Structural alterations in peripheral arteries during experimental heart failure : opposing effects of vasoconstrictors and vasodilators

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Proefschrift

ter verkrijging van de graad van doctor,
aan de Universiteit Maastricht,
op gezag van de Rector Magnificus, Prof. Mr. M.J. Cohen,
volgens het besluit van het College van Decanen,
in het openbaar te verdedigen
op donderdag 22 mei 1997 om 14.00 uur

door

Sylvia Heeneman

geboren te 's-Gravenhage in 1968

The most exciting phrase ever heard in science,
is not 'eureka' (I found it), but 'that's funny'...

- Isaac Asimov -

Contents

AA	Abdominal aorta
ACE	Angiotensin converting enzyme
ANG I	Angiotensin I
ANG II	Angiotensin II
ANP	Atrial natriuretic peptide
BNP	Brain natriuretic peptide
CA	Carotid aorta
CNP	C-type natriuretic peptide
AT1 receptor	Angiotensin II Type-1 receptor
AT2 receptor	Angiotensin II Type-2 receptor
CSA	Cross-sectional area
cGMP	cyclic 3',5'-guanosine monophosphate
ET	Endothelin
GFR	Glomerular filtration rate
IA	Iliac artery
LAD	Left anterior descending artery
L-NAME	N^{ω}-nitro-l-arginine methyl ester
MAP	Mean arterial pressure
MI	Myocardial infarction
mRNA	messenger RNA
mRA	Mesenteric resistance arteries
NO	Nitric Oxide
eNOS	endothelial NO synthase
iNOS	inducible NO synthase
nNOS	neuronal NO synthase
NS	not significant
pRA	Pulmonary resistance arteries
RA	Renal artery
RAS	Renin Angiotensin System
sMA	superior Mesenteric artery
TA	Thoracic aorta

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Introduction

General

The clinical syndrome of heart failure reflects the inability of the heart to pump sufficient amounts of blood to meet the metabolic needs of the body at normal filling pressures (1). The initial event can occur in the form of coronary artery disease, hypertension, valve dysfunction or diabetes mellitus. The syndrome is characterized by circulatory congestion, edema and exercise intolerance (2, 3). Despite the relative success of new therapeutic strategies, as demonstrated for the angiotensin-converting enzyme (ACE) inhibitors in the CONSENSUS, SOLVD and SAVE trials (4, 5), death rates and hospital admissions continue to increase, both in the Netherlands and the United States. In the Netherlands, the annual number of hospital admissions because of heart failure increased by 69% from 14441 in 1980 to 24368 in 1992 (6). Cardiovascular disease remains the leading cause of death in the United States and heart failure afflicts 1% of all men and women under the age of 50 and more than 10% of those over the age of 80 years (6-9).

The loss of cardiac function induces a series of compensatory mechanisms to maintain blood pressure and the perfusion of vital organs. These mechanisms include the development of cardiac hypertrophy, ventricular dilatation, activation of neurohormonal systems and peripheral vascular alterations. Although activation of these compensatory mechanisms may be beneficial in the initial stage of cardiac dysfunction, prolonged and/or abnormal activation may result in a vicious circle, with further deterioration of cardiac function. The final result is heart failure (1).

In the past, much research has been focussed on the changes in the heart muscle itself, but in recent years it has become clear that other factors are also important in the development of heart failure. This thesis concentrates on changes in peripheral vascular structure.

Peripheral vascular alterations during heart failure

Aortic compliance
Afterload is an important determinant of cardiac output. Afterload consist of a steady resistance component (determined by the resistance arteries) and a pulsatile component (determined by the elastic properties of the large conduit arteries) (10). Aortic compliance is a contributing factor to aortic impedance. It depends on the composition and geometry of the aorta and is a measure of distensibility. The dependence of left ventricular performance on aortic impedance has been shown in a dog model in which the intrathoracic aorta was bypassed by a stiff plastic tube (11). This resulted in an augmented cardiac dysfunction during acute coronary artery occlusion. Indeed, a positive linear relationship between the

ejection fraction of the left ventricle and systemic arterial compliance suggests that arterial compliance is an important determinant of left ventricular afterload (12).

Aortic compliance is decreased during ventricular failure in animal models of heart failure (13, 14) and human heart failure (15-18). It is unclear whether this vascular response is an early change or a late manifestation of the disease. Eaton *et al.* studied the aortic impedance spectrum 48 h after ventricular pacing (dog) and suggested that the reduced conduit vessel compliance may contribute to the progression of abnormal myocardial energetics and thus systolic dysfunction. Interestingly, this study also showed that changes in compliance may precede alterations in the peripheral vascular resistance (13). Although the impairment of arterial compliance is more marked in severe congestive heart failure, it is also manifest in mild heart failure (19). However, there are also studies which show no changes in aortic impedance during heart failure (20-22). The reasons for these inconsistent observations is not clear; some of these studies used indirect techniques to measure aortic impedance, but even the results from studies using similar techniques are not comparable (15, 21, 22). Some of the discrepancy may be explained by the extent of cardiac failure in the different patient groups or by the medication used.

Peripheral vasoconstriction
Next to the possible changes in the pulsatile component during heart failure, changes in the resistance component are well known to occur and were first described by Zelis *et al.* in 1969. This study showed that in patients with heart failure both rest and exercise arm blood flow were reduced due to an increase in vascular resistance (23). The same group also showed that the vasodilator responses to adrenergic blockade, ischemia and direct arterial vasodilators were decreased in patients with heart failure (24). Also, attenuated increases in cardiac output and stroke volume following exercise were found (25). All these observations are indicative for excessive peripheral vasoconstriction during heart failure, since vasoconstriction and a reduced capacity to vasodilation (24) may prevent the development of severe hypotension in situations of prominent vasodilation as seen during exercise (26).

Endothelial dysfunction
Recently, attention has been focussed on an impairment of endothelium-dependent vasodilation during heart failure. The vascular endothelium modulates smooth muscle relaxation via the release of nitric oxide (NO) (27). The constitutive basal release of NO from the endothelium has been shown to contribute to the regulation of basal vascular tone (reviewed in references 28 and 29). Next to this basal release, endothelial NO synthesis can be induced by several agonists such as acetylcholine and bradykinin (reviewed in references 30 and 31).

Ontkean *et al.* (32) showed impaired endothelium-dependent vasodilation in rats 10 weeks after induction of a myocardial infarction. Vasodilation in response to acetylcholine and adenosine diphosphate (both endothelium-dependent), but not to nitroglycerin (endothelium-independent) were decreased in isolated vessel rings of the thoracic aorta of rats with a myocardial infarction. These results suggest an impairment of acetylcholine mediated production of NO following myocardial infarction. Subsequently, other studies also showed impaired agonist-mediated vasodilation in isolated aortic vessel rings of myocardial infarcted rats (33-35). In man, the endothelium dependent vasodilation is impaired in the coronary, skeletal muscle and skin circulation of patients with heart failure (36-41). Interestingly, in an animal model for heart failure, this endothelial dysfunction has been shown to be present in the absence of hemodynamic compromise (34).
In contrast, reports on changes in basal production of NO during human and experimental heart failure heart are conflicting. Human studies reported an intact (41, 42) or even enhanced basal release of NO (43, 44). Animal studies using isolated vascular rings, however, show decreased basal release of NO (32, 35). Thus, the status of the basal release of NO during heart failure is not clear yet.

Possible mechanisms for peripheral alterations

The mechanisms responsible for the above mentioned peripheral alterations are still incompletely understood. There is no doubt, however, that these mechanisms are complex and most likely form a network of events interrelating and influencing each other. In this outline, possible mechanisms will be discussed, like (a) neurohumoral activation and (b) structural vascular alterations. The neurohumoral mechanisms are divided in (a1) activation of the sympathetic nervous system, (a2) activation of the natriuretic peptides (a3) activation of endothelium derived vasoactive factors and (a4) activation of the renin-angiotensin system.

Neurohormonal activation : The sympathetic nervous system (a1)

Introduction
Evidence for sympathetic activation during left ventricular dysfunction comes from both clinical and experimental studies. First, plasma concentrations of norepinephrine are increased in patients with heart failure (45-48). Kinetic studies using tritiated norepinephrine have shown that these elevated norepinephrine levels result from an increased release and spillover, in combination with a decreased clearance (46). The increase in plasma norepinephrine reflects increased sympathetic activity. Also, direct microneurographic recordings of peroneal nerve activity have demonstrated increased sympathetic activity to skeletal muscle in patients with heart failure (49). Furthermore, studies in patients with severe untreated heart failure also show high plasma concentrations of

norepinephrine, indicating that these high plasma concentrations are unrelated to drug therapy (50).

Elevated plasma concentrations of norepinephrine and epinephrine are correlated with poor prognosis (51, 52), and Cohn *et al.* reported that plasma norepinephrine was an independent predictor of mortality in heart failure (53). Moreover, a subgroup analysis of the results of the CONSENSUS trial showed that the ACE-inhibitor enalapril was effective in prolonging life expectancy, especially for patients with increased plasma concentrations of norepinephrine (52).

Also, a variety of physiological and pharmacological forms of stress has been used to test the integrity of adrenergic reflex control during heart failure. Upright orthostatic tilt normally leads to a rise in plasma norepinephrine and plasma renin. The receptors responsible are probably the low-pressure mechanoceptors, which are unloaded by the fall in central venous pressure during the tilt. In patients with heart failure, the response to tilt is significantly attenuated. Plasma norepinephrine concentrations do not rise and the normal decrease in forearm bloodflow is not seen (54-56). Francis *et al.* showed that, although plasma norepinephrine concentrations were higher in patients with heart failure, compared to normal controls, patients were unable to increase plasma norepinephrine levels during comparable percentages of peak exercise O_2 consumption, indicating blunting of sympathetic drive (57). Also, other stimuli of the activity of the sympathetic nervous system, such as the cold pressor test (58) and electrical stimulation (59, 60) do not result in the expected sympathetic peripheral vasoconstriction.

The arterial baroreflex during heart failure

It is still unclear what causes the overactivity of the sympathetic nervous system during heart failure. One mechanism that has been proposed is a depression of the baroreflex (61, 62). Normally, cardiopulmonary baroreceptors, located in the heart and arterial baroreceptors, located in the aortic arch, carotid sinuses and pulmonary vasculature, respond to stretch during increased blood pressure by initiating reflexes that promote parasympathetic stimulation and restrain sympathetic efferent activity (63). Signals from the arterial baroreceptors inhibit the vasoconstrictor center and excite the vagal center. The results are a general vasodilation in the peripheral vascular bed and a decreased heart rate. The arterial baroreflex can have major effects on sympathetic and vagal outflow (64). As early as 1972, Higgins *et al.* showed a depressed baroreflex in a dog model of heart failure. Before the induction of heart failure, carotid occlusion resulted in an increase in arterial pressure, heart rate and mesenteric resistance. A clear depression of this response was seen in the heart failure state (65). Other studies reported a similar depressed baroreflex function in patients with heart failure (58, 63, 66, 67). Blunted baroreceptor responses to high cardiac filling pressures or depressed cardiac function reduce afferent signals that normally inhibit

sympathetic efferent activity, vasopressin release and renin secretion. As a result, neurohormonal activity will increase (62). Creager *et al.* showed that patients with heart failure have a comprised ability to increase their blood pressure during arterial baroreceptor unloading. They do, however remain able to reduce blood pressure during baroreceptor stimulation. Thus, reduced inhibitory signals from the arterial baroreflexes may contribute to the increased sympathetic activity during heart failure (63). However, not all studies show a decrease in baroreflex activity. Meyrelles *et al.* demonstrated preserved short-term baroreflex control of heart rate and even exaggerated baroreflex control one month after the induction of a myocardial infarction in rats (68). Schwartz *et al.* studied the evolution of baroreflex sensitivity in patients during the first year after myocardial infarction. Although the baroreflex sensitivity was decreased 3 months after infarction in a subgroup of patients, it returned to normal 13 months after the infarction (69).

Although most studies show that sympathetic tone and arterial baroreflex sensitivity are altered during heart failure, there have been no studies showing a cause-and-effect relationship. Arguments in favour of such a relationship are observations that baroreflex abnormalities constitute an early event after induction of heart failure in dogs (70) and that discontinuation of pacing in dogs or cardiac transplantation in humans restores the baroreflex control (66, 70-72). However, there are also studies in canine heart failure, induced by an aorta-caval fistula, that show persistent abnormalities of baroreflex control after closing the fistula (73), possibly due to loss of arborization in receptor endings in these dogs (74).

There is however, one preliminary study showing that the arterial baroreflex may not be responsible for the increase in sympathetic drive. Brandle *et al.* showed that sino-aortic denervation of paced dogs resulted in the same increase in plasma norepinephrine, compared to paced dogs without denervation. Also, there were no differences in hemodynamics and the progression of heart failure in both groups. Thus, the elimination of the baroreflex resulted in the same sympathetic response to heart failure, suggesting that the baroreflex is not the sole mechanism for the increase in sympathetic drive in heart failure (75).

The vascular α and β adrenergic receptor during heart failure

The arterial vasoconstriction in different vascular beds is mediated by a mixed population of post-junctional vascular adrenoreceptors, mainly of the α1 subtype (76, 77). The α2 receptors are also located extrajunctionally, where they serve as the receptors activated by spill over norepinephrine (78, 79). The density of α1 and α2 receptor populations vary widely within different organs and vascular beds. In most species, large conduit arteries mainly possess the α1 receptor, while distal arteries may have equivalent or higher numbers of α2 receptors (80, 81). Presynaptic α2 receptors, however can inhibit norepinephrine release and blunt the vasoconstricting response by attenuating norepinephrine release (80).

Studies on changes in the vascular α-adrenergic system during heart failure are

limited. Goldsmith *et al.* infused norepinephrine in patients with heart failure. Plasma norepinephrine concentrations increased to 2500 pg/ml without any effects on blood pressure and heart rate. Similar increases in norepinephrine in normal subjects increased blood pressure and decreased heart rate. The investigators concluded that the peripheral α_1-receptor pathway may be partially desensitized in heart failure (82). Angus *et al.* studied skin resistance arteries from patients with congestive heart failure and showed an impaired contraction and relaxation to a variety of agonists, including norepinephrine, angiotensin II and potassium chloride (39). On the other hand, Kubo *et al.* reported that the α2 receptor blocker yohimbine produced comparable increases in forearm blood flow in patients with heart failure in comparison to normal controls (83). A recent study by Indolfi *et al.* showed that phenylephrine (a selective α1 receptor agonist) and BHT933 (a selective α2 receptor agonist) induced equivalent vasoconstriction of the forearm vascular bed in patients with heart failure and in normal subjects (84). Animal studies on the vascular responsiveness to adrenergic stimuli are numerous, but very inconsistent. Much seems to depend on the type of arteries investigated, the studied time point after induction of heart failure and the model used. Results vary from increased sensitivity and responsiveness to α1 receptor stimulation to decreased sensitivity to α2 receptor stimulation in large arteries in the paced dog model for heart failure (85). In rats with experimental heart failure, following the induction of myocardial infarction, Bergdahl *et al.* showed no changes in absolute contractile effect to phenylephrine (α1 receptor agonist) and a decreased responsiveness to α2 receptor stimulation in the large arteries one month after the induction of myocardial infarction (85). Stassen *et al.* also found no alterations in contractile reactivity to phenylephrine and norepinephrine of the thoracic aorta (86). One week after induction of myocardial infarction, however, a hyporesponsiveness of the thoracic aorta was observed by Teerlink *et al.* (35). For the resistance arteries, Feng *et al.* showed a decreased vascular α2 receptor responsiveness (87), while Stassen *et al.* also observed a hyporesponsiveness of the mesenteric resistance arteries for α1 receptor stimulation (86, 88).

The peripheral vasculature also contains β-adrenergic receptors, mainly of the β2 subtype, which mediate vascular smooth muscle cell relaxation (89, 90). A well known feature of heart failure is the downregulation of cardiac β receptors, concomitant with a decoupling of these receptors from their second messenger adenylyl cyclase (91-93). Much less is known about the regulation of peripheral vascular β2 receptors during heart failure. Creager *et al.* infused isoproterenol (β receptor agonist) into patients with heart failure and into normal subjects. They found that maximal forearm blood flow responses to isoproterenol were comparable in both groups (94). Thus there appears to be no desensitization of the peripheral vascular β receptor pathway during human heart failure. In an animal model for heart failure (dogs subjected to cardiac pacing), however, β adrenergic receptor density was significantly decreased in membrane

preparations from mesenteric vessels, without a change in affinity (95).

In summary, data on the status of vascular α and β adrenergic receptors during heart failure are far from complete. All together, the data suggest that heart failure is followed by changes in α or β adrenergic receptor regulation, but alterations seem to be different depending on the species and the vascular bed examined, as well as the time frame used.

Thus, we are left with the apparent paradox of an increase in sympathetic nervous system activity but attenuated responses to sympathetic stimuli such as orthostatic tilt and a depressed baroreceptor function. Vascular α or β adrenergic receptors may change, but data on this topic are still incomplete. Also, other mediators, such as the renin-angiotensin system, may also participate in sympathetic activation during heart failure.

Neurohormonal activation : The natriuretic peptides (a2)

Introduction
In 1981, DeBold *et al.* reported a rapid and potent natriuretic response to intravenous injections of an atrial extract in rats (96). This study formed the basis for the detection of a new peptide family, the natriuretic peptides. It has become clear that this family of natriuretic peptides plays an important role in cardiovascular homeostasis, since its three members, atrial natriuretic peptide (ANP), brain natriuretic peptide (BNP) and C-type natriuretic peptide (CNP), exhibit natriuretic-diuretic, vasorelaxing and other functions operative in lowering blood pressure and the control of electrolyte homeostasis (97).

All three natriuretic peptide are first synthesized as pre-pro-hormones, which are cleaved and activated to yield the biological active peptides (98). The heart is the major source of both plasma ANP and BNP. In the atria, 1-3% of all mRNA transcripts is ANP mRNA (99). The ANP levels in the ventricles are 50-100 fold lower than in the atria, but ventricular ANP synthesis is increased during cardiac development and disease (100). In the latter situation, it becomes the principal source of plasma ANP, due to the greater mass of the ventricles (101). The level of atrial BNP is 4% of that of ANP, while the BNP/ANP ratio is approximately 30% in the ventricles (102). CNP is predominantly found in the brain and only very little CNP is found in the heart (100). Recently, it was shown that vascular endothelial cells produce CNP in vitro (103) and the concept of CNP as a 'vascular natriuretic peptide system' has been proposed (103, 104).

Biological actions of natriuretic peptides
The biological actions of the natriuretic peptides are mediated through specific high-affinity receptors, which are divided into two major subtypes, and activate (R1) or not (R2) activate guanylyl cyclase (GC). The R1 receptors are subdivided in GC-A (high affinity for ANP) and GC-B (high affinity for BNP and CNP). The R2

receptors (equal affinity for ANP, BNP and CNP) account for 90% of the natriuretic receptors on vascular smooth muscle and endothelial cells and were first regarded as clearance receptors, since they were not coupled to GC and had little natriuretic effect. Subsequent research, however, showed that they were coupled to the adenylyl/cAMP signal transduction system and are more than just clearance receptors (105).

Both ANP and BNP possess potent natriuretic and diuretic effects through actions on the kidney. ANP and BNP increase glomerular filtration rate and filtration fraction by relaxation of afferent arterioles and mild constriction of efferent arterioles, which lead to an increase in glomerular capillary pressure (97, 106). In addition to their renal effect, the natriuretic peptides control blood pressure via their reducing effects on cardiac output (105) (by reducing central venous and left atrial pressure (107)) and relaxing effects on vascular smooth muscle cells (108, 109). Other evidence for the important role of the natriuretic peptides on blood pressure regulation comes from a significant correlation between plasma ANP levels and blood pressure variations throughout the day (110).

As mentioned above, CNP may be a more vascular peptide than ANP and BNP. CNP has only minor natriuretic diuretic activity (111, 112). Infusion of CNP into dogs caused a more pronounced decrease in blood pressure when compared to ANP (111), but this effect was not seen in humans (113). It is possible that CNP may be a local regulator of vascular tone and growth as transforming growth factor β and other cytokines increased the mRNA-expression of CNP in endothelial cells (103, 104).

Natriuretic peptides during heart failure

During heart failure, plasma concentrations of ANP (114-117) and BNP (114, 118-120) increase in proportion to the clinical severity of the syndrome (51, 120). Also, ANP secretion is no longer limited to the atria, but also involves the ventricles (121, 122). Plasma CNP concentrations are normally low and do not seem to increase during heart failure (114).

Infusion of ANP or BNP in humans and animals with heart failure results in a reduction of systemic vascular resistance, enhanced natriuresis and diuresis, inhibition of the activity of the renin angiotensin system and an improved exercise performance (123-126). Several studies however also show attenuated responses to ANP infusion (124, 127-129). In fact, the elevated plasma concentrations are at odds with the tendency of patients and animals with heart failure to retain salt and water. One explanation for this apparent discrepancy is a generalized hyporesponsiveness to the effects of ANP/BNP during heart failure. Hirooka *et al.* showed an attenuated forearm vasodilatory response to intra-arterial ANP infusions in patients with severe heart failure (130). Kubo *et al.* demonstrated a maintenance of vasodilatory response to ANP in the same experimental setting, but their patient group had less severe heart failure (131).

Abassi *et al.* showed the same trend in rats with experimental heart failure due to an aorta-caval fistula. Severely decompensated rats showed progressive sodium retention and edema formation, whereas compensated rats did not. The natriuretic response to exogenous ANP was attenuated only in rats with decompensated heart failure. It was suggested by this group that the renal effects of ANP were overridden by an activation of the renin-angiotensin system in the severely decompensated, sodium-retaining state, as administration of the angiotensin type I-receptor antagonist losartan restored the natriuretic effects of ANP in severely decompensated rats (132). Another explanation may be the possible downregulation of ANP-receptors under high concentrations of ANP (133, 134). Tsutamoto *et al.* showed that the molar ratio of cGMP production to ANP extraction in the peripheral circulation (femoral vascular bed) was significantly lower in patients with severe congestive heart failure and suggested that this may be related to a guanylate cyclase-coupled receptor down regulation (127).

In conclusion, although the natriuretic peptides are a potential counter-regulatory system for the natriuretic and vasoconstricting state seen during heart failure, the relative attenuation of renal responsiveness and the possible downregulation of ANP-receptors may be factors contributing to the development of decompensated heart failure.

Neurohormonal activation : Endothelium derived vasoactive factors (a3)

The endothelium of blood vessels plays an important role in the regulation of vascular tone. A number of vasoactive substances produced by endothelial cells has been identified, not only relaxing factors such as nitric oxide (NO) and prostacyclin, but also contracting factors such as endothelin, and cyclooxygenase products (thromboxane A_2, prostaglandin H_2 and superoxide anions) (135, 136). In this outline, endothelin (a3-1), NO (a3-2) and their effects during heart failure are discussed, as it has become clear that imbalances in the production and action of these factors could contribute to the pathophysiology of heart failure.

Endothelium derived vasoactive factors, the endothelins (a3-1)
The endothelins are a family of three related peptides, endothelin (ET) 1, 2 and 3, each 21 amino acids large, of which ET-1 is the best known. ET-2 and ET-3 are closely related to ET-1 (80% homology), with ET-1 and ET-2 being the more potent vasoconstrictors (137). The source of ET-3 is unknown, but may be either neural or endocrine derived. ET-2 has not been detected in human plasma and appears to be synthetised predominantly in the kidney and intestine (138).

ET-1 produces potent and sustained vasoconstrictor and vasopressor actions in both humans and animals. Exposure of vascular endothelial cells to a variety of stimuli, such as adrenaline, thrombin (139), angiotensin II (133), cytokines or

shear stress (138) causes increased transcription of prepro-ET-1, which is cleaved to yield big ET-1 (140). Big ET-1 is then cleaved to ET-1 by several isoforms of a membrane bound neutral metalloproteinase, endothelin-converting enzyme (ECE) (141). Vascular smooth muscle cells also produce ET-1 in vitro, but at a significantly lower rate than endothelial cells. The relatively greater mass of smooth muscle cells in large arteries probably makes them a major contributor to the local production of ET-1 (142). Plasma concentrations of ET-1 are substantially lower than the concentrations needed to produce direct vasoconstriction in vivo (143). In addition, vascular endothelial cells release more ET-1 towards the basement membrane (abluminally) than luminally (144). Thus in the normal situation, ET-1 appears to be a locally acting paracrine hormone instead of a circulating endocrine hormone.

Biological actions of ET-1

There are several specific high affinity binding sites for the ETs. The ET-A receptor, is expressed in human aorta (vascular smooth muscle cells), lung, heart and kidney (145) and the ET-B receptor is mainly expressed in the cerebral cortex, cerebellum and endothelial cells (146), but also in human vascular smooth muscle cells (147). The ET-A receptor has a high affinity for ET-1 (binding potency ET-1>ET-2>>ET-3), the ET-B receptor has equal affinity for all three ETs (138). The ET-A receptor appears to be the major receptor mediating arterial vasoconstriction, while the ET-B receptor mediates the release of endothelium-dependent vasodilating substances. In some resistance and large conduit arteries, the ET-B receptor may also contribute to the vasoconstriction (138, 148), but this markedly depends on the species, vessel type and vessel size (147, 149).

ET-1 produces a long-lasting contraction of larger arteries (139) and contributes to basal vascular tone in man (148). Additionally, infusion of ET-1 in man increases the blood pressure (150). ET-2 and ET-3 also constrict isolated large arteries, although ET-3 is less potent (137).

The vasoconstrictor effect of the endothelins is preceded by an endothelium-dependent transient vasodilation, although this response may be more pharmacological than physiological and does not seem to occur in conditions in which the ET concentration gradually rises, such as after administration of proendothelins (151). Besides its vascular effect, ET-1 has a potent positive inotropic effect (152).

ET-1 also interacts with other local mediators of vascular tone. The ETs stimulate nitric oxide (NO) production in isolated vessels (153) suggesting that there is an autocrine feedback mechanism to modulate the vasoconstricting properties of ET-1. ET-1 infusions also increase circulating ANP in rats and dogs (154, 155), but not in humans (150) and ET-1 potentiates the volume-induced cardiac secretion of ANP (156). In turn, ANP can, like NO, dose-dependently relax ET-1 precontracted vascular strips (157). Also, the production of ET-1 is inhibited by

ANP and NO (158, 159).

Endothelins during heart failure
Plasma ET-1 concentrations are increased in animal models of heart failure (160-164) and patients with heart failure (165-168). ET-1 plasma concentrations correlate with the clinical severity of the syndrome (165-167) and were shown to be a prognostic indicator of mortality (169). However, there is some reservation about the meaning of these correlations as several authors indicated that plasma big ET-1 concentrations are also increased during heart failure (167, 168, 170). Wei *et al.* showed that the increase in plasma ET is mainly due to an increase of big ET-1, which is less biologically active. The authors also showed that the increase in total circulating ET is a late phenomenon in human congestive heart failure and correlates with cardiac dysfunction only in the moderately and severely symptomatic heart failure (167). There are at least two studies, showing a relationship between plasma ET-1 and pulmonary, but not central hemodynamics (e.g. cardiac index, total peripheral resistance) (171, 172). One of these studies also indicated that the main source of circulating ET-1 is increased ET-1 spillover in the pulmonary vascular bed (172). Although these studies suggest that ET-1 is simply a marker of heart failure, there are several other studies contradicting this assumption. Pacher *et al.* recently showed that plasma big ET is a better prognostic indicator of mortality in patients with severe congestive heart failure than hemodynamic variables and plasma levels of ANP (170), and several studies showed that plasma ET-1 levels were not normalized after cardiac transplantation (173, 174). Also, Kiowski *et al.* showed evidence that the high ET-1 plasma concentrations in patients with heart failure contribute to the maintenance of vasoconstriction (175). Studies with ET-1 antagonist also indicate that ET-1 could be more than a marker of heart failure. Sakai *et al.* demonstrated that long term administration of an ET-A receptor antagonists to rats with myocardial infarction improved survival and cardiac function (176).

In conclusion, an elevation in plasma ET-1 is seen in both human and experimental heart failure. It is not yet clear whether this increase is just a marker for heart failure or is of pathophysiological importance.

Endothelium derived vasoactive substances, nitric oxide (a3-2)
In 1983, Furchgott and Zawadzki were the first to describe the obligatory role of the vascular endothelium in the vasodilating response by acetylcholine. In isolated rabbit aorta segments, precontracted with norepinephrine, acetylcholine produced a relaxation but only if the endothelium was present (177). Subsequently, in 1987 Palmer *et al.*, showed that nitric oxide was (one of) the factor(s) responsible for this endothelium-derived relaxation (27).

The radical NO, with a half live of only 3-5 sec, is generated by oxidation of one of the terminal guanidine nitrogen atoms of the amino acid L-arginine (178). The

reaction is catalyzed by NO-synthase (NOS) of which three isoforms, the endothelial constitutive (ecNOS), the neuronal (nNOS) and the inducible (iNOS) have been identified to date. These NOS's were isolated from vascular endothelial cells, neurons and cytokine-activated macrophages, respectively (179) but the three isoforms are now thought to be present in many tissues and cells, which are also able to express more than 1 isoform (180).

Although expressed in other cells, expression of ecNOS is predominantly found in vascular endothelial cells (179). ecNOS is responsible for a continuously low basal release of NO from the endothelium (181). Next to this basal release, both receptor-dependent and receptor-independent mechanisms enhance the release of NO. Among the receptor-dependent factors are acetylcholine and bradykinin, while factors such as shear stress and hypoxia are receptor-independent (182).

iNOS can also be expressed in a number of cells, including macrophages (183) and vascular smooth muscle cells (184-186). iNOS is activated by inflammatory cytokines such as tumor necrosis factor and interleukin 1 (178). In contrast to ecNOS, iNOS is calcium independent due to the presence of a calmodulin-unit in the iNOS enzyme (187).

Biological actions of NO

The intravenous administration of analogues of L-arginine which competitively inhibit NO synthase, rapidly increases the arterial blood pressure in animals (188-191). This pressor effect is the result of an inhibition of the basal release of NO from the endothelium, indicating that this basal release of NO is important for the regulation of basal vascular tone and blood flow (29, 192).

