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Arterial Reactivity and Cardiac Natriuretic Peptides in Genetic Hypertension, Nitric Oxide Deficiency and Renal Failure

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To Annukka

CONTENTS

LIST OF ORIGINAL COMMUNICATIONS

This thesis is based on the following original communications, which are referred to in the text by Roman numerals I-VI:

I   Kähönen M, Tolvanen J-P, Kalliovalkama J, Wu X, Karjala K, Mäkynen H and Pörsti I (1999): Losartan and enalapril therapies enhance vasodilatation in the mesenteric artery of spontaneously hypertensive rat. European Journal of Pharmacology 368:213-222.

II   Kalliovalkama J, Kähönen M, Tolvanen J-P, Wu X, Voipio J, Pekki A, Doris PA, Ylitalo P and Pörsti I (2000): Arterial responses *in vitro* and plasma digoxin immunoreactivity after losartan and enalapril treatments in experimental hypertension. Pharmacology & Toxicology 86:36-43.

III   Kalliovalkama J, Jolma P, Tolvanen J-P, Kähönen M, Hutri-Kähönen N, Wu X, Holm P and Pörsti I (1999): Arterial function in nitric oxide-deficient hypertension: influence of long-term angiotensin II receptor antagonism. Cardiovascular Research 42:773-782.

IV   Kalliovalkama J, Jolma P, Tolvanen J-P, Kähönen M, Hutri-Kähönen N, Saha H, Tuorila S, Moilanen E and Pörsti I (1999): Potassium channel-mediated vasorelaxation is impaired in experimental renal failure. American Journal of Physiology 277:H1622-H1629.

V   Magga J, Kalliovalkama J, Romppanen H, Vuolteenaho O, Pörsti I, Kähönen M, Tolvanen J-P and Ruskoaho H (1999): Differential regulation of cardiac adrenomedullin and natriuretic peptide gene expression by AT_1 receptor antagonism and ACE inhibition in normotensive and hypertensive rats. Journal of Hypertension 17:1543-1552.

VI   Suo M, Kalliovalkama J, Pörsti I, Jolma P, Tolvanen J-P, Vuolteenaho O and Ruskoaho H: Chronic L-NAME-induced hypertension and activation of natriuretic peptide gene expression: Inhibition by AT_1 receptor antagonism. Submitted.

ABBREVIATIONS

AA	Arachidonic acid
ACE	Angiotensin converting enzyme
ACh	Acetylcholine
ADM	Adrenomedullin
ADMA	asymmetrical dimethylarginine
Ang I	Angiotensin I
Ang II	Angiotensin II
Ang-(1-7)	Angiotensin-(1-7)
ANOVA	Analysis of variance
ANP	Atrial natriuretic peptide
AT_1	Subtype 1 angiotensin II receptor
AT_2	Subtype 2 angiotensin II receptor
ATP	Adenosine 5'-triphosphate
ATPase	Adenosine 5'-triphosphatase
BNP	B-type natriuretic peptide
$[Ca^{2+}]_i$	Intracellular free Ca^{2+} concentration
Ca^{2+} pump	Ca^{2+}-ATPase
cAMP	Cyclic adenosine 3',5'-monophosphate
cGMP	Cyclic guanosine 3',5'-monophosphate
CNP	C-type natriuretic peptide
CNS	Central nervous system
COX	Cyclooxygenase
DAG	1,2-diacylglycerol
ECFV	Extracellular fluid volume
EDCF	Endothelium-derived contracting factor
EDHF	Endothelium-derived hyperpolarizing factor
ET	Endothelin
G protein	Guanosine 5'-triphosphate-binding protein
GMP	Guanosine 3',5'-monophosphate
GTP	Guanosine 5'-triphosphate
HPLC	High performance liquid chromatography
5-HT	5-hydroxytryptamine, serotonin
ir	Immunoreactive
IP_3	Inositol 1,4,5-trisphosphate
K_{ATP}	ATP-sensitive K^+ channels
K_{Ca}	Ca^{2+}-activated K^+ channels
K_{IR}	Inward rectifier K^+ channels
K_V	Voltage-dependent K^+ channels

L-NAME	N^G-nitro-L-arginine methyl ester
MLCK	Myosin light chain kinase
NA	Noradrenaline
NEP	Neutral endopeptidase
NO	Nitric oxide
NOS	Nitric oxide synthase
NOx	Nitrite and nitrate
PG	Prostaglandin
PGI_2	Prostacyclin
PIP	Phosphatidylinositol 4,5-bisphosphate
PLC	Phospholipase C
PSS	Physiological salt solution
RAS	Renin-angiotensin system
ROC	Receptor operated Ca^{2+} channel
SHR	Spontaneously hypertensive rats
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
SR	Sarcoplasmic reticulum
SSC	Saline sodium citrate
TEA	Tetraethylammonium
TXA_2	Thromboxane A_2
VOC	Voltage operated Ca^{2+} channel
VSMC	Vascular smooth muscle cell
WKY	Wistar-Kyoto

INTRODUCTION

Hypertension is the most common risk factor for myocardial infarction, stroke, and end-stage renal disease. Approximately one quarter of the entire population in industrialised societies and more than half of the population aged over 65 years are hypertensive (Ruoff 1998). Despite extensive studies carried out in the past decades to elucidate the mechanisms involved in the pathogenesis of hypertension, the origin of high blood pressure remains unknown in 95 % of the patients. It still appears that none of the main genes for essential hypertension have been identified or even mapped to genomic regions (Colhoun 1999). In general, elevated blood pressure has been thought to result from an increase in peripheral arterial resistance, while cardiac output remains normal (Kaplan 1998).

The individual variations in polygenetic disposition and environmental factors in hypertensive patients are substantial, and various pathomechanisms have been suggested to associate with essential hypertension (Lindpaintner et al. 1992). Therefore, numerous experimental models have been used to study the cause and progression of human cardiovascular disease as well as potential therapeutic interventions (Doggrell and Brown 1998). The most commonly used model of essential hypertension is the spontaneously hypertensive rat (SHR). The genetic hypertension in SHR follows the same progression as human hypertension (Adams et al. 1989, Folkow 1993), and as in essential hypertension, the actual cause of the disease in SHR is unknown (Dzau et al. 1995). Furthermore, the defects in the production or action of nitric oxide (NO) may contribute to the essential hypertension (Moncada and Higgs 1993). Therefore, the chronic inhibition of NO synthase (NOS) has been introduced as one of the models for experimental hypertension (Baylis et al. 1992). Moreover, the most common cause of secondary hypertension is renal parenchymal disease (Preston 1999). On the other hand, sustained essential hypertension predisposes to the development of renal failure (Frohlich 1997, Luke 1998, Rahn 1998). Therefore, experimental renal failure is a fascinating model of cardiovascular disease.

The vascular endothelium regulates the contractile state of the underlying smooth muscle by releasing relaxing and contracting factors. The endothelium is therefore an important regulator of local blood flow and peripheral arterial resistance. Abnormal endothelium-dependent vasodilatation has been observed in both patients with essential hypertension and renal failure, as well as in experimental models of these diseases. Furthermore, the natriuretic peptides synthesised in cardiac myocytes contribute to the regulation of blood pressure and fluid homeostasis by forming a humoral link between the heart and kidney (Ruskoaho 1992, Wilkins et al. 1997). These hormones lower blood pressure by promoting natriuresis and vasorelaxation, and by decreasing cardiac output. The atrial and ventricular synthesis, and cardiac and plasma levels of these hormones increase in cardiac hypertrophy and in response to volume expansion and pressure overload of the heart (Levin et al. 1998). Moreover, adrenomedullin (ADM) is a recently discovered peptide that participates in the physiological regulation of circulation. It is a potent vasodilatator, causes diuresis and

natriuresis, and has positive inotropic and chronotropic effects on the heart (Szokodi et al. 1998, Charles et al. 1999, Samson 1999). The synthesis of ADM has been observed to be increased in several cardiovascular diseases (Charles et al. 1999).

The present study was designed to examine arterial reactivity and structure and cardiac hormones in genetic hypertension, NO deficiency and renal failure. In addition, the effects of long-term inhibition of the renin-angiotensin system (RAS) on arteries and cardiac hormones were evaluated in SHR and N^G-nitro-L-arginine methyl ester (L-NAME)-treated Wistar rats.

REVIEW OF THE LITERATURE

I Control of blood pressure

1 General aspects

1.1 Autonomic nervous system

Adaptation of the cardiovascular system to an organism's requirements is achieved by the interaction between central and peripheral regulatory mechanisms (Culman and Unger 1992). The central nervous system (CNS) modifies blood pressure by adjusting the heart rate and contractility as well as peripheral vascular resistance. This occurs mainly via the sympathetic and parasympathetic pathways of the autonomic nervous system, but neuroendocrine pathways, such as the hypothalamo-pituitary axis, are also involved (Reid 1994). The effects of CNS on blood pressure are modified by arterial baroreceptors, which in hypertension are desensitised and reset to a higher level of blood pressure (Sleight 1991, Grassi et al. 1998). Although it is generally accepted that the primary purpose of the cardiac and arterial baroreflexes is to keep blood pressure close to a particular set point over a relatively short period of time, the contribution of the baroreceptor dysfunction to long-term essential hypertension is unclear (Panfilov and Reid 1994, Head 1995).

SHR have consistently shown indications of a centrally mediated increased sympathetic outflow (Reid 1994). In SHR, the release and synthesis of noradrenaline (NA) was found to be enhanced in the posterior hypothalamus (Pacák et al. 1993), an area known to produce marked cardiovascular pressor responses when stimulated (Bunag et al. 1975). In addition, the activity of the sympathoinhibitory neurons was reported to be decreased in both juvenile and adult SHR (Fujino 1984, Chalmers et al. 1992). These results suggest that the enhanced sympathetic activity is involved in the development and maintenance of hypertension in SHR. However, contradictory observations have also been published, indicating that an increase in the sympathetic tone may not always contribute to the development of spontaneous hypertension (Shah and Jandhyala 1995).

Although animal models of hypertension have indicated that high blood pressure is associated with an increase in sympathetic cardiovascular influences, a similar demonstration in humans has been more difficult to obtain for methodological reasons (Mancia et al. 1999). There is now evidence, however, of increased sympathetic activity in essential hypertension (Goldstein 1983, Guzzetti et al. 1988, Mancia et al. 1999). There seems to be a clear relationship between cardiac sympathetic activity and the progression of hypertension in its early stages (Julius 1996, Sakata et al. 1999). Furthermore, plasma NA levels are elevated, the rate of NA spillover from sympathetic nerve terminals increased, and the muscle sympathetic nerve activity enhanced in patients with essential hypertension as compared with normotensive control subjects (Goldstein 1983, Floras and Hara 1993, Rahn et al. 1999).

Sympathetic activity appears to be particularly high in young subjects with borderline hypertension, and increased sympathetic activity has even been found in the normotensive offspring of hypertensive patients, supporting the idea that increased sympathetic outflow is the cause, rather than the consequence, of blood pressure elevation (Floras and Hara 1993, Esler 1995, Noll et al. 1996). Thus, the dysfunction of the autonomic nervous system may contribute to the development of essential and experimental hypertension.

1.2 Kidneys

The kidneys regulate long-term blood pressure by adjusting sodium balance, extracellular fluid volume (ECFV), and blood volume (Navar 1997). When arterial pressure is elevated, pressure natriuresis will result in the renal excretion of sodium and water until the blood volume is decreased sufficiently to return the arterial pressure to normal levels (Guyton et al. 1972). Therefore, hypertension develops when the relationship between sodium excretion and arterial pressure is shifted towards higher pressures (Navar 1997). Furthermore, derangements that compromise the ability of the kidneys to maintain sodium balance can result in the kidney's need for an elevated arterial pressure to re-establish net salt and water balance (Navar 1997). Resetting of pressure natriuresis mechanism has actually been associated with the development of both human and experimental hypertension (Guyton et al. 1972, Cowley and Roman 1996, Cowley 1997).

The associations between renal dysfunction and hypertension are complex and these diseases may coexist at least in three distinct clinical settings (Preston 1999). First, renal parenchymal disease with impaired renal sodium excretion leading to ECFV expansion is a well-established cause of secondary hypertension. In fact, renal parenchymal disease is the most common cause of secondary hypertension, accounting for 2.5 % to 5 % of all cases of systemic hypertension (Preston 1999). Furthermore, hypertension is present in virtually all renal failure patients by the time renal-replacement therapy is initiated (Rabelink and Koomans 1997, Luke 1998). Moreover, disturbed hormonal profiles with increased sympathetic activity, activated RAS, increased levels of catecholamines, vasopressin and endothelin (ET), and decreased NO activity may participate in the high incidence of hypertension in renal failure patients (Bellinghieri et al. 1999, Ligtenberg et al. 1999). Second, it is well known that sustained, poorly controlled essential hypertension induces renal vascular injury and nephrosclerosis and predisposes to the development of renal failure (Frohlich 1997, Luke 1998, Rahn 1998). Hypertension also accelerates the progression of renal injury if it is inadequately controlled (Herrera-Acosta 1994). Hypertension has been suggested to be the cause of end-stage renal disease in 8 % of dialysis patients (Bellinghieri et al. 1999). The third major circumstance in which hypertension and renal failure occur simultaneously is ischemic renal disease followed by bilateral or unilateral arteriosclerotic renal artery stenosis (Preston 1999). Therefore, hypertension may be either a cause or a consequence of renal disease.

1.3 Renin-angiotensin system

RAS has been extensively studied in the past decades as an important mediator of hypertension and hypertensive end-organ damage. Originally, RAS was described as an endocrine system that exerts its action through the effector peptide, angiotensin II (Ang II). Recently, tissue-based RAS, which acts through paracrine-autocrine mechanisms, has been proposed to be a more important pathway (Stock et al. 1995, Rothermund and Paul 1998). The existence of local Ang II production has been demonstrated in many tissues including the brain, kidney, adrenal cortex, heart, and blood vessel wall (Cockcroft et al. 1995, Mulrow and Franco-Saenz 1996, Kubo et al. 1999). However, local synthesis of renin may be limited to very small amounts and it is very likely that there is uptake of renal renin from the circulation (Stock et al. 1995, Danser 1996). This uptake may occur via mannose 6-phosphate receptor (Danser et al. 1999).

Ang II is derived from angiotensinogen, which is synthesised in the liver. Angiotensinogen is cleaved to form angiotensin I (Ang I) by renin, an enzyme synthesised and released by the kidney. Ang I is then converted to Ang II through the action of angiotensin converting enzyme (ACE), which is located in the luminal surface of vascular endothelium (Riordan 1995, Wright et al. 1995). In addition to the conversion of Ang I to Ang II, ACE degrades bradykinin and related kinins to inactive peptides (Vanhoutte et al. 1993). In human, primate and dog tissues the conversion of Ang I to Ang II is also catalysed by chymase, while rat and mouse chymases degrade Ang II (Fukami et al. 1998, Wei et al. 1999). Ang II is not the only active peptide of RAS (Ardaillou 1997), since angiotensin III, angiotensin IV and angiotensin-(1-7) [Ang-(1-7)] also possess biological functions, but the debate continues over their relative importance compared with Ang II (Riordan 1995, Wright et al. 1995, Ardaillou 1997).

Ang II exerts its effects via binding to specific receptors on the cell surface. Ang II receptors of the cardiovascular system are divided into two main subtypes: AT_1 and AT_2, which are specifically blocked by losartan and PD123177, respectively (de Gasparo et al. 1995). To date, most of the known effects of Ang II in adult tissues are attributable to the AT_1 receptor (Horiuchi et al. 1999). These include the contraction of the vascular smooth muscle, positive inotropic and chronotropic actions in the heart, induction of vascular and cardiac hypertrophy, remodelling of arterial wall matrix tissue, reduction of baroreceptor activity in aorta, stimulation of ET production in endothelial cells, activation of superoxide generation in the vascular smooth muscle, enhancement of the Ca^{2+} sensitivity of the contractile apparatus of the smooth muscle, induction of inflammatory response in vascular wall, stimulation of catecholamine release in the adrenal medulla, facilitation of aldosterone biosynthesis and secretion in the adrenal cortex, facilitation of NA biosynthesis and release accompanied by inhibition of its reuptake in sympathetic nerve terminals, augmentation of tubular sodium reabsorption in the kidney and inhibition of renin release (Henrion et al. 1992, Lüscher et al. 1992, Falkenhahn et al. 1994, Griendling et al. 1994, Kang et al. 1994, Cockcroft et al. 1995,

Dieguez-Lucena et al 1996, dos Santos et al. 1998, Otsuka et al. 1998, Unger et al. 1998, Mervaala et al. 1999). In addition, the stimulation of AT_1 receptors in CNS has been reported to activate the sympathetic nervous system and the release of vasopressin from the pituitary gland (Steckelings et al. 1992, Höhle et al. 1995), and to excite pressor neurons in the rostral ventrolateral medulla, thereby increasing arterial pressure (Tagawa and Dampney 1999). (The main cardiovascular effects of Ang II are summarised in table 1).

Until recently, the functional role of the AT_2 receptor in cardiovascular system has been unknown. Mice lacking the AT_2 receptor have been shown to have slightly elevated systolic blood pressure compared with that of wild-type mice (Siragy et al. 1999). In rats, the stimulation of AT_2 receptors has been proposed to reduce blood pressure via vasodilatation (Scheuer and Perrone 1993, Carey et al. 2000), increase cyclic guanosine 3',5'-monophosphate (cGMP) production in the aorta (Munzenmaier and Greene 1994), inhibit proliferation of cultured endothelial cells (Stoll et al. 1995) and reduce arterial hypertrophy and fibrosis *in vivo* (Levy et al. 1996). The AT_2 receptor may thus play a counterregulatory role against the actions of Ang II at the AT_1 receptor (Horiuchi et al. 1999, Siragy et al. 1999).

Table 1. The main cardiovascular effects of angiotensin II mediated via AT_1 and AT_2 receptors.

AT_1 receptor		
	Vasculature	Contraction
		Hypertrophy
		Remodelling of vascular wall tissue
		Increased synthesis of extracellular matrix
		Inflammatory response
	Heart	Positive chronotropic action
		Positive inotropic action
		Hypertrophy
		Increased synthesis of extracellular matrix
	Adrenal medulla	Catecholamine release
	Adrenal cortex	Aldosterone release
	Kidney	Increased sodium reabsorption
		Decreased renin secretion
AT_2 receptor		
	Vasculature	Dilatation
		Inhibition of cell proliferation
		Reduction of hypertrophy
		Reduction of fibrosis
		Induction of apoptosis

It has been suggested that RAS is involved in the pathophysiology of genetic hypertension (Timmermans et al. 1993, Riordan 1995). The cultured vascular smooth muscle cells (VSMCs) from SHR, but not from normotensive Wistar-Kyoto (WKY) rats, produce

Ang II (Fukuda et al. 1999). In addition, VSMCs derived from SHR have a higher affinity for AT_1 receptor ligands and a greater AT_1 receptor density than those derived from WKY rats (Bunkenburg et al. 1992). In agreement, the Ang II-induced increase of extracellular Ca^{2+} uptake and release of intracellular Ca^{2+} have been found to be amplified in VSMCs from SHR when compared with those from WKY rats (Orlov et al. 1993). When evaluated by plasma measurements of Ang II and renin activity, a hyperactive RAS is not a consistent feature of essential hypertension, and in SHR the activity of the circulating RAS is not elevated (Paran et al. 1995). In contrast, SHR appear to have higher arterial ACE activities and concentrations than WKY rats (Nakata et al. 1987, Saavedra et al. 1992, Vicaut and Hou 1994). The arterial reactivity to Ang II has been reported to be either unaffected or enhanced in SHR and in essential hypertensive patients when compared with the respective normotensive controls (Chatziantoniou et al. 1990, Angus et al. 1992, Vicaut and Hou 1994, Tschudi and Lüscher 1995). It has even been suggested that the vascular reactivity to Ang II is enhanced in the initial phase of the development of hypertension in SHR, but it becomes attenuated as hypertension progresses (Endemann et al. 1999).

The findings in rats whereby the production of Ang II plays a significant role in the maintenance of basal vasomotor tone (Caputo et al. 1995), and arterial ACE activity positively correlates with blood pressure, support the crucial role of RAS in blood pressure control (Nakata et al. 1987, Cockcroft et al. 1995). Furthermore, an interruption of RAS activity at the genetic level, by a single intracardiac injection of AT_1 receptor antisense cDNA by a retrovirally mediated delivery system, causes a permanent cardiovascular protection against hypertension as a result of genomic integration of the transgene and germ line transmission in the SHR offspring (Reaves et al. 1999). Moreover, ACE antisense treatment prevents alterations in renal vascular pathophysiology and causes a modest lowering of blood pressure in SHR (Gelband et al. 2000). In male subjects, allelic variation in the ACE gene has been suggested to influence interindividual blood pressure variability (Fornage et al. 1998). In addition, the effectiveness of RAS inhibitors in reducing blood pressure in SHR and in patients with essential hypertension strongly supports the view that RAS is involved in the pathophysiology of genetic hypertension (Timmermans et al. 1993, Riordan 1995).

1.4 Natriuretic peptides and adrenomedullin

The natriuretic peptide family consists of three peptides, atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP) and C-type natriuretic peptide (CNP). The natriuretic peptides contribute to the regulation of blood pressure and fluid homeostasis by forming a humoral link between the heart and kidney (Ruskoaho 1992, Wilkins et al. 1997, Levin et al. 1998). ANP is mainly synthesised by the atrial myocytes of normal adult heart, but synthesis in ventricles and various extracardiac tissues including CNS, lung, adrenal gland, kidney and vasculature, have also been demonstrated (Gardner et al. 1986, Gerbes et al. 1994). BNP is synthesised by both atria and ventricles, although it is also produced in CNS, and CNP is

synthesised in CNS, kidney and vascular endothelium (Levin et al. 1998). The major determinant of ANP secretion is atrial wall stretch, while for BNP secretion it is both atrial and ventricular wall stretch. In addition, several hormones and neurotransmitters, such as ET, Ang II, vasopressin, and catecholamines, stimulate the secretion of ANP (Ruskoaho 1992, Levin et al. 1998, Thibault et al. 1999). The ventricular synthesis of ANP and BNP and the plasma concentrations of these peptides increase in cardiac hypertrophy and in response to volume expansion and pressure overload of the heart (Levin et al. 1998). However, the plasma concentration of CNP changes very little with cardiac overload (Furuya et al. 1991, Levin et al. 1998).

In mammalian tissues, the effects of natriuretic peptides are mediated by three receptors (A, B, and C) (Levin et al. 1998). The A receptor binds both ANP and BNP, with a preference for ANP, while CNP is the natural ligand for the B receptor. The A receptor is the most abundant type in large blood vessels, but there are also some B receptors. The B receptors predominate in the brain. Both receptors are present in the adrenal glands and kidneys. Receptors A and B mediate many of the cardiovascular and renal effects of the natriuretic peptides, and receptor C seems to be involved in clearance of the peptides and thus regulates the plasma natriuretic peptide concentrations (Maack et al. 1987). All three natriuretic peptides are also cleared by neutral endopeptidase (NEP) in several tissues (Wilkins et al. 1997).

The cardiovascular effects of ANP and BNP are very similar, but the effects of CNP are different, since it is not natriuretic but it possesses vasorelaxant activity (Wilkins et al. 1997, Chun et al. 1997). Natriuretic peptides counterbalance the effects of RAS, defend against excess salt and water retention, inhibit the production and action of vasoconstrictor peptides, promote vascular relaxation and inhibit sympathetic outflow (Levin et al. 1998). Thus, the blood pressure-lowering action of ANP is based on the reduction of the peripheral vascular resistance and decrease in cardiac output and is most apparent in states of volume excess (Levin et al. 1998). The natriuretic and diuretic actions of the natriuretic peptides are due to both renal haemodynamic and direct tubular actions (Levin et al. 1998). ANP causes vasodilatation of afferent renal arterioles and vasoconstriction of efferent arterioles, leading to increased filtration pressure within the glomerular capillaries (Marin-Grez et al. 1986). Natriuretic peptides have also antimitogenic and antitrophic activity in the cardiovascular system, and are suggested to inhibit the structural remodelling of heart and blood vessels in hypertension (Ruskoaho and Leppäluoto 1988, Furuya et al. 1991). Abnormalities of genes for the natriuretic peptides or their receptors have not been clearly linked to cardiovascular disease in human beings (Wilkins et al. 1997). However, studies with transgenic mice have shown that overexpression of the ANP gene decreases arterial blood pressure, while the deletion of the ANP gene leads to hypertension and cardiac hypertrophy (Steinhelper et al. 1990, John et al. 1995, Oliver et al. 1997).

