a significant reduction in capillary density was seen in the hypertrophied spared myocardium of MI-hearts, with unchanged capillary to myocyte ratio's. Indeed, an insufficient adaptation of the coronary vascular bed to the increase in cardiac muscle mass that has to be supplied, is consistent with the decreased capillary density found in the rat MI-model [1,23]. Prevention of hypertrophy in moxonidine treated MI-hearts was associated with a normalization of capillary density. The effects of moxonidine on capillary density can be attributed to prevention of hypertrophy itself, rather than an angiogenic effect of moxonidine. This explanation was supported by the values of the capillary to myocyte ratio, which were similar for untreated as well as moxonidine treated MI-hearts. Improved coronary microcirculation attributed to decreased hypertrophy and collagen content was also observed in spontaneously hypertensive rats treated with moxonidine [10].

The relationship between cardiac function and capillarization is not fully elucidated. In the present study, LV dysfunction of untreated MI-hearts was reflected by a decreased LV systolic pressure and increased *in vitro* heart rate. Furthermore, depressed LV function in MI-hearts was confirmed by a significantly decreased peak velocity of contraction and relaxation.

Tachycardia in isolated perfused MI-hearts cannot be explained by an enhanced sympathetic tone, as was the case for the *in vivo* tachycardia observed in conscious MI-rats [12], but may rather be related to changes in intrinsic activation of the sinus node [24]. Accordingly, whereas moxonidine was shown to normalize *in vivo* heart rate by suppression of sympathetic tone, it did not affect *in vitro* heart rate. Although prevention of hypertrophy with restored capillary density in high-dose moxonidine treated MI-hearts could be regarded as beneficial to the heart, this finding was not associated with an improved LV function. This may be explained by the fact that LV systolic pressure in moxonidine treated MI-hearts has to be developed from significantly less contractile tissue. In addition, LV function may be the net result of improved oxygenation to existing contractile elements and the amount of contractile tissue.

4.4.2. Ischemic sensitivity

MI induced cardiac hypertrophy [3] has been associated with enhanced ischemic vulnerability of the spared myocardium [25], which could be attributed to differences in cardiac perfusion, and increased oxygen diffusion distance with a relatively inadequate increase of capillary surface [3]. Sensitivity to ischemia was evaluated from ATP breakdown products in the coronary effluent and maximal coronary flow during reperfusion. During ischemia, decreased ATP regeneration can result in the loss of ATP catabolites from the cell [14]. This has been shown to be a sensitive marker for ischemia, since the concentration of the purines in the coronary effluent is lineary correlated with the degree of ATP breakdown and, hence, severity of ischemia [16,26]. In order to preserve intracellular ATP levels during ischemia, anaerobic glycolysis can be activated. This would be associated with the release of lactate in the coronary effluent, which is used as an indicator of anaerobic glycolysis activity of the

cardiomyocyte. Lactate production causes proton accumulation within the myocyte, which hampers cellular function.

In the present study, 8 times lower coronary flow was associated with on average 14 times higher purines and 20 times higher lactate concentrations in the coronary effluent. This indicates actual ischemia-induced increased concentrations rather than flow-related increased concentrations. Whereas ischemic purines concentration from MI-hearts was not different from sham-hearts, improved tolerance to ischemia with high-dose moxonidine was supported by substantially lower purines concentrations. Since ventricular weight of high-dose moxonidine treated rats is lower compared to untreated MI-hearts, ischemic purines concentration may be corrected for ventricular weight and coronary flow; purine release. Nevertheless, this ischemic purine release was also lower in high-dose moxonidine treated MI-hearts (data not shown).

A reduced loss of purines in high-dose moxonidine treated MI-hearts could be compensated for by a greater ATP production by anaerobic glycolysis. However, since lactate concentration in those hearts was lower as well, this does not seem feasible. Therefore, the explanation may rather lie in a lower ATP consumption in high-dose moxonidine treated MI-hearts. Indeed, moxonidine was shown to decrease myocardial oxygen consumption per unit weight of myocardium, thereby improving economics of cardiac function [27]. Furthermore, the above findings are confirmed by the results of the maximal coronary flow during reperfusion. The heart is responding to transient ischemia with reactive vasodilation (reactive hyperemia) due to release of vasodilatory substances, such as nitric oxide [28] and adenosine [29]. Increased maximal coronary flow during reperfusion, therefore, is lineary correlated to an increased sensitivity to ischemia [30,31]. Whereas an increased sensitivity to ischemia was observed in untreated MI-hearts, moxonidine decreased sensitivity to ischemia, as reflected by a reduced maximal coronary flow during reperfusion. On the other hand, increased ischemic sensitivity in MI-hearts was not correlated to higher purines or lactate values, which is probably due to a more efficient energetic adaptation of MI-hearts. Furthermore, the observation that maximal coronary flow after sodium nitroprusside was not different for the different experimental groups, implies that the changes in maximal coronary flow during reperfusion indeed reflect

responses to ischemia rather than indicate absolute coronary capacity. Improved ischemic tolerance with moxonidine is also supported by clinical studies, which showed restoration of coronary vasodilator reserve and improved maximal coronary blood flow in patients with coronary artery disease [32].

4.4.3. Conclusions

Remodeling of the spared myocardium after MI may increase sensitivity to ischemia, and hence the risk of additional morbidity, and mortality. Reduced capillary density as a consequence of myocyte hypertrophy may limit oxygen supply and lead to ischemia during periods of increased coronary flow demand. Therefore, reconstitution of cardiac mass with sufficient oxygen supply to the cells may be one of the therapeutic goals to improve the functional capacity of the ventricle in myocardial infarcted patients. The findings of the present study show that moxonidine therapy can prevent cardiac hypertrophy and restore capillary density in chronically, infarcted rat hearts. Furthermore, prevention of post-MI remodeling with moxonidine may contribute to a more aerobic metabolism during ischemia, thereby improving ischemic tolerance. Preliminary clinical observations so far, suggest that the efficacy of moxonidine in protecting myocardial structure is comparable to currently used drugs such as ACE-inhibitors [7,33] or ß-adrenoceptor antagonists [34].

Based on our present and previous findings, it should be emphasized that therapeutic efficacy of moxonidine highly depends on the used dosage. Whereas the lower dose of moxonidine only suppressed sympathetic activation, a dose which is only 2 times higher will also completely prevent the post-MI remodeling required for functional compensation. Indeed, although beneficial to the heart itself, complete prevention of hypertrophy after MI may deteriorate LV hemodynamics as shown in rats [5] and patients [35] treated with ACE-inhibitors. Accordingly, the promising effects of moxonidine on cardiac remodeling and microcirculation were not associated with improved LV function.

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4.5. References

1. Anversa P, Beghi C, Kikkawa Y, Olivetti G. Myocardial infarction in rats. Infarct size, myocyte hypertrophy, and capillary growth. *Circ Res.* 1986;58:26-37.
2. Vatner SF, Hittinger L. Coronary vascular mechanisms involved in decompensation from hypertrophy to heart failure. *J Am Coll Cardiol.* 1993;22:34A-40A.
3. Anversa P, Loud AV, Levicky V, Guideri G. Left ventricular failure induced by myocardial infarction. II. Tissue morphometry. *Am J Physiol.* 1985;248:H883-9.
4. Pfeffer JM, Pfeffer MA, Braunwald E. Hemodynamic benefits and prolonged survival with long-term captopril therapy in rats with myocardial infarction and heart failure. *Circulation.* 1987;75:I149-55.
5. Schoemaker RG, Debets JJ, Struyker-Boudier HA, Smits JF. Delayed but not immediate captopril therapy improves cardiac function in conscious rats, following myocardial infarction. *J Mol Cell Cardiol.* 1991;23:187-97.
6. Fowler MB. Beta-blockers in heart failure. Do they improve the quality as well as the quantity of life? *Eur Heart J.* 1998;19:17-25.
7. Schieffer B, Wirger A, Meybrunn M, Seitz S, Holtz J, Riede UN, Drexler H. Comparative effects of chronic angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade on cardiac remodeling after myocardial infarction in the rat. *Circulation.* 1994;89:2273-82.
8. Sladek T, Sladkova J, Kolar F, Papousek F, Cicutti N, Korecky B, Rakusan K. The effect of AT1 receptor antagonist on chronic cardiac response to coronary artery ligation in rats. *Cardiovasc Res.* 1996;31:568-76.
9. Kalkman EA, Bilgin YM, van Haren P, van Suylen RJ, Saxena PR, Schoemaker RG. Determinants of coronary reserve in rats subjected to coronary artery ligation or aortic banding. *Cardiovasc Res.* 1996;32:1088-95.
10. Mall G, Greber D, Gharenhbaghi H, Wiest G, Amann K, Mattfeldt T. Effects of nifedipine and moxonidine on cardiac structure in spontaneously hypertensive rats (SHR)--stereological studies on myocytes, capillaries, arteries, and cardiac interstitium. *Basic Res Cardiol.* 1991;86:33-44.
11. Ollivier JP, Christen MO. I1-imidazoline-receptor agonists in the treatment of hypertension: an appraisal of clinical experience. *J Cardiovasc Pharmacol.* 1994;24:S39-48.
12. Van Kerckhoven R, van Veen TA, Boomsma F, Saxena PR, Schoemaker RG. Chronic administration of moxonidine suppresses sympathetic activation in a rat heart failure model. *Eur J Pharmacol.* 2000;397:113-20.
13. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. *Am J Pathol.* 1978;90:57-70.

14. Schrader J, Haddy FJ, Gerlach E. Release of adenosine, inosine and hypoxanthine from the isolated guinea pig heart during hypoxia, flow-autoregulation and reactive hyperemia. *Pflugers Arch.* 1977;369:1-6.
15. Achterberg PW, Harmsen E, de Tombe PP, de Jong JW. Balance of purine nucleotides and catabolites in the isolated ischemic rat heart. *Adv Exp Med Biol.* 1984;165:483-6.
16. Harmsen E, Schoemaker R, Yu J, Ruzicka M, Leenen FH. Sensitivity to ischaemic ATP breakdown in different models of cardiac hypertrophy in rats. *J Hypertens.* 1994;12:49-57.
17. Smolenski RT, Lachno DR, Ledingham SJ, Yacoub MH. Determination of sixteen nucleotides, nucleosides and bases using high-performance liquid chromatography and its application to the study of purine metabolism in hearts for transplantation. *J Chromatogr.* 1990;527:414-20.
18. Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT, Clark WA. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. *Circ Res.* 1989;65:657-70.
19. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ Res.* 1979;44:503-12.
20. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. *Circ Res.* 1980;47:1-9.
21. Kalkman EA, van Haren P, Saxena PR, Schoemaker RG. Regionally different vascular response to vasoactive substances in the remodelled infarcted rat heart; aberrant vasculature in the infarct scar. *J Mol Cell Cardiol.* 1997;29:1487-97.
22. Tomanek RJ, Searls JC, Lachenbruch PA. Quantitative changes in the capillary bed during developing, peak, and stabilized cardiac hypertrophy in the spontaneously hypertensive rat. *Circ Res.* 1982;51:295-304.
23. Turek Z, Grandtner M, Kubat K, Ringnalda BE, Kreuzer F. Arterial blood gases, muscle fiber diameter and intercapillary distance in cardiac hypertrophy of rats with an old myocardial infarction. *Pflugers Arch.* 1978;376:209-15.
24. Kohl P, Noble D. Mechanosensitive connective tissue: potential influence on heart rhythm. *Cardiovasc Res.* 1996;32:62-8.
25. Snoeckx LH, van der Vusse GJ, Coumans WA, Willemsen PH, Reneman RS. Differences in ischaemia tolerance between hypertrophied hearts of adult and aged spontaneously hypertensive rats. *Cardiovasc Res.* 1993;27:874-81.
26. Jennings RB, Steenbergen C. Nucleotide metabolism and cellular damage in myocardial ischemia. *Annu Rev Physiol.* 1985;47:727-749.
27. Motz W, Scheler S, Mox B. The potential clinical application of moxonidine in congestive heart failure. *Rev. Contemp. Pharmacother.* 1998;9:473-79.
28. Kostic MM, Schrader J. Role of nitric oxide in reactive hyperemia of the guinea pig heart. *Circ Res.* 1992;70:208-12.
29. Saito D, Steinhart CR, Nixon DG, Olsson RA. Intracoronary adenosine deaminase reduces canine myocardial reactive hyperemia. *Circ Res.* 1981;49:1262-7.

30. Peters KG, Wangler RD, Tomanek RJ, Marcus ML. Effects of long-term cardiac hypertrophy on coronary vasodilator reserve in SHR rats. *Am J Cardiol.* 1984;54:1342-8.

31. McAinsh AM, Turner MA, O'Hare D, Nithythyananthan R, Johnston DG, O'Gorman DJ, Sheridan DJ. Cardiac hypertrophy impairs recovery from ischaemia because there is a reduced reactive hyperaemic response. *Cardiovasc Res.* 1995;30:113-21.

32. Motz W, Scheler S, Strauer BE. Coronary microangiopathy in hypertensive heart disease: pathogenesis, diagnosis and therapy. *Herz.* 1995;20:355-64.

33. Sun Y, Cleutjens JP, Diaz-Arias AA, Weber KT. Cardiac angiotensin converting enzyme and myocardial fibrosis in the rat. *Cardiovasc Res.* 1994;28:1423-32.

34. Bristow MR. beta-adrenergic receptor blockade in chronic heart failure. *Circulation.* 2000;101:558-69.

35. Swedberg K, Held P, Kjekshus J, Rasmussen K, Ryden L, Wedel H. Effects of the early administration of enalapril on mortality in patients with acute myocardial infarction. Results of the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS II). *N Engl J Med.* 1992;327:678-84.

CHAPTER 5

Chronic vasopressin V_{1A}- but not V_2 receptor antagonism prevents heart failure in chronically infarcted rats

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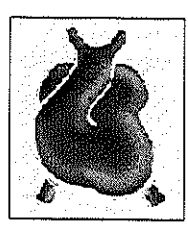

CHRONIC VASOPRESSIN V_{1A}- BUT NOT V_2 RECEPTOR ANTAGONISM PREVENTS HEART FAILURE IN CHRONICALLY INFARCTED RATS

Abstract

Evidence is increasing that therapeutic modulation of neurohormonal activation with vasopressin antagonists via V_{1A} and V_2 receptors may favourably affect prognosis of heart failure. This study was designed to compare *in vivo* hemodynamic effects of *early* treatment (1-21 days after infarction) with a V_{1A}- (SR-49059; 0.3 mg/kg/day) and a V_2 (SR-121463B; 0.5 mg/kg/day) receptor antagonist in infarcted (MI) rats, chronically instrumented for hemodynamic measurements. Left ventricular (LV) dysfunction in conscious MI-rats, which was evidenced by a significantly decreased cardiac output (MI: 70±3 vs Sham: 81±3 ml/min) and stroke volume (MI: 190±10 vs Sham: 221±7 µl), was restored by the vasopressin V_{1A} (81±2 ml and 224±5µl, respectively), but not V_2 antagonist. Improved cardiac output with the vasopressin V_{1A} antagonist resulted from an increased stroke volume at a reduced MI-induced tachycardia. In addition to the hemodynamic measurements, LV hypertrophy and capillary density were determined histologically measured as the cross-sectional area of Gomori stained myocytes and Lectin stained capillaries per tissue area, respectively. The observed LV concentric hypertrophy (MI: 525±38 vs Sham: 347±28 μm^2; $P<0.05$) and reduced capillary density (MI: 2068±162 vs Sham: 2800±250 number/mm^2; $P<0.05$) in the spared myocardium of MI-rats, remained unaffected by the vasopressin V_{1A} or V_2 antagonist. Thus, chronic vasopressin V_{1A}-, but not V_2 receptor blockade prevents heart failure in 3 week old infarcted rats. Moreover, the improved cardiac function could not be attributed to changes in LV hypertrophy and/or capillary density.

5.1. Introduction

Neurohomonal activation after acute MI refers to increased activity of the sympathetic nervous system, renin-angiotensin system, atrial natriuretic peptide and arginine-vasopressin [1,2]. Although initially compensatory in nature, prolonged neurohormonal activation after myocardial infarction (MI) has been shown to negatively affect prognosis of heart failure [3,4]. Although angiotensin-converting enzyme inhibitors [5] and β-adrenoceptors antagonists [6] have been shown to interfere with postinfarction remodeling resulting in improved prognosis of heart failure, current therapy is still not optimal.