Another important feature of NO is its effect on myocardial function and contractility. The myocardial endothelium regulates cardiac contractility (193, 194) and various studies have suggested a negative inotropic effect of NO or its intracellular messenger cGMP (195-197), although other studies show that physiological concentrations do not exert a major regulatory effect on myocardial contractility (198). The inotropic effects of NO may depend on its concentration (positive inotropy after low concentrations and negative inotropy after high concentration) (199, 200).

As for the interactions of NO with other hormonal systems, NO is a negative modulator of ANP secretion (201) and down-regulates the ANP R2 receptor (202). NO is believed to mediate the initial vasodilatory response to ET (203). On the other hand, NO can suppress the production of ET-1 (158) and reduce the affinity of the ET receptor for its ligand (204).

NO during heart failure

Changes in NO metabolism have been linked to the endothelial dysfunction seen during heart failure. Thus, the stimulated release of NO from the endothelium by agonists such as acetylcholine is impaired in animals and patients with heart failure, whereas the basal release is intact or may be enhanced. Recently, Habib

et al. infused an inhibitor of NOS, N^G-monomethyl-l-arginine (L-NMMA) in patients with heart failure and observed that the increase in systemic vascular resistance in response to L-NMMA was greatest in those patients with heart failure who had the highest basal systemic vascular resistance. This suggests that these patients have an enhanced basal production of NO (205). In addition, Winlaw *et al.* measured plasma nitrate levels, as a stable end-product of NO production, and showed significant increases in patients with heart failure (43). Plasma nitrate levels correlated with the clinical severity of heart failure (44). It should be noted that the study of Habib *et al.* only included a small number of patients (n=8). Also there is still debate whether plasma nitrate is really a good reflection of the actual NO concentrations in plasma.

Recent reports also show changes in the expression of the various NO synthases during heart failure. Haywood *et al.* found increased expression of iNOS mRNA in the ventricular myocardium of patients with idiopathic dilated cardiomyopathy and in patients with ischemic, and valvular heart disease. The myocardium of these patients also showed diffuse staining with an iNOS-antibody, confirming the presence of iNOS protein (206). Habib *et al.* confirmed the presence of immunoreactivity of iNOS in the hearts of patients with dilated cardiomyopathy and ischemic heart disease (207). DeBelder *et al.* showed increased activity of the iNOS and ecNOS in ventricular tissue samples of patients with dilated cardiomyopathy (208). However, Thoenes *et al.* failed to show iNOS-protein expression (by immunoblotting) in patients with idiopathic dilated cardiomyopathy and ischemic heart disease, whereas iNOS-expression was increased in failing hearts of septic patients (209). Recently, Patel *et al.* showed a decreased expression of nNOS in the hypothalamus and brainstem of rats with a myocardial infarction. The authors hypothesized that the resulting decrease in NO production in these brain areas could be associated with the increased sympathetic activity during heart failure (210).

In the vasculature, Smith *et al.* showed a reduced expression of the ecNOS gene in the vascular endothelium of the thoracic aorta of dogs with heart failure due to ventricular pacing (211). Comini *et al.* also showed a reduced ecNOS protein expression in the endothelium in the aorta of rats with heart failure (due to pulmonary hypertension), but an increase of ecNOS protein expression in the smooth muscle layer. The authors suggested that there may be a shift in ecNOS protein expression from the endothelium towards the underlying smooth muscle layer (212).

If the basal release of NO is enhanced during heart failure, it may represent in concert with ANP, a counter-regulatory mechanism to the vasoconstrictive forces of ET-1, norepinephrine and angiotensin II. The impaired stimulated release of NO may attenuate the above mentioned beneficial effects of NO during heart failure.

Neurohormonal activation : The renin-angiotensin system (a4)

Introduction
Traditionally, the renin angiotensin system (RAS) is regarded as a circulating hormonal system, important in blood pressure control and sodium and potassium homeostasis. Recently, however, components of the enzymatic RAS cascade have been identified in a variety of tissues such as the brain, kidney, reproductive tract, heart and blood vessels (213-216). It is thought that this tissue RAS substantially controls blood pressure, as well as structure and function of heart and vessels (215, 217, 218).

The circulating renin-angiotensin system
The first reaction in the renin-angiotensin cascade is the cleavage of liver-derived angiotensinogen by renin in the plasma, whereby angiotensin I (ANG I) is released. The angiotensin-converting enzyme (ACE) catalyzes the cleavage of ANG I into the vasoactive substance angiotensin II (ANG II). ACE is widely distributed in organs and vascular endothelium. In physiological conditions, a major site for the conversion of circulating ANG I is the lung (219, 220).

The RAS is activated through an increased secretion of renin from the kidneys. The secretion of renin from the juxtaglomerular cells is triggered by renal hypoperfusion, by low sodium loading to the macula densa region of the renal tubuli and by an increase in renal sympathetic tone induced by baroreceptor unloading in response to arterial hypotension or hypovolemia (219, 221).

Functions of ANG II
The most important physiological effects of ANG II are listed in table 1.1. The main function of ANG II is maintenance of blood pressure and electrolyte homeostasis. ANG II increases the blood pressure in several ways. ANG II induces a systemic vasoconstriction, resulting in a pressure increase. In the kidneys, it constricts the efferent and (to a lesser extent) the afferent arterioles; as a result the glomerular filtration pressure will increase; concomitant reduction of the glomerular filtration coefficient results in a net decrease of the glomerular filtration rate. At the same time, it acts on the renal proximal tubules to increase sodium reabsorption. Additionally, ANG II stimulates the adrenal cortex to secrete aldosterone, which acts to retain sodium in the distal nephron, in exchange for potassium. The sodium retention is associated with water retention, thereby increasing whole body fluid volume and increasing arterial blood pressure (219). Finally, ANG II is a potent growth-factor of various cell types, such as cardiac myocytes, endothelial cells and vascular smooth muscle cells (222, 223). Infusion of ANG II in normal rats induces a hypertrophic response in peripheral arteries (224-226) even at a non-hypertensive dose (227, 228).

Table 1.1   Functions of ANG II

Function of ANG II	Physiological effect	Reference
A. Systemic vasoconstriction ↑	Blood pressure ↑	(232, 233)
Vasoconstriction in the kidney ↑	Renal flow and GFR ↓ and sodium retention	(219)
Aldosterone secretion ↑	Sodium retention and kaliuresis	(219, 232)
Renin release ↓	Negative feedback on ANG II release	(232)
Sympathetic activity ↑	Increased noradrenaline release	(64, 231)
B. Stimulation of growth	Hypertrophy of e.g. VSMC, endothelial cells, cardiac myocytes	(222, 223)
	Extracellular matrix synthesis	(229, 230)

↑ = stimulation, ↓ = inhibition, GFR = Glomerular filtration rate, VSMC = vascular smooth muscle cell

Interactions with other neurohumoral systems

There are extensive interactions between the RAS and other neurohumoral and blood pressure controlling systems, including the sympathetic nervous system. ANG II resets the baroreflex (reviewed in references 64 and 234) and increases the release of epinephrine and norepinephrine (64, 231). Additionally, ANG II may act centrally to enhance sympathetic output (235). Local renal ANG II formation may also enhance the action of the sympathetic nerves through pre-synaptic mechanisms (236) and the sympathetic nervous system is a regulator of renin release (237).

The interaction between the sympathetic nervous system and ANG II in peripheral hemodynamics is still unclear. Hilgers *et al.* reported that neither ACE-inhibition nor ANG II receptor antagonism affected the pressor response to nerve stimulation in rats (238). Kline *et al.* showed that rats with hypertension induced by chronic ANG II infusion had similar norepinephrine turnover rates in several peripheral organs, suggesting these rats do not have enhanced sympathetic tone (239). Also, selective α1 receptor blockade in rats with ANG II induced hypertension did not decrease the blood pressure (240, 241). One study found no interaction between ANG II and the sympathetic nervous system in vasoconstrictor reflexes of the (human) forearm (242). Other studies however, did suggest an interaction between the two systems. Lyons *et al.* showed that the vasoconstrictor action of exogenous ANG II is sympathetically mediated, as

phentolamine (postsynaptic α receptor blockade) attenuated the vasoconstrictor response to infused ANG II into the brachial artery of healthy volunteers (243). Seidelin *et al.* also suggested that ANG II is involved in the sympathetic vasoconstriction in forearm vessels in man (244). Some of the controversy can be explained by the difference between endogenous and chronic infusions of exogenous ANG II. Reid (64) suggested that enhance sympathetic activity does not significantly contribute to the pressor response to exogenous ANG II, while the actions of endogenous ANG II on the sympathetic nervous system enhance the cardiovascular responses elicited by an activation of the sympathetic nervous system.

The endothelium is an important regulator of the activity of the RAS. ET-1 infusions in animals increase renin, aldosterone and adrenaline concentrations (155, 245). Furthermore, NO decreases ANG II binding to cultured vascular smooth muscle cells (246), whereas ANG II can inhibit the cytokine induced expression of iNOS in vascular smooth muscle cells (184).

Another important counterregulatory system of the RAS is formed by the natriuretic peptides. Keinert *et al.* showed that ANP is more effective in antagonizing contractions in rabbit aorta induced by ANG II than those induced by norepinephrine (108). Both in vivo and in vitro, ANP directly reduces basal secretion of renin (247). In humans, ANP inhibits the ANG II stimulated sympathetic nerve activity (248). Also, ANP and BNP inhibit the pressor response to ANG II and blunt the aldosterone response to ANG II in humans (249, 250).

The circulating RAS during heart failure

Activation of the circulating RAS has been shown in patients with heart failure (251, 252), but plasma renin, ANG II levels or ACE-activity may be normal in the compensated or moderate state of heart failure (253, 254). The literature is not consistent on the status of activation of the circulating RAS in experimental heart failure. Most studies show normal plasma levels of plasma renin, ANG II or ACE-activity (255-261), but others demonstrate increased levels of these compounds (256, 262, 263). The discrepancies may be related to hemodynamic changes, elapsed time after induction of heart failure, the use of medication and the severity of heart failure.

The angiotensin receptors

At least two distinct types of membrane bound ANG II receptors have been identified, the angiotensin receptor type I and II (AT1 and AT2). All functions of ANG II listed in table 1.1 are AT1 mediated (264-267). There has been a long-standing discussion on the function of the AT2 receptor. The abundance and location of AT2 receptors in young mice suggest that this receptor is involved in growth and fetal development (268). However, 'knock-out' mice with targeted disruption of the AT2 receptor gene show normal fetal development and growth (269, 270), which questions the putative role of AT2 receptor in growth and

development. Attention has also been focussed on a counterbalancing role of the AT2 receptor opposing the blood-pressure and proliferative effects of the AT1 receptor. Data from the AT2 receptor 'knock-out' mice showed that basal blood pressure was increased and that the pressor response to ANG II was enhanced in these animals, suggesting that the AT2 receptor opposed the pressor responses to ANG II (269, 270). In the kidney, the AT2 receptor may blunt the blood pressure induced sodium excretion (271). Furthermore, the AT2 receptor mediates an antimitogenic effect on rat coronary endothelial cells (272) and R3T3 cells (273) and may mediate apoptosis (programmed cell death) (274). It has been hypothesized that ANG II can only exert its proliferative effects through the AT1 receptor, but only when either the AT2 receptor is absent (as in certain culture conditions) or inactivated (as during pretreatment with an AT2 antagonist) (275).

Both receptor subtypes belong to the seven-transmembrane family of G-protein coupled proteins. Depending on the cell type, the AT1 receptor is coupled to the activation of phospholipase A2, C and D, as well as voltage-dependent Ca^{2+} channels and inhibition of adenylate cyclase (276). In addition, the AT1 receptor is directly coupled to kinases initiating tyrosine phosphorylation cascades, like the MAP kinase cascade and the JAK/STAT pathway (277-279). The AT2 receptor is also coupled to a G protein in the rat fetus (280) and in vascular smooth muscle cells transfected with the intracellular third loop domain of the AT2 receptor (281). Siragy and Carey have shown that the AT2 regulates renal cyclic guanosine 3', 5'-monophosphate (cGMP) in the rat kidney (282).

AT-receptors in the cardiovascular system
Binding studies have identified the AT1 and AT2 receptor in heart tissue. Sechi *et al.* showed that both receptors were widely distributed throughout the rat heart, with each receptor subtype accounting for approximately 50% of the specific binding (283, 284). In the human heart, receptor density decreases from the right atrium to the left ventricle, with the AT1 receptor accounting for 60% in both chambers in one study (285, 286). A study from Regitz-Zagrosek *et al.* showed a predominance of the AT2 receptor in the normal human right atrium (287).
The aortic wall of a normal adult rat contains the AT1 and AT2 receptor subtypes in a 4:1 ratio (288, 289).

AT receptors in the cardiovascular system during heart failure
In humans, Regitz-Zagrosek *et al.* showed a significant loss of cardiac AT receptors in end stage, but not in moderate heart failure. The loss of receptors was equal for both receptor subtypes (287). The group of Wang and de Gasparo, however, found a selective downregulation of the AT1 receptor, but not the AT2 receptor in the hearts of transplant recipients (285). Rogg *et al.* recently reported a decrease in AT1 receptor density and an increased density of the AT2 receptor in atrial tissue in patients with valvular or coronary artery disease (290). Thus, in

human heart failure, there appears to be a downregulation of the AT1 receptor. The data on the AT2 receptor is controversial, but this may be related to the type of cardiac tissue examined (e.g. atrial versus ventricular) or the stage of heart failure. In animal studies, rats with cardiac hypertrophy due to aortic banding showed AT1 receptor downregulation (291) as did rats with experimental heart failure due to volume overload (aorta caval fistula) (292). Most studies in rats with experimental heart failure due to coronary artery ligation, however, show an upregulation of cardiac AT1 and AT2 receptors (284, 293-295).

There are no studies describing possible changes in the AT receptors in the peripheral vasculature during heart failure.

Local renin-angiotensin systems

The first indication for the existence of a local-RAS came from a study by Unger *et al.* who observed a prolonged hypotensive response in rats after withdrawal of chronically administered ACE-inhibitors, despite the early return of plasma ACE activity to normal values (296). Subsequent evidence for a local-RAS in the peripheral vasculature was derived from the detection of renin, angiotensinogen and ACE in the media and adventitia of rat aorta (213, 297, 298), allowing the local production of ANG II. Campbell and Habener quantified the amount of angiotensinogen mRNA in rat aorta and calculated that the aortic concentration was approximately one-third of the concentration in the liver and significantly higher than that found in the kidney, heart, adrenals or brain (299). ACE concentrations vary markedly in different arteries. Jandeleit *et al.* showed that the highest concentration of ACE is found in homogenates from mesenteric resistance arteries, with lower concentrations in large conduit arteries, such as the aorta (298).

A role for the local vascular RAS in the regulation of peripheral vascular tone and blood pressure has been proposed (215, 300-302). The perfused rat hindlimb releases ANG II under basal conditions (303). In this preparation, it was also shown that ANG II could be generated from a tetradecapeptide renin substrate in the absence of circulating renin (302, 304). In humans, local inhibition of ACE in the forearm did not change resting blood flow in volunteers on a normal sodium diet. When the subjects were sodium-depleted, local inhibition of ACE did cause an increase in forearm blood flow. Thus, salt depletion activated not only the circulating RAS but also the local vascular RAS (305). Whether this local vascular RAS is as effective as the circulating RAS is not entirely clear. Vicaut and Hou showed that, in the perfused rat cremaster muscle, circulating renin and angiotensinogen were more effective in inducing arteriolar constriction, than local renin and angiotensinogen (306).

The local RAS of the cardiovascular system during heart failure

The circulating RAS is mainly activated in the acute phase of heart failure (e.g. shortly after myocardial infarction) (252). In compensated or mild heart failure,

Figure 1.1 : Relative contributions of tissue and circulating RAS during heart failure (217)

Clinical State of Heart Failure
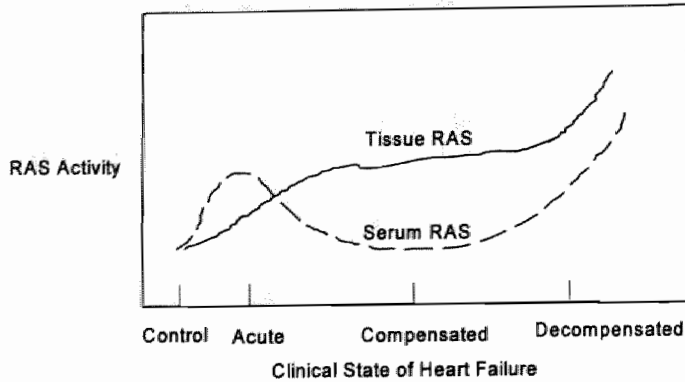
normal or near-normal plasma renin activities and ANG II values have been reported (253, 254). Nevertheless, ACE-inhibition has long term beneficial effects on cardiac remodelling and function in animals and patients with heart failure (307, 308). It has been hypothesized by the group of Dzau that the circulating RAS serves to maintain circulatory homeostasis during acute cardiac failure, while the tissue RAS contributes to homeostatic responses during chronic sustained impairment of cardiac function (217, 309) (see figure 1.1).

Several studies show the activation of cardiac RAS during heart failure. Expression of the ACE-gene was increased in the left ventricle in patients with heart failure (310). Animal studies using rats with myocardial infarction also showed an upregulation of cardiac ACE and renin mRNA and ACE activity (258, 311, 312). In rats with heart failure due to volume overload, similar increases in cardiac mRNA expression for ACE and renin were observed (292).

There is only one study on changes of the components of the vascular RAS during heart failure; Hirsch et al. showed no changes in ACE mRNA expression in the thoracic aorta of myocardial infarcted rats (258).

Possible mechanisms : Vascular structural alterations (b)

The relation between vascular smooth muscle tone and vascular smooth muscle proliferation

It is important to recognize that the activated neurohormonal systems during heart failure not only regulate vascular tone but also influence proliferation of cells in the vascular wall. The relationships which exist between ANG II, ET-1, NO and ANP in the regulation of vascular tone, also exist for the regulation of vascular growth. Thus, ANG II is a vasoconstrictor and a potent growth-factor for several cardiovascular cell-types, even at low, non-hypertensive doses (222-224, 226-228). Norepinephrine induces polyploidization in vascular smooth muscle cells in vitro (313) and infusion of catecholamines increases medial cross-sectional areas in vivo in the rat (314). ET-1 also regulates DNA and protein synthesis,

expression of protooncogenes (c-fos), cell proliferation and hypertrophy in different celltypes (e.g. cardiomyocytes and vascular smooth muscle cells (315-317)). Also, ET-1 acts synergistically with other growth-factors, including platelet-derived-growth-factor and transforming growth factors α and β to potentiate cellular proliferation (reviewed in reference 317).

Vasodilators, such as NO and ANP inhibit vascular endothelium and smooth muscle cell proliferation. Thus, for NO, studies have shown that both exogenous nitrogen vasodilators and inducers of endogenous NO such as interferon-γ, decrease vascular smooth muscle cell proliferation (318, 319). These studies also show that cell proliferation correlated inversely with cyclic GMP (cGMP), making guanylate cyclase and cGMP important second messengers in the antiproliferative effect of NO (320, 321). Furthermore, NO's antimitogenic effects on vascular smooth muscle cells can be modulated by several growth factors. Platelet-derived-growth-factor decreases the induction of NOS and thus the antimitogenic effects of NO, while basic fibroblast growth factor enhances induction of NOS and promotes the antimitogenic effect of NO (320).

The natriuretic peptides are also important counterregulators of the hypertrophic effects of ANG II. The group of Dzau *et al.* showed that ANP is a potent inhibitor of endothelial and vascular smooth muscle cell growth induced by serum, transforming growth factor ß and ANG II (322, 323). This anti-proliferative effect is thought to be mediated by the R2-receptor (324). In vivo, infusions of non-hypotensive doses of ANP reduced the structural remodelling (e.g. increase in medial thickness) of the thoracic aorta of spontaneously hypertensive rats (325).

More recently, it was shown by the group of Suga *et al.* that various cytokines, such as transforming growth factor β and tumor necrosis factor, both known as potent vascular smooth muscle cell growth-factors, increase endothelial production of CNP (103, 104) and this vascular natriuretic peptide system may play an important role in the vascular growth interaction of endothelial and smooth muscle cells (326).

Vascular structural changes during heart failure
Vascular structural changes or vascular remodelling have received much attention in hypertension research. Well known is the hypothesis of Folkow (251), that wall thickening in resistance arteries of patients with hypertension takes place at the expense of the lumen. Histological data suggests that vascular remodelling leads to a rearrangement of the tissue mass around a smaller lumen. Thus, cross-sectional areas of normotensive and hypertensive vessels may be the same, in spite of a decrease in both internal and external diameters (327).

On the basis of early work of Zelis *et al.* (328), in which an increase in the arterial vascular sodium content was shown during experimental heart failure, a 'vascular stiffness' component was suggested to explain the reduced maximal vasodilatory response in heart failure. Subsequently, Sinoway *et al.* showed that

in patients with heart failure diuretic therapy enhances metabolic vasodilation during exercise. Approximately one-third of the reduced vasodilation could be attributed to increased sodium and water content (329). However, structural alterations may also be involved. Again, Zelis et al. reported increased basement membrane thickness of skeletal muscle capillaries in patients with heart failure (330). Wroblewski et al. also reported microangiopathic alterations in patients with heart failure, resulting from hyalinosis of the basement membranes of terminal skin arterioles (331, 332). Lindsay et al., however, measuring diameters, cross-sectional areas and endothelial cell areas of these skeletal capillaries, did not find differences between patients with heart failure and normal subjects (333).

Other studies reporting vascular structural changes are limited and mainly consist of measurements of arterial diameters. Arnold et al. showed decreased brachial artery diameters in patients with heart failure (18), while Gabella et al. also reported a decrease in media thickness of a large conduit artery (carotid artery) in rats with myocardial infarction due to coronary artery ligation (14). In contrast, reports on structural changes in the resistance arteries are controversial. In myocardial infarcted rats, Schieffer et al. reported an increase in the medial thickness of resistance arteries of the skeletal muscle bed after 1 year (334). However, in the same model and time frame, there were no changes in medial cross-sectional areas of the femoral and mesenteric resistance arteries (335, 336).

In conclusion, studies on changes in vascular structure during heart failure are limited, but do point to alterations in diameters of large conduit and resistance arteries during heart failure.

Hypothesis

Peripheral vascular abnormalities during heart failure consist of reduced aortic compliance, excessive peripheral vasoconstriction and endothelial dysfunction. To date, there are few data available on the magnitude of and mechanisms leading to these peripheral vascular abnormalities in heart failure. There is indirect evidence for structural vascular alterations in heart failure, which could explain, at least in part, the observed peripheral functional vascular changes. Since changes in vascular structure depend among others of the balance between growth-stimulating and growth-inhibiting factors, the increased concentrations of several vaso-active agents with different growth-regulating properties could have an important impact on the peripheral vascular structure during heart failure.

Our working hypothesis was that peripheral vascular structural alterations do develop during heart failure and that a resetting of the neurohumoral balance of vasoconstrictors/growth stimulators and vasodilators/growth inhibitors plays an important role in the development of peripheral vascular structural alterations. In

view of the increased activity of the RAS, the well-known beneficial effects of ACE-inhibitor therapy, the recently observed abnormalities in NO-related endothelial dysfunction during heart failure and the interaction between the RAS and NO, we focussed on the potential roles of ANG II and NO on peripheral structural vascular alterations.

This thesis

In this thesis we studied the development and possible underlying mechanisms of peripheral vascular alterations in experimental heart failure by measuring several morphometric parameters of peripheral vessels (e.g. cross-sectional area, diameters and media-to-lumen ratio) in a rat model for experimental heart failure. The role of neurohormones was investigated by using pharmacological interventions.

In chapter 2, the development of peripheral vascular structural changes of several large conduit arteries and resistance arteries was studied in rats with myocardial infarction and compared to sham operated rats. In chapters 3, 4 and 5, several pharmacological interventions were used to study the possible role of neurohormones. In chapter 3, the hemodynamic and structural effects of ANG II were studied in myocardial infarcted and sham operated rats. In chapter 4, the role of angiotensin receptors was analysed by co-infusion of ANG II and AT1 and AT2 antagonists and in chapter 5, the possible role of NO was examined by infusing an inhibitor of NO synthase. The findings of these studies are discussed in chapter 6.

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Peripheral Vascular Alterations during Experimental Heart Failure in the Rat. Do They Exist?

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Abstract

Background: Structural changes of the peripheral vascular component as seen during hypertension and atherosclerosis have been suggested during heart failure but have never been reported. Therefore, we studied possible structural alterations in the peripheral vasculature in an experimental model of heart failure, induced by ligation of the left coronary artery in rats.

Methods and results: Both conduit and resistance type arteries were excised at 1, 3, 5 and 12 weeks after induction of a myocardial infarction or sham surgery. Vessel dimensions (medial cross-sectional area, internal and external diameter and media-to-lumen ratios) as well as medial collagen and elastin volume fractions were measured by computerized morphometry. The hydroxyproline assay was used to determine collagen and elastin content biochemically. In separate groups of rats, peripheral tissue flows were measured using radioactive microspheres 5 and 12 weeks after myocardial infarction. To evaluate the effects of the degree of heart failure, rats of the 12 weeks group (n=10) were subdivided in subgroups of moderate (<45% infarct size) and large (>45% infarct size) infarction. At all time points, body weights of sham-operated and myocardial infarcted (MI) rats were comparable. Lung weights of MI rats were increased proportional to infarct size. No major changes in vessel dimensions were seen at earlier time points. 12 Weeks after coronary artery ligation, significantly smaller medial cross-sectional areas were observed in several large conduit arteries as the thoracic aorta, carotid artery and superior mesenteric artery. These changes coincided with reductions in both internal and external diameters. In contrast, internal and external diameters of mesenteric and pulmonary resistance arteries were increased after 12 weeks of coronary artery ligation. Collagen and elastin volume fractions of large conduit arteries were comparable in both sham-operated and MI rats during the entire experimental period. This latter observation was confirmed by the results of the hydroxyproline assay, which showed no differences in collagen and elastin content. In general, no major changes could be observed in absolute blood flow to peripheral tissues measured in resting conditions, at 5 and 12 weeks after coronary artery ligation.

Conclusions. The data indicate that myocardial infarction induces diverse adaptive changes in peripheral arteries. Medial cross-sectional areas, internal and external diameters of large conduit arteries of MI rats are smaller 12 weeks after the operation compared to sham-operated rats. These parameters show a tendency to increase in resistance type arteries.

Introduction

Changes in structure of the vascular wall have received ample attention in hypertension research. Increases in wall thickness have been reported both in large conduit arteries and resistance arterioles (reviewed in 1 and 2). This increase in wall thickness is the result of hypertrophy (in large vessels) or hyperplasia (in small vessels), as well as changes in extracellular matrix content. Both absolute collagen and elastin contents increase (3), without a change in relative volume fractions, indicating little change in the composition of the vascular wall (4). Structural changes of the vessel wall are also seen in other cardiovascular diseases such as atherosclerosis (5) and restenosis after balloon angioplasty (6). The magnitude and role of possible structural changes in another cardiovascular disease, heart failure, are unknown.

Functional peripheral vascular alterations have been reported both in patients and in animal models for heart failure and consist of an increased resistance due to excessive vasoconstriction (7, 8). Maximal vasodilator capacity of various peripheral vascular beds is decreased (9, 10), probably caused by increased vascular stiffness, due to increased arterial sodium content (11). Distensibility of the aorta is also markedly reduced in patients with coronary artery disease (12). These functional vascular changes are thought to be partly related to the observed reduction in blood flow to peripheral tissues in rest and during exercise (13, 14).

Analogous to what is seen during hypertension, structural remodeling has been suggested as a possible mechanism for the above described changes in vascular resistance and distensibility during heart failure (reviewed in 15 and 16). However, there are, to our knowledge, no studies documenting such structural changes. Also, data on the time-course of possible structural changes in the vasculature during heart failure are lacking.

In this study we determined possible structural vascular alterations in experimental heart failure in the rat produced by coronary artery ligation. At specified times, several large conduit and resistance arteries were excised and vessel dimensions (including medial cross-sectional areas, internal and external diameters and media-to-lumen ratios) and collagen and elastin volume fractions were measured using histological staining methods and computerized morphometry. The hydroxyproline assay was used to determine collagen and elastin content biochemically. This study provides evidence for structural alterations of peripheral arteries in an experimental model of heart failure, which were, however, only present at the later time points examined and showed a heterogenous pattern. While the medial cross-sectional area and internal and external diameters of several large conduit vessels were smaller in MI rats, these parameters showed at least a tendency to increase in 2 resistance type arteries. There were no major quantitative changes in the extracellular matrix within the experimental time span.

Materials and Methods

Animals
Male Wistar rats (290-320 g, Winkelmann, Borchen, Germany) were housed under standard conditions and fed standard rat chow (RMH-TM, Hope Farms, Woerden, The Netherlands) and water ad libitum. The experiments were performed in accordance with local institutional guidelines.

Coronary artery ligation
Myocardial infarction (MI) was induced by ligation of the left anterior descending coronary artery (LAD) according to the method of Fishbein (17) as described in detail previously (18). After intraperitoneal induction of anesthesia with sodium pentobarbital (60 mg/kg), positive pressure respiration was started through an endotracheal tube. The thorax was opened in the fourth left intercostal space and the LAD was occluded near the origin of the pulmonary artery by a 6-0 silk ligature. The thorax was closed in layers. In the sham-procedure a superficial suture was placed in the epicardium of the left ventricle, near the LAD. All rats received post-operative analgesics (0.3 mg/ml Temgesic, 250 µl twice subcutaneously, Reckitt & Colman Products Ltd, Kingston-upon-Hill, UK).

Experimental protocol I: Structural changes in the vascular wall after coronary artery ligation

Myocardial infarction was induced at 1 week (n=7)), 3 weeks (n=8), 5 weeks (n=7) and 12 weeks (n=10) before euthanization. At all time intervals, sham-operated rats served as controls (1 week (n=6), 3 weeks (n=7), 5 weeks (n=7) and 12 weeks (n=9)).

Tissue processing
The rats were euthanized in ether anesthesia. The heart was arrested in diastole, by injecting 1-2 ml $CdCl_2$ (0.1 M) into the inferior caval vein. The rats were perfused with Phosphate Buffered Saline (PBS), followed by perfusion with 5% phosphate buffered formalin (10 min each) at a pressure of 100 mmHg, via a catheter in the right carotid artery. To ensure maximal vasodilation, nitroprusside (1 mg/ml, Sigma, St Louis, MO, USA) was added to both perfusion solutions.

