PASI JOLMA

Calcium Metabolism and Vascular Tone in Experimental Hypertension and Renal Failure

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ACADEMIC DISSERTATION

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for public discussion in the main auditorium of Building B,
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University of Tampere, Medical School, Department of Pharmacological Sciences
Tampere University Hospital, Department of Internal Medicine
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Supervised by
Docent Ilkka Pörsti
University of Tampere

Reviewed by
Docent Ilkka Kantola
University of Turku
Docent Eero Mervaala
University of Helsinki

Distribution

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Bookshop TAJU
P.O. Box 617
33014 University of Tampere
Finland

Tel. +358 3 215 6055
Fax +358 3 215 7685
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II Kähönen M, Näppi S, Jolma P, Hutri-Kähönen N, Tolvanen J-P, Saha H, Koivisto P, Krogerus L, Kalliovalkama J and Pörsti I (2003): Vascular influences of calcium supplementation and vitamin D-induced hypercalcaemia in NaCl-hypertension. Journal of Cardiovascular Pharmacology, 42(3):319-328.

III 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(4 Pt 2): H1622-H1629.

IV Jolma P, Kalliovalkama J, Tolvanen J-P, Kööbi P, Kähönen M, Saha H and Pörsti I (2002): Preserved endothelium-dependent but impaired isoprenaline-induced vasorelaxation of the resistance vessels in experimental renal failure. Experimental Nephrology, 10(5-6): 348-354.

V Jolma P, Kööbi P, Kalliovalkama J, Saha H, Fan M, Jokihaara J, Moilanen E, Tikkanen I and Pörsti I (2003): Treatment of secondary hyperparathyroidism by high calcium diet is associated with enhanced resistance artery relaxation in experimental renal failure. Nephrology Dialysis Transplantation, 18(12):2560-2569.

ABBREVIATIONS

AA	Arachidonic acid
ACh	Acetylcholine
Ang I	Angiotensin I
Ang II	Angiotensin II
ANOVA	Analysis of variance
$[Ca^{2+}]_i$	Intracellular free Ca^{2+} concentration
Ca^{2+} pump	Ca^{2+}-ATPase
cAMP	Cyclic adenosine 3',5'-monophosphate
cGMP	Cyclic guanosine 3',5'-monophosphate
COX	Cyclooxygenase
CRF	Chronic renal failure
EDCF	Endothelium-derived contracting factor
EDHF	Endothelium-derived hyperpolarizing factor
ESRD	End-stage renal disease
ET	Endothelin
G protein	Guanosine 5'-triphosphate-binding protein
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
L-NAME	N^G-nitro-L-arginine methyl ester
NA	Noradrenaline
NaCl	Sodium chloride
NO	Nitric oxide
NOS	Nitric oxide synthase
NOx	Nitrite and nitrate
PG	Prostaglandin
PGI_2	Prostacyclin
PTH	Parathyroid hormone
RAS	Renin-angiotensin system
SH	Secondary hyperparathyroidism
SHR	Spontaneously hypertensive rats
SNP	Sodium nitroprusside
SOD	Superoxide dismutase
VSMC	Vascular smooth muscle cell
WKY	Wistar-Kyoto

INTRODUCTION

The most common risk factor for myocardial infarction, cerebral stroke, and end-stage renal disease (ESRD) is elevated blood pressure. Almost one fourth of the entire population in industrialised societies and more than half of the population aged over 65 years have elevated blood pressure (Ruoff 1998). Recent guidelines for high blood pressure detection and treatment contained key messages for physicians dealing with blood pressure evaluation (Chobanian et al. 2003). The goal blood pressure should be <140/90 mmHg, or <130/80 mmHg for patients with diabetes or chronic kidney disease and in persons older than 50 years, systolic blood pressure or more than 140 mmHg is a more important cardiovascular disease risk factor than diastolic blood pressure (Chobanian et al. 2003). Extensive studies have been carried out to elucidate the complex pathogenesis of hypertension, yet the origin of high blood pressure still remains unknown in 95 % of the patients. It is generally suggested that elevated blood pressure mainly results from increased peripheral arterial resistance, while cardiac output often remains unaltered (Kaplan 1998). Moreover, the leading cause of mortality in patients with ESRD are cardiovascular disease and stroke (Slatopolsky 2003). Cardiovascular disease in uraemia includes disorders of the heart (left ventricular hypertrophy, cardiomyopathy) and disorders of the vascular system (atherosclerosis, arteriosclerosis) (London 2003). Even after stratification by age, gender, race, and the presence or absence of diabetes, cardiovascular mortality in dialysis patients is 10 to 20 times higher than in the general population (Foley et al. 1998, London 2000). This excess cardiovascular risk and mortality seems to already be demonstrable in early renal disease and chronic renal failure (CRF) (London 2003).

In the past, numerous experimental models have been used to study the cause and progression of human cardiovascular disease (Doggrell and Brown 1998). The complex pathomechanims, the individual variation in polygenetic disposition as well as the environmental factors regarding the development of essential hypertension are among the parameters that need to be considered in studying cardiovascular phenomena such as hypertension (Lindpaintner et al. 1992). Defects in the production or action of nitric oxide (NO) (Moncada and Higgs 1993), as well as elevated levels of salt intake may contribute to the pathogenesis of essential hypertension. NaCl-induced hypertension exhibits the cardiovascular effects of long-term salt administration, whereas chronic inhibition of nitric oxide synthase (NOS) represents a more novel model for animal hypertension (Baylis et al. 1992). The most common cause of secondary hypertension is renal parenchymal disease (Preston 1999) whereas, on the other hand, sustained essential hypertension predisposes to the development of renal failure (Frohlich 1997, Luke 1998, Rahn 1998). Therefore, experimental renal failure represents 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. Small arteries are the main regulators of peripheral vascular resistance and blood pressure and are therefore often referred to as resistance vessels (lumen diameter < 400μm) (Bohlen 1986, Schiffrin 1997). Endothelial dysfunction is a sensitive indicator of cardiovascular disease, predicts its prognosis, and is closely associated with the development of arteriosclerosis (Galle et al. 2003). Abnormal endothelium-dependent vasodilatation of small and large arteries has been observed in patients with essential hypertension, and there is accumulating evidence suggesting that CRF is also associated with impaired endothelial function.

More than two decades ago calcium supplementation was shown to lower the blood pressure of spontaneously hypertensive rats (SHR) (Ayachi 1979) and this finding sparked a burst of activity exploring the relationships between dietary calcium intake and blood pressure regulation in human and animal models of hypertension. While the question whether elevated calcium intake lowers blood pressure in humans remains controversial, data showing an inverse correlation between calcium intake and blood pressure in several models of hypertension in the rat and dog very are consistent (Bukoski 2001). Over the past two decades, multiple mechanisms have been postulated to explain how positive calcium balance results in lower blood pressure, yet the final conclusion remains unclear. Oral calcium carbonate is also used as a phosphate binding agent in renal failure to reduce the elevated circulating levels of phosphate and parathyroid hormone (PTH) (Rostand and Drüeke 1999), but the effect of high calcium diet on vascular function during impaired kidney function has not been studied.

The present study was designed to examine the reactivity of large arteries in NO deficiency, sodium chloride (NaCl)-induced hypertension and renal failure. The vascular effects of long-term calcium supplementation in N^G-nitro-L-arginine methyl ester (L-NAME)-hypertension and the influences of high calcium intake and vitamin D-induced hypercalcaemia were evaluated in NaCl fed Wistar rats. Moreover, the reactivity of small mesenteric arteries, as well as the influences of phosphate binding by high calcium intake on the control of microvascular tone in renal failure were studied.

REVIEW OF THE LITERATURE

1 Control of blood pressure

1.1 General aspects

1.1.1 Central and autonomic nervous system

Blood pressure is regulated by a myriad of mechanisms that feature complex interaction between central and peripheral regulatory mechanisms adapting the cardiovascular system to the surrounding requirements (Culman and Unger 1992, MacGregor 1998). Under normal conditions, the kidney plays the dominant role in setting long-term arterial pressure, and the central and autonomic nervous systems act primarily as short-term regulators, stabilizing perfusion pressure in the face of disturbances in circulatory homeostasis (e.g., standing, running, and stress) (Wyss and Carlson 1999). The central nervous system modulates blood pressure via adjusting heart rate and contractility as well as controlling peripheral vascular resistance. In executing the required cardiovascular adatations, the sympathetic and parasympathetic pathways of the autonomic nervous system play the key role, but neuroendocrine pathways, such as the hypothalamo-pituitary axis, are also involved (Reid 1994, de Wardener 2001). Moreover, the sympathetic nervous system also exerts long-term trophic action on the vasculature (Tsuru et al. 2002). Anatomically, areas like the medulla oblongata and its nucleus tractus solitarius are of primary importance in the cardiorespiratory reflex integration and regulation of sympathetic neuronal activity (Colombari et al. 2001).

The effects of central nervous system on blood pressure are modified by arterial baroreceptors located at the aortic arch and the carotid sinuses, which in hypertension appear to be desensitised and reset to a higher level of blood pressure (Sleight 1991, Grassi et al. 1998, Stauss 2002). Although it is generally accepted that the primary purpose of the cardiovascular baroreflexes is to keep blood pressure close to a particular set point over a relatively short period of time, the contribution of baroreceptor dysfunction to long-term essential hypertension remains unclear (Panfilov and Reid 1994, Wyss and Carlson 1999). Nevertheless, in several animal models and in subsets of hypertensive human patients, the nervous system seems to play a more significant role in the chronic elevation of arterial pressure (Wyss and Carlson 1999). SHR have consistently shown indications of a centrally mediated increased sympathetic outflow (Reid 1994). Moreover, both juvenile and adult SHR have shown decreased activity of sympatoinhibitory neurons, suggesting that enhanced sympathetic activity is involved in the development and maintenance of hypertension in SHR (Fujino 1984, Chalmers et al. 1992). However, there are reports questioning the presence of increased sympathetic tone in the development of spontaneous hypertension (Shah and Jandhyala 1995).

Methodological difficulties have obstructed research in humans on the importance of increased sympathetic activity as a cause for long-term elevation of blood pressure (Mancia et al. 1999). Nevertheless, the sympathetic nervous system could be a key factor in the genesis of essential hypertension, and additionally it may also promote the development of its complications (Guzzetti et al 1988, Mancia et al. 1999, Esler 2000). In humans, there has been a clear relationship between cardiac sympathetic activity and the progression of hypertension in its early stages (Julius 1996).

Furthermore, plasma noradrenaline (NA) levels are reported to be elevated, rate of NA spillover from sympathetic nerve terminals could be increased, and 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 outflow has even been found in the normotensive offspring of hypertensive patients, supporting the idea that increased sympathetic tone could be cause, rather than the consequence, of elevated blood pressure (Floras and Hara 1993, Noll et al 1996). Altogether, dysfunction of the autonomic nervous system may contribute to the development of both essential and experimental hypertension.