ADM is a peptide recently identified in extracts of cultured pheochromocytoma cells, and it participates in the control of fluid and electrolyte homeostasis and circulation (Kitamura

et al. 1993, Autelitano et al. 1999). In the vasculature, ADM is synthesised and secreted from both smooth muscle and endothelial cells in response to shear stress, hypoxia and various growth factors and hormones (Chun et al. 1997, Cormier-Regard et al. 1998). Its bioactivity is mediated through different mechanisms including cyclic adenosine 3',5'-monophosphate (cAMP), NO, Ca^{2+} and tissue prostaglandins (Kato et al. 1995, Kureishi et al. 1995, Miura et al. 1995, Yang et al. 1996). It also inhibits ET-1 production (Kohno et al. 1995), and acts as an antiproliferative (Kano et al. 1996) and anti-smooth muscle migratory factor (Kohno et al. 1997). In the vasculature, ADM elicits a potent and long lasting hypotensive effect (Kangawa et al. 1996, Charles et al. 1999). Within the kidney it is diuretic and natriuretic and plays a role in the regulation of renal artery tone (Seguchi et al. 1995), and the control of mitogenesis in the glomeruli (Charles et al. 1999). ADM also inhibits water intake, salt appetite and aldosterone secretion, and thus the peptide facilitates the loss of plasma volume. Moreover, it elevates sympathetic tone and has positive inotropic and chronotropic effects on the heart (Szokodi et al. 1996, Szokodi et al. 1998, Romppanen 1999, Samson 1999). ADM is suggested to be degraded by NEP (Lisy et al. 1998).

Plasma ADM is elevated in association with increases in sympathetic nervous activity and body fluid volume (Ishimitsu et al. 1994). ADM levels in plasma are raised in patients with impaired renal function, in some patients with essential hypertension or primary aldosteronism, in poorly controlled diabetes, after acute myocardial infarction, in congestive heart failure, in sepsis and in thyreotoxicosis. ADM levels are also increased through normal pregnancy (Charles et al. 1999). The ADM may participate, along with ANP and BNP, in mechanisms counteracting a further elevation of blood pressure and the development of left ventricular hypertrophy in patients with essential hypertension (Kato et al. 1999a, Morimoto et al. 1999).

Vasopeptidase inhibitors, which simultaneously inhibit NEP and ACE, are effective in lowering blood pressure in various models of experimental hypertension and in essential hypertension. They also seem to offer advantages over ACE inhibition in improving cardiac performance, providing target organ protection and prolonging survival in essential hypertension and in heart failure. Therefore, ANP, BNP, CNP and ADM all seem to be involved in the pathophysiology of hypertension (Burnett 1999).

2 Vascular endothelium

The vascular endothelium, ideally situated at the interface between blood and the underlying smooth muscle, regulates vascular function by sensing the physical and chemical environment and releasing factors, which act on itself or on other vascular cells. The endothelium takes part in the regulation of various physiological functions including coagulation, lipid transport, immunological reactivity, vascular structure and vascular tone (Busse and Fleming 1993, Haynes and Webb 1998). The degree of contraction or relaxation of VSMCs characterises the general vasomotor tone, which governs the local blood pressure level and distributes the flow

according to metabolic needs. Therefore, by releasing vasoactive substances such as NO, hyperpolarizing factor, cyclooxygenase (COX) metabolites, ET and other contracting factors the endothelium continuously adjusts the balance between vasoconstriction and vasodilatation and maintains an adequate blood flow (Busse and Fleming 1993, Boulanger 1999).

A number of studies have demonstrated that endothelium-mediated arterial relaxation is impaired in patients with essential hypertension (Panza et al. 1990, Taddei et al. 1997a, Schmieder et al. 1997) although this view is not supported by all investigators (Cockcroft et al. 1994, Hutri-Kähönen et al. 1999). The impairment of endothelium-mediated vasodilatation has also been observed in various models of experimental hypertension including SHR (Cohen 1995, Küng and Lüscher 1995, Nava and Lüscher 1995). The manifestation of endothelial dysfunction has been shown to occur before the development of hypertension in SHR (Jameson et al. 1993), and endothelial function has been reported to be impaired even in the normotensive offspring of hypertensive parents (Taddei et al. 1996). On the contrary, the endothelium-dependent vasodilatation has been found to be preserved during the developmental phase of hypertension in SHR, suggesting that endothelial dysfunction provides no significant pathogenetic contribution to the onset of hypertension (Radaelli et al. 1998). In addition, endothelial dysfunction in humans seems to be independent of the degree of vascular structural alterations and of the aetiology of hypertension, and it is probably more linked to the haemodynamic load (Rizzoni et al. 1998a). The pathophysiological basis of endothelial dysfunction is still largely unknown, and it is even uncertain whether there is any association between endothelial dysfunction and hypertension (Van Zwieten 1997). However, endothelial dysfunction in cardiovascular disease may be reversed by drug treatments (Muiesan et al. 1999), and the risk of acute cardiovascular events can thus be decreased (Celermajer 1997).

2.1 Endothelium-derived vasodilatory factors

2.1.1 Nitric oxide

The discovery of endothelium-derived relaxing factor and its identification as NO, a highly reactive free radical gas, was one of the most exciting discoveries of biomedical research in the 1980s (Furchgott and Zawadzki 1980, Palmer et al. 1987). In 1992, Science picked NO as the Molecule of the Year, and over the past years NO has become established as a universal intercellular messenger that serves a variety of biomodulatory functions in many physiological and pathophysiological states (Beck et al. 1999). In addition to being a potent vasodilator, endothelium-derived NO also inhibits the proliferation and induces apoptosis of smooth muscle cells, reduces platelet activation and aggregation, and prevents leukocyte adhesion to the vascular surface. Thus NO protects blood vessels against remodelling, atherosclerosis and thrombosis (Moncada and Higgs 1993, Fukuo et al. 1996, Rudic et al. 1998, Taddei et al. 1998a, Papapetropoulos et al. 1999).

NO is synthesised from L-arginine by the NOSs. Three distinct NOS isoforms, endothelial, neuronal and inducible, exist in mammalian cells (Palmer et al. 1988, Singh and Evand 1997). The endothelial subtype accounts for the majority of the basal and stimulated NO synthesis in endothelial cells throughout the vasculature (Umans and Levi 1995). The synthesis of NO can be stimulated by an increase in endothelial Ca^{2+} concentration following physical and chemical stimuli such as shear stress and hypoxia, activation of cell surface receptors by a variety of endogenous substances like acetylcholine (ACh) and bradykinin, or application of Ca^{2+} channel agonists (Busse and Fleming 1995, Vanhoutte et al. 1995; Figure 1).

NO relaxes vascular smooth muscle via the activation of soluble guanylate cyclase that converts guanosine 3',5'-monophosphate (GMP) to cGMP (Moncada et al. 1991; Figure 1). In smooth muscle cells, cGMP has been reported to activate the cGMP-dependent protein kinase that regulates several pathways involved in Ca^{2+} homeostasis, the end result being a reduction in the concentration of Ca^{2+} available for contraction and a decrease in the sensitivity of contractile proteins to Ca^{2+} (Lincoln et al. 1994, Busse and Fleming 1995). In addition, NO has been reported to hyperpolarize vascular smooth muscle via cGMP-dependent mechanisms as well as by directly activating Ca^{2+}-activated K^+ channels (K_{Ca}) and Na^+-K^+ adenosine 5'-triphosphatase (ATPase) (Bolotina et al. 1994, Gupta et al. 1994, Cohen 1995). Furthermore, NO attenuates NOS activity by a negative feedback mechanism (Ignarro 1996). The activity of NOS can also be inhibited by analogues of L-arginine such as L-NAME, N^G-monomethyl-L-arginine and the asymmetrical dimethylarginine (ADMA) (Moncada et al. 1991, Vallance et al. 1992).

There appears to be a continuous basal release of NO from the endothelium, which significantly contributes to the maintenance of arterial tone (Schulz and Triggle 1994). This view is supported by the findings that chronic treatment of experimental animals with NOS inhibitors leads to a persistent hypertension (Baylis et al. 1992, Deng et al. 1993), and the disruption of endothelial NOS gene induces hypertension in mice (Huang et al. 1995, Shesely et al. 1996). Moreover, it has been suggested that there is diminished basal NO synthesis in the vasculature of patients with essential hypertension as well as in experimental hypertension (Calver et al. 1992, Cohen 1995, Forte et al. 1997). In contrast, studies in genetically hypertensive rats suggest that the vascular synthesis and release of NO is unaffected or it may even be enhanced (Grunfeld et al. 1995, Tschudi et al. 1996, Wu et al. 1996, Le Marquer-Domagala and Finet 1997). However, in these animals the excessive vascular production of superoxide anion has been reported to increase the decomposition of NO and, thus, attenuate endothelium-dependent vasodilatation (Jameson et al. 1993, Grunfeld et al. 1995, Tschudi et al. 1996). Potential enzymatic sources of superoxide in blood vessels include the mitochondrial electron transport chain, xanthine oxidase, COX, lipoxygenase, NOS, and NADH and NADPH oxidases, in endothelial, vascular smooth muscle and adventitial cells (Kojda and Harrison 1999). In physiological conditions, the concentration of superoxide radicals remains low within the organism as a result of its reaction with the superoxide

dismutase (SOD) enzyme. However, in pathological situations, such as hypertension, there may be an increase in the production of these radicals or a deficiency of SOD (De Artinano and Gonzalez 1999).

2.1.2 Prostacyclin

Prostacyclin (PGI_2) is a member of the prostaglandin (PG) family. It causes vasodilatation and inhibition of platelet aggregation (Busse et al. 1994). It also inhibits VSMC proliferation *in vitro* (Weber et al. 1998a). The formation of PGs begins with the liberation of arachidonic acid (AA) from cell membrane phospholipids by phospholipase A_2. COX then converts AA into PGG_2 and PGH_2. Finally, PGH_2 is converted into PGI_2 by PGI_2 synthase (Cohen 1995, Gryglewski 1995; Figure 1). Although other PGs (PGE_2, $PGF_{2\alpha}$ and PGD_2) are also synthesised in the endothelial cells, PGI_2 is the major prostanoid produced in all the vascular cells (Busse et al. 1994). The production of PGs can be blocked by non-steroidal anti-inflammatory drugs, which inhibit COX (Vane and Botting 1993).

Endothelial cells release PGI_2 in response to shear stress, hypoxia, and stimulation of various receptors such as muscarinic, B_2-kinin and purinergic P_{2Y} receptors (Gryglewski 1995, Lüscher and Noll 1995). Physiologically, PGI_2 is a local rather than a circulating hormone, because its blood levels are too low to have any general effects (Vane and Botting 1993). PGI_2 exerts its actions by binding to membrane receptors on the smooth muscle, which activate adenylate cyclase and subsequently increase the intracellular concentration of cAMP (Busse et al. 1994). The elevation of intracellular cAMP leads to the hyperpolarization of cell membrane, reduction of intracellular Ca^{2+} concentration, and to a decrease in the sensitivity of contractile proteins to Ca^{2+} (Bülbring and Tomita 1987, Ushio-Fukai et al. 1993, Cohen and Vanhoutte 1995; Figure 1). When compared with the inhibition of NOS, the blockade of COX has negligible impact on blood pressure (Ruoff 1998). However, the inhibition of COX has been found to enhance endothelium-mediated vasodilatation in SHR as well as in essential hypertensive patients (Jameson et al. 1993, Taddei et al. 1993, Takase et al. 1994, Taddei et al. 1997b). In addition, it has been proposed that in the hypertensive rat endothelium, the conversion of PGH_2 into PGI_2 is diminished, resulting in the accumulation of the vasoconstrictor PGH_2 (Cohen 1995). Therefore, genetic hypertension appears to be associated with an imbalance in the endothelial production of COX-derived vasodilator and vasoconstrictor factors, with the balance tilting to vasoconstriction.

2.1.3 Endothelium-derived hyperpolarizing factor

Endothelium-derived hyperpolarizing factor (EDHF) is defined as that substance, which produces vascular smooth muscle hyperpolarization and relaxation that cannot be explained by NO or PGI_2 (Edwards and Weston 1998). EDHF has been reported to be a diffusible factor, which causes the opening of K^+ channels in the smooth muscle membrane (Bray and Quast

1991, Garland et al. 1995). Involvement of adenosine 5'-triphosphate (ATP)-sensitive K^+ channels (K_{ATP}) and Na^+,K^+-ATPase has been reported in some vessels, but in the majority of the studies EDHF has been suggested to act through K_{Ca} (Quayle and Standen 1994, Cohen and Vanhoutte 1995, Garland et al. 1995, Kagota et al. 1999). The action of EDHF can be inhibited by K^+ channel blockers or by depolarizing the smooth muscle with high K^+ concentrations (Adeagbo and Triggle 1993).

In recent years, the most popular candidates for EDHF have been the nonprostanoid products of the metabolism of AA, namely epoxyeicosatrienoic acids (Cohen and Vanhoutte 1995, Garland et al. 1995, Campbell et al. 1996, Vanhoutte and Mombouli 1996, Fisslthaler et al. 1999, Bolz et al. 2000). They are produced by the endothelium, released in response to vasoactive hormones and elicit vasorelaxation via stimulation of K_{Ca} (Quilley et al. 1997). Studies have also indicated that endocannaboid agonists, such as anandamide, may be EDHFs, since they induce K^+ channel activation-mediated vasorelaxation in isolated vascular preparations (Randall and Kendall 1998). The K^+ ion itself has also been suggested to be EDHF in small mesenteric resistance arteries of rats (Edwards et al. 1998), although not all investigators are convinced of this claim (Lacy et al. 2000). Moreover, the electrotonic propagation of endothelial cell hyperpolarization via gap junctions between endothelial and smooth muscle cells has been suggested to play some role in the EDHF response in rat hepatic and mesenteric arteries, and to be the sole mechanism underlying the EDHF response in the guinea-pig internal carotid artery (Edwards et al. 1999). Therefore, the nature of the responses attributed to EDHF seem still to be unsolved, but the evidence from several sources suggests that there are multiple EDHFs, and that the chemical mediator of the EDHF response may vary with the vascular bed (Edwards and Weston 1998, Campbell and Harder 1999).

The release of EDHF, like that of NO and PGI_2, is initiated by an increase in intracellular free Ca^{2+} concentration ($[Ca^{2+}]_i$) in the endothelial cell (Busse et al. 1994, Cohen 1995; Figure 1). Consequently, many autacoids and hormones that release NO and PGI_2 have also been shown to release EDHF (Cohen and Vanhoutte 1995). The mechanisms whereby hyperpolarization causes relaxation remain controversial. Most likely, the hyperpolarization of the smooth muscle cell membrane reduces Ca^{2+} influx through voltage dependent Ca^{2+} channels, which allows the Ca^{2+} sequestration and removal mechanisms to lower $[Ca^{2+}]_i$ (Busse et al. 1994, Cohen 1995). However, it has been suggested that the inhibition of voltage dependent Ca^{2+} channels does not entirely account for the relaxations attributed to EDHF in all the vessels (Cohen and Vanhoutte 1995). The hyperpolarization induced by potassium channel openers has been suggested to decrease the phospholipase C (PLC)-mediated inositol trisphosphate (IP_3) production, reduce the sensitivity of contractile elements to Ca^{2+}, enhance Ca^{2+} extrusion via Na^+/Ca^{2+} exchanger, and increase the binding of intracellular Ca^{2+} to the internal side of the plasmalemma (Quast et al. 1994).

The importance of EDHF in endothelium-dependent relaxations has been reported to increase as the artery size decreases (Shimokawa et al. 1996, Urakami-Harasawa et al. 1997). NO has been shown to inhibit both the production and action of EDHF (Bauersachs et al.

1996, McCulloch et al. 1997). Therefore, in pathophysiological states, such as hypertension, which may be associated with the decreased bioavailability of endothelium-derived NO, EDHF-mediated vasorelaxation could be enhanced and of greater importance than under physiological conditions (Bauersachs et al. 1996). Nevertheless, decreased endothelium-mediated hyperpolarization has been observed in many forms of experimental hypertension including genetic hypertension (Van de Voorde et al. 1992, Fujii et al. 1992, Mäkynen et al. 1996, Sunano et al. 1999).
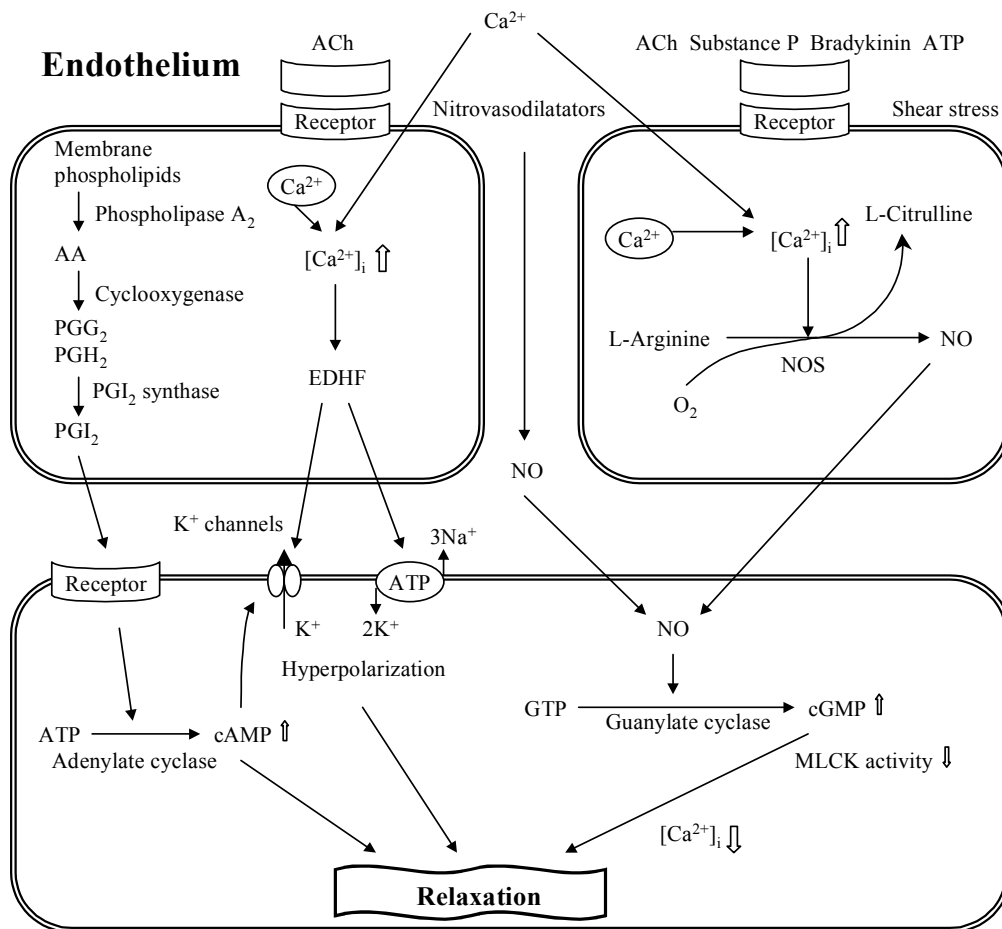
Figure 1. Schematic diagram shows major mechanisms of endothelium-dependent arterial relaxation. Abbreviations: AA, arachidonic acid; ACh, acetylcholine; ATP, adenosine 5-triphosphate; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration; cAMP, cyclic adenosine 3',5'-monophosphate; cGMP, cyclic guanosine 3',5'-monophosphate; EDHF, endothelium-derived hyperpolarizing factor; GTP, guanosine 5'-triphosphate; MLCK, myosin light chain kinase; NO, nitric oxide; NOS, nitric oxide synthase; PGG_2, prostaglandin G_2; PGH_2, prostaglandin H_2; PGI_2, prostacyclin; ⇑, increase; ⇓, decrease.

2.2 Endothelium-derived contractile factors

In addition to being a source of relaxing factors, endothelial cells also produce contractile factors such as vasoconstrictor prostanoids, ET-1 and Ang II. The production of endothelium-derived contracting factors (EDCFs) is induced by hypoxia, stretch, pressure, and various local and circulating hormones, and a marked heterogeneity of these responses exists among species, strains, and different vascular beds (Lüscher et al. 1992, Rubanyi 1993).

2.2.1 Cyclooxygenase-derived contractile factors

COX produces several EDCFs, the most potent of which are thromboxane A_2 (TXA_2) and the PG endoperoxide intermediates PGG_2 and PGH_2, which all act via the same receptor (Cohen 1995). Several agonists including ACh and 5-hydroxytryptamine (5-HT) can induce the release of COX-derived contractile factors in SHR (Lüscher and Vanhoutte 1986, Auch-Schwelk and Vanhoutte 1991, Ito and Carretero 1992), whereas such responses are absent in WKY rats (Lüscher and Vanhoutte 1986, Jameson et al. 1993). The release of COX-derived contractile factors can be inhibited by COX inhibitors such as diclofenac (Auch-Schwelk and Vanhoutte 1991).

Numerous studies in SHR and some in patients with essential hypertension have found that endothelium-dependent vasodilatation is impaired due to the production of COX-derived factors (Jameson et al. 1993, Taddei et al. 1993, Takase et al. 1994, Küng and Lüscher 1995, Taddei et al. 1997a). However, the blockade of TXA_2/PG endoperoxide receptor has no or only a minimal effect on systemic blood pressure in SHR or in patients with essential hypertension (Ritter et al. 1993, Tesfamariam and Ogletree 1995, Ruoff 1998). The COX inhibition has been found to enhance endothelium-mediated dilatation in essential as well as in experimental hypertension (Jameson et al. 1993, Taddei et al. 1993, Takase et al. 1994, Taddei et al. 1997b). However, the production of COX-dependent EDCFs does not seem to occur in young essentially hypertensive patients, suggesting that COX-derived EDCFs do not participate in the development of human hypertension (Taddei et al. 1997a).

2.2.2 Endothelin-1

ETs (ET-1, ET-2, ET-3) are peptides that possess characteristically sustained vasoconstrictor properties (Haynes and Webb 1998). They are synthesised from larger precursors, preproendothelins via the formation of proendothelin, which in turn is cleaved to form the mature ET-1 by ET converting enzymes (Haynes and Webb 1998). The predominant member of the ET family appears to be ET-1 generated by vascular and cardiac endothelial and smooth muscle cells, kidney, posterior pituitary and CNS (Lüscher et al. 1992, Haynes and Webb 1998, Rothermund and Paul 1998). ET-2 produced in endothelial cells, heart and kidney, and ET-3 produced in the endocrine, gastrointestinal and central nervous systems are probably less

important mediators of the cardiovascular effects of ETs (Haynes and Webb 1998, Masaki 1998).

The production and release of ET-1 occurs in response to many stimuli, including adrenaline, Ang II, vasopressin, interleukin-1, transforming growth factor-β, cyclosporine, lipoproteins, free radicals, thrombin, endotoxin, and physical factors such as stretch, hypoxia and low shear stress (Kuchan and Frangos 1993, Lüscher and Noll 1995, Haynes and Webb 1998, Rothermund and Paul 1998). On the other hand, high shear stress and the formation of cGMP and cAMP within the endothelium have been shown to inhibit ET-1 production (Haynes and Webb 1998, Rothermund and Paul 1998). Endothelial cells secrete synthesised ET-1 mainly onto the abluminal side towards the vascular smooth muscle rather than into the lumen (Wagner et al. 1992, Rothermund and Paul 1998). This has lead to the concept that ETs predominantly act locally in a paracrine or autocrine manner (Rothermund and Paul 1998).

The haemodynamic effects of ETs can be explained by the activation of two receptor subtypes, ET_A and ET_B (Sakurai et al. 1990, Nava and Lüscher 1995). The intracellular mechanisms of action of ETs involve the activation of phosphatidylinositol metabolism, mobilisation of intracellular Ca^{2+} stores, and promotion of Ca^{2+} entry through plasmalemmal Ca^{2+} channels (Schiffrin 1995). The ET_A receptor has a high affinity for ET-1, is present in VSMCs and cardiomyocytes, and predominantly mediates contraction (Rothermund and Paul 1998). The ET_B receptor is equally sensitive to the three ET isoforms, is present both in the endothelium, where it releases NO and PGI_2, resulting in vasodilatation, and in VSMCs, where it mediates vasoconstriction (Clozel et al. 1992, Lüscher et al. 1993a, Masaki 1998).