Vessel segments (2-3 mm) were obtained using anatomic landmarks. A vessel segment of the thoracic aorta was sampled between the first and second intercostal artery, the left carotid artery was sampled 0.5 cm cranial from the aortic arch, the abdominal aorta from the right iliolumbar artery to the bifurcation, the superior mesenteric artery from its origin at the aorta to its first branching point, the right renal artery from the suprarenal artery to its bifurcation at the hilus of the kidney and the right iliac artery from its origin at the bifurcation of the abdominal aorta to its bifurcation of the femoral arteries. Three mesenteric resistance arteries draining the jejunum were excised. Also, the right lung was

excised to study the pulmonary resistance arteries.

After excision of the heart and removal of the atria, the ventricles were blotted dry and weighed. For determination of the wet/dry weight ratio, the apex was removed and weight separately. Apex dry weight was determined after freeze drying. The percentage water in the heart (apex) was calculated as 100*(wet weight-dry weight)/wet weight. Lungs were also excised and weighed. All tissues were fixed overnight in 10% phosphate buffered formalin. Fixed tissues were processed and embedded in paraplast via routine histological procedures.

Morphometric measurements.

For elastin, rehydrated 4 µm sections were incubated for 30 min in Lawson's solution (Klinipath, Zevenaar, The Netherlands), differentiated in 70% alcohol, dehydrated and mounted with Entellan. For collagen, rehydrated 4 µm sections were incubated for 5 min with 0.2% (wt/vol) aqueous phosphomolybdic acid (19) and then incubated for 90 min with 0.1% Sirius Red F3BA (C.I. 35780, Polysciences, Northampton, UK) in saturated aqueous picric acid, washed for 2 min with 0.01 N HCl, dehydrated and mounted with Entellan (Merck, Darmstadt, F.R.G). Volume fractions of collagen and elastin of the media of large conduit arteries were evaluated with a computerized morphometric system (Quantimet 570, Leica, Cambridge, U.K.). Approximately 6 fields of the medial area (defined as the area between internal and external elastica laminae) of 2 to 3 cross sections of each vessel were analyzed using a 400x magnification and the percentage of total tissue surface occupied by collagen or elastin was calculated. Intra- and interobserver variations of this method are less then 5% for the large conduit arteries (data not shown). The analyses were performed in a blinded fashion by three experienced investigators. Lawson's stained sections were also used to measure the medial cross-sectional areas, the internal and external diameters and media to lumen ratios (defined as the ratio of medial area and lumen area times*100%). Infarct size was determined on AZAN stained sections (4 µm) of the heart using the same morphometric system. Infarct size was expressed in percent of left ventricular circumference (17). Rats with an infarct size less than 20% were excluded from all the studies described in this thesis, as previous hemodynamic studies in our laboratory indicated that these rats exhibited no signs of cardiac failure (20).

Experimental protocol II: Biochemical changes in extracellular matrix of the vascular wall 12 weeks after coronary artery ligation.

In a second group of 12 weeks MI (n=7) and sham (n=9) rats, total collagen and elastin concentrations were determined biochemically using the hydroxyproline assay (21). Rats were euthanized in ether anesthesia. Large segments of conduit vessels were excised, again using anatomic landmarks. A segment of the thoracic aorta was sampled from the first to the eight intercostal artery. Both carotid arteries were taken from their origin at the aortic arch to the bifurcation in the

internal and external carotid branches. A 2-cm segment of the superior mesenteric artery was taken from its origin at the aorta. Both renal arteries were excised from their origin at the aorta to the bifurcation at the kidney. Finally, a 2-cm segment of the abdominal aorta was taken out.

The vessels were washed free of blood and cleared of adhering tissues. Using fine forceps, the medial layer of the thoracic aorta, the abdominal aorta and both carotid arteries was carefully separated from the adventitial layer. The renal arteries and the mesenteric artery were analyzed intact. Vessel segments of 2-3 rats were pooled for subsequent analyses.

Pooled vessel were lyophilized and weighed, 1.0 ml/ 4 mg dry weight of hot 0.01M phosphate buffer containing 1.0% sodium dodecyl sulphate (SDS), was added and the mixture was boiled for 15 min, followed by overnight extraction at 20°C in the same buffer. The SDS extract was dialyzed against distilled water and lyophilized. Separation of elastin and collagen exploits the fact that elastin contains no methionine residues and thus resist digestion by CNBr (22). The residue after SDS extraction was digested with cyanogen bromide (CNBr, 50 mg/ml in 70% formic acid) at 20°C for 24 hours. The CNBr extract, which contains collagen and other solubilized proteins was lyophilized. Insoluble residues remaining after CNBr extraction of the tissues were taken as elastin, lyophilized and weighed.

The amount of tissue elastin and collagen were measured by quantification of the amount of hydroxyproline in the CNBr residue (elastin) and the SDS extracts and CNBr extracts (total collagen) (21). All separate lyophilized fractions were hydrolyzed in 200 µl 6 M HCl for 16 hours at 105°C. The samples were dried under vacuum and reconstituted in 200 µl double distilled water. 5-100 µl of each sample was taken and the volume was adjusted to 100 µl with distilled water. Subsequently, 300 µl of acetate-citrate-isopropanol buffer and 100 µl oxidant solution (84.5 mg chloramine T/ml) was added and incubated during 5 min at 25 °C. Finally, 1.3 ml 3.5 M p-dimethylamino-benzaldehyde in 72% perchloric acid was added and incubated during 30 min at 65°C. Oxidation of hydroxyproline results in the formation of a pyrrole, which reacts with p-dimethylaminobenzaldehyde to form a colored compound. Absorbance was measured at 558 nm (Ultrospec III, Pharmacia Biotech, Brussels, Belgium). Calculation was performed using a calibration curve. Results are expressed as µg hydroxyproline/ mg dry weight for both elastin and collagen fractions. Finally, DNA concentrations in these segments were determined using the Hoechst assay.

Experimental protocol III: Regional blood flow measurements 5 and 12 weeks after coronary artery ligation

In a third group consisting of 5 and 12 weeks MI (n=11 and n=11 respectively) or sham rats (n=9 and n=7 respectively), radioactive microspheres (Sn^{113}, 15±5 µm in diameter, Dupont, NEN products, Boston, MA) were used to measure regional

Table 2.1 Characteristics of experimental groups at different time points after coronary artery ligation or sham surgery.

		1 Week	3 Weeks	5 Weeks	12 Weeks
BW (g)	sham[#]	312 ± 4 (n=6)	361 ± 8 (n=7)	351 ± 10 (n=7)	459 ± 12 (n=9)
	MI[□#]	306 ± 10 (n=7)	343 ± 7 (n=8)	369 ± 11 (n=7)	443 ± 11 (n=10)
	MI-MOD				460 ± 11 (n=5)
	MI-LARGE				427 ± 19 (n=5)
HW (g)	sham[#]	0.99 ± 0.03	1.15 ± 0.05	1.06 ± 0.09	1.30 ± 0.06
	MI[#]	0.92 ± 0.04	1.08 ± 0.04	1.20 ± 0.03*	1.47 ± 0.04*
	MI-MOD				1.43 ± 0.04
	MI-LARGE				1.51 ± 0.07‡
LW (g)	sham	1.45 ± 0.04	1.51 ± 0.17	1.75 ± 0.18	1.70 ± 0.07
	MI[#]	1.83 ± 0.15*	2.31 ± 0.32*	2.79 ± 0.42*	3.20 ± 0.33*
	MI-MOD				2.98 ± 0.60†
	MI-LARGE				3.41 ± 0.38‡
HW/BW ratio (g/kg)	sham	3.16 ± 0.06	3.18 ± 0.12	3.00 ± 0.21	2.84 ± 0.10
	MI	3.01 ± 0.08	3.15 ± 0.09	3.27 ± 0.12	3.33 ± 0.14*
	MI-MOD				3.13 ± 0.09†
	MI-LARGE				3.54 ± 0.13‡
Infarct size (%)	MI	41 ± 4	37 ± 3	37 ± 4	39 ± 4
	MI-MOD				30 ± 2
	MI-LARGE				49 ± 4§

Data are expressed as mean ± sem. BW=Body weight, HW=Heart weight, LW=Lung weight, MI-MOD=Moderate MI, MI-LARGE=Large MI. □: NOTE:MI 12 weeks is the combined data of MI-MOD and MI-LARGE. Statistical analysis: Individual group comparison by Mann-Whitney *p<0.05 sham versus MI, † p<0.05 sham versus MI-MOD, ‡ p<0.05 sham versus MI-LARGE, § p<0.05 MI-MOD versus MI-LARGE. Effects in time by ANOVA: # p<0.05.

blood flow according to the reference sample technique, adapted for use in the rat (23). Microspheres were suspended in saline, 0.01% Tween 80 and thoroughly mixed and agitated by sonification before each injection to prevent clumping.

Instrumentation
Under ether anaesthesia, polyethylene catheters were placed into the left ventricle (through the right carotid artery, PE50) and into the tail artery (PE10). The catheter for the left ventricle catheter was connected to a pressure transducer (Honeywell microswitch, Dépex, De Bilt Netherlands). Placement of the left ventricular catheter was verified by the change in pressure waveform upon entering the ventricle. Both catheters were exteriorized in the neck. After closure of all incisions, rats were given a minimum of 3 hours to recover from surgery.

Blood flow measurements
Sn[113] labeled microspheres (total number ± 200.000) were injected in the left

Figure 2.1   Example of lawson stained cross-sections of the thoracic aorta of a sham-operated rat (top panel) and a MI rat (bottom panel) 12 weeks after the operation. Note the difference in medial thickness (magnification 400x)

ventricle in a 0.3 ml volume over a 15 sec period, followed by a 0.1 ml flush of 0.9% NaCl over another 15 sec period. Blood withdrawal was started 30 sec before injection through the caudal arterial catheter at a rate of 0.656 ml/min by a Harvard suction pump and continued for 3 min after injection. Rats were killed by pentobarbital injection in the left ventricle, organs and tissues were excised. All tissues were blotted, weighed and counted in a two-channel gamma scintillation counter. Absolute blood flow was calculated by the reference sample method (23) and expressed as ml/min/g tissue. Relative flow was calculated as percentage of cardiac output.

Statistics
Data are expressed at means±SEM. The level of significance was set at $p<0.05$. For statistical analysis the following tests were used: The Mann-Whitney test (non-parametric) was used for individual comparison of the group means at

superior Mesenteric Artery
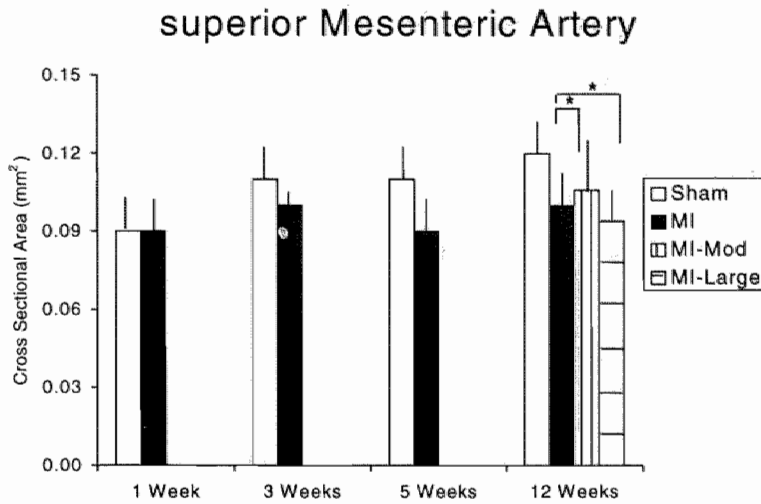
Figure 2.2 :   Medial cross-sectional area of the superior mesenteric artery at different time points after coronary artery ligation or sham-surgery. Statistical analysis: Effect over time by oneway ANOVA; sham rats p=0.07, MI rats p=0.85, * p<0.05 by Mann-Whitney test.

specific time points. For the comparison of group means of sham-operated rats with rats of the MI-MOD and MI-LARGE groups, the Mann-Whitney with Bonferroni correction was used. One way ANOVA was used to test for effect in time.

Results

Characteristics of experimental groups: Characteristics of rats at the different time points are listed in table 2.1. Both MI and sham-operated controls increased in body weight during the experimental period. No differences were observed between groups. The mean infarct size for all MI rats was 38±2% (n=32) and comparable between all MI groups. To evaluate the effects of the degree of heart failure, rats of the 12 weeks group (n=10) were subdivided in subgroups of moderate (30±2%, n=5) and large (49±4%, n=5) infarction.

Heart weights and heart-to-body weight ratios increased 5 and 12 weeks after coronary artery ligation (table 2.1). There was no evidence of oedema in the heart, as the mass fraction of water was comparable at all time points (data not shown). Lung weights of MI rats were increased compared to sham-operated controls at all time points, suggesting that induction of a myocardial infarction resulted in a substantial pulmonary oedema, indicative of heart failure. Also, changes in heart and lung weight became more pronounced as infarct size increased.
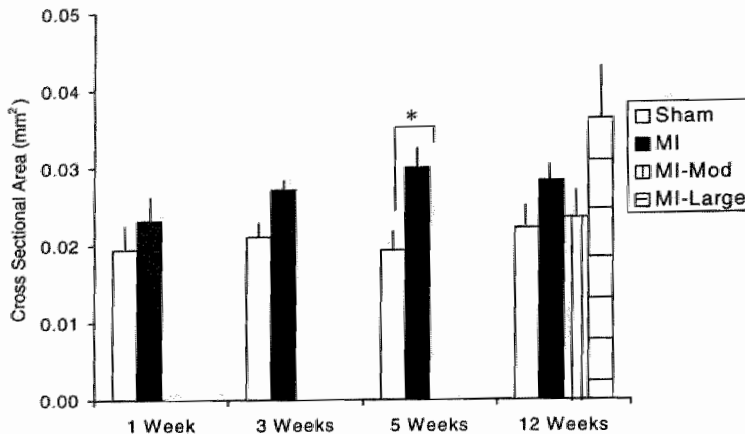
Pulmonary Resistance Arteries

Figure 2.3 :   Medial cross-sectional area of the pulmonary resistance arteries at different time points after coronary artery ligation or sham surgery. Statistical analysis: Effects over time by oneway ANOVA; sham rats and MI rats p=not significant, * p<0.05 by Mann-Whitney test.

Vessel dimensions:

Large conduit arteries (internal diameter >600 µm): Changes in vessel dimensions of the large conduit arteries became evident as heart failure progressed. Only the data from the 12 weeks time point are shown (table 2.2). However, small changes were observed at earlier time points. Five weeks after coronary artery ligation, these changes consisted of smaller medial cross-sectional areas of the abdominal aorta (MI versus sham -9%, p=0.06), carotid artery (-25%, p=0.08) and renal artery (-13%, p<0.05). Also, smaller external diameters of the thoracic aorta (-5%, p=0.07), mesenteric artery (-25%, p<0.05)) and renal artery (-15%, p=0.06) were observed as well as smaller internal diameters of the mesenteric artery (-13%, p=0.06) and renal artery (-16%, p=0.09). Twelve weeks after coronary artery ligation, smaller medial cross-sectional areas of the thoracic aorta and superior mesenteric artery were observed, as illustrated in figures 2.1 and 2.2, accompanied by a reduction in internal and external diameters (table 2.2). Similar changes were seen in the renal artery, carotid artery and the abdominal aorta (table 2.2). These changes coincided with a reduction in media-to-lumen ratio of the abdominal aorta, carotid and iliac artery (table 2.2).

Resistance arteries (internal diameter < 300 µm) : Internal and external diameters mesenteric and pulmonary resistance arteries (table 2.2, see also figure 2.3) were increased 12 weeks after coronary artery ligation, while media-to-lumen ratios

Table 2.2   Medial cross-sectional areas (CSA, mm^2), internal (ID, mm) and external (OD, mm) and media-to-lumen ratio (M/L, %) 12 weeks after coronary artery ligation or sham surgery.

		TA	AA	RA	MA	CA	IA	MRA	PRA
CSA	sham	0.49 ± 0.01	0.23 ± 0.01	0.10 ± 0.005	See figure 2.2	0.11 ± 0.007	0.15 ± 0.01	0.015 ± 0.001	See figure 2.3
	MI	0.42 ± 0.02	0.21 ± 0.01*	0.08 ± 0.004*		0.09 ± 0.002*	0.14 ± 0.01	0.017 ± 0.002	
	MI-MOD	0.44 ± 0.04	0.20 ± 0.02	0.08 ± 0.009		0.09 ± 0.003†	0.15 ± 0.02	0.019 ± 0.002	
	MI-LARGE	0.41 ± 0.03‡	0.21 ± 0.02	0.07 ± 0.004‡		0.08 ± 0.002‡	0.13 ± 0.01	0.016 ± 0.003	
ID	sham	1.76 ± 0.04	1.10 ± 0.02	0.68 ± 0.01	0.79 ± 0.04	0.88 ± 0.03	0.92 ± 0.03	0.23 ± 0.01	0.22 ± 0.02
	MI	1.65 ± 0.02*	1.11 ± 0.04	0.63 ± 0.01*	0.68 ± 0.03*	0.83 ± 0.06	0.95 ± 0.03	0.28 ± 0.02*	0.26 ± 0.02*
	MI-MOD	1.66 ± 0.01†	1.08 ± 0.05	0.62 ± 0.03	0.68 ± 0.05	0.84 ± 0.01	0.97 ± 0.06	0.27 ± 0.03	0.23 ± 0.04
	MI-LARGE	1.63 ± 0.05	1.13 ± 0.06	0.64 ± 0.03	0.67 ± 0.03‡	0.82 ± 0.02	0.93 ± 0.02	0.30 ± 0.03	0.29 ± 0.03
OD	sham	2.02 ± 0.05	1.23 ± 0.02	0.77 ± 0.03	0.99 ± 0.05	0.79 ± 0.03	0.80 ± 0.03	0.27 ± 0.01	0.27 ± 0.02
	MI	1.81 ± 0.03*	1.23 ± 0.04	0.71 ± 0.01*	0.77 ± 0.03*	0.76 ± 0.01	0.84 ± 0.03	0.33 ± 0.02*	0.32 ± 0.02*
	MI-MOD	1.83 ± 0.03†	1.20 ± 0.05	0.71 ± 0.02	0.77 ± 0.05†	0.77 ± 0.01	0.85 ± 0.05	0.34 ± 0.03	0.29 ± 0.04
	MI-LARGE	1.79 ± 0.05‡	1.25 ± 0.07	0.71 ± 0.02	0.76 ± 0.03‡	0.75 ± 0.02	0.83 ± 0.03	0.32 ± 0.03	0.35 ± 0.03
M/L	sham	20.5 ± 1.1	25.2 ± 0.7	26.3 ± 1.4	25.6 ± 1.9	22.6 ± 1.1	31.0 ± 1.5	37.0 ± 3.7	59.8 ± 3.9
	MI	19.8 ± 1.1	21.8 ± 0.9*	25.2 ± 1.1	28.2 ± 2.2	18.9 ± 0.8*	25.6 ± 1.4*	26.3 ± 1.4*	53.5 ± 6.0
	MI-MOD	20.0 ± 1.7	21.9 ± 1.0†	27.1 ± 1.9	29.5 ± 3.2	18.5 ± 1.0†	26.8 ± 2.3	26.8 ± 3.1†	45.6 ± 4.4†
	MI-LARGE	19.7 ± 1.9	21.7 ± 2.0	24.1 ± 1.4	26.7 ± 3.6	19.2 ± 1.3	24.4 ± 1.9‡	25.9 ± 1.6‡	61.4 ± 11.1

Data are expressed as mean ± sem. sham n=9, MI n=10, MI-MOD n=5, MI-large n=5. TA = Thoracic aorta, AA = Abdominal aorta, RA = Renal artery, MA = superior Mesenteric artery, CA = Carotid artery, IA = Iliac artery, MRA = Mesenteric Resistance arteries and PRA = Pulmonary resistance arteries. Statistical analysis: individual group comparison by Mann-Whitney *p<0.05 sham versus MI, † p<0.05 sham versus MI-MOD, ‡ p<0.05 sham versus MI-LARGE.

Table 2.3   Relative medial collagen and elastin content (% positive area) of MI and sham rats, 12 weeks after surgery.

	TA	AA	sMA	CA	IA
Collagen					
Sham	20.6 ± 2.0	16.4 ± 1.5	21.2 ± 1.9	10.1 ± 1.2	13.8 ± 1.2
MI	22.1 ± 1.0	19.9 ± 0.4	24.4 ± 1.2	12.1 ± 1.2	16.1 ± 1.1
MI-MOD	21.1 ± 1.4	19.8 ± 1.3	23.3 ± 3.8	12.0 ± 2.8	13.3 ± 1.9
MI-LARGE	23.1 ± 3.3	19.9 ± 1.2	26.0 ± 2.3	12.2 ± 1.2	18.9 ± 1.3
Elastin					
Sham	26.6 ± 2.0	15.3 ± 0.8	22.3 ± 1.7	28.5 ± 1.5	16.4 ± 1.6
MI	24.5 ± 1.2	17.5 ± 1.3	21.6 ± 1.5	28.7 ± 0.9	16.8 ± 0.8
MI-MOD	20.3 ± 1.8	17.3 ± 3.9	20.4 ± 4.3	30.1 ± 1.3	16.1 ± 2.2
MI-LARGE	29.8 ± 1.7	17.6 ± 2.9	22.9 ± 2.6	27.3 ± 3.0	17.7 ± 1.4

Data are expressed as mean ± sem. sham n=9, MI n=10, MI-MOD n=5, MI-large n=5. TA = Thoracic aorta, AA = Abdominal aorta, sMA = superior Mesenteric artery, CA = Carotid artery, IA = Iliac artery. Statistical analysis: individual group comparison by Mann-Whitney test, no significant differences.

decreased (table 2.2). Cross-sectional areas of these arteries increased slightly, but this difference did not reach statistical significance.

Extracellular matrix
Volume fractions of collagen and elastin (table 2.3, only 12 week time point is shown) measured by morphometric analysis were comparable in both sham and MI rats in the large conduit arteries during the entire experimental period. The hydroxyproline assay of these vessels confirmed this observation for the large conduit arteries and the renal artery (table 2.4). Also, medial DNA concentrations (expressed as ng/mg dry weight (table 2.4) or as ng/mm vessel (data not shown)) were similar between groups.

Regional flow measurements
Blood flow at rest was measured 5 and 12 weeks after coronary artery ligation. Again, infarcted groups were divided in moderate and large MI's. At 5 weeks, a small decrease in cardiac output was observed (sham 401±30 ml/min/g versus moderate MI 303±42 ml/min/g (p=0.09) and sham versus large MI 307±35 ml/min/g (p=0.07)). Twelve weeks after coronary artery ligation, resting cardiac output did not differ between groups (sham 330±38 ml/min/g, moderate MI 308±38 ml/min/g, large MI 341±44 ml/min/g). In general, no major changes could

Table 2.4   Absolute elastin and collagen content (μg hydroxyproline/μg dry weight) and DNA concentration per vessel (ng DNA/mg dry weight) 12 weeks after coronary artery ligation or sham surgery.

	ELASTIN		COLLAGEN		DNA	
	Sham	*MI*	*Sham*	*MI*	*Sham*	*MI*
TA	13.8 ± 0.9	12.9 ± 0.9	na	4.9 ± 0.4	18.8 ± 2.2	21.5 ± 6.2
CA	9.9 ± 1.8	9.3 ± 1.0	3.7 ± 0.6	4.6 ± 1.9	87.6 ± 27.7	89.9 ± 21.9
AA	9.9 ± 0.8	11.1 ± 0.6	3.9 ± 0.5	3.4 ± 1.1	51.8 ± 20.5	65.3 ± 25.7
sMA	6.1 ± 1.1	5.9 ± 1.0	4.7 ±1.8	5.9 ± 1.0	53.0 ± 9.7	75.7 ± 26.2
RA	6.7 ± 1.3	7.5 ± 2.1	8.6 ± 0.8	6.3 ± 1.7	44.1 ± 5.9	65.2 ± 30.8

Data are expressed as mean ± sem. Pooled vessel segments, sham n= 3, MI n=3, measured in duplo. TA = Thoracic aorta, AA = Abdominal aorta, sMA = superior Mesenteric artery, CA = Carotid artery, RA = Renal artery. TA, CA and AA represent medial elastin and collagen concentrations, MA and RA represent total vessel (=medial and adventitial) elastin and collagen content. na=not available. Infarct size = 45 ± 3%. TA = Thoracic aorta, AA = Abdominal aorta, RA = Renal artery, MA = superior Mesenteric artery, CA = Carotid artery. Statistical analysis: Individual group comparison by Mann-Whitney test; MI rats compared to sham rats; no significant differences.

be observed in absolute or relative peripheral flow (not shown) in the examined tissues at both time points (5 weeks; table 2.5, 12 weeks; table 2.6). After 12 weeks, flow to gastro-intestinal tissues increased slightly, but liver flow was decreased.

Discussion

This study provides the first evidence for structural alterations of peripheral arteries in an experimental model of heart failure. Changes in vessel dimensions were, however, only present at the later time points examined. This might indicate that coronary artery ligation produced only mild heart failure. However, lung weights of MI rats were increased proportional to infarct size at all time points, suggesting a substantial pulmonary oedema, indicative of heart failure. Also, infarct sizes comparable to the ones presented in this study (38±2%) are known to induce a significant decrease in stroke volume and stroke work (18), a decrease in cardiac output after a volume overload (24) and elevated left ventricular end diastolic pressures (25, 26).

In contrast to the smaller diameters and medial cross-sectional areas of large conduit arteries, internal and external diameters of resistance arteries (mesenteric

and pulmonary arteries) increased. Schieffer *et al.* (27) reported an increase in medial thickness of muscular resistance arteries one year after myocardial infarction. In our study, media-to-lumen ratios of the resistance arteries decreased, which is unexpected in view of the reported increase in peripheral resistance indicating resistance artery vasoconstriction during heart failure (7, 8). It should be reminded that perfusion fixation of the cardiovascular system was performed under maximal vasodilation. Thus, our data indicate that resistance arteries still have the potential for maximal vasodilation, and suggest that the observed vasoconstriction of resistance arteries during heart failure has a functional rather than a structural basis. An alternative possibility is that the vasoconstriction is primarily regulated in vascular beds other than the ones studied here. However, as the two resistance type arteries measured in this study showed the same phenomenon, this seems unlikely.

In contrast to sham rats, the medial cross-sectional area of large conduit arteries of MI rats did not increase during the experimental period. There are several possibilities to explain the smaller medial cross-sectional areas of the large conduit arteries. The first possibility is that the growth of MI rats was retarded as compared to sham-operated rats. Medial cross-sectional areas of large arteries in sham rats increased over the 12 week time period, but the large conduit arteries of MI rats did not show this apparently normal growth pattern, as illustrated in figure 2.3 for the mesenteric artery. Body weights of MI rats, however, were comparable to shams, indicating that this growth retardation in MI rats is not a general phenomenon, but specific for the large conduit arteries. The unchanged elastin and collagen contents (tables 2.3 and 2.4) and DNA concentration (table 2.4) after 12 weeks of coronary artery ligation in the media of these large conduit arteries suggest alterations in the control of vessel wall mass in MI rats, leading to the observed inhibition of growth of the large conduit arteries.

A reduction in peripheral flow may explain the inhibition of vascular growth of large conduit arteries. A decrease in flow has been shown to reduce vessel diameters and medial cross-sectional area (28, 29). During heart failure, a decrease in flow has been mentioned as a possible cause for abnormal skeletal muscle metabolism, but studies on measurements of peripheral flow changes during heart failure have yielded conflicting results. Most of these studies show decreased peripheral blood flow (7, 13, 14, 26, 30, 31), but some show unchanged blood flow (32-35). In our study, no major changes in resting peripheral blood flow were observed, despite the decrease in cardiac output after 5 weeks of heart failure (table 2.5) and a substantial ventricular damage of approximately 50% of left ventricular circumference. It should be noted that blood flow measurements were taken at rest, and therefore possible flow changes during e.g. exercise cannot be excluded. Also, blood flow measurements were taken with a technique, that could be sensitive to potential errors (23). Taken together, it seems unlikely that the observed vascular changes are induced by

Table 2.5   Regional Blood Flow (ml/min/g) 5 weeks after coronary artery ligation or sham surgery.

	Sham	*Moderate MI (25 ± 3%)*	*Large MI (47 ± 4%)*
Left Ventricle	6.63 ± 1.85	9.23 ± 0.68	9.5 ± 0.61
Right Ventricle	5.18 ± 1.33	6.08 ± 1.37	5.98 ± 0.48
Heart	11.0 ± 2.83	15.3 ± 1.52	14.5 ± 1.26
Small Intestine	4.12 ± 0.40	3.33 ± 0.69	4.93 ± 0.65
Large Intestine	1.03 ± 0.12	0.90 ± 0.22	1.10 ± 0.11
Liver	0.61 ± 0.08	0.96 ± 0.12 *	0.93 ± 0.16
Spleen	1.55 ± 0.33	1.15 ± 0.32	1.45 ± 0.52
Kidneys	9.53 ± 1.13	9.78 ± 2.15	10.2 ± 2.41
Soleus muscle	2.07 ± 0.27	2.38 ± 0.24	2.06 ± 0.46
Gastrocnemicus muscle	0.53 ± 0.09	0.36 ± 0.15	0.27 ± 0.05
Skin	0.18 ± 0.03	0.20 ± 0.04	0.22 ± 0.04
Brain	1.41 ± 0.13	1.67 ± 0.22	1.46 ± 0.22

Data are expressed as mean ± sem. sham n=9, MI-MOD n=5, MI-LARGE n=6. Statistical analysis: individual group comparison by Mann-Whitney and Bonferroni correction *p<0.025 sham versus MI-MOD, † p<0.025 sham versus MI-LARGE.

Table 2.6   Regional Blood Flow (ml/min/g) in rats 12 weeks after coronary artery ligation or sham surgery.