1.1.2 Kidneys

Long-term regulation of arterial blood pressure is closely linked to volume homeostasis through renal body fluid feedback mechanisms (Lohmeier 2001). A key feature of the renal body fluid feedback control system is the pressure natriuresis or the ability of the kidneys to respond to changes in arterial pressure by altering the renal excretion of salt and water, i.e. sodium balance, extracellular fluid volume (ECFV) and blood volume (Navar 1997, Lohmeier 2001). In addition to playing a major role in long-term blood pressure adjustment, the sodium transporters along the nephron are very dynamic, even responding quickly to normal fluctuations of blood pressure (McDonough et al. 2003). 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, when the relationship between sodium excretion and arterial pressure is shifted towards higher pressures, hypertension develops (Navar 1997). Moreover, any derangements that compromise the ability of the kidneys to maintain sodium balance potentially result in the kidney's need for an elevated arterial pressure to re-establish net salt and water balance (Navar 1997, Lohmeier 2001). Such resetting of the pressure-natriuresis mechanism has been linked to the development of both human and experimental hypertension (Guyton et al. 1972, Cowley and Roman 1996, Cowley 1997). Recent studies in humans have provided quite surprising evidence of how many genetic forms of hypertension or

hypotension solved to date converge an a final common pathway, altering blood pressure by changing net renal salt balance (Lifton et al. 2001). These findings establish the role of altered salt homeostasis in blood pressure variation in humans and underscore the key role of the kidney in the long-term determination of blood pressure.

The sensitivity of the pressure-natriuresis mechanism can be modified by extrarenal hormonal regulatory systems, such as the renin-angiotensin system (RAS) (Lohmeier 2001). As arterial pressure or sodium intake increases, the RAS is suppressed, which enhances the ability of the kidneys to excrete salt and water (Lohmeier 2001). Moreover, the sympathetic nervous system can alter the pressure-natriuresis mechanism and contribute to long-term regulation of arterial pressure through changes in renal sympathetic nerve activity (Lohmeier 2001). Interestingly, one of the classical explanations used for the blood pressure lowering effect of dietary calcium has been the detected natriuretic action of calcium in both humans and animals (Pörsti et al. 1991, Akita et al. 2003). There is considerable evidence from acute studies that baroreflex-mediated changes in renal sympathetic nerve activity influence pressure natriuresis and contribute to short-term regulation of body fluid volumes (Van Vliet et al. 1996, Dibona and Kopp 1997). The long-term effects of the compensatory changes in renal sympathetic nerve activity on body fluid volumes and arterial pressure remain somewhat unclear. However, certain human studies have demonstrated that renal norepinephrine overflow, an index of renal sympathetic nerve activity, is increased in the early stages of essential hypertension (Esler 1995, 2000). Furthermore, experimental studies have shown that chronic renal adrenergic stimulation may result in sustained hypertension (Van Vliet et al. 1996, Dibona and Kopp 1997, Lohmeir 200). It seems that chronic decreases, as well as increases, in renal adrenergic stimulation have a sustained modulatory influence on pressure-natriuresis (Lohmeier 2001).

Renal disease in type 2 diabetes has become an important clinical problem as the prevalence of type 2 diabetes is rising in all Westernized societies. ESRD in patients with diabetes (mostly type 2) as a co-morbid condition has also risen dramatically in the past decade (Ritz and Tarng 2001). This constellation has become the single most common cause of ESRD in most countries (Ritz and Tarng 2001, Deferrari et al. 2002). Aggressive treatment of hypertension and meticulous glycaemic control are the most important clinical strategies in preventing the increase in number of diabetic patients with ESRD (Ritz and Tarng 2001).

The linkage between kidney failure and the elevation of blood pressure is complex and these conditions may coexist at least in three clinical settings (Preston 1999). First, renal parenchymal disease with impaired renal sodium excretion leads to ECFV expansion, which is the most common form of secondary hypertension, accounting for 2.5 % to 5 % of systemic hypertension cases (Preston 1999). Practically every kidney failure patient suffers from hypertension by the time of renal-replacement therapy (Rabelink and Koomans 1997, Luke 1998). Furthermore, pathologically altered hormonal profiles with increased sympathetic

activity, activated RAS, increased levels of catecholamines, vasopressin and endothelin (ET), enhanced oxidative stress, and decreased NO activity or production may contribute to the high incidence of hypertension in renal failure patients (Bellinghieri et al. 1999, Ligtenberg et al. 1999, Schmidt and Baylis 2000, Annuk et al. 2001, Vaziri et al. 2002, Annuk et al. 2003). Second, it is agreed that sustained, uncontrolled essential hypertension damages the renal vasculature, causes nephrosclerosis and markedly predisposes to the development of renal failure (Frohlich 1997, Luke 1998, Rahn 1998). It has been suggested that hypertension has been the cause of end-state renal disease in 8 % of dialysis patients (Bellinghieri et al. 1999). However, some reports suggest that sufficient blood pressure control in patients with essential hypertension inhibits the development of progressive decline in kidney function (Herrera-Acosta 1994, Siewert-Delle et al. 1998). The third significant clinical circumstance in which hypertension and renal failure occur simultaneously, is ischemic renal disease following by bilateral or unilateral arteriosclerotic renal artery stenosis (Preston 1999). Hence, hypertension can either be a result of renal failure or the leading cause for impaired kidney function.

1.1.3 Renin-angiotensin system

In the 1970s, a series of observations demonstrated that angiotensin II (Ang II) has deleterious effects on the heart and kidney and that patients with high levels of plasma renin activity are at a higher risk of developing stroke or myocardial infarction than those with low plasma renin activity (Gavras et al. 1971, Brunner et al. 1972). Thereafter, the development of pharmacological probes that block the RAS have helped to define the contribution of this system to blood pressure control and to the pathogenesis of diseases such as hypertension, congestive heart failure and CRF (Burnier 2001).

RAS is a regulatory cascade that plays an essential role in the regulation of blood pressure, electrolyte, and volume homeostasis (Li et al. 2002). The first and rate-limiting component of this endocrine cascade is renin, a protease synthesized and secreted predominantly by the juxtaglomerular apparatus in the nephron (Li et al. 2002). Renin cleaves angiotensin I (Ang I) from liver-derived angiotensinogen, which is then converted to Ang II by the angiotensin-converting enzyme (ACE) located in the luminal surface of the vascular endothelium (Riordan 1995, Wright et al. 1995). Although there are other angiotensin peptides with biological effects, Ang II is the major end product of the system and through binding to its receptors Ang II exerts diverse actions that affect the electrolyte, volume and blood pressure homeostasis (Ballerman et al. 1991, Burnier 2001). Ang II has numerous actions on vascular smooth muscle: it modulates vasomotor tone through its potent vasoconstrictor effects, regulates cell growth and apoptosis, influences cell migration and extracellular matrix deposition, it is proinflammatory, and it stimulates production of other growth factors and vasoactive agents (Touyz 2003). Regulation of Ang II-induced vascular

contraction is generally attributed to a G protein-mediated increase in cytoplasmic $[Ca^{2+}]_i$, which is the signal activating the contractile machinery of vascular smooth muscle cells (Touyz and Schiffrin 2000). Ang II is also a major stimulus for activation of NAD(P)H oxidases, which are a predominant source of reactive oxygen species, such as superoxide, that consume NO in vascular cells resulting in endothelial dysfunction (Cai et al. 2003).

Remodeling of small arteries in hypertension is associated with increased VSMC growth (Touyz et al. 2002), and Ang II is one of the most important active agents that have been implicated in this in this vascular process (Geisterfer et al. 1988, Touyz et al. 1999). Ang II appears to have direct growth-promoting effects independent of blood pressure changes, which may contribute to the vascular remodeling in hypertension (Morishita et al. 1994, Kim et al. 1999). Signal transduction pathways underlying Ang II-mediated growth actions involve phosphorylation of tyrosine kinases, such as c-Src, and activation of mitogen-activated protein (MAP) kinases (Takahashi and Berk 1998, Touyz et al. 1999, Touyz 2003). Alterations in tyrosine and MAP kinase signaling are suggested to play a role in the pathological cellular processes that are associated with vascular remodeling in hypertension (Touyz et al. 2002, Touyz 2003).

At first, RAS was described as an endocrine system exerting its action through Ang II, but recently, tissue-based RAS, which acts through paracrine-autocrine mechanisms, has been suggested to be of more importance (Stock et al. 1995, Rothermund and Paul 1998). Local Ang II production has been demonstrated in many tissues including the brain, kidney, adrenal cortex, heart, and blood vessel wall (Cockroft et al. 1995, Mulrow and Franco-Saenz 1996, Kubo et al. 1999). The local synthesis of renin seems to be limited to small amounts and it is likely that uptake of renal renin occurs from the circulation (Stock et al. 1995, Danser 1996). Ang I and Ang II can also be generated by other enzymatic pathways (Urata et al. 1990, Dzau et al. 1993). Ang I can be formed by nonrenin enzymes such as tonin or cathepsin, and Ang I can be converted to Ang II by enzymes such as trypsin, cathepsin, or the heart chymase (Burnier 2001). Today, the quantitative contribution of these alternative pathways to the generation of Ang II remains unclear.

ACE is also called kininase II, and it participates in metabolising bradykinin to inactive peptides. The inhibition of ACE produces an increase in plasma bradykinin levels (Linz et al. 1995, Nussberger et al. 1998), which contributes to the side effects of ACE inhibitors (eg, angioedema) and might play a role in the organ-specific effects of ACE inhibitors (Nussberger et al. 1998).

The discovery of specific Ang II receptor antagonists has confirmed the existence of various subtypes of Ang II receptors (Timmermans et al. 1993). Ang II type 1 (AT_1) receptors are selectively inhibited by losartan, whereas type 2 (AT_2) receptors are inhibited by PD 123177 and related compounds (Burnier 2001). In rodents, AT_1 receptors have been further subdivided into AT_{1A} and AT_{1B}. In amphibians and in neuroblastoma cell lines, an Ang II

receptor inhibited neither by losartan nor by PD 123177 has been classified as AT_3 (Burnier 2001). AT_1 receptors have been localized in the kidney, heart, vascular smooth muscle cells (VSMCs), brain, adrenal gland, platelets, adipocytes, and placenta (Burnier 2001). AT_2 receptors are abundant in the fetus, but their number decreases in the postnatal period (Timmermans et al. 1993, Burnier 2001). In adult tissues, AT_2 receptors are present only at low levels, mainly in the uterus, the adrenal gland, the central nervous system, the heart (cardiomyocytes and fibroblasts), and the kidney (Timmermans et al. 1993, Burnier 2001). AT_2 receptors seem to be re-expressed or upregulated in experimental cardiac hypertrophy, myocardial infarction, and vascular and wound healing (Janiak et al. 1992, Nio et al. 1995, Ohkubo et al. 1997).

However, most of the known clinical effects of Ang II are mediated by the AT_1 receptor (Horiuchi et al. 1999, Burnier 2001). The actions of Ang II via the AT_1 receptor include vasoconstriction, increased sodium retention, suppressed renin secretion, increased ET secretion, increased vasopressin release, activation of sympathetic nervous system, promotion of myocyte hypertrophy, stimulation of vascular and cardiac fibrosis and increased myocardial contractility (Burnier 2001). The physiological role of the AT_2 receptor is only partially understood. In recent years, several new functions have been attributed to AT_2 receptors, including inhibition of cell growth, promotion of cell differentation, and apoptosis (Nakajima etl. 1995, Stoll et al. 1995, Meffert et al. 1996, Morrisey and Klahr 1999). Thus, AT_2 receptors could have an important role in counterbalancing some of the effects of Ang II mediated by AT_1 receptors (Horiuchi et al 1999, Siragy et al. 1999). However, this remains a matter of debate because controversial results have been reported (Li et al. 1998, Cao et al. 1999). There is also data suggesting that AT_2 receptors could mediate the production of bradykinin, NO, and perhaps prostaglandins (PGs) in the kidney (Siragy and Carey 1996).

1.2 Vascular endothelium

Vascular endothelium serves not merely as a passive barrier between flowing blood and the vascular wall but uses this strategic location to maintain vascular homeostasis. It plays a pivotal role in modulating vascular tone, calibre, and blood flow in response to humoral, neural, and mechanical stimuli by synthesizing and releasing vasoactive substances (Behrendt and Ganz 2002). The endothelium also takes part in the regulation of various physiological functions including coagulation, lipid transport, immunological reactivity and vascular structure (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, superoxide 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).