ET-1 is one of the most potent known endogenous vasoconstrictors, and it has inotropic, trophic and mitogenic properties, influences the homeostasis of salt and water, alters central and peripheral sympathetic activity, and stimulates RAS and sympathetic nervous system (Yanagisawa et al. 1988, Haynes and Webb 1998, Moreau 1998). Moreover, ET-1 is able to potentiate the contractile responses to other vasoconstrictors such as 5-HT and NA at subthreshold concentrations, and it may also provoke the release of free radicals and COX-derived vasoconstrictor factors (Lüscher et al. 1993b, De Artinano and Gonzalez 1999).

In view of the multiple cardiovascular actions of ET-1, there has been much interest in its contribution to the pathogenesis of hypertension and renal disorders (Haynes and Webb 1998, Barton and Lüscher 1999). ET-1 is overexpressed in the vascular wall in certain models of experimental hypertension including deoxycorticosterone acetate salt-treated rats, stroke-prone SHR, Ang II-infused rats and 1-kidney 1 clip Goldblatt rats, but it is not overexpressed in SHR, 2-kidney 1-clip hypertensive rats or L-NAME-treated rats (Schiffrin 1999). The vascular ET activity in patients with essential hypertension has also been found to be increased and could be of pathophysiological relevance to their increased vascular tone (Cardillo et al. 1999). ET-1 may also have a role in the onset of hypertension-induced remodelling in conduit arteries (Cattaruzza et al. 2000). Furthermore, ET-1 has been involved in the progression of renal failure in rats with subtotal nephrectomy, and ET receptor blockade has reduced protein excretion in these rats (Wolf et al. 1999). Moreover, the administration of

anti-ET agents produces vasodilatation and lowers blood pressure in hypertensive humans and experimental animals, and reduces end-organ damage in animal studies, irrespective of whether vascular ET activity is increased in hypertension (Brunner 1998, Haynes and Webb 1998, Verhagen et al. 1998, Webb and Strachan 1998). Therefore, ET-1 may contribute to the functional and structural changes associated with hypertension and renal failure (Barton and Lüscher 1999).

3 Vascular smooth muscle

3.1 Contraction

Vasoconstricting neurotransmitters and hormones bind to their receptors on the cell surface and initiate a series of processes leading to the contraction of vascular smooth muscle. Most receptors activate various types of guanosine 5-triphosphate (GTP) binding proteins (G proteins), which are coupled to different ion channels and enzymes, and modulate their activities. These enzymes include both phospholipase C (PLC), which metabolises phosphatidylinositol 4,5-bisphosphate (PIP) and produces inositol 1,4,5-triphosphate (IP$_3$) and 1,2-diacylglycerol (DAG), and adenylate cyclase, which metabolises ATP to produce cAMP (Abdel-Latif 1986, Nishizuka 1995). IP$_3$ releases Ca^{2+} from intracellular stores whereas DAG activates protein kinase C (PKC), which phosphorylates a number of proteins (Nahorski et al. 1994). In addition to the activation of the phosphatidylinositol metabolism, vasoconstrictors, such as NA and 5-HT, have been shown to depolarize the arterial smooth muscle and consequently activate voltage operated Ca^{2+} channels (VOCs) in the plasma membrane of the smooth muscle, leading to an increased influx of Ca^{2+}. Moreover, the existence of receptor operated Ca^{2+} channels (ROCs) has been proposed in VSMCs (Nelson et al. 1990). The activation of the above mechanisms increases $[Ca^{2+}]_i$, which is the primary signal for smooth muscle contraction (Allen and Walsh 1994).

As a consequence of the elevated $[Ca^{2+}]_i$ concentration, Ca^{2+} binds to calmodulin to form a Ca^{2+}-calmodulin complex, which removes the autoinhibition of myosin light chain kinase (MLCK; Allen and Walsh 1994). The activated MLCK phosphorylates reversibly the light chain of myosin and activates the myosin ATPase (Walsh 1994, Winder et al. 1998). The phosphorylated myosin cyclically binds to actin filaments producing force or the shortening of the smooth muscle (Walsh 1994). On the other hand, a fall in $[Ca^{2+}]_i$ inactivates the MLCK, permits dephosphorylation of myosin by myosin light chain phosphatase and causes relaxation (Stull et al. 1991, Cirillo et al. 1992, Rembold 1992, Allen and Walsh 1994). The contractile force does not, however, depend directly on $[Ca^{2+}]_i$, since the force may be enhanced by augmenting the responsiveness of the contractile machinery or the sensitivity of the myofilaments to $[Ca^{2+}]_i$ (Andrea and Walsh 1992, Ruegg 1999). Changes in free calmodulin concentrations, myosin light chain phosphorylation elicited by small G proteins (e.g. Rho A) and the enzymes associated with them (Rho-associated kinase), regulation of myosin

phosphatase activity and thin filament-associated proteins, such as calponin and caldesmon, are the possible mechanisms for regulation of Ca^{2+} sensitivity (Hori and Karaki 1998, Winder et al. 1998). These modulatory mechanisms for changing the Ca^{2+} sensitivity, together with the major regulatory mechanism for cellular Ca^{2+} metabolism, play an important role in the regulation of vascular smooth muscle tone (Walsh 1994, Takuwa 1996, Somlyo et al. 1999).

Increased receptor-mediated arterial smooth muscle contractility has often been observed in experimental hypertension (Longhurst et al. 1988, Perry and Webb 1988, Brodde and Michel 1992, Orlov et al. 1993). However, in many recent reports only minor differences have been detected in the contractile responses between hypertensive and normotensive animals (Bockman et al. 1992, Tolvanen et al. 1996). Enhanced responsiveness of G proteins (Kanagy and Webb 1994, Feldman et al. 1995), increased turnover and accumulation of inositol phosphates (Vila et al. 1993) and augmented release of Ca^{2+} from sarcoplasmic reticulum (SR) by IP_3 (Kawaguchi et al. 1993) have been suggested to mediate increased vascular contractility in experimental hypertension. In addition, the generation of DAG stimulated by vasoconstrictor agents and subsequent activation of protein kinase C have been reported to contribute to the enhanced vascular reactivity (Turla and Webb 1990, Okamura et al. 1992, Nguyen et al. 1993, Nahorski et al. 1994). Therefore, an overactive receptor-mediated contraction pathway may play some role in the pathogenesis of hypertension.

3.2 Cellular calcium regulation

The Ca^{2+} metabolism plays a central role in many cellular functions including growth, proliferation and contraction of the vascular smooth muscle. Contraction is initiated by an increase in $[Ca^{2+}]_i$ (Karaki and Weiss 1988). Since the Ca^{2+} stores of the vascular smooth muscle are both extracellular and intracellular (Cirillo et al. 1992), the $[Ca^{2+}]_i$ is adjusted by a complex interaction between Ca^{2+} entry and extrusion across the plasmalemma, and Ca^{2+} release from and uptake to SR (Marks 1992). Both the plasmalemma and SR form a barrier to an approximately 10 000-fold Ca^{2+} concentration gradient. The plasmalemmal Ca^{2+} permeability is under the control of membrane potential and various agonists, whereas SR is controlled by second messengers (Van Breemen and Saida 1989; The major mechanisms involved in the cellular Ca^{2+} regulation are summarised in figure 2).

Ca^{2+} influx across the plasmalemma under physiological conditions occurs through ion channels or via exchangers. Plasmalemmal Ca^{2+} channels are either voltage-gated or receptor-operated (Horowitz et al. 1996). Voltage-gated Ca^{2+} channels have been divided by electrophysiological and pharmacological techniques into two different subgroups: one type is activated by small depolarizations and is inactivated quickly (T-type), whereas the other requires stronger depolarizations and inactivates more slowly (L-type) (Spedding and Paoletti 1992). The L-type Ca^{2+} current can selectively be blocked by dihydropyridine Ca^{2+} channel antagonists like nifedipine, and T-type Ca^{2+} channels can be blocked by mibefradil (Nelson et al. 1990, Spedding and Paoletti 1992, Mishra and Hermsmeyer 1994). Some Ca^{2+} ions enter

VSMCs also due to the low passive permeability of the plasma membrane to Ca^{2+} (Cirillo 1992). Moreover, the plasma membrane has a capacity to bind Ca^{2+} and thus buffer an increase in $[Ca^{2+}]_i$, and it has been suggested that an elevation in both extra- and intracellular Ca^{2+} makes the plasma membrane less permeable to Ca^{2+} (Dominiczak and Bohr 1990, Cirillo et al. 1992). In vascular smooth muscle, Ca^{2+} can be extruded from the cell by the plasmalemmal Ca^{2+}-ATPases (Ca^{2+} pump) or the Na^+/Ca^{2+} exchanger (Allen and Walsh 1994, Horowitz et al. 1996). The Ca^{2+} pump uses energy provided by ATP hydrolysis and accounts for most of the Ca^{2+} efflux at normal $[Ca^{2+}]_i$. The Na^+/Ca^{2+} exchange is an antiporter which under basal conditions permits the efflux of one Ca^{2+} ion coupled with the influx of three Na^+ ions (Cirillo 1992).

A large capacity for total Ca^{2+} storage in VSMCs is achieved mainly by SR, although special Ca^{2+} binding molecules are also present (Karaki and Weiss 1988, Horowitz et al. 1996). SR can actively sequester Ca^{2+} (Martonosi et al. 1990, DeLong and Blasie 1993) and release it following plasmalemmal receptor activation (Minneman 1988). IP_3 formed in response to the activation of cell surface receptors releases Ca^{2+} by binding to IP_3-receptors in SR (Marks 1992, Allen and Walsh 1994, Somlyo et al. 1999). Intracellular Ca^{2+} stores can also be mobilised by Ca^{2+}-induced Ca^{2+} release, a mechanism in which the influx of a small amount of Ca^{2+} releases more Ca^{2+} from SR via ryanodine receptors (Marks 1992, Allen and Walsh 1994, Horowitz et al. 1996). The physiological significance of Ca^{2+}-induced Ca^{2+} release may be the amplification of IP_3-induced Ca^{2+} release, since Ca^{2+} is a coagonist of IP_3-induced Ca^{2+} release (Finch et al. 1991, Nahorski et al. 1994). The membrane of SR contains also a Ca^{2+} pump, which transports Ca^{2+} ions from the cytosol into SR (Van Breemen and Saida 1989, Allen and Walsh 1994, Horowitz et al. 1996).

The $[Ca^{2+}]_i$ has been found to be abnormally high in blood cells, cultured aortic and mesenteric arterial smooth muscle cells, and also in intact aortas and renal arteries of hypertensive animals (Spieker et al. 1986, Jelicks and Gupta 1990, Sada et al. 1990, Sugiyama et al. 1990, Oshima et al. 1991, Papageorgiou and Morgan 1991, Bendhack et al. 1992, Arvola et al. 1993, Ishida-Kainouchi et al. 1993), whereas some studies have failed to observe this abnormality (Liu et al. 1994, Neusser et al. 1994). However, since the studies on VSMCs from resistance arteries have shown no difference in the basal $[Ca^{2+}]_i$ between SHR and WKY rats (Storm et al. 1992, Bukoski et al. 1994, Bian and Bukoski 1995), the elevations in $[Ca^{2+}]_i$ found in aortic smooth muscle cells and in several other cell types of hypertensive animals are unlikely to contribute to the heightened peripheral resistance in SHR (Dominiczak and Bohr 1990, Bian and Bukoski 1995).

Contractile responsiveness of VSMCs from large arteries of SHR have been shown to be enhanced to depolarization and Bay K 8644, an agonist of dihydropyridine-sensitive Ca^{2+} channel, when compared with WKY rats (Aoki and Asano 1986, Aoki and Asano 1987, Bruner and Webb 1990). In addition, increased vascular sensitivity to the effects of nifedipine has been found in prehypertensive and adult SHR (Aoki and Asano 1986, Aoki and Asano 1987, Asano et al. 1995). In resistance arteries, an increase in the Ca^{2+} influx by the voltage-

dependent Ca^{2+} channels has been found in the early hypertensive stage, but not in prehypertensive SHR (Arii et al. 1999). The increased amplitude of the whole-cell Ca^{2+} current in the arterial smooth muscle cells from SHR compared with WKY rats has been suggested to be attributed to enhanced sensitivity of dihydropyridine receptors in the channels in SHR, while the opening of single Ca^{2+} channels has been suggested to be unaltered (Kubo et al. 1998). These results indicate that Ca^{2+} entry through VOCs is enhanced in SHR when compared with WKY rats, which could partially account for altered Ca^{2+} homeostasis and increased vascular reactivity, and thus contribute to the genesis of hypertension and increased peripheral resistance (Arii et al. 1999).

In SHR and in patients with essential hypertension, less Ca^{2+} than normal seems to be bound to the plasma membrane, and the Ca^{2+} permeability of plasma membrane seems to be increased (Lamb et al. 1988, Dominiczak and Bohr 1990). The extrusion of Ca^{2+} through the plasmalemma by the Na^+/Ca^{2+} exchange has been reported to be enhanced in aortic VSMCs of SHR (Ashida et al. 1989), whereas depressed activities have been found in tail arteries (Thompson et al. 1990). Studies on the Ca^{2+} pump-mediated Ca^{2+} efflux in VSMCs of SHR have also yielded contradictory results (Kwan and Daniel 1982, Ashida et al. 1989, Monteith et al. 1996, Monteith et al. 1997). Furthermore, the ability of SR to sequester Ca^{2+} has been proposed to be attenuated in SHR (Dohi et al. 1990, Kojima et al. 1991). In addition, SR of SHR appears to have a larger capacity to store Ca^{2+}, but the filling of SR is slower when compared with WKY rats (Kanagy et al. 1994). These findings could result from a reduced activity of SR Ca^{2+} pump. Nevertheless, the activity and density of SR Ca^{2+} pump have been reported to be increased in VSMCs of SHR (Levitsky et al. 1993), and the levels of SR Ca^{2+} pump mRNA were shown to be higher in VSMCs from SHR than in those from WKY rats (Monteith et al. 1997).

Taken together, if $[Ca^{2+}]_i$ is to be elevated, then the Ca^{2+} entry must be increased, or the storage of Ca^{2+} into SR must be decreased, or the extrusion of Ca^{2+} must be decreased. There is no clear evidence whether any of these abnormalities are present in hypertensive VSMCs (Gonzalez and Suki 1995). Finally, it has recently been suggested that one link between the metabolism of Ca^{2+} and the control of arterial tone could be the extracellular Ca^{2+} receptor in the perivascular sensory nerves, the activation of which can cause vasorelaxation via the release of a hyperpolarizing mediator (Bukoski 1998, Ishioka and Bukoski 1999).

3.3 Na^+-K^+-ATPase

A significant part of the membrane potential in VSMC can be attributed to the electrogenic action of the Na^+,K^+-ATPase, which generates a hyperpolarizing current by extruding three Na^+ ions for every two K^+ ions pumped in. Vascular Na^+,K^+-ATPase can be inhibited by cardiac glycosides such as digoxin and ouabain (Blaustein 1993, O'Donnell and Owen 1994). The activity of Na^+,K^+-ATPase is proposed to influence $[Ca^{2+}]_i$ in VSMC via VOCs and Na^+/Ca^{2+} exchange. Thus, decreased Na^+,K^+-ATPase activity leads to membrane

depolarization and increased Ca^{2+} influx through VOCs. In addition, decreased activity promotes Na^+ retention in VSMC, which reduces the driving force of Na^+/Ca^{2+} exchange leading to attenuated Ca^{2+} extrusion (Bova et al. 1990, Rayson and Gilbert 1992). However, it has been suggested that the latter mechanism does not play an important role in the modification of $[Ca^{2+}]_i$ in VSMCs of SHR (Zhu et al. 1994).

Altered Na^+,K^+-ATPase function has been proposed to play a role in the pathogenesis of both essential and experimental hypertension (Hermsmeyer 1987), but the findings supporting this view are still very controversial. The activity of Na^+,K^+-ATPase has been reported to be either decreased, normal, or increased in VSMCs of SHR when compared with those of WKY rats (Tamura et al. 1986, Manjeet and Sim 1987, Rinaldi and Bohr 1989, Kuriyama et al. 1992, Orlov et al. 1992, Redondo et al. 1995). The reason for depressed activity of Na^+,K^+-ATPase in hypertension may either be an inherent defect or a circulating digitalis- or ouabain-like inhibitor of the pump (Blaustein 1993, Hamlyn et al. 1996). The plasmas from patients with essential hypertension have been claimed to contain ouabain-like factors (Masugi et al. 1987), and the concentrations of these Na^+,K^+-ATPase inhibitors may be increased, especially in chronic renal insufficiency (Kelly et al. 1986). Circulating sodium pump inhibitor concentrations have even been suggested to be sufficient to affect the Na^+,K^+-ATPase in human mesenteric artery, thus the interaction of digitalis-like Na^+,K^+-pump inhibitors with the Na^+,K^+-ATPase could be of importance (Bagrov and Fedorova 1998). However, the existence of such circulating Na^+,K^+-ATPase inhibitors in human plasma is still not clear (Pidgeon et al. 1996), and the level of endogenous ouabain is reported to be lower in SHR when compared with WKY rats (Doris 1994). Moreover, in both rats and humans, studies on the effects of ouabain on blood pressure have produced inconsistent results, and therefore, it is not clear whether the inhibition of Na^+,K^+-ATPase could elevate blood pressure (Pidgeon et al. 1996).

Increased activity of the Na^+,K^+-ATPase has been linked to the increased vascular tone and smooth muscle growth found in SHR (Berk et al. 1989, Davies et al. 1991, Kemp et al. 1992). The activity of Na^+,K^+-ATPase may be enhanced in hypertension because of an increase in passive membrane permeability to Na^+, and since the pump does not appear to fully compensate for this, the vascular tone can be elevated (O'Donnell and Owen 1994). Taken together, these contradictory results on vascular Na^+,K^+-ATPase in hypertension possibly reflect the fact that the pump's functional, enzymatic and biochemical properties may not be uniformly altered in hypertension (Young et al. 1988), and that the type and duration of hypertension profoundly affect these results. It remains to be clarified whether the altered activity of the Na^+,K^+-ATPase in vascular smooth muscle is a key factor in hypertension (O'Donnell and Owen 1994).

3.4 K$^+$ channels

The intracellular concentration of K$^+$ in the vascular smooth muscle is approximately 25-fold higher when compared with the extracellular concentration of K$^+$, and therefore the opening of K$^+$ channels is accompanied by an efflux of K$^+$ from the cytosol, resulting in a loss of positive charge and hyperpolarization of the cell membrane (Quast et al. 1994, Ackerman and Clapham 1997). The K$^+$ channels play an essential role in the regulation of smooth muscle membrane potential, which in turn controls [Ca^{2+}]$_i$ and thus contraction of the vascular smooth muscle (Kitazono et al. 1995, Standen and Quayle 1998). Therefore, the factors that regulate the activity of K$^+$ channels in arterial smooth muscle significantly influence arterial tone, arterial diameter, vascular resistance, blood flow and blood pressure (Standen and Quayle 1998). Four types of K$^+$ channels have been described in arterial smooth muscle on the basis of their electrophysiological and pharmacological properties: K$_{Ca}$, K$_{ATP}$, voltage-activated K$^+$ channels (K$_V$) and inward rectifier K$^+$ channels (K$_{IR}$) (Figure 2). The modulation and expression of K$^+$ channels varies within vascular bed and between larger arteries and resistance vessels (Nelson and Quayle 1995).

K$_{Ca}$ oppose the pressure induced depolarization and myogenic tone of arterial smooth muscle by causing re- and hyperpolarization, the triggers being increased intracellular Ca^{2+} and membrane depolarization (Edwards and Weston 1990, Guia et al. 1999). K$_{Ca}$ are divided into large, intermediate and small according to their conductance. Large-conductance K$_{Ca}$ appear to be the most important K$^+$ channels in the regulation of arterial tone and are inhibited by tetraethylammonium (TEA), charybdotoxin and iberiotoxin whereas the small-conductance K$_{Ca}$ are blocked by apamin (Nelson 1993, Kitazono et al. 1995; Figure 2). The activity of K$_{Ca}$ has been shown to be increased in VSMCs of SHR when compared with those of WKY rats (Shoemaker and Worrel 1991, Asano et al. 1993a, Asano et al. 1993b, England et al. 1993, Rusch and Runnells 1994). In SHR, this phenomenon has been suggested to be caused by increased Ca^{2+} influx through L-type voltage-dependent Ca^{2+} channels, increased Ca^{2+} sensitivity of K$_{Ca}$, and increased expression of K$_{Ca}$ (Shoemaker and Worrel 1991, Asano et al. 1993a, Asano et al. 1993b, England et al. 1993, Liu et al. 1998). This elevated activation of K$_{Ca}$ has been proposed to represent a compensatory mechanism activated during the development of hypertension to counteract abnormal arterial excitability and prevent further vasoconstriction (England et al. 1993, Rusch and Runnels 1994, Rusch et al. 1996). Furthermore, EDHF is suggested to function via K$_{Ca}$ in many blood vessels (Cohen and Vanhoutte 1995).

K$_{ATP}$ have been identified by and named after their sensitivity to inhibition by intracellular ATP (Nelson 1993). Elevation in cytosolic adenosine 5'-diphosphate or intracellular acidification increases the open state probability of these channels (Nelson 1993, Ishizaka et al. 1999). K$_{ATP}$ can be inhibited by glibenclamide and tolbutamide, and activated by a variety of compounds such as cromakalim, levcromakalim, diazoxide and pinacidil (Quast et al. 1994; Figure 2). K$_{ATP}$ appear to contribute to the vasodilatory effects of several

endogenous and exogenous substances, in particular to those acting at receptors linked to cAMP-dependent protein kinase (Quayle and Standen 1994, Prieto et al. 1996). These include adenosine, calcitonin gene-related peptide, PGI_2 and β-adrenoceptor agonists (Standen and Quayle 1998). In addition, β-adrenoceptor agonists and PGI_2 have been reported to stimulate K_{ATP} independently of cAMP (Randall and McCulloch 1995, Vanhoutte and Mombouli 1996). Moreover, the vasorelaxation induced by adenosine, NO and EDHF involves the activation of K_{ATP} in some vessels whereas the vasoconstrictors Ang II and ET appear to inhibit these channels (Quayle and Standen 1994, Murphy and Brayden 1995). The basal opening state of coronary vascular K_{ATP} appears to be activated to a greater extent in SHR than WKY rats (Numaguchi et al. 1996), and K_{ATP} have been found to show reduced sensitivity to ATP in VSMCs of SHR (Furspan and Webb 1993). In addition, increased sensitivity to the effects of cromakalim and diazoxide and decreased sensitivity to the action of glibenclamide have been reported in the arteries of SHR when compared with those of WKY rats (Miyata et al. 1990, Furspan and Webb 1993). Therefore, SHR have been suggested to express a different subtype of K_{ATP} from WKY rats (Furspan and Webb 1993). In contrast, an impaired action of levcromakalim on K_{ATP} was recently found in SHR, and this impairment was restored by the normalisation of blood pressure with hydralazine. This finding suggests that the impairment can be attributed to high blood pressure rather than to the existence of a different subtype of K_{ATP} (Ohya et al. 1996).

K_V are activated by depolarization, but unlike K_{Ca}, the open state probability of these channels is decreased by elevation of intracellular Ca^{2+} concentration (Nelson and Quayle 1995, Cox and Petrou 1999). K_V can be inhibited by 4-aminopyridine. In addition to K_{Ca}, also K_V appear to oppose the depolarization of arterial smooth muscle by returning the membrane potential towards the resting level (Kitazono et al. 1995). K_{IR} are opened by pronounced hyperpolarization and exhibit a sustained inward current and are inhibited by extracellular Ba^{2+} (Nelson and Quayle 1995; Figure 2). These channels may play a role in maintaining resting membrane potential and regulating excitability of VSMCs (Kitazono et al. 1995, Brayden 1996). The roles of K_V and K_{IR} in the regulation of membrane potential in hypertension remain to be elucidated.