	Sham	*Moderate MI (36 ± 3%)*	*Large MI (46 ± 1%)*
Left Ventricle	6.46 ± 1.12	6.42 ± 1.14	7.53 ± 1.39
Right Ventricle	4.68 ± 0.38	4.65 ± 1.03	5.88 ± 0.49
Heart	11.2 ± 1.36	11.1 ± 1.73	12.2 ± 2.04
Small Intestine	2.99 ± 0.28	4.22 ± 0.56	4.28 ± 0.39†
Large Intestine	0.80 ± 0.08	0.89 ± 0.16	1.25 ± 0.25†
Liver	0.86 ± 0.11	0.75 ± 0.20	0.44 ± 0.09†
Spleen	1.22 ± 0.09	1.57 ± 0.41	1.42 ± 0.28
Kidneys	9.60 ± 1.50	9.93 ± 1.21	9.89 ± 2.24
Soleus muscle	2.34 ± 0.42	1.96 ± 0.41	1.90 ± 0.29
Gastrocnemicus muscle	0.40 ± 0.13	0.32 ± 0.03	0.33 ± 0.08
Skin	0.15 ± 0.02	0.15 ± 0.02	0.18 ± 0.03
Brain	1.29 ± 0.12	1.58 ± 0.28	1.66 ± 0.17

Data are expressed as mean ± sem. sham n=7, MI-MOD n=5, MI-LARGE n=6. Statistical analysis: individual group comparison by Mann-Whitney and Bonferroni correction *p<0.025 sham versus MI-MOD, † p<0.025 sham versus MI-LARGE.

changes in flow.

The smaller medial cross-sectional area of the large conduit arteries is remarkable in view of the described elevated plasma levels of several potential stimulators of vessel wall growth during heart failure. Human heart failure is associated with an increase in plasma levels of angiotensin II (36, 37), catecholamines (38, 39) and endothelin (40, 41). This neurohumoral activation is considered to be important in cardiovascular adaptations during heart failure. For instance, angiotensin II is one of the regulators of cardiac remodeling and blood pressure after infarction (18, 24). The peptide also promotes vascular smooth muscle cell proliferation (42, 43) and synthesis of various extracellular matrix components (44, 45). Catecholamines and endothelin have also growth-promoting effects (46, 47). The apparent paradox, i.e. increased circulating levels of several potential stimulators of vessel wall growth, without increases medial cross-sectional area, may be explained by a concomitant increase in plasma levels of other factors that have growth inhibitory effects, like atrial natriuretic peptide and nitric oxide (NO), which are also elevated during heart failure (48-50). Both peptides have antimitogenic effects in cultured vascular smooth muscle cells (51-53). In vivo, infusion of non-pressor doses of atrial natriuretic peptide decreases medial thickness in spontaneous hypertensive rats (54), while NO is thought to be important in inhibition of neointima formation by angiotensin-converting enzyme-inhibitors in the rat balloon injury model (55). Elevations of both vasoconstrictor and vasodilating mediators during heart failure may serve functional hemodynamic purposes (e.g. maintenance of blood pressure) but may also have a diverse effect on the structure of the vascular system. One could hypothesize that during progressing heart failure a neurohumoral unbalance between counteracting systems becomes evident, with a dominating effect of potential inhibitors of vessel wall growth. This could result in a inhibition of large artery growth. Furthermore, the existence of different smooth muscle pheno- and/or genotypes may explain the observed different response of conduit and resistance arteries during heart failure (56).

In summary, this study showed alterations in vessel dimensions of large conduit arteries during experimental heart failure. Moreover, changes became more pronounced as infarct size increased. Medial cross-sectional areas, internal and external diameters of large conduit arteries were smaller after 12 weeks in MI rats, compared to sham rats of the same age and weight, suggesting an inhibition of vascular growth. In contrast, internal and external diameters of resistance arteries were increased. It is suggested that following myocardial infarction both growth-stimulatory and inhibitory neurohormonal mechanisms are activated which over time lead to regional diverse adaptive changes in peripheral arteries. This diverse adaptation could have consequences for cardiac function during experimental heart failure.

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Chapter

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Effects of Angiotensin II on Cardiac Function and Peripheral Vascular Structure during Compensated Heart Failure in the Rat

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Abstract

Background: The present experiments were designed to test the hypothesis that activation of the renin-angiotensin system during compensated heart failure may have adverse effects on cardiac function and change peripheral vascular structure. ANG II (250 ng/kg/min) or saline (0.9% NaCl) were infused in myocardial infarcted and sham rats. After 2 weeks, cardiac function and peripheral vascular changes were investigated. *Results:* ANG II infusion reduced baseline cardiac index in sham rats, but did not further reduce this index in ANG II infused MI rats. Total peripheral resistance was similarly increased in ANG II infused infarcted and sham rats and also plasma ANG II concentrations were comparable. ANG II elevated systolic blood pressure by approximately 70 mmHg in sham rats and increased medial cross-sectional area of the superior mesenteric artery by 33%. However, ANG II infusions in MI rats resulted in only a minor increase in blood pressure whereas the cross-sectional area of the superior mesenteric artery did not change. ANG II infusion had no effect on vessel dimensions of resistance arteries of the pulmonary and mesenteric vascular bed of either group. Calculated ED_{50} and peak pressor response to acute ANG II injections were comparable in all groups, confirming the presence of functionally intact AT1 receptors. The increases in plasma atrial natriuretic peptide (ANP) and nitric oxide (NO) synthase activity (estimated by aortic cyclic GMP concentrations) were higher in ANG II infused MI rats, than in ANG II infused sham rats. *Conclusion:* ANG II infusion in rats with and without MI has comparable negative effects on cardiac function, but has different effects on blood pressure and vascular structure. The concomitant increases in plasma ANP and NO synthase activity in ANG II infused MI rats suggest that growth-stimulatory and hypertensive actions of ANG II in sham rats may be counte-regulated by activation of inhibitory neurohumoral systems such as ANP or NO in MI rats.

Introduction

The renin-angiotensin system (RAS) is one of the most extensively studied neurohumoral systems in the pathophysiology of congestive heart failure. Angiotensin II (ANG II), the effector peptide of the RAS, is involved in the regulation of cardiovascular function and volume homeostasis by raising peripheral resistance through vasoconstriction, stimulating aldosterone release and enhancing renal sodium reabsorption (1). Given its important role in maintaining circulatory homeostasis, one can expect an activation of the RAS in a situation of a decreased cardiac output, as seen during heart failure. An interesting aspect of RAS activation during heart failure is the timing. Plasma concentrations of ANG II are increased in the acute phase after myocardial infarction, normalize in the compensated phase and increase again in overt heart failure (2-4). This biphasic activation is considered to be a compensatory mechanism in response to decreased cardiac function. Although this may well be true for the first phase, it may not be true for the second phase in which activation of the renin angiotensin system may even have adverse effects on function and/or structure of the cardiovascular system. Indeed, a significant positive correlation between mortality and plasma levels of ANG II has been shown in patients with heart failure (5, 6) and it has been suggested that the effect of ACE-inhibition is related to neurohormonal activation in general and the RAS in particular (6).

To test the hypothesis that increased plasma concentrations of ANG II have adverse effects on cardiac function in conditions of an already reduced cardiac output, we infused ANG II in rats 2 weeks after induction of a myocardial infarction and studied cardiac function. This timing and model were chosen since we knew already from our own work as well as that from other groups that cardiac function is decreased in this model and that plasma levels of ANG II are not elevated at this time (2, 7-9). Since it is also known that peripheral vascular alterations do occur after induction of a myocardial infarction and that ANG II has strong effects on vascular structure (10, 11), we also studied the effects of ANG II on vascular structural parameters.

The results show that infusion of ANG II in MI rats had similar effects on cardiac function in MI and sham rats. The increases in mean arterial pressure and vessel wall mass were, however, smaller in MI rats than in sham rats. We suggest that activation of inhibitory neurohumoral systems such as ANP and NO may be responsible for suppression of hypertensive and growth stimulatory effects of ANG II in MI rats.

Materials and methods

Animals
Male Wistar rats (n=161, 200-250g at the start of training on day -14,

Winkelmann, Borchen, Germany) were housed under standard conditions and fed standard rat chow (RMH-TM, Hope Farms, Woerden, The Netherlands) and water ad libitum. The experiments were performed in accordance with local institutional guidelines. The randomization scheme is shown in figure 3.1. Of the 161 rats used, 26 died following coronary artery ligation and 2 following sham surgery. Of the 133 remaining rats, 39 were used for measurement of cardiac function and hemodynamics (experiment 1), 46 were used in the final evaluations of experiment 2 (tail cuff plethysmography and morphometric measurements), and 39 rats for experiment 3 (blood sampling and excision of thoracic aorta). The remaining 9 rats had infarct sizes less than 20% of left ventricular circumference, or showed non-transmural infarcts.

Coronary artery ligation

Myocardial infarction (MI) was induced by ligation of the left anterior descending coronary artery (LAD) according to the method of Fishbein (12) as described in chapter 2.

Experimental protocol

Rats were randomly assigned to either the protocol for measurement of hemodynamics and cardiac function (experiment 1, n=39), tail cuff plethysmography and morphometric measurements (experiment 2, n=46) or blood sampling (experiment 3, n=39) as outlined in figure 3.1. For each study, rats were assigned to one of the following 4 groups: *SH-NaCl* underwent a sham operation and were infused with saline (0.9% NaCl). *MI-NaCl* were infused with saline following infarct induction. *SH-ANG* and *MI-ANG* were subjected to sham surgery and myocardial infarction respectively, and infused with ANG II. The number of animals per group is presented in figure 3.1.

Infusions: ANG II (human [^{5}Val] ANG II, 250 ng/kg/min, Brunschwig, Amsterdam, The Netherlands) dissolved in saline or saline were infused subcutaneously for 2 weeks by osmotic minipumps (Alzet model 2002, Alza Corporation, Palo Alto, California), implanted subcutaneously between the shoulder blades. Minipumps were inserted at day 0.

Experiment 1: In this study, hemodynamic measurements were performed on saline and ANG II infused sham and MI rats. These rats were implanted with an electromagnetic probe and various catheters to determine cardiac and peripheral hemodynamics.

Implantation of measuring equipment: 7 Days after coronary artery ligation or sham surgery and implantation of osmotic minipump, all rats were equipped with an electromagnetic flow probe (2.7 mm diameter; Skalar, Delft, The Netherlands) on the ascending aorta according to previously described methods (7). Rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.). After endotracheal intubation and start of positive pressure respiration, the thorax was opened in the
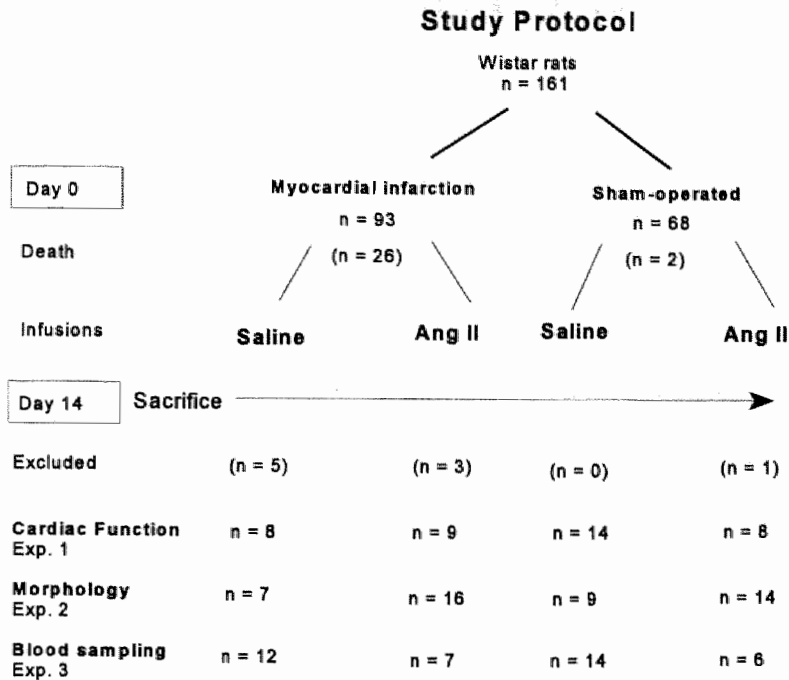
Study Protocol

Figure 3.1   Outline of research protocol with randomization scheme

third right intercostal space and the ascending aorta was dissected from surrounding tissue. An electromagnetic flow probe was placed around the aorta at 1-2 mm above the heart. The thorax was closed in layers, the cable was fixed to the ribs, and the connector was exteriorized in the neck where it was sutured to the skin.

Four days later (day 11), rats were anesthetized with ether and implanted with a PE-10 catheter in the abdominal aorta through the right femoral artery to measure arterial blood pressure. Furthermore, through the right femoral vein, a PE-10 catheter was implanted into the abdominal vena cava for infusions. A Silastic (602-175, Dow Corning, Midland, MI, USA) catheter was placed in the thoracic vena cava for measurement of central venous pressure. All catheters were exteriorized in the neck, filled with saline, and closed with metal plugs. After these operations, rats were allowed to recover for 3 days before hemodynamic measurements.

Measurements and protocol: On day 14, the electromagnetic flow probe was connected to a sinewave flowmeter (model MDL 401, Skalar) to measure blood flow through the ascending aorta. Although this flow comprises cardiac output

minus coronary blood flow, we refer to it as cardiac output. The baseline was established by taking late diastolic blood flow as zero. The arterial and central venous catheters were connected to low-volume displacement pressure transducers (CP01; Century Technology, Inglewood, Ca, USA). Mean values for arterial blood pressure and central venous pressure were obtained by digital integration. Stroke volume was calculated from the flow signal by integration of each beat. Total peripheral resistance was calculated as (mean arterial pressure - central venous pressure)/ cardiac output. Stroke work was estimated by multiplying stroke volume with the difference between mean arterial pressure and central venous pressure. All derivations were made on-line and stored on disk for later processing.

After 45-60 min, baseline recordings were made for 15 min. Then a rapid infusion of 12 ml of a warm (37°C) Ringer's solution was given in 1 min through the abdominal caval vein catheter. This has been shown to increase cardiac output to a plateau level, which can be used as an indicator of maximal cardiac function. (7) During this period, hemodynamics were monitored continuously. The plateau cardiac output was obtained during the final 10-15 s of the volume loading and is termed "maximal cardiac output during volume loading". The values for cardiac output and stroke volume (at baseline and during volume loading) were normalized for body weight and termed cardiac index and stroke volume index.

After the measurements, rats were killed using an overdose of pentobarbital, after which the heart was arrested in diastole by injecting $CdCl_2$ (0.1M) into the inferior caval vein. The heart was excised and weighed after removal of the atria. Lungs were also excised and weighed. The heart was then fixed overnight in 10% phosphate buffered formalin and processed and embedded in paraplast via routine histological procedures. Infarct size was determined on AZAN stained sections (4 µm) on a computerized morphometric system (Quantimet 570, Leica, Cambridge, U.K.) of a slice of the heart taken at the level of the papillary muscle. Infarct size was expressed in percent of left ventricular circumference.

Experiment 2 : In this study, systolic blood pressures were measured by tail cuff plethysmography throughout the protocol. Prior to sacrifice on day 14, mean arterial blood pressures were measured through an arterial catheter, and some of the rats were used for a cumulative ANG II pressor dose response curve.

Tail cuff plethysmography : Systolic blood pressures were measured by tail-cuff plethysmography (IITC Inc, Life Science Instruments, Woodland Hills, CA, USA) in conscious rats. During 7 days (day -14 to day -8), rats were trained for the procedure. Measurements started 1 week before surgery (day -7 to day 0) and continued during infusions. Blood pressures and heart rates were measured three times a week. The mean of 4-5 measurements per animal in one session was used for calculations.

Assessment of mean arterial pressures and heart rates on day 14. On day 13, rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and provided

with a polyethylene (PE10) catheter in the abdominal aorta through the left femoral artery to measure mean arterial blood pressure. The catheter was exteriorized in the neck, filled with saline and closed with a metal plug. On day 14, the arterial catheter was connected to low-volume displacement pressure transducers (CP01; Century Technology, Inglewood, Ca, USA). All signals were fed into a microcomputer, sampling all signals at 500 Hz each. Mean values for arterial blood pressure and heart rates were obtained by digital integration.

Assessment of cumulative ANG II pressor response curve : On day 14, some of the rats assigned to this study protocol (SH-NaCl n=7, MI-NaCl n=7, SH-ANG n=6, MI-ANG n=6) were anesthetized with pentobarbital (60 mg/kg intraperitoneal) and a PE10 catheter was implanted in the right jugular vein. The previously implanted arterial catheter was connected to the same system as described above. An ANG II dose-blood pressure response curve in a dose ranging from 0.3 ng to 30 µg ANG II was determined, while body temperature was maintained at 37°C using heating pads. To minimize interference of tachyphylaxis, doses were given cumulatively, with each successive injection given immediately after the maximum effect of the preceding dose was achieved (10-20 s). The dose at the half-maximal effect (ED_{50}) was computed by fitting the mean arterial pressure response to ANG II to a sigmoidal curve using the equation

$$\Delta MAP = \frac{\Delta MAP_{max} \times D^n}{Ed^n_{50} + D^n}$$

where ΔMAP_{max} = maximal increase in mean arterial pressure, D = dose of ANG II and n = Hill-coefficient

For tissue processing (heart and vessels) and morphometric measurements, see materials and methods of chapter 2.

Experiment 3 : In this protocol, blood samples were taken for determination of plasma ANG II and atrial natriuretic peptide (ANP) in ANG II and saline infused MI and sham rats. Also, the thoracic aorta was excised for determination of cyclic GMP (cGMP) concentrations (13). In these groups of rats, mean arterial pressures were measured on day 15.

Blood sampling. On day 13, rats were provided with a polyethylene (PE10) catheter in the abdominal aorta through the left femoral artery, as described above. On day 14, blood (2 ml) was sampled from the arterial catheter in non-disturbed rats and collected in chilled heparinized tubes, containing 1.4 µM enalaprilate. Tubes were centrifuged at 3000 RPM at 4°C for 15 min and stored at -70°C until plasma ANG II and ANP measurements.

Due to blood sampling, measurement of mean arterial pressures in these rats was postponed to day 15. After these measurements (see experiment 2), rats were killed using an overdose of pentobarbital. As in experiment 1, the heart was arrested in diastole, by injecting 1-2 ml $CdCl_2$ (0.1M) into the inferior caval vein.

The thoracic aorta was rapidly excised, rinsed in cold buffered saline, frozen in liquid nitrogen and stored at -70°C. Heart and lungs were excised and weighed. The heart was then prepared for infarct size measurement as described in experiment 1.

Detection of plasma angiotensin II and ANP concentrations. Plasma ANG II and ANP concentrations were measured using a radioimmunoassay. For ANG II, plasma samples (0.5 ml) were extracted using ethanol. For ANP, plasma samples (0.5 ml) were acidified with 1.5 ml 4% acetic acid and ANP was eluted from a C^{18}column (Millipore, Waters Chromotography, Etten-Leur, The Netherlands) by applying 3 x 1 ml 4% acetic acid in 86% ethanol at the top of the column. Both collected eluates were evaporated to dryness in a vacuum evaporator. The dried extracts were reconstituted by adding assay buffer to each tube and then stored at -15°C.

Radiolabeled ANG II (Dupont, NEN products, Dordrecht, the Netherlands) and ANP (Nichols Institute, Diagnostics B.V., Wijchen, The Netherlands) competed with unlabeled ANG II and ANP in the test samples and standards for a limited number of specific antibody binding sites. At the end of the incubation period (42 h), antibody-bound ANG II and ANP were separated from unbound ANG II and ANP using anti-rabbit coated cellulose (Nichols Institute, Diagnostics B.V., Wijchen, The Netherlands) in suspension as a solid phase. Following a brief incubation and centrifugation, the unbound ANG II and ANP were decanted and the antibody-bound radiolabeled ANG II and ANP measured in a gamma counter. A standard curve was prepared and the test sample concentrations were read from the curve. Intra- and interassay variations are 4.9% and 7.9% for ANG II and 6.9% and 12.7% for ANP.

Tissue sampling: Thoracic aorta's were powered with a mortar and pestle, placed in liquid nitrogen. Tissues were further homogenized in 6% trichloroacetic acid (TCA) with an ultra turrax at 4°C, centrifuged at 4000 g for 10 minutes at 4°C and the supernatant was transferred to a clean test tube. TCA was removed from the supernatant by extracting three times with three volumes of water-saturated diethyl ether. Samples were then dried under nitrogen and stored at -20°C until assayed.

Determination of cyclic GMP concentration: The cGMP content of thoracic aorta segments was determined with a commercially available kit (^{125}I-cGMP-RIA, IBL, Hamburg, Germany). The residues were dissolved in 300 µl assay buffer of which 100 µl was used for the assay. 100 µl ^{125}I- tracer and 200 µl antiserum were added. Samples for the standard curve and non-specific- binding were prepared according to the same procedure. After an incubation of 24 hours at 4°C, cooled separation reagent was added after which the tubes were centrifuged. The supernatant was discarded and residual radioactivity was counted in a gamma counter. The protein content was measured using a commercially available assay (Biorad protein assay, Biorad Lab. München, Germany) and cGMP values are presented as femtomoles per mg protein.

Table 3.1   General characteristics and cardiac function of groups in experiment 1.

Variable	Saline infused		ANG II infused	
	Sham	MI	Sham	MI
BW day 14	293 ± 5	278 ± 5*	245 ± 4*	254 ± 6†
Gain of BW (g)	-24 ± 3	-31 ± 7	-40 ± 4*	-35 ± 8
HW (g)	0.85 ± 0.02	0.80 ± 0.03	0.86 ± 0.02	0.93 ± 0.06
HW/BW (g/kg)	2.9 ± 0.1	2.9 ± 0.1	3.5 ± 0.1*	3.7 ± 0.3
LW/BW (g/kg)	ND	ND	5.3 ± 0.3	7.7 ± 1.1
Infarct size (%)		46 ± 4		47 ± 4
HR (beats/min)	360 ± 14	376 ± 9	387 ± 13	391 ± 25
SW (mmHg/min)	23 ± 1	16 ± 1*	26 ± 2	16 ± 1‡
MAP (mmHg)	97 ± 2	88 ± 4	166 ± 8*	122 ± 7†,‡
TPR (mmHg/min/ml)	1.2 ± 0.1	1.5 ± 0.2	2.9 ± 0.3*	2.4 ± 0.2†
CVP (cm H$_2$O)	0.2 ± 0.4	-0.7 ± 0.7	-1.1 ± 0.7	-3.2 ± 1.2
n	14	8	8	9

Table shows gain of body weights (BW) during 14 days of infusions, heart weight (HW), heart-to-body weight ratios (HW/BW), lung-to-body weight ratios (LW/BW), infarct size, heart rates (HR), stroke work (SW), mean arterial pressures (MAP), total peripheral resistance (TPR) and central venous pressure (CVP). ND = not determined. Data are expressed as mean ± sem. Statistical analysis: Intergroup differences were tested by Mann-Whitney tests with a Bonferroni correction for multiple group comparison; *p<0.0125 compared to saline infused sham rats, †p<0.0125 compared to saline infused MI rats and ‡p<0.0125 compared to ANG II infused sham rats.

Statistics

Data are expressed as means±SEM. Intergroup differences were evaluated with a non-parametric Mann-Whitney test with a Bonferroni correction for multiple group comparison. The Bonferroni procedure corrects the p value for each pairwise group comparison, thus with 4 pairwise comparisons (SH-NaCl versus MI-NaCl, SH-NaCl versus SH-ANG, MI-NaCl versus MI-ANG and SH-ANG versus MI-ANG), statistical significance is defined as p<0.0125 (p<0.05 divided by 4). For the evaluation of systolic blood pressure measurements during the experimental period, areas under the curve were determined and used for subsequent Mann-Whitney test procedure.

Table 3.2   General characteristics and blood pressure measurements in
                   experiment 2 and 3

Variable	Saline infused		ANG II infused	
	Sham	MI	Sham	MI
BW day 14	303 ± 4	295 ± 6	238 ± 5*	255 ± 4†
Gain of BW (g)	2 ± 3	-11 ± 4	-44 ± 6*	-28 ± 3
HW (g)	0.96 ± 0.03	0.96 ± 0.02	0.93 ± 0.04	0.96 ± 0.03
HW/BW (g/kg)	3.2 ± 0.1	3.2 ± 0.1	3.9 ± 0.1*	3.8 ± 0.1†
LW/BW (g/kg)	4.6 ± 0.2	5.9 ± 0.6*	5.6 ± 0.2*	9.1 ± 0.8†,‡
Infarct size (%)		42 ± 3		46 ± 2
MAP (mmHg)	129 ± 5	104 ± 3*	189 ± 5*	126 ± 4†,‡
HR (beats/min)	333 ± 22	345 ± 14	382 ± 10	353 ± 8
n	23	19	20	23

Table shows gain of body weights (BW) in 14 days, heart weight (HW), heart-to- body weight ratios (HW/BW), lung-to-body weight ratios (LW/BW), infarct size, heart rates (HR) and mean arterial pressures (MAP). Data are expressed as mean ± sem. Statistical analysis: Intergroup differences were tested by Mann-Whitney test with a Bonferroni correction for multiple group comparison; *$p<0.0125$ compared to saline infused sham rats, †$p<0.0125$ compared to saline infused MI rats and ‡$p<0.0125$ compared to ANG II infused sham rats.

Results

Effect of a myocardial infarction (SH-NaCl versus MI-NaCl):
Ligation of the LAD resulted in an infarction of approximately 45% of left ventricular circumference. Infarct sizes were comparable in all experiments (tables 3.1 and 3.2). Body weights at day 0 were comparable in all groups. Induction of a MI decreased body weight in saline infused rats (table 3.1 and 3.2). Of note, the weight loss of saline infused rats in experiment 1 (measurement of cardiac function, see table 3.1) was more excessive compared to rats used in experiment 2 and 3 (table 3.2), presumably due to intensive operation procedures. There were no differences in absolute heart weight or heart-to-bodyweight ratios between saline infused sham and MI rats (table 3.1 and 3.2). Lung-to-body-weight ratio increased in the MI rats (table 3.2).

The measurements of cardiac function in **experiment 1** demonstrated that MI induction resulted in a decrease in baseline and maximal cardiac index, stroke volume index (figure 3.2) and baseline stroke work (table 3.1). Total peripheral
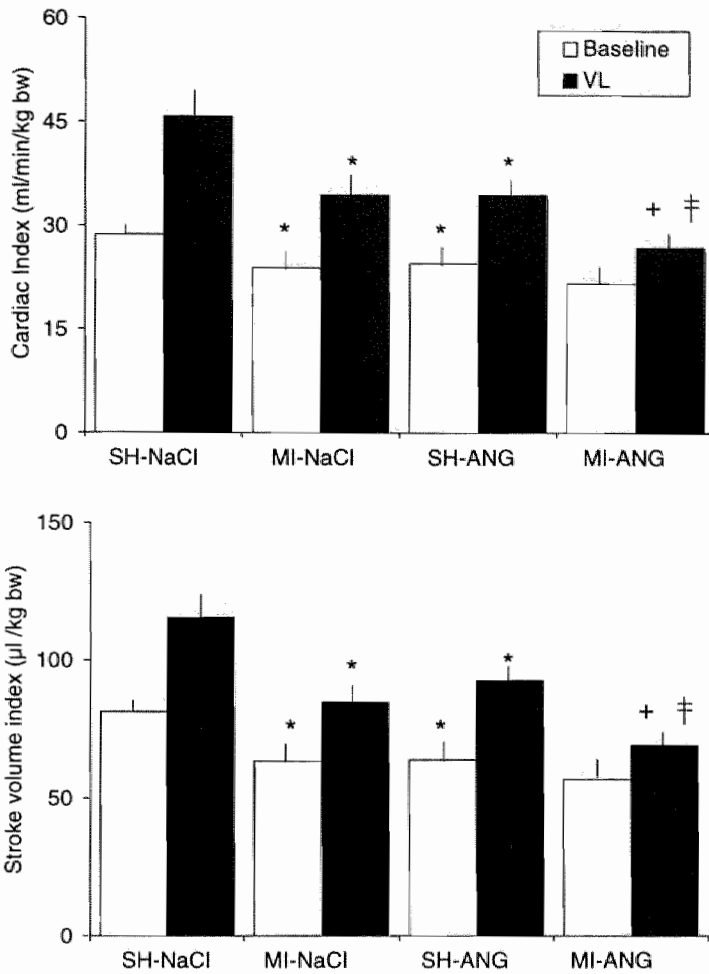
Figure 3.2   Graph showing the effects of saline and ANG II infusions on cardiac index (top
panel, ml/min/kg body weight) and stroke volume index (bottom panel, µl/kg
body weight) at baseline and during rapid volume loading (VL) in sham and MI
rats. * $p<0.0125$ compared to SH-NaCl, + $p<0.0125$ compared to MI-NaCl, ‡
$p<0.0125$ compared to SH-ANG by Mann-Whitney test with Bonferroni
correction.

resistance and central venous pressure did not change in MI-NaCl rats (table
3.1).

Systolic blood pressures, measured in **experiment 2,** were comparable at
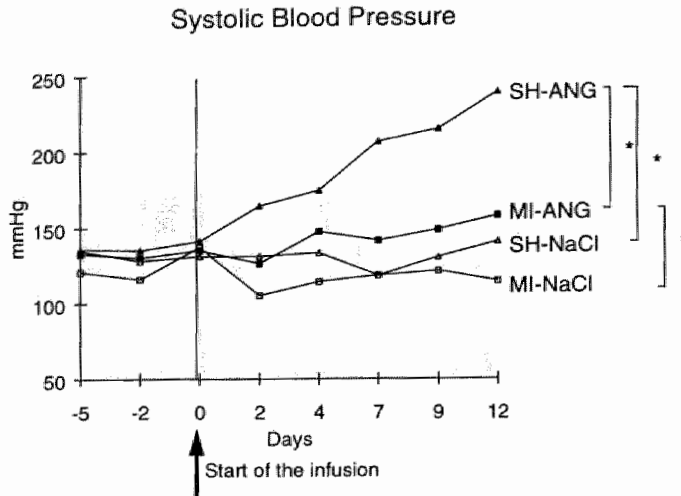day 0 in all groups. Although at the end of the experimental period systolic blood

Systolic Blood Pressure

Figure 3.3   Graph showing the effects of a saline or ANG II infusion on systolic blood pressure (mmHg) in sham and MI rats. * $p<0.0125$ by Mann-Whitney with Bonferroni correction.

pressures had decreased by 25 mmHg in MI-NaCl rats compared to SH-NaCl rats (figure 3.3), this did not reach statistical significance ($p=0.04$). However, direct intra-arterial mean arterial blood pressure measurement on day 14 demonstrated a significant difference between saline infused sham and MI rats (tables 3.2). No differences between saline infused sham and MI rats were found in the cumulative ANG II pressor response curve as presented in table 3.3.