Another less investigated approach to achieve inhibition of prolonged neurohormonal activation could be obtained by blockade of the arginine vasopressin system. The antidiuretic hormone vasopressin plays a pivotal role in blood pressure control and salt and water homeostasis through its effects at the vasopressin V_{1A}

receptor [7] to cause vasoconstriction and at the renal vasopressin V_2 receptor to mediate antidiuresis [8]. The recent development of vasopressin antagonists such as YM087, SR-49059 and OPC-31260, have allowed reevaluation of the precise role of vasopressin in the development of heart failure [9]. However, currently there are only a limited number of studies examining the use of vasopressin antagonists as therapy for heart failure. Selective inhibition of the V_1 and V_2 receptor led to immediate improvement in hemodynamic parameters [10] and increased diuresis [8].

Whereas previous studies have mainly focused on the acute and short-term hemodynamic and renal effects of V_{1A} and V_2 antagonism [11-13], the present study was designed to compare the *in vivo* hemodynamic effects of *chronic* treatment with a V_{1A} and V_2 receptor antagonist in conscious infarcted rats. These rats have been shown to provide a well established postinfarction heart failure model [14]. Furthermore, to investigate whether hemodynamic changes could be attributed to effects on postinfarction remodeling, LV hypertrophy and capillary density in the surviving myocardium were determined histologically using image analysis.

5.2. Materials and methods

5.2.1. Animals

Male Wistar rats (Harlan, Zeist, The Netherlands) weighing 260-300 g were housed in groups of 2 or 3 on a 12 h light-dark cycle with standard rat chow and water available *ad libitum*. The experimental protocol was approved by the University ethics committee for the use of experimental animals and conformed with the *Guide for Care and Use of Laboratoy Animals*.

5.2.2. Myocardial infarction

Rats were subjected to sham surgery or coronary artery ligation. Under pentobarbital anesthesia (60 mg/kg i.p.), myocardial infarction was induced by ligation of the left anterior descending coronary artery [14]. Briefly, after intubation of the trachea an incision was made in the skin overlying the fourth intercostal space, with the overlying muscles separated and kept aside. The animals were put on positive pressure ventilation (frequency 65-70/min, tidal volume 3 ml) and the

thoracic cavity was opened by cutting the intercostal muscles. The heart was carefully pushed to the left and 6-0 silk suture was looped under the left descending coronary artery near the origin of the pulmonary artery. After returning the heart to its normal position, the suture was tied. Intercostal space was closed by pulling the ribs with 3-0 silk, the muscles were returned to their normal position and the skin incision was sutured. Sham-operated animals underwent the same surgical procedure, without the actual coronary artery ligation. Proper occlusion of the coronary artery resulted in an extensive transmural infarction comprising a major part of the left ventricular free wall, with small variations in size [15]. Infarct size was determined by planimetry at mid-ventricular levels in transverse slices [16] as the percentage of LV circumference [17].

5.2.3. Treatments and doses

Vasopressin antagonist treatment was administered from day 1 to 21 after MI. Therefore, rats were randomized to receive subcutaneous implantation of osmotic minipumps (Alzet® 2001, ALZA Pharmaceuticals, Palo Alto, CA) filled with the vasopressin V_{1A} receptor antagonist SR-49059 or vasopressin V_2 receptor antagonist SR-121463B (generous gifts from Sanofi, Montpellier, France). Minipumps were replaced each week under light ether anesthesia. Sham-rats and non-treated myocardial infarcted rats underwent the same anesthesia and surgical procedure without the actual implantation of the minipumps. The V_{1A} receptor antagonist SR-49059((2*S*)1-[(2*R*3*S*)-5-chloro-3-(2-chlorophenyl)-1-(3,4-dimethoxybenzenesulfonyl)-3-hydroxy-2,3-dihydro-1*H*-indole-2-car-bonyl]-pyrrolidine-2-carboxamide) (Serra-deil-Le Gal, 1994) and V_2 receptor antagonist SR-121463B were dissolved in dimethyl sulfoxide at 3.75 mg/ml and 1.25 mg/ml, respectively, to provide a final daily dose of 0.3 mg/kg/day and 0.5 mg/kg/day. In pilot experiments the used dose of the V_{1A} receptor antagonist for 3 weeks, caused a rightward shift of the vasopressin pressure-response curve one log unit, while the used dose of the V_2 receptor antagonist chronically increased 24 h urine production by 20% (data not shown).

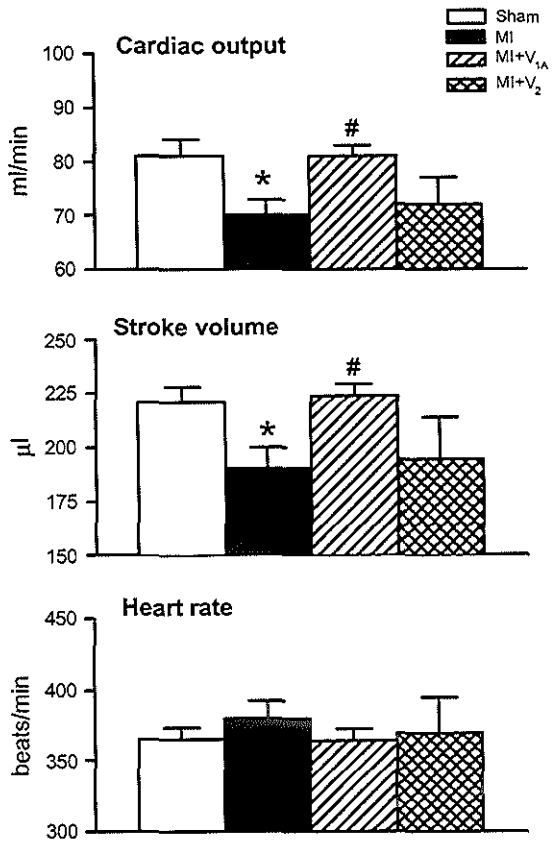

Fig. 1. Cardiac output *(upper panel)*, stroke volume *(middle panel)* and heart rate *(lower panel)* measurements obtained from sham, untreated infarcted and vasopressin antagonists treated infarcted rats. *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

5.3.3. Cardiac remodeling

Representative photomicrographs of Gomori stained sections in the LV viable wall are shown in Fig.2 (*Panel A*). MI-induced LV hypertrophy at cellular level, which was confirmed by a significantly increased myocyte cross-sectional area, was not affected by vasopressin V_{1A} or V_2 antagonist treatment.

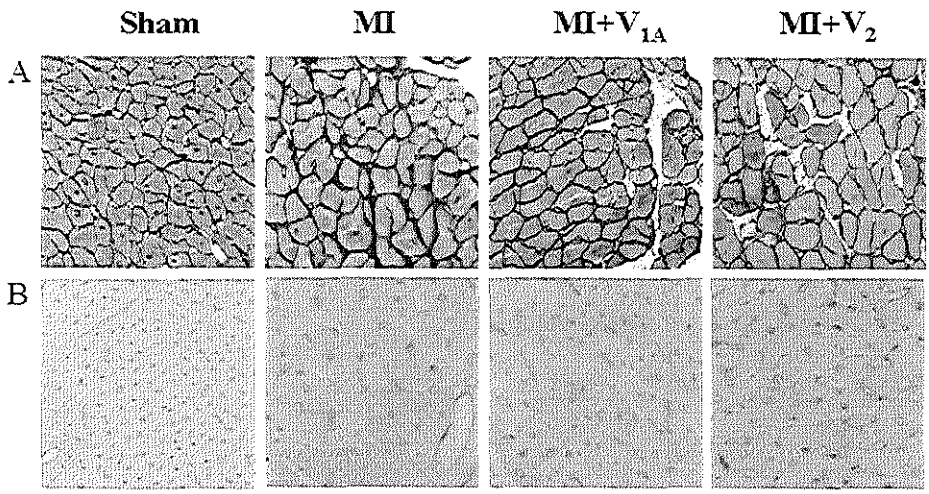

Fig. 2. Representative micrographs of Gomori as well as Lectin stained sections in the left ventricular viable wall of the different experimental groups, showing individual myocytes *(Panel A)* and, respectively, individual capillaries (small dark brown circles) *(Panel B)*. The bar in left upper photomicrograph indicates 100 µm, and accounts for all micrographs. MI: untreated infarcted rats; MI+V_{1A}: vasopressin V_{1A} antagonist treated infarcted rats; MI+V_2: vasopressin V_2 antagonist treated infarcted rats.

These observations were substantiated by the actual measurements as presented in Fig.3 (*upper panel*). Figure 2 (*Panel B*) shows representative photomicrographs of Lectin stained sections in the LV viable wall showing individual capillaries. Reduced capillary density, which was observed in MI-induced hypertrophied hearts, remained unaffected by treatment with vasopressin antagonsists. This was confirmed by the actual measurements in Fig.3 *(lower panel)*. In addition, capillary to myocyte ratio was similar in all groups (Sham: 1.31±0.12; MI: 1.37±0.14; MI+V_{1A}: 1.27±0.08; MI+V_2: 1.41±0.19).

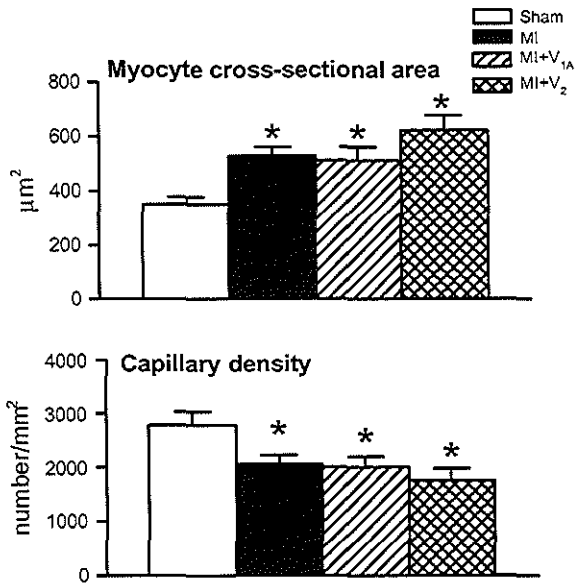

Fig. 3. Actual measurements for myocyte cross-sectional area *(upper panel)* and capillary density *(lower panel)* in the different experimental groups. *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

5.4. Discussion

5.4.1. In vivo hemodynamics

The important role of neurohormonal activation in the progression of heart failure and LV dysfunction is well established [22]. That vasopressin may contribute importantly to this process, is supported by prior studies demonstrating elevated arginin vasopressin levels in acute as well as chronic heart failure [23-25]. When antivasopressor V_1-receptor antagonists became available for intravenous use in humans, it was shown to produce a hemodynamic improvement with transient decrease in systemic vascular resistance and increased cardiac output [26]. Moreover, understanding of the functional significance of vasopressin in heart failure have also been achieved with V_2-receptor antagonists, which were shown to improve diuresis and increase free water clearance in experimental models [27,28] as well as humans [29].

In contrast to previous studies, which mainly examined the acute effects of vasopressin receptor antagonism, the present results demonstrate that *chronic* treatment with the V_{1A} receptor antagonist SR-49059, but not the V_2 receptor antagonist SR-121463B, could improve *in vivo* hemodynamics in a postinfarction rat model of heart failure. Whereas LV dysfunction in untreated conscious MI-rats was reflected by a significantly decreased stroke volume and cardiac output, chronic V_{1A} receptor blockade restored these functional parameters. Furthermore, whereas acute administration of V_{1A} and V_2 antagonists in MI-rats significantly reduced mean arterial blood pressure and more than doubled urine production [13], *chronic* treatment did not alter cardiac loading conditions as reflected by mean arterial pressure, central venous pressure and total peripheral resistance. Thus, an improved stroke volume and cardiac output with the V_{1A} antagonist, but not V_2 antagonist, could not be attributed to changes in pre- or afterload. In addition, a lowered *in vivo* tachycardia as observed with the vasopressin V_{1A} antagonist may be advantageous in terms of improving myocardial oxygen delivery by enhanced tissue perfusion through longer diastolic time [30]. The hemodynamic results of this study are fully supported by observations in a rat model of postinfarction heart failure in which short-term therapy with a combined V_{1A}/V_2 receptor antagonist resulted in a increased cardiac output however at substantial increased urine output [31]. Hemodynamic improvement with this combined V_{1A}/V_2 receptor antagonist could, based on the findings of the present study, be mainly attributed to the effects of V_{1A} antagonism.

5.4.2. Cardiac remodeling

Alterations in the cardiac structure may be responsible for improved cardiac function in vasopressin V_{1A} antagonist treated MI-rats. Therefore, its effects on LV hypertrophy and capillary density were investigated. Postinfarction remodeling is associated with alterations in shape and size of the injured left ventricle and compensatory hypertrophy of the spared myocardium with reduced capillary density [32]. Although ACE-inhibitors [33] and β-blockers [34] are now extensively used to treat functional and structural consequences following large MI, little is known about the effects of vasopressin-antagonists on cardiac remodeling.

In the present study, LV hypertrophy associated with a lower capillary density was observed in the spared myocardium of untreated MI-rats, which was not affected by treatment with the vasopressin V_{1A} or V_2 antagonist. The lower capillary density directly results from LV hypertrophy since capillary to myocyte ratio remained unchanged. Hemodynamic improvement at a preserved hypertrophic response during early post-MI treatment supports our hypothesis that complete prevention of MI-induced compensatory hypertrophy, as was observed with *early* ACE-inhibitor therapy, deteriorates rather than improves LV hemodynamics in MI-rats [18]. Recent studies have demonstrated that in addition to other neurohormones, vasopressin increases the rate of protein synthesis in the myocardium, leading to myocyte hypertrophy, a direct effect mediated by the V_{1A} receptor [35,36]. However, in the present study antagonizing the V_{1A} or V_2 vasopressin receptors did not affect myocyte hypertrophy and, without a direct effect on capillary growth, capillary density remained reduced. A minor role of vasopressin compared to the renin-angiotensin-system in promoting LV hypertrophy might be one explanation. From these results, we may assume that improved cardiac function observed with the vasopressin V_{1A} antagonist is not due to changes in hypertrophic response and myocardial capillarity.

5.4.3. Conclusions

It is conceivable that in the setting of neurohormonal blockade with ACE-inhibitors, β-blockers, and aldosterone-antagonists, other neurohormones such as vasopressin begin to play a more prominent role in the subsequent progression of heart failure. Currently there are only a limited number of studies examining the use of vasopressin antagonists in human heart failure. In general, vasopressin antagonism has been shown to improve hemodynamics and diuresis in several models of heart failure. The results of the present study showed that chronic vasopressin V_{1A}-, but not V_2 receptor blockade prevented heart failure in chronically infarcted rats. Interestingly, in our hands this is the very first treatment administered during the first 3 weeks, that actually improved cardiac output. Previous work resulted at the most in a sustained depressed cardiac output, but at a lower heart rate and improved stroke volume [37]. The observed improvement of cardiac function

in these rats could not be attributed to changes in LV hypertrophy and capillary density. However, in line with the observations with ACE-inhibitor therapy [18], effect of *early* treatment with vasopressin receptor antagonists may not predict effects of more *delayed* treatment. These aspects need further investigation.