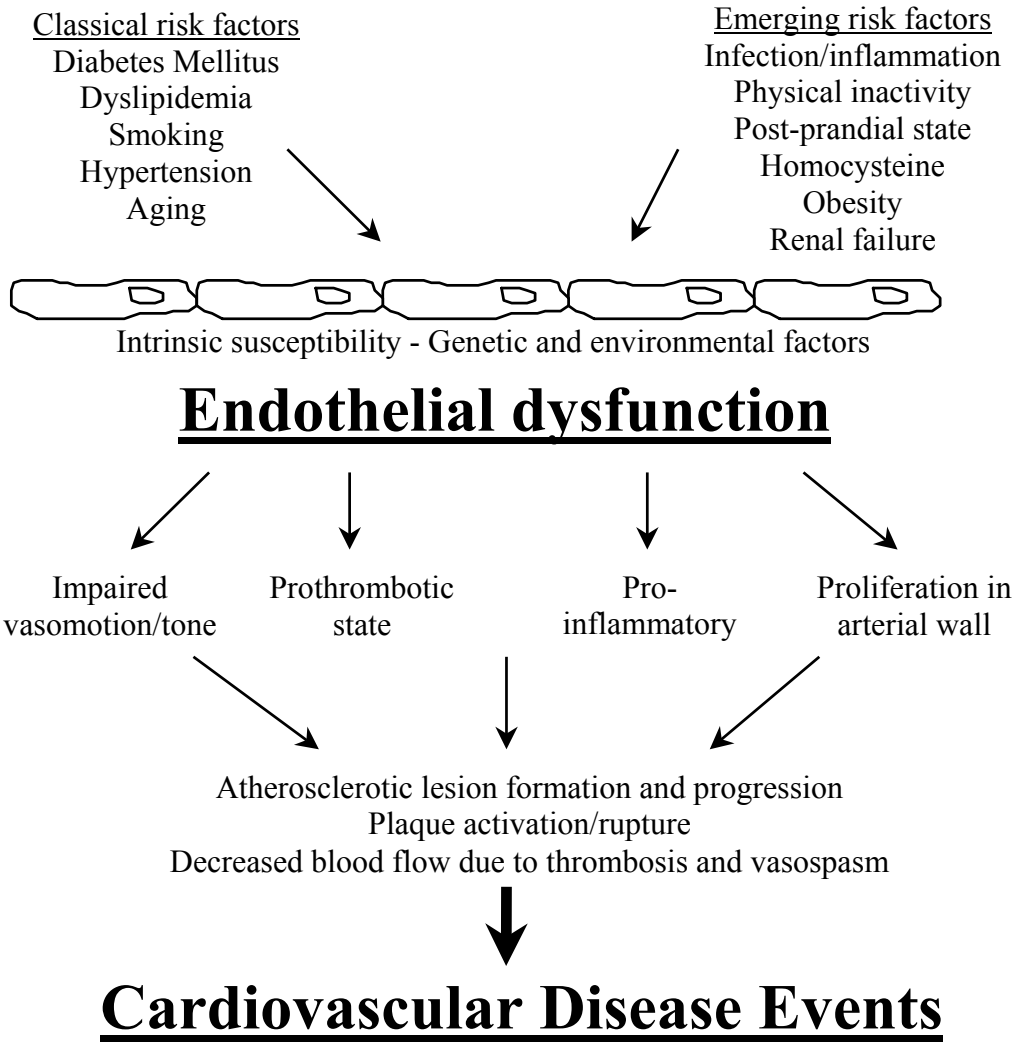
A number of studies have demonstrated that endothelium-mediated arterial relaxation is impaired in patients with essential hypertension (Panza et al. 1990, Taddei et al. 1997a, Schmieder et al. 1997) although this view is not supported by all investigators (Cockcroft et al. 1994, Hutri-Kähönen et al. 1999). Moreover, measurement of endothelial function in patients has recently emerged as a useful tool for atherosclerosis research (Widlansky et al. 2003). Clinical syndromes other than hypertension, such as stable and unstable angina, acute myocardial infarction, claudication, and stroke relate, in part, to a loss of endothelial control of vascular tone, thrombosis, and the composition of the vascular wall (Widlansky et al. 2003). Recent studies have shown that the severity of endothelial dysfunction relates to the risk for an initial or recurrent cardiovascular event, yet a growing number of interventions known to reduce cardiovascular risk also improve endothelial function (Celermaier 1997, Muiesan et al. 1999, Widlansky et al. 2003). Therefore, speculation has been prompted that endothelial function may serve as a "barometer" for cardiovascular health that could be used for patient care and evaluation of new therapeutic strategies (Vita and Keaney 2002). Given this possible causal pathway from endothelial dysfunction to atherosclerosis (Figure 1), numerous methods have been employed to measure endothelial dysfunction in humans. These *in vivo* methods include intracoronary agonist infusion with with quantitative angiography, brachial artery catheterization with venous occlusive plethysmography, vascular tonometry and measurements of vascular stiffness, and brachial artery ultrasound with flow-mediated dilatation (Widlansky et al. 2003).

In addition to vasomotor dysfunction, circulating blood markers of endothelial dysfunction also have prognostic value. For instance, in patients without known cardiovascular disease, elevated levels of soluble intercellular adhesion molecule (ICAM), and tissue plasminogen activator are independent predictors of future cardiovascular events (Ridker et al. 1998, Thogersen et al. 1998). In patients with known coronary disease, soluble ICAM, von Willebrand factor, tissue plasminogen activator, plasminogen activator inhibitor, and endothelin all have prognostic value (Hamsten et al. 1987, Omland et al. 1994, Thompson et al. 1995, Haim et al. 2002). Furthermore, markers of systemic inflammation, including increased levels of C-reactive protein, are also associated with endothelial dysfunction in human subjects (Fichtlscherer et al. 2000, Hingorani et al. 2000, Prasad et al. 2002, Vita and Loscalzo 2002). These studies illustrate that identifying endothelial phenotype using systemic markers carries prognostic value. However, it remains unknown which individual marker or combination of markers will prove most useful.

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. 1998). The pathophysiological basis of endothelial dysfunction is still largely unknown, and some reports have even questioned whether there is any association between endothelial dysfunction and hypertension (Van Zwieten 1997).

Figure 1. The role of endothelial dysfunction in the pathogenesis of cardiovascular disease events.



1.2.1 Endothelium-derived vasodilatory factors

1.2.1.1 Nitric oxide

The pioneering experiments by Furchgott and Zawadski showed that presence of intact endothelium is essential for acetylcholine (ACh) to induce dilation of isolated arteries (Furchgott and Zawadski 1980). In contrast, if endothelium was removed, the arteries constricted in response to ACh. Subsequent studies revealed that ACh stimulated the release of a potent vasodilating substance by the endothelium, identified as NO (Ignarro et al. 1987, Furchgott 1996). When NO is lost – as after mechanical denudation of the endothelium or due to pathologic disease states affecting the endothelium – the normal vasodilator response to ACh is replaced by paradoxical constriction resulting from the direct effect of ACh on vascular smooth muscle (Ignarro et al. 1987, Furchgott 1996). In experimental models of vascular disease, increased superoxide production and the subsequent inactivation of NO, seems to be critically involved in reduced NO bioactivity and endothelial dysfunction (Ohara et al. 1993).

NO is generated by conversion of the amino acid L-arginine to NO and L-citrulline (Palmer et al. 1988) by the enzyme NOS. The isoform NOSIII (eNOS) is constitutively expressed by the endothelium (Behrendt and Ganz 2002). NO exerts its relaxing effect on vascular smooth muscle by activation of guanylate cyclase leading to increased production of cyclic guanosine 3',5'-monophosphate (cGMP) and a reduction in intracellular calcium (Behrendt and Ganz 2002). NO actively mediateds many of the functions exerted by intact endothelium. In addition to its potent vasodilating effect, NO counteracts leukocyte adhesion to the endothelium (Gauthier et al. 1995, Kubes et al. 1991), vascular smooth musle proliferation (Cornwell et al. 1994), and platelet aggregation (de Graaf etal. 1992). These biologic actions of NO emphasize its significance in protection against vascular injury, inflammation, and thrombosis, which are all key events involved throughout the process of atherosclerosis (Behrendt and Ganz 2002).

1.2.1.2 Prostacyclin

Prostacyclin (PGI_2) is a member of the PG family. PGI_2 causes vasodilatation, inhibits platelet aggregation (Busse et al. 1994) and also reduces VSMC proliferation *in vitro* (Weber et al. 1998). PG formation is initiated by the liberation of arachidonic acid (AA) from cell membrane phospholipids by phospholipase A_2. AA is converted into PGG_2 and PGH_2 by COX, and PGH_2 is then further converted into PGI_2 by PGI_2 synthase (Cohen 1995, Gryglewski 1995). PGI_2 is the major prostanoid produced in the vascular cells, although other

PGs are also synthesised in the endothelium (Busse et al. 1994). Production of vascular PGs can be blocked by non-steroidal anti-inflammatory drugs that inhibit COX, such as diclofenac (Vane and Botting 1993).

The vascular 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). The blood levels of PGI_2 are too low to have any general physiological effects, and therefore PGI_2 is regarded as a local rather than a circulating hormone (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 cyclic adenosine 3',5'-monophosphate (cAMP) (Busse et al. 1994). The increased intracellular cAMP leads to hyperpolarization of cell membrane, reduction of intracellular Ca^{2+} concentration, and to decreased sensitivity of contractile proteins to Ca^{2+} (Bülbring and Tomita 1987, Ushio-Fukai et al. 1993, Cohen and Vanhoutte 1995). Compared with the effects of NOS inhibition, blockade of COX has negligible impact on blood pressure (Ruoff 1998). Nevertheless, the inhibition of COX seems to enhance endothelium-mediated vasodilation in SHR as well as in essential hypertensive patients (Jameson et al. 1993, Taddei et al. 1993, Takase et al. 1994, Taddei et al. 1997). Moreover, the conversion of PGH_2 into PGI_2 seems to be diminished in the hypertensive rat endothelium, which results in accumulation of vasoconstrictor PGH_2 (Cohen 1995) and consequently imbalances the endothelial production of COX-derived vasodilator and vasoconstrictor factors. In humans, vasodilator prostanoids actively contribute to the maintenance of basal vascular tone, whereas vasoconstrictor products of COX limit the endothelium-dependent vasodilations (Campia et al. 2002). However, COX products do not appear to play a major role in the endothelial dysfunction of hypertensive patients (Campia et al. 2002).

1.2.1.3 Endothelium-derived hyperpolarizing factor

In various blood vessels, endothelium-dependent relaxations can be accompanied by the endothelium-dependent hyperpolarization of smooth muscle cells (Bolton et al. 1984, Félétou and Vanhoutte 1988, Taylor et al. 1988, Chen et al. 1988, Huang et al. 1988). These endothelium-dependent relaxations and hyperpolarizations can be partially or totally resistant to inhibitors of COXs and NO synthases (Garland and McPherson 1992, Nagao and Vanhoutte 1992), and can occur without an increase in intracellular levels of cyclic nucleotides in the smooth muscle cells (Taylor et al. 1988, Cowan and Cohen 1991, Mombouli JV et al. 1992). Therefore, the existence of an additional pathway that involved smooth muscle hyperpolarization was suggested and attributed to a non-characterized endothelial factor called endothelium-derived hyperpolarizing factor (EDHF) (Mcguire et al. 2001).