In summary, almost every physiological vasodilator and vasoconstrictor has been shown to modulate K^+ channels of one type or another in arterial smooth muscle (Standen and Quayle 1998). Most vasodilators and vasoconstrictors have multiple pathways of action, and the K^+ channels contribute to the changes in contractile tone by altering the cellular membrane potential. Such effects usually occur in concert with changes in Ca^{2+} channel activity, intracellular Ca^{2+} release and Ca^{2+} sensitivity of contractile proteins (Standen and Quayle 1998).
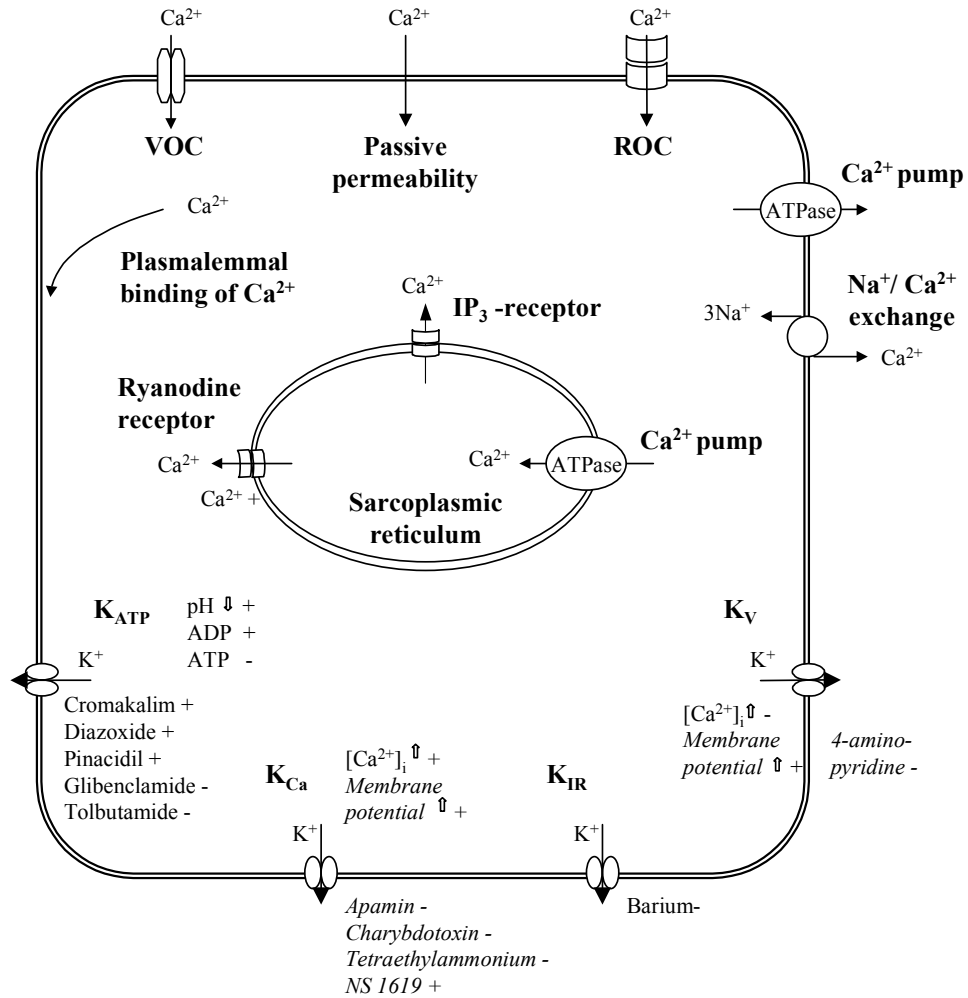
Figure 2. Schematic diagram shows the major mechanisms involved in the cellular Ca^{2+} regulation and some physiological and pharmacological properties of smooth muscle K^+ channels. Abbreviations: ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; IP_3, inositol 1,4,5-trisphosphate; ROC, receptor operated Ca^{2+} channel; VOC, voltage operated Ca^{2+} channel; $[Ca^{2+}]_i$, intracellular free Ca^{2+} concentration, K_{ATP}, ATP-sensitive K^+ channels; K_{Ca}, Ca^{2+}-activated K^+ channels; K_{IR}, inward rectifier K^+ channels; K_V, voltage-dependent K^+ channels; -, inhibition; + stimulation; ⇑, increase; ⇓, decrease.

II Arterial tone and structure in experimental hypertension and renal failure

Animals have been used by humans for centuries to understand our own biology. In cardiovascular research, animal models allow the study of the mechanisms of the pathogenesis of cardiovascular disease and the effects of drug interventions. The aim of the studies is to provide clear concepts for selected investigations in humans. Human hypertension is difficult to study as there is substantial individual variation in the two triggering elements of hypertension, polygenetic disposition and excitatory environmental factors, leading to many variations in the direct and indirect effects on the cardiovascular system that are difficult to differentiate (Lindpaintner et al. 1992). The use of relevant models for human cardiovascular disease provides useful information allowing an understanding of the cause and progression of the disease state as well as increasing our knowledge of potential therapeutic interventions (Doggrell and Brown 1998).

1 Genetic hypertension

The most commonly used model of essential hypertension is SHR often with WKY rat as the normotensive control. SHRs are descendants of an outbred Wistar male with spontaneous hypertension from a colony in Kyoto, Japan, mating with a female with an elevated blood pressure, and then brother - sister mating continued with selection for spontaneous hypertension (Okamoto and Aoki 1963, Dzau et al. 1995). From 1968, this inbred strain of SHRs was further developed in the USA. The WKY controls were established later, in 1971, as a normotensive control strain by the National Institutes of Health as an inbreed of the Wistar Kyoto colony (Kurtz and Morris 1987).

Within each colony, SHRs have uniform polygenetic disposition and excitatory factors, leading to uniform changes in the cardiovascular system. This lack of inter-individual variation is one of the major advantages of the SHR (Lindpaintner et al. 1992). Another advantage of the SHR is that it follows the same progression of hypertension as human hypertension with pre-hypertensive, developing and sustained hypertensive phases (Adams et al. 1989, Folkow 1993). In the early stages of hypertension, SHRs have an increased cardiac output with normal total peripheral resistance. As SHR progresses into the established hypertension state, the cardiac output returns to normal and the hypertrophied and dysfunctional blood vessels produce an increase in the total peripheral resistance (Smith and Hutchins 1979). However, SHR differs from human hypertension in that SHRs develop hypertension in young adulthood rather than in middle age as is the case in humans (Folkow and Svanborg 1993). Importantly, the compounds that lower blood pressure in SHR also lower blood pressure in hypertensive humans.

A common criticism of SHR is that it can only model one of many possible causes of human hypertension, and it has been available for a long time but we still know little about the cause of the onset of hypertension (Dzau et al. 1995). The functional alterations of RAS and

sympathetic nervous system are suggested to be involved in hypertension in SHR (K-Laflamme et al. 1997). On the other hand, the development of hypertension in SHR does not seem to be due to an impairment of NO production, since the L-arginine/NO pathway is upregulated, and the NO production is actually increased in SHR both before and after the onset of hypertension (Vaziri et al. 1998a). However, endothelium-dependent relaxations are impaired in SHR arteries. This can, in part, be explained by reduced endothelium-dependent hyperpolarization (Fujii et al. 1992). Thus, alterations in the EDHF system may play a pivotal role in endothelial dysfunction in SHR (Onaka et al. 1998). Furthermore, enhanced sensitivity to vasoconstrictors in vascular smooth muscle from SHR has been found (Keaton et al. 1998).

The mesenteric resistance arteries from young and adult SHR have shown increased stiffness and media/lumen ratio, and smaller lumen diameter than arteries from WKY rats (Intengan et al. 1999). The wall components of resistance arteries further stiffen with age in SHR (Intengan et al. 1999). An increase in collagen/elastin ratio is also seen in SHR (Intengan et al. 1999). Moreover, decreased incidence of cellular apoptosis has been observed in the wall of mesenteric arteries from SHR compared with WKY rats, which could be responsible for the larger media volume found in older SHR and contribute to the development of hypertension in these animals (Dickhout and Lee 1999). Furthermore, interrupting the RAS has normalising effects on the stiffness and growth of the arterial wall in SHR (Intengan et al. 1999).

2 Hypertension induced by nitric oxide deficiency

Variants of the endothelial NOS gene may be associated with elevated blood pressure (Wang et al. 1997), although some recent reports do not support this view (Kato et al. 1999b, Takami et al. 1999). Nevertheless, the endothelial production of NO appears to be essential for the maintenance of normal blood pressure (Huang et al. 1995), and defects in the production or action of NO may be associated with essential hypertension (Moncada and Higgs 1993). Thus, chronic inhibition of NOS by L-NAME is an interesting model of hypertension (Baylis et al. 1992). Chronic administration of L-NAME has been shown to result in sustained hypertension in normotensive rats (Baylis et al. 1992, Deng et al. 1993), with an associated impairment of endothelium-dependent arterial relaxations (Küng et al. 1995, Dowell et al. 1996, Henrion et al. 1996, Henrion et al. 1997), and arterial remodelling (Deng et al. 1993, Li and Schiffrin 1994, Takemoto et al. 1997). The findings concerning endothelium independent arterial relaxations and contractile responses have been inconsistent (Küng et al. 1995, Dowell et al. 1996, Henrion et al. 1996). Changes in cardiac chamber geometry and myocardial diastolic mechanical properties may also be induced in L-NAME hypertension (Akuzawa et al. 1998, Matsubara et al. 1998). Moreover, chronic administration of L-NAME produces nephrosclerosis and impairs renal function in rats (Hropot et al. 1994, Akuzawa et al. 1998).

Substantial levels of constitutive NOS activity have been shown to remain in the aorta of WKY rats during chronic L-NAME administration (Zhao et al. 1999). Therefore, other

mechanisms than simple inhibition of endothelium-derived NO synthesis may be involved in the long-term cardiovascular effects of L-NAME in the rat arteries (Zhao et al. 1999). Recently the pathogenesis of cardiovascular remodelling in this model has been suggested to result from increased oxidative stress in endothelium (Usui et al. 1999). Excessive production of superoxide radicals may thus underlie the vascular RAS activation and in particular the increase in local ACE expression in rats with long-term NOS inhibition (Takemoto et al. 1997, Usui et al. 1999). Furthermore, hypertension and cardiovascular remodelling induced by NOS blockade have been suggested to be in part due to an elevation of plasma aldosterone concentration secondary to increased Ang II type 1 receptor expression in the adrenal gland (Usui et al. 1998). However, since the role of decreased NO production in human hypertension is unclear, it remains to be established whether NOS inhibition is an appropriate model of hypertension.

3 Renal failure

The patients with renal failure are characterised by abnormal elastic properties of large arteries, reflected as decreased distensibility and compliance (Barenbrock et al. 1994, London et al. 1996). These changes are independent of the level of blood pressure and tensile stress but are related to the uremic state itself (Luik et al. 1997, Mourad et al. 1997). Furthermore, in patients with end-stage renal disease, carotid arterial stiffness is a strong independent predictor of mortality (Blacher et al. 1998). In experimental renal failure, the increase in arterial wall thickness has been suggested to result primarily from an increase in extracellular matrix, although smooth muscle cell hyperplasia may also be involved (Amann et al. 1997). Plasma ET levels are elevated in patients with renal failure (Warrens et al. 1990), which is suggested to underlie the associated vascular remodelling (Demuth et al. 1998). However, in addition to the structural changes, alterations in arterial function contribute to increased vascular stiffness (Mourad et al. 1997). The functional changes have been attributed to the impaired NO-mediated endothelium-dependent vasodilatation as observed in brachial arteries of hemodialysis patients (Joannides et al. 1997, van Guldener et al. 1997). In the arteries of reduced renal mass hypertensive rats, not only the endothelium-dependent relaxations mediated by NO, but also those evoked by EDHF, have been attenuated (Kimura and Nishio 1999). Nevertheless, unaltered reactivity to endothelium-dependent vasodilators has also been observed (Verbeke et al. 1994, Liu et al. 1997). The increased oxygen-derived free radical activity and reduced enzymatic antioxidant defence mechanisms have been suggested to result, in part, from the altered arterial function and hypertension in renal failure (Durak et al. 1994, Vaziri et al. 1998b). Nevertheless, increased plasma levels of SOD and catalase have also been observed in renal failure (Martin-Mateo et al. 1998). It is noteworthy that endothelial dysfunction may also contribute to the development and progression of renal failure, and because endothelial dysfunction is detected at an early phase in the process of renal injury, it appears to be an attractive target for therapy (Rabelink and Koomans 1997).

In recent years several reports have discussed the role of NO synthesis and its inhibition in the development and progression of renal failure. The NO synthesis can be inhibited by analogues of arginine, including endogenous ADMA (Vallance et al. 1992). ADMA is present in normal human plasma, but it accumulates in renal failure (Vallance et al. 1992, Kielstein et al. 1999), suggesting changes in biosynthesis or excretion (Marescau et al. 1997). In chronic renal failure, circulating concentrations of ADMA are thought to rise sufficiently to inhibit NO synthesis, the inhibition of which might contribute to the changes in arterial function and to the hypertension associated with chronic renal failure (Vallance et al. 1992, MacAllister et al. 1996, Kielstein et al. 1999). However, the clinical role of ADMA is still questionable (Anderstam et al. 1997). Moreover, dietary L-arginine supplementation has been suggested to increase NO generation and enhance vasodilatation (Peters and Noble 1996), and to prevent the progression of glomerular sclerosis by ameliorating glomerular capillary hypertension in experimental models of kidney disease (Katoh et al. 1994). Nevertheless, elevated or unaltered plasma levels of L-arginine have been observed in uremic patients even without dietary supplementation (Noris et al. 1993, Kielstein et al. 1999), and both increased and decreased basal NO production have been reported in the vasculature of rats with reduced renal mass (Aiello et al. 1997, Vaziri et al. 1998b). The enhanced endothelial NOS expression and the larger amount of NO formed in arteries of reduced renal mass rats can serve as a defence mechanism to limit systemic blood pressure elevation in experimental renal failure (Aiello et al. 1997).

III Arterial tone and cardiovascular structure following inhibition of the renin-angiotensin system

The ideal antihypertensive drug should be effective in lowering blood pressure, well tolerated, safe in the long term and easy to use. Most importantly, it should reduce the risk of the adverse effects of high blood pressure, such as myocardial infarction, heart failure, stroke, and renal damage (Kendall 1998). One of the most important issues in the field of hypertension research centres on the therapeutic use of inhibitors of the RAS (Blaine et al. 1998). One reason for the interest in treating hypertension with drugs that not only reduce blood pressure but also have an inhibitory effect on the RAS is that hypertensive patients with inappropriately high renin levels have an increased risk of cardiovascular events (Weber 1997). Blockade of the RAS has proved to be a powerful therapeutic tool for lowering blood pressure and improving kidney function in disorders such as hypertension and chronic renal disease (Hall et al. 1999). Moreover, inhibitors of the RAS have potent antihypertensive effects, even in experimental models of hypertension and in essential hypertension, where the activity of the peripheral RAS is low or normal (Blaine et al. 1998).

1 Angiotensin converting enzyme inhibition

One possible intervention to interrupt the deleterious effects of the RAS is suppression of Ang II formation by the inhibition of ACE. The first ACE inhibitor was introduced more than twenty years ago (Nicholls et al. 1994). Since then, ACE inhibitors have been widely used and an enormous number of studies concerning their cardiovascular effects have been published. ACE inhibitors have proved to be effective in the treatment of essential hypertension, and in various models of experimental hypertension including genetic, L-NAME, reduced renal mass, and two-kidney-one-clip hypertension (Bao et al. 1992, Kanagy and Fink 1993, Novosel et al. 1994, Takase et al. 1996, Brown and Vaughan 1998). Long-term treatment with ACE inhibitors lowers blood pressure even in normotensive WKY rats (Bunkenburg et al. 1991, Kähönen et al. 1995b), but not in normotensive humans (Mancini et al. 1996). Furthermore, age, gender or plasma renin activity do not influence the antihypertensive response to ACE inhibition in humans (Weir and Saunders 1998).

The inhibition of Ang II formation by ACE inhibitors is far from complete, and the reduction of blood pressure by ACE inhibitors is better correlated with the inhibition of local ACE activity in tissues than with its activity in the plasma (Stock et al. 1995). ACE inhibitors also decrease the degradation of bradykinin, prolonging and potentiating its effects in endothelial cells (Auch-Schwelk et al. 1993, Auch-Schwelk et al. 1995, Hornig et al. 1997, Kohno et al. 1999). The modulation of the interaction between bradykinin and its receptor has been suggested (Hecker et al. 1994), and the ACE inhibitor ramiprilat has been shown to interfere with the sequestration of the B_2 kinin receptor within the plasma membrane of porcine aortic endothelial cells (Benzing et al. 1999). Bradykinin may also contribute to the acute effects of ACE inhibition on blood pressure in hypertensive humans, because coadministration of a specific B_2-receptor antagonist, icatibant, significantly attenuated the antihypertensive effect of captopril (Gainer et al. 1998). However, kinins do not appear to have a significant role in the chronic blood pressure-lowering effect of ACE inhibitors in SHR or in aortic coarctation hypertensive mice, since B_2 kinin receptor blockade or absence of the B_2 receptor gene have no effect on the antihypertensive action of long-term ACE inhibition (Bao et al. 1992, Gohlke et al. 1994, Cachofeiro et al. 1995, Rizzoni et al. 1998b, Rhaleb et al. 1999).

Both COX and NOS inhibition attenuate the long-term blood pressure-lowering effect of ramipril in SHR and of enalapril in patients with essential hypertension (Salvetti et al. 1987, Cachofeiro et al. 1995, Dijkhorst-Oei et al. 1998). Long-term lisinopril treatment also increases plasma levels of NO and 6-keto $PGF_1\alpha$, a stable metabolite of PGI_2, in hypertensive patients (Kohno et al. 1999). Furthermore, Ang II blockade diminishes the production of the superoxide anion, an inactivator of NO (De Artinano and Gonzalez 1999). Therefore, endogenous PGs and NO may be involved in the antihypertensive effect of ACE inhibitors. In addition, plasma catecholamine concentrations and cardiac sympathetic nervous activity have been shown to decrease in essential hypertensive patients responsive to ACE inhibitor therapy

(Prats et al. 1996, Sakata et al. 1998). Moreover, in cultured endothelial cells and cardiac myocytes ACE inhibitors can upregulate β-adrenoceptors (Graf et al. 1993, Yonemochi et al. 1998). The chronic antihypertensive action of ACE inhibitors may thus be partly mediated through the normalisation of sympathetic hyperreactivity and the restoration of β-adrenergic signalling pathway sensitivity (K-Laflamme et al. 1997). Furthermore, the inhibition of ACE leads to the accumulation of Ang I and its vasoactive metabolite Ang-(1-7) (Yamamoto et al. 1992, Kohara et al. 1993), which may also contribute to the effects of ACE inhibitors, since Ang-(1-7) has been found to release NO and vasodilatory PGs from the endothelium (Jaiswal et al. 1992, Pörsti et al. 1994, Brosnihan et al. 1996), inhibit endothelial ACE activity (Li et al. 1997b), and potentiate the effects of bradykinin (Ferrario and Iyer 1998, Oliveira et al. 1999). Ang-(1-7) has also been suggested to exhibit opposite effects on the regulation of VSMC growth than Ang II (Freeman et al. 1996).

Numerous studies have shown that long-term ACE inhibitor treatment improves endothelium-dependent vasodilatation in rats with experimental hypertension (Novosel et al. 1994, Bennett et al. 1996, Henrion et al. 1997, Sharifi et al. 1998), and in patients with essential hypertension (Schiffrin and Deng 1995, Rizzoni et al. 1997, Millgard et al. 1998, Taddei et al 1998b, Prasad et al. 1999). However, the improvement has not been detected in all studies (Creager and Roddy 1994, Bijlstra et al. 1995, Kiowski et al. 1996). It is noteworthy that chronic treatment with perindopril, quinapril or enalapril improved endothelium-mediated vasorelaxation in SHR, whereas no effect was found with hydralazine or amlodipine despite equal lowering of blood pressure (Bennett et al. 1996, Onaka et al. 1998). Moreover, ACE inhibition with quinapril, which did not reduce blood pressure, has been reported to improve endothelial dysfunction in normotensive patients with coronary artery disease (Mancini et al. 1996). An increase in NO production has been suggested to underlie the improvement in the endothelium-dependent relaxation by ACE inhibitors in both experimental and essential hypertension (Wiemer et al. 1997, Higashi et al. 1998). However, in the mesenteric arteries of SHR, the ACE inhibitor enalapril especially seems to improve EDHF-mediated hyperpolarization (Onaka et al. 1998). Thus, alterations in the EDHF system may play a pivotal role in the improvement of endothelial dysfunction with ACE inhibitor therapy (Onaka et al. 1998). Collectively, the above results suggest that ACE inhibitors may have beneficial effects on endothelial function which are independent of the reduction of blood pressure.

Long-term ACE inhibition has also been reported to modulate arterial responses to different vasoconstrictors in SHR. The majority of the studies have shown that contractions to NA are reduced after ACE inhibitor therapy (Lee et al. 1991, Arvola et al. 1993, Major et al. 1993, Kähönen et al. 1995b, Bennett et al. 1996), though some workers have failed to observe this effect (Dohi et al. 1994, Novosel et al. 1994). Contractile responses to another receptor-mediated agonist 5-HT have also been found to be either attenuated (Major et al. 1993, Berkenboom et al. 1995, Keaton et al. 1998) or unaffected (Tschudi et al. 1994, Hutri-Kähönen et al. 1997). In contrast, ACE inhibitor-treated SHR have shown no changes in

vasoconstriction induced by ET-1 (Dohi et al. 1994, Novosel et al. 1994, Rodrigo et al. 1997) or depolarization with KCl (Sada et al. 1990, Major et al. 1993, Dohi et al. 1994, Berkenboom et al. 1995). However, an enhancement of the response to ET-1 was reported in the blood vessels of patients with essential hypertension (Schiffrin and Deng 1995). In addition to the withdrawal of Ang II-induced amplification of vasoconstriction (Story and Ziogas 1987, Henrion et al. 1992, Qiu et al. 1994), the mechanisms underlying the attenuation of the contractile responses after ACE inhibition may involve normalised function of L-type Ca^{2+} channels (Sada et al. 1990, Arvola et al. 1993), decreased smooth muscle cell membrane permeability to ions (Arvola et al. 1993), and improved modulatory action of the endothelium on vasoconstriction (Kähönen et al. 1995b).

Arterial wall thickness and stiffness appear to be increased during hypertension and renal failure, which may contribute to the increased risk of cardiovascular complications. In essential hypertension and in animal models of genetic hypertension, the resistance vessels are considered to undergo inward eutrophic remodelling, e.g. rearrangement of the normal amount of vascular tissue (normal-sized smooth muscle cells, extracellular matrix) around a smaller lumen diameter (Mulvany 1999; Figure 3). On the other hand, in human renal hypertension, an inward hypertrophic response has been reported (Rizzoni et al. 1996). Reduced radial artery wall hypertrophy and improved carotid artery compliance, and decreased media to lumen ratio in the subcutaneous small resistance arteries have been observed in hypertensive patients treated with ACE inhibitors (Rizzoni et al. 1997, Girerd et al. 1998). Reduced media to lumen ratio has also been found in mesenteric resistance arteries of SHR after ACE inhibitor treatment (Rizzoni et al. 1998b). The correction of hypertensive structural remodelling by ACE inhibitors has been suggested to be either outward hypotrophic or eutrophic (Rizzoni et al. 1998c, Mulvany 1999). The structural matrix changes in the arterial wall after ACE inhibition may include decreased collagen accumulation and reduced collagen/elastin ratio (Benetos et al. 1997, Sharifi et al. 1998, Intengan et al. 1999). ACE inhibitors have also been suggested to enhance apoptosis in the vascular wall in SHR (Sharifi and Schiffrin 1998). Taken together, long-term treatment with ACE inhibitors seems to be effective in correcting vascular structural alterations in both SHR and patients with essential hypertension.