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5.5. References

1. McAlpine HM, Morton JJ, Leckie B, Rumley A, Gillen G, Dargie HJ. Neuroendocrine activation after acute myocardial infarction. *Br Heart J.* 1988;60:117-24.
2. Rouleau JL, Moye LA, de Champlain J, Klein M, Bichet D, Packer M, Dagenais G, Sussex B, Arnold JM, Sestier F, et al. Activation of neurohumoral systems following acute myocardial infarction. *Am J Cardiol.* 1991;68:80D-86D.
3. Packer M. Pathophysiology of chronic heart failure. *Lancet* 1992;340:88-92.
4. Francis GS. Neurohumoral activation and progression of heart failure: hypothetical and clinical considerations. *J. Cardiovasc. Pharmacol.* 1998;32:S16-21.
5. Pfeffer MA, Braunwald E, Moye LA, Basta L, Brown EJ, Jr., Cuddy TE, Davis BR, Geltman EM, Goldman S, Flaker GC, et al. Effect of captopril on mortality and morbidity in patients with left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. The SAVE Investigators. *N. Engl. J. Med.* 1992;327:669-77.
6. Gu XH, Kompa AR, Summers RJ. Regulation of beta-adrenoceptors in a rat model of cardiac failure: effect of perindopril. *J. Cardiovasc. Pharmacol.* 1998;32:66-74.
7. Burrell LM, Phillips PA, Stephenson JM, Risvanis J, Johnston CI. Vasopressin and a nonpeptide antidiuretic hormone receptor antagonist (OPC-31260). *Blood Press.* 1994;3:137-41.
8. Wong LL, Verbalis JG. Vasopressin V2 receptor antagonists. *Cardiovasc Res.* 2001;51:391-402.
9. Naitoh M, Suzuki H, Murakami M, Matsumoto A, Arakawa K, Ichihara A, Nakamoto H, Oka K, Yamamura Y, Saruta T. Effects of oral AVP receptor antagonists OPC-21268 and OPC-31260 on congestive heart failure in conscious dogs. *Am J Physiol.* 1994;267:H2245-54.
10. Creager MA, Faxon DP, Cutler SS, Kohlmann O, Ryan TJ, Gavras H. Contribution of vasopressin to vasoconstriction in patients with congestive heart failure: comparison with the renin-angiotensin system and the sympathetic nervous system. *J Am Coll Cardiol.* 1986;7:758-65.
11. Yatsu T, Tomura Y, Tahara A, Wada K, Tsukada J, Uchida W, Tanaka A, Takenaka T. Pharmacological profile of YM087, a novel nonpeptide dual vasopressin V1A and V2 receptor antagonist, in dogs. *Eur J Pharmacol.* 1997;321:225-30.
12. Burrell LM, Phillips PA, Risvanis J, Chan RK, Aldred KL, Johnston CI. Long-term effects of nonpeptide vasopressin V2 antagonist OPC-31260 in heart failure in the rat. *Am J Physiol.* 1998;275:H176-82.
13. Lankhuizen IM, van Veghel R, Saxena PR, Schoemaker RG. Vascular and renal effects of vasopressin and its antagonists in conscious rats with chronic myocardial infarction; evidence for receptor shift. *Eur J Pharmacol.* 2001;423:195-202.
14. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. *Am J Pathol.* 1978;90:57-70.

15. Kalkman EA, Bilgin YM, van Haren P, van Suylen RJ, Saxena PR, Schoemaker RG. Determinants of coronary reserve in rats subjected to coronary artery ligation or aortic banding. *Cardiovasc. Res.* 1996;32:1088-95.

16. Nelissen-Vrancken HJ, Kuizinga MC, Daemen MJ, Smits JF. Early captopril treatment inhibits DNA synthesis in endothelial cells and normalization of maximal coronary flow in infarcted rat hearts. *Cardiovasc Res.* 1998;40:156-64.

17. Kalkman EA, van Suylen RJ, van Dijk JP, Saxena PR, Schoemaker RG. Chronic aspirin treatment affects collagen deposition in non-infarcted myocardium during remodeling after coronary artery ligation in the rat. *J. Mol. Cell. Cardiol.* 1995;27:2483-94.

18. Schoemaker RG, Debets JJ, Struyker-Boudier HA, Smits JF. Delayed but not immediate captopril therapy improves cardiac function in conscious rats, following myocardial infarction. *J. Mol. Cell. Cardiol.* 1991;23:187-97.

19. Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT, Clark WA. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. *Circ Res.* 1989;65:657-70.

20. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ. Res.* 1979;44:503-12.

21. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. *Circ. Res.* 1980;47:1-9.

22. Packer M. The neurohormonal hypothesis: a theory to explain the mechanism of disease progression in heart failure. *J. Am. Coll. Cardiol.* 1992;20:248-54.

23. Szatalowicz VL, Arnold PE, Chaimovitz C, Bichet D, Berl T, Schrier RW. Radioimmunoassay of plasma arginine vasopressin in hyponatremic patients with congestive heart failure. *N Engl J Med.* 1981;305:263-6.

24. Goldsmith SR, Francis GS, Cowley AW, Jr., Levine TB, Cohn JN. Increased plasma arginine vasopressin levels in patients with congestive heart failure. *J Am Coll Cardiol.* 1983;1:1385-90.

25. Rouleau JL, Packer M, Moye L, de Champlain J, Bichet D, Klein M, Rouleau JR, Sussex B, Arnold JM, Sestier F, et al. Prognostic value of neurohumoral activation in patients with an acute myocardial infarction: effect of captopril. *J. Am. Coll. Cardiol.* 1994;24:583-91.

26. Nicod P, Waeber B, Bussien JP, Goy JJ, Turini G, Nussberger J, Hofbauer KG, Brunner HR. Acute hemodynamic effect of a vascular antagonist of vasopressin in patients with congestive heart failure. *Am J Cardiol.* 1985;55:1043-7.

27. Yamamura Y, Nakamura S, Itoh S, Hirano T, Onogawa T, Yamashita T, Yamada Y, Tsujimae K, Aoyama M, Kotosai K, Ogawa H, Yamashita H, Kondo K, Tominaga M, Tsujimoto G, Mori T. OPC-41061, a highly potent human vasopressin V2-receptor antagonist: pharmacological profile and aquaretic effect by single and multiple oral dosing in rats. *J Pharmacol Exp Ther.* 1998;287:860-7.

28. Yatsu T, Tomura Y, Tahara A, Wada K, Kusayama T, Tsukada J, Tokioka T, Uchida W, Inagaki O, Iizumi Y, Tanaka A, Honda K. Cardiovascular and renal effects of conivaptan hydrochloride

(YM087), a vasopressin V1A and V2 receptor antagonist, in dogs with pacing-induced congestive heart failure. *Eur J Pharmacol.* 1999;376:239-46.

29. Kalra PR, Anker SD, Coats AJ. Water and sodium regulation in chronic heart failure: the role of natriuretic peptides and vasopressin. *Cardiovasc Res.* 2001;51:495-509.
30. Stewart JT, Simpson IA, Smith RE, Callicott C, Gray HH, Camm AJ. Left ventricular energetics: heat production by the human heart. *Cardiovasc. Res.* 1993;27:1024-32.
31. Mulinari RA, Gavras I, Wang YX, Franco R, Gavras H. Effects of a vasopressin antagonist with combined antipressor and antiantidiuretic activities in rats with left ventricular dysfunction. *Circulation.* 1990;81:308-11.
32. Anversa P, Beghi C, Kikkawa Y, Olivetti G. Myocardial infarction in rats. Infarct size, myocyte hypertrophy, and capillary growth. *Circ Res.* 1986;58:26-37.
33. Sanbe A, Tanonaka K, Kobayasi R, Takeo S. Effects of long-term therapy with ACE inhibitors, captopril, enalapril and trandolapril, on myocardial energy metabolism in rats with heart failure following myocardial infarction. *J Mol Cell Cardiol.* 1995;27:2209-22.
34. Fowler MB. Beta-blockers in heart failure. Do they improve the quality as well as the quantity of life? *Eur. Heart J.* 1998;19:P17-25.
35. Fukuzawa J, Haneda T, Kikuchi K. Arginine vasopressin increases the rate of protein synthesis in isolated perfused adult rat heart via the V1 receptor. *Mol Cell Biochem.* 1999;195:93-8.
36. Nakamura Y, Haneda T, Osaki J, Miyata S, Kikuchi K. Hypertrophic growth of cultured neonatal rat heart cells mediated by vasopressin V(1A) receptor. *Eur J Pharmacol.* 2000;391:39-48.
37. Schoemaker RG, Saxena PR, Kalkman EA. Low-dose aspirin improves in vivo hemodynamics in conscious, chronically infarcted rats. *Cardiovasc. Res.* 1998;37:108-14.

CHAPTER 6

Lower *intrinsic* rather than actual *in vivo* heart rate contributes to capillary angiogenesis in infarcted rat hearts

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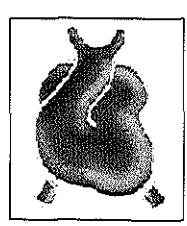

LOWER *INTRINSIC* RATHER THAN ACTUAL *IN VIVO* HEART RATE CONTRIBUTES TO CAPILLARY ANGIOGENESIS IN INFARCTED RAT HEARTS

Abstract

Postinfarction remodeled hearts display a lower mechanical efficiency amplified by tachycardia. Furthermore, in these hearts a lower capillary density negatively affects myocardial oxygen supply. The present study investigated the relation between heart rate (*in vivo* and *intrinsic*) and capillary angiogenesis in the surviving myocardium of chronically infarcted (MI) rats treated with aspirin (25mg/kg/day; i.p., n=6-12), methylprednisolone (5mg/kg/day; i.p.;n=7-12), moxonidine (6mg/kg/day; osmotic minipumps; n=7-14) and captopril (2g/l drinkwater; n=7-10). While actual *in vivo* heart rate was measured in conscious rats as the frequency of aortic pressure signal, *intrinsic* heart rate was measured from spontaneously beating isolated perfused hearts. Infarcted rats manifested a significant *in vivo* tachycardia (mean values; MI: 386±9 vs. Sham: 354±7 beats/min) as well as increased *intrinsic* heart rate (mean values; MI: 274±8 vs. Sham: 229±8 beats/min) when compared to sham-rats. Whereas aspirin, methylprednisolone and moxonidine significantly reduced infarction induced *in vivo* tachycardia, captopril further increased *in vivo* tachycardia. Increased *intrinsic* heart rate in infarcted hearts was reduced to sham-values with aspirin and methylprednisolone, but not with moxonidine and captopril. Concentric left ventricular hypertrophy, defined as cross-sectional area of transversally cut Gomori stained myocytes, was indicated by an almost double myocyte area (mean values; MI: 586±45 vs. Sham: 320±26 μm^2; $P<0.05$), and prevented by methylprednisolone, moxonidine and captopril ($P<0.05$), but not aspirin. Capillary density, which was defined as the number of Lectin stained capillaries per tissue area, significantly decreased in infarcted hearts (mean values; MI: 1949±27 vs. Sham: 3363±26 number/mm^2) at maintained capillary to myocyte ratio, but was increased by all treatments ($P<0.05$). Moreover, treatment with aspirin and methylprednisolone, but not moxonidine and captopril, resulted in an even increased capillary to myocyte ratio when compared to untreated infarcted hearts ($P<0.05$). The present results show that prevention of left ventricular hypertrophy normalizes capillary density without affecting capillary number. Moreover, normalized *intrinsic* rather than *in vivo* tachycardia is associated with actual capillary growth in chronically infarcted hearts.

6.1. Introduction

Large myocardial infarction (MI) is known to induce remodeling of the surviving myocardium, which includes compensatory hypertrophy of cardiomyocytes, interstitial fibrosis, and limited adaptation of the capillary microvasculature. Early investigations have reported a decreased capillary supply to the surviving hypertrophic myocytes in the early as well as late phases after MI [1]. Normalization of the relation between cardiac muscle growth and capillarization is associated with improved function of non-infarcted myocardium, and may thus beneficially affect clinical outcome [2].

Vascularization in remodeled infarcted hearts can be improved by inhibition of reactive hypertrophy, as was shown for ACE-inhibtors [3]. However, immediate and complete prevention of compensatory hypertrophy after MI with captopril was shown to have deleterious effects on cardiac function of infarcted rats [4]. Alternative strategies to stimulate capillary growth in the infarcted heart include the use of growth factors, such as vascular endothelial growth factor or basic fibroblast growth factor [5], and long-term reduction of heart rate [6]. Bradycardia has been demonstrated to improve tissue perfusion by increasing diastolic time [7,8] and appears to induce capillary angiogenesis irrespective of the cause of heart rate reduction [6].

A major consequence of MI is the activation of the sympathetic nervous system, leading to increased heart rate and plasma catecholamines levels [9,10]. However, besides MI-induced *in vivo* tachycardia, *in vitro or intrinsic* heart rate of isolated perfused MI-hearts was also found to be increased [11,12]. Chronic therapy with aspirin and moxonidine in the early compensatory phase after MI, reduced the *in vivo* tachycardia independent [11,12] and, respectively, dependent on inhibition of the sympathetic nervous system[11,12]. Surprisingly, when rat hearts treated with aspirin were isolated, heart rate was still found to be reduced [13], indicating a lower intrinsic heart rate. However, whether reduction of MI-induced tachycardia can induce capillary growth in the infarcted heart needs to be established.

The present study was designed to compare the chronic effects of four different treatments on *in vivo* and *intrinsic* heart rate related to capillary angiogenesis in a well established rat postinfarction heart failure model [14]. From our previous studies with infarcted rats, the following treatments were selected: 1) Low-dose aspirin was shown to reduce *in vivo* heart rate without effect on left ventricular (LV) hypertrophy [11]. 2) Methylprednisolone, an anti-inflammatory drug, has previously been shown to prevent hypertrophy after MI. 3) Moxonidine, a centrally-sympatholytic, was shown to suppress sympathetic outflow with normalized MI-induced *in vivo* tachycardia, and prevent hypertrophy [12] 4) The ACE-inhibitor captopril increased MI-induced *in vivo* tachycardia and has been reported to prevent LV hypertrophy [15].

6.2. Methods

Male Wistar rats (Harlan, Zeist, The Netherlands) weighing 260-330 g were housed in groups of 2 or 3 on a 12 h light-dark cycle with standard rat chow and water available *ad libitum*. The experimental protocol was approved by the University ethics committee for the use of experimental animals and conformed with the *Guide for Care and Use of Laboratoy Animals*.

6.2.1. Myocardial infarction

Rats were subjected to sham surgery or coronary artery ligation. Under pentobarbital anesthesia (60 mg/kg i.p.), MI was induced by ligation of the left anterior descending coronary artery. Briefly, after intubation of the trachea an incision was made in the skin overlying the fourth intercostal space, with the overlying muscles separated and kept aside. The animals were put on positive pressure ventilation (frequency 65-70/ min, tidal volume 3 ml) and the thoracic cavity was opened by cutting the intercostal muscles. The heart was carefully pushed to the left and 6-0 silk suture was looped under the left descending coronary artery near the origin of the pulmonary artery. After returning the heart to its normal position, the suture was tied. Intercostal space was closed by pulling the ribs with 3-0 silk, the muscles were returned to their normal position and the skin incision was sutured. Sham-operated animals underwent the same surgical procedure, without the actual coronary artery ligation. Proper occlusion of the coronary artery resulted in an extensive transmural infarction comprising a major part of the LV free wall, with small variations in size [15]. Infarct size was determined by planimetry at mid-ventricular levels in transverse slices [16] as the percentage of LV circumference [13].

6.2.2. Treatments

MI-rats were randomized to receive treatment with low-dose aspirin, methylprednisolone, moxonidine or captopril. Low-dose aspirin (25 mg/kg; lysine-acetylsalicylic, Aspégic®, Lorex B.V., Maarssen, The Netherlands) [13] and methylprednisolone (5 mg/kg; methylprednisolone sodiumsuccinate, Solu-Medrol®, Pharmacia&Upjohn, The Netherlands) [17] were dissolved in saline and administered as

daily i.p. injections of 1 ml/kg. This dose of aspirin is shown to reduce platelet production of pro-aggregatory and vasoconstrictor thromboxane in favour of anti-aggregatory and vasodilator prostaglandins without exerting anti-inflammatory activity [18]. Whereas aspirin treatment was started 2 days before surgery and continued until the end of the experiment at 21 days after surgery, methylprednisolone was started at the end of the acute inflammatory phase [14] at 7 days post-MI and also continued to 21 days after surgery. Untreated control rats were receiving once daily saline injections of 1ml/kg i.p. from 2 days before until 21 days after surgery (control for asprin treatment) or from 7 days to 21 days after surgery (control for methylprednisolone treatment).

The centrally-acting sympatholytic moxonidine (Solvay Pharma, Hannover, Germany) was dissolved in accidified saline to provide a final daily dose of 6 mg/kg/day using subcutaneously implanted osmotic minipumps (Alzet® 2001, ALZA Pharmaceuticals, Palo Alto, CA, USA) [12]. Administration of moxonidine was started 24 h following MI and continued until the end of the experiment at 21 days after surgery. Minipumps were replaced each week under ether anesthesia. Sham-rats and untreated MI-rats underwent the same anesthesia and surgical procedure without the actual implantation of the minipumps.

The ACE-inhibitor, captopril (Squibb, Princeton, NJ, USA), was dissolved in the drinking water (2g/l) [19,20], 24 h after MI and continued until the end of the experiment, at 21 days after surgery.