Hyperpolarization of smooth muscle induces relaxation by reducing both the open probability of voltage-dependent Ca^{2+} channels, and the turnover of intracellular phosphatidylinositides, thus decreasing the intracellular Ca^{2+} concentration {$[Ca^{2+}]_i$}(Nelson et al. 1990). As the blood vessel size decreases the contribution of EDHF-mediated responses to endothelium-dependent relaxations increases (Shimokawa et al. 1996), except for the coronary and renal vascular beds in which EDHF plays a major role in conduit arteries as well (Quilley et al 1997, Félétou and Vanhoutte 1999).

EDHF-mediated relaxations, in response to agonists that stimulate G-protein-coupled receptors, are associated with an increase in $[Ca^{2+}]_i$ in the endothelial cell (Johns et al. 1988, Lückhoff et al. 1988) and are also generated by substances that increase endothelial $[Ca^{2+}]_i$ in a receptor-independent manner (e.g. Ca^{2+} ionophores, and the sarcoplasmic reticulum Ca^{2+}-ATPase inhibitors) (Illiano et al. 1992, Fukao et al. 1995). Conversely, a decrease in the extracellular Ca^{2+} concentration attenuates EDHF responses (Chen and Suzuki 1990), suggesting that for EDHF-mediated responses, as for many other endothelial functions, the increase in endothelial $[Ca^{2+}]_i$ is a crucial step (Nilius and Droogmans 2001).

Previously, several studies have suggested that the endothelium-dependent hyperpolarization of the smooth muscle cells involved an increase in K^+ conductance and that EDHF was an endothelium-derived K^+ channel opener (Chen et al. 1988, Taylor et al. 1988, Chen and Suzuki 1989). This interpretation has been somewhat modified by new experimental observations. A hallmark of the EDHF-mediated response is its abolition by the combination of apamin [a specific inhibitor of K_{Ca} channels of small-conductance (SK_{Ca} channels)] plus charybdotoxin [a nonselective inhibitor of large-conductactance (BK_{Ca}) and intermediate-conductance (IK_{Ca}) channels, and of some voltage-dependent K^+-channels] (Corriu et al. 1996, Garland and Plane 1996, Zygmunt and Hoggestatt 1996). Further evidence from studies on endothelial and smooth muscle K^+ channels suggest that the toxin combination of apamin and charybdotoxin targets two types of K_{Ca} channels (IK_{Ca} and SK_{Ca} channels) expressed on endothelial cells (i.e. prevent endothelial cell hyperpolarization) rather than K^+ channels activated by an EDHF and located on smooth muscle cells. Iberiotoxin is a novel drug that selectively inhibits the BK_{Ca} of the VSMCs (Marchenko and Sage 1996, Cai et al. 1998, Edwards et al. 1998, Doughty et al. 1999, Edwards et al. 1999b, Edwards et al. 2000, Coleman et al. 2001, Burnham et al. 2002,).

In recent years, the most popular candidates for EDHF have been the arachinoid-acid-derived products of cytochrome P450 epoxygenases (fibrates induce cypP450 in Dahl rats), namely epoxyeicosatrienoic acids (EETs) (Cohen and Vanhoutte 1995, Garland et al. 1995, Campbell et al. 1996, Vanhoutte and Mombouli 1996, Fisslthaler et al. 1999, Bolz et al. 2000, Coats et al. 2001, Archer et al. 2003). They are produced by the endothelium, released in response to vasoactive hormones and elicit vasorelaxation via stimulation of K_{Ca} (Quilley et al. 1997) and therefore appear to play an important role in the regulation of vascular

homeostasis (Fleming et al. 2001). Moreover, hyperpolarization of endothelial cells might be partly regulated by activation of the cytochrome P450, because EETs might modulate endothelial Ca^{2+} influx in response to Ca^{2+}-store depletion (Hoebel et al. 1997) and might facilitate the activation of endothelial K^+ channels by increasing their sensitivity to Ca^{2+} (Baron et al. 1997, Li and Campbell 1997). Thefore, EETs and other products of cytochrome P450 seem to be crucial for the initiation and transmission of endothelial cell hyperpolarization, and consequently to EDHF-mediated hyperpolarization and relaxation of VSMCs.

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. 1999a). It has also been suggested that the number of these heterocellular myo-endothelial gap junctions increases with the diminution in the size of the artery (Sandow and Hill 2000), a finding that would support the view of more significant contribution of EDHF to the control of vascular tone in smaller arteries (Shimokawa et al. 1996).

A moderate increase in the myo-endothelial K^+ concentration can induce the hyperpolarization of VSMCs by activating the inward rectifier K^+ channels (K_{IR}) and the Na^+-K^+-ATPase (Nelson and Quayle 1995, Prior et al. 1998). Therefore, the K^+ ion itself has also been suggested to be EDHF in small mesenteric resistance arteries of rats (Edwards et al. 1998, Dora and Garland 2001), although not all investigators are convinced of this claim (Doughty et al. 2000, Lacy et al. 2000). Thus, 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). Previously, 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 and renal failure, which may feature decreased bioavailability of endothelium-derived NO, EDHF-mediated vasorelaxation could be enhanced and of greater importance than under physiological conditions (Bauersachs et al. 1996). However, this hypothesis has not been supported by reports where decreased endothelium-mediated hyperpolarization was observed in many forms of experimental hypertension (Fujii et al. 1992, Van de Voorde et al. 1992, Mäkynen et al. 1996, Sunano et al. 1999).

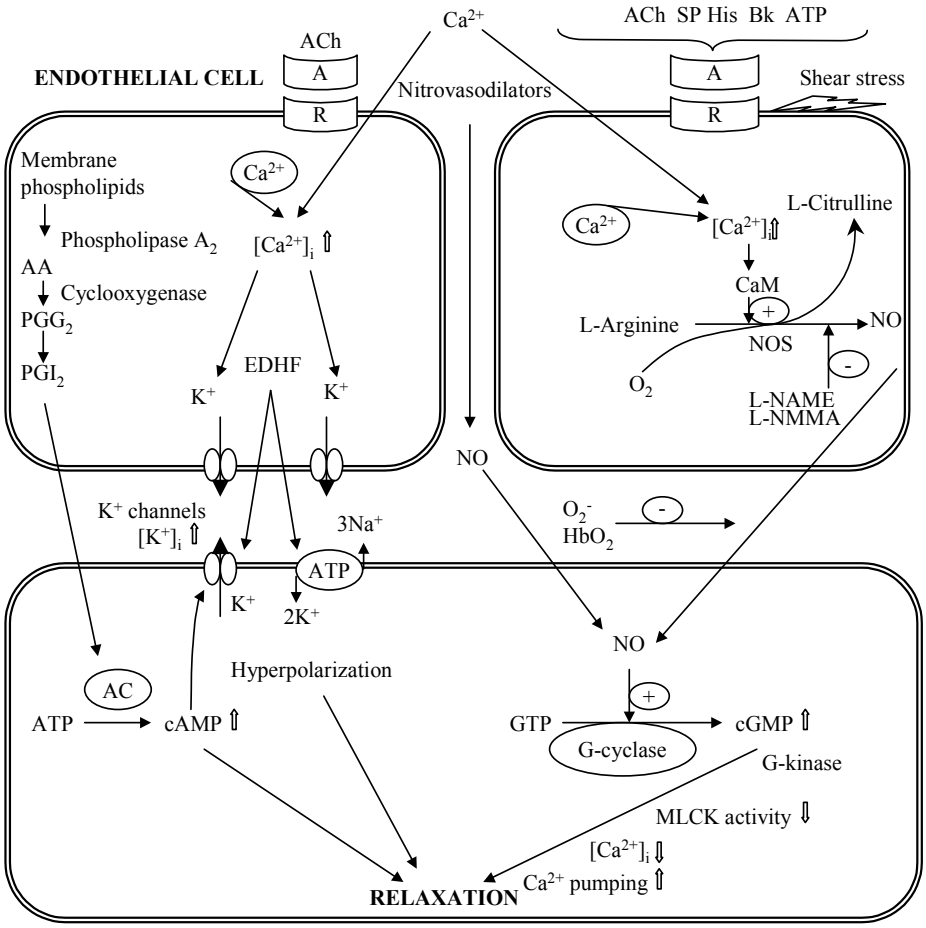


Figure 2. Schematic diagram shows major mechanisms of endothelium-dependent arterial relaxation. Abbreviations: A, agent; AA, Arachidonic acid; AC, adenylate cyclase; ACh, Acetylcholine; ATP, adenosine 5'-triphosphate; Bk, Bradykinin; $[Ca^{2+}]_i$, intracellular free calcium concentration; $[K^+]_I$, free potassium concentration; cAMP, cyclic adenosine 3',5'-monophosphate; cGMP, cyclic guanosine 3',5'-monophosphate; CaM, calmodulin; Ca^{2+} pump, Ca^{2+}-Mg^{2+} ATPase; EDHF, endothelium-derived hyperpolarizing factor; G-cyclase, guanylyl cyclase; GTP, guanosine 5'-triphosphate; Hb, haemoglobin; His, histidine; L-NAME, N^G-nitro-L-arginine; L-NMMA, N^G-monomethyl-L-arginine; MLCK, myosin light chain kinase; NO, nitric oxide; NOS, nitric oxide synthase; PGH_2, prostaglandin G_2; PGI_2, prostacyclin; R, receptor; SP substance P. -, inhibition; +, stimulation; ⇧ , increase; ⇩, decrease.

1.2.2 Endothelium-derived contractile factors

The control of blood vessel wall homeostasis is achieved via production of vasorelaxants and vasoconstrictors. 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 contractile 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, Schiffrin 2001).

1.2.2.1 Cyclooxygenase-derived contractile factors

COX produces several EDCFs, the most potent of which are endothelial thromboxane A_2 and the PG endoperoxide intermediates PGG_2 and PGH_2, which all act via the same receptor (Cohen 1995). In SHR several agonists including ACh and 5-hydroxytryptamine can induce the release of COX-derived contractile factors (Lüscher and Vanhoutte 1986, Auch-Schwelk and Vanhoutte 1991, Ito and Carretero 1992), whereas in Wistar-Kyoto (WKY) rats such responses seem to be absent (Lüscher and Vanhoutte 1986, Jameson et al. 1993). Production of vascular COX-derived contractile factors can be blocked by non-steroidal anti-inflammatory drugs that inhibit COX, such as diclofenac (Auch-Schwelk and Vanhoutte 1991).

Studies in SHR and in patients with essential hypertension have suggested 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. 1997). However, the blockade of thromboxane A_2/PG endoperoxide receptor has no or only a marginal effect on systemic blood pressure in SHR or in patients with essential hypertension (Ritter et al. 1993, Tesfamariam and Ogletree 1995, Ruoff 1998). 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. 1997). 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. 1997).

2.2.2 Endothelin-1

ETs (ET-1, ET-2, ET-3) are vasoconstrictor peptides that are synthesised from larger precursors, preproendothelins via the formation of proendothelin, which is further cleaved to form the mature ET-1 by ET converting enzymes (Haynes and Webb 1998). Of the three forms characterized the endothelial cells only seem to produce ET-1 (Lüscher et al. 1992, Agapitov and Haynes 2002). ET is formed from the precursor preproendothelin via the formation of proendothelin (big ET). Whether the release of ET requires *de novo* intracellular

protein synthesis initiated by physiological stimuli or chemical substances remains to be elucidated (Kuchan and Frangos 1993, Lüscher et al. 1993a, Lüscher et al. 1993b, Vanhoutte 1993, Lüscher and Noll 1995). Stretching of the vascular wall, hypoxia, low shear stress and substances such as NA, Ang II and thrombin seem to induce the production of ET, whereas high shear stress and endothelial formation of cGMP and cAMP have been shown to inhibit the synthesis of ET (Kuchan and Frangos 1993, Lüscher et al. 1993b, Lüscher and Noll 1995).