ACE inhibitors are also effective in normalising cardiac mass in SHR (Rizzoni et al. 1998b, Rizzoni et al. 1998c, Sharifi et al. 1998), and in patients with essential hypertension (Gonzalez-Juanatey et al. 1998). They appear to limit the hypertension-induced remodelling of both myocytes and interstitial tissues, with a subsequent reduction in myocardial passive stiffness (Grimm et al. 1998, Matsubara et al. 1998). Furthermore, ACE inhibitors protect against renal damage in experimental and essential hypertension (Akuzawa et al. 1998, Linz et al. 1998, Shionoiri et al. 1998). This renoprotective effect has been attributed to the reduction in Ang II formation (Lafayette et al. 1992). In addition, the beneficial effect of ACE inhibitors on cardiovascular structure and renal function in hypertension may be due to the effect of these drugs on the proliferation/cell death balance (Buemi et al. 1999).
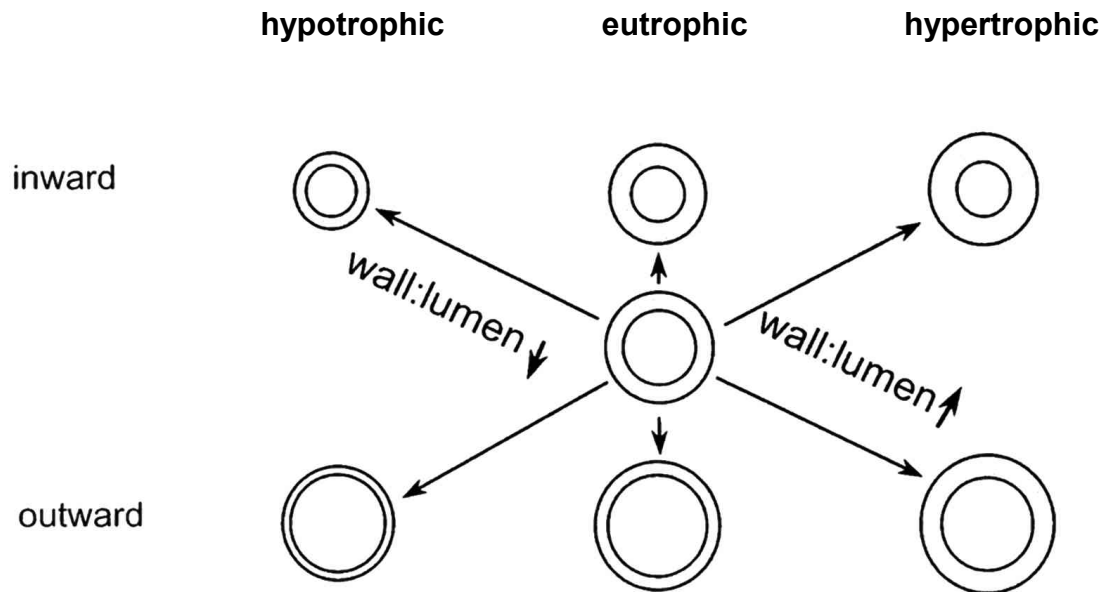
hypotrophic **eutrophic** **hypertrophic**

inward

outward

wall:lumen

wall:lumen

Figure 3. The figure shows the manner in which remodelling can modify the cross-sections of blood vessels. The starting point is the vessel at the centre. Remodelling can be hypertrophic (e.g. increase of cross-sectional area, vessels in right column), eutrophic (no change in cross-sectional area, vessels in centre column), or hypotrophic (e.g. decrease of cross-sectional area, vessels in left column). These forms of remodelling can be inward (i.e. reduction in lumen diameter, vessels in top row), or outward (i.e. increase in lumen diameter, vessels in bottom row. Along a diagonal axis from top left to bottom right the media:lumen ratio of vessels does not change. Upward and to the right of the axis, the media:lumen ratio increases. Below and to the left of the axis, the media:lumen ratio decreases.

2 Angiotensin II receptor antagonism

AT_1 receptors are believed to mediate the known deleterious effects of Ang II. Therefore, AT_1 receptor antagonists have been introduced to the therapy of hypertension. Losartan, the prototype of this class of drugs, was synthesised in 1990 (Nicholls et al. 1994). Since then, numerous novel AT_1 receptor antagonists have been developed, and the literature about the cardiovascular effects of these compounds has expanded rapidly. These drugs induce a dose-dependent blockade of Ang II effects, resulting in reduced blood pressure, decreased urinary protein excretion, and prevention of glomerular sclerosis (Unger et al. 1998). The blood pressure-lowering effect has been observed in patients with essential hypertension and in various forms of experimental hypertension, including genetic, L-NAME and reduced renal mass hypertension (Pollock et al. 1993, Timmermans and Smith 1996). Similarly to ACE inhibition, long-term AT_1 receptor antagonism reduces blood pressure in normotensive WKY rats (Bunkenburg et al. 1991, Gillies et al. 1997, Medina et al. 1997), but not in normotensive humans (Kang et al. 1994). When compared with ACE inhibitors, the AT_1 receptor antagonists have been suggested to be more effective antihypertensive agents (Unger et al.

1996). However, in general, the antihypertensive effect of different AT_1 receptor antagonists has been comparable to that of different ACE inhibitors (Bunkenburg et al. 1991, Tschudi et al. 1994, Rodrigo et al. 1997).

The antihypertensive effect of AT_1 receptor antagonists has mainly been attributed to the inhibition of the binding of Ang II to AT_1 receptor. In addition, recent studies have pointed to the involvement of PGs and NO in the antihypertensive effect of losartan in SHR (Cachofeiro et al. 1995, Maeso et al. 1996, Gohlke et al. 1996). The mechanisms underlying the participation of PGs and NO in this effect have not been established, but losartan stimulates the synthesis of PGE_2 and PGI_2 in cultured endothelial cells (Jaiswal et al. 1991), and has antagonistic effect on the vascular TXA_2/PGH_2 receptor (Li et al. 1997c, Li et al. 1998). Furthermore, an interference with the negative feedback control of renin release by AT_1 receptor antagonists results in the accumulation of Ang II (Bunkenburg et al. 1991, Goldberg et al. 1993). This increases the stimulation of unopposed Ang II receptors, such as the AT_2 receptor (Chung et al. 1996), and the AT_2 receptor stimulation may increase the vascular production of bradykinin, NO and cGMP (Wiemer et al. 1993, Munzenmaier and Greene 1994, Seyedi et al. 1995, Gohlke et al. 1998). However, in mice AT_1 receptor blockade decreases tissue Ang II despite increased plasma levels, suggesting that Ang II may be protected from metabolisation by binding to its receptor (Mazzolai et al. 2000). During AT_1 receptor blockade the Ang I metabolite Ang-(1-7) can also release NO and vasodilatory PGs from the endothelium, an effect which is not mediated via AT_1 or AT_2 receptors (Jaiswal et al. 1992, Pörsti et al. 1994, Brosnihan et al. 1996).

AT_1 antagonists also inhibit Ang II-mediated catecholamine release from presynaptic sympathetic nerves and the adrenal medulla (Dendorfer et al. 1998). Moreover, the excitatory synaptic inputs to pressor neurons in the rostral ventrolateral medulla arising from the activation of the paraventricular nucleus are mediated predominantly by AT_1 receptors, the activation of which results in an increase in arterial pressure (Tagawa and Dampney 1999). The inhibition of these receptors may partly account for the blood pressure-lowering action of AT_1 receptor antagonists, since systemically administered irbesartan and losartan have access to central angiotensin receptors (Culman et al. 1999).

Long-term AT_1 receptor antagonism has been shown to beneficially influence arterial vasodilator function in experimental hypertension (Soltis 1993, Dohi et al. 1994, Tschudi et al. 1994, Vacher et al. 1996, Rodrigo et al. 1997), and when comparisons with ACE inhibitors have been made, the effects on vasorelaxation have been surprisingly similar (Dohi et al. 1994, Tschudi et al. 1994, Rodrigo et al. 1997). In addition to the reduced blood pressure, the enhanced endothelium-dependent vasodilatation following long-term AT_1 receptor antagonism has been attributed to increased endothelial production of NO (Dohi et al. 1994, Tschudi et al. 1994) and decreased production of EDCFs (Rodrigo et al. 1997).

The studies that have elucidated the effects of long-term AT1 receptor antagonism on vasoconstrictor responses in SHR have yielded inconsistent results. Vasoconstrictions elicited by 5-HT have either been reported to be unaffected (Tschudi et al. 1994), increased (Vacher et

al. 1996), or decreased (Soltis 1993) whereas the responses to KCl have either been unaffected (Dohi et al. 1994, Rodrigo et al. 1997) or increased (Vacher et al. 1996). NA-induced contractions have been found to be unaffected (Dohi et al. 1994, Gillies et al. 1997) or reduced (Soltis 1993), while those induced by ET have been reported to remain unchanged (Dohi et al. 1994, Rodrigo et al. 1997). Nevertheless, contractile responses to Ang II have always been found to be attenuated after AT_1 receptor antagonist therapy (Soltis 1993, Dohi et al. 1994, Rodrigo et al. 1997).

Chronic AT_1 receptor antagonist treatment, even at a dose that only moderately reduces blood pressure, induces regression of cardiovascular hypertrophy in hypertensive rats (Li et al. 1997a). It has been postulated that the selective blockade will directly decrease the growth-promoting actions of Ang II via the AT_1 receptor, while leaving the growth-inhibitory effects of the AT_2 receptor unaffected (Weber 1997, Unger et al. 1998). However, the blockade of AT_1 receptor-mediated effects of Ang II appears to be the main mechanism in the prevention of hypertensive cardiovascular remodelling (Mazzolai et al. 2000). Furthermore, losartan and irbesartan have normalising effects on stiffness, growth, and the collagen/elastin ratio of the mesenteric resistance artery wall in SHR (Benetos et al. 1997, Intengan et al. 1999). Losartan therapy also induces outward remodelling of mesenteric resistance arteries in SHR (Rizzoni et al. 1998c).

A reduction in left ventricular mass in hypertensive patients and in SHR has been observed after losartan therapy (Tedesco et al. 1998, Varo et al. 1999). The remodelling of cardiac tissue induced by AT_1 receptor antagonist treatment seems to be associated with a decrease in the synthesis of collagen and a subsequent reduction in tissue fibrosis (Varo et al. 1999). AT_1 receptor antagonists also exert clear renoprotective effects and have an antiproteinuric action in various models of experimental hypertension (Heller and Hellerova 1998, Kobayashi et al. 1999).

AIMS OF THE PRESENT STUDY

The objective of the present study was to examine arterial function and structure and cardiac natriuretic peptides in genetic hypertension, NO deficiency and renal failure. In addition, the effects of long-term inhibition of RAS on arteries and cardiac natriuretic peptides were evaluated in SHR and L-NAME-treated Wistar rats.

The detailed aims were:

1. To compare the effects of losartan and enalapril therapies, the two inhibitors of RAS with different mechanisms of action, on arterial relaxation in SHR.

2. To study the effects of losartan and enalapril treatments on mesenteric arterial structure, dihydropyridine-sensitivity, potassium relaxation and circulating sodium pump inhibitor in SHR.

3. To examine the influences of losartan treatment on the control of arterial tone in NO deficiency.

4. To investigate the effects of renal failure on the function of the vascular endothelium and arterial smooth muscle and on cardiac natriuretic peptides in WKY rats.

5. To compare the effects of AT_1 receptor antagonism and ACE inhibition on the regulation of ADM and natriuretic peptide gene expression in SHR and WKY rats.

6. To examine the effects of AT_1 receptor antagonism on cardiac natriuretic peptides in NO deficiency.

MATERIALS AND METHODS

1 Experimental animals

Normotensive Wistar rats were obtained from the colony of the Medical School at the University of Tampere (III, VI), whereas SHR (Okamoto-Aoki strain) and WKY rats were obtained from Møllegaard's Breeding Centre, Ejby, Denmark (I, II, V), and WKY rats also from M&B A/S, Ry, Denmark (IV). The rats were housed two (IV) or four (I, II, III, V, VI) to a cage in a standard animal laboratory room (temperature +22°C, a controlled environmental 12 h light-dark cycle). The studies were approved by the Animal Experimentation Committee of the University of Tampere, and by the Provincial Government of Western Finland, Department of Social Affairs and Health (IV).

2 Drug treatments

All the rats in study IV, and the untreated rats in studies I, II, III, V and VI were freely provided with tap water. Losartan (15 mg/kg/day) (I, II, V) and (20 mg/kg/day) (III, VI), enalapril (4 mg/kg/day) (I, II, V), and L-NAME (20 mg/kg/day) (III, VI) were administered in drinking fluid. The daily prepared drug solutions were kept in light-proof bottles. To obtain the desired daily drug dose, the concentration in drinking water was adjusted according to 24 h fluid consumption measurements.

3 Blood pressure measurements

The systolic blood pressures of the conscious rats restrained in plastic holders were measured indirectly by the tail cuff method at +28°C. All the measurements were performed with an IITC Inc. Model 129 Blood Pressure Meter (Woodland Hills, CA, USA) equipped with a photoelectric pulse detector. The blood pressure of each rat was obtained by averaging three reliable recordings.

4 Urine collection and measurement of fluid intake

Urine was collected for 24 h individually in metabolic cages where animals had free access to food and water (IV). Urine volumes were measured and samples stored at -20°C. The consumption of drinking fluid was measured by weighing the bottles in two consecutive mornings.

5 Blood and heart samples

The rats were anaesthetised by the intraperitoneal administration of urethane (1.3 g/kg) and

the carotid arteries cannulated. Blood samples were drawn into chilled tubes on ice containing 2.7 mM ethylenediaminetetraacetic acid (III, VI), and into tubes and glass capillaries containing heparin (IV), after which the samples were centrifuged, and the plasma stored at -70°C until analysis. After exsanguination, the thoracic and abdominal cavities of the animals were opened, the hearts removed and weighed. The tissue samples were frozen in liquid nitrogen and stored at -70°C until analyses.

6 Biochemical determinations

6.1 Total peroxyl radical-trapping and vitamin E (III)

Total peroxyl radical-trapping capacity was determined by the chemiluminescense method which has been described elsewhere (Alanko et al. 1993). Briefly, the production of peroxyl radicals by 2,2'-azobis(2-amidinopropane) hydrochloride induces luminol-enhanced chemiluminescence, and the time during which the added test sample extinguishes the reaction is directly proportional to its peroxyl radical-trapping antioxidant capacity. The test reaction was initiated by mixing 475 µl of oxygen-saturated sodium phosphate buffer (100 mM, pH 7.4) with 50 µl of 400 mM 2,2'-azobis (2-amidinopropane) hydrochloride (prepared in 100 mM phosphate-buffer) and 50 µl of 10 mM luminol in 20 mM boric acid-Borax-buffer in a cuvette (pH 9.0). The cuvette was placed in a luminometer (37°C), and the plasma sample (25 µl) was injected after the luminescence had stabilised, whereafter the chemiluminescence was measured at 40 second intervals. The water-soluble tocopherol was used as a standard. Vitamin E concentration was determined by modified high performance liquid chromatography (HPLC) (Catignani and Bieri 1983), and in our modification the UV detection of antioxidants was replaced by an LC-4 amperometric detector (Bioanalytical Systems Inc. West Lafayette, USA).

6.2 Nitrite and nitrate (IV)

To measure nitrite and nitrate (NOx) concentrations in plasma and urine, vanadium chloride in HCl was used to convert nitrite and nitrate to NO, which was quantitated by the ozone-chemiluminescence method (Braman and Hendrix 1989). The samples were first treated with ethanol at -20°C for two hours to precipitate proteins. Then a 20 µl sample was injected into a cylinder containing saturated VCl_3 solution (0.8 g VCl_3 per 100 ml of 1 M HCl) at 95°C, and NO formed under these reducing conditions was measured by the NOA 280 analyser (Sievers Instruments Inc., Boulder, CO, USA) using sodium nitrate as the standard.

6.3 Sodium, potassium, urea nitrogen, phosphate, creatinine, calcium and haemoglobin (IV)

Plasma sodium and potassium concentrations were measured by potentiometric direct dry

chemistry, urea nitrogen by colorimetric enzymatic dry chemistry, and phosphate by colorimetric end-point dry chemistry (Vitros 950 analyzer, Johnson & Johnson Clinical Diagnostics, Rochester, NY, USA). Creatinine was determined by the kinetic colorimetric assay according to Jaffe (Cobas Integra analyzer, F. Hoffman-La Roche Ltd, Diagnostics Division, Basel, Switzerland). Ionised calcium was measured by an ion selective electrode (Ciba Corning 634 Ca^{2+}/pH Analyzer, Ciba Corning Diagnostics, Sudbury, UK). Haemoglobin was determined by photometric analysis using Technicon cyanide free haemoglobin reagent (Technicon H*2™, Technicon Instruments Corporation, Tarrytown, NY, USA).

6.4 Digoxin-like immunoreactivity (II)

Plasma digoxin-like immunoreactivity was determined by ELISA of C18 (Bond Elut, Varian, Harbor City, CA, USA) extracts of plasma. The ELISA employed a digoxin antibody with cross-reactivity characteristics which have been reported previously (Doris 1992). The antibody was raised in rabbits against digoxin coupled via reductive amination after periodate oxidation to bovine serum albumin. ELISA plates were coated with digoxin coupled to ovalbumin. The assay was incubated at room temperature for 2 h. Unbound antibody was removed by washing. Peroxidase-coupled goat-anti-rabbit gammaglobulin was added to each well. After a further 30 min incubation the plates were rinsed and TMB reagent (Kirkegaard and Perry, Gaithersburg, MD, USA) added as a colour indicator of peroxidase activity. Assays were read at end-point after the addition of phosphoric acid to stop the peroxidase reaction. Standard curves were fitted to a 4-parameter logistic model and unknown values interpolated using Delta-Soft software.

6.5 Isolation and analysis of cytoplasmic RNA (V, VI)

RNA was isolated from ventricular and atrial samples by the guanidine thiocyanate-CsCl method (Chirgwin et al. 1979). For the RNA Northern blot and dot blot analysis, 3 μg samples of the RNA from atria and 22 μg (V) or 20 μg (VI) from ventricles were transferred to the Schleicher & Schuell BAS 85 nitrocellulose membrane (V) or to the Hybond N+ nylon membrane (VI) (Amersham Pharmacia Biotech). A 390 bp fragment of rat BNP cDNA probe (Ogawa et al. 1991), full-length rat ANP cDNA probe (Flynn et al. 1985), full-length cDNA probe complementary to glyceraldehyde 3-phosphate-dehydrogenase (Fort et al. 1985), an oligonucleotide probe complementary to rat 18S ribosomal RNA, and cDNA probe for ADM made by RT-PCR (Romppanen et al. 1997) were labelled with [^{32}P]-dCTP with Quick Prime Kit (Pharmacia LKB Biotechnology, Uppsala, Sweden). The membranes were hybridised overnight at +42°C in 5 x SSC (saline sodium citrate, 1 x SSC = 0.15 mol/l NaCl, 0.015 mol/l trisodium citrate, pH 7), 0.5 % sodium dodecyl sulphate, 5 x Denhardt's solution, 50 % formamide and 100 μg/ml sheared herring sperm DNA. After hybridisation, the membrane

was washed in 0.1 x SSC, 0.1 % sodium dodecyl sulphate three times for 20 min at +50°C (V) or +60°C (VI) and exposed to Phosphor Screen (Molecular Dynamics, Sunnyvale, USA) at room temperature. Phosphor Screens were scanned with Phosphor Imager (Molecular Dynamics, Sunnyvale, USA). The hybridisation signal of ANP, BNP and ADM mRNA was normalised to that of glyceraldehyde 3-phosphate-dehydrogenase mRNA (V) and that of ANP and BNP mRNA to 18S (VI) for each sample to correct for potential differences in loading and/or transfer. The samples in the renal failure experiments were treated similarly as samples in study VI.

6.6 Radioimmunoassays (V, VI)

Tissue and plasma samples were extracted by Sep-Pak C_{18} cartridges as previously described (Kinnunen et al. 1993, Magga et al. 1994). Eluates were lyophilised and redissolved in radioimmunoassay buffer. Plasma immunoreactive (ir-) NT-proANP and atrial ir-ANP were determinated by radioimmunoassay without prior extraction. For the BNP radioimmunoassay, the atrial and ventricular guanidine thiocyanate extracts were diluted 200- (VI) or 100-fold (V) and 50-fold, respectively. For the ANP radioimmunoassay, the atrial and ventricular guanidine thiocyanate extracts were diluted 5×10^4-fold and 50- (VI) or 400-fold (V), respectively. The extracted samples were incubated in duplicate with the specific rabbit BNP (Kinnunen et al. 1993) or ANP antiserum (Vuolteenaho et al. 1985). Synthetic rat BNP_{51-95}, synthetic rat ANP_{99-126}, and synthetic human pro- ANP_{79-98} were incubated as standards. The BNP, ANP and NT-proANP tracers were prepared by chloramine-T iodination of synthetic rat $[Tyr_0]$-BNP_{51-95}, rat ANP_{99-126} and human $[Tyr_0]$-human pro- ANP_{79-98} followed by reverse phase HPLC purification. After incubation for 48 h at +4°C, the ^{125}I -labelled rat $[Tyr_0]$- BNP_{51-95} and rat ANP_{99-126} with normal rabbit serum were added (VI). After incubation for another 24 h (VI) or for 48 h (V) at +4°C the immunocomplexes were precipitated with sheep antiserum directed against rabbit gammaglobulin in the presence of 8 % polyethylene glycol 6000, pH 7, followed by centrifugation at 3000 g for 30 min. The plasma samples were incubated with the rabbit NT-proANP antiserum and ^{125}I -labeled $[Tyr_0]$-human pro- ANP_{79-98} overnight at +4°C and the bound and free fractions were separated with double antibody in the presence of polyethylene glycol. The sensitivities of the BNP, ANP and NT-ANP assays were 0.5 (VI) or 2 fmol/tube (V), 1 fmol (V) or 2 pg/tube (VI), and 0.75 fmol/tube, respectively. 50 % displacements of the respective standard curve occurred at 16 (V) or 34.5 (VI) fmol/tube for ANP, at 4.3 (VI) or 25 fmol/tube (V) for BNP and at 0.5 fmol/tube for NT-ANP. The intra- and interassay variations were less than 15 %. Serial dilutions of tissue extracts showed parallelism with the standards. The ANP antiserum recognised ANP and proANP with equal avidity but did not cross-react with BNP or CNP. The BNP antiserum did not recognise ANP or CNP.

For the ADM radioimmunoassay, guanidine thiocyanate supernatants were diluted 100-fold with 0.1 % trifluoroacetic acid and extracted with Sep-Pak C_{18} cartridges (Magga et al.

1994). The extracts were dried and redissolved in radioimmunoassay buffer. Synthetic rat ADM-(1-50) standards (Phoenix Pharmaceuticals Inc., Mountain View, CA, USA) and the tissue extracts were incubated 16-24 h at +4°C with rabbit anti-rat ADM serum. ^{125}I-rat ADM-(1-50) was added and the incubation was continued for another 16-24 h. The free and bound fractions were separated by double antibody precipitation. The rat ADM antiserum (Phoenix Pharmaceuticals) does not cross-react with rat ADM-(1-20), human amylin or ET-1. The sensitivity of the ADM assay was 1 fmol/tube and the intra- and interassay coefficients of variation were <10 % and <15 %, respectively. The samples in the renal failure experiments were treated similarly as samples in study VI.

7 Mesenteric arterial responses *in vitro*

7.1 Arterial preparations and organ bath solutions

The superior mesenteric arteries were carefully cleaned of adherent connective tissue, excised, and placed on a Petri dish containing physiological salt solution (PSS) (pH 7.4) of the following composition (mM): NaCl 119.0, NaHCO$_3$ 25.0, glucose 11.1, KCl 4.7, CaCl$_2$ 1.6, KH$_2$PO$_4$ 1.2 and MgSO$_4$ 1.2, and aerated with 95 % O$_2$ and 5 % CO$_2$. Standard sections of the mesenteric artery (3 mm in length) were cut, beginning 5 mm distally from the mesenteric artery-aorta junction. The endothelium was either left intact or removed by gently rubbing it with a jagged injection needle (Arvola et al. 1992). The rings were placed between stainless steel hooks (diameter 0.3 mm) and mounted in an organ bath chamber (volume 20 ml) in PSS described above. The preparations were aerated with 95 % O$_2$ and 5 % CO$_2$ at +37°C, and rinsed with fresh solutions at least every 20 min, during which time the pH in the baths remained stable. In solutions containing high concentrations of K$^+$ (20-125 mM), NaCl was replaced with KCl on an equimolar basis. In Ca^{2+}-free solutions, CaCl$_2$ was omitted without substitution. K$^+$-free solutions were prepared by replacing KH$_2$PO$_4$ and KCl with NaH$_2$PO$_4$ and NaCl, respectively, on an equimolar basis.

7.2 Arterial contractile and relaxation responses

The rings were initially equilibrated for 1 h at +37°C with a resting force of 1.5 g. The force of contraction was measured with an isometric force-displacement transducer and registered on a polygraph (FT 03 transducer and Model 7 E Polygraph; Grass Instrument Co., Quincy, MA, USA). The presence of the functional endothelium in vascular preparations was confirmed by a clear relaxation response to 1 μM ACh in 1 μM NA-precontracted arterial rings, and the absence of endothelium by the lack of this response. If any relaxation was observed in the endothelium-denuded rings, the endothelium was further rubbed.