6.2.3. In vivo heart rate and mean arterial pressure

At day 19 after coronary artery ligation, rats were re-anesthetized with sodium pentobarbital and a catheter (PE-10 heat-sealed to PE-50) was inserted in the abdominal aorta through the femoral artery. The heparinized saline filled catheter was tunneled under the skin, exteriorized at the back of the neck and closed with a metal plug. The animals were housed separately and allowed to recover 2 days before measurements. On the experimental day, the arterial catheter was connected to a pressure transducer (Viggo-spectramed, DT-XX, Bilthoven, The Netherlands) and signal was fed into a 68B09-based microprocessor and compatible computer, sampling at 500 Hz. After 1 h stabilization, baseline values of mean arterial blood

pressure and heart rate were obtained. Heart rate was measured as the frequency of the pulsatile aortic pressure signal.

6.2.4. Isolated heart perfusion

At the end of the protocol, the hearts were rapidly excized under pentobarbital anesthesia and mounted for perfusion with an oxygenated Krebs-Henseleit buffer (composition in mM: NaCl 125, KCl 4.7, $CaCl_2$ 1.35, $NaHCO_3$ 20, NaH_2PO_4 0.4, D-glucose 10; pH=7.4; 37°C) at a constant pressure of 85 mmHg, using the Langendorff technique. Isolated hearts were allowed to beat spontaneously. LV function was measured as the isovolumetric developed pressure against a fluid-filled latex balloon placed in the left ventricle, connected to a miniature low-volume displacement pressure transducer. LV end-diastolic pressure was set to 5 mmHg by adjusting the balloon volume. Coronary flow was measured by a flow probe (Transonic Systems, Ithaca, NY, USA) placed in the tubing just before the ostia of the coronary arteries. Cardiac perfusion was defined as the coronary flow corrected for ventricular weight. Coronary vasodilator reserve, reflected by maximal coronary flow, was obtained with 0.1 ml of 10^{-2} M sodium nitroprusside solution (University Hospital pharmacy) injected into the perfusing buffer just before entering the coronary arteries. After 15 min stabilization, baseline heart rate and LV function were recorded.

6.2.5. Left ventricular hypertrophy

After completion of the functional measurements, hearts were removed from the Langendorff preparation and weighed after exclusion of the atria and large vessels. Ventricular hypertrophy was indicated as the ratio of ventricular weight to body weight. Ventricles were cut into 4 transversal slices from apex to base and fixated with 3.6% phosphate-buffered formaldehyde for at least 24 h. After fixation, the slices were dehydrated and paraffin-embedded. Deparaffinized 5 μm thick sections were stained with a Gomori's silver staining [21] in order to visualize individual myocytes of the viable LV wall. Concentric myocyte hypertrophy in the viable LV free wall, remote from the infarcted area, was measured as the cross-sectional area of transversally cut myocytes showing a nucleus using image analysis (Zeiss KS 400,

Germany). Myocyte density was calculated as the average number of myocytes per tissue area.

6.2.6. Capillary density

To visualize capillaries in the myocardium, endothelial cells were stained with Lectin GSI (Sigma-Aldrich Chemie©, Zwijndrecht, The Netherlands), as previously described by Nelissen-Vrancken *et al.* [16]. Sections of 5 μm thickness were deparaffinized and rehydrated, and endogenous peroxidase was inhibited by methanol/H_2O_2 (0.3%) for 15 minutes. The sections were incubated overnight with the biotinylated Lectin GSI (1:100) at room temperature. Then, in a second step, the signal was intensified with an ABC-complex containing peroxidase labeled biotins (1:100) (Lab vision, CA, USA). Finally, the sections were incubated with a Ni-Co amplified DAB solution to which a stable peroxide substrate buffer was added (Pierce©, USA). Endothelial cells of capillaries and larger vessels are visualized in the myocardium as a brown precipitate. A background staining was not used in order to avoid interference with the Lectin staining. The number of capillaries were counted in the same region of the viable LV free wall in which myocyte area was determined. Image analysis (Zeiss KS 400, Germany) was used to measure capillary density, calculated as the number of capillaries per tissue area in the viable LV wall. Capillary to myocyte ratio was calculated as capillary density divided by myocyte density.

6.2.7. Data analysis

All data are presented as means ± S.E.M. Data of infarcted rats were only included if the infarction comprized the major part of the LV free wall, since small infarctions (<20%) are found to be hemodynamically fully compensated [4,22]. Estimation of infarct size by macroscopic appearance has proven to be a reliable method to recognize too small infarctions (<20 %) [15]. Because of temporal variation and different control administration, each treatment group had its own sham and untreated infarct controls. Statistical analysis of effects of treatment was performed within each group using one-way analysis of variance (ANOVA) (SigmaStat™, Jandel Scientific, Erkrath, Germany) followed by Bonferroni's post-hoc *t*-tests for multiple group comparisons [23]. Differences were considered statistically significant if $P<0.05$.

6.3. Results

Overall mortality following MI was 39 % and did not depend on treatment, since death mainly occurred within the first 24 h after coronary artery ligation. No other than surgery related death was observed during the treatment period. Data from four different treatment groups were collected: low-dose aspirin (n=6-12), methylprednisolone (n=7-12), moxonidine (n=7-14) and captopril (n=7-10) treated MI-rats .

6.3.1. Functional parameters

Hemodynamics obtained from conscious rats as well as isolated perfused hearts are summarized in Table 1. Mean arterial pressure was similar for all groups, except captopril treated MI-rats, which displayed a lower mean arterial pressure compared to untreated MI-rats. *In vitro* LV dysfunction of MI-hearts was reflected by a significantly reduced LV systolic pressure, which was not affected by treatment. Coronary flow corrected for ventricular weight (cardiac perfusion) as well as maximal coronary flow obtained with sodium nitroprusside were similar in all experimental groups.

Table 1. LV functional parameters obtained from conscious rats and isolated perfused hearts at 21 days after surgery

Experimental groups		ASP	MP	MOX	CAP
n		6-12	7-12	7-14	7-10
In vivo					
MAP (mmHg)	*Sham*	106±2	115±2	112±2	133±2
	MI	99±4	108±4	98±3	127±7
	MI+	96±2	99±3	100±5	101±4 [a b]
In vitro					
LV systolic pressure (mmHg)	*Sham*	75±5	89±6	77±6	94±6
	MI	51±4 [a]	58±6 [a]	51±7 [a]	74±7 [a]
	MI+	49±4 [a]	78±7	44±5 [a]	77±14 [a]
Cardiac perfusion (ml/min.g)	*Sham*	10.6±0.6	10.3± 0.7	8.6±0.9	9.5±1.4
	MI	8.9±1.0	8.7±1.0	6.6±.0.8	7.4±0.6
	MI+	9.8±1.4	7.3± 0.6	9.4±0.8	8.1±1.1
Maximal $CF_{nitroprusside}$ (ml/min)	*Sham*	19.1±1.1	21.9±1.5	19.9±1.0	20.7±0.7
	MI	17.8±2.2	22.7±1.4	21.8±0.9	19.9±0.9
	MI+	17.8±1.1	19.8±1.1	19.2±1.3	20.1±0.8

Data are presented as means±S.E.M. MI: untreated infarcted rats; MI+: treated infarcted rats; ASP: aspirin ; MOX: moxonidine; MP: methylprednisolone; CAP: captopril; MAP: mean arterial pressure; LV: left ventricular; CF: coronary flow; [a] $P<0.05$ vs. SHAM; [b] $P<0.05$ vs. MI

Values for *in vivo (upper panel)* and *intrinsic* (*lower panel)* heart rate are represented in Fig. 1. Untreated conscious MI-rats of all groups were characterized by a marked *in vivo* tachycardia, which was normalized to sham-values with aspirin, moxonidine, and methylprednisolone. In case of captopril a further increase rather than normalization of *in vivo* heart rate was observed. Accordingly, *intrinsic* heart rate measured in isolated perfused hearts was significant higher in untreated MI- than sham-hearts. Treatment with aspirin and methylprednisolone, but not moxonidine or captopril, resulted in normalization of *intrinsic* heart rate to sham-values.

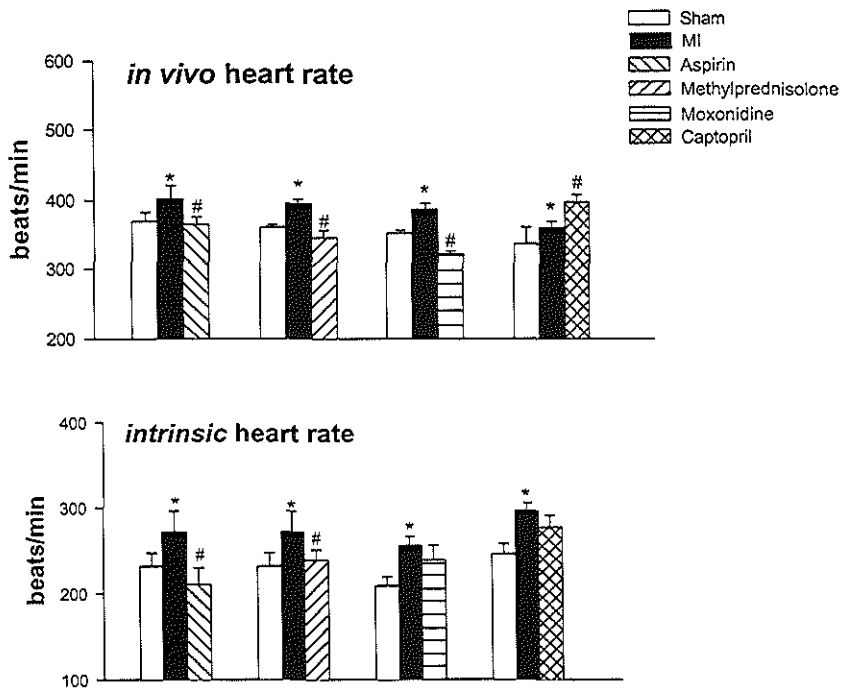

Fig. 1. *In vivo (upper panel)* and *intrinsic* heart rate *(lower panel)* obtained from conscious rats and isolated heart perfusions, respectively. *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

6.3.2. LV hypertrophy

Three weeks after surgery, infarct size as well as body and ventricular weight were measured for the different groups (Table 2). Infarct size in untreated and treated MI-hearts was similar in all groups. Whereas a lower body weight was observed in untreated MI-rats of the aspirin group compared to sham-rats, moxonidine and captopril treated MI-rats manifested a lower body weight compared to untreated MI-rats. Ventricular weight of untreated MI-rats in the methylprednisolone, moxonidine and captopril group was significantly higher than sham-operated control hearts despite replacement of the major part of the left ventricular free wall by lighter scar tissue. Moreover, treatment with methylprednisolone, moxonidine and captopril, but not aspirin, normalized ventricular weight to sham-values. In addition, ventricular weight to body weight ratio, reflecting

total ventricular hypertrophy, was increased in untreated MI-rats of all groups, and normalized with moxonidine. Increased ventricular weight to body weight ratio in untreated MI-rats of the aspirin group is explained by a lower body weight of these rats 3 weeks after MI.

Table 2. Infarct size, body and ventricular weight measured in the different experimental groups 21 days after surgery

Experimental groups		ASP	MP	MOX	CAP
n		6-12	7-12	7-14	7-10
Infarct size (%)	*Sham*	-	-	-	-
	MI	37± 1	43± 2	45± 3	41± 5
	MI+	39± 3	41±3	44± 3	39± 4
Body weight (g)	*Sham*	376± 6	346± 8	333± 7	368±5
	MI	340±7 [a]	347± 8	320±10	359±4
	MI+	335±8	300± 10 [b]	299± 9 [b]	334±4 [b]
Ventricular weight (mg)	*Sham*	1060±19	1061±52	1174±37	1071± 21
	MI	1098±35	1211±54 [a]	1543±75 [a]	1232±56 [a]
	MI+	1147±39	1059±64 [b]	1076±24 [b]	1033±41 [b]
Ventricular weight / body weight (mg/g)	*Sham*	2.8±0.1	3.1±0.1	3.5±0.1	2.9±0.1
	MI	3.3±0.1 [a]	3.5±0.1 [a]	4.7±0.3 [a]	3.5±0.2 [a]
	MI+	3.4±0.1	3.5±0.1	3.6±0.2 [b]	3.1±0.1

Data are presented as means±S.E.M. MI: untreated infarcted rats; MI+: treated infarcted rats; ASP: aspirin ; MOX: moxonidine; MP: methylprednisolone; CAP: captopril; LV: left ventricular; [a] $P<0.05$ vs. SHAM; [b] $P<0.05$ vs. MI

Representative photomicrographs of Gomori stained sections in the LV viable wall are shown in Fig.2. MI-induced LV hypertrophy at cellular level, which was confirmed by a significantly increased myocyte cross-sectional area, was prevented in moxonidine, methylprednisolone and captopril treated MI-hearts.

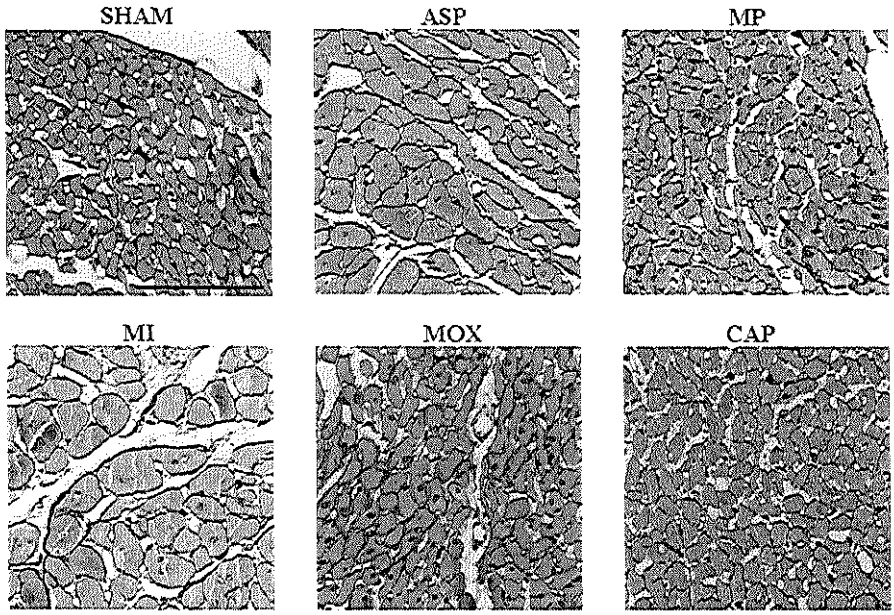

Fig. 2 Gomori stained sections in the LV viable wall of sham-hearts, untreated and treated MI-hearts, showing individual myocytes. The bar in left upper photomicrograph indicates 100 μm, and accounts for all micrographs. ASP: aspirin ; MOX: moxonidine; MP: methylprednisolone; CAP: captopril.

These observations were substantiated by the actual measurements as presented in Fig.3.

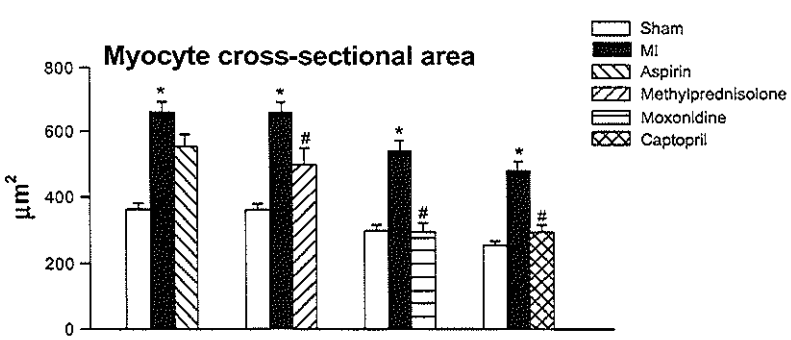

Fig. 3. Actual measurements for myocyte cross-sectional area in the different experimental groups. *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

6.3.3. Capillary density

Photomicrographs of Lectin stained sections in the LV viable wall showing individual capillaries are represented in Fig.4. A significantly reduced capillary density observed in MI-induced hypertrophied hearts, was normalized to sham-values with moxonidine, methylprednisolone and captopril and even increased above sham-values with aspirin.