Endothelium-derived ET-1 is a potent vasoconstrictor, which acts through smooth muscle ET_A and ET_B receptors that mainly mediate vasoconstriction, and endothelial ET_B receptors that oppose ET_A- and ET_B –mediated vasoconstriction by stimulating NO and PGI_2 formation (Lüscher et al. 1993a, Nava and Lüscher 1995, Taddei et al. 2000). The intracellular mechanims of action of ETs involve activation of phosphatidylinositol metabolism, mobilization of intracellular Ca^{2+} stores, promotion of calcium entry through plasmalemmal Ca^{2+} channels and protein kinase C (Schiffrin 1995a, Black et al. 2003).

Most studies in essential hypertension have reported normal or slightly elevated plasma ET levels, which may be due to the fact that most of the ET-1 is released from the endothelium abluminally towards the vascular smooth muscle rather than into the lumen and circulation (Wagner et al. 1992, Lüscher et al. 1993b, Schiffrin 1995b). Furthermore, even subthreshold concentrations of ET-1 potentiate the effect of other vasoconstrictors such as 5-hydroxytryptamine and NA (Lüscher et al. 1993c). Therefore, the concentration of circulating ET-1 may not accurately reflect its role as a local modulator of vascular tone (Vanhoutte 1993a).

In experimental hypertension the findings concergning the influence of ET-1 on both blood pressure and vascular reactivity have been somewhat inconclusive (Vanhoutte 1993, Nava and Lüscher 1995, Schiffrin 1995b). Although there is evidence for a role of ET-1 in blood pressure elevation in some experimental forms of hypertension, particularly sodium-sensitive hypertension, the significance of ET-1 may be more in accentuating rather than initiating blood pressure elevation (Schiffrin 2001a). However, some studies suggest that hypertensive rats treated with ET antagonists are protected from stroke and renal injury (Schiffrin 2001b). Furthermore, in essential hypertensive patients, the activity of exogenous ET-1 is either increased, similar or decreased as compared to normotensive subjects, depending on which vascular district or scheme of administration is considered (Taddei et al. 2001). However, recent studies suggest that essential hypertension is characterized by increased ET-1 vasoconstrictor tone (Taddei et al. 2001). ET-1 also features a range of other local actions – in the kidney, the nervous system and other hormone systems – that could play a part in the genesis of hypertension (Goddard and Webb 2000). Therefore, potentially the ET antagonists could become effective disease-modifying agents in different forms of cardiovascular disease (Schiffrin 2001b).

1.3 Vascular smooth muscle

1.3.1 Contraction

Contraction of vascular smooth muscle is a complex process that is initiated when vasoconstricting neurotransmitters or hormones activate the cell surface receptors. Most receptors activate different types of guanosine 5'-triphosphate-binding proteins (G proteins) that are coupled to different ion channels and enzymes, and regulate their activities. Among these enzymes are phospholipase C, which metabolises phosphatidylinositol 4,5-bisphosphate and produces inositol 1,4,5-trisphosphate (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 store 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-hydroxytryptamine, have been shown to depolarize the arterial smooth muscle and consequently activate voltage-operated Ca^{2+} channels in the plasma membrane of the smooth muscle, leading to an increased influx of Ca^{2+}. The activation of the described mechanisms increases $[Ca^{2+}]_i$, which is the primary signal for smooth muscle contraction (Allen and Walsh 1994).

Following the elevation of $[Ca^{2+}]_i$, Ca^{2+} binds to calmodulin and forms a Ca^{2+}-calmodulin complex, which removes the autoinhibition of myosin light chain kinase (Allen and Walsh 1994). The activated myosin light chain kinase 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). Should the $[Ca^{2+}]_i$ fall, the myosin light chain kinase is inactivated, which allows dephosphorylation of myosin by myosin light chain phosphatase and causes the smooth muscle to relax (Stull et al. 1991, Cirillo et al. 1992, Rembold 1992, Allen and Walsh 1994). However, the contractile force does not 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 mechanisms that change the Ca^{2+} sensitivity, together with the major regulatory mechanisms 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).

A multitude of studies in experimental hypertension have demonstrated increased receptor-mediated arterial smooth muscle contractility in these models (Perry and Webb 1991, Brodde and Michel 1992, Orlov et al. 1993). Nevertheless, some investigations have detected only slight differences in the contractile responses between hypertensive and normotensive

animals (Bockman et al. 1992, Tolvanen et al. 1996). Possible mediators for the increased vascular contractility in experimental hypertension include enhanced responsiveness of G proteins (Kanagy and Webb 1994), 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). Furthermore, the generation of DAG stimulated by vasoconstrictor agents and subsequent activation of protein kinase C have been reported to contribute to the enhanced vascular responsiveness (Okamura et al. 1992, Nguyen et al. 1993, Nahorski et al. 1994). Thus, an overactive receptor-mediated contraction pathway could contribute to the pathogenesis of hypertension.

1.3.2 Cellular calcium regulation

Numerous cellular functions are highly influenced by Ca^{2+} metabolism. These include VSMC growth and proliferation as well as contraction of vascular smooth muscle, which is initiated by increased $[Ca^{2+}]_i$ (Karaki and Weiss 1988). There are both extracellular and intracellular Ca^{2+} stores in the vascular smooth muscle (Cirillo et al. 1992), and therefore 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 maintain 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 Ca^{2+} permeability of SR is controlled by second messangers (Van Breemen and Saida 1989).

Under physiological conditions the Ca^{2+} influx across the plasmalemma takes place either via ion channels or exchangers. The Ca^{2+} channels in plasmalemma are either voltage-gated or receptor-operated (Horowitz et al. 1996). The voltage-gated Ca^{2+} channels have been sorted by electrophysiological and pharmacological techniques into two different subgroups: one type is activated by small depolarizations and is rapidly inactivated (T-type), whereas the other requires stronger depolarizations and is more slowly inactivated (L-type) (Spedding and Paoletti 1992). Evidence suggests that sustained depolarization of smooth muscle underlies the increase in arterial tone during hypertension by increasing the open probability of voltage-dependent L-type Ca^{2+} channels (Wellman et al. 2001), which increases $[Ca^{2+}]_i$ and contributes to vasoconstriction (Knot and Nelson 1998, Amberg et al. 2003). The L-type channels can be selectively blocked by dihydropyridine Ca^{2+} channel antagonists like nifedipine, and the T-type channels by mibefradil (Nelson et al. 1990, Spedding and Paoletti 1992, Mishra and Hermsmeyer 1994). Some Ca^{2+} ions enter the VSMCs also due to the passive permeability of the plasma membrane to Ca^{2+} (Cirillo 1992). Furthermore, the plasma membrane binds Ca^{2+} and buffers increases in $[Ca^{2+}]_i$, and it may become less permeable to Ca^{2+} following an increase in the extra- and intracellular $[Ca^{2+}]_i$ (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+} pump or the Na^+/Ca^{2+} exchanger (Allen and Walsh 1994, Horowitz et al. 1996). The Ca^{2+} pump uses energy from 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).

The SR plays a major role in Ca^{2+} storage in VSMCs, although special Ca^{2+} binding molecules are also present (Karaki and Weiss 1988, Horowitz et al. 1996). Ca^{2+} is actively sequestered and released by SR following plasmalemmal receptor activation (Minneman 1988, Martonosi et al. 1990, DeLong and Blasie 1993). Activation of cell surface receptors forms IP_3, which 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, where 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 this mechanism 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).

Recently, Ca^{2+} oscillations and gradients in vascular smooth muscle have been studied more closely due to the physiological phenomenom of Ca^{2+} ion selectively triggering varying responses in the same cell, for instance Ca^{2+} -mediated responses being different in smooth muscle cells located at different sites (Lee et al. 2002). This is suggested to result from temporal fluctuations and spatial variations of cytoplasmic $[Ca^{2+}]_i$, which depend on the interaction of ion transport proteins of plasma membrane and membranes of SR, nuclear envelope and mitochondria (Lee et al. 2002). All smooth muscle $[Ca^{2+}]_i$ oscillations depend on plasma membrane-SR interactions, but there are two fundamentally different types of $[Ca^{2+}]_i$ oscillations, depending on their immediate source of Ca^{2+}. When $[Ca^{2+}]_i$ rises more or less evenly across the entire cell, no apparent Ca^{2+} waves are observed (Peng et al. 2001), but when the endoplasmic reticulum /SR is the immediate Ca^{2+} source for each Ca^{2+} spike, $[Ca^{2+}]_i$ initially rises in a specific cellular locus, and this regional elevation in $[Ca^{2+}]_i$, propagates in a wagelike fashion throughout the length of the cell (Lee et al. 2001). In VSMCs, both non-wavelike and wavelike $[Ca^{2+}]_i$ oscillations are observed.

The vascular smooth muscle regulates blood flow through selective vasoconstriction and vasomotion, of which the latter is associated with $[Ca^{2+}]_i$ oscillations and the tonic contraction has been thought to be initiated by SR Ca^{2+} release and then maintained by elevated Ca^{2+} influx (Lee et al. 2002). However, confocal microscopy has shown that in many blood vessels agonist-induced contractions are maintained by asynchronous wavelike $[Ca^{2+}]_i$ oscillations in single smooth muscle cells, which summate to give a steady-state elevation in $[Ca^{2+}]_i$ for the whole tissue (Ruehlmann et al. 2000). Moreover, the asynchronous wavelike $[Ca^{2+}]_i$ oscillations appear to be instrumental in the initiation of vasomotion in the rat mesenteric artery (Peng et al. 2001). Ca^{2+} waves have also been associated with the induction of dilatation of cerebral resistance arteries, where the wavelike Ca^{2+} release is thought to stimulate K_{Ca} on the plasma membrane and the relaxing effect of the resulting hyperpolarization-induced closing of voltage-gated Ca^{2+} channels outweighs the local

stimulation of contraction (Jaggar 2001). This dual function of Ca^{2+} waves presents an intriguing yet a complex example of vascular heterogeneity. It seems that the manner in which localized Ca^{2+} signals are coupled to either contraction or relaxation is to a large extent determined by the specific ion pumps and channels contained within the plasma membrane – SR junctional complexes (Lee et al. 2002).

Abnormally high $[Ca^{2+}]_i$ has been found in blood cells, cultured aortic and mesenteric arterial smooth muscle cells, and 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. 1993b, Ishida-Kainouchi et al. 1993), but not all reports confirm this abnormality (Liu et al. 1994, Neusser et al. 1994). Importantly, studies on VSMCs from resistance arteries have exhibited comparable basal $[Ca^{2+}]_i$ between SHR and WKY rats (Storm et al. 1992, Bukoski et al. 1994, Bian and Bukoski 1995), suggesting that the elevations in $[Ca^{2+}]_i$ found in aortic smooth muscle cells and in other cell types of hypertensive animals are unlikely to contribute to the heightened peripheral vascular resistance in SHR (Dominiczak and Bohr 1990, Bian and Bukoski 1995).

Contractile responsiveness of VSMCs from conduit arteries of SHR are enhanced to depolarization and Bay K 8644, an agonist of dihydropyridine-sensitive Ca^{2+} channels, when compared with WKY (Aoki and Asano 1986, Aoki and Asano 1987, Bruner and Webb 1990). Furthermore, augmented 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 may be attributed to enhanced sensitivity of dihydropyridine receptors in the Ca^{2+} channels in SHR, while the opening properties of a single Ca^{2+} channel have been suggested to be unaltered (Kubo et al. 1998). These results indicate that Ca^{2+} entry through voltage-operated Ca^{2+} channel 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 increased peripheral resistance and the genesis of hypertension (Arii et al. 1999).