Agonist-induced contractions. The contractions of the endothelium-intact preparations to NA were studied in the absence (I, IV) and presence of L-NAME (0.1 mM) (III, IV), and in

the presence of diclofenac (3 μM) plus L-NAME (I, III, IV). In study IV, the contractions to NA were also elicited in the presence of L-arginine (1 mM). The contractions elicited by 5-HT were investigated in the endothelium-intact preparations in the absence and presence of diclofenac, and in the presence of diclofenac plus L-NAME (II).

Depolarization-induced contractions. The concentration-response curves of the endothelium-denuded rings to KCl were determined in the absence (I, II, IV) and presence (II) of phentolamine (1 μM) and atenolol (10 μM). In study III, the responses to KCl were performed in the presence of L-NAME.

Ca^{2+} *contractions*. The contractile responses of the endothelium-denuded rings to cumulative addition of Ca^{2+} to the organ bath after precontraction with KCl (125 mM) in Ca^{2+}-free buffer in the absence (III) and presence (II, IV) of phentolamine (1 μM) and atenolol (10 μM) were studied. Thereafter, the effects of nifedipine (0.5 nM) (II, III, IV), and nifedipine plus mibefradil (0.05 μM) (III) on these responses were examined. In study III, these responses were performed in the presence of L-NAME.

Endothelium-dependent relaxations to ACh. Mesenteric arterial relaxations were studied in response to ACh in rings precontracted with NA (1 μM) (I, IV). The ACh-induced relaxations after NA-precontraction were also elicited in the presence of diclofenac (I); L-NAME (III, IV); diclofenac and L-NAME (I, III, IV); diclofenac, L-NAME and TEA (1 mM) (I); diclofenac, L-NAME, and apamin (50 nM) plus charybdotoxin (0.1 μM) (IV). The responses to ACh were further studied in the presence of SOD (50 U/ml) (IV); L-NAME plus SOD (III); SOD plus catalase (100 U/ml) (IV); and in the presence of L-NAME, SOD plus catalase (III). The ACh-induced relaxations were also examined in the presence of L-arginine (IV). Moreover, the relaxations to ACh were investigated in rings precontracted with KCl (50 mM) in the absence (I) and presence (I, III) of diclofenac plus L-NAME.

Endothelium-independent relaxations to sodium nitroprusside (SNP), isoprenaline and cromakalim. The relaxation responses of NA-precontracted endothelium-denuded (I, III, IV) rings to SNP were examined. The responses to SNP were also studied in KCl-precontracted endothelium-denuded preparations (I, III) and in NA-precontracted rings in the presence of TEA (I). The vasorelaxations elicited by isoprenaline and cromakalim were studied in endothelium-denuded rings precontracted with NA (I, III, IV) and with KCl (I).

Potassium relaxation. The relaxations of K^+-free solution-contracted endothelium-denuded rings to the return of K^+ (1 mM) were determined in the absence and presence of 1 mM ouabain (II).

8 Morphological studies

In studies III and VI, and in unpublished experiments with the renal failure rats, the third order mesenteric arterial branches were chosen from the vascular bed that feeds the small intestine 2-6 cm prior to the ileocecal junction. A segment (2 mm in length) of the mesenteric artery was isolated under a dissection microscope (Nikon SMZ-2T, Nikon Inc., Japan) and

transferred to the myograph chamber (Living Systems Instrumentation Inc., Burlington, VT, USA) containing 8 ml of PSS (pH 7.4) of the following composition (mM): NaCl 119.0, NaHCO$_3$ 25.0, glucose 11.1, CaCl$_2$ 2.5, KCl 4.7, KH$_2$PO$_4$ 1.2, MgSO$_4$ 1.2, Na$_2$EDTA 0.4. The proximal end of the vessel was cannulated with a micropipette and flushed to remove the remaining blood before the cannulation of the distal end. At the beginning of the study, the arterial wall thickness and lumen diameter of the unpressurised vessels were recorded by the use of a video monitoring system (Video dimension analyzer, Living Systems Instrumentation Inc., Burlington, VT, USA). The artery was superfused at a rate of 10 ml/min and aerated with 95 % O$_2$ and 5 % CO$_2$. Thereafter the intraluminal pressure was slowly raised to 60 mmHg (III) and to 100 mmHg (VI and renal failure experiments). The pressure in the proximal end of the artery segment was monitored by a pressure transducer and controlled by a servo perfusion system (Pressure servo control, Living Systems Instrumentation Inc., Burlington, VT, USA). After the vessel had equilibrated for 40 min the arterial dimensions were recorded.

From five animals in each group in study II, vascular rings were prepared for light microscopy from the most proximal part of the remaining section of each superior mesenteric artery. The rings were fixed in 2 % glutaraldehyde at +4°C and postfixed in 2 % osmiumtetroxide. After washing, they were stained with 1 % uranyl acetate and dehydrated with acetone series. Thereafter the samples were embedded in Epon (LX-112 Resin, Ladd, Burlington, VT, USA). Thin (2 μM) transverse sections were stained with 1 % toluidine blue, examined, and photographed under light microscopy (Nikon Microphot-FXA, Japan). In each vascular ring, the diameter of lumen and thickness of medial smooth muscle were measured from the photographs.

9 Compounds

The following drugs and chemicals were used: acetylcholine chloride, apamin, catalase, charybdotoxin, cromakalim, 5-hydroxytryptamine, isoprenaline hydrochloride, noradrenaline bitartrate, N^G-nitro-L-arginine methyl ester hydrochloride, superoxide dismutase, ouabain, tetraethylammonium chloride (Sigma Chemical Co., St. Louis, MO, USA), phentolamine, diclofenac (Voltaren$^®$ injection solution, Ciba-Geigy AG, Basel, Switzerland), atenolol (Leiras Pharmaceutical, Turku, Finland), enalapril maleate, losartan potassium (Merck Pharmaceutical Company, Wilmington, DE, USA), ethylenediaminetetraacetic acid, noradrenaline hydrogentartrate, sodium nitroprusside (Fluka Chemie AG, Buchs SG, Switzerland), diazepam, nifedipine (Orion Pharma Ltd., Espoo, Finland), ketamine (Parke-Davis Scandinavia, Solna, Sweden), and buprenorphine (Reckitt and Colman, Hull, UK). The stock solutions of the compounds used in the *in vitro* studies were made by dissolving the compounds in distilled water, with the exception of ouabain (directly in physiological salt solution), and cromakalim and nifedipine (in 50 % ethanol). Drinking fluids containing losartan, enalapril and N^G-nitro-L-arginine methyl ester hydrochloride were made by dissolving the compounds in tap water. All solutions were freshly prepared before use and

protected from light. The chemicals used in the preparation of PSS were of the highest grade available and obtained from E. Merck AG (Darmstadt, Germany).

10 Analysis of results

The statistical analysis was performed using a one-way analysis of variance (ANOVA) supported by Bonferroni test when carrying out pairwise comparisons between the study groups. For comparison of statistical significance between two test groups in natriuretic peptide and ADM studies Student's *t* test was used. ANOVA for repeated measurements was applied for data consisting of repeated observations at successive time points. All the results are expressed as mean $\pm$ SEM. The data were analysed with BMDP Statistical Software version PC90 (Los Angeles, CA, USA).

Table 2. Summary of the experimental design of the studies on arterial reactivity and cardiac natriuretic peptides.

Study	Rats Treatment	E+ relaxations (precontraction)	E- relaxations (precontraction)	Contractions	Natriuretic peptides
Genetic hypertension					

I	SHR and WKY rats
	
	Losartan
	Enalapril
		ACh (NA)
		 + diclofenac
		 + L-NAME
		 + TEA
		ACh (KCl)
		 + diclofenac and
		  L-NAME
			Isoprenaline
			(NA, KCl)
			Cromakalim
			(NA, KCl)
			Nitroprusside (KCl)
			Nitroprusside (NA)
			 + TEA
				NA
				 + diclofenac and
				  L-NAME
				KCl	

II			K$^+$ (K$^+$-free) + ouabain	KCl + phentolamine and atenolol Calcium + nifedipine K$^+$-free + ouabain 5-HT + diclofenac + L-NAME	
V					ANP, BNP, ADM
Nitric oxide deficiency					

III	L-NAME
	hypertensive
	Wistar rats
	
	Losartan
		ACh (NA)
		 + L-NAME and
		  diclofenac
		 + (KCl)
		ACh (NA)
		 + SOD
		 + catalase
			Isoprenaline (NA)
			Cromakalim (NA)
			Nitroprusside
			(NA and KCl)
				KCl
				Calcium
				 + nifedipine
				 + mibefradil
				NA
				 + L-NAME and
				  diclofenac	

VI					ANP, BNP
Renal failure					
IV	5/6 nephrectomized WKY rats	ACh (NA) + L-NAME + diclofenac + apamin and charybdotoxin ACh (NA) + SOD + catalase ACh (NA) + L-arginine	Nitroprusside (NA) Isoprenaline (NA) Cromakalim (NA)	NA + L-NAME + diclofenac KCl Calcium + nifedipine	
					ANP, BNP, ADM

ACh, acetylcholine; ADM, adrenomedullin; ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; E+, endothelium-dependent; E-, endothelium-independent; 5-HT, 5-hydroxytryptamine; L-NAME, N^G-nitro-L-arginine methyl ester; NA, noradrenaline; SHR, spontaneously hypertensive rats; SOD, superoxide dismutase; TEA, tetraethylammonium; WKY, Wistar-Kyoto.

RESULTS

1 Blood pressure, arterial morphology, heart weight, drinking fluid and urine volumes

Blood pressure. The systolic blood pressures of untreated SHR and WKY rats were 234 and 143 mmHg, respectively, when measured at the end of the follow up periods (I, II, V). The long-term administration of L-NAME resulted in the elevation of blood pressure up to 198 mmHg in Wistar rats (III, VI). Both losartan and enalapril completely prevented the development of hypertension in SHR, but had no effect on blood pressure in WKY rats (I, II, V). Losartan treatment also prevented the development L-NAME induced hypertension (III, VI). The chronic renal failure in WKY rats was not associated with the elevation of blood pressure (IV).

Arterial morphology. Chronic L-NAME hypertension resulted in an increase in small mesenteric arterial wall to lumen ratio, but losartan treatment was without an effect on it (III, VI). An increased media to lumen ratio was also observed in mesenteric arteries of untreated SHR when compared with WKY rats (II). However, in SHR both losartan and enalapril treatments effectively corrected the observed arterial remodelling (II). The chronic renal failure was not associated with alterations in mesenteric arterial morphology (Unpublished observation, Table 3).

Table 3. Morphological characteristics of mesenteric arteries at 100 mmHg.

	Control (n=8)	Renal failure (n=7)
Wall thickness (μm)	28±2	29±2
External diameter (μm)	398±12	375±10
Wall to lumen ratio	0.08±0.01	0.09±0.01
Wall cross-sectional area (μm^2)	33288±2524	32248±2789

Values are mean ± SEM.

Heart weight. Genetic hypertension was associated with clear cardiac hypertrophy (I, II, V). Losartan and enalapril therapies completely prevented the development of cardiac hypertrophy in SHR, and reduced the relative heart weights also in WKY rats (I, II, V). In contrast, the heart-body weight ratios were comparable in L-NAME hypertensive and normotensive Wistar rats, whereas losartan therapy reduced relative heart weights in both of these groups (III, VI). Furthermore, the chronic renal failure did not change heart weights in WKY rats (IV).

Drinking fluid and urine volumes. At the end of the study, the intake of drinking fluid and the output of urine were higher in the rats with chronic renal failure when compared with the control WKY rats (IV).

2 Total peroxyl radical-trapping, vitamin E, sodium, potassium, urea nitrogen, phosphate, creatinine, calcium, haemoglobin, nitrite and nitrate, and digoxin-like immunoreactivity

L-NAME hypertension or losartan treatment did not influence the plasma total peroxyl radical-trapping capacities or vitamin E concentrations (III). In rats with chronic renal failure, the plasma creatinine and urea nitrogen values were increased, while plasma sodium, haemoglobin and calcium concentrations were decreased when compared with control rats (IV). Renal failure did not have any influence on plasma potassium, phosphate, pH or NOx (IV). Plasma digoxin-like immunoreactivity did not differ between untreated SHR and WKY rats, and neither losartan nor enalapril treatment did affect plasma digoxin-like immunoreactivity in SHR or WKY rats (II).

3 Control of arterial tone *in vitro*

3.1 Arterial tone in genetic hypertension and the influences of long-term angiotensin receptor antagonism and angiotensin converting enzyme inhibition (I, II)

3.1.1 Arterial contractile responses

Maximal tissue dry weight-related contractile force generation to NA in the absence and presence of L-NAME and diclofenac was attenuated in endothelium-intact rings of untreated SHR when compared with WKY, although the sensitivity to NA was comparable in these groups (I). In contrast, the maximal contractions to NA in losartan- and enalapril-treated SHR (both in the absence and presence of L-NAME and diclofenac) did not differ from those of WKY (I). There were no significant differences in the sensitivity to NA between untreated SHR and losartan- and enalapril-treated SHR, while in the presence of diclofenac and L-NAME, the sensitivity was somewhat lower in enalapril-treated SHR when compared with the untreated controls (I). Furthermore, enalapril-treated SHR showed lower sensitivity to NA than losartan-treated SHR both in the absence and presence of L-NAME and diclofenac (I). Maximal contractile force generation of endothelium-denuded arterial rings to KCl was comparable in all SHR and WKY groups, and untreated SHR showed increased sensitivity to KCl when compared with all WKY groups (I). Losartan, but not enalapril, further diminished the sensitivity to KCl in WKY, while in SHR the treatments were without effect on the sensitivity to KCl (I).

Differing from study I, the arterial contractile responses in study II were expressed as maximal wall tensions in mN/mm instead of expressing forces as g/mg. Maximal wall tension and sensitivity of endothelium-denuded arterial rings to KCl were increased in untreated SHR when compared with WKY rats (II). Losartan and enalapril treatments decreased maximal wall tension to KCl in SHR, but not in WKY rats (II). Furthermore, neither of the treatments

affected the sensitivity to KCl in SHR, while losartan, but not enalapril, diminished the sensitivity to KCl in WKY rats (II). When the responses to KCl were induced in the presence of phentolamine and atenolol, no differences in maximal wall tension or sensitivity were found between SHR and WKY rats (II).

The endothelium-intact vascular rings of untreated SHR showed comparable maximal wall tension and sensitivity in response to 5-HT in the absence and presence of diclofenac and L-NAME when compared with WKY rats (II). Neither losartan nor enalapril treatment significantly affected the maximal wall tension elicited by 5-HT when compared with untreated controls, while the response was higher in enalapril- than in losartan-treated SHR, and this difference was also observed in the presence of L-NAME and diclofenac (II). Losartan, but not enalapril, slightly increased the sensitivity to 5-HT in SHR (II). Diclofenac elicited a more pronounced decrease in sensitivity to 5-HT in losartan-treated SHR than in untreated and enalapril-treated SHR, while L-NAME comparably increased the sensitivity to 5-HT in all groups (II).

In the absence of the dihydropyridine Ca^{2+} entry blocker nifedipine, the contractile sensitivity of isolated mesenteric arterial rings induced by cumulative addition of Ca^{2+} did not differ between SHR and WKY rats, and losartan and enalapril were without effect on these responses (II). However, in the presence of nifedipine, the responses were clearly less effectively inhibited in losartan- and enalapril-treated SHR and WKY rats than in untreated SHR (II). Thus, the contractile response induced by the addition of Ca^{2+} showed higher dependency on dihydropyridine-sensitive Ca^{2+} entry in untreated SHR (II). The maximal wall tension induced by Ca^{2+} cumulation was higher in untreated SHR when compared with losartan- and enalapril-treated SHR and untreated WKY rats, and this difference was also abolished by nifedipine (II). Furthermore, maximal wall tension elicited by K^{+}-free solution in the absence and presence of ouabain did not differ in untreated SHR and WKY rats, and losartan and enalapril were without effect on these responses (II).

3.1.2 Arterial relaxation responses

Endothelium-independent relaxations. The relaxations of NA-precontracted endothelium-denuded mesenteric arterial rings to SNP were attenuated in SHR when compared with WKY rats (I). The responses to SNP remained decreased in SHR in the presence of K_{Ca} blocker TEA (I). However, when the arterial rings were precontracted with KCl the relaxations induced by SNP were comparable to those of WKY rats (I). The vasodilatations of NA-precontracted rings to isoprenaline and cromakalim were also attenuated in SHR when compared with WKY rats (I). When the precontractions were elicited by KCl, the responses to isoprenaline and cromakalim were completely abolished in SHR and WKY rats (I).

Losartan and enalapril therapies improved the relaxations of NA-precontracted rings to SNP in SHR (I). However, the addition of TEA to the organ bath as well as KCl-induced precontractions abolished this improvement in losartan- and enalapril-treated rats (I). The

vasodilatations induced by isoprenaline and cromakalim were also improved in losartan- and enalapril-treated SHR (I) (see table 4). Furthermore, in the normotensive WKY rats losartan or enalapril treatments were without effect on endothelium-independent relaxation (I).

After the return of potassium to the organ bath upon the K^+-free precontractions the rate of the subsequent relaxation was faster in WKY rats than in SHR (II). Furthermore, both losartan and enalapril enhanced the potassium relaxation in SHR (II) (see table 4). This relaxation was effectively inhibited by the Na^+,K^+-ATPase inhibitor ouabain in all groups, although a significant difference was still detected between untreated SHR and WKY rats (II). The calculated ouabain-sensitive part of the potassium relaxation was initially (during the first 4 min after K^+ repletion) lower in untreated SHR when compared with WKY rats (II). Furthermore, both losartan and enalapril treatments especially enhanced the ouabain-sensitive part of the potassium relaxation in SHR (II).

Endothelium-dependent relaxations. The relaxations induced by ACh in the absence and presence of diclofenac were clearly attenuated in NA-precontracted mesenteric arterial rings of SHR when compared with those of WKY rats (I). The addition of L-NAME to the organ bath practically abolished the relaxation to ACh in SHR, whereas distinct relaxations were observed in WKY rats (I). The addition of TEA further reduced the diclofenac and L-NAME-resistant relaxations to ACh in WKY rats, but the relaxations in WKY rats still remained more pronounced than those in SHR (I). When the relaxations to ACh were elicited in KCl-precontracted rings, no differences were observed between SHR and WKY rats (I). In addition, none of the relaxations induced by ACh in losartan- and enalapril-treated SHR differed from those in control WKY rats (I) (see table 4). Furthermore, losartan and enalapril treatments did not affect endothelium-dependent relaxations in WKY rats.

3.2 Arterial tone in L-NAME hypertension and the influence of long-term angiotensin receptor antagonism (III)

3.2.1 Arterial contractile responses

The maximal contractile force generation and contractile sensitivity in response to NA in the presence of L-NAME was comparable in L-NAME hypertensive and control rats. Losartan treatment decreased the maximal contractile force generation to NA in control rats, but not in L-NAME treated rats. In Wistar rats, L-NAME hypertension or losartan treatment did not alter the maximal contractile force generation to KCl. However, losartan treatment increased the contractile sensitivity to KCl in L-NAME treated rats.

The contractile sensitivity of arterial smooth muscle to the cumulative addition of Ca^{2+} during depolarization with KCl was similar in L-NAME treated rats as in control rats and losartan treatment did not affect this response. Moreover, the L-type Ca^{2+} entry blocker nifedipine equally decreased arterial contractile sensitivity to Ca^{2+} in the control and L-NAME treated rats, and the effect of nifedipine was smaller in losartan treated L-NAME rats. In

contrast, the T-type Ca^{2+} entry blocker mibefradil, when added after nifedipine, reduced the Ca^{2+} sensitivity of arterial smooth muscle more effectively in the L-NAME hypertensive rats than in control rats. Losartan treatment was without effect on the inhibitory effect of mibefradil on the Ca^{2+} sensitivity in either group.

3.2.2 Arterial relaxation responses

Endothelium-independent relaxations. The relaxations of endothelium-denuded NA-precontracted rings to SNP, isoprenaline and cromakalim, were impaired in L-NAME hypertensive rats when compared with normotensive control rats. In addition, when hyperpolarization of smooth muscle was prevented by precontractions with KCl, the relaxations to SNP were still impaired in L-NAME rats. Furthermore, in L-NAME treated rats, but not in control rats, the vasodilatations to SNP, isoprenaline and cromakalim were clearly improved by the losartan treatment (see table 4).

Endothelium-dependent relaxations. The relaxations induced by ACh in NA-precontracted arterial rings in the presence of L-NAME were markedly impaired in the L-NAME treated rats when compared with the control rats, while these responses were clearly improved by losartan treatment in both of these groups (see table 4). The addition of diclofenac to the organ bath improved the relaxations to ACh in all other groups, but not in the losartan treated normotensive group, and abolished the difference between the control and losartan treated groups, while the relaxations still remained impaired in the L-NAME treated group when compared with the other groups. On the other hand, diclofenac caused a 5.2-fold increase in the maximum relaxation to ACh in the L-NAME hypertensive group when compared with the 1.6-fold change in the losartan treated L-NAME group. The responses to ACh were almost abolished in all groups when induced in KCl-precontracted rings in the presence of L-NAME and diclofenac.

When SOD was added to the organ bath, the relaxations to ACh were enhanced in all other groups, but not in the losartan-treated normotensive group, while the responses to ACh remained impaired in the L-NAME treated group when compared with the other groups (see table 4). However, the addition of SOD caused a 3.4-fold increase in the maximum response to ACh in the L-NAME group when compared with the 1.5-fold change in the losartan-treated L-NAME group. In addition, SOD abolished the difference in response to ACh between the control rats and losartan-treated control rats. The further addition of catalase had no significant effects on the relaxation to ACh in any of the study groups.

3.3 Effects of renal failure on the control of arterial tone (IV)

3.3.1 Arterial contractile responses

Vasoconstrictor responses. The chronic renal failure did not modulate arterial contractile

sensitivity to NA or KCl. The maximal contractions to NA in the absence and presence of L-NAME were also comparable between renal failure and control rats. However, in the presence of L-NAME and diclofenac the maximal contractile force generation induced by NA was higher in the renal failure rats, and the maximal contractions to KCl were also more pronounced in the renal failure rats when compared with the control rats.

3.3.2 Arterial relaxation responses

Endothelium-independent relaxations. The chronic renal failure did not alter arterial relaxations to SNP. However, the relaxations to isoprenaline and cromakalim were impaired in rats with chronic renal failure when compared with the control rats (see table 4).

Endothelium-dependent relaxations. The relaxations induced by higher concentrations of ACh (1-10 µM) in NA-precontracted arterial rings were impaired in the renal failure rats when compared with the control rats. L-NAME diminished the relaxations in both study groups, but the attenuation was more pronounced in the renal failure group than in the control group. Diclofenac was without significant effects on ACh-induced relaxations in both groups. The addition of apamin and charybdotoxin further reduced the relaxations to ACh in the control rats but not in the renal failure rats, and in that way the difference between the groups in the remaining relaxation to ACh was abolished (see table 4).

4 Regulation of adrenomedullin and natriuretic peptides

4.1 Cardiac synthesis of adrenomedullin in losartan- and enalapril-treated spontaneously hypertensive and Wistar-Kyoto rats (V)

The concentrations of ADM mRNA and ir-ADM in the left ventricle and atrium were similar in untreated SHR and WKY rats. Losartan or enalapril treatments had no effect on ADM mRNA or ir-ADM levels in the left ventricle in either strain. However, in SHR both losartan and enalapril increased left atrial concentration of ir-ADM. Furthermore, an increase in left atrial ADM mRNA levels was noted in enalapril-treated SHR and in losartan-treated WKY rats when compared to their respective control groups (V).

4.2 Cardiac synthesis of atrial natriuretic peptide and B-type natriuretic peptide in losartan- and enalapril-treated spontaneously hypertensive and Wistar-Kyoto rats (V)

The left ventricular concentrations of ANP mRNA and ir-ANP were similar in untreated SHR and WKY rats. In WKY rats, losartan treatment reduced left ventricular levels of ANP mRNA and ir-ANP. Similar decreases in levels of ANP mRNA and ir-ANP were noted in enalapril-treated WKY rats. In SHR, both losartan and enalapril treatments reduced levels of ANP mRNA. This reduction in left ventricular ANP mRNA levels was greater in the losartan-

treated SHR than in the WKY rats. Furthermore, left ventricular ir-ANP levels were lower in losartan- and enalapril-treated SHR than in the untreated SHR.