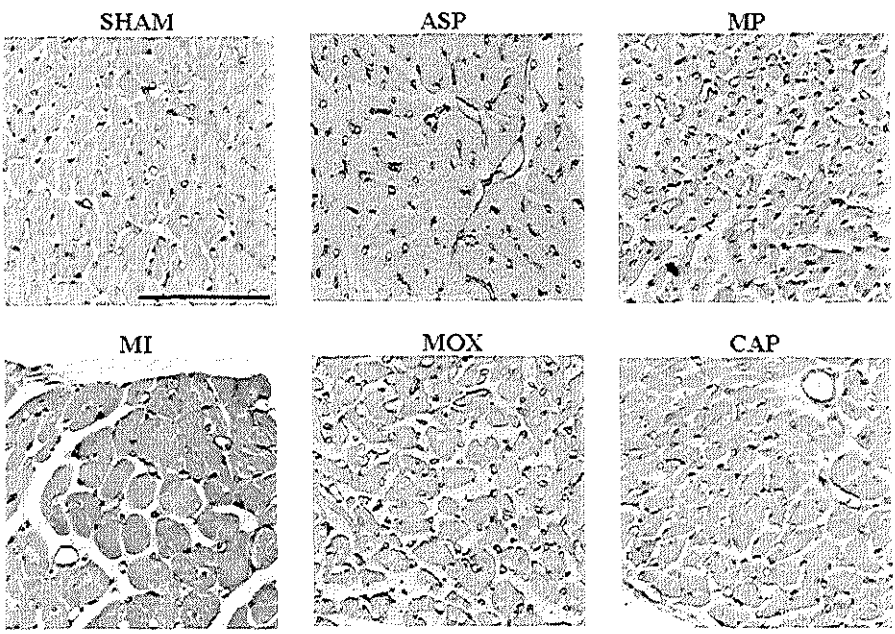

Fig. 4. Lectin stained sections in the LV viable wall of sham-hearts, untreated and treated MI-hearts, showing individual capillaries (small dark brown circles). The bar in left upper photomicrograph indicates 100 µm, and accounts for all micrographs. ASP: aspirin ; MOX: moxonidine; MP: methylprednisolone; CAP: captopril.

These observations were substantiated by the actual measurements in Fig.5 *(upper panel)*. Whereas capillary to myocyte ratio remained unchanged in moxonidine and captopril treated MI-hearts, it was almost doubled in aspirin and methylprednisolone treated MI-hearts (Fig.5, *lower panel)*.

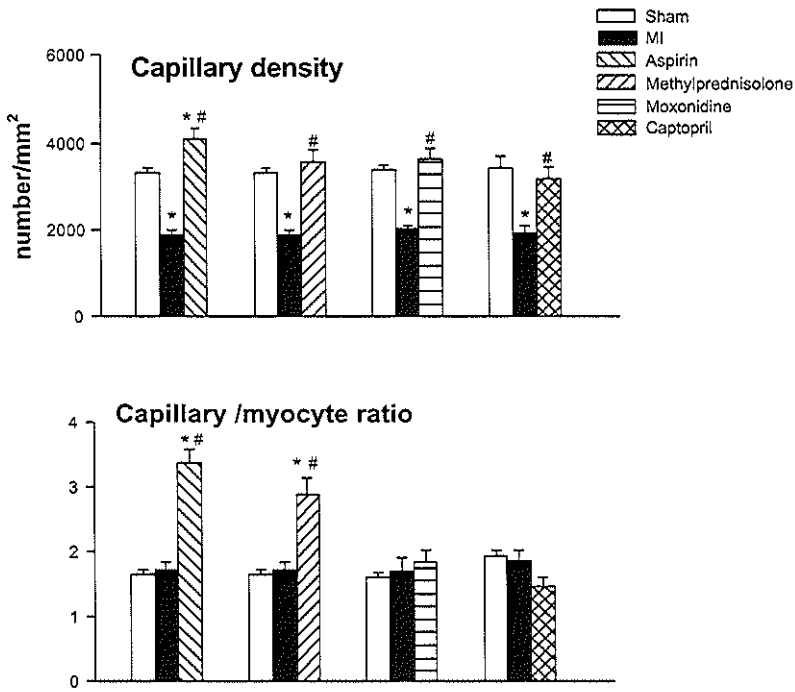

Fig. 5. Actual measurements for capillary density *(upper panel)* and capillary to myocyte ratio *(lower panel)* in the different experimental groups. *: $P<0.05$ vs Sham; #: $P<0.05$ vs MI.

6.4. Discussion

6.4.1. LV function and heart rate

In the present study, LV dysfunction of isolated MI-hearts was reflected by a decreased *in vitro* LV systolic pressure, which was not significantly improved by either treatment. Furthermore, chronically infarcted rats were characterized by a marked *in vivo* tachycardia. Whereas treatment with aspirin, methylprednisolone and moxonidine normalized MI-induced *in vivo* tachycardia, captopril further increased *in vivo* heart rate of MI-hearts. The latter observation with captopril is in accordance with previous functional observations in early captopril treated rats [4], whose hearts displayed a decreased LV developed pressure and depressed peak velocity of

contraction [20]. In order to maintain cardiac output at a lower LV function in these hearts, heart rate needs to be increased.

Interestingly, when hearts of untreated MI-rats were isolated and perfused, thereby circumventing influence of sympathetic nerve activity and circulating catecholamines as the major determinants of *in vivo* heart rate, their heart rate was found to be still higher than in sham-operated hearts. This may indicate changes in *intrinsic* activation of the sinus node as was suggested by Schoemaker et al. [11]. The presence of local sympathetic activity that could have remained after isolation was excluded by the absence of heart rate response to 10^{-6} M propranolol (data not shown). Chronic treatment with aspirin and methylprednisolone, but not moxonidine and captopril, normalized the increased *intrinsic* heart rate of isolated MI-hearts. Hence, the lower *in vivo* heart rate observed with aspirin and methylprednisolone might be due to a reduced *intrinsic* heart rate rather than to a suppressed sympathetic nervous system activity. This explanation is further supported by unchanged plasma catecholamine concentatrions in aspirin [11] and methylprednisolone treated rats (data not shown). In case of moxonidine, a clear relation between sympathetic suppression and reduced *in vivo* heart rate has been previously demonstrated in experimental [12] and clinical studies [24,25]. However, moxonidine was not found to reduce *intrinsic* tachycardia in isolated MI-hearts.

The mechanism by which the *intrinsic* heart rate in aspirin and methylprednisolone treated MI-hearts was reduced, might be explained as a reflection of the effects of treatment on the cardiac remodeling process [13]. In one study, a correlation between sinus node activity and collagen modulated by stretch was suggested [26]. Since aspirin and methylprednisolone have been shown to interfere with collagen deposition in the surviving myocardium of infarcted rats [17], we cannot exclude the possibility that collagen of the sinus node was also affected. However, also moxonidine [12] and ACE-inhibitors [27] have been shown to inhibit collagen deposition in the spared myocardium, without reducing *intrinsic* heart rate.

6.4.2. Left ventricular hypertrophy and capillary adaptation

The heart responds to overload following MI with an adaptive hypertrophic reaction, which is aimed to restore the myocardial function and normalize increased

wall stress. Hypertrophied cardiomyocytes in the spared myocardium are characterized by a decreased capillary surface and increased diffusion distance for oxygen. The present results concerning morphometric indices, clearly demonstrated a MI-induced hypertropic response of the spared myocardium, which was associated with a decreased number of capillaries per tissue area. This is in accordance with previous observations in infarcted rats, which were characterized by a decreased capillary and myocyte density [2,28]. It appears that despite the increased cardiomyocyte cell volume, capillary number per myocyte remains unaltered, and, hence, probably insufficient for adequate oxygenation of hypertrophied cardiomyocytes [29]. Microvascularization in remodeled MI-hearts can be improved by inhibition of reactive compensatory hypertrophy. This was recently confirmed in moxonidine treated MI-rats, which manifested a restored capillary density associated with improved ischemic tolerance as a consequence of prevented LV hypertrophy (Van Kerckhoven, submitted). Whereas in the present study, prevention of compensatory hypertrophy was confirmed by a normalized myocyte area in methylprednisolone, moxonidine and captopril treated MI-hearts, aspirin did not affect LV hypertrophy at all. Normalization of hypertrophy in methylprednisolone, moxonidine and captopril MI-hearts was associated with a restored capillary density in the spared myocardium. Surprisingly, although aspirin did not prevent compensatory hypertrophy, capillary density was even increased above sham-values suggesting capillary angiogenesis rather than restored capillary density. These findings were fully supported by the values of capillary to myocyte ratio, which were doubled when compared sham-hearts. Capillary angiogenesis indicated by a significantly increased capillary to myocyte ratio, was also observed in methylprednisolone treated MI-hearts, although to a lesser extent than aspirin treated MI-hearts

6.4.3. Interaction between heart rate and capillary growth

Independent of its origin, bradycardia is associated with capillary growth [6], which could provide high clinical benefit. Capillary angiogenesis has been shown by either bradycardial pacing [7,30] or infusion of bradycardic drugs such as alinidine [5,31]. A lower *in vivo* heart rate was shown to improve the balance between oxygen demand and

supply by enhanced tissue perfusion through longer diastolic time [32] in addition to the increased myocardial capillarization [6,7]. However, a distinction should be made between restored capillary density as a direct result of prevention of LV hypertrophy and actual growth of new capillaries. The latter phenomenon was most pronounced in aspirin and subsequently, methylprednisolone treated MI-hearts. These hearts happen to display lower *intrinsic* heart rates. Restored capillary density resulting from prevented hypertrophy seems to be the case in both moxonidine and captopril treated MI-hearts, since capillary to myocyte ratio remained unchanged. Similar findings were observed in spontaneously hypertensive rats with the ACE-inhibitor temocapril [33]. An interaction between lower *intrinsic* heart rate and cardiac capillarization has been suggested from studies with exercise training induced bradycardia [34,35]. Whereas heart rate after autonomic blockade (*intrinsic* heart rate) was shown to be reduced as a part of adaptation to training [36], morphometric measurements in exercise induced hypertrophied pig hearts have revealed compensated capillary proliferation [37]. From the present study, we may conclude that a lower *intrinsic* heart rate in aspirin and methylprednisolone treated MI-hearts could be responsible for observed capillary angiogenesis, as indicated by an increased capillary to myocyte ratio. In addition, capillary angiogenesis in aspirin treated MI-hearts may be an attractive explanation for the improved *in vivo* hemodynamics, which was observed in aspirin treated MI-rats [11]. However, the exact mechanism by which a lower *intrinsic* heart rate is associated with capillary angiogenesis, remains yet unclear. Both mechanical factors, such as stretch of the capillary wall, or treatment induced release of growth factors stored in the capillary basement membrane [6] may play a role. Indeed, the vascular endothelial growth factor or VEGF, was shown to play a key role in bradycardia induced angiogenesis [5]. Furthermore, possible synergism between steroids and basic fibroblast growth factor was shown to improve capillary density in rats with spinal cord injury [38].

6.4.4. Conclusions

The present results show that pharmacological induced prevention of left ventricular hypertrophy normalizes capillary density without affecting capillary

number. Furthermore, normalized *intrinsic* rather than *in vivo* tachycardia may contribute to actual capillary angiogenesis in chronically infarcted hearts.

Acknowledgements

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6.5. References

1. Anversa P, Loud AV, Levicky V, Guideri G. Left ventricular failure induced by myocardial infarction. II. Tissue morphometry. *Am J Physiol.* 1985;248:H883-9.
2. Sladek T, Sladkova J, Kolar F, Papousek F, Cicutti N, Korecky B, Rakusan K. The effect of AT1 receptor antagonist on chronic cardiac response to coronary artery ligation in rats. *Cardiovasc Res.* 1996;31:568-76.
3. Liu YH, Yang XP, Sharov VG, Nass O, Sabbah HN, Peterson E, Carretero OA. Effects of angiotensin-converting enzyme inhibitors and angiotensin II type 1 receptor antagonists in rats with heart failure. Role of kinins and angiotensin II type 2 receptors. *J Clin Invest.* 1997;99:1926-35.
4. Schoemaker RG, Debets JJ, Struyker-Boudier HA, Smits JF. Delayed but not immediate captopril therapy improves cardiac function in conscious rats, following myocardial infarction. *J Mol Cell Cardiol.* 1991;23:187-97.
5. Zheng W, Brown MD, Brock TA, Bjercke RJ, Tomanek RJ. Bradycardia-induced coronary angiogenesis is dependent on vascular endothelial growth factor. *Circ Res.* 1999;85:192-8.
6. Hudlicka O, Brown MD, Walter H, Weiss JB, Bate A. Factors involved in capillary growth in the heart. *Mol Cell Biochem.* 1995;147:57-68.
7. Brown MD, Davies MK, Hudlicka O. The effect of long-term bradycardia on heart microvascular supply and performance. *Cell Mol Biol Res.* 1994;40:137-42.
8. Gordon RJ. A general mathematical model of coronary circulation. *Am J Physiol.* 1974;226:608-15.
9. Packer M. Pathophysiology of chronic heart failure. *Lancet* 1992;340:88-92.
10. Francis GS. Neurohumoral activation and progression of heart failure: hypothetical and clinical considerations. *J. Cardiovasc. Pharmacol.* 1998;32:S16-21.
11. Schoemaker RG, Saxena PR, Kalkman EA. Low-dose aspirin improves in vivo hemodynamics in conscious, chronically infarcted rats. *Cardiovasc. Res.* 1998;37:108-14.
12. Van Kerckhoven R, van Veen TA, Boomsma F, Saxena PR, Schoemaker RG. Chronic administration of moxonidine suppresses sympathetic activation in a rat heart failure model. *Eur J Pharmacol.* 2000;397:113-20.
13. Kalkman EA, van Suylen RJ, van Dijk JP, Saxena PR, Schoemaker RG. Chronic aspirin treatment affects collagen deposition in non-infarcted myocardium during remodeling after coronary artery ligation in the rat. *J. Mol. Cell. Cardiol.* 1995;27:2483-94.
14. Fishbein MC, Maclean D, Maroko PR. Experimental myocardial infarction in the rat: qualitative and quantitative changes during pathologic evolution. *Am J Pathol.* 1978;90:57-70.
15. Kalkman EA, Bilgin YM, van Haren P, van Suylen RJ, Saxena PR, Schoemaker RG. Determinants of coronary reserve in rats subjected to coronary artery ligation or aortic banding. *Cardiovasc Res.* 1996;32:1088-95.

16. Nelissen-Vrancken HJ, Kuizinga MC, Daemen MJ, Smits JF. Early captopril treatment inhibits DNA synthesis in endothelial cells and normalization of maximal coronary flow in infarcted rat hearts. *Cardiovasc Res.* 1998;40:156-64.

17. Van Kerckhoven R, Kalkman EA, Saxena PR, Schoemaker RG. Altered cardiac collagen and associated changes in diastolic function of infarcted rat hearts. *Cardiovasc Res.* 2000;46:316-23.

18. Patrignani P, Filabozzi P, Patrono C. Selective cumulative inhibition of platelet thromboxane production by low-dose aspirin in healthy subjects. *J Clin Invest* 1982;69:1366-72.

19. Pfeffer JM, Pfeffer MA, Braunwald E. Hemodynamic benefits and prolonged survival with long-term captopril therapy in rats with myocardial infarction and heart failure. *Circulation.* 1987;75:I149-55.

20. Kalkman EA, van Haren P, Saxena PR, Schoemaker RG. Regionally different vascular response to vasoactive substances in the remodelled infarcted rat heart; aberrant vasculature in the infarct scar. *J Mol Cell Cardiol.* 1997;29:1487-97.

21. Benjamin IJ, Jalil JE, Tan LB, Cho K, Weber KT, Clark WA. Isoproterenol-induced myocardial fibrosis in relation to myocyte necrosis. *Circ Res.* 1989;65:657-70.

22. Pfeffer MA, Pfeffer JM, Fishbein MC, Fletcher PJ, Spadaro J, Kloner RA, Braunwald E. Myocardial infarct size and ventricular function in rats. *Circ Res.* 1979;44:503-12.

23. Wallenstein S, Zucker CL, Fleiss JL. Some statistical methods useful in circulation research. *Circ Res.* 1980;47:1-9.

24. Ziegler D, Haxhiu MA, Kaan EC, Papp JG, Ernsberger P. Pharmacology of moxonidine, an I1-imidazoline receptor agonist. *J. Cardiovasc. Pharmacol.* 1996;27:S26-37.

25. Motz W, Scheler S, Mox B. The potential clinical application of moxonidine in congestive heart failure. *Rev. Contemp. Pharmacother.* 1998;9:473-79.