The vessel dimensions of the superior mesenteric artery, mesenteric and pulmonary resistance arteries are presented in table 3.4 and 3.5. Compared to SH-NaCl rats, MI induced no changes in vessel dimensions of these three arteries.

Finally, plasma ANP concentrations were increased in saline infused MI rats, while plasma ANG II concentrations did not differ between groups. Also, aortic cGMP concentrations did not differ between the two groups (**experiment 3**, table 3.6).

Effect of ANG II infusion in sham-operated rats (SH-NaCl versus SH-ANG)
ANG II infusion in sham rats resulted in a substantial weight loss (table 3.1 and 3.2). Although heart weight did not differ between the groups, ANG II infusion increases heart-to-body-weight ratios in SH-ANG rats. There was no evidence of cardiac oedema, as mass fraction of water was comparable in all groups (SH-

Table 3.3   Characteristics of the acute ANG II pressor response curve

Variable	Saline infused		ANG II infused	
	Sham	MI	Sham	MI
ED_{50} for ANG II (µg/kg)	0.39 ± 0.09	0.48 ± 0.05	0.30 ± 0.09	0.46 ± 0.07
Peak pressor response to ANG II (mmHg)	+ 71 ± 6	+ 59 ± 5	+ 94 ± 9	+ 74 ± 10
n	7	7	6	6

Table shows characteristics of the acute ANG II pressor response curve. Data are expressed as mean ± sem. Statistical analysis: Intergroup differences were tested by Mann-Whitney test with a Bonferroni correction for multiple group comparison; *p<0.0125 compared to saline infused sham rats, †p<0.0125 compared to saline infused MI rats and ‡p<0.0125 compared to ANG II infused sham rats.

NaCl 76±2%, data of other groups not shown). Lung-to-body weight ratio increased during ANG II infusion in sham rats (table 3.2).

Compared to SH-NaCl rats, ANG II infusions in sham rats decreased both baseline and maximal cardiac index and stroke volume index (**experiment 1**, figure 3.2). Stroke work, however, did not change. Total peripheral resistance significantly increased in these rats, accounting for the increase in mean arterial pressure (table 3.1).

ANG II treatment gradually increased systolic blood pressures in SH-ANG rats by approximately 70 mmHg at day 12 (**experiment 2**, figure 3.3). The same increase was seen in mean arterial pressures, measured at day 14 (table 3.1 and 3.2). Characteristics for the acute ANG II dose-blood pressure responses were comparable in ANG II and saline infused sham rats (table 3.3).

ANG II infusion resulted in 33% increase in medial cross-sectional area of the superior mesenteric artery in sham-operated rats (table 3.4). Vessel dimensions of both mesenteric and pulmonary resistance arteries were similar in saline and ANG II infused sham-operated rats.

ANG II infusion increased plasma ANG II and ANP in sham rats (**experiment 3**, table 3.6). Aortic cGMP concentrations tended to decrease from 559±87 to 306±59 fmol/mg protein in SH-NaCl and SH-ANG rats (p=0.02), respectively (table 3.6).

Effect of ANG II in myocardial infarcted rats (MI-NaCl versus MI-ANG)
ANG II infusion in MI rats resulted in a substantial weight loss (table 3.2). Similar to SH-ANG rats, absolute heart weights of ANG II infused MI rats were

Table 3.4   Vessel dimensions of the superior mesenteric artery

	Saline infused		ANG II infused	
	Sham	MI	Sham	MI
Medial cross-sectional area	0.15 ± 0.01	0.14 ± 0.02	$0.20 \pm 0.01^*$	$0.14 \pm 0.01\ddagger$
Lumen area	0.29 ± 0.03	0.25 ± 0.06	0.34 ± 0.02	0.28 ± 0.02
Media-to-lumen ratio	54 ± 6	44 ± 5	59 ± 2	50 ± 4
n	9	7	14	16

Table shows cross-sectional area (mm^2), lumen area (mm^2) and media-to-lumen ratio (cross-sectional area/lumen area * 100) of superior mesenteric artery. Data are expressed as mean ± sem. Statistical analysis: Intergroup differences were tested by Mann-Whitney test with a Bonferroni correction for multiple group comparison; $^*p<0.0125$ compared to saline infused sham rats, $\dagger p<0.0125$ compared to saline infused MI rats and $\ddagger p<0.0125$ compared to ANG II infused sham rats.

comparable to saline infused MI rats, while heart-to-body-weight ratios of ANG II infused MI rats were increased (table 3.1 and 3.2). ANG II infusions in MI rats increased lung-to-body weight ratios.

Cardiac function measurements (**experiment 1**) demonstrated that baseline cardiac index of ANG II infused MI rats was not different from SH-ANG rats and also not from MI-NaCl rats (figure 3.2). Stroke work was reduced compared to SH-ANG, but not different from MI-NaCl (table 3.1). Cardiac index and stroke volume index during volume loading of MI-ANG rats were significantly reduced compared to SH-ANG rats, but not different from MI-NaCl rats (figure 3.2). Thus, while ANG II infusion in sham rats significantly reduced cardiac function compared to saline infused sham rats, infusion of ANG II in MI rats did not further reduce cardiac function. This is illustrated by calculations of the relative decrease of cardiac index during volume loading. Thus, cardiac index during volume loading of ANG II infused MI rats decreased 22±7% compared to SH-ANG and 22±7% compared to MI-NaCl rats. This decrease in cardiac function was fully comparable to the decrease of 25±4% in cardiac index during volume loading in SH-ANG rats compared to SH-NaCl rats. Total peripheral resistance increased by 62±15% in MI-ANG rats and by 146±24% in SH-ANG rats (p<0.001). Absolute values, however, were comparable in both groups (table 3.1).

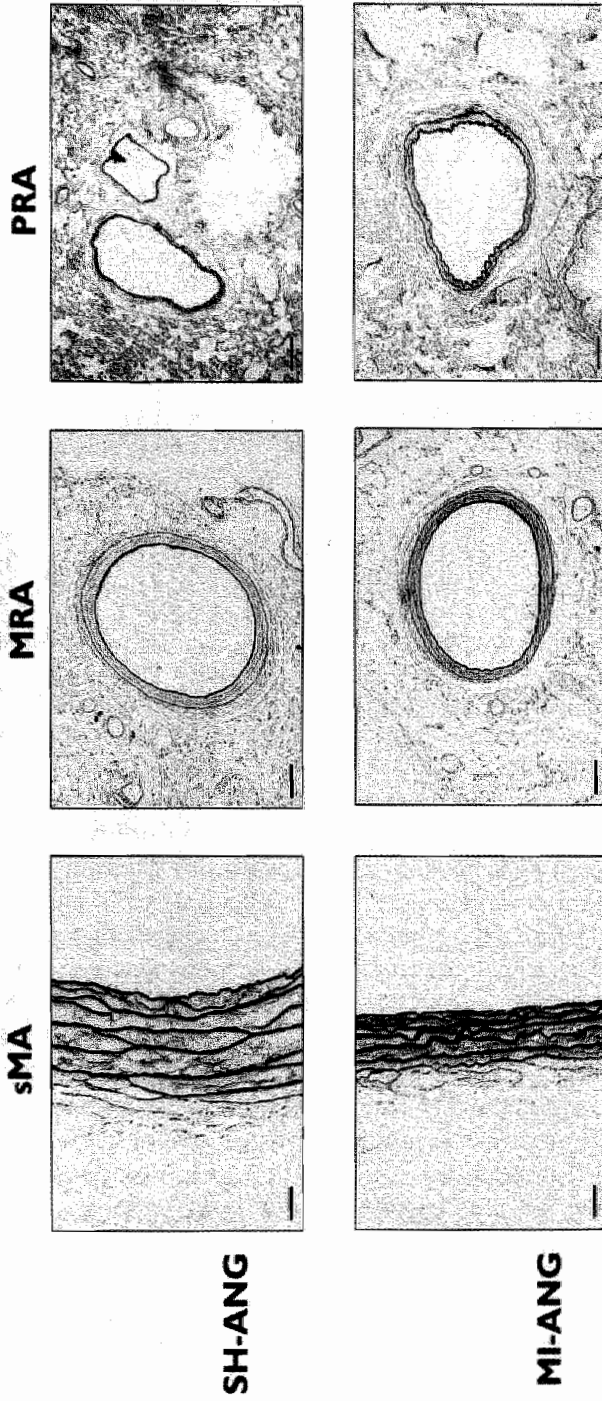Despite comparable effects on cardiac function, infusion of ANG II in a dose

Figure 3.4   Representative photographs of Lawson stained medial cross-cross sections of vessels of ANG II infused sham-operated rats (SH-ANG) and MI rats (MI-ANG). Left panel : superior mesenteric arteries, bar represents 31 µm. Middle panel : mesenteric resistance arteries, bar represents 80 µm. Right panel: pulmonary resistance arteries, bar represents 143 µm.

Table 3.5   Vessel dimensions of resistance arteries

	Saline infused		ANG II infused	
	Sham	MI	Sham	MI
Medial cross-sectional area				
mRA	22366 ± 2623	23737 ± 2942	25143 ± 1787	22629 ± 1916
pRA	31483 ± 2319	35041 ± 2023	29403 ± 2115	39665 ± 3192
Lumen area				
mRA	27679 ± 7318	35072 ± 8167	27511 ± 4772	30555 ± 5793
pRA	64826 ± 7970	67608 ± 3555	692ï0 ± 7398	76431 ± 5274
Media-to-lumen ratio				
mRA	78 ± 13	53 ± 9	97 ± 11	75 ± 11
pRA	60 ± 9	57 ± 6	51 ± 6	57 ± 7
n	9	7	14	16

Table shows cross-sectional areas, lumen areas (both in μm^2) and media-to-lumen ratio (cross-sectional area/lumen area * 100) of the mesenteric and pulmonary resistance arteries (mRA and pRA, respectively). Data are expressed as mean ± sem. Statistical analysis: Intergroup differences were tested by Mann-Whitney test with a Bonferroni correction for multiple group comparison; *p<0.0125 compared to saline infused sham rats, †p<0.0125 compared to saline infused MI rats and ‡p<0.0125 compared to ANG II infused sham rats.

that increased systolic blood pressures in ANG II infused sham rats, resulted in a much smaller increase of 30 mmHg in MI rats (**experiment 2**, figure 3.3). In fact, blood pressures in ANG II infused MI rats were not different from SH-NaCl rats during the entire experimental period. The same pattern of changes was seen in mean arterial pressures of the rats used in both experiment 1 and 2, measured at day 14. While ANG II infusion in sham rats increased mean arterial pressure by approximately 60 mmHg as compared to SH-NaCl rats (relative increase of 47±5%), mean arterial pressure in MI-ANG rats was only 20 mmHg higher than in MI-NaCl rats (relative increase of 21±4%) and not different from SH-NaCl rats (table 3.2). ANG II infusion in MI rats, in contrast to its infusion in sham rats, did not increase medial cross-sectional area of the superior mesenteric artery (figure 3.4 and table 3.4). Furthermore, the ANG II infusion tended to increase medial cross-sectional area of the pulmonary resistance arteries, but this was not statistical significant (p=0.06, table 3.5).

ANG II infusion in MI rats increased plasma concentrations of ANG II and ANP (**experiment 3**, table 3.6). Plasma ANG II concentrations did not differ in MI-ANG and SH-ANG rats. Plasma ANP levels showed a clear tendency to increase in MI-ANG rats (SH-ANG 58±13 pg/ml and MI-ANG 152±48, p=0.07). ANG II

Table 3.6 Plasma ANG II and ANP concentrations and aortic cGMP concentrations

Variable	Saline infused		ANG II infused	
	Sham	MI	Sham	MI
Plasma ANG II	22 ± 4	22 ± 6	84 ± 21*	75 ± 23†
Plasma ANP	21 ± 1	42 ± 10*	58 ± 13*	152 ± 48†
Aortic cGMP	559 ± 87	422 ± 72	306 ± 59	635 ± 106‡
n	14	12	6	7

Table shows plasma concentrations of ANG II (pg/ml) and atrial natriuretic peptide (ANP, pg/ml) and aortic cGMP concentrations (fmol/mg protein). Statistical analysis: Intergroup differences were tested by Mann-Whitney test with a Bonferroni correction for multiple group comparison; *p<0.0125 compared to saline infused sham rats, †p<0.0125 compared to saline infused MI rats and ‡p<0.0125 compared to ANG II infused sham rats.

infusions in MI rats tended to increase cGMP concentrations in ANG II infused MI rats (from 422±72 to 635±106 fmol/mg protein, p=0.15). As a result of the decrease in cGMP concentrations in SH-ANG rats, aortic cGMP concentrations significantly increased in ANG II infused MI rats, compared to SH-ANG (table 3.6).

Discussion

The results of the present study indicate that infusion of ANG II decreased cardiac function in both sham and MI rats. Thus, although an increased plasma ANG II concentration is correlated with a poor prognosis in humans (5, 6), it did not result in the expected adverse effects on cardiac function in MI rats. Other expected findings of the exogenous ANG II infusion, i.e. increased mean arterial blood pressure and hypertrophy of the peripheral vascular wall, were also not observed in MI rats. The data further suggests that during infusion of ANG II in compensated heart failure, other hormonal systems are activated and suppress the hypertensive and hypertrophic effects of ANG II.

Infusion of ANG II in experimental animals is known to reduce cardiac output (14, 15). This is confirmed in the present study as ANG II infusion reduced cardiac function in sham rats. However, in the ANG II infused MI rats, the reduction of baseline cardiac index and stroke volume index were not different from those observed in SH-ANG rats and, more importantly, not different from MI-

NaCl rats. As outlined in the results section, relative reductions in indexes during volume loading of MI-ANG rats were comparable to relative reductions observed in ANG II infused sham rats. Also, MI-ANG animals were able to generate a peak pressor response following injections of ANG II comparable to SH-ANG (table 3.3, MI-NaCl +59±5 mmHg, MI-ANG +74±10 mmHg, p=NS), indicating that combination of myocardial infarction and ANG II infusion did not result in a further deterioration of cardiac function. Finally, absolute values of total peripheral resistance did not differ in ANG II infused sham and MI rats, indicating that ANG II infusions did result in similar increases in total peripheral resistance in MI and sham rats.

ANG II infusion had comparable negative effects on cardiac function in rats with and without MI. In ANG II infused MI rats, however, it did not result in the large increase in blood pressure as seen in ANG II infused sham rats nor did it induce the typical hypertrophic response of the vascular wall of large conduit arteries.

At least three phenomena may be responsible for suppression of pressor and structural vascular effects of ANG II in MI rats. Firstly, ANG II may deteriorate cardiac function in MI rats, which did not occur. Secondly, different ANG-receptor subtypes may be involved in the acute and long-term hypertensive and hypertrophic actions of the peptide. The aortic wall of a normal adult rat contains the angiotensin subtype 1 (AT1) and subtype 2 (AT2) receptor subtype in a 4:1 ratio (16). AT1 receptors mediate vasoconstriction and vascular smooth muscle cell growth (17). AT2 receptors are thought to have a functional role during embryonal development (18) and may mediate antiproliferative and apoptotic effects (19, 20). The subtypes may differ in the extent to which they are regulated by elevated levels of the agonist (17) and a switch from the AT1 to the AT2 receptor in peripheral arteries of MI rats might explain the suppression of pressor and structural effects of ANG II infusions. In the heart, a change in expression of AT1 and AT2 receptors has been shown to occur during heart failure (21-23). In the present study, however, both ED_{50} and maximal elevation of pressure following acute injections of ANG II, were not affected in ANG II infused MI rats, indicating the presence of functionally active vascular AT1 receptors after a two week ANG II infusion. A possible upregulation of AT2 receptors, however, cannot be excluded.

Another possibility is that in MI rats the pressor and mitogenic effects of ANG II may have been counteracted by a concomitant activation of other neurohumoral systems. Candidate vasodilating and vascular smooth muscle cell growth suppressor systems are ANP and NO, which have increased plasma levels during heart failure (24, 25). In cultured vascular smooth muscle cells, both ANP and NO have antimitogenic effects (26, 27). In vivo, infusions of non-depressor doses of ANP decrease medial thickness in spontaneously hypertensive rats (28), while inhalation of NO inhibits neointimal formation after balloon-induced arterial injury

(29). In our experimental model of heart failure, ANG II infusion tended to increase plasma ANP concentrations. Moreover, although ANG II had divergent effects on aortic cGMP concentrations (i.e. tendency to decrease in SH-ANG rats and to increase in MI-ANG rats), aortic cGMP concentrations were significantly higher in ANG II infused MI rats, compared to ANG II infused sham rats. The in vivo basal aortic cGMP concentration seems to mainly depend on NO synthase (30), since contribution of endogenous ANP to basal aortic cGMP generation, via stimulation of the particulate guanylate cyclase, appears to be minor compared to that of NO via soluble guanylate cyclase. Thus, it is attractive to hypothesize that during infusion of ANG II in experimental heart failure, the growth-stimulatory and hypertensive effects of ANG II are counteracted by concomitant activation of vasodilating and growth-inhibitory systems, such as ANP and NO.

It should be noted that the lack of hypertrophic response of the superior mesenteric artery in MI rats may also be explained by the normal, non hypertensive blood pressures after two weeks of ANG II infusions. However, infusion of ANG II in normal rats induces a hypertrophic response in peripheral vessels (11, 31), and this effect has been shown to be independent of the increase in blood pressure (32). Also, ANG II has growth-stimulating effects for vascular smooth muscle cells in culture (31, 33). Thus, we assume that the dose of ANG II given in this study was high enough to potentially induce a hypertrophic vascular response, and that this effect is independent from blood pressure.

A different response to ANG II was seen in the resistance arteries. MI alone or in combination with ANG II infusion did not induce changes in dimensions of the mesenteric resistance arteries. ANG II has been shown to have different effects on large conduit arteries and resistance arterioles (reviewed in 34 and 35). While the increase in wall thickness in large vessels is the result of hypertrophy, changes in wall thickness in small vessels appear to result from hyperplasia. Also, ANG II has been shown to induce increases in vessel wall thickness in the mesenteric circulation of young and adult rats (32, 36) at similar infusion rates as the ones used in this study. However, in those studies (32, 36), medial thickness was measured after myograph experiments (no in situ fixation) and in smaller branches of the mesenteric circulation (lumen diameter 200 μm versus 300 μm in the present study), which might explain the differences with results of the present study. Also, mesenteric resistance arteries have been reported to have only weak contractions to ANG II (compared to e.g. femoral resistance arteries (37)). It is also possible that, in this study, the time span was too short to induce hyperplastic changes in mesenteric resistance arteries.

Next to its effects on peripheral vascular structure, ANG II also increased heart-to-body weight ratio. Many studies have shown a development of cardiac hypertrophy during chronic ANG II infusions (38, 39). Most studies define 'cardiac hypertrophy' as an increase in heart-to-body weight ratio's. According to this definition, infused MI and sham rats in the present study would also display

cardiac hypertrophy. However, given the fact that the absolute heart weights did not differ, and as the animals lost a significant amount of body weight, the heart-to-body weight ratio does not give a fair representation of the amount of cardiac hypertrophy. Thus, in the present study, a two week ANG II infusion of 250 ng/kg/min did not result in the development of a pronounced cardiac hypertrophy.

In conclusion, this study shows that elevation of plasma ANG II infusion 2 weeks after infarction results in a comparable reduction of cardiac function in both sham and MI rats. Interestingly, in contrast to ANG II infused sham rats, ANG II infused MI rats do not develop hypertension or structural vascular alterations. We suggest that the reason for this divergent response in sham and MI rats is the activation of counterregulatory hormonal systems in MI rats, which are, at least in the compensated phase of heart failure, responsible for suppression of the hypertensive and hypertrophic effects of ANG II. This suggests that adverse effects of ANG II on function and structure of the cardiovascular system become evident only when counter-regulatory hormonal systems are failing, as in decompensated heart failure.

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Chapter 4

Opposing Effects of Angiotensin type 1 and type 2 receptor on Blood Pressure and Peripheral Vascular Structure in Rats with a Myocardial Infarction

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Abstract

In this study, a model for compensated heart failure with increased plasma concentrations of angiotensin II (ANG II) was used to investigate the involvement of ANG II receptors (AT) type 1 and 2 in the regulation of blood pressure and peripheral vascular structure. Therefore, ANG II (250 ng/kg/min) in combination with the AT1 receptor antagonist GR138950 (2 mg/kg/day) or the AT2 receptor antagonist PD123319 (10 mg/kg/day) was infused in myocardial infarcted rats. After two weeks, blood pressure and indices of peripheral vascular structure were measured. *Results:* ANG II infusion increased mean arterial blood pressure and medial cross-sectional area (CSA) of several large conduit arteries in sham-operated rats, but had no effect in MI rats. PD123319 infusion in ANG II infused sham rats did not change mean arterial blood pressure. In ANG II infused MI rats, however, PD123319 did increase blood pressure further, while GR138950 significantly lowered blood pressure. The effects of AT2 receptor antagonism on the structure of peripheral arteries were very heterogeneous. PD123319 increased medial CSA of the thoracic aorta of ANG II infused sham rats, but did not change medial CSA of the superior mesenteric and carotid arteries. It had no major effects on vessel dimensions of the resistance arteries in this group of rats. In ANG II infused MI rats, PD123319 had no effects on vessel dimensions of both large conduit and resistance arteries. Co-infusion of ANG II and GR138950 in MI rats decreased medial CSA of both large conduit and resistance arteries as compared to ANG II (and PD) infused MI rats. *Conclusion:* During blockade of the AT1 receptor, ANG II will selectively stimulate the AT2 receptor. Thus, the reduction of both blood pressure and medial CSAs during co-infusion of ANG II and GR138950 suggests that the AT2 receptor opposes the effects of the AT1 receptor in the regulation of blood pressure and vascular structure in MI rats. Furthermore, the observations that AT2 receptor blockade does increase blood pressure in ANG II infused MI rats, but not in sham rats confirms the conclusion on the role of the AT2 receptor in blood pressure regulation in MI rats. In contrast to sham rats, AT2 receptor blockade in MI rats does not affect medial CSAs of peripheral arteries. It is hypothesized that these variations in vascular remodeling during AT2 receptor blockade may be explained by either different ratio's of vascular AT1 and AT2 receptors or the involvement of other regulatory mechanisms.

Introduction

The responses of heart and blood vessels to angiotensin II (ANG II) are mediated by two receptor subtypes, the angiotensin type 1 (AT1) and the angiotensin type 2 (AT2) receptor. The AT1 receptor is well-characterized and present in cardiac and vascular tissue. It mediates the effects of ANG II on peripheral vasoconstriction, aldosterone release and cardiac myocyte and vascular smooth muscle cell proliferation (1-4). Much less is known about the function of the AT2 receptor, but the results of recent studies suggest that the AT2 receptor has opposing actions on the blood pressure regulating and growth-stimulating effects of the AT1 receptor (5, 6).

During heart failure, the activity of the renin-angiotensin system and plasma concentrations of ANG II have been shown to increase (7, 8). In the acute phase, ANG II mediates vasoconstriction and fluid-retention thereby maintaining cardiac output and sustaining organ perfusion. However, despite these beneficial effects, increased plasma concentrations are also correlated with a poor prognosis (9, 10). Thus, in view of the important role of ANG II on vascular tone and structure and the opposing actions of the AT2 receptor on the AT1 receptor, the AT2 receptor could be important in determining the effects of ANG II during heart failure. In chapter 3, it was shown that infusion of exogenous ANG II in sham and MI rats influenced systemic blood pressure and peripheral vascular structure quite differently. While ANG II increased the blood pressure and induced hypertrophy of the peripheral vascular wall in sham-operated rats, it failed to increase the systemic blood pressure in MI rats above the normotensive control level and failed to affect peripheral vascular structure. In the present study, the hypothesis was tested that suppression of hypertensive and peripheral hypertrophic effects of ANG II in MI rats, is caused by the effects of the AT2 receptor on blood pressure and peripheral vascular structure. To test this hypothesis, blood pressure and parameters of peripheral vascular structure were measured in MI rats that were co-infused with ANG II and either the AT1 receptor antagonist GR138950 or the AT2 receptor antagonist PD123319.

Materials and methods

Animals
Male Wistar rats (n=57, 270-275 g at day 0, Iffa Credo, Someren, the Netherlands) were housed under standard conditions and fed standard rat chow (RMH-TM, Hope Farms, Woerden, The Netherlands) and water ad libitum. The experiments were performed in accordance with local institutional guidelines. Of the 57 rats used, 14 died

following coronary artery ligation and none following sham surgery. Five rats had infarct sizes less than 20% of left ventricular circumference, or showed non-transmural infarcts.

Rats were randomly assigned to the following groups, *SH-ANG* (n=9) were subjected to sham surgery and were infused with angiotensin II (ANG II). *MI-ANG* (n=10) were infused with ANG II following induction of a myocardial infarction (MI). *SH-ANG-PD* (n=7) and *MI-ANG-PD* (n=7) were subjected to sham and MI surgery, respectively, and infused with ANG II and the AT2 receptor antagonist PD123319. MI-ANG-GR (n=5) were subjected to MI surgery and infused with ANG II and the AT1 receptor antagonist GR138950. Sham-operated rats infused with the AT1 antagonist GR138950 were not included, as previous studies in our laboratory showed that combined infusion of ANG II and AT1 blockade did not increase blood pressure (4). Also, the blood-pressure reducing effects of AT1 receptor blockade during infusions of ANG II are well known from literature (4, 11, 12).

Coronary artery ligation
Myocardial infarction was induced by ligation of the left anterior descending coronary artery (LAD) according to the method of Fishbein (13) as described in chapter 2.

Drug treatment
Drugs were infused by minipumps (Alzet model 2002 for ANG II and PD123319 and Alzet 2ml1 for GR138950, Alza Corporation, Palo Alto, California), implanted subcutaneously between the shoulder blades on the day of MI or sham surgery. ANG II (human [^{5}Val] ANG II, 250 ng/kg/min, Brunschwig, Amsterdam, The Netherlands) was dissolved in 0.9% NaCl. The AT2 receptor antagonist PD123319 (10 mg/kg/day, firma) was dissolved in 0.9% NaCl and the AT1 receptor antagonist GR138950 (2mg/kg/day) was dissolved in 0.8% $NaHCO_3$ and 10% ethanol. For GR138950, the osmotic minipump was connected to a PE10 catheter, to infuse GR138950 directly into the left jugular vein.

The doses of the two AT receptor antagonist were based on pilot studies. Rats (n=4-6) were infused for two weeks with NaCl, GR138950 and PD123319. At the end of the infusion period, rats were anesthetized with sodium pentobarbital (60 mg/kg i.p.) and the ANG II dose-pressor relationship was assessed in a cumulative fashion as described in chapter 3.

GR138950 in a dose of 2 mg/kg/day was found to induce a 20-fold right-ward shift of the dose-pressor response to ANG II. PD123319 in a dose of 10 mg/kg/day did not influence ED_{50}, suggesting a total lack of inhibition of the pressor response to ANG II. Infusion of PD123319 in a dose of 10 mg/kg/min in the rat results in plasma concentrations around 300 nM (14). Since the IC_{50} of PD123319 for the AT2 receptor is approximately 10 nM, this dose of PD123319 should result in an effective AT2 receptor blocking, without affecting the AT1 receptor.

Assessment of mean arterial pressures and heart rates on day 14.
On day 14, mean arterial blood pressures and heart rates were measured as described in the materials and methods in chapter 3.
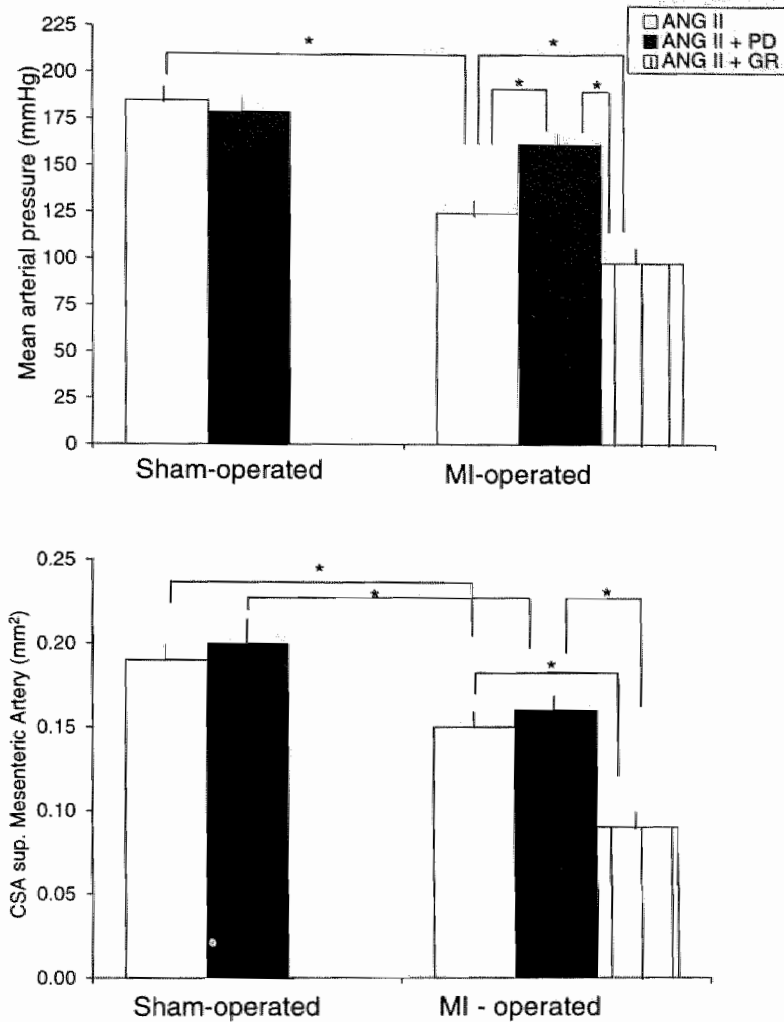
Figure 4.1   Graph showing the effects of the different infusion protocols on mean arterial blood pressure (mmHg, top panel) and medial cross-sectional area of the superior mesenteric artery (mm^2, bottom panel). * p<0.01 by Mann-Whitney test with Bonferroni correction.