In patients with essential hypertension and in SHR, less Ca^{2+} seems to be bound to the plasma membrane, and the Ca^{2+} permeability of the 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 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 also 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).

Collectively, 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, of the extrusion of Ca^{2+} must be decreased. There is no clear evidence whether any of these abnormalities are present in hypertensive VSMCs (Gonzales and Suki 1995). Finally, it has been suggested that one more 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).

1.3.3 Na^+-K^+-ATPase

The Na^+-K^+-ATPase carries out the coupled extrusion and uptake of Na^+ and K^+ across the plasma membrane of VSMCs (Kaplan 2002). The Na^+-K^+-ATPase creates a significant part of the membrane potential in VSMC by extruding three Na^+ ions for every two K^+ ions pumped inwards, consequently generating a hyperpolarizing current (Kaplan 2002). Cardiac glycosides such as digoxin and ouabain inhibit vascular Na^+-K^+-ATPase (Blaustein 1993, O'Donnell and Owen 1994). Na^+-K^+-ATPase activity is suggested to influence $[Ca^{2+}]_i$ in VSMC via voltage-operated Ca^{2+} channels and Na^+/Ca^{2+} exchange. Therefore, decreased Na^+-K^+-ATPase activity leads to membrane depolarization and increased Ca^{2+} influx through voltage-operated Ca^{2+} channels. 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).

Disturbed function of Na^+-K^+-ATPase has been suggested to contribute to the pathogenesis of both essential and experimental hypertension (Hermsmeyer 1987), but reports on this matter remain controversial. Na^+-K^+-ATPase activity 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 decreased activity of Na^+-K^+-ATPase in hypertension could be an inherent defect of a circulating digitalis- or ouabain-like inhibitor of the sodium pump (Blauestein, Hamlyn et al. 1996). The plasmas from patients with essential hypertension have been proposed to contain ouabain-like factors (Masugi et al. 1987), and the concentrations of these Na^+-K^+-ATPase inhibitors may be elevated, 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 arteries, thus the interaction of digitalis-like Na^+,K^+-pump inhibitors with

the Na^+-K^+-ATPase could be of importance (Bagrov and Fedorova 1998), but this remains unclear (Pidgeon et al. 1996). In both rats and humans, reports on the effects of ouabain on blood pressure have been inconsistent and therefore it is not known whether the inhibition of Na^+-K^+-ATPase actually elevates blood pressure (Pidgeon et al. 1996).

The function of Na^+,K^+-pump affects nucleid acid synthesis and smooth muscle cell proliferation, which could contribute to vascular remodelling (Orlov et al. 2001). Moreover, vascular superoxide seems to impair smooth muscle cell Na^+-K^+-ATPase activity (Gupta et al. 2002), which could be of importance in both hypertension and renal failure. Previously, increased activity of 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 due to an increase in passive membrane permeability of Na^+, and since the pump does not appear to fully compensate for this, the vascular tone may be elevated (O'Donnell and Owen 1994). 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 affect these results. Thus, it remains to be clarified whether the altered Na^+-K^+-ATPase activity in VSMCs is a key factor in hypertension (O'Donnell and Owen 1994).

1.3.4 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, Jackson 2000). Potassium channels are the dominant ion conductive pathways in VSMCs (Nelson and Quayle 1995, Jackson 1998), therefore significantly contributing to the determination and regulation of smooth muscle membrane potential, which in turn controls $[Ca^{2+}]_i$ and thus contraction of vascular smooth muscle (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 and blood pressure (Standen and Quayle 1998).

VSMCs express at least four different types of K^+ channels in their plasma membranes (Nelson and Quayle 1995, Jackson 1998). The identified subtypes of K^+ channels in vascular smooth muscle are ATP-sensitive K^+ channels (K_{ATP}), K_{Ca}, voltage-dependent K^+ channels, and K_{IR} channels (Jackson et al. 1997, Jackson 1998, Jackson and Blair 1998, Jackson 2000). The K_{ATP} channels were first found in cardiac myocytes (Noma 1983) and they close as intracellular ATP concentration increases. The open state probability of these channels increases via elevation in cytosolic adenosine 5'-diphosphate or intracellular acidification (Nelson 1993, Ishizaka 1999). However, K_{ATP} are also regulated by several signal transduction pathways independent from changes in ATP. Glibenclamide, a selective K_{ATP} channel blocker, causes arterial constriction in a number of species, including humans,

whereas K_{ATP} channel agonists such as cromakalim, levcromakalim, diazoxide and pinacidil dilate arteries (Quast et al. 1994, Jackson 2000). Functional experiments have suggested that there are open K_{ATP} channels in the VSMCs, and this has been confirmed by electrophysiological measurements (Jackson et al. 1997). 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). An impaired action of levcromakalim on K_{ATP} was found in SHR, which was restored by the normalisation of blood pressure. This suggests that the impairment can be attributed to high blood pressure (Ohya et al. 1996).

K_{Ca} channels are found in most cells and are activated by increases in $[Ca^{2+}]_i$ and membrane depolarization (Nelson and Quayle 1995, Carl et al. 1996). K_{Ca} are divided into large, intermediate and small according to their conductance. Large-conductance K_{Ca} (BK_{Ca}) seem to be the most important K^+ channels in the regulation of arterial tone (Amberg et al. 2003). In arteries that display myogenic tone, activity of BK_{Ca} channels has been reported to contribute to resting membrane potential: blockade of the channels with iberiotoxin (selective inhibitor of BK_{Ca}), charybdotoxin (non-selective inhibito of BK_{Ca}), iberiotoxin or tetraethylammonium (non-selective K^+ channel inhibitor) ions leads to membrane depolarization and vasoconstriction. The small-conductance K_{Ca} can be blocked by apamin (Nelson et al. 1990, Brayden and Nelson 1992, Kitazono et al. 1995, Nelson and Quayle 1995, Nelson et al. 1995). BK_{Ca} channels seem to play a central role in the regulation of vascular tone due to focal increases in subsarcolemmal Ca^{2+} (i.e. Ca^{2+} sparks) by Ca^{2+} released through ryanodine receptors in the SR (Nelson et al. 1995, Jaggar et al. 1998, Knot et al. 1998, Amberg et al. 2003). Furthermore, BK_{Ca} channels play a negative feedback role to limit active vasoconstriction and prevent vasospasm (Jackson 2000, Amberg et al. 2003). BK_{Ca} channels may be activated by vasodilators acting through the cGMP and cAMP cascades (Nelson and Quayle 1995, Paterno et al. 1996), epoxides of AA (Campbell et al. 1996), and carbon monoxide (Wang and Wu 1997). Furthermore, vasodilators and vasoconstrictors may influence the frequency and amplitude of Ca^{2+} sparks and thus effect BK_{Ca} channel activity (Jaggar et al. 1998, Porter et al. 1998, Amberg et al. 2003). Expression of BK_{Ca} channels in membranes of VSMCs was found to be increased in hypertension (Liu et al. 1997b,) which has been suggested to occur as a negative feedback response to the increased vascular reactivity observed in hypertension (Rusch and Liu 1997). However, it was recently reported that the expression of the ß1 subunit of the BK_{Ca} channels is downregulated in hypertension (Amberg et al. 2003) warranting future studies on the expression of BK_{Ca} channels in the VSMCs.

Voltage-activated K^+ channels, inhibited by 4-aminopyridine, are another ubiquitous class of K^+ channels expressed by VSMCs (Nelson and Quayle 1995). These channels are activated by membrane depolarization with threshold potentials for substantial activation of -30mV, and they may participate in the regulation of resting membrane potential and vascular tone (Nelson and Quayle 1995, Jackson et al. 1997, Jackson 1998). Their role in vivo has not been explored, largely because of the lack of availability of inhibitors selective for the channels expressed in vascular muscle cells. However, electrophysiological studies indicate a

decreased functional expression of voltage-dependent K^+ channels in vascular muscle cells from hypertensive animals, which may contribute to depolarization and predispose to increased vascular tone in hypertension (Martens and Gelband 1996). K_{IR} pass inward K^+ current much more readily than outward current with physiological ion gradients and also show a parallel rightward shift in the potential at which rectification appears (activation potential) and a large increase in conductance with increases in the extracellular K^+ concentration (Jackson 2000). The role of K_{IR} in the regulation of resting membrane potential and smooth muscle tone remains somewhat unclear. Some reports suggest that K_{IR} channels mediate K^+-induced vasodilation in cerebral and coronary resistance arteries (Nelson and Quayle 1995, Knot et al. 1996, Quayle et al. 1997), but the role if K_{IR} in K^+-induced vasodilation of skeletal muscle arteries is yet to be clarified.

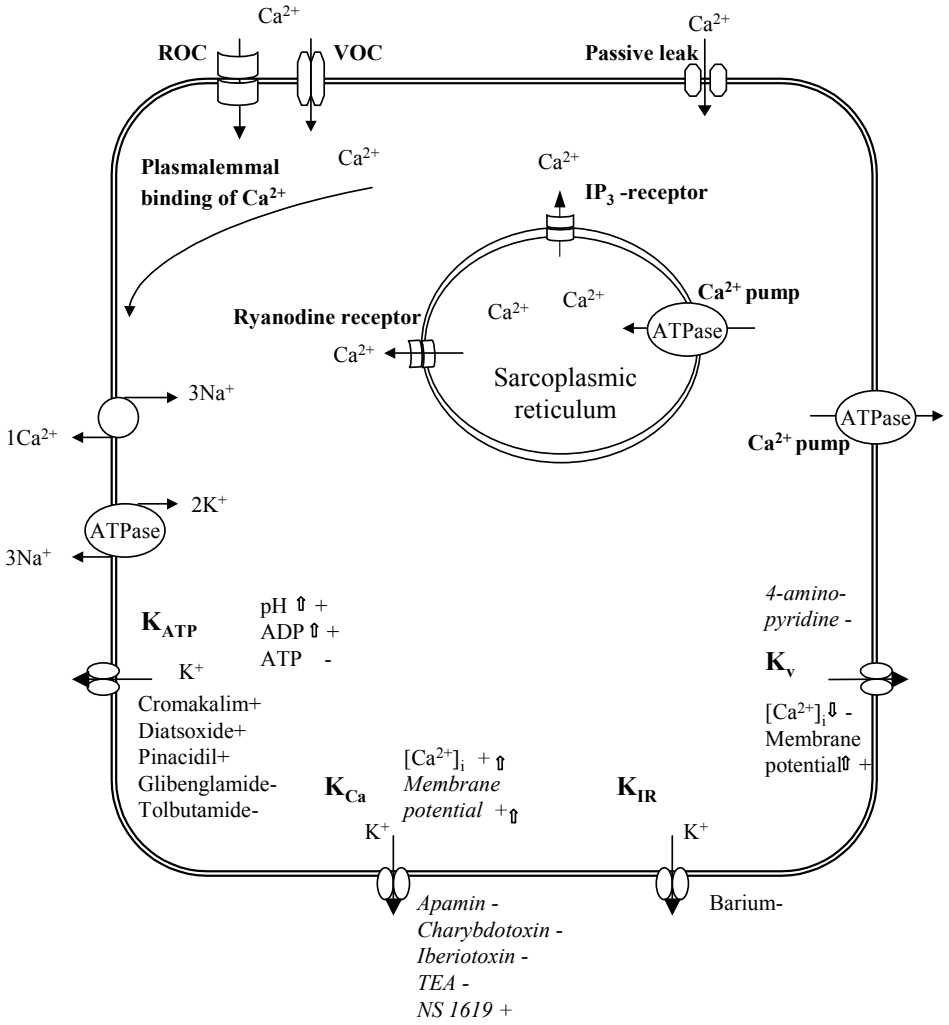


Figure 3. Schematic diagram shows the major mechanisms involved in the cellular Ca^{2+} regulation and some physiological and pharmacological properties of smooth muscle K^+ channels. Abbreviations: ADP, adenosine 5'-diphosphate; ATP, adenosine 5'-triphosphate; Ca^{2+} pump, Ca^{2+}-Mg^{2+} ATPase; IP_3 , inositol 1,4,5-trisphosphate; ROC, receptor-operated calcium channel; VOC, voltage-operated calcium channel; $[Ca^{2+}]_i$, intracellular free calcium concentration, E_K, potassium equilibrium potential; K_{ATP}, ATP-sensitive K^+ channel; K_{Ca}, Ca^{2+}-activated K^+ channel; K_{IR}, inward rectifier K^+ channel; K_V, voltage-dependent K^+ channel; TEA, tetraethylammonium; V_m, membrane potential. -, inhibition; + stimulation; ⇧ , increase; ⇩ , decrease.