26. Kohl P, Noble D. Mechanosensitive connective tissue: potential influence on heart rhythm. *Cardiovasc Res.* 1996;32:62-8.

27. Volders PG, Daemen MJ. Interstitial fibrosis and angiotensin-converting enzyme inhibition in patients with end-stage myocardial infarction. *J Am Coll Cardiol.* 1998;32:552-3.

28. Turek Z, Grandtner M, Kubat K, Ringnalda BE, Kreuzer F. Arterial blood gases, muscle fiber diameter and intercapillary distance in cardiac hypertrophy of rats with an old myocardial infarction. *Pflugers Arch.* 1978;376:209-15.

29. Anversa P, Beghi C, Kikkawa Y, Olivetti G. Myocardial infarction in rats. Infarct size, myocyte hypertrophy, and capillary growth. *Circ Res.* 1986;58:26-37.

30. Wright AJ, Hudlicka O. Capillary growth and changes in heart performance induced by chronic bradycardial pacing in the rabbit. *Circ Res.* 1981;49:469-78.

31. Granetzny A, Schwanke U, Schmitz C, Arnold G, Schafer D, Schulte HD, Gams E, Schipke JD. Pharmacologic heart rate reduction: effect of a novel, specific bradycardic agent on the heart. *Thorac Cardiovasc Surg.* 1998;46:63-9.

32. Stewart JT, Simpson IA, Smith RE, Callicott C, Gray HH, Camm AJ. Left ventricular energetics: heat production by the human heart. *Cardiovasc. Res.* 1993;27:1024-32.

33. Xie Z, Gao M, Horimoto M, Togashi H, Saito H, Koyama T. Rearrangement of the ventricular capillary network in stroke-prone spontaneously hypertensive rats (SHRSP) following a late start of treatment with the angiotensin converting enzyme blocker temocapril. *Jpn Heart J* 1998;39:699-706.

34. Tharp GD, Wagner CT. Chronic exercise and cardiac vascularization. *Eur J Appl Physiol Occup Physiol.* 1982;48:97-104.

35. Gao M. [Arterialization of the capillary network in the left ventricular subendocardium in young rats subjected to exercise training]. *Hokkaido Igaku Zasshi.* 1997;72:225-34.

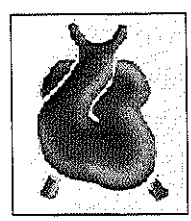
36. Areskog NH. Effects and adverse effects of autonomic blockade in physical exercise. *Am J Cardiol.* 1985;55:132D-134D.

37. White FC, McKirnan MD, Breisch EA, Guth BD, Liu YM, Bloor CM. Adaptation of the left ventricle to exercise-induced hypertrophy. *J Appl Physiol.* 1987;62:1097-110.

38. Baffour R, Achanta K, Kaufman J, Berman J, Garb JL, Rhee S, Friedmann P. Synergistic effect of basic fibroblast growth factor and methylprednisolone on neurological function after experimental spinal cord injury. *J Neurosurg.* 1995;83:105-10.

CHAPTER 7

General discussion



7.1. General discussion

Heart failure is no longer conceptualized as a pure hemodynamic disorder. Rather, the syndrome of heart failure is a more complex interplay of hemodynamic changes, neurohormonal activation and structural changes in the heart itself. The loss of ventricular function is usually preceded by an initiating event such as myocardial infarction, which leads to activation of specific neurohormonal systems such as sympathetic nervous system, renin-angiotensin system and arginin vasopressin system. Myocardial infarction is a leading cause of heart failure, which is associated with a 10-fold greater risk of developing heart failure compared with a normal population [1]. The neurohormonal hypothesis postulates that neurohormones are initially adaptive by maintaining cardiac output and blood pressure, but later the same compensatory responses become pathological by increasing cardiac loading conditions and contribute to adverse remodeling, progressive ventricular failure, and ultimately to the syndrome of heart failure [2].

Acute loss of myocardium after myocardial infarction results in an abrupt increase in loading conditions that induces a unique pattern of remodeling involving the infarcted border zone and remote non-infarcted myocardium. These structural changes or *remodeling* include abnormal myocyte growth (hypertrophy), proliferation of cells in the extracellular matrix, and myocyte cell loss which finally contribute to deterioration of ventricular function. These processes of remodeling have taken on increasing importance with the recognition that pharmacological interference could favourably affect the remodeling process and thereby reduce cardiovascular morbidity and mortality when administered in the *later* postinfarction period. Postinfarction remodeling is a time-dependent process, but the time course and effects of interference, particulary the *early* time course, has not been completely elucidated. Therapy during the different phases of the remodeling process require different strategies. In the past, pharmacological research has mainly focused on therapeutic intervention in the decompensation phase when congestive heart failure is diagnosed and prognosis becomes very poor. However, although still patients at the decompensation stage are clinically the most important ones, inclination towards intervention during the *early* postinfarction period (in which a major part of

remodeling occurs) is growing. One of the problems that may arise, however, is that *earlier* treatment is aimed at the opposite direction as the later therapy. This problem is substantiated by the results of the 2 CONSENSUS trials, in which *delayed* treatment with ACE-inhibitors had clearly beneficial effects (CONSENSUS I), whereas similar treatment during *early* phases had detrimental effects (CONSENSUS II)[3]. During the early stages after myocardial infarction, cardiac hypertrophy and remodeling have become a major target for therapy since they are associated with improved prognosis at later stages (SOLVD investigators). When treatment should be started *earlier*, that is with ventricular dysfunction but before signs of heart failure, it implies interference with cardiac hypertrophy and remodeling, which during the *early phase* are regarded a pathophysiological response to compensate for the loss of contractile tissue. This indicates that pharmacological therapy should therefore be selected carefully [3,4]. In the light of these observations we hypothesized that treatment during this early phase should be directed to preserved total amount of contractile tissue of the non-infarcted myocardium, with optimal perfusion. Pharmacological interference with *early* postinfarction remodeling may induce major changes in cardiac architecture such as the collagen network, hypertrophic reponse of cardiomyocytes, microcirculation, and subsequently metabolic regulation.

7.1.1. Interference with collagen network

Collagen accumulation in the non-infarcted tissue is a potential target for treatment since it has been related to increased myocardial stiffness and impaired diastolic heart function. In *early* postinfarction remodeling, structural alterations of the collagen network are associated with infarct expansion and left ventricular dilation [5,6]. Later in the course of postinfarction remodeling, infarcted tissue is replaced by scar tissue with a high collagen content while in the non-infarcted tissue collagen content is increased as well [7,8]. However, a too large reduction of the tensile strength of the left ventricular collagen network could result in aggravation of chamber dilation, as has been reported with non-steroid anti-inflammatory drugs and steroids [9,10]. The above suggestions were confirmed in chapter 2, in which *early* aspirin and methylprednisolone treatment were shown to interfere with collagen deposition in the non-infarcted myocardium rather than in the infarct scar, resulting

in an altered left ventricular diastolic function. Furthermore, prevention of interstitial collagen build-up in the non-infarcted myocardium after myocardial infarction, as observed with methylprednisolone but not aspirin, promoted left ventricular dilation without affecting diastolic stiffness, but also improved active relaxation. Thus, based on these results we conclude that *early* interference with the collagen build-up in the non-infarcted myocardium may induce detrimental effects by promoting left ventricular dilation, but also beneficial effects by improving left ventricular relaxation.

7.1.2. Interference with compensatory hypertrophy

Pharmacological treatment during the *early* phase after myocardial infarction may have profound effects on the compensatory hypertropic response of the non-infarcted myocardium and cardiac function as was previously reported with ACE-inhibitors. In a study that compared effects of *early* and *late* treatment with captopril, it was shown that *late* treatment had beneficial effects but *early* treatment even may have deleterious hemodynamic effects [4]. This was supported by the clinical enalapril studies using mortality as outcome (CONSENSUS I an II). *Early* captopril treatment prevented hypertrophy, interstitial fibrosis in the spared myocardium [11], and reduced coronary reserve. Because *in vivo* hemodynamic measurements even showed deterioration after this *early* treatment, it was concluded that preventing all processes during this *early* phase may not be very effective in preventing heart failure. Maybe part of the remodeling can be regarded as beneficial and part as detrimental, and a more subtle intervention is required. That prevention of early compensatory hypertrophy may not be advantageous in terms of improving cardiac function was supported by the functional results of early treatment with moxonidine (Chapter 3) and captopril (Chapter 6). On the contrary, preserved compensatory hypertrophy, as was observed with aspirin (Chapter 2) and V_{1A} vasopressin antagonist (Chapter 5) could be one of conditions to restore cardiac function in the infarcted heart.

7.1.3. Interference with vascularization

A second part of our hypothesis stated that preserved hypertrophy should be accompanied with optimal perfusion. Early investigations have reported a decreased

capillary supply to the surviving hypertrophic myocytes in the *early* as well as *late* phases after myocardial infarction [12]. Normalization of the relation between cardiac muscle growth and vascular growth is associated with improved function of the non-infarcted myocardium, and may thus beneficially affect clinical outcome [13]. Vascularization in remodeled infarcted hearts can be improved by inhibition of reactive hypertrophy, direct stimulation of vascular growth using growth factors or indirectly by reducing heart rate. The present thesis showed that all treatments which did prevent *early* compensatory hypertrophy (captopril, moxonidine, methylprednisolone) (Chapter 6) were, hence, able to normalize capillary density (number of capillaries per tissue area). Restored capillary density in the non-infarcted myocardium of these hearts could be explained as result of prevented hypertrophy rather than actual growth of capillaries, since capillary to myocyte ratio remained the same. This explanation was further supported by other experimental studies using AT_1 receptor antagonists [14,15]. A restored capillary density following prevention of hypertrophy may have beneficial effects on the ischemic tolerance of the non-infarcted myocardium, as was shown for moxonidine (Chapter 4) and captopril [16].

Since a preserved hypertrophic response after myocardial infarction may be essential to support cardiac function, optimal perfusion must be achieved by stimulation of capillary growth (angiogenesis). Several ways to stimulate capillary growth in the non-infarcted myocardium have been described:

1. Locally administration of growth factors :

A number of growth factors are candidates for therapeutic augmentation of myocardial perfusion. These include vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), transforming growth factor-β (TGF-β) and insulin-like growth factor. Although angiogenesis is therapeutically desirable in the infarcted heart, one problem that may arise is that these growth factors need to be delivered locally, and not systemically, to avoid possible tumor formation [17]. Another strategy to administer growth factors involves the introduction of genes encoding these factors [18]. However, preliminary evidence suggests that prolonged local production of potent growth factors such as VEGF and bFGF may cause unwanted hemangioma formation or fibromatosis [19]. Moreover, the theoretical advantage of gene therapy

approaches with respect to long-term angiogenic factor exposure depends on effective local expression, which is not optimal yet because of high variability in the level and duration of gene expression.

2. Heart rate reduction or bradycardia :

Bradycardia appears to be a particularly good way of stimulating angiogenesis, because the magnitude of capillary growth is impressive. Independent of its origin, bradycardia is associated with capillary growth [20] which could provide high clinical benefit. Capillary angiogenesis has been shown by either bradycardial pacing [21] or infusion of bradycardic drugs such as alinidine [22]. Long-term reduction of *in vivo* heart rate has been acknowledged to improve tissue perfusion by increasing diastolic time, while energetic efficiency is improved [23] in addition to the increased myocardial capillarization [24]. This capillary angiogenesis in response to bradycardia appears to be associated with upregulation of VEGF mRNA and followed by enhanced VEGF protein synthesis [25].

Since postinfarction remodeled hearts display a lower mechanical efficiency which may be amplified by tachycardia, pharmacological induced normalization of this tachycardia may have favourable effects. *In vivo* heart rate following myocardial infarction is mainly attributed to increased sympathetic nervous system activation and plasma noradrenaline levels. However, if these infarcted hearts are isolated and perfused, heart rate is also found to be increased indicating changes in *intrinsic* heart rate. Besides lowering *in vivo* tachycardia after myocardial infarction, reduction of this *intrinsic* heart rate by modulation of the sinus node activity may stimulate angiogenesis and thus, improve cardiac function. Agents such as zatebradine and zeneca ZD7288 have been demonstrated to slow sinus node rate, thereby decreasing *intrinsic* heart rate [26] and improving cardiac mechanics and energetics in patients with left ventricular dysfunction [27]. Furthermore, from the present thesis lowering *intrinsic* rather than *in vivo* tachycardia seems to contribute to capillary angiogenesis in the non-infarcted myocardium as was shown for aspirin and, to a lesser extent, methylprednisolone (Chapter 6). Indeed, normalization of *intrinsic*, but not in *vivo* tachycardia, was exclusively related to capillary growth. At least in case of *early* aspirin treatment this could explain the improved hemodynamics 3 weeks after

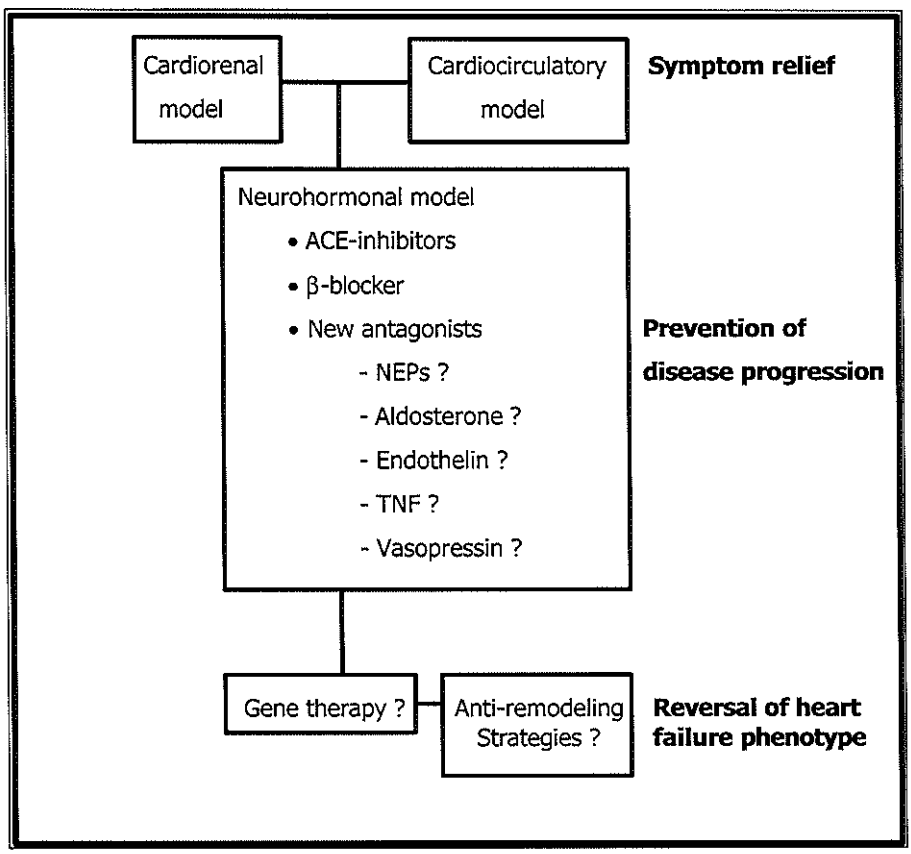
myocardial infarction. Thus, we conclude that strategies which result in reduced *intrinsic* heart rate may have benefit to optimize perfusion in the failing heart. However, normalization of tachycardia and associated capillary angiogenesis may not be the only determinant factor for improving cardiac function. This was confirmed by *early* V_{1A} vasopressin antagonist treatment in which improved cardiac function could not be attributed to capillary growth.