Structural methods

After the measurements of mean arterial blood pressures and heart rates, rats were prepared for perfusion fixation. For tissue processing and morphometric measurements, see the materials and methods in chapter 2.

Statistics
Data are expressed as means±SEM. Intergroup differences were evaluated with a non-parametric Mann-Whitney test with a Bonferroni correction for multiple group comparison. The Bonferroni procedure corrects the p value for each pairwise group comparison, thus with 5 pairwise comparisons (SH-ANG versus MI-ANG, SH-ANG versus SH-ANG-PD, MI-ANG versus MI-ANG-PD, MI-ANG versus MI-ANG-GR and MI-ANG-PD versus MI-ANG-GR), statistical significance is defined as p<0.01 (p<0.05 divided by 5).

Results

Characteristics of experimental groups.
The mean infarct size was 46±4%, 41±4% and 42±2% in MI-ANG, MI-ANG-PD and MI-ANG-GR rats, respectively. Body weights at day 0 were comparable in all groups. The weight loss was approximately 40 g in all groups, except in GR138950 infused MI rats, in which a small weight gain was observed during the experimental period (table 4.1).

Heart weights tended to decrease in SH-ANG-PD rats (heart weight 0.92±0.06 g in SH-ANG and 0.80±0.03 g in SH-ANG-PD, p=0.06), but were not changed in MI-ANG and MI-ANG-PD rats. Infusion of GR138950 in MI-ANG rats however, significantly
decreased heart weight and heart-to-body weight ratios compared to ANG II and ANG II-PD infused MI rats (table 4.1).

Lung weight (and lung-to-body weight ratios, data not shown) were increased in ANG II and ANG II-PD infused MI rats, compared to the appropriate control group of sham rats. AT1 receptor antagonism by GR138950 in ANG II infused MI rats, however, completely normalized lung weights (table 4.1).

Blood pressure measurements. At the end of the experimental period, mean arterial pressures were significantly lower in MI-ANG rats, compared to SH-ANG rats (table 4.1, figure 4.1). AT2 receptor antagonism by PD123319 did not affect mean arterial blood pressure in ANG II infused sham rats. However, blockade of the AT1 receptor in ANG II infused MI rats significantly decreased, and blockade of the AT2 receptor significantly increased mean arterial blood pressure from 124±6 mmHg in MI-ANG rats to 162±9 mmHg in MI-ANG-PD rats (table 4.1, figure 4.1). Heart rates were comparable in all groups .

Vessel dimensions.
Large conduit arteries: The medial cross-sectional areas of the large conduit arteries were increased in ANG II infused sham rats, compared to ANG II infused MI rats (table 4.2, figure 4.1).

AT1 receptor blockade by GR138950 in ANG II infused MI rats reduced medial

Table 4.1   Characteristics of experimental groups

	Sham-operated		MI-operated		
	ANG II	ANG II + PD	ANG II	ANG II + PD	ANG II + GR
Gain of BW (g)	-33 ± 7	-41 ± 7	-40 ± 7	-37 ± 5	8 ± 4†,§
BW day 14 (g)	244 ± 5	241 ± 7	232 ± 5	242 ± 5	287 ± 7†,§
HW (g)	0.92 ± 0.06	0.80 ± 0.03	0.93 ± 0.06	0.97 ± 0.06‡	0.77 ± 0.03§
LW (g)	1.5 ± 0.2	1.2 ± 0.07	2.4 ± 0.3	2.2 ± 0.3‡	1.2 ± 0.04†,§
HW/BW (g/kg)	3.8 ± 0.3	3.3 ± 0.1	4.0 ± 0.2	4.0 ± 0.2‡	2.7 ± 0.1†,§
MAP (mmHg)	184 ± 7	178 ± 7	124 ± 6*	162 ± 9†	98 ± 4†,§
HR (beats/min)	370 ± 6	376 ± 6	336 ± 11	345 ± 25	355 ± 10
n	9	7	10	7	5

Table shows gain of body weights (BW) in 14 days, body weight after 14 days of infusions, heart weight (HW), lung weight (LW), heart-to-body weight ratios (HW/BW), mean arterial pressures (MAP) and heart rates (HR) in sham and MI rats infused with ANG II (250 ng/kg/min) or co-infused with either PD123319 (PD, 10 mg/kg/day) or GR138950 (GR, 2 mg/kg/day). Data are expressed as mean±sem. Groups comparisons by Mann-Whitney test with a Bonferroni correction; *=P<0.01 compared to SH-ANG, †=P<0.01 compared to MI-ANG, ‡=P<0.01 compared to SH-ANG-PD and §=P<0.01 compared to MI-ANG-PD.

cross-sectional areas of the large conduit arteries investigated (table 4.2).

Blockade of the AT2 receptor by PD123319 in ANG II infused sham rats, further increased medial cross-sectional area of the thoracic aorta, but had no effect on medial cross-sectional areas of the superior mesenteric artery (sMA) and carotid artery (CA). The lumen areas of the two later vessels, however tended to increase during the co-infusion of ANG II and PD (lumen area sMA; SH-ANG 0.34±0.02 mm^2 versus SH-ANG-PD 0.44±0.04 mm^2, p=0.10 and lumen area CA; SH-ANG 0.31±0.03 mm^2 versus SH-ANG-PD 0.37±0.04 mm^2, p=0.06). The increase in lumen diameter resulted in a decrease in the media-to-lumen ratio of the superior mesenteric artery, although the difference did not reach statistical significance (SH-ANG 57±3% versus SH-ANG-PD 46±3%, p=0.02).

Blockade of the AT2 receptor by PD123319 in ANG II infused MI rats did not increase medial cross-sectional area of the large conduit arteries (table 4.2, figure 4.1), although the medial cross-sectional area of the carotid artery tended to increase (MI-ANG 0.11 ± 0.01 mm^2 versus MI-ANG-PD 0.13 ± 0.01 mm^2, p=0.02; table 4.2). As the lumen diameter of the superior mesenteric artery of rats co-infused with PD and ANG II increased compared to MI rats infused with ANG II alone, the media-to-lumen ratio tended to decrease (MI-ANG 51±4% versus MI-ANG-PD 40±2, p=0.03, table 4.2).

Resistance arteries: There were no differences between ANG II infused sham and MI rats for the different types of resistance arteries.

AT1 receptor antagonism in ANG II infused MI rats had major effects on vessel dimensions of the resistance arteries. Medial cross-sectional areas of the pulmonary vascular bed were significantly smaller and medial cross-sectional areas of the mesenteric resistance arteries tended to be smaller (MRA^{2nd} order MI-ANG 21351±2077 mm^2 versus MI-ANG-GR 13772±2000 mm^2, p=0.03). Also, media-to-lumen ratios of the 2nd order mesenteric resistance arteries were significantly smaller compared to ANG II infused MI rats.

AT2 receptor antagonism in ANG II infused sham rats had no effect on medial cross-sectional areas of all investigated resistance arteries. The media-to-lumen ratio of the 2nd order mesenteric resistance arteries of PD123319 treated ANG II infused sham rats tended to decrease (media-to-lumen-ratio SH-ANG 61±10 versus SH-ANG-PD 43±2, p=0.06). AT2 receptor antagonism in ANG II infused MI rats had no effects on vessel dimensions of the resistance arteries (table 4.3).

Discussion

In this study, infusion of exogenous ANG II after myocardial infarction was used to create a model of compensated heart failure with increased plasma concentrations of ANG II. Previous work showed that this infusion of ANG II resulted in a comparable decrease in cardiac function in MI and sham rats. Further effects of this

Table 4.2   Vessel dimensions of large conduit arteries after 2 weeks of infusions.

	Sham-operated		MI-operated		
	ANG II	ANG II + PD	ANG II	ANG II + PD	ANG II + GR
Cross-sectional Area					
TA	0.67 ± 0.02	0.75 ± 0.01*	0.53 ± 0.04*	0.53 ± 0.02‡	0.41 ± 0.02 §
sMA	0.19 ± 0.01	0.20 ± 0.02	0.15 ± 0.02*	0.16 ± 0.01‡	0.09 ± 0.005 †,§
CA	0.14 ± 0.008	0.15 ± 0.007	0.11 ± 0.01*	0.13 ± 0.01	0.08 ± 0.007 §
Lumen Area					
TA	1.72 ± 0.09	1.87 ± 0.06	1.47 ± 0.08	1.52 ± 0.21	0.93 ± 0.29
sMA	0.34 ± 0.02	0.44 ± 0.04	0.30 ± 0.02	0.39 ± 0.03	0.28 ± 0.05
CA	0.31 ± 0.03	0.37 ± 0.04	0.25 ± 0.02	0.30 ± 0.04	0.27 ± 0.06
Media-to-lumen ratio					
TA	39 ± 2	40 ± 1	37 ± 4	38 ± 6	56 ± 13
sMA	57 ± 3	46 ± 3	51 ± 4	40 ± 2	34 ± 4
CA	42 ± 3	42 ± 6	49 ± 12	44 ± 4	41 ± 17
n	9	7	10	7	5

Table shows cross-sectional area (mm^2), lumen area (mm^2) and media-to-lumen ratio (cross-sectional area/lumen area * 100) of the thoracic aorta (TA), superior mesenteric artery (sMA) and the carotid artery (CA) of sham and MI rats infused with ANG II (250 ng/kg/min) or co-infused with either PD123319 (PD, 10 mg/kg/day) or GR138950 (GR, 2 mg/kg/day). Data are expressed as mean±sem. Groups comparisons by Mann-Whitney test with a Bonferroni correction;*=P<0.01 compared to SH-ANG, †=P<0.01 compared to MI-ANG, ‡=P<0.01 compared to SH-ANG-PD and §=P<0.01 compared to MI-ANG-PD.

infusion in sham, but not in MI rats, were a substantial increase in blood pressure and hypertrophy of the peripheral vascular wall. In the present study, the hypothesis was tested that changes in AT receptor subtypes are responsible for this phenomenon.

It is well established that the AT1 receptor mediates vasoconstriction and that blockade of this receptor reduces blood pressure (4, 11, 12). The results of the present study agree with this finding as blockade of the AT1 receptor in ANG I infused MI rats significantly reduced blood pressure. In contrast to the effects of AT1 receptor blockade, other studies have shown that infusion of PD123319 alone did no increase blood pressure (4, 11). However, studies in mice with targeted disruption of the AT2 receptor gene exhibited an increased basal blood pressure (5) and an enhanced response to ANG II (5, 15), suggesting that the AT2 receptor may oppose the pressor responses to ANG II. Although the present study also showed no effects of the AT2 blockade on the blood pressure of ANG II infused sham rats, co-infusion of ANG II and PD123319 did increase blood pressure in MI rats. This may depend upon a change in the AT receptor sub-populations in the peripheral vasculature.

Opposing effects of the AT1 and AT2 receptors have also been described for the regulation of vascular structure. The proliferative and hypertrophic effects of ANG I are predominantly regulated by the AT1 receptor, but the AT2 receptor has been shown to induce an antimitogenic effect on rat coronary endothelium cells (6) and R3T3 cells (16). Also, the AT2 receptor mediates apoptosis in a rat pheochromo cytoma cell line (17). Overexpression of the AT2 receptor by transfecting an AT2 receptor vector in the balloon-injured carotid artery attenuated neointimal formation indicating similar effects in vascular smooth muscle cells (18).

The peripheral structural effects of the blockade of the AT1 receptor were very homogenous in the large conduit arteries, since all 3 exhibited a decreased media cross-sectional area. During co-infusion of ANG II and an AT1 antagonist, ANG II is expected to exert a selective stimulation of the AT2 receptor. Thus, the decrease in medial cross-sectional areas of large conduit arteries during the blockade of the AT1 receptor, indicates that in MI rats, the AT2 receptor down-regulates vascular growth

In contrast, the structural vascular effects of blockade of AT2 receptor were heterogenous in both sham and MI rats. During co-infusion of ANG II and an AT2 antagonist, ANG II is expected to selectively stimulate the AT1 receptor. Thus, as infusion of PD123319 induced an additional increase in medial cross-sectional area of the thoracic aorta of sham rats, this suggests that the AT2 receptor is involved in regulation of vascular wall structure of this vessel. In the superior mesenteric and carotid arteries, no effects on medial cross-sectional area were seen during infusion of PD123319 in sham rats. In MI rats, blockade of the AT2 receptor did not change medial cross-sectional area of the thoracic aorta or the other large conduit arteries investigated.

The heterogenous response of the AT1 and AT2 receptor blockades on blood

Table 4.3 Vessel dimensions of resistance arteries after 2 weeks of infusions.

	Sham-operated		MI-operated		
	ANG II	ANG II + PD	ANG II	ANG II + PD	ANG II + GR
Cross-sectional Area					
mRA [3nd-4th order]	8520 ± 1038	9903 ± 2135	7838 ± 2065	6097 ± 1339	3800 ± 249
mRA [2nd order]	22569 ± 2236	24358 ± 758	21351 ± 2077	19556 ± 2210	13772 ± 2000
pRA	24215 ± 2096	23397 ± 6596	34716 ± 2896	21365 ± 3165	17963 ± 2240
Lumen Area					
mRA [3nd-4th order]	19983 ± 3315	16523 ± 4130	19929 ± 4255	10595 ± 3318	10977 ± 2324
mRA [2nd order]	37204 ± 6742	56208 ± 3597	36328 ± 5761	39137 ± 8130	46702 ± 7401
pRA	59554 ± 2590	49258 ± 10941	59564 ± 7057	36281 ± 4618	48786 ± 9285
Media-to-lumen ratio					
mRA [3nd-4th order]	45 ± 6	65 ± 15	43 ± 7	60 ± 10	44 ± 8
mRA [2nd order]	61 ± 10	43 ± 2	62 ± 10	49 ± 7	29 ± 2 †,§
pRA	47 ± 7	51 ± 7	71 ± 8	61 ± 8	45 ± 10
n	8	12	10	12	4

Table shows cross-sectional area (μm^2), lumen area (μm^2) and media-to-lumen-ratio (cross-sectional area/lumen area * 100) of mesenteric resistance arteries of the third-fourth order side branches (mRA [3nd-4th order]), mRA of the second order side branches (mRA [2nd order]) and pulmonary resistance arteries (pRA) of sham and MI rats infused with ANG II (250 ng/kg/min) or co-infused with either PD123319 (PD, 10 mg/kg/day) or GR138950 (GR, 2 mg/kg/day). Groups comparisons by Mann-Whitney test with a Bonferroni correction; *=$P<0.01$ compared to SH-ANG, †=$P<0.01$ compared to MI-ANG, ‡=$P<0.01$ compared to SH-ANG-PD and §=$P<0.01$ compared to MI-ANG-PD.

pressure and vascular structure in MI and sham rats may be explained by different ratios of AT1 and AT2 receptors along the vascular tree on the one hand and between MI and sham rats on the other. A wide variety in the relative proportion of AT receptor subtypes has been shown, not only among different tissues of the same species, but also within the same tissue of different species (19). This is also reflected by the wide variety of contractile responses to ANG II of aortic rings from different vessels. Juul *et al.* showed that ANG II in the rat induced the strongest contraction in the femoral artery, followed by a 2.5 fold reduction in contractile force of the cerebral artery, 5.3 fold reduction of the mesenteric artery and 15 fold reduction of the renal arteries (20). Differences which could be explained by a smaller amount of AT1 receptors, or a higher amount of AT2 receptors, opposing the contractile effects of the AT1 receptor.

During heart failure, changes in the cardiac AT-receptor population have been described, but, data are lacking on the status of vascular AT-receptors. Preliminary data from our laboratory on AT1 and AT2 receptor mRNA expression in peripheral arteries (carotid and superior mesenteric artery) determined by the competitive reverse transcriptase polymerase chain reaction (21, 22) show several interesting findings. Firstly, significant amounts of AT2 receptor mRNA transcripts are present in both the carotid and superior mesenteric artery (~ 1000 fg/pg RNA), and its expression exceeded that of AT1 receptor transcripts (~ 100 fg/pg RNA). This is surprising as most studies detecting vascular AT1 and AT2 receptors on the protein level by autoradiography showed a relative abundancy of AT1 receptors (70-80%) over AT2 receptors (20-30%) (19, 23, 24). Secondly, there is a tendency for higher AT2 receptor mRNA expression in the ANG II infused MI rats compared to ANG II infused sham rats, suggesting a change in regulatory mechanisms in the turnover of this receptor. The levels of AT1 receptor are variable, but unchanged. These preliminary data, which must be completed with autoradiographic studies, combined with the antagonist studies suggest that an upregulation of vascular AT2 receptors could be involved in the suppression of hypertensive and hypertrophic effects of ANG II during heart failure.

Changes in the AT1 and AT2 receptors may not be the sole explanation for the divergent effects of AT2 receptor blockade on vascular structure. The renin-angiotensin system is known to interact with several other neurohormonal systems, which may have an effect on the vascular structure. A candidate in this respect is the Nitric Oxide system (NO). ANG II has been shown to modulate NO release from the vascular endothelium (25, 26) and recent results from our laboratory show that co-infusion of ANG II and an inhibitor of NO synthase (L-NAME) in MI rats increases the medial cross-sectional area of the thoracic aorta, the superior mesenteric artery and the carotid artery, but has, in contrast to PD123319, no effect on mean arterial blood pressure (see chapter 5). The activation of other neuro-hormonal systems, such as NO, during infusion of ANG II in MI rats could also explain the dissociation of the effects of the AT receptor blockers on blood pressure and peripheral vascular

structure.

In conclusion, the reduction of both blood pressure and medial cross-sectional areas during co-infusion of ANG II and the AT2 receptor antagonist GR138950 suggests that the AT2 receptor opposes the effects of the AT1 receptor in the regulation of blood pressure and peripheral vascular structure in MI rats. Furthermore, the observation that AT2 receptor blockade does increase blood pressure in ANG II infused MI rats, but not in sham rats, confirms that the AT2 receptor is involved in blood pressure regulation in MI rats. Moreover, as AT2 receptor blockade did not influence medial cross-sectional areas, this suggests that there are either variations in the AT1 and AT2 receptor populations of different vessels or that other mechanisms are also involved in the regulation of peripheral vascular structure in MI rats.

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Chapter

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6

Vascular Structure and Basal Release of Nitric Oxide during Experimental Heart Failure in the Rat

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Abstract

Chronic heart failure is associated with an endothelial dysfunction and impaired vascular responsiveness to vasoconstrictors. The first objective in the present study, was to determine whether heart failure induces changes in the basal release of nitric oxide (NO), and therefore an inhibitor of NO synthase, N^{ω}-nitro-l-arginine methyl ester (L-NAME, 25 mg/kg/day) or saline (0.9% NaCl), were infused for two weeks in rats with a myocardial infarction (MI). Secondly, it was shown in previous studies that infusion of angiotensin II (ANG II) did not result in the expected hypertension and hypertrophy of the peripheral vascular wall in MI rats. Therefore, a co-infusion of ANG II and L-NAME was given in an additional group of MI rats to investigate whether the release of NO is involved in the effects of ANG II on blood pressure and peripheral vascular structure. *Results:* Aortic cyclic GMP (cGMP) concentrations as an indicator of NO synthase activity, did not differ in saline infused MI or sham rats, while L-NAME infusions significantly reduced aortic cGMP contents in both groups. L-NAME elevated mean arterial blood pressure by 31±5% in sham-operated rats. In MI rats, a similar increase in mean arterial pressure (22±4%) was observed, although the blood pressure did not increase above the level of normotensive control rats. The changes in peripheral vascular structure during L-NAME infusions differed in sham and MI rats. L-NAME infusions caused a marked increase in medial cross-sectional area (CSA) of the large conduit arteries in sham rats. In contrast, in MI rats, L-NAME increased the CSA of the carotid artery only, but had no effect on the CSAs of the thoracic aorta and superior mesenteric artery. Medial CSAs of the pulmonary and mesenteric resistance arteries were unaffected by L-NAME infusions in both groups. In the additional group of ANG II infused MI rats, L-NAME did not increase blood pressure, but had profound effects on peripheral vascular structure as the medial CSA of both large conduit and resistance arteries increased significantly.

In conclusion, L-NAME infusions induced similar blood pressure elevations in sham and MI rats. Since aortic cGMP concentrations were comparable in saline infused sham and MI rats, these results suggest basal release of NO is intact during experimental heart failure. However, the response on vascular structure differed markedly in L-NAME infused MI and sham rats. The co-infusion of ANG II and L-NAME in MI rats had no further effect on blood pressure but did increase the CSA of both large conduit and resistance arteries. This suggests that NO modulates the hypertrophic but not the hypertensive effects of ANG II in MI rats.

Introduction

Nitric oxide (NO), released from the vascular endothelium, plays an important role in endothelium-dependent vasodilation (1, 2). The continuous basal release of NO has been shown to contribute to the regulation of basal vascular tone (reviewed in reference 3 and 4). In addition to this basal release, endothelial NO release can be induced by several agonists such as acetylcholine and bradykinin (reviewed in reference 1 and 2). Recently, abnormalities of the vascular endothelium during heart failure have received considerable attention. Various reports, both in animals (5-7) and patients (8-10), show that agonist induced endothelium-dependent vasodilation is impaired in heart failure, indicating a reduced release of endothelial NO. In contrast, reports on changes in the basal production during heart failure are conflicting. Human studies report an intact (8, 11) or even enhanced basal release of NO (12, 13). Ex vivo animal studies using isolated vascular rings, however, show decreased basal release of NO (6, 14).

Besides its important role in the regulation of vascular tone, NO is also involved in the regulation of vascular structure as it inhibits vascular smooth muscle cell proliferation (15, 16). Little is known about this effect of NO during heart failure. In previous work, we have shown that (i) induction of a myocardial infarction in rats resulted in a reduced peripheral vascular growth (chapter 2) and that (ii) ANG II infusion in MI rats did not induce hypertension nor vascular hypertrophy (chapter 3). Since ANG II has been shown to modulate NO release from the vascular endothelium (17, 18), we speculate that NO might antagonize the hypertensive and vascular hypertrophic effects of ANG II in MI rats.

In the present study, we therefore wanted to determine (i) whether experimental heart failure induces changes in the basal release of NO, (ii) whether NO is involved in the regulation of peripheral vascular structure and (iii) whether NO antagonizes the effects of ANG II during heart failure. Therefore, an inhibitor of NO synthase, N^{ω} - nitro-l-arginine methyl ester (L-NAME) or saline (0.9% NaCl) were continuously infused for two weeks in MI and sham-operated rats. In an additional group of MI rats, a co-infusion of L-NAME and ANG II was given. The data indicate that the basal release of NO is intact during experimental heart failure and that NO antagonizes the vascular hypertrophic but not the hypertensive effects of ANG II in MI rats.

Materials and methods

Animals

Male Wistar rats (n=113, 200-250g at start of training on day -14, Iffa Credo, Someren, the Netherlands) were housed under standard conditions and fed standard rat chow (RMH-TM, Hope Farms, Woerden, The Netherlands) and water ad libitum. The experiments were performed in accordance with local institutional guidelines. The randomization scheme is shown in figure 5.1. From the 113 rats used, 13 died
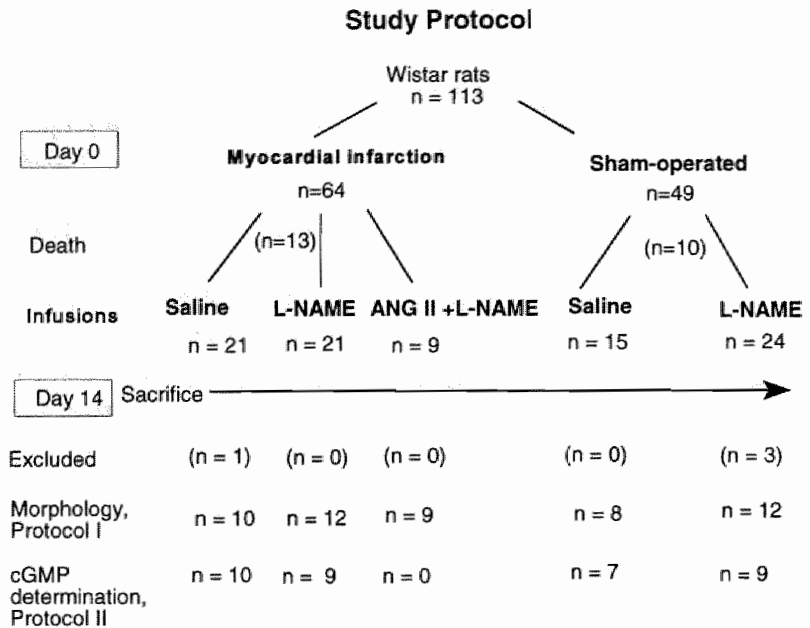
Study Protocol

Figure 5.1   Outline of the research protocol with randomization scheme

following coronary artery ligation and 10 following sham surgery. From the 90 remaining rats, 51 were used for blood pressure and morphometric measurements (protocol 1), and 35 rats for the determination of aortic cGMP (protocol 2). The remaining 4 rats had infarct sizes less than 20% of left ventricular circumference, or showed non-transmural infarcts.

Coronary artery ligation
Myocardial infarction was induced by ligation of the left anterior descending coronary artery (LAD) according to the method of Fishbein (19) as described in chapter 2.

Experimental protocol
Rats were randomly assigned to either the protocol for blood pressure and morphometric measurements (protocol 1, n=51) or determination of aortic cGMP content (protocol 2, n=35) as outlined in figure 5.1. For each study, rats were subsequently randomized to the following groups: *SH-NaCl* (protocol 1: n=8, protocol 2: n=7) were subjected to sham surgery and infused with saline (0.9% NaCl). *MI-NaCl* (protocol 1: n=10, protocol 2: n=10) were infused with saline following induction of a myocardial infarction (MI). *SH-NAME* (protocol 1: n=12, protocol 2 : n=9) and *MI-*

NAME (protocol 1: n=12, protocol 2 : n=9) were subjected to sham and MI surgery respectively, and infused with N^{ω} -nitro-l-arginine methyl esther (L-NAME, Sigma). In a previous study (chapter 3), ANG II was given to MI rats using a dose (250 ng/kg/min) that increased mean arterial blood pressure in sham-operated animals to approximately 190 mmHg after 14 days of infusions. However, in preliminary experiments, MI rats co-infused with L-NAME and this dose of ANG II became very ill and had excessive weight loss (average of 90 g during two weeks). These rats had no significant increase in mean arterial blood pressure (137±18 mmHg after 14 days of infusions (n=5)). Therefore, a lower dose of ANG II was used (100 ng/kg/min) in the co-infusion experiment with L-NAME (MI-ANG-NAME, protocol 1, n=9). This dose of ANG II has been reported to increase blood pressure in normal rats (20, 21).

Infusions: Osmotic minipumps (Alzet model 2002, Alza Corporation, Palo Alto, California) were prepared containing saline, or L-NAME (25 mg/kg/day) dissolved in saline, or ANG II (human [^{5}Val] ANG II, 100 ng/kg/min, Brunschwig, Amsterdam, The Netherlands). Minipumps were implanted subcutaneously between the shoulder blades, immediately following induction of a myocardial infarction or sham surgery. The duration of the infusions was 14 days.

Protocol 1 : In this protocol, mean arterial pressures and heart rates were measured prior to sacrifice on day 14. After perfusion fixation of the vascular tree, several vessels were excised for morphological measurements. For the assessment of mean arterial pressures and heart rates on day 14, see material and methods in chapter 3. For tissue processing after sacrifice and morphometric measurements, see material and methods in chapter 2.

Protocol 2 : In this protocol, the thoracic aorta was excised for the determination of cyclic GMP (cGMP) concentrations (22). The in vivo basal aortic cGMP concentration depends mainly on NO synthetase (23), as the contribution of endogenous ANP, via stimulation of the particulate guanylate cyclase, to basal aortic cGMP generation appears to be minor to that of NO via soluble guanylate cyclase.

For the tissue processing and determination of cyclic GMP concentration, see the materials and methods in chapter 3.

Statistics
Data are expressed as means±SEM. Intergroup differences were evaluated with a non-parametric Mann-Whitney test with a Bonferroni correction for multiple group comparison. The Bonferroni procedure corrects the p value for each pairwise group comparison, thus with 5 pairwise comparisons (SH-NaCl versus MI-NaCl, SH-NaCl versus SH-NAME, MI-NaCl versus MI-NAME, SH-NAME versus MI-NAME and MI-NAME versus MI-ANG-NAME), statistical significance is defined as p<0.01 (p<0.05 divided by 5).
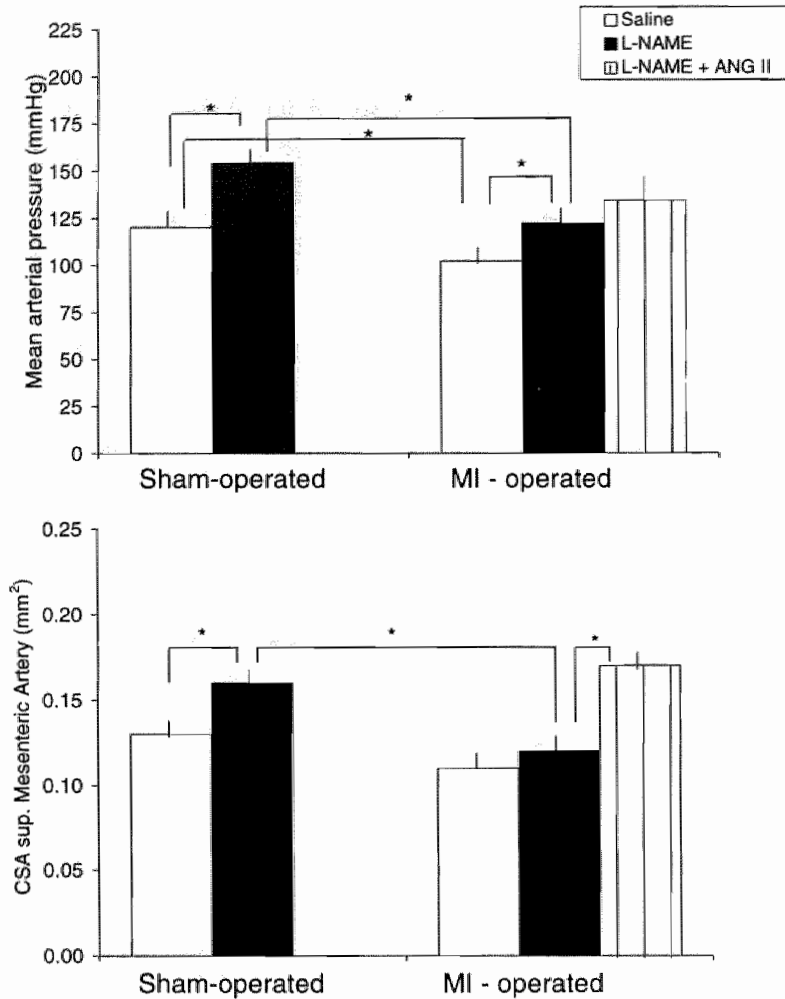
Figure 5.2   Graph showing the effects of the different infusion protocols on mean arterial blood pressure (mmHg, top panel) and medial cross-sectional area of the superior mesenteric artery (mm², bottom panel). * $p < 0.01$ by Mann-Whitney test with Bonferroni correction.