2 Arterial tone and structure in experimental hypertension and renal failure

2.1 Hypertension induced by NO-deficiency

Variants of the endothelial NOS gene may be associated with elevated blood pressure (Wang et al. 1997), although some reports do not support this view (Kato et al. 1999, Takami et al. 1999). However, sufficient production of NO in the vascular endothelium seems to be essential for the maintenance of normal blood pressure (Huang et al. 1995), and defects either in the production or action of NO are likely to be associated with essential hypertension (Moncada and Higgs 1993). Therefore, chronic inhibition of NOS by L-NAME is theoretically an interesting model of experimental hypertension (Baylis et al. 1992). Previously, chronic administration of L-NAME has been shown to result in sustained and dose- and time-dependent hypertension in normotensive rats (Baylis et al. 1992, Deng et al. 1993, Arnal et al. 1992, Ribeiro et al 1992, Zatz and Baylis 1998). Furthermore, inhibiting NOS by L-NAME consistently leads to impaired endothelium-dependent arterial relaxations (Küng et al. 1995, Dowell et al. 1996, Henrion et al. 1996, Henrion et al. 1997, Kalliovalkama et al. 1998) as well as arterial remodelling (Deng et al. 1993, Li and Schiffrin 1994, Takemoto et al. 1997, Kalliovalkama et al. 1998, Sakamoto et al. 2002). Data regarding the endothelium-independent arterial relaxations and vascular contractility during NO-deficiency appear somewhat inconsistent (Küng et al. 1995, Dowell et al. 1996, Henrion et al. 1996, Kalliovalkama et al 1998). L-NAME hypertension has also elicited changes in cardiac chamber geometry, myocardial diastolic mechanical properties and increased the amount of myocardial fibrosis (Akuzawa et al. 1998, Matsubara et al. 1998, Bernatova et al 2002). These alterations typically lead to left ventricular hypertrophy, which may further be augmented by metabolic disturbances and ultrastructural alterations such as increased permeability of the cardiac capillaries followed by extracellular and mitochondrial edema (Tribulova et al. 2000, Bernatova et al. 2002). Moreover, in rats exposure to chronic administration of L-NAME seems to produce nephrosclerosis and hyaline arteriopathy as well as impair renal function, which resemble the changes and complications caused by advanced essential hypertension (Hropot et al. 1994, Akuzawa et al. 1998, De Gracia et al. 2000).

The pathogenesis of cardiovascular remodeling in this model has been suggested to result from increased oxidative stress in the endothelium (Usui et al. 1999). Excessive production of superoxide radicals may result from local vascular RAS activation and, in particular, from an increase in local ACE expression in rats with long-term NOS inhibition (Takemoto et al. 1997, Usui et al. 1999). Blockade of the synthesis of NO seems also to upregulate the expression of vascular endothelial growth factor in the coronary arterial smooth muscle cells, which potentially could contribute to the increased wall-to-lumen ratios (Sakamoto et al. 2002). 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). There is also evidence that the autonomic nervous system plays a major role in the L-NAME–induced hypertension via both sympathetic overactivity and vagal suppression (Cunha et al. 1993, Souza et al. 2001). It is of note, that 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 involvement of Rho-kinase in vascular remodeling in L-NAME hypertension was reported, which is of interest because the Rho-kinase pathway has been demostrated to play a role in various models of vascular disease (Ikegaki et al. 2001). Nevertheless, since the exact role of diminished NO production in human hypertension is not absolutely solved, it remains to be established whether NOS inhibition is a relevant experimental model of human forms of hypertension.

2.2 Hypertension induced by NaCl

The causal relation between salt intake and high blood pressure has been widely recognized, but the exact pathophysiological mechanisms and the reason for this relation are less clear (Law et al. 1991, MacGregor and Sever 1996, Cutler et al. 1997, Kurokawa 2001). Blood pressure is affected by dietary salt in many, but not in all subjects (Dustan et al. 1986, Weinberger et al.1986, Haddy 1991). Nevertheless, a large international study showed that the amount of salt consumed related to blood pressure levels, both within populations and between populations, and was responsible for much of the increase in blood pressure that occurs with increasing age in Western populations (Elliot et al. 1996). Moreover, a Finnish prospective study suggested that high sodium intake predicts mortality and risk of coronary heart disease, independent of other cardiovascular risk factors, including blood pressure (Tuomilehto et al. 2001). Intervention studies have also demonstrated the importance of salt intake in regulating human blood pressure (Forte et al. 1989, Geleijnse et al. 1997). An experiment in chimpanzees, a group of which were fed their natural diet, i.e. similar to that which humans and chimpanzees ate during evolution (10 mmol sodium per day), was compared to a group where salt intake was increased to the same amount that we now consume, 150-200 mmol per day. Over the 2 years of the study, there was a progressive increase in blood pressure (systolic blood pressure increasing by >30 mmHg) in those chimpanzees on the higher salt intake diet (Denton et al. 1995). Human and animal studies have shown a genetic predisposition to develop hypertension in response to an increase in dietary NaCl intake (Sanders 1996). Dissection of the etiologic factors causing salt sensitivity has proved difficult in large, genetically heterogenous populations of rats and humans. For this reason, inbred models of hypertension (such as the Dahl rats or transgenic mice) have

been used to examine the mechanism of salt sensitivity (Sanders 1996). In humans, the African-American population has a disproportionately high age-adjusted prevalence of hypertension and many are particularly sensitive to dietary salt (Burt et al. 1995).

In a physiological sense, almost all NaCl is present in the extracellular fluid (ECF) and NaCl is the main determinant of ECF tonicity. Therefore, extracellular fluid volume (ECFV) is determined by the NaCl content of the body, and thus varies with salt intake (Kurokawa 2001). In rats, the expansion of plasma and blood volume as well as of total body water seems to be greater in the young animals subjected to salt-loading than of those in adulthood (Zicha and Kunes 1999). The renin-angiotensin-aldosterone-system (RAAS) participates in the regulation of ECFV by reacting to changes in ECFV and leading to reabsorption of Na^{2+} to maintain ECFV (Kurokawa 2001). The RAAS is activated in states of low salt intake and suppressed by high salt intake (Aguilera and Catt 1981, Schmid C et al. 1997). Renal renin activity is suppressed to a greater extent by high salt intake in younger rats, and the inactivation of renal RAS is accompanied by decreased resistance in the afferent arterioles which leads to increased glomerular capillary pressure predisposing to glomerular damage (Dworkin et al. 1984, Jelínek et al. 1990). Moreover, as the ECFV increases during salt loading, more shear stress subsequently acts in parallel to the blood vessel surface activating the endothelium and altering the expression of various genes in the arterial wall (Ying and Sanders 2002). There is also evidence suggesting that a neurogenic mechanism is involved in salt hypertension, via an early interaction between vasopressinergic and adrenergic neurons in the central nervous system. This leads to a persistent hyperadrenergic state, which together with a suggested baroreceptor reflex dysfunction serve as neurogenic alterations that increase peripheral resistance and further amplify the salt-induced hypertension (Gavras and Gavras 1989, Tanoue et al. 2002).

Both structural changes of the vascular bed, including vascular rigidity and hypertrophy of large arteries, and abnormal control of vascular tone contribute to the hemodynamic effects exerted by increased salt intake (Zicha and Kunes 1999, Et-taouil et al. 2001). High salt intake results in changes in the microvascular structure and function even in the absence of increased arterial blood pressure (Boegehold 2002). Moreover, endothelium-dependent vasodilation and vascular smooth muscle hyperpolarization are impaired by high salt diet (Sylvester et al. 2002, Lombard et al. 2003) and salt loading is reported to downregulate various NOS isotypes, including endothelial, which could contribute to the development and maintenance of elevated peripheral resistance (Ni and Vaziri 2001). Due to the many age-related abnormalities of BP regulation in experimental NaCl hypertension, it should be noted that the maturation of the structure and biochemical composition of rat arteries is achieved relatively late in ontogeny. Aortic collagen biosynthesis reaches the adult level at the age of 7-9 wk, whereas the morphological and mechanical properties of conduit arteries become stabilized in rats aged 10-12 wk and small arteries at 9-11 wk (Zicha and Kunes 1999).

2.3 Renal failure

Cardiovascular complications are the leading cause of mortality in patients with ESRD and the excess cardiovascular risk and mortality is already demonstrable in early renal disease and CRF (London 2003). Even after stratification by age, gender, race, and the presence or absence of diabetes, cardiovascular mortality in dialysis patients is 10 to 20 times higher than in the general population (Foley et al. 1998). Cardiovascular disease in uraemia includes disorders of the heart (left ventricular hypertrophy, cardiomyopathy) and disorders of the vascular system (atherosclerosis, arteriosclerosis). The vascular characteristics of patients with renal failure seem to exhibit abnormal elastic properties of large arteries, reflected as decreased distensibility and compliance (Barenbrock et al. 1994, London et al. 1996). These alterations are independent of the level of blood pressure and tensile stress but appear to be related to the uremic state *per se* (Luik et al. 1997, Mourad et al. 1997). Furthermore, in patients with ESRD, both aortic and carotid arterial stiffness and presence of vascular calcification are reported to be strong independent predictors of mortality (Blacher et al. 1998, Blacher et al. 1999, Blacher et al. 2001, London 2003). Increased intima-media thickness of carotid arteries is already present in patients with renal failure before they start hemodialysis, supporting the concept that the arterial alterations are not due to the hemodialysis treatment, but due to renal failure *per se* or the associated secondary metabolic abnormalities (Shoji et al. 2002). Coronary-artery calcification is common even among young adults with ESRD (Goodman et al. 2000). In experimental renal failure, the increased arterial wall thickness is suggested to result mainly from an increase in extracellular matrix, although hyperplasia of the VSMCs could also be involved (Amann et al. 1997). Plasma ET levels are elevated in patients with kidney failure (Warrens et al. 1990), which could partially underlie the associated vascular remodelling (Demuth et al. 1998). Nevertheless, in addition to the structural changes, alterations in arterial function contribute to increased vascular stiffness as well (Mourad et al. 1997).