7.2. Conclusions and future outlook

In the past, pharmacological research has mainly focused on relief of symptoms in the *late* decompensation phase, when congestive heart failure is diagnosed and prognosis becomes very poor. Except for treatment with ACE-inhibitors, prognosis has not benefited substantially from all these research. Instead of aiming the regression of remodeling in the *late* phase, therapy *early* after myocardial infarction can be an alternative approach to prevent heart failure, but may include strong interference with the cardiac remodeling process. Moreover, different aspects of this remodeling process can be affected separately, providing a unique opportunity to study them distinctively. Based on previous observations with *early* and *late* ACE-inhibitor treatment, the results of the present thesis support our hypothesis that optimal treatment during the *early* compensatory stage after myocardial infarction may not interfere with left ventricular hypertrophy. Indeed, a distinction should be made between favourable and unfavourable remodeling, since the former may be a necessary physiological response to the primary myocardial damage. However, pharmacological induced prevention of compensatory hypertrophy may also have advantageous effects to the non-infarcted hypertrophied myocardium. These include restored capillary density and reduced ischemic vulnerability of the non-infarcted myocardium [28]. Although these effects are undoubtedly beneficial to the myocardium, *in vivo* cardiac function seems rather to depend on the total amount of viable contractile tissue left. Therefore to provide optimal perfusion of the hypertrophied non-infarcted myocardium, improving vascularization could be one approach. *Early* pharmacological therapy that preserves hypertrophy and stimulates vascular growth in the non-infarcted myocardium seems to have beneficial effects on

hemodynamics, as shown for aspirin. However, other mechanisms besides enhancing vascularization may play an important role in the improvement of the failing heart. This was confirmed by *early* V_{1A} vasopressin antagonist treatment which improved cardiac function independent of capillary growth. Both treatments restored infarction induced tachycardia, but aspirin also normalized *intrinsic or in vitro* tachycardia. This intrinsic heart rate, rather than the *in vivo* heart rate, was associated with actual capillary growth. In addition to the effects of *early* treatment on hypertrophic response and capillary growth, interference with collagen depositon in the non-infarcted myocardium are associated with changes in left ventricular chamber geometry as well as diastolic function. *Early* treatment which interferes too much with collagen depostion in the non-infarcted myocardium, leads to increased left ventricular dilation, but at the same time can improve left ventricular relaxation. In conclusion, the findings of this thesis confirm our hypothesis that *early* treatment after myocardial infarction should not simply prevent cardiac remodeling. It requires subtle and specific interference with the remodeling process in order to improve contractile function of the failing heart. In the light of these observations, the role of new pharmacological agents interfering with specific aspects of neurohormonal activation and remodeling process may be promising. These include vasopressin antagonists, cytokine and endothelin blockers and neutralendopeptidase inhibitors (Fig.1).

Fig.1 Future treatment strategies for chronic heart failure.



Even alternative therapies with the same goals, i.e. increasing viable cardiac tissue and optimal cardiac perfusion, are now emerging to finally improve the function of the failing heart. These include gene therapy enabling adenoviral gene transfection with vascular endothelial growth factor [1,29] or phenotypic transformation of embryonic stem cells into cardiomyocytes or facilitate cardiomyocyte regeneration and engraftment in regions of fibrosis and thinning to restore wall thickness and myocardial mass. Thus, all therapeutic approaches which lead to a preserved contractile tissue with stimulation of vascular growth may well be amenable to clinical application in the future.

7.3. References

1. Yousef ZR, Redwood SR, Marber MS. Postinfarction left ventricular remodeling: a pathophysiological and therapeutic review. *Cardiovasc Drugs Ther.* 2000;14:243-52.
2. Packer M. Pathophysiology of chronic heart failure. *Lancet.* 1992;340:88-92.
3. Swedberg K, Held P, Kjekshus J, Rasmussen K, Ryden L, Wedel H. Effects of the early administration of enalapril on mortality in patients with acute myocardial infarction. Results of the Cooperative New Scandinavian Enalapril Survival Study II (CONSENSUS II) [see comments]. *N Engl J Med.* 1992;327:678-84.
4. Schoemaker RG, Debets JJ, Struyker-Boudier HA, Smits JF. Delayed but not immediate captopril therapy improves cardiac function in conscious rats, following myocardial infarction. *J. Mol. Cell. Cardiol.* 1991;23:187-97.
5. Whittaker P, Boughner DR, Kloner RA. Role of collagen in acute myocardial infarct expansion. *Circulation.* 1991;84:2123-34.
6. Weber KT, Sun Y, Tyagi SC, Cleutjens JP. Collagen network of the myocardium: function, structural remodeling and regulatory mechanisms. *J Mol Cell Cardiol.* 1994;26:279-92.
7. Smits JF, van Krimpen C, Schoemaker RG, Cleutjens JP, Daemen MJ. Angiotensin II receptor blockade after myocardial infarction in rats: effects on hemodynamics, myocardial DNA synthesis, and interstitial collagen content. *J Cardiovasc Pharmacol.* 1992;20:772-8.
8. McCormick RJ, Musch TI, Bergman BC, Thomas DP. Regional differences in LV collagen accumulation and mature cross- linking after myocardial infarction in rats. *Am J Physiol.* 1994;266:H354-9.
9. Mannisi JA, Weisman HF, Bush DE, Dudeck P, Healy B. Steroid administration after myocardial infarction promotes early infarct expansion. A study in the rat. *J. Clin. Invest.* 1987;79:1431-9.
10. Jugdutt BI, Basualdo CA. Myocardial infarct expansion during indomethacin or ibuprofen therapy for symptomatic post infarction pericarditis. Influence of other pharmacologic agents during early remodelling. *Can J Cardiol.* 1989;5:211-21.
11. van Krimpen C, Schoemaker RG, Cleutjens JP, Smits JF, Struyker-Boudier HA, Bosman FT, Daemen MJ. Angiotensin I converting enzyme inhibitors and cardiac remodeling. *Basic Res. Cardiol.* 1991;86:149-55.
12. Anversa P, Loud AV, Levicky V, Guideri G. Left ventricular failure induced by myocardial infarction. I. Myocyte hypertrophy. *Am. J. Physiol.* 1985;248:H876-82.
13. Sutton MG, Sharpe N. Left ventricular remodeling after myocardial infarction: pathophysiology and therapy. *Circulation.* 2000;101:2981-8.
14. Schieffer B, Wirger A, Meybrunn M, Seitz S, Holtz J, Riede UN, Drexler H. Comparative effects of chronic angiotensin-converting enzyme inhibition and angiotensin II type 1 receptor blockade on cardiac remodeling after myocardial infarction in the rat. *Circulation.* 1994;89:2273-82.

15. Sladek T, Sladkova J, Kolar F, Papousek F, Cicutti N, Korecky B, Rakusan K. The effect of AT1 receptor antagonist on chronic cardiac response to coronary artery ligation in rats. *Cardiovasc Res.* 1996;31:568-76.

16. Kalkman EA, Bilgin YM, van Haren P, van Suylen RJ, Saxena PR, Schoemaker RG. Determinants of coronary reserve in rats subjected to coronary artery ligation or aortic banding. *Cardiovasc. Res.* 1996;32:1088-95.

17. Carmeliet P. VEGF gene therapy: stimulating angiogenesis or angioma-genesis? *Nat Med.* 2000;6:1102-3.

18. Simons M, Bonow RO, Chronos NA, Cohen DJ, Giordano FJ, Hammond HK, Laham RJ, Li W, Pike M, Sellke FW, Stegmann TJ, Udelson JE, Rosengart TK. Clinical trials in coronary angiogenesis: issues, problems, consensus: An expert panel summary. *Circulation.* 2000;102:E73-86.

19. Schwarz ER, Speakman MT, Patterson M, Hale SS, Isner JM, Kedes LH, Kloner RA. Evaluation of the effects of intramyocardial injection of DNA expressing vascular endothelial growth factor (VEGF) in a myocardial infarction model in the rat--angiogenesis and angioma formation. *J Am Coll Cardiol.* 2000;35:1323-30.

20. Hudlicka O, Brown MD, Walter H, Weiss JB, Bate A. Factors involved in capillary growth in the heart. *Mol. Cell. Biochem.* 1995;147:57-68.

21. Wright AJ, Hudlicka O. Capillary growth and changes in heart performance induced by chronic bradycardial pacing in the rabbit. *Circ Res.* 1981;49:469-78.

22. Weihs H, Mutschler E, Walland A. Alinidine prevents hypoperfusion-induced transmural flow redistribution in isolated paced rat hearts. *Clin Exp Pharmacol Physiol.* 1994;21:471-6.

23. Stewart JT, Simpson IA, Smith RE, Callicott C, Gray HH, Camm AJ. Left ventricular energetics: heat production by the human heart. *Cardiovasc. Res.* 1993;27:1024-32.

24. Brown MD, Davies MK, Hudlicka O. The effect of long-term bradycardia on heart microvascular supply and performance. *Cell Mol Biol Res.* 1994;40:137-42.

25. Zheng W, Brown MD, Brock TA, Bjercke RJ, Tomanek RJ. Bradycardia-induced coronary angiogenesis is dependent on vascular endothelial growth factor. *Circ Res.* 1999;85:192-8.

26. Rouse W, Johnson IR. Haemodynamic actions of a novel sino-atrial node function modulator, ZENECA ZD7288, in the anaesthetized dog: a comparison with zatebradine, atenolol and nitrendipine. *Br J Pharmacol.* 1994;113:1064-70.

27. Shinke T, Takeuchi M, Takaoka H, Yokoyama M. Beneficial effects of heart rate reduction on cardiac mechanics and energetics in patients with left ventricular dysfunction. *Jpn Circ J* 1999;63:957-64.

28. Canby CA, Tomanek RJ. Regression of ventricular hypertrophy abolishes cardiocyte vulnerability to acute hypoxia. *Anat Rec.* 1990;226:198-206.

29. Francis GS. Can we prevent congestive heart failure? Excerpts from a symposium. *Clin Cardiol.* 2000;23:IV1-3.

Summary

The aim of this thesis was to investigate the effects of pharmacological interference with the different aspects of postinfarction remodeling during the *early compensatory stage* in a rat heart failure model. Ligation of the left anterior descending coronary artery in rats, resulting in extensive transmural infarction of the LV free wall, was used to study the structural and functional consequences of a large myocardial infarction. The rat heart failure model, which has been well established, is associated with left ventricular dysfunction, neurohormonal activation and *remodeling* of the cardiac structure. We hypothesized that pharmacological therapy should be directed to preservation of the total amount of contractile tissue with optimal perfusion. The former implicates that reactive hypertrophy should not be inhibited in this compensatory stage, whereas perfusion should be enhanced by increased vascularization, or optimizing the use of existing vascularization by prolonged diastolic time or improved cardiac relaxation. In addition, interference with collagen deposition afer myocardial infarction should be minimized in order to preserve sufficient tensile strength and prevent left ventricular dilation.

In **Chapter 1**, the pathophysiology of postinfarction induced heart failure has been described. The different aspects of myocardial infarction induced neuroendocrine activation, including sympathetic nervous system, renin-angiotensin-aldosterone system as well as arginine vasopressin system, and postinfarction remodeling in both infarcted and non-infarcted myocardium as well as their possible role in the transition from left ventricular dysfunction towards heart failure are discussed in detail.

In **Chapter 2**, the effects of *early* interference with postinfarction induced collagen deposition related to left ventricular diastolic function were studied by means of chronic aspirin or methylprednisolone therapy. It was shown that pharmacological interference with aspirin and methylprednisolone may affect the deposition of collagen in the non-infarcted myocardium, resulting in an altered left ventricular diastolic function. Furthermore, changes in interstitial rather than perivascular

collagen seemed to be important in the regulation of passive as well as active relaxation of the isolated heart.

In **Chapter 3**, the effects of *early* interference with postinfarction induced sympathetic activation and left ventricular remodeling (hypertrophy and interstitial collagen) of the non-infarcted myocardium were evaluated after treatment with the imidazoline I_1 receptor agonist moxonidine. It could be demonstrated that moxonidine effectively suppressed sympathetic activation following myocardial infarction in a dose-dependent way as was indicated by reduced heart rate and plasma noradrenaline levels. Furthermore, postinfarction left ventricular remodeling may be attenuated at a higher dose-range of moxonidine as shown by normalization of ventricular weight to body weight ratio and interstitial collagen deposition in the non-infarcted myocardium.

In **Chapter 4**, the effects of *early* interference with myocyte hypertrophy and capillary density in relation to ischemic sensitivity were studied in isolated infarcted hearts by means of chronic moxonidine therapy. Histological analysis revealed that concentric hypertrophy of the non-infarcted myocardium following myocardial infarction, as indicated by almost double cross-sectional area of Gomori stained myocytes, was completely prevented by moxonidine. Although capillary to myocyte ratio was similar in all groups, prevention of hypertrophy with moxonidine was accompanied by a a restored number of Lectin stained capillaries per tissue area. Furthermore, ischemic sensitivity of infarcted hearts, as reflected by increased maximal coronary flow during reperfusion, was reduced with moxonidine. This was supported by substantially lower purines and lactate concentrations in the coronary effluent during ischemia. It was concluded that prevention of myocyte hypertrophy with moxonidine may preserve capillary density without affecting actual capillary number, thereby improving ischemic tolerance of the spared myocardium.

In **Chapter 5**, the *in vivo* hemodynamic effects of *early* treatment with a V_{1A}- (SR-49059) and V_2 (SR-121463B) receptor antagonist in chronically infarcted rats were studied. Improved cardiac output with the vasopressin V_{1A} antagonist resulted both from an increased stroke volume and slightly reduced postinfarction tachycardia.

Moreover, left ventricular concentric hypertrophy associated with reduced capillary density in the spared myocardium of infarcted rats, remained unaffected by therapy with the vasopressin V_{1A} or V_2 antagonist. Thus, chronic vasopressin V_{1A}-, but not V_2 receptor blockade was able to prevent heart failure in 3 week old infarcted rats. Nevertheless, the improved cardiac function in these rats could not be attributed to changes in left ventricular hypertrophy and/ or capillary density.

In **Chapter 6**, the relation between heart rate (*in vivo* and *intrinsic*) and capillary angiogenesis in the non-infarcted myocardium of rats treated with aspirin, methylprednisolone, moxonidine and captopril in the *early* compensatory stage was investigated. While actual *in vivo* heart rate was measured in conscious rats, *in vitro or intrinsic* heart rate was obtained from isolated perfused hearts. Infarcted rats manifested a significant *in vivo* tachycardia as well as increased *intrinsic* heart rate when compared to sham-rats. Whereas aspirin, methylprednisolone and moxonidine significantly reduced infarction induced *in vivo* tachycardia, captopril further increased *in vivo* tachycardia. Increased *intrinsic* heart rate in infarcted hearts was reduced to sham-values with aspirin and methylprednisolone, but not with moxonidine and captopril. In addition, prevention of left ventricular hypertrophy with moxonidine and captopril was shown to normalize capillary density without affecting capillary number, whereas aspirin and methylprednisolone normalized capillary density by increasing capillary number. In conclusion, normalized *intrinsic* (aspirin and methylprednisolone) rather than *in vivo* tachycardia after myocardial infarction was the suggested mechanism associated with actual capillary growth in infarcted hearts.

In **Chapter 7**, the general discussion with concluding remarks and implications for future research was described. Based on the results of this thesis, we concluded that *early* treatment after myocardial infarction should not simply prevent cardiac remodeling. It requires subtle and specific interference with the remodeling process in order to improve contractile function of the failing heart. *Early* interference with postinfarction remodeling may not affect compensatory hypertrophy, but rather stimulate capillary growth in the non-infarcted myocardium in order to improve hemodynamics (aspirin). Indeed, stimulation of angiogenesis in the non-infarcted

hypertrophied myocardium achieved by reduction of *intrinsic* tachycardia in isolated infarcted hearts rather than *in vivo* tachycardia, resulted in improved hemodynamics (aspirin/methylprednisolone). However, other mechanisms independent of capillary growth may play an important role in the improvement of the failing heart, as observed with *early* V_{1A} vasopressin antagonist therapy. In addition to the effects of *early* treatment on hypertrophic response and capillary growth, interference with collagen depositon was shown to affect diastolic function as well as active relaxation. According to our hypothesis, the results of the present thesis indicate that *early* pharmacological interference with the postinfarction remodeling process should preserve contractile tissue and/or stimulate vascular growth in order to improve the function of the failing heart.

Samenvatting

Deze thesis had tot doel de effecten van farmacologische interferentie met de verschillende aspecten van het postinfarct remodeling proces in de *vroege* compensatoire fase te bestuderen en dit aan de hand van een rat hartfalen model. Ligatie van de linker kransslagader resulteert in ratten in een omvangrijk transmuraal infarct van de linker ventrikel vrije wand, en kan gebruikt worden als model om de functionele en structurele gevolgen van een myocardinfarct te bestuderen. Dit rat hartfalen model is een goed bestudeerd experimenteel model gekarakteriseerd door neurohormonale activatie en *remodeling* van de hartstructuur zoals die bij patiënten met hartfalen waargenomen worden.