The concentration of ir-ANP in the left atrium was lower in SHR than in WKY rats. In SHR, losartan, but not enalapril, increased left atrial ir-ANP concentration. Left atrial ir-ANP concentrations remained unchanged in WKY rats in response to losartan and enalapril treatments. Furthermore, the left atrial levels of ANP mRNA were similar in SHR and WKY rats, and drug-treatments had no effects on left atrial ANP mRNA levels in either strain.

The left ventricular BNP mRNA level was higher in SHR than in WKY rats, whereas the ir-BNP concentration in the left ventricle was lower in SHR than in WKY rats. Losartan and enalapril treatments decreased significantly the BNP mRNA concentration in SHR, but had no effect on left ventricular BNP mRNA levels in WKY rats. Furthermore, the decrease in left ventricular BNP mRNA levels was more pronounced in losartan-treated SHR than in enalapril-treated SHR. Overall, the changes in left ventricular concentrations of ir-BNP during drug treatments were small when compared to those induced by losartan and enalapril in left ventricular ir-ANP concentrations. A slight decrease in the concentration of ir-BNP in the left ventricles was noted in both losartan- and enalapril-treated WKY rats, while the left ventricular ir-BNP concentration was higher in enalapril-treated SHR than in untreated SHR.

The left atrial ir-BNP concentration was higher in SHR than in WKY rats, whereas there was no difference in left atrial BNP mRNA levels between the strains. Losartan and enalapril decreased consistently left atrial ir-BNP levels in both strains, but had no effect on left atrial BNP mRNA levels. In WKY rats, losartan and enalapril treatments reduced left atrial ir-BNP concentration when compared with untreated WKY rats. Similar decreases in levels of ir-BNP during losartan and enalapril treatments were noted in SHR.

4.3 Effect of losartan on cardiac and plasma natriuretic peptide levels in L-NAME-treated rats (VI)

To characterise the role of Ang II in the NO deficiency-induced ANP gene activation, the effect of losartan on L-NAME-stimulated changes in ANP mRNA and ir-ANP levels were studied. Ventricular ANP mRNA and ir-ANP levels increased by L-NAME treatment. Losartan treatment attenuated the increase in ANP gene expression induced by L-NAME, but did not affect the ir-ANP levels in the ventricles. Losartan treatment decreased ventricular ir-ANP concentration in control rats. L-NAME hypertensive rats showed decreased ir-ANP concentrations in left and right atria when compared with the control group. These changes in atrial ir-ANP concentrations were prevented by concomitant losartan therapy. Furthermore, the left and right atrial ANP mRNA levels were not affected by L-NAME or losartan.

L-NAME hypertensive rats showed an increase in ventricular BNP mRNA and ir-BNP levels when compared with the control rats. In losartan treated L-NAME rats, ventricular BNP mRNA levels did not differ from control, although losartan therapy did not prevent the increase of ir-BNP levels induced by L-NAME. However, losartan decreased ventricular

ir-BNP concentrations in control rats. No changes in the right or left atrial BNP mRNA levels were observed. In the left atria, L-NAME treatment decreased ir-BNP concentrations when compared with the control rats.

Losartan treatment decreased plasma ir-NT-proANP concentrations in both control and L-NAME rats. In L-NAME rats, plasma ir-BNP concentrations were increased when compared with the control rats, the increase of which was prevented by concomitant losartan treatment.

4.4 Synthesis of natriuretic peptides and adrenomedullin in experimental renal failure

Plasma concentrations of NT-ANP were increased in rats with renal failure when compared with the control rats. Ventricular ANP and BNP mRNA levels were also increased in rats with renal failure. Furthermore, renal failure rats showed increased ventricular ir-BNP concentrations when compared with control rats. However, the ir-ANP concentrations in the ventricles were not significantly changed in experimental renal failure (Unpublished observation, Figure 4). Moreover, ANP mRNA levels in both right and left atrium and ir-ANP levels in right atrium were comparable in control and renal failure rats. However, ir-ANP levels in left atrium were increased in rats with renal failure. The concentrations of ir-ADM in ventricles and atria were comparable in renal failure and control rats. However, the ventricular levels of ADM mRNA were increased in renal failure rats. Furthermore, ADM mRNA levels in left, but not in right, atrium were decreased in rats with renal failure when compared with control rats (Unpublished observation, Figure 5).
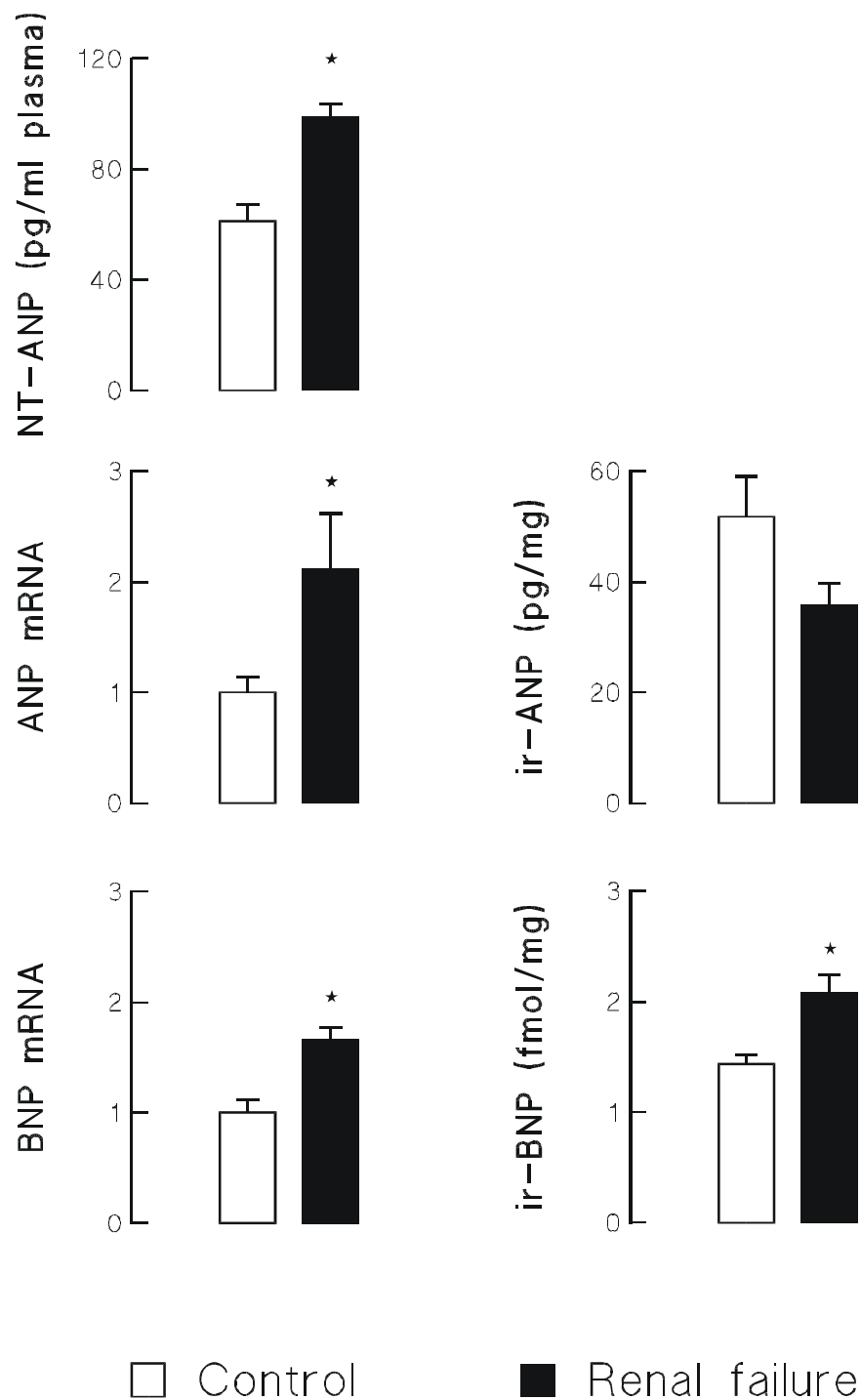
Figure 4. Plasma levels of NT- atrial natriuretic peptide (ANP), and ventricular levels of ANP mRNA, B-type natriuretic peptide (BNP) mRNA, immunoreactive (ir)-ANP, and ir-BNP in Control and Renal failure rats. The ANP and BNP mRNA results are expressed as the ratio of ANP mRNA to 18S ribosomal mRNA, and BNP mRNA to 18S ribosomal mRNA, respectively, as determined by northern blot analysis. Data represent mean ± SEM, $n = 7$ in Renal failure and 12 in Control group; *$P<0.05$ versus Control group (Student's t-test).
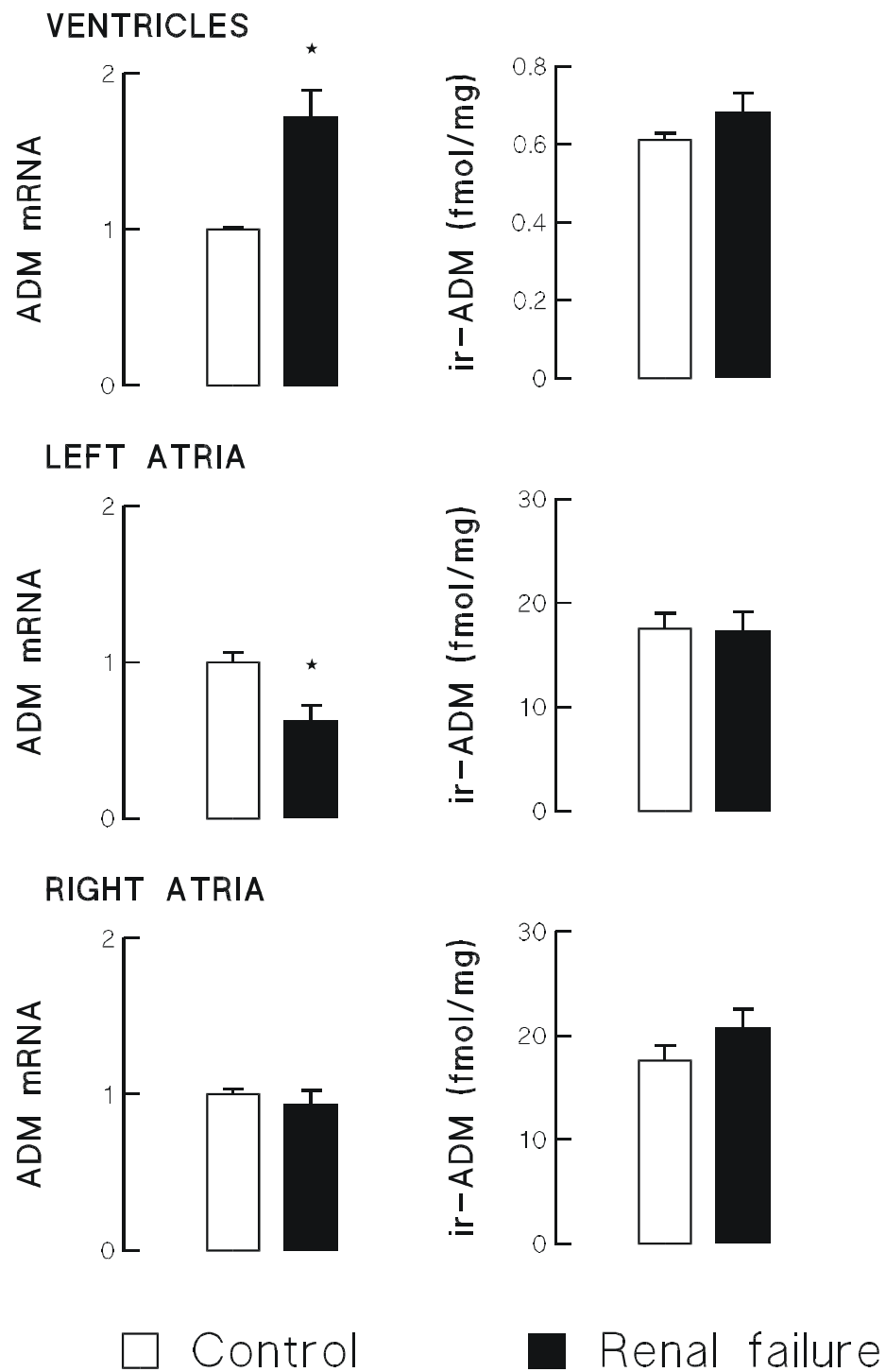
Figure 5. The levels of adrenomedullin (ADM) mRNA and immunoreactive (ir)-ADM in ventricles and atria in Control and Renal failure rats. The mRNA results are expressed as the ratio of ADM mRNA to 18S ribosomal mRNA as determined by northern blot analysis. Data represent mean ± SEM, $n = 7$ in Renal failure and 12 in Control group; *$P<0.05$ versus Control group (Student's t-test).

Table 4. Summary of the alterations in arterial relaxations in hypertensive rats after losartan and enalapril treatments when compared with untreated hypertensive controls, and in renal failure rats when compared with sham-operated controls.

Variable	Genetic hypertension Losartan	Enalapril	Nitric oxide deficiency Losartan	Renal failure
E+ relaxations (precontraction)				
Acetylcholine (NA)	↑	↑		↓
+ L-NAME			↑	↓
+ diclofenac	↑	↑		
+ L-NAME and diclofenac	↑	↑	↑	↓
+ L-NAME, diclofenac, tetraethylammonium	↑	↑		
+ L-NAME, diclofenac, apamin and charybdotoxin				↔
+ superoxide dismutase			↑	↓
Acetylcholine (KCl)	↔	↔		
+ diclofenac and L-NAME	↔	↔	↔	
E- relaxations (precontraction)				
Nitroprusside (NA)	↑	↑	↑	↔
+ tetraethylammonium	↔	↔		
Nitroprusside (KCl)	↔	↔	↑	
Isoprenaline (NA)	↑	↑	↑	↓
Isoprenaline (KCl)	↔	↔		
Cromakalim (NA)	↑	↑	↑	↓
K^+ (K^+-free)	↑	↑		
+ ouabain	↔	↔		

E+, endothelium-dependent; E-, endothelium-independent; L-NAME, N^G-nitro-L-arginine methyl ester; NA, noradrenaline. ↑, ↓ and ↔ indicate an increase, reduction and no change when compared with the corresponding control group, respectively.

DISCUSSION

The present investigation examined the effects of long-term AT_1 receptor antagonism and ACE inhibition on arterial responses and on cardiac natriuretic peptide and ADM gene expression in SHR, and the effects of long-term AT_1 receptor antagonism on arterial function and on cardiac natriuretic peptide production in L-NAME hypertension. In addition, the influence of chronic renal failure on the control of arterial tone and on cardiac natriuretic peptide and ADM synthesis in WKY rats was evaluated.

1 Experimental models of the study

SHR is the most commonly studied animal model of human essential hypertension often with WKY as the normotensive control (Okamoto and Aoki 1963). Although SHR and WKY rats have been derived from the same colony, the amount of genetic variation between these strains is nowadays comparable to the maximum divergence possible between unrelated humans (Johnson et al. 1992). Therefore, some of the differences observed between SHR and WKY rats are likely to be unrelated to hypertension (St. Lezin et al. 1992). The present study showed that treatment with either losartan or enalapril completely prevented the development of hypertension in SHR. The result agrees with previous investigations which have stressed the importance of the RAS in the pathogenesis of high blood pressure in this model of genetic hypertension (Oddie et al. 1993, Schoemaker et al. 1994). The other model of experimental hypertension in this study was chronic inhibition of NOS (Baylis et al. 1992). NO deficiency is an interesting model of hypertension, since the endothelial production of NO is essential for the maintenance of normal blood pressure (Huang et al. 1995), and several disease states including essential hypertension have been associated with defects in the production or action of NO (Moncada and Higgs 1993). In this study, in agreement with previous experiments, oral administration of L-NAME resulted in a marked hypertension, which reached its maximum within four weeks, whereas losartan therapy totally prevented the elevation of blood pressure (Ribeiro et al. 1992, Jover et al. 1993). The third experimental model in this study was 5/6 nephrectomy in rats. The WKY rat strain is known to be resistant to the elevation of blood pressure (Bidani et al. 1990), and it was chosen in order to avoid the development of hypertension after the induction of renal failure and the subsequent changes in vascular function caused by elevated blood pressure. Accordingly, the moderate renal failure was not associated with the development of hypertension in WKY rats in this study.

2 Cardiovascular remodelling in experimental hypertension and renal failure

Cardiac hypertrophy is the primary chronic compensatory mechanism to increased haemodynamic overload in hypertension, and it was also observed in SHR in this study. In agreement with previous findings, losartan and enalapril therapies prevented cardiac

hypertrophy in SHR and decreased heart weights also in WKY rats (Mizuno et al. 1992, Oddie et al. 1993, Soltis 1993). Hypertension induced by chronic L-NAME administration would also be expected to induce cardiac hypertrophy. However, the heart weight-body weight ratios did not differ between L-NAME hypertensive and normotensive control rats. This surprising finding agrees with recent reports, and it is probably explained by the negative metabolic effects of L-NAME on protein synthesis (Arnal et al. 1993, Bartunek et al. 2000). Despite the absence of cardiac hypertrophy in the L-NAME rats, losartan therapy reduced heart weights in both L-NAME-treated and normotensive control rats. The influence of losartan on cardiac weight can be explained by the blockade of the AT_1 receptor-mediated growth-promoting actions of Ang II (Mazzolai et al. 2000).

A characteristic morphological change in the resistance arteries of patients with essential hypertension and SHR is inward eutrophic remodelling, i.e. increased wall to lumen ratio with unaltered media cross-sectional area (Mulvany 1999), which was also seen in SHR in this study. In concert with previous observations, both losartan and enalapril therapies decreased the media to lumen ratio, but were without significant effect on the media cross-sectional area in the mesenteric artery of SHR (Rizzoni et al. 1998c). Therefore, the changes in vascular structure induced by losartan and enalapril are compatible with outward eutrophic remodelling of arteries. Chronic L-NAME hypertension induced inward hypertrophic remodelling of the mesenteric resistance arteries in Wistar rats, i.e. increased wall to lumen ratio and media cross-sectional area. Losartan therapy did not reduce arterial hypertrophy in the L-NAME rats even though it normalised their blood pressure. Therefore, vascular hypertrophy in this model of hypertension appears to be independent of blood pressure, and may result from decreased vascular NO production, since a defect in the NOS/NO pathway has been suggested to promote abnormal remodelling and to facilitate pathological changes in vessel wall morphology associated with hypertension (Rudic et al. 1998). However, it is noteworthy that NOS inhibitors cannot completely block the production of endothelium-derived NO (Simonsen et al. 1999, Ge et al. 2000).

The patients with renal failure are characterized by abnormal elastic properties of large arteries, reflected as decreased distensibility and compliance (Barenbrock et al. 1994, London et al. 1996). The increased stiffness of the arteries has even been seen in the absence of structural changes (Mourad et al. 1997). Consistent with this, the experimental renal failure in our study was not associated with morphological changes of the mesenteric arteries. Moreover, no changes in cardiac weight were observed in rats with renal failure when compared with control rats.

3 Arterial contractions in experimental hypertension and the influences of long-term antihypertensive treatments

Various approaches can be applied to investigate the vasoconstrictor responses. The arterial contractile forces can be related to segment length, segment weight, media cross sectional

area, or lumen diameter, and thus the findings depend on the experimental setting which has been applied (Mulvany et al. 1991, Arvola et al. 1993, Bennett et al. 1996). Different approaches to report arterial contractions were also applied in separate original communications of this study. In study I, the contractile forces were related to artery segment dry weight (g/mg), in studies II and III the contractions were expressed as the actual forces that were recorded (g), and in study IV the contractile forces were related to the arterial segment length and expressed as wall tensions (mN/mm). Therefore, the numerical values obtained from the separate studies cannot be compared as such. Furthermore, in the present literature there is no complete consensus regarding the method of the expression of arterial contractile forces.

In the present study, losartan and enalapril treatments normalised the maximal contractile force generated by NA in arterial preparations from SHR. The NA-induced vasoconstrictor responses have also previously been beneficially influenced by chronic ACE inhibition in SHR (Bennett et al. 1996). The maximal contractile force to KCl was comparable in SHR and WKY rats, and neither losartan nor enalapril affected the responses to KCl in SHR. In agreement with this finding, maximal contractions to KCl have been found to remain unaffected by both Ang II antagonist and ACE inhibitor treatment in the aorta of SHR (Rodrigo et al. 1997). However, when maximal contractions were expressed as wall tensions, the arterial preparations from SHR showed increased contractile force generation when compared with WKY rats. Both losartan and enalapril treatments normalised the increased maximal wall tensions to KCl in SHR. Thus, the present results suggest that both losartan and enalapril therapies restored the impaired receptor-mediated contractions to NA and normalised the increased depolarization-mediated contractions to KCl in the mesenteric artery of SHR.

Some differences in vasoconstrictor sensitivity following the present treatments were observed. In the presence of diclofenac and L-NAME the sensitivity to NA was somewhat lower in enalapril-treated SHR when compared with the untreated controls. Furthermore, enalapril-treated SHR showed lower sensitivity to NA than losartan-treated SHR both in the absence and presence of L-NAME and diclofenac. However, the individual effects of diclofenac and L-NAME on NA-induced contractions were not studied and cannot be estimated. Moreover, losartan, but not enalapril, increased the sensitivity to 5-HT in SHR, and diclofenac elicited a more pronounced decrease in sensitivity to 5-HT in both losartan-treated groups than in the other groups, suggesting that the products of the COX pathway differentially modulated the responses to 5-HT in the study groups. On the other hand, L-NAME induced a corresponding shift in constrictor sensitivity to 5-HT in all groups, which suggests that endothelium-derived NO similarly modulated arterial constrictor responses in all study groups. Moreover, the contractile sensitivity to KCl was higher in SHR when compared with WKY, but neither of the therapies affected the responses to KCl in SHR. Taken together, since the long-term antihypertensive therapies in the present study somewhat differently modulated the sensitivity to NA and 5-HT in SHR, other factors in addition to the reduction of blood pressure and inhibition of Ang II may have contributed to these effects, one candidate

being the potentiation of the actions of bradykinin following the enalapril treatment (Minshall et al. 1997, Benzing et al. 1999).

The arteries of SHR are more sensitive to the actions of dihydropyridine Ca^{2+} channel blockers than those of WKY rats (Arvola et al. 1992, Kähönen et al. 1996), which was also confirmed in the present study. Moreover, the proportions of voltage-dependent Ca^{2+} currents are different in blood vessels of hypertensive and normotensive rats: in SHR the L-type current is greater whereas in WKY rats the T-type predominates (Rusch and Hermsmeyer 1988). Accordingly, nifedipine less effectively inhibited the Ca^{2+}-induced contractions in losartan- and enalapril-treated SHR and WKY rats than in untreated SHR in the present study. Thus, losartan and enalapril treatments normalised the function of voltage-dependent Ca^{2+} channels in the vascular smooth muscle of SHR. The present study also confirmed the earlier finding whereby L-NAME hypertension does not affect vasoconstrictor responses to NA (Küng et al. 1995). The constrictor sensitivity of arterial smooth muscle to cumulative addition of Ca^{2+} was also comparable in L-NAME hypertensive and control rats, and the response remained unaffected by losartan. The L-NAME rats did not show any increased sensitivity to the action of nifedipine, while the T-type Ca^{2+} entry blocker, mibefradil, elicited a more pronounced shift in the Ca^{2+} sensitivity of arterial rings from L-NAME rats, which suggests increased Ca^{2+} influx via T-type channels in this model of experimental hypertension.

4 Arterial relaxations in experimental hypertension and the influences of long-term antihypertensive treatments

ACh relaxes arterial smooth muscle by releasing several dilatory factors from the vascular endothelium, the most prominent of these being NO, PGI_2 and EDHF (Busse and Fleming 1993). In the present study, the relaxations to ACh in NA-precontracted rings were attenuated in SHR, whereas these responses were completely normalised by losartan and enalapril, in accordance with previous findings (Tschudi et al. 1994). The relaxations of arterial rings to ACh were also impaired in the L-NAME hypertensive rats, and effectively improved by losartan therapy. The finding concerning the impaired ACh-induced relaxation in L-NAME hypertension is not new. However, the influence of losartan on arterial relaxations in L-NAME hypertension has not been previously reported, although ACE inhibitor trandolapril has improved ACh-induced relaxations in L-NAME hypertension (Küng et al. 1995). Since both losartan and enalapril treatments augmented endothelium-dependent relaxations, this beneficial effect on the vasculature was probably mediated via the reduction of blood pressure and inhibition of the actions of Ang II.