Results

Characteristics of experimental groups (protocol 1 and 2; table 5.1). The mean infarct size was 42±4% and 42±3% in MI-NaCl and MI-NAME rats respectively and 35±6% in the MI rats co-infused with L-NAME and ANG II. The infusion of L-NAME resulted

Table 5.1   Characteristics of experimental groups

	Sham-operated		MI-operated		
	Saline	L-NAME	Saline	L-NAME	L-NAME+ANG II
Gain of BW (g)	1 ± 2	-5 ± 3	-8 ± 3	-16 ± 3 ‡	-30 ± 10 †
BW day 14 (g)	282 ± 3	276 ± 6	287 ± 5	272 ± 5	251 ± 10 †
HW (g)	0.86 ± 0.02	0.88 ± 0.02	0.95 ± 0.02	0.81 ± 0.02 †,‡	0.97 ± 0.04 §
LW (g)	1.3 ± 0.1	1.3 ± 0.03	1.9 ± 0.2*	1.6 ± 0.1 ‡	1.8 ± 0.1
HW/BW (g/kg)	2.9 ± 0.1	3.2 ± 0.1*	3.2 ± 0.1	3.0 ± 0.1	3.9 ± 0.1 ‡,§
MAP (mmHg)	120 ± 4	154 ± 4 *	102 ± 3*	122 ± 4 †,‡	134 ± 10 †
HR (beats/min)	346 ± 9	344 ± 6	339 ± 6	347 ± 11	356 ± 13
n	15	23	20	21	9

Table shows gain of body weights (BW) in 14 days, body weight after 14 days of infusions, heart weight (HW), lung weight (LW), heart-to-body weight ratios (HW/BW), mean arterial pressures (MAP) and heart rates (HR). Data are expressed as mean ± sem. Group comparisons by Mann-Whitney test with Bonferroni correction; *P<0.01 compared to SH-NaCl, † P<0.01 compared to MI-NaCl, ‡ P<0.01 compared to SH-NAME and § P<0.01 compared to MI-NAME.

in a decreased body weight, which was more pronounced in ANG II infused MI rats. Infusion of L-NAME in sham rats and the subsequent hypertension increased heart-to-body weight ratios, but did not affect absolute heart weight. Heart weights of MI-NAME rats, however, were lower compared to MI-NaCl, SH-NAME and MI-ANG-NAME rats. Lung weights (table 5.1) and lung-to-body weight ratios (data not shown) of all infarct groups were increased compared to sham-operated rats.

Aortic cGMP concentrations. cGMP concentrations were not different in the saline infused rats (SH-NaCl 559±87 versus MI-NaCl 422±72 fmol/mg protein). L-NAME treatment significantly lowered cGMP concentrations in both MI and sham rats (SH-NAME 247±36 and MI-NAME 269±27 fmol/mg protein, p<0.05 versus respective saline infused rats), confirming the inhibition of NO synthase by L-NAME.

Blood pressure measurements. At the end of the experimental period, mean arterial pressure was reduced by 20 mmHg in MI-NaCl rats, compared to SH-NaCl rats (table 5.1, figure 5.2). L-NAME infusions increased mean arterial blood pressure by 31±5% (from 120±4 mmHg to 154±4 mmHg) in sham-operated rats. Although the absolute mean arterial blood pressure values of MI-NAME rats were not different from 'normotensive' SH-NaCl rats, the relative increase in mean arterial blood pressure by L-NAME was similar (22±4%, from 102±3 mmHg to 122±4 mmHg). Co-infusions of L-NAME and ANG II in MI rats did not increase mean arterial blood pressure (table 5.1, figure 5.2). Heart rates were comparable in all groups at the end of the experimental period (table 5.1).

Vessel dimensions. Compared to SH-NaCl rats, induction of a myocardial infarction induced no major changes in vessel dimensions of the large conduit (table 5.2, figure 5.2), but decreased media-to-lumen ratio of the pulmonary resistance arteries (table 5.3).
    L-NAME infusion in sham rats induced hypertrophy of the thoracic aorta, superior mesenteric artery and carotid artery, as medial cross-sectional area of all three vessels increased compared to SH-NaCl rats (table 5.2, figure 5.2). Infusion of the same dose of L-NAME in MI rats did not increase medial cross-sectional area, or media-to-lumen ratio of either the superior mesenteric artery or the thoracic aorta, but did increase the medial cross-sectional area of the carotid artery. In MI rats, the combination of ANG II and L-NAME induced the same increase in the medial cross-sectional area of the large conduit arteries as L-NAME infusion alone did in the sham-operated rats, but without the increase in mean arterial blood pressure (table 5.2, figure 5.2).
    L-NAME infusions in sham and MI rats did not result in major changes in the vessel dimensions of pulmonary and mesenteric resistance arteries (table 5.3). The lumen area of the 2nd order mesenteric resistance arteries of MI-NAME rats decreased compared to MI-NaCl rats. The media-to-lumen ratio of the pulmonary resistance arteries decreased in SH-NAME rats compared to SH-NaCl rats. The co-

Table 5.2   Vessel dimensions of large conduit arteries after 2 weeks of infusions.

	Sham-operated		MI-operated		
	Saline	L-NAME	Saline	L-NAME	L-NAME+ANG II
Cross-sectional Area					
TA	0.41 ± 0.03	0.54 ± 0.02 *	0.39 ± 0.02	0.40 ± 0.02 ‡	0.58 ± 0.02 †,§
sMA	0.13 ± 0.01	0.16 ± 0.01 *	0.11 ± 0.01	0.12 ± 0.01 ‡	0.17 ± 0.01 †,§
CA	0.073 ± 0.003	0.091 ± 0.003 *	0.069 ± 0.003	0.083 ± 0.004 †	0.107 ± 0.005 †,§
Lumen area					
TA	1.17 ± 0.20	1.30 ± 0.10	1.57 ± 0.13	1.22 ± 0.15	1.70 ± 0.11
sMA	0.33 ± 0.02	0.29 ± 0.02	0.30 ± 0.02	0.27 ± 0.03	0.36 ± 0.03
CA	0.23 ± 0.03	0.25 ± 0.02	0.26 ± 0.04	0.23 ± 0.03	0.31 ± 0.04
Media-to-lumen ratio					
TA	27 ± 2	41 ± 4 *	26 ± 2	29 ± 3 ‡	35 ± 2 †
sMA	38 ± 2	56 ± 3 *	36 ± 3	41 ± 3	47 ± 3
CA	42 ± 3	48 ± 1	38 ± 3	48 ± 4	39 ± 7
n	8	10	10	12	9

Table shows cross-sectional area (mm^2), lumen area (mm^2) and media-to-lumen ratio (cross-sectional area/lumen area * 100) of the thoracic aorta (TA), superior mesenteric artery (sMA) and the carotid artery (CA). Data are expressed as mean ± sem. Group comparisons by Mann-Whitney test with Bonferroni correction; *P<0.01 compared to SH-NaCl, † P<0.01 compared to MI-NaCl, ‡ P<0.01 compared to SH-NAME and § P<0.01 compared to MI-NAME.

infusion of L-NAME and ANG II in MI rats, however, increased medial cross-sectional area of the mesenteric resistance arteries (table 5.3).

Discussion

This study shows that the vasoconstrictor effect of L-NAME is preserved during experimental heart failure. In MI rats, L-NAME gradually increased mean arterial pressure, an increase which was proportional the same as that seen in sham rats. Other studies also show an increase in blood pressure during either acute or chronic L-NAME infusions in MI rats (24, 25). As L-NAME evokes increases in vascular resistance by continuous inhibition of the basal release of NO (3), these results, combined with the unchanged aortic cGMP concentrations in saline infused MI rats, suggest that the basal release of NO from the endothelium is preserved during experimental heart failure.

Alterations in endothelial function during heart failure have been subject of many investigations. Despite the intact basal release of NO, most reports agree upon an impaired agonist-induced release of NO. Ontkean (6) showed an impaired endothelium-dependent vasodilation in rats 10 weeks after MI. Vasodilation in response to acetylcholine and adenosine diphosphate (both endothelium-dependent), but not to nitroglycerin (endothelium-independent) were decreased in isolated vessel rings of MI rats. Subsequently, other studies also showed impaired agonist-mediated vasodilation in isolated vascular rings of rats after MI (7, 14, 26). In man, the endothelium dependent vasodilation is impaired in the coronary, skeletal muscle and skin circulation of patients with congestive heart failure, (8, 10, 27-30).

The present study demonstrates that, despite the similar proportional increase in mean arterial blood pressure, absolute values were significantly lower compared to L-NAME infused sham rats. In a previous study, we observed a suppressed response to the hypertensive effects of ANG II in MI rats (chapter 3 and 4). While ANG II increased the blood pressure in sham-operated rats after 14 days of infusions (189 ± 5 mmHg, relative increase of $47\pm4\%$), it did not increase the blood pressure of MI rats above the level of normotensive controls (126 ± 4 mmHg, relative increase of $21\pm4\%$). This decrease in responsiveness to vasoconstrictors in MI rats may be related to changes in contractility of the vascular smooth muscle cells. However, Stassen *et al.* showed that the contractility of the thoracic aorta and mesenteric resistance arteries in MI rats was comparable to that in sham rats, three weeks after surgery (31, 32). In addition, the present study shows that despite the intact basal release of NO in MI-rats, inhibition of NO release by L-NAME in ANG II infused MI rats did not further increase the blood pressure, indicating that NO is not involved in counterregulating the effects of ANG II on blood pressure in MI rats.

Another interesting aspect of this study was the differential effects of the infusions on peripheral vascular structure. For NO, studies have shown that both exogenous nitrogen vasodilators and inducers of endogenous NO, such as interferon-γ,

Table 5.3   Vessel dimensions of resistance arteries after 2 weeks of infusions.

	Sham-operated		MI-operated		
	Saline	L-NAME	Saline	L-NAME	L-NAME+ANG II
Cross-sectional Area					
mRA 3rd-4th order	5231 ± 561	5084 ± 496	4856 ± 407	5114 ± 465	8204 ± 1066 †
mRA 2nd order	15393 ± 1194	15119 ± 1248	16817 ± 2212	15020 ± 964	24399 ± 2048 †,§
pRA	26943 ± 2294	25655 ± 1319	30226 ± 2122	27642 ± 2258	29182 ± 3611
Lumen Area					
mRA 3rd-4th order	10868 ± 2689	9838 ± 1805	7673 ± 1676	7466 ± 2436	19435 ± 5844
mRA 2nd order	31516 ± 3519	24160 ± 5020	36326 ± 2485	21198 ± 4459	47461 ± 5726 §
pRA	46528 ± 7510	61414 ± 12101	66985 ± 4667	57998 ± 9831	60201 ± 8626
Media-to-lumen ratio					
mRA 3rd-4th order	49 ± 7	62 ± 11	64 ± 11	80 ± 18	45 ± 10
mRA 2nd order	62 ± 14	66 ± 14	52 ± 9	67 ± 14	46 ± 4
pRA	74 ± 6	36 ± 4 *	50 ± 10 *	54 ± 6	73 ± 11
n	8	12	10	12	9

Table shows cross-sectional area (μm^2), lumen area (μm^2) and media-to-lumen-ratio (cross-sectional area/lumen area * 100) of mesenteric resistance arteries of the third-fourth order side branches (mRA $^{3rd-4th\ order}$), mRA of the second order side branches (mRA $^{2nd\ order}$) and pulmonary resistance arteries (pRA). Data are expressed as mean ± sem. Groups comparisons by Mann-Whitney test with Bonferroni correction; *P<0.01 compared to SH-NaCl, † P<0.01 compared to MI-NaCl, ‡ P<0.01 compared to SH-NAME and § P<0.01 compared to MI-NAME.

decrease vascular smooth muscle cell proliferation (15, 16). NO's antimitogenic effects on vascular smooth muscle cells can be modulated by several growth factors. Platelet-derived-growth-factor decreases the induction of NOS and thus the antimitogenic effects of NO, while fibroblast growth factor enhances the induction of NOS and promotes the antimitogenic effect of NO (33).

In the L-NAME treated sham rats, suppression of NO-mediated anti-mitogenic and/or apoptotic effects of NO (34) resulted in a vascular hypertrophy of the large conduit arteries. Other studies have also shown this increase in cross-sectional area of large conduit arteries during L-NAME infusions (35, 36). In the resistance arteries, however, the existence of structural vascular alterations during L-NAME infusions is still controversial. Some studies do report structural changes in resistance vessels in L-NAME treated rats (37-39), while the present and other studies cannot confirm these findings (36, 40). The reason for these discrepancies could be well due to the methodology used, as different techniques for in vitro investigations of these small vessels were used (wire-myograph versus the pressurized small artery preparation) (41). The present study used perfusion fixation of the intact vascular tree at maximal vasodilation and demonstrates that a two week L-NAME infusion did not induce vascular hypertrophy of the resistance arteries. The different findings in large conduit and resistance arteries is not an unknown phenomenon. NO relaxes the pulmonary conduit arteries more than pulmonary resistance arteries (42), due to the existence of morphologically distinct smooth muscle phenotypes (43-45). Also, it has been shown that in large conduit arteries such as the aorta and superior mesenteric artery, NO synthase inhibition eliminated endothelium-dependent relaxations to acetylcholine, while in the mesenteric resistance arteries and hindlimb circulation, the endothelium-dependent relaxation was resistant to NO synthase inhibition (46-48).

In previous work, we have shown that MI rats had smaller medial cross-sectional areas of several large conduit arteries, compared to sham-operated rats of the same age and weight (chapter 2). If NO is involved in this suppression of vascular growth, L-NAME should have some effect on the peripheral vascular structure in MI rats. L-NAME alone had no effect on medial cross-sectional area of the thoracic aorta and superior mesenteric artery of the MI rats, but it did increase the medial cross-sectional area of the carotid artery (table 5.2). Although the time of infusion may have been too short to induce changes in all vessels, this indicates that NO may be involved in peripheral vascular alterations during heart failure. The changes in peripheral vascular structure during co-infusion of L-NAME and ANG II in MI rats supports this observation. Thus, endogenous NO may be involved in counteracting the growth-stimulating effects of ANG II during experimental heart failure. This is surprising, as NO was ineffective in counterregulating the effects of ANG II on blood pressure. One possible explanation for this dissociation is that during heart failure other neuro-humoral factors are dominant over the effect of NO on blood pressure regulation or that NO as a regulator of vascular structure is predominantly active at the local level.

In conclusion, this study showed that (i) the basal release of NO is intact during experimental heart failure, (ii) the peripheral vasculature of MI rats is relatively unresponsive to potential stimulators of vascular smooth muscle cell proliferation and (iii) the indifferent response to these growth-stimulating factors may be explained by a concomitant upregulation of inhibitors of vascular smooth muscle cell proliferation.

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Chapter 1 2 3 4 5 6

General Discussion

General

The primary goal of the studies described in this thesis was to investigate the involvement of the peripheral arteries in the pathophysiology of heart failure. Using the myocardial infarcted rat as a model for experimental heart failure, the studies in this thesis show that

(i) myocardial infarction induces alterations in the structure of the peripheral arterial wall.
(ii) the activation of vasodilating/growth-inhibiting systems is an important regulator of the structural alterations of the peripheral arterial wall in experimental heart failure

Background of the studies

The initial research question was based on the recognition of the importance of the close physiological relation between the heart and arteries. The small resistance arteries determine the afterload of the heart and their primary role is to deliver the blood in such a way that each tissue is provided with the correct volume and at the correct pressure (1). In contrast to resistance arteries, it is generally agreed that large conduit arteries impose low resistance to blood flow. However, large arteries are more than passive conduits. The large conduit arteries contribute to cardiac load by conversion of pulsatile flow to virtually continuous tissue perfusion by absorbing the energy of pulsatile flow in the elastic components of their walls and releasing this energy during late systole and diastole (2). Large arteries thus make an important contribution to the mechanical efficiency of the circulation, which may be relevant in a situation of disturbed heart function. The mechanics of both resistance and large conduit arteries may be altered acutely by changes in smooth muscle cell tone and chronically by changes in wall structure (2). As described in chapter 1, an increase in peripheral resistance and a decrease in arterial distensibility and compliance is often seen in patients with heart failure, suggesting changes in both resistance and large conduit artery component of the circulation. The contribution of alterations in wall structure to these peripheral changes were unknown at the start of the experiments.

Structural alterations of peripheral arteries during heart failure

Therefore, the objective of the first study, described in chapter 2, was to determine possible structural alterations in peripheral arteries during heart failure. At specified timepoints after induction of a myocardial infarction (MI) in rats, several large conduit arteries and resistance arteries of the mesenteric and pulmonary vascular beds were excised. The experiments were done in young-adult Wistar rats, which gained weight during the experimental period of 3 months. In sham-operated rats, the increase in body weight was accompanied by a gradual increase in vessel wall mass (medial cross-sectional area), internal and external diameters of the large conduit arteries. Despite similar increases in body weight, this was not observed in MI rats,

suggesting a relative reduction of vascular growth. The question is whether this is a specific adaptation of the vascular system to cardiac failure. In general, the distensibility characteristics of arteries depend on the extent to which they are stretched, which in turn depends on transmural pressure (difference between intraluminal and tissue pressure). At low pressures and small diameters, arteries are very distensible, whereas with increasing pressure and diameter they gradually become stiffer (3). Experimental data indicate that MI rats have normalized intra luminal (arterial) pressures 3 months after infarction (4) but smaller diameters and no change in collagen and elastin content (chapter 2). This could result in an increase in distensibility. If so, this would indicate that in this model for heart failure, arterial distensibility is not (yet) decreased, in contrast to what is seen in patients with heart failure (5, 6). Regardless of its possible function, it is not clear which mechanism is responsible for the observed lack of vascular growth. In chapter 2, it was hypothesized that it could be the result of the prevailing actions of vasodilators/growth-inhibitors. Subsequent studies showed that 3 months after induction of the infarct, plasma concentrations of the vasodilator/growth-inhibitor atrial natriuretic peptide were still elevated (sham rats: 16.2 ± 1.0 pg/ml versus MI rats 36.2 ± 4.6 pg/ml, $p<0.05$), while plasma concentrations of the vasoconstrictor/growth-stimulator angiotensin II were comparable (sham rats: 14.6 ± 4.4 pg/ml versus MI rats 19.6 ± 4.4 pg/ml, $p=NS$). In order to further investigate this hypothesis, infusion of angiotensin II in MI rats was used to create a model of compensated heart failure with increased plasma concentrations of angiotensin II in which the effects of angiotensin II on vascular structure and blood pressure could be studied.

Angiotensin II and structural vascular alterations during heart failure

Surprisingly, while infusion of angiotensin II substantially increased mean arterial blood pressure in sham-operated rats, it did not increase mean arterial blood pressure of MI rats above the pressure measured in normotensive control rats. In addition, angiotensin II increased medial cross-sectional area of the large conduit arteries of sham-operated rats, but failed to do so in MI rats. Changes in vessel wall mass and diameter can be defined in terms of remodeling, a term introduced by Baumbach and Heisted (7). The term remodeling originally defines structural changes in resistance vessels of hypertensive animals and patients and is associated with a reduction of the lumen and an increase of the media-to-lumen ratio, but is not necessarily associated with net growth. As the term remodeling is now used to describe any change in vascular (or myocardial) structure, Mulvany *et al.* proposed an adaptation of terminology to allow a precise description of the structural changes that can occur in the vasculature (8). Figure 6.1 shows the manner in which remodeling can change the cross-sections of blood vessels. From the vessel in the centre (shaded), the medial cross-sectional can increase, resulting in hypertrophic remodeling. A lack of change in medial cross-sectional area results
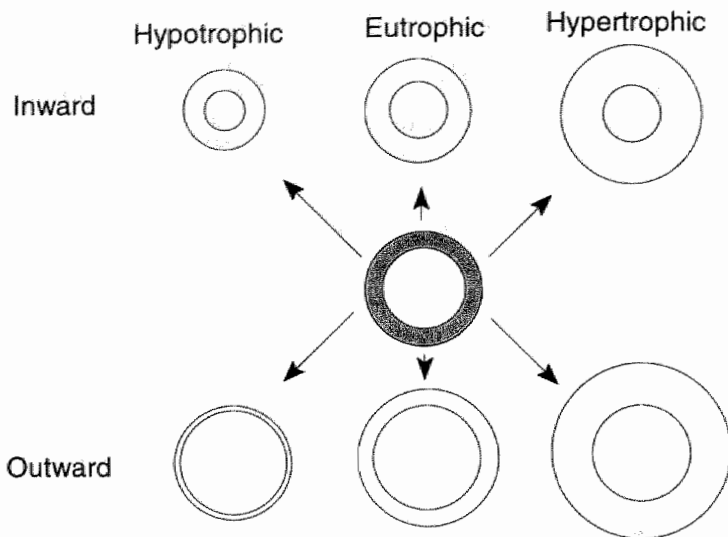
Figure 6.1: Vascular remodeling (Mulvany *et al.* (8)

in eutrophic remodeling and a decrease in medial cross-sectional area is termed hypothrophic remodeling. These forms of remodeling can be inward with a reduction in lumen (or internal) diameter or outward with an increase in lumen diameter. Thus, in the angiotensin II infused sham rats, the increase in medial cross-sectional area, combined with a slight increase in internal diameter can be defined as hypertrophic outward remodeling. In the MI rats, however, these vessels showed no remodeling (table 6.1). These paradoxical findings do support the above mentioned hypothesis, that the primary activation of counterregulatory hormonal systems in MI rats is, at least in the compensated phase of heart failure, responsible for suppression of the hypertensive and hypertrophic effects of angiotensin II. In fact, plasma atrial natriuretic peptide and an indicator of NO synthase activity (measured as aortic cGMP concentrations) were proportionally more increased in the angiotensin II infused MI rats, compared to angiotensin II infused sham rats.

As discussed in chapter 3, the role of angiotensin II receptors (AT receptors) in this process is not clear. The two types of AT-receptors also have the yin-yang relation of vasoconstrictors/growth-stimulators and vasodilators/growth-inhibitors of vascular growth (9). Thus, the AT1 receptor mediates vasoconstriction and stimulates proliferation of vascular smooth muscle and endothelial cells (10, 11) and protects them from apoptosis (12), whereas it has become clear that the AT2 receptor has opposing effects. It lowers blood pressure, mediates apoptosis and possesses anti-proliferative effects (13-16). Stoll *et al.* hypothesized that angiotensin II can only exert its effects through the AT1 receptor when the AT2 receptor is either absent or inactivated (17).

Table 6.1: Type of remodeling in the different experimental groups after 14 days of infusions

Group	Chapter	Vessel	Change in Int. Diameter (%)	Change in CSA (%)	Type of remodeling according to Mulvany *et al.* (8)
ANG II infusion in sham rats	3	sMA	+10 ± 3	+38 ± 5	Hypertrophic outward remodeling
ANG II infusion in MI rats	3	sMA	+8 ± 5	+3 ± 6	No remodeling
ANG II + AT2 block in sham rats	4	sMA	+13 ± 6	+3 ± 9	Eutrophic outward remodeling
ANG + AT2 block in MI rats	4	sMA	+15 ± 5	+5 ± 7	Eutrophic outward remodeling
ANG II + AT2 block in MI rats	4	TA	+1 ± 7	+0 ± 4	No remodeling
L-NAME inf. in sham rats	5	sMA	-7 ± 3	+27 ± 6	Hypertrophic inward remodeling
L-NAME inf. in MI rats	5	sMA	-6 ± 5	+9 ± 6	No remodeling
L-NAME inf. in MI rats	5	MRA	-28 ± 8	-11 ± 6	Eutrophic inward remodeling
ANG II+L-NAME inf. in MI rats	5	sMA	+9 ± 4	+53 ± 10	Hypertrophic outward remodeling

Table shows changes in internal diameter and medial cross-sectional area (CSA) of the superior mesenteric artery (sMA), thoracic aorta (TA), and mesenteric resistance arteries (MRA) in the different experimental groups. Inf. = infusion. Data are mean±SEM.

In chapter 4, the role of these AT-receptors was investigated by co-infusing an AT1 antagonist (GR138950) or an AT2 antagonist (PD123319) in angiotensin II infused MI rats. These studies showed that during blockade of the AT1 receptor in MI rats, blood pressure decreased significantly compared to angiotensin II infusion alone. Blockade of the AT2 receptor in angiotensin II infused rats, however, did not further increase blood pressure in sham rats, but did increase the blood pressure in MI rats, which may be explained by an upregulation of the AT2 receptors in the peripheral vasculature of these animals. The structural vascular alterations during the GR138950 infusions can be best defined as hypotrophic inward remodeling, with exception of the carotid artery, which showed a decrease in medial cross-sectional area, but no change in internal diameter. During co-infusion of angiotensin II and an AT1 antagonist, angiotensin II is expected to exert a selective stimulation of the AT2 receptor. Thus, the decrease in medial cross-sectional areas of the large conduit arteries during blockade of the AT1 receptor, indicates that in MI rats the AT2 receptor attenuates vascular structural alterations. The effects of stimulation of the

AT1 receptor (during blockade of the AT2 receptor) on vascular structure of the large conduit arteries were more heterogenous. As shown in chapter 4 and table 6.1, the effects of PD123319 on the thoracic aorta can be defined as hypertrophic outward remodeling, but only in sham rats as MI rats showed no remodeling. In contrast, the superior mesenteric artery of both sham and MI rats showed an eutrophic inward remodeling as the internal diameter increased, without changes in medial cross-sectional area. These variations in vascular remodeling during AT2 receptor blockade may be explained by (i) different ratio's of vascular AT1 and AT2 receptors or (ii) the involvement of other regulatory mechanisms.

Vasodilators and vascular structure during heart failure

Possible counterregulatory neurohormonal systems on the effects of angiotensin II in MI rats include the natriuretic peptides and nitric oxide. Both are vasodilators and inhibitors of proliferation of cells in the peripheral vascular wall (18, 19). Atrial natriuretic peptide (ANP) was a potential candidate, especially in view of its increased plasma concentration after 3 months of infarction and during angiotensin II infusions (chapter 3). Unfortunately, the one ANP receptor-antagonist described in literature, HS142-1, was not available to us. Studies by others using this compound in animals with heart failure indicate that endogenous ANP contributes to suppression of the activity of the renin angiotensin system and sympathetic nervous system and plays a critical role in maintaining renal hemodynamic function (20, 21). ANPs vasodilator actions, however, are attenuated as HS142-1 did not increase mean arterial blood pressure (20).

In chapter 5, L-NAME an inhibitor of NO synthase, was used to study the potential role of nitric oxide in the suppression of hypertensive and structural effects of angiotensin II. Although the basal release of NO was intact in MI rats, co-infusion of L-NAME and angiotensin II did not increase blood pressure in these animals. L-NAME infusion in sham rats did induce hypertrophy of the vascular wall of the large conduit arteries. However, in contrast to the hypertrophic outward remodeling seen in angiotensin II infused sham rats, L-NAME decreased the internal diameter slightly, resulting in a type of remodeling, which can be best classified as hypertrophic inward remodeling (table 6.1). Recent data from a study in mice with a disrupted endothelial NO synthase (ecNOS) gene demonstrate a similar phenomenon. These knock-out mice showed a significantly smaller lumen compared to normal mice, suggesting that ecNOS is an important determinant of the caliber of the arterial lumen (22). Interestingly, a reduction in ecNOS gene and protein expression has been shown in the vascular endothelium of animals with heart failure (23, 24). This reduction of ecNOS expression in vascular endothelial cells may have been important in the smaller diameters observed in the large conduit arteries of MI rats 3 months after ligation of the coronary artery (chapter 2). Alternatively, in contrast to the decrease in ecNOS of vascular endothelial cells, Comini *et al.* demonstrated an increase in ecNOS of the vascular smooth muscle cell layer in rats with heart failure. This could have important consequences for peripheral vascular structure. If NO is important

in the process of vascular remodeling during heart failure, inhibition of NO synthase should have an effect on the peripheral vascular structure in MI rats. As shown in chapter 5, L-NAME alone had no effect on medial cross-sectional areas of the thoracic aorta and superior mesenteric artery of MI rats, but did increase medial cross-sectional area of the carotid artery and decreased internal diameter of the 2nd order side branches of the mesenteric resistance arteries (table 6.1). The time of infusion may have been too short to induce changes in all vessels, but the diverse response may also be explained by regional differences of NOS expression in vascular smooth muscle and endothelial cells. The changes in vessel dimensions of L-NAME infused MI rats indicates that NO may be involved in peripheral vascular alterations during heart failure. The change in peripheral vascular structure during co-infusion of L-NAME and angiotensin II in MI rats supports this hypothesis, and suggest that NO is involved in structural vascular alterations during infusion of angiotensin II in MI rats.

Vascular growth: A balance between cell growth and cell death
As mentioned previously, the candidate counterregulatory hormonal systems ANP and NO are potential inhibitors of proliferation of cells in the vascular wall. During the initial experiments with angiotensin II infusions in sham and MI rats, BrdU incorporation (5-bromo-2'-deoxyuridine as a marker of vascular smooth muscle cell DNA synthesis (25, 26)) was used to study the effects of angiotensin II on vascular smooth muscle cell proliferation. Preliminary results showed that angiotensin II infusion in sham rats resulted in the expected BrdU incorporation increase in the superior mesenteric artery (26). In angiotensin II infused MI rats however, BrdU incorporation was virtually absent, indicating an inhibition of vascular smooth muscle cell DNA synthesis.