Onze vooropgestelde hypothese luidde dat optimale farmacologische therapie zich moet richten op het behoud van de totale hoeveelheid contractiel weefsel met een optimale perfusie. Dit impliceert dat reactieve hypertrofie in de *vroege* compensatoire fase niet dient te worden geremd maar eerder dat perfusie van het resterend myocardweefsel verbeterd dient te worden door stimulatie van vaatgroei. Anderzijds, dient interferentie met collageen afzetting na myocardinfarct minimaal te zijn teneinde voldoende reksterkte te garanderen alsook verdere dilatatie van het linker ventrikel te voorkomen.

In **hoofdstuk 1** werd de pathofysiology van postinfarct geïnduceerd hartfalen beschreven. De verschillende aspecten van neuroendocriene activatie (sympathisch zenuwstelsel, renine-angiotensine-aldosterone en arginine-vasopressine systeem) na myocardinfarct, en de verschillende aspecten van het postinfarct remodeling proces in het geïnfarceerd en niet-geïnfarceerd myocard alsook de mogelijke rol in de evolutie van linker ventrikel dysfunctie naar hartfalen worden gedetailleerd beschreven.

In **hoofdstuk 2** werden de effecten bestudeerd van *vroege* farmacologische interventie met de collageen afzetting na myocardinfarct en gerelateerd aan linker ventrikel diastole functie aan de hand van chronische therapie met een lage dosis

aspirine of steroïdbehandeling met methylprednisolone. Er werd aangetoond dat *vroege* farmacologische (aspirine en methylprednisolone) interferentie de collageen afzetting in het niet-geïnfarceerd myocard beïnvloed en hierdoor linker ventrikel diastole functie verandert. De veranderingen in het interstitieel eerder dan perivasculair collageen bleken belangrijk te zijn in de regulatie van zowel passieve als actieve relaxatie van het geïsoleerde hart.

In **hoofdstuk 3** werden de effecten geëvalueerd van een *vroege* interferentie met sympathische activatie en linker ventrikel remodeling (met name hypertrofie en interstitieel collageen afzetting) na myocardinfarct aan de hand van chronische behandeling met de imidazoline I1 receptor agonist moxonidine. Er werd aangetoond dat moxonidine op een dosis afhankelijke manier de postinfarct geïnduceerde sympathische activatie na myocardinfarct kan onderdrukken, dewelke bevestigd werd door een verlaging van tachycardie en plasma noradrenaline spiegels. Verder werd aangetoond dat postinfarct remodeling geattenueerd kan worden met een hogere dosis moxonidine dewelke bevestigd werd door een normalizatie van de ventrikel- en lichaamsgewicht ratio alsook interstitiële collageen afzetting in het niet-geïnfarceerd deel van het myocard.

In **hoofdstuk 4** werden de effecten bestudeerd van *vroege* interferentie met cellulaire hypertrofie en capillaire dichtheid in relatie tot de gevoeligheid voor ischemie in geïsoleerde infarct harten en dit aan de hand van chronische behandeling met moxonidine. Histologische analyse bracht aan het licht dat concentrische hypertrofie in het niet-geïnfarceerd myocard, dewelke bevestigd werd door een bijna verdubbeling van de hartspiercelloppervlakte, vollledig geremd kon worden door moxonidine therapy. Hoewel de capillair myocyte ratio ongewijzigd bleef in de verschillende groepen, ging preventie van hypertrofie gepaard met een herstel in het aantal capillairen per weefseloppervlakte. De gevoeligheid voor ischemie in de onbehandelde infarct harten, dewelke bevestigd werd door een toename in maximale coronair flow gedurende reperfusie, werd gereduceerd na chronische behandeling met moxonidine. Dit werd verder ondersteund door de lagere purine en lactaat concentraties in het coronair effluent gedurende ischemie. Er werd geconcludeerd

dat remming van hypertrofie na moxonidine behandeling gepaard gaat met een herstel in capillaire dichtheid zonder wijziging van het aantal capillairen en als gevolg hiervan de gevoeligheid voor ischemie vermindert in het gespaarde deel van het myocard.

In **hoofdstuk 5** werden de *in vivo* hemodynamische effecten bestudeerd van een *vroege* behandeling met een V_{1A} (SR-49059) en V_2 (SR-121463B) receptor antagonist in chronisch geïnfarceerde ratten. Een verbetering van het hartdebiet (=hartfrekwentie x slagvolume) waargenomen met de V_{1A} antagonist resulteerde uit een toename in slagvolume en reductie in postinfarct tachycardie. Verder werd aangetoond dat linker ventrikel hypertrofie die gepaard gaat met een lagere capillaire dichtheid in het gespaarde deel van het myocard, niet beïnvloed werd door *vroege* behandeling met de V_{1A} of V_2 antagonist. Gebaseerd op deze resultaten werd geconcludeerd dat chronische V_{1A}- ,maar niet V_2 blokkade, in staat is myocardinfarct geïnduceerd hartfalen te remmen. De opmerkelijke verbetering van hartfunctie in deze ratten kon niet worden toegeschreven aan eventuele structurele veranderingen in linker ventrikel hypertrofie en/of capillaire dichteid .

In **hoofdstuk 6** werd de relatie tussen hartfrekwentie (*in vivo* en *intrinsieke*) en capillaire groei in het gespaarde deel van het myocard bestudeerd in infarct ratten behandeld met aspirine, methylprednisolone, moxonidine en captopril in de *vroege* compensatoire fase. *In vivo* hartfrekwentie werd gemeten in wakkere ratten en de *in vitro* of *intrinsieke* hartfrekwentie in geïsoleerde harten. Onbehandelde infarct ratten werden gekenmerkt door een opmerkelijke *in vivo* tachycardie en toename in *intrinsieke* hartfrekwentie. Vroege behandeling met aspirine, methylprednisolone en moxonidine resulteerde, in tegenstelling tot captopril,in een verlaging van postinfarct *in vivo* tachycardie. De toename in *intrinsieke* hartfrekwentie na myocardinfarct kon gereduceerd worden met *vroege* aspirine en methylprednisolone behandeling maar niet met captopril en moxonidine. In deze vergelijkende studie werd tevens aangetoond dat preventie van linker ventrikel hypertrofie gepaard gaat met normalisatie van de capillaire dichteid zonder de hoeveelheid capillairen te beïnvloeden (in geval van moxonidine en captopril behandeling), en dat *vroege*

aspirine en methylprednisolone behandeling de capillaire dichteid kan normaliseren als gevolg van effectieve capillaire groei. Er werd geconcludeerd dat normalisatie van *intrinsieke* (in geval van aspirine en methylprednisolone) eerder dan *in vivo* tachycardie na myocardinfarct een mogelijk mechanisme is om capillaire groei in geïnfarceerde harten te stimuleren.

In **hoofdstuk 7** werd de algemene discussie met conclusies en implicaties voor de toekomst beschreven. Gebaseerd op de resultaten van deze thesis werd geconcludeerd dat *vroege* behandeling na myocardinfarct zich niet louter mag focuseren op remming van het remodeling proces. Het vereist een subtiele en specifieke interferentie met het remodeling proces teneinde de functie van het falende hart te verbeteren. *Vroege* interferentie met het postinfarct remodeling proces dient niet gefocuseerd te zijn op de remming van compensatoire hypertrofie, maar eerder op behoud van totaal weefsel en stimulatie van capillaire groei. Alle vroege behandelingen die reactieve hypertrofie na myocardinfarct remden, konden afgezien van een herstelde capillaire dichtheid, de hartfunctie niet echt verbeteren. In dit proefschrift werd tevens aangetoond dat capillaire groei in het niet-geïnfarceerde myocard als gevolg van een gereduceerde *intrinsieke* eerder dan *in vivo* tachycardie, resulteerde in verbetering van de hemodynamiek (aspirine en methylprednisolone). Nochtans bleek duidelijk uit de resultaten van *vroege* behandeling met de V_{1A} vasopressine antagonist dat nog andere mechanismen onafhankelijk van capillaire groei een belangrijke rol spelen in de verbetering van de functie van het falende hart. Naast de effecten van *vroege* behandeling op de hypertrofie en capillaire groei, werd aangetoond dat farmacologische interferentie met de collageenafzetting een belangrijk effect heeft op diastole hartfunctie en actieve relaxatie van de hartspier. Tot slot kunnen we in overeenstemming met onze vooropgestelde hypothese en resultaten besluiten dat *vroege* farmacologische interferentie met het postinfarct remodeling proces dient gericht te zijn op het behoud van de hoeveelheid contractiel weefsel en stimulatie van vaatgroei teneinde de functie van het falende hart te verbeteren.

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Curriculum vitae

Roeland Van Kerckhoven zag het levenslicht op 11 december 1970 in Ekeren, te België. Na zijn klassieke humaniorastudies te hebben volbracht aan het Onze-Lieve-Vrouwecollege te Anwerpen, startte hij met de opleiding biologie aan het Rijksuniversitair Centrum Antwerpen (RUCA). In 1994 behaalde hij het licentiaatsdiploma in de moleculaire biologie aan de Universitaire Instelling Antwerpen (UIA) met als thesis *'Expressie van Multidrugresistente (mdr) Genen in Coloncarcinoomcellen'* verricht in de onderzoeksgroep klinische oncologie o.l.v. Prof. dr. A.T. van Oosterom. In de periode van 1994-1997 volgde hij de doctoraatsopleiding medische wetenschappen aan de UIA in het Laboratorium voor Experimentele Heelkunde, Cardiovasculaire Onderzoekseenheid met als onderzoeksproject " *Farmacologische modulatie van de in vivo myocardfunctie : Fysiologische aspecten en clinische implicaties".* In 1998 begon hij als assistent in opleiding bij het instituut Farmacologie van de Erasmus Universiteit Rotterdam onder de supervisie van Prof. dr. P.R. Saxena en Dr. R.G. Schoemaker. De resultaten van deze 4 jaar durende onderzoeksopleiding vindt u in dit proefschrift.

Publications

Full papers

De Mulder PA, Van Kerckhoven R, Adriaensen HF, Gillebert TC, De Hert SG. Continuous total intravenous anesthesia, using propofol and fentanyl in an open-thorax rabbit model: evaluation of cardiac contractile function and biochemical assessment. *Laboratory Animal Science 1997;47(4):367-75.*

De Mulder PA, De Hert SG, Van Kerckhoven R, Adriaensen HF, Gillebert TC. Sodium nitroprusside enhances in vivo left ventricular function in beta-adrenergically stimulated rabbit hearts. *Cardiovascular Research 1998 ;38(1):133-9.*

Van Kerckhoven R, Kalkman EAJ, Saxena PR, Schoemaker RG. Altered cardiac collagen and associated changes in diastolic function of infarcted rat hearts.*Cardiovascular Reserach 2000 :46(1):316-23.*

Van Kerckhoven R, van Veen TAB, Boomsma F, Saxena PR, Schoemaker RG. Chronic administration of moxonidine suppresses sympathetic activation in a rat heart failure model. *European Journal of Pharmacology 2000;397:113-120.*

Van Kerckhoven R, Saxena PR, Schoemaker RG. Restored capillary density improves ischemic tolerance in infarcted rat hearts. *Journal of Cardiovascular Pharmacology 2001, accepted.*

Van Kerckhoven R, Lankhuizen I, van Veghel R Saxena PR, Schoemaker RG.Chronic vasopressin V_{1A}- but not V_2 antagonism prevents heart failure in chronically infarcted rats. *Europan Journal of Pharmacology 2002, accepted.*

Van Kerckhoven R, Lankhuizen I, van Veghel R Saxena PR, Schoemaker RG Lower *intrinsic* rather than *in vivo* heart rate contributes to capillary growth in infarcted rat hearts. *American Journal of Physiology 2002, submitted.*

Abstracts

De Mulder PA, Van Kerckhoven R, Adriaensen HF, Gillebert TC, De Hert SG. Continuous total intravenous anesthesia, using propofol and fentanyl in an open-thorax rabbit model: evaluation of cardiac contractile function and biochemical assessment. 16 th Scientific meeting of the Belgian society of cardiology, Brussels, Belgium. *Acta Cardiologica 1995; 6 (Vol LI): 572-3.*

De Mulder PA, Van Kerckhoven R, Adriaensen HF, Gillebert TC, De Hert SG. Sodium nitroprusside and left ventricular function: a combined clinical and experimental study. 17 th Scientific meeting of the Belgian society of cardiology, Brussels, Belgium. *Acta Cardiologica 1996*

De Mulder PA, Van Kerckhoven R, Adriaensen HF, Gillebert TC, De Hert SG. Sodium nitroprusside enhances in vivo left ventricular function in beta-adrenergically stimulated rabbit hearts. 18 th Scientific meeting of the Belgian society of cardiology, Brussels, Belgium. *Acta Cardiologica 1997*

Van Kerckhoven R, Kalkman EAJ, Saxena PR, Schoemaker RG. Effects of aspirin and methylprednisolone on cardiac collagen and diastolic function in chronically, infarcted hearts. *Fundamental and Clinical Pharmacology 1999, Vol.13, 133.*
American Heart Association (AHA), Atlanta, USA. *Circulation 2000, Vol.100,I-117.*

Van Kerckhoven R, van Veen TAB, Boomsma F, Saxena PR, Schoemaker RG. Chronic administration of moxonidine suppresses sympathetic activation in a rat heart failure model. International Society for Heart Research (ISHR), Maastricht, The Netherlands. *Journal of Molecular and Cellular Cardiology 1999, Vol. 31, nr.6, A 79.*
Fundamental and Clinical Pharmacology 2000, Vol.14, 51.

Prevention of hypertrophy restores capillary density and ischemic tolerance in infarcted rats. International Society for Heart Research (ISHR), Winnipeg, Canada. *Journal of Molecular and Cellular Cardiology 2001, Vol. 33, A 58.*

Lower *intrinsic* rather than *in vivo* heart rate contributes to capillary growth in infarcted rat hearts. Dutch Pharmacological Society, Lunteren, The Netherlands. *Fundamental Clinical Pharmacology 2002, Vol.16.*

Chronic vasopressin V_{1A} but not V_2 antagonism prevents heart failure in chronically infarcted rats. International Society for Heart Research (ISHR), Szeged, Hungary. *Journal of Molecular and Cellular Cardiology 2002, not yet published.*

Lower *intrinsic* rather than *in vivo* heart rate contributes to capillary angiogenesis in infarcted rats. International Society for Heart Research (ISHR), Szeged, Hungary. *Journal of Molecular and Cellular Cardiology 2002, not yet published.*

Abbreviations

ACE:	Angiotensin I converting enzyme
ANOVA:	Analysis of variance
ASP:	Aspirin
ATP:	Adenosine triphosphate
AT-R:	Angiotensin receptor
bFGF:	Basic fibroblast growth factor
BW:	Body weight
CAP:	Captopril
CF:	Coronary flow
CO:	Cardiac output
CVP:	Central venous pressure
DNA:	Desoxyribonucleic acid
dP/dt:	Pressure change per unit time
HW:	Heart weight
HR:	Heart rate
i.p.:	Intraperitoneal
i.v.:	Intravenous
LV:	Left ventricular
LVEDP:	Left ventricular end-diastolic pressure
LVSP:	Left ventricular systolic pressure
MAP:	Mean arterial pressure
MI:	Myocardial infarction
MOX:	Moxonidine
MP:	Methylprednisolone
mRNA:	Messenger ribonucleic acid
NSAID:	Non-steroid anti-inflammatory drug
PV:	Pressure-volume
$V_{1A/2}$:	Vasopressin 1A/2 receptor
VEGF:	Vascular endothelial growth factor
VW:	Ventricular weight

Myocardial infarction is a leading cause of heart failure. The neurohormonal hypothesis postulates that neurohormones are initially adaptive by maintaining cardiac output and blood pressure, but later the same compensatory responses become pathological and contribute to adverse remodeling, progressive ventricular failure, and ultimatly to the syndrome of heart failure. In the past, pharmacological research has mainly focused on the relief of symptoms when congestive heart failure is diagnosed and prognosis becomes very poor. Except for treatment with angiotensin converting enzyme inhibitors, prognosis has not benefited substantially from all these research. Instead of aiming the regression of remodeling in the late phase, therapy early after myocardial infarction can be an alternative approach to prevent heart failure, but may include strong interference with the cardiac remodeling process. The aim of this thesis was to investigate the effects of pharmacological interference with the different aspects of postinfarction remodeling during the early compensatory stage in an experimental heart failure model. The findings of this thesis confirm our hypothesis that early treatment after myocardial infarction should not simply prevent cardiac remodeling. It requires subtle and specific interference with the remodeling process in order to improve contractile function of the failing heart.