One explanation to the attenuated relaxations in hypertension is enhanced release of EDCFs (Lüscher and Vanhoutte 1986, Küng et al. 1995). Previously, endothelium-dependent vasoconstrictor responses have been shown to be blocked by COX inhibition in SHR (Takase et al. 1994). In the present study, the COX inhibitor, diclofenac, enhanced the relaxations to

ACh in SHR and in L-NAME hypertensive rats. This suggests that there is an imbalance in the production of vasoconstrictor and vasodilator prostanoids in the vessels of hypertensive rats, which favours vasoconstriction. Since diclofenac had no effect on the response to ACh in losartan- or enalapril-treated SHR, the present antihypertensive therapies appeared to correct this imbalance of the COX pathway in SHR. However, diclofenac enhanced the relaxations to ACh in losartan-treated L-NAME rats, although less than in untreated L-NAME hypertensive rats. Therefore, losartan appeared to improve the imbalance of the COX pathway also in L-NAME rats.

The chemical antagonism between superoxide and NO is recognised as an important modulator of vascular tone. In addition, superoxide has been reported to inhibit the vascular synthesis of PGI_2 without affecting that of the vasoconstrictor TXA_2 (Katusic 1996). Therefore, superoxide production or decreased antioxidant activity in the cardiovascular system could increase arterial tone and contribute to the development of hypertension. In the present study, the total peroxyl radical-trapping capacity and vitamin E concentration in plasma were comparable in L-NAME hypertensive rats and in control rats, suggesting that L-NAME hypertension is not associated with changes in circulating antioxidant capacity. In contrast, losartan treatment appeared to reduce the production of superoxide in the arteries of L-NAME rats, since the enhancing effect of SOD, the superoxide anion scavenger, on the relaxations to ACh was more pronounced in the L-NAME hypertensive rats when compared with losartan-treated L-NAME rats. Moreover, the difference in the relaxation induced by ACh between untreated and losartan-treated normotensive control rats was abolished by SOD, suggesting that losartan therapy reduced the production of superoxide also in control rats. The view of reduced superoxide production by losartan is in agreement with previous results whereby losartan treatment decreased the production of superoxide and normalised the ACh-induced arterial relaxations in Ang II hypertensive rats (Rajagopalan et al. 1996). It is noteworthy that decreased superoxide production may also have contributed to the enhanced endothelium-mediated vasodilatation after diclofenac administration, since COX is a significant source of superoxide (Katusic 1996).

ACE inhibition has been suggested to potentiate endothelium-dependent dilatation in the aorta of normotensive and hypertensive animals by enhancing the availability of NO (Berkenboom et al. 1995). In addition, Ang II antagonism and ACE inhibition have been found to comparably improve endothelial function in the aorta and coronary arteries of SHR, the underlying mechanism possibly being increased availability of NO (Tschudi et al. 1994, Rodrigo et al. 1997). In the present study, however, the mesenteric arteries of losartan- and enalapril-treated SHR and all WKY groups showed distinct NOS inhibitor-resistant relaxations to ACh, suggesting that endothelial products other than NO were mediating the enhanced endothelium-dependent vasodilatations.

Recent investigations have indicated that endothelium-mediated relaxations which remain resistant to both NOS and COX inhibitions are mediated by EDHF (Cohen and Vanhoutte 1995). The action of EDHF can be eliminated by K^+ channel blockers or by cell

membrane depolarization with high K^+ concentration (Adeagbo and Triggle 1993). In this study, losartan and enalapril treatments enhanced ACh-induced relaxations in NA-precontracted preparations in SHR, but these treatments did not alter ACh-induced relaxations in KCl-precontracted preparations. This suggests that the improvement of endothelium-dependent relaxation following long-term Ang II antagonism and ACE inhibition was largely mediated via hyperpolarization mechanisms. Furthermore, the NOS and COX inhibitor-resistant relaxations to ACh were attenuated in the L-NAME hypertensive rats when compared with the control rats, while the responses in the losartan-treated L-NAME rats did not differ from the normotensive control rats. Thus, losartan prevented the impairment of endothelium-dependent hyperpolarization in L-NAME-treated rats. The precontraction of arterial rings with KCl virtually abolished the remaining NOS and COX inhibitor-resistant relaxations to ACh, suggesting that these responses were mediated by EDHF. It is noteworthy that NO has been shown to inhibit the production and action of EDHF (Bauersachs et al. 1996, McCulloch et al. 1997). Therefore, in pathophysiological states like hypertension, which may be associated with decreased bioavailability of NO, EDHF-mediated vasorelaxation could be of greater importance than under normal conditions (Bauersachs et al. 1996). Nevertheless, decreased endothelium-dependent hyperpolarization has been reported in genetic, renal, and mineralocorticoid-NaCl forms of experimental hypertension (Van de Voorde et al. 1992, Fujii et al. 1992, Mäkynen et al. 1996), and the results of this study suggest that the same also holds true for L-NAME hypertension.

Impaired endothelium-dependent hyperpolarization could result from reduced sensitivity of smooth muscle to EDHF or from decreased endothelial release of EDHF. The present results whereby the relaxations induced by the K_{ATP} opener cromakalim were attenuated in SHR and in L-NAME hypertensive rats suggest that the sensitivity of smooth muscle to hyperpolarizing factors was decreased. In addition, isoprenaline has been reported to hyperpolarize arterial smooth muscle via K_{ATP} and K_{Ca} (Randall and McCulloch 1995, Song and Simard 1995). Thus, the present finding whereby isoprenaline-induced relaxation was impaired in SHR and in L-NAME hypertensive rats is also in agreement with the view of reduced hyperpolarization of smooth muscle in these rats.

In our study, untreated SHR and L-NAME hypertensive rats showed attenuated endothelium-independent relaxations to SNP in both NA- and KCl-precontracted vascular rings, suggesting that the sensitivity of arterial smooth muscle to NO was decreased. Since the relaxations induced by isoprenaline and cromakalim were also impaired in SHR and L-NAME rats, these models of experimental hypertension were associated with attenuated vasorelaxant responses via cGMP, cAMP and the opening of K^+ channels, suggesting a general impairment of relaxation in arterial smooth muscle. Because these impairments were observed both in SHR and in L-NAME hypertensive rats, they are likely to result from the long-term elevation of blood pressure. This view is supported by the fact that the present antihypertensive effects of losartan and enalapril were accompanied by a complete normalisation of these changes. Furthermore, the improved vasodilator function of arterial smooth muscle of losartan- and

enalapril-treated rats could well explain the augmented endothelium-dependent relaxations in this study.

Vascular Na^+,K^+-ATPase function was evaluated indirectly by the readdition of potassium to the organ bath upon K^+-free medium-induced precontractions (Arvola et al. 1992). The return of potassium activates Na^+,K^+-ATPase which repolarizes the cell membrane and initiates smooth muscle relaxation (Bonaccorsi et al. 1977). Although general smooth muscle relaxation mechanisms are also involved in this response, previous results suggest that potassium relaxation reflects Na^+,K^+-ATPase activity in arterial smooth muscle (Arvola *et al.* 1992). The present results showed that potassium relaxation was slower in SHR than WKY rats, and enhanced in hypertensive rats by losartan and enalapril. The finding whereby potassium relaxation was inhibited by ouabain in all groups indicates the involvement of the sodium pump in this response. Moreover, the fact that especially the ouabain-sensitive part of the relaxation was augmented after the treatments suggests increased recovery rate of ionic gradients across the cell membrane via Na^+,K^+-ATPase in SHR following losartan and enalapril therapies. Some ouabain-resistant relaxation was detected particularly in the WKY rats, whereby the function of the sodium pump was not completely inhibited by ouabain, or additional mechanisms took part in the response. However, the fact that ouabain abolished the losartan- and enalapril-induced improvement of potassium relaxation supports the notion that Na^+,K^+-ATPase function was augmented in SHR following these treatments.

Several investigations have provided evidence for elevated levels of circulating sodium pump inhibitors in hypertensive subjects (Hamlyn and Manunta 1992) and experimental animals (Wauquier et al. 1988, Doris 1994). This digitalis-like factor augments natriuresis, but it also depolarizes smooth muscle via Na^+,K^+-ATPase inhibition, and increases Ca^{2+} influx through VOC. Subsequently, peripheral arterial resistance is elevated (Zhu et al. 1994). The non-pharmacological treatment of hypertension has lowered plasma ouabain-like activity in hypertensive rats (Doris 1988, Doris 1994), and quinapril therapy has reduced plasma digoxin-like immunoreactivity in SHR, possibly via an alteration of sodium balance (Kähönen et al. 1995a). In the present study, however, both losartan and enalapril treatments were without significant effects on plasma digoxin-like immunoreactivity in SHR. Thus, augmented potassium relaxation after losartan and enalapril therapies could not be explained by decreased plasma digoxin-like material, instead it resulted from the lowering of blood pressure. Previously, quinaprilat has increased the availability of NO in the forearm circulation of patients with chronic heart failure, whereas enalaprilat was without effect (Hornig et al. 1998). Therefore, it seems possible that differences exist in the long-term vascular effects of enalapril and quinapril, and on the basis of the present results the influences of these treatments on plasma digoxin-like immunoreactivity also appear to be dissimilar. It is noteworthy that the impaired potassium relaxation in arteries of untreated SHR was practically abolished by ouabain. Thus, the arteries of SHR were probably also more sensitive to the actions of circulating sodium pump inhibitors, which in turn could result in

smooth muscle depolarization, enhanced Ca^{2+} entry, and elevation of peripheral arterial resistance.

5 Arterial reactivity in experimental renal failure

In the rats with chronic renal failure, the contractile sensitivity to NA and KCl was not altered, the finding of which corresponds to previous observations in acute renal failure (Yates et al. 1985). In addition, the sensitivity of the KCl-induced constrictor responses to increasing organ bath Ca^{2+} concentrations in the absence and presence of nifedipine remained unchanged in the renal failure. However, some differences in the arterial contractions were found, since the maximal forces induced by KCl in endothelium-denuded rings, and by NA in endothelium-intact rings in the presence of L-NAME and diclofenac, were increased in chronic renal failure rats.

The relaxation to ACh was attenuated in the rats with renal failure, and although L-NAME diminished the relaxations in both groups, this effect was more pronounced in the renal failure rats than in controls. Hence, endothelium-mediated relaxations in the renal failure rats were predominantly mediated by NO, whereas the controls showed distinct L-NAME resistant relaxations to ACh. In contrast to L-NAME, diclofenac had no effect on ACh-induced relaxations in either group, suggesting that the products of the COX pathway were not playing a significant role in the responses to ACh in the blood vessels of these animals.

The distinct NOS and COX inhibitor-resistant relaxations to ACh were more pronounced in the sham-operated rats. The combination of apamin and charybdotoxin was without effect on the L-NAME and diclofenac-resistant relaxation to ACh in the renal failure rats, but it significantly inhibited the response in the control rats, such that the difference in the remaining relaxation to ACh between the groups was abolished. This suggests that decreased endothelium-dependent vasodilatation in the renal failure rats was associated with reduced relaxation via a mechanism which included the activation of K^+ channels and the subsequent hyperpolarization of arterial smooth muscle.

Several recent reports have discussed the role of reduced constitutive NO synthesis in the development of renal failure. The NO is synthesised from L-arginine by NOS (Moncada and Higgs 1993), and dietary L-arginine supplementation has been suggested to increase NO generation and enhance vasodilatation in experimental models of kidney disease (Peters and Noble 1996). However, elevated plasma levels of L-arginine have been observed in uremic patients even without dietary supplementation (Noris et al. 1993), and both increased and decreased basal NO production have been reported in the vasculature of rats with reduced renal mass (Aiello et al. 1997, Vaziri et al. 1998c). In this study, the total body NO generation was not altered by chronic renal failure, since the plasma concentration and urinary excretion of NO metabolites were similar in control rats and in rats with renal failure. However, this does not exclude the possibility that local changes in constitutive NO generation could have occurred in the arterial wall, since the contribution of NO to the endothelium-dependent

relaxation appeared to be more pronounced in the mesenteric artery of the renal failure rats than controls. This could represent a compensatory change in the vasculature in order to keep the blood pressure within the normal limits.

As previously reported, the sensitivity of arterial smooth muscle to NO was not altered in renal failure (Verbeke et al. 1994). However, the endothelium-independent relaxations induced by isoprenaline and cromakalim were impaired in the rats with renal failure. In addition to the elevation of intracellular cAMP, isoprenaline has been reported to open K_{ATP} in the smooth muscle of rat mesenteric artery (Randall and McCulloch 1995). Therefore, the impaired function of K^+ channels in smooth muscle could explain the reduced relaxations to the endothelium-independent agonists and the impaired endothelium-mediated hyperpolarization in experimental renal failure.

6 The synthesis of natriuretic peptides in experimental hypertension and renal failure and the influences of antihypertensive treatments

The changes in cardiac mass produced by losartan and enalapril treatments were accompanied by markedly reduced left ventricular ANP mRNA and ir-ANP levels in SHR and WKY rats. This reduction in ANP synthesis may reflect a direct effect of Ang II on ventricular ANP gene expression. Our results agree with a previous study in which ACE inhibitors and AT_1 receptor antagonists were shown to decrease ANP gene expression in hypertrophied myocardium independent of blood pressure levels (Kim et al. 1996). We also found that the behaviour of atrial ANP synthesis differs from that of ventricular ANP. The finding of decreased left atrial ANP storage in SHR as well as increased ANP concentration in the left atrium of SHR after losartan treatment can be explained by changes in ANP release from the atria secondary to alterations in left atrial pressure, because atrial ANP mRNA levels did not change in drug-treated SHR and the treatments did not influence atrial ANP synthesis in normotensive rats. These results on ventricular and atrial ANP gene expression are in line with earlier results obtained with several other models of experimental hypertension (Ruskoaho 1992, de Bold et al. 1996). The finding that left atrial ir-ANP levels in SHR increased less in response to ACE inhibitor treatment than during AT_1 receptor blocker treatment may be related to differential actions of these drugs on tissue levels of bradykinin, PGs and NO.

BNP gene expression in both atria and ventricles reacts more rapidly to haemodynamic overload than ANP (Magga et al. 1994). In the present study, the effect of chronic administration of ACE inhibitors or AT_1 receptor antagonists on ventricular BNP gene expression was different between SHR and WKY rats. This suggests, as previously reported (Ogawa et al. 1996), that the reduction in ventricular BNP gene expression could be more closely related to changes in systolic blood pressure than that of ventricular ANP gene expression. However, Ang II may directly contribute to the long term regulation of atrial BNP synthesis, since left atrial levels of ir-BNP were decreased in both rat strains in response to drug treatments. This potential differential role of Ang II in regulating atrial and ventricular

BNP gene expression is consistent with a recent study, in which ET-1 was reported to be an important paracrine regulator of BNP gene expression in the atria, but not in the left ventricle (Magga et al. 1997). Taken together, autocrine/paracrine factors may play a greater role in the regulation of ventricular ANP and atrial BNP gene expression than atrial ANP and ventricular BNP gene expression.

There was a dissociation between cardiac BNP mRNA and ir-BNP levels. The reason for this is not clear, but a similar dissociation between BNP mRNA levels and BNP peptide levels has been previously reported in response to acute and chronic haemodynamic overload in rats (Magga et al. 1994, Ogawa et al. 1996, Magga et al. 1997). The elevation of BNP mRNA levels could result from transcriptional and posttranscriptional mechanisms or from a combination of both mechanisms whereas the rate of release influences tissue peptide levels. Therefore, decreased BNP peptide levels together with increased BNP mRNA levels could be explained by an increased rate of release of BNP from the atria and ventricles. In the present study, we also found that ANP mRNA and ir-ANP levels were similar in SHR and WKY rats, although ventricular re-induction of the ANP gene has been demonstrated to occur simultaneously with the progression of hypertension and ventricular hypertrophy in the SHR strain (Arai et al. 1988, Ruskoaho et al. 1989, Matsubara et al. 1990). This observation may be due to the fact that the rats were studied in the early phase of hypertrophy and the difference in ANP mRNA levels between the strains was not yet statistically significant, as also described previously (Kinnunen et al. 1991, Ruskoaho 1992).

Despite the lack of the development of compensatory myocardial hypertrophy during L-NAME-induced hypertension, ventricular ANP mRNA and BNP mRNA levels and ir-ANP and ir-BNP levels increased significantly. These results show an interesting dissociation between the natriuretic peptide gene expression and myocardial hypertrophy during L-NAME-induced hypertension. Administration of losartan abolished the L-NAME-induced activation of the ventricular ANP and BNP expression, but was without effect on the increases in tissue natriuretic peptide levels. However, it is not clear whether the effect of Ang II on ANP release is secondary to the haemodynamic changes or due to direct receptor stimulation (Ruskoaho et al. 1997). Furthermore, AT_1 receptor antagonism has no effect on mechanical load-induced early activation of ventricular BNP gene expression, and thus Ang II is not obligatorily required for stretch to trigger the increased BNP expression in ventricular myocytes *in vivo* (Magga et al. 1997).

The experimental renal failure of this study was neither associated with elevated blood pressure nor cardiac hypertrophy. However, plasma NT-ANP levels and ventricular ANP mRNA, BNP mRNA and ir-BNP levels were increased. Therefore, the synthesis of natriuretic peptides appears to be stimulated in chronic renal failure, possibly reflecting increased ventricular wall stretch caused by volume retention. Furthermore, increased rate of ANP release from the ventricles in rats with renal failure seems likely due to the dissociation between ANP mRNA and ir-ANP levels.

7 Adrenomedullin in genetic hypertension and renal failure and the influences of long-term angiotensin receptor antagonism and angiotensin converting enzyme inhibition

The possible regulatory effects of AT_1 receptor antagonism or ACE inhibition on cardiac ADM gene expression were examined in SHR and WKY rats. ADM has been considered as representing tissue marker for left ventricular hypertrophy, because a positive correlation has been observed between left ventricular mass index and left ventricular ADM peptide concentration (Jougasaki et al. 1997, Morimoto et al. 1999). In this study, losartan and enalapril treatments had no effect on ventricular ADM gene expression suggesting that ventricular ADM synthesis is an insensitive marker of chronic changes in haemodynamic load or myocyte hypertrophy. In support of this, there was no correlation in SHR between cardiac mass reduction and ADM mRNA or ir-ADM levels, whereas a significant correlation between the decrease in cardiac hypertrophy and both ANP mRNA and ir-ANP levels was noted. Furthermore, there were no differences in ventricular ADM gene expression between SHR and WKY rats. This finding is consistent with the lack of change in ventricular ADM mRNA levels in other experimental models of pressure-induced hypertrophy including aortic banding (Kaiser et al. 1998) or hypertensive transgenic TGR(mREN-2)27 rats (Romppanen et al. 1997). Therefore, ventricular ADM gene expression appears not to be activated during the chronic phase of pressure-induced cardiac hypertrophy in rats.

The lack of change in ventricular ADM expression in the setting of significant alterations in cardiac mass suggests that ADM is not involved in the long term cardiac adaptive response to pressure overload in rats. Moreover, the absence of increased ADM expression in response to chronic pressure overload suggests that increased wall stress is not directly involved. However, acute cardiac pressure overload, produced by vasopressin infusion, significantly stimulates ADM gene expression in the left ventricle in normotensive rats suggesting that haemodynamic overload may regulate ADM gene expression (Romppanen et al. 1997). Cardiac overload produced by Ang II infusion also stimulates ventricular ADM gene expression (Romppanen et al. 1997). Therefore, the induction of ADM expression may be a part of a selective change in cardiac gene expression in response to acute, but not chronic haemodynamic load, and may play an important role in early cardiac failure and left ventricular hypertrophy.

Interestingly, the experimental renal failure of this study was associated with increased ventricular ADM expression although no increases in blood pressure or cardiac weight were observed. However, in the rats with renal failure, the expression of ADM in left atrium was decreased and in right atrium unchanged when compared with control rats. The synthesis of ADM in renal failure seems to differ from that in chronic pressure overload, because no changes in ADM synthesis were observed in genetic hypertension. The reason for increased ventricular ADM synthesis in renal failure is not clear. Chronic volume overload or endocrine changes associated with renal dysfunction may contribute to the synthesis of ADM in this condition (Ishimitsu et al. 1994).

SUMMARY AND CONCLUSIONS

The present study was designed to examine the effects of the long-term losartan and enalapril treatments on arterial function and on cardiac natriuretic peptide and ADM gene expression in SHR, as well as the effects of the long-term losartan treatment on arterial function and on the regulation of cardiac natriuretic peptides in NO-deficient hypertension in Wistar rats. The influence of renal failure on the control of arterial tone and cardiac natriuretic peptides and ADM in WKY rats was also evaluated.

The major findings and conclusions are:

1. The attenuated mesenteric arterial relaxations in different forms of experimental hypertension and volume overload seemed to be particularly related to reduced vasodilatation via potassium channels:

> 1.1. Long-term AT_1 receptor antagonist and ACE inhibitor therapies prevented the development of hypertension in SHR, an effect which was associated with enhanced arterial dilatation. The endothelium-mediated relaxation in enalapril- and losartan-treated SHR was augmented in the absence and presence of NOS inhibition but not under conditions preventing hyperpolarization, and endothelium-independent relaxation was enhanced during receptor- but not during depolarization-mediated precontractions. Therefore, the improved vasodilatation of mesenteric arteries following losartan and enalapril therapies could be attributed to enhanced hyperpolarization of arterial smooth muscle in genetic hypertension.

> 1.2. Chronic L-NAME hypertension was associated with the impairment of endothelium-dependent and -independent vasorelaxation in Wistar rat. Long-term AT_1 receptor antagonism clearly improved arterial dilatation in NO deficiency. The mechanisms underlying the augmented vasodilatation following losartan therapy in this model of experimental hypertension may have included enhanced arterial hyperpolarization, increased sensitivity to NO in smooth muscle, and decreased vascular production of superoxide and vasoconstrictor prostanoids.

> 1.3. Endothelium-mediated relaxation in rats with chronic renal failure was impaired in the absence and presence of NOS inhibition but not under conditions where hyperpolarization was blocked. In addition, endothelium-independent relaxations via the activation of β-adrenoceptors and the opening of K^+ channels were reduced. Therefore, impaired arterial relaxation in experimental renal failure could be attributed to reduced vasodilatation via potassium channels.

2. Long-term AT_1 receptor antagonism and ACE inhibition corrected the mesenteric arterial structural alterations, and normalised the increased dihydropyridine-sensitivity in arterial segments of SHR. Treatment with losartan and enalapril also augmented arterial potassium relaxation in SHR, suggesting an enhanced function of Na^+,K^+-ATPase, but this effect could not be attributed to changes in circulating sodium pump inhibitor concentration.

3. AT_1 receptor antagonism and ACE inhibition for 10 weeks resulted in the regression of cardiac hypertrophy associated with the increase in left ventricular ANP gene expression in normotensive and hypertensive rats. The changes in systolic blood pressure and cardiac hypertrophy induced by losartan and enalapril treatments had no effect on ventricular ADM gene expression, suggesting that ventricular ADM synthesis is an insensitive marker of chronic changes in haemodynamic load or myocyte hypertrophy in genetic hypertension. The expression of ADM, ANP and BNP was differently regulated both in the left ventricle and atria in response to AT_1 receptor antagonism and ACE inhibition.

4. Despite the lack of the development of compensatory myocardial hypertrophy during L-NAME-induced hypertension, ventricular ANP and BNP mRNA levels and ir-ANP and ir-BNP levels were significantly increased. Therefore, the natriuretic peptide gene expression and myocardial hypertrophy during L-NAME-induced hypertension appear to dissociate. Furthermore, the chronic L-NAME-induced hypertension and the associated activation of ventricular ANP and BNP gene expression are, at least in part, mediated by Ang II.

5. In chronic renal failure, the plasma NT-ANP levels and ventricular ANP mRNA, BNP mRNA and ADM mRNA levels were increased although no changes in blood pressure or cardiac weight were observed. Therefore, the synthesis of natriuretic peptides and ADM appears to be stimulated in chronic renal failure, possibly reflecting increased ventricular wall stretch caused by volume retention. Furthermore, the ventricular synthesis of ADM appears to be a marker of chronic volume overload associated with renal dysfunction, since its synthesis increases in renal failure but not in chronic pressure overload.

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