Although a decrease in DNA synthesis could explain the smaller cross-sectional areas in MI rats, a role for programmed cell death (apoptosis) cannot be excluded. It was recently shown that angiotensin II and NO are antagonists in inducing apoptosis of vascular smooth muscle cells in vitro (12). This mechanism could also be important in the studies described in this thesis, but there are no results (yet) to confirm this.

Heterogenous response of large conduit and resistance arteries
An important aspect of the studies described in this thesis, is the heterogenous response of the different vessels. There were differences among the different large conduit arteries themselves as well as between large conduit and resistance arteries. These differences can be explained, not only on the basis of structural differences, but also by the heterogenous functions of different vascular beds and variations in receptor populations and innervation.

Structural differences among vessels were first recognized by Burton (1944), who showed a variety of sizes, wall thicknesses and proportions of four basic tissues

(endothelium, elastin, collagen and smooth muscle cells) of different blood vessels (27). The measurement of relative elastin and collagen content of the large arteries in chapter 2, already indicated variations in the proportion of these extracellular matrix components in different large conduit arteries. The carotid artery was shown to contain more elastin compared to the other large conduit arteries investigated. The results in chapter 5 (L-NAME infusions) also demonstrate that the carotid artery exhibited a different pattern of remodeling compared to other large conduit arteries. Since in vitro studies with vascular smooth muscle cells indicate that extracellular matrix components are important in growth-regulation (28, 29), it is possible that besides differences in receptor population and innervation, these structural differences participated in the divergent response of the carotid artery.

An unexpected finding in the studies described in this thesis was the lack of structural alterations in the (mesenteric) resistance arteries during the various infusions. First, as shown in chapter 2, the increase in internal diameter and unchanged medial cross-sectional area in MI rats 3 months after surgery can be best defined as eutrophic outward remodeling. Secondly, as shown in chapters 3 and 5, infusion of both angiotensin II and L-NAME did not increase medial cross-sectional areas of mesenteric resistance arteries despite the increase in blood pressure. In chapters 3 and 5, possible explanations such as different fixation methods and time of infusion were already discussed. Interestingly, the same paradigm has also been developed in hypertension research. In hypertension, upstream small arteries exhibit growth and remodeling, resulting in a decrease of the luminal area, but it is not yet clear whether this contributes to changes in resistance (30). It is now thought that during hypertension an increase in intrinsic myogenic tone of the smaller arterioles contributes to the increase in peripheral resistance and blood pressure (30). In the present studies, changes in myogenic tone were not investigated, but such changes may have contributed to the increase in peripheral resistance and maintenance of blood pressure.

Finally, arteries, arterioles and veins have been shown to contain different populations of for instance α-adrenergic receptor subtypes (31, 32) and ANP receptors (33), which could also be involved in different responses of large conduit and resistance arteries.

Conclusions

The studies presented in this thesis show a complex interaction between activated neurohormonal systems and structural vascular alterations during heart failure. In view of the complexity, it is not easy to deduce the possible implications of these structural and regulatory changes for the patient with heart failure. A complicating factor is that patients frequently display other peripheral vascular alterations, such as atherosclerosis, prior to the development of heart failure. Thus, the vascular alterations induced by heart failure are hard to evaluate. The changes in the rat model, that is smaller medial cross-sectional areas and internal diameters, and its possible beneficial consequences for distensibility and compliance of the large

conduit arteries may, in patients with heart failure, be overruled by an increase in arterial stiffness due to atherosclerotic plaques. In line with this consideration is a study by Arnold *et al.*, who observed reduced internal diameters in the brachial artery in patients with New York Heart Association Class III and IV heart failure. The expected increase in distensibility, however, was not seen. The authors suggested that the effect of increased stiffness was greater than the effect of decreased diameter, resulting in reduced and not increased arterial compliance (5).

Another important feature is that the rats subjected to myocardial infarction, with or without the various infusions, still have compensated heart failure. Thus, the (primary) activation of counterregulatory hormonal systems in MI rats is well balanced and able to suppress the potential adverse effects of angiotensin II on function and structure of the cardiovascular system. Consequently, these effects of angiotensin II will become evident only when counterregulatory hormonal systems are failing, e.g. in the phase of decompensated heart failure.

Despite these limitations, the present studies show that the interaction between vasoconstrictors and vasodilators is important in peripheral vascular structure during heart failure. Other evidence that neurohormonal factors such as angiotensin II, are potentially important in peripheral vascular alterations comes from studies in patients and animals with heart failure which have been treated with ACE-inhibitors. ACE-inhibition has been shown to improve the vasodilatory capacity of forearm conduit and resistance vessels (34, 35) and radial compliance in patients with heart failure (36). Also, in rats with myocardial infarction, there were no changes in medial cross-sectional area of the mesenteric resistance arteries, 12 months after surgery. Twelve months of ACE-inhibition, however, decreased medial cross-sectional area and collagen density of these resistance arteries and restored endothelial dysfunction (37, 38). These beneficial effects of ACE-inhibition on endothelial function were also observed in patients with heart failure (39). These studies indicate that part of the beneficial effects of ACE-inhibition during heart failure may be derived from its favourable effects on peripheral vascular structure, function and regulation.

In conclusion, structural changes in peripheral vessels during experimental heart failure do exist. There is evidence for the same decrease in diameter of large conduit arteries in patients as in the rat model for heart failure (5). The observations in this thesis that (i) structural changes in peripheral arteries develop slowly and that (ii) potential stimulators of the proliferation of cells in the vascular wall do not induce the anticipated changes in vascular structure, lead to the conclusion that these vascular structural alterations are the result of a well balanced, parallel activation of growth-inhibiting/vasodilating systems, rather than a specific adaptation of the vascular system to the cardiac dysfunction.

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Summary

Heart failure is not a disease but a syndrome, in which the heart is unable to pump sufficient blood to meet the metabolic demands of the peripheral tissues. The initial event can be ischemic heart disease, chronic hypertension or valvular dysfunction. The deterioration of cardiac function is compensated by cardiac hypertrophy and ventricular dilatation. However, the syndrome of heart failure involves more than the heart itself. When the perfusion pressure to peripheral organs drops, a number of neurohumoral systems will be activated, resulting in peripheral vasoconstriction and water retention. Also, the heart is an integral part of the systemic circulation and peripheral vascular tone and resistance are important determinants of afterload and cardiac function. An important aspect of the neurohumoral systems is their dual role in peripheral vascular function and structure. The effectors of the sympathetic nervous system and the renin-angiotensin system, norepinephrine and angiotensin II, mediate vasoconstriction, but also stimulate vascular smooth muscle cell growth. Vasodilating systems, such as the natriuretic peptides and endothelial-derived nitric oxide (NO), on the other hand, are inhibitors of vascular smooth muscle cell growth. The increase in plasma concentrations of norepinephrine, angiotensin II, atrial natriuretic peptide and the increase in local activity of NO as seen in heart failure may therefore not only alter the hemodynamics, but also the peripheral vascular structure. The aim of this thesis was to investigate whether structural alterations in peripheral arteries develop during experimental heart failure and to determine the role of neurohumoral activation in the regulation of peripheral vascular structure.

To address these questions, an animal model (rat) was used, in which heart failure was induced by ligation of the left coronary artery. Sham-operated rats (no ligation) served as controls. In the first experiment, the development of peripheral structural vascular alterations was studied. One, 3, 5 or 12 weeks after induction of myocardial infarction (MI) or sham operation, several large and small arteries were excised and medial cross-sectional area and media-to-lumen ratios of vessel rings were measured. During the experimental period, body weight gain was equal in both sham and MI rats. In the sham rats, this was accompanied by a parallel increase in the medial cross-sectional area of several large conduit arteries. This increase in medial cross-sectional area was not observed in the MI rats, which may be explained by a decrease in peripheral bloodflow. However additional experiments, in which the blood flow to several peripheral organs was measured 5 and 12 weeks after the operation, showed no major differences between sham and infarct rats. Determination of plasma concentrations of atrial natriuretic peptide and angiotensin II showed increased concentrations of plasma atrial natriuretic peptide and no differences for plasma angiotensin II in the MI rats, 12 weeks after the operation. Thus, the persisted increase in the plasma concentrations of a potential inhibitor of vascular smooth muscle cell growth could

explain the lack of increase in medial cross-sectional area of the large conduit arteries in MI rats.

The renin-angiotensin system is involved in the regulation of cardiovascular function and volume homeostasis through peripheral vasoconstriction and by water retention in the kidney, respectively. Given this important role in maintaining circulatory homeostasis, it is not surprising that the renin-angiotensin system is activated during heart failure. Plasma angiotensin II concentrations are increased in the acute phase after MI, normalize in the compensated phase and increase again in overt heart failure. However, angiotensin II may have adverse effects on cardiovascular function and/or function in the phase of overt heart failure. Indeed, a significant positive correlation between mortality and plasma concentrations of angiotensin II have been reported in patients with heart failure. To test the hypothesis that increased plasma concentrations of angiotensin II have adverse effects in conditions of an already reduced cardiac output, angiotensin II was infused in sham and MI rats for 14 days (chapter 3). A control infusion of saline was given to separate groups of sham and MI rats for 14 days. To determine the effects of angiotensin II on cardiac function, several hemodynamic parameters (cardiac output, stroke volume, total peripheral resistance, mean arterial blood pressure) were measured in conscious rats. The results show that cardiac function was significantly reduced in saline infused MI rats. Infusion of angiotensin II significantly decreased cardiac function in sham rats. Thus, as both the induction of a MI and the infusion of angiotensin II decreased cardiac function, a substantial decrease in cardiac function was expected in the angiotensin II infused MI rats. This however, was not found. The infusion of angiotensin II in MI rats resulted in comparable decreases in cardiac function compared to saline infused MI rats. Also, other expected findings of the exogenous angiotensin II infusion, e.g. an increase in mean arterial blood pressure and hypertrophy of the peripheral vascular wall were observed in the sham rats, bot not in the MI rats. To determine whether an activation of vasodilating and growth-inhibiting systems such as the natriuretic peptides and NO could be involved in this suppression of the hypertensive and hypertrophic effects of angiotensin II, plasma atrial natriuretic peptide and local vascular NO activity (determined by aortic cyclic GMP concentration) were measured. It was found that plasma atrial natriuretic peptide and aortic cyclic GMP concentrations were higher in the angiotensin II infused MI rats, compared to both saline infused MI rats and angiotensin II infused sham rats. This suggests that the activation of vasodilating and growth-inhibiting systems is involved in the suppression of the hypertensive and hypertrophic effects of angiotensin II in MI rats.

Another possible explanation for the suppression of hypertensive and hypertrophic effects of angiotensin II in MI rats, is a change in angiotensin (AT) receptor expression. The AT subtype 1 (AT1) receptor mediates the vasocon-stricting and growth-stimulating effects of angiotensin II. The AT subtype 2 (AT2) receptor antagonizes these effects and mediates vasodilation and growth-

inhibition. Thus, a relative upregulation of the AT2 receptor in the angiotensin II infused MI rats, could explain the suppression of hypertensive and hypertrophic effects of angiotensin II in MI rats. To examine this hypothesis, angiotensin II infused sham and MI rats were treated with an AT2 receptor antagonist (chapter 4). Mean arterial blood pressure and medial cross-sectional areas were measured after 14 days of infusion. Results showed that the infusion of the AT2 antagonist did not further increase the blood pressure of angiotensin II infused sham rats. Also, medial cross-sectional areas of most large conduit arteries did not change in these rats. In the MI rats, however, the co-infusion of angiotensin II and the AT2 antagonist substantially increased mean arterial blood pressure, although it had no effect on the medial cross-sectional areas of large conduit arteries. From these data, it can be concluded that the AT2 receptor is involved in the suppression of blood pressure during the infusion of angiotensin II in MI rats, but does not mediate the suppression of its hypertrophic effect on the peripheral vascular wall.

In the last experimental chapter, the possible involvement of NO was investigated (chapter 5). The vascular endothelium is involved in the synthesis of NO, which is important in the regulation of basal vascular tone. This NO synthesis can be suppressed by specific NO synthase inhibitors. One of these inhibitors L-NAME, was infused in sham and MI rats for 14 days. In addition, a co-infusion of L-NAME and angiotensin II was given to MI rats. Results showed that L-NAME increased the blood pressure and increased the medial cross-sectional area of several large conduit arteries in sham rats. In the MI rats, L-NAME infusion resulted in a small increase in mean arterial blood pressure, but blood pressure levels were still significantly lower compared to L-NAME infused sham rats. Measurements of peripheral vascular structure demonstrated a significant increase in medial cross-sectional area of the carotid artery but no changes in the other large conduit arteries investigated. The co-infusion of L-NAME and angiotensin II did not result in a further increase of mean arterial blood pressure, but the medial cross-sectional area of the large conduit arteries increased significantly. Thus, the results of chapter 4 and 5 demonstrate that different systems are involved in the suppression of hypertensive and hypertrophic effects of angiotensin II in MI rats. The AT2 receptor seems to be involved in the blood pressure regulation in these rats, while the NO system seems to play a role in the regulation of peripheral vascular structure.

In conclusion, structural alterations in peripheral arteries during experimental heart failure do exist. The observations in this thesis that (i) structural alterations in peripheral arteries develop slowly and that (ii) stimulators of vascular growth do not induce the anticipated remodeling of vascular structure, lead to the conclusion that these vascular structural alterations are the result of a well balanced, parallel activation of growth-inhibiting/vasodilating systems, rather than a specific adaptation of the vascular system to the cardiac dysfunction.

Samenvatting

Hartfalen wordt gekenmerkt door een afgenomen pompfunctie van het hart. Hierdoor schiet de circulatie tekort in het transport van voedingsstoffen naar en afvalstoffen vanuit weefsels en organen. De klachten van de patiënt bestaan uit kortademigheid bij inspanning, en naarmate de pompfunctie verder achteruit gaat, ook in rust. Andere klachten zijn vermoeidheid en vochtretentie. De oorzaken van dit complexe ziektebeeld kunnen zijn; ischemische hartziekte (hartinfarct), langdurige hypertensie, klepafwijkingen of een combinatie van deze 3 factoren. Als de pompfunctie van het hart afneemt, probeert het lichaam het verlies aan orgaanperfusie te compenseren. Het hart zelf compenseert het verlies aan kracht door een toename van de wandmassa (hypertrofie). Daarnaast worden verschillende neurohormonale systemen geactiveerd. Het best bekend zijn de activatie van het sympathisch zenuwstelsel, het renine-angiotensine systeem, de natriuretische peptiden en het stikstof-oxide (NO) 'systeem'.

In dit kader is het belangrijk te beseffen dat hartfalen niet alleen wordt bepaald door afwijkingen van het hart zelf. Een deel van de pompfunctie van het hart wordt bepaald door de eigenschappen van de ermee verbonden vaatboom (perifere slagaders=arteriën). Zo zal bij een vernauwing van deze perifere arteriën de pompkracht van het hart moeten toenemen om de orgaanperfusie op peil te houden. De effecten van de zojuist genoemde neurohormonale systemen zijn voor een belangrijk deel gericht op de perifere organen en arteriën. De activatie van het sympathisch zenuwstelsel en renine angiotensine systeem leidt onder andere tot perifere vaatvernauwing. Het renine-angiotensine systeem is daarnaast ook betrokken bij het vasthouden van water en zouten in de nieren (vochtretentie). Een natuurlijke tegenhanger voor de effecten van deze systemen zijn de natriuretische peptiden, die zorgen voor vaatverwijding en afscheiding van water door de nieren. NO is eveneens een belangrijke vaatverwijder van perifere arteriën.

Een ander belangrijk aspect van deze neurohormonale systemen is dat ze, naast hun functionele effecten op perifere arteriën, ook een rol spelen in groei van de cellen in de vaatwand. De belangrijkste celtypen in de perifere vaatwand zijn gladde spiercellen en de vaatwand-bekledende endotheelcellen. Tijdens een activatie van het renine-angiotensine systeem zal de concentratie van angiotensine II in het bloed stijgen; hetzelfde geldt voor noradrenaline tijdens een activatie van het sympathische zenuwstelsel. Deze hormonen stimuleren de groei van de gladde spiercellen in de vaatwand. Ook hier zijn de natriuretische peptiden en NO natuurlijke tegenhangers; zij remmen de celgroei.

Compensatie-mechanismen op het niveau van de perifere arteriën zouden belangrijk kunnen zijn in het verloop van het klinisch ziektebeeld en de uiteindelijke prognose van hartfalen. Hartfalen wordt gekenmerkt door een verhoging van de perifere weerstand, vermoedelijk veroorzaakt door perifere vaatvernauwing. Echter gezien de potentiële effecten van de neurohormonale

systemen op vaatgroei, is het niet uitgesloten dat ook structurele veranderingen van de vaatwand een rol spelen in deze functionele veranderingen. Tot nu toe, is echter weinig onderzoek verricht naar mogelijke structurele veranderingen in de perifere arteriën tijdens hartfalen. In dit proefschrift worden experimenten beschreven waarin (1) het optreden van structurele veranderingen in de perifere arteriën tijdens hartfalen en (2) de potentiële rol van de neurohormonale activatie hierin, onderzocht zijn. In de experimenten is een diermodel (rat) gebruikt, waarbij hartfalen geïnduceerd werd door afbinden van de linker kransslagader van het hart. Deze operatie resulteert in een hartinfarct dat ongeveer de helft van de linker hartkamer omspant. In ieder experiment werden eveneens controle dieren geopereerd, die een soortgelijke operatie ondergaan, maar waarbij niet de linker kransslagader werd afgebonden (schijn-geopereerde dieren).

Allereerst is gekeken of in dit model voor experimenteel hartfalen structurele veranderingen in de perifere vaatboom optreden (hoofdstuk 2). Na de operatie waarin de dieren wel of geen hartinfarct kregen, werden na 1, 3, 5 en 12 weken, verschillende grote en kleine slagaders bestudeerd. Structurele veranderingen van de perifere vaatwand werden gemeten door, van een dwarsdoorsnede van een vat, het vaatwandoppervlak en de verhouding tussen vaatwand- en lumenoppervlak te meten. Tijdens de experimentele periode, nam het lichaamsgewicht van beide groepen toe. In de controle dieren ging dit gepaard met een toename in vaatwandoppervlak van de grote arteriën. In de infarct dieren, echter, trad deze toename in vaatwandoppervlak van de grote arteriën niet op. Bovendien bleek dat de verhouding tussen vaatwand- en lumenoppervlak van de kleinere arteriën na 12 weken kleiner was dan in de schijn-geopereerde dieren. Zoals al gezegd waren de lichaamsgewichten van schijn-geopereerde en infarct dieren niet verschillend, dus werd gezocht naar andere mogelijke verklaringen voor deze bevindingen. Het wordt algemeen aangenomen dat veranderingen in de bloeddoorstroming van een vat (=doorbloeding) invloed heeft op het vaatwandoppervlak. Verlaging van de doorbloeding zal het vaatwandoppervlak verkleinen. Aangezien de infarct dieren een slechtere pompfunctie van het hart hebben, is een verlaging van de doorbloeding in de perifere arteriën niet uitgesloten. Om dit te onderzoeken is in aparte groepen van schijn-geopereerde en infarct dieren 5 en 12 weken na de operatie de doorbloeding naar verschillende perifere organen gemeten. Uit de resultaten bleek dat de perifere doorbloeding niet belangrijk verschilde tussen infarct en schijn-geopereerde dieren. Dus ook de doorbloeding lijkt geen factor van belang te zijn in het uitblijven van groei in het vaatwandoppervlak van de grote arteriën in infarct dieren. Een tweede mogelijke verklaring kan berusten op neurohormonale activatie. Het bleek dat 12 weken na operatie de plasma concentratie van één van de natriuretische peptiden (atrium-natriuretisch peptide) verhoogd was in de infarct dieren, de plasma concentratie van angiotensine II verschilde niet tussen infarct en schijn-geopereerde dieren. Aangezien de natriuretische peptiden de groei van gladde spiercellen in de perifere vaatwand remmen, zou de blijvende

activatie van de natriuretische peptiden een rol kunnen spelen in het achterblijven van vaatgroei van de grote arteriën in infarct dieren.

Het renine-angiotensine systeem speelt een belangrijke rol in de regulatie van perifere weerstand en bloedvolume. In de vroege fase na een hartinfarct draagt angiotensine II bij aan het op peil te houden van de verminderde orgaanperfusie door middel van vaatvernauwing en waterretentie. Als de verschillende compensatiemechanismen in balans zijn en de orgaanperfusie op peil is, zal de activiteit van het renine-angiotensine systeem weer afnemen. In een later stadium is beschreven dat het renine-angiotensine systeem weer geactiveerd wordt en de concentratie van circulerend angiotensine II stijgt. Het is niet bekend of de stijging van circulerend angiotensine II verband houdt met de verslechtering van de pompfunctie van het hart in deze fase. Om dit te onderzoeken werd gedurende 2 weken een continu infuus gegeven van angiotensine II aan infarct en schijn-geopereerde dieren (hoofdstuk 3). Naast deze 2 groepen (schijn-geopereerde en infarct dieren met angiotensine II infuus) werd ter controle ook een infuus van fysiologisch zout gegeven in zowel schijn-geopereerde als infarct dieren. Allereerst werden in de 4 groepen verschillende hemodynamische parameters gemeten, zoals hartminuutvolume (volume dat het hart uitpompt per minuut), slagvolume (volume dat het hart uitpompt per slag), perifere weerstand, hartfrequentie en bloeddruk. De resultaten toonden aan dat, zoals verwacht, de pompfunctie na een infarct verminderd is. Ook bleek dat de infusie van angiotensine II in de schijn-geopereerde dieren een substantiële verlaging van de pompfunctie tot gevolg had. Dus, zowel een infarct als de infusie van angiotensine II verlagen de pompfunctie. Met deze resultaten in het achterhoofd werd verwacht dat de combinatie van angiotensine II infuus en de aanwezigheid van een infarct, de pompfunctie sterk zou verlagen. Dit was niet het geval. Het bleek dat de angiotensine II infuus in infarct dieren zorgde voor een vergelijkbare daling in pompfunctie als in zout geïnfundeerde infarct dieren. Een stijging van de plasma concentratie angiotensine II in infarct dieren lijkt dus niet verantwoordelijk voor een verslechtering van de pompfunctie. De infusie van angiotensine II in de schijn-geopereerde dieren had bovendien een substantiële bloeddrukstijging én een toename van het vaatwandoppervlak van de grote arteriën tot gevolg. Echter, angiotensine II infuus in infarct dieren had slechts een lichte bloeddrukstijging tot gevolg en het vaatwandoppervlak veranderde niet. Een mogelijke verklaring hiervoor zou kunnen liggen in de neurohormonale activatie tijdens de angiotensine II infuus. In dit experiment werden naast de plasma concentratie van angiotensine II, ook de plasma concentratie van atrium-natriuretisch peptide en een afgeleide van NO activiteit in de vaatwand gemeten. Uit deze bepalingen bleek dat de plasma angiotensine II concentratie in het plasma niet verschilde in zout geïnfundeerde schijn-geopereerde en infarct dieren en dat het angiotensine II infuus resulteerde in een vergelijkbare stijging van de plasma angiotensine II concentraties in schijn-geopereerde en infarct dieren. De meting aan de verschillende vaatverwijdende systemen toonde aan dat zowel de atrium-

natriuretisch peptide plasma concentratie als locale activiteit van NO in de vaatwand hoger waren in de angiotensine II geïnfundeerde infarct dieren. Deze resultaten suggereren dat de sterkere activatie van natriuretische peptiden en NO (als vaatverwijdende en groei remmende systemen) verantwoordelijk zijn voor het laag houden van bloeddruk en het onveranderde vaatwandoppervlak in infarct dieren. Uit de resultaten van hoofdstuk 2 en 3 wordt geconcludeerd dat de activatie van de vaatverwijdende en groei-remmende systemen belangrijk is bij de regulatie van bloeddruk en structurele vaatwandveranderingen in infarct dieren.

Hormonale systemen zoals het renine-angiotensine systeem oefenen hun invloed op perifere arteriën en organen uit via specifieke celgebonden receptoren. Voor angiotensine II zijn dit de angiotensine (AT) receptoren. Uit onderzoek blijkt dat de AT receptoren heterogeen zijn, dat wil zeggen, dat verschillende subtypen bestaan met distincte functies. De belangrijkste en best beschreven AT receptoren zijn tot dusver de AT subtype 1 (AT1) en de AT subtype 2 (AT2) receptoren. Vaatvernauwing en groeistimulatie komen tot stand via de AT1 receptor. Recentelijk is duidelijk geworden dat de AT2 receptor een natuurlijke tegenhanger is van de AT1 receptor. Dus, de AT2 receptor reguleert vaatverwijding en groeiremming van de gladde spiercellen in de vaatwand. De resultaten van hoofdstuk 3, namelijk dat angiotensine II infuus in infarct dieren geen substantiële bloeddrukverhoging geeft en geen effect heeft op het perifere vaatwandoppervlak, zou verklaard kunnen worden door grotere rol van de AT2 receptoren. Om dit te onderzoeken is gebruik gemaakt van specifieke substanties die óf de AT1 óf de AT2 receptor remmen (zogenaamde receptor antagonisten). In dit experiment (hoofdstuk 4) werd opnieuw een angiotensine II infuus gegeven aan infarct of schijn-geopereerde dieren, al dan niet gecombineerd met een infusie met een specifieke AT2 antagonist. Opnieuw werden bloeddruk en perifeer vaatwandoppervlak na 2 weken infusie gemeten. Uit de resultaten blijkt dat de AT2 antagonist in angiotensine II geïnfundeerde schijn-geopereerde dieren geen effect heeft op de bloeddruk (deze wordt onveranderd verhoogd) en in de meeste arteriën geen effect heeft op het vaatwandoppervlak. In de infarct dieren echter had de infusie met AT2 antagonist een substantiële bloeddrukstijging tot gevolg, maar veranderingen in het perifere vaatwandoppervlak werden niet gevonden. Uit de resultaten van dit experiment blijkt dat in infarct dieren, de AT2 receptor, als natuurlijke antagonist van de AT1 receptor, wél betrokken is bij het laag houden van de bloeddruk gedurende een infusie van angiotensine II in infarct dieren, maar niet bij de veranderingen in de perifere vaatwandstructuur.

Wat overblijft is de vraag, welke systemen betrokken zijn in de regulatie van perifere vaatwandstructuur. In het laatste experiment is de potentiële rol van NO onderzocht (hoofdstuk 5). Stikstof-oxide of NO wordt onder andere gemaakt door het endotheel dat de vaatwand bekleedt. Dit door het endotheel gesynthetiseerde NO is een belangrijke vaatverwijder en betrokken bij de bloeddrukregulatie. Daarnaast remt NO de groei van de gladde spiercel in de vaatwand. De synthese van NO kan geremd worden door specifieke substanties die de activiteit van het

verantwoordelijke NO-synthetiserende enzym verlagen. Een dergelijke remmer (L-NAME) werd gedurende 2 weken geïnfundeerd in infarct en schijn-geopereerde ratten. Bovendien werd gekeken naar het effect van een combinatie van angiotensine II en L-NAME infuus in infarct dieren. De resultaten toonden aan dat de infusie van L-NAME in schijn-geopereerde dieren de bloeddruk verhoogde. Deze verhoging werd verwacht aangezien tijdens de remming van NO synthese een belangrijk vaatverwijdend systeem wegvalt. Hierbij viel op dat de bloeddruk absoluut en relatief minder verhoogd was dan tijdens een angiotensine II infuus in schijn-geopereerde dieren. Een tweede effect van het L-NAME infuus in schijn-geopereerde dieren was een verhoging van het vaatwandoppervlak van verschillende perifere grote arteriën. In de infarct dieren zorgde de L-NAME infusie eveneens voor een bloeddrukstijging die ditmaal relatief gezien vergelijkbaar was met de stijging in de L-NAME geïnfundeerde schijn-geopereerde dieren. Absoluut was de bloeddruk van L-NAME geïnfundeerde infarct dieren nog steeds lager dan in L-NAME geïnfundeerde schijn-geopereerde dieren. Wat het vaatwandoppervlak van de perifere arteriën betreft, bleek dat bij een van de onderzochte arteriën (de halsslagader=arteria carotis) het vaatwandoppervlak wel vergroot was in L-NAME geïnfundeerde infarct dieren, terwijl in andere grote arteriën geen structurele veranderingen optraden. Alhoewel zowel angiotensine II als L-NAME een lichte verhoging van de bloeddruk tot gevolg had in infarct dieren, had de combinatie van beide geen effect op de bloeddruk (d.w.z. vergelijkbaar met L-NAME geïnfundeerde infarct dieren). Echter, het vaatwandoppervlak van alle bestudeerde perifere arteriën was substantieel verhoogd in de infarct dieren geïnfundeerd met zowel angiotensine II als L-NAME. Uit de resultaten van hoofdstuk 4 en 5 wordt geconcludeerd dat verschillende systemen betrokken zijn bij het laag houden van de bloeddruk en het optreden van structurele vaatwandveranderingen tijdens een angiotensine II infuus in infarct dieren. De AT2 receptor lijkt betrokken te zijn bij het laag houden van de bloeddruk in angiotensine II geïnfundeerde infarct dieren. Een vaatverwijdend systeem als NO lijkt betrokken te zijn bij de veranderingen in de perifere vaatwandstructuur. Immers, de infusie van de NO-synthase remmer L-NAME bleek het vaatwandoppervlak van de halsslagader al te verhogen, terwijl de combinatie van zowel angiotensine II als L-NAME het vaatwandoppervlak van diverse grote arteriën vergrootte.

Samenvattend, in dit proefschrift wordt aangetoond dat perifere structurele vaatwandveranderingen zich pas in een laat stadium na het optreden van een hartinfarct in de rat ontwikkelen. Bovendien blijkt dat perifere arteriën in infarct dieren relatief ongevoelig zijn voor hormonen met een groei stimulerende werking. Uit de experimenten beschreven in dit proefschrift blijkt dat dit met name het gevolg is van een activatie van groei remmende en vaatverwijdende systemen.

Curriculum Vitae

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