Accumulating evidence suggests that CRF is associated with impaired endothelial function (Annuk et al. 2003). 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). Even in patients with mild renal insufficiency, endothelial function is abnormal in the absence of atherosclerotic vascular disease, which suggests that uraemia may directly promote the development of atherosclerosis early in the progression of CRF (Thambyrajah et al. 2000). Potential candidates for an atherogenic “uremic factor“ include homocysteine, increased oxidative stress, endogenous inhibitors of NO synthase such as ADMA and N^G-monomethyl-L-arginine (L-NMMA), chronic inflammation and accumulation of oxLDL (Steinvinkel et al. 1999, Morris et al. 2001, Annuk et al. 2003). 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. 1997a). 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. 1998). In fact, there is increasing evidence that elevated vascular superoxide production plays a central role in the development of vascular endothelial dysfunction in renal failure (Annuk et al. 2001, Hasdan et al. 2002, Annuk et al. 2003) Nevertheless, increased plasma levels of superoxide dismutase (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 either biosynthesis or excretion (Marescau et al. 1997). In CRF, 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 CRF (Vallance et al. 1992, MacAllister et al. 1996, Kielstein et al. 1999). The clinical role of ADMA remains questionable (Anderstam et al. 1997) and a lack of relationship was recently reported between the plasma ADMA and creatinine levels in CRF patiens, that exhibited low total NO production levels (Schmidt and Baylis 2000). In isolated human uremic resistance arteries, impaired endothelial function has been reported, but the sole responsibility of endogenous inhibitors of NO synthase, such as ADMA, for the deficient vasorelaxations remains uncertain (Morris et al. 2001). 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. 1998). 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).

3 Blood pressure and arterial tone following high calcium intake

More than 20 years ago Ayachi showed that feeding calcium to the spontaneously hypertensive rat lowered the animal's blood pressure (Ayachi 1979). This finding, along with other similar observations (McCarron et al. 1981, McCarron et al. 1982, Resnick et al. 1983), sparked a burst of activity exploring the relationship between dietary calcium intake and blood pressure regulation in human and animal models of hypertension. While the question of whether elevated calcium intake lowers blood pressure in humans remains controversial (Appel 1997, Taubes 1998), data showing an inverse correlation between calcium intake and blood pressure in several models of hypertension in the rat and dog are highly consistent (McCarron et al. 1985, Peuler et al. 1987, Kageyama et al. 1987, Kotchen et al. 1989, Bukoski et al. 1989, Mäkynen et al. 1996).

3.1 Dietary calcium in experimental hypertension

In animal models of hypertension the antihypertensive effect of a high calcium diet has been consistent (Hatton et al. 1994, Mäkynen et al. 1996), and calcium supplementation has been found to be especially effective in sodium volume-dependent hypertension (Arvola et al. 1993a, Mäkynen et al. 1996). Some experimental studies have suggested that the blood pressure lowering effect of dietary calcium could be due to increased natriuresis following calcium supplementation (Pörsti et al. 1991, Butler et al. 1995). Calcium has been shown to alter sodium reabsorption in the proximal tubule, loop of Henle and cortical collecting tubules (Butler et al. 1995). Calcium has also been shown to blunt the pressure effects of dietary sodium in human hypertension (McCarron 1997). A multitude of mechanisms have been postulated to explain how increased calcium intake lowers blood pressure. Studies have focused on neural, humoral, and renal effects, whereas others have attempted to relate the antihypertensive action of calcium to improved vascular function (Hatton and McCarron 1994, Hatton et al. 1995).

Plausible antihypertensive mechanisms of a high calcium diet include suppression of sympathetic vasoconstrictor tone via modulation of central (Peuler et al. 1987) or peripheral (α_1-adrenoceptor responsiveness) nervous system activity (Hatton et al. 1993, Hatton and McCarron 1994), improved function of cell membrane Na^+-K^+ATPase, reduced voltage-dependent Ca^{2+} entry in arterial smooth muscle (Arvola et al. 1993a), augmented arterial sensitivity to NO, enhanced hyperpolarization of vascular smooth muscle (Mäkynen et al. 1996), and suppression of serum levels of calciotropic hormones, including $1,25(OH)_2$ vitamin D_3 and PTH (Bukoski et al. 1995). An interesting link between the intake and metabolism of calcium and the control of arterial tone may be the extracellular Ca^{2+} receptor

in the perivascular sensory nerves, the activation of which causes vasorelaxation via the release of a hyperpolarizing mediator (Bukoski 1998, Ishioka and Bukoski 1999). The Ca^{2+} receptor seems to be present in the perivascular nerve network of a variety of rat tissues, including the mesenteric vasculature, intrarenal arteries and cerebral arteries (Wang and Bukoski 1998).

3.2 Dietary calcium in human hypertension

The concept that dietary calcium could be of significance in blood pressure regulation emerged in the early 1980s (McCarron et al. 1982, McCarron et al. 1984). The reports suggested that low intake of calcium-containing foods was associated with hypertension and that dietary calcium consumption by adults was inversely related to the probability of being hypertensive. Subsequently, there is a wealth of evidence supporting the view that an adequate intake of calcium protects against high blood pressure in humans (Van Leer et al. 1995, McCarron 1997). After 20 years of investigation, a consensus is at hand: a large body of recent data consistently prove the antihypertensive effect of increased intake of calcium (McCarron and Reusser 1999, Vaskonen 2003). The Recommended Dietary Allowance (RDA) for calcium has long been 800 mg/day, but the recognition of the many health benefits of calcium has led to increases in dietary calcium recommendations up to 1500 mg/day, depending on sex and age group (NIH Consensus Panel 1994, Bryant et al. 1999). Dietary calcium intake up to 2000 mg/day is generally regarded as safe (NIH Consensu Panel 1994).

Calcium supplementation has been suggested to lower blood pressure in patients with essential hypertension (Bucher et al. 1996) and dietary calcium seems to even reduce the effect of a high sodium chloride intake on blood pressure (Haddy 1991, McCarron 1997). However, findings from these and other meta-analyses demonstrate considerable heterogeneity in the blood pressure response to increased calcium. This may be explained by several factors, including a threshold effect, consistent with the suggested 600-700 mg/day calcium threshold (McCarron et al. 1984, Zemel 2001). A key factor contributing to the heterogeneity of response is the baseline blood pressure status of the study group. The systolic blood pressure response to calcium supplementation was –3.9 mmHg in the hypertensive patients versus –0.15 mmHg in the normotensive individuals in the six studies that provided separate analyses based on blood pressure status (Bucher et al. 1996, Zemel 2001). Thus, the inclusion of normotensives may have diluted the effect of the dietary intervention.

The heterogeneity in blood pressure response to calcium may also be explained by the intake of other nutrients, interactions among nutrients, and the source of dietary calcium. Indeed, studies that utilized dietary sources of calcium demonstrated approximately twofold greater, and more consistent, effects on blood pressure compared to those that utilized supplements (McCarron and Reusser 1999). The Dietary Approaches to Stop Hypertension (DASH) study compared dietary food patterns and recognized the significance of adding dairy products in the diet of the hypertensive subgroup (Appel et al. 1997). The results from the

DASH study suggested that significant population-wide reductions in coronary heart disease and stroke could be achieved by switching from a typical U.S. diet to the DASH combination diet (fruit and vegetable/dairy products) (Svetky et al. 1999). This would probably be due to achieved reduction in blood pressure but the decreased circulating homocysteine levels might also be of importance (Appel et al. 1997, Appel et al. 2000, Zemel 2001).

4 Calcium metabolism in renal failure

CRF is associated with disturbances of calcium and phosphate metabolism (Drüeke 2001). Patients with CRF tend to develop secondary hyperparathyroidism (SH) (Llach 1995), which is characterized by hyperplasia of the parathyroid glands and enhanced synthesis of PTH (Mihai and Farndon 2000, Slatopolsky 2001, Silver et al. 2002). SH develops because phosphate excretion is decreased in renal failure, and elevated plasma phosphate together with reduced synthesis of 1,25-dihydroxyvitamin D_3 (1,25D) contribute to the development of SH (Slatopolsky et al. 2001). Furthermore, the elevated plasma calcium and phosphate levels in SH could play important roles in the uremic cardiovascular disease (Rostand and Drüeke 1999).

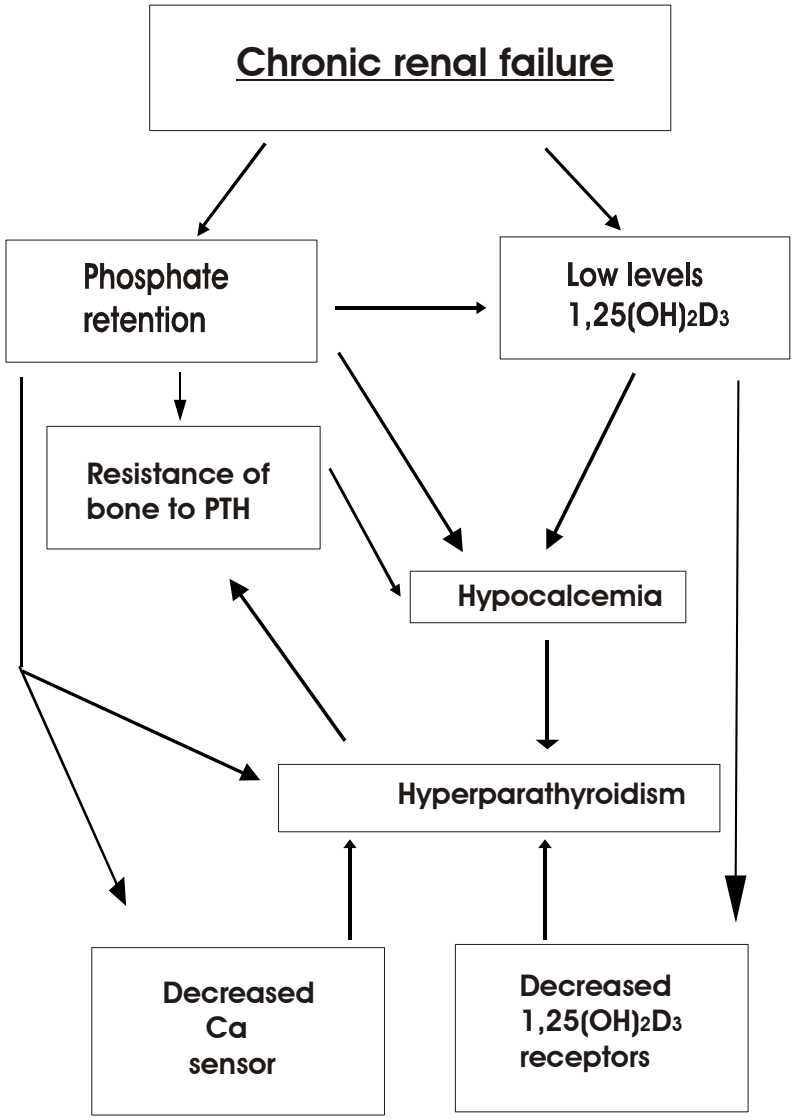
In early renal failure, deficient 1,25D synthesis is an important factor leading to slightly decreased plasma calcium levels, but reduced expression of vitamin D receptors and the Ca^{2+}-sensing receptor may also be present in the parathyroid cells and contribute to hypocalcemia (Korkor 1987, Mihai and Farndon 2000). Later, in advanced renal failure, hyperphosphataemia becomes an important pathogenic factor augmenting the development of SH (Llach and Forero 2001). Increased serum levels of PTH occur even in patients with mild to moderate renal impairment and the three main up-regulators of PTH in man are low serum 1,25D, low ionised calcium and high phosphorus (Slatopolsky et al. 1999). Plasma phosphorus level per se, independent of the levels of Ca^{2+} and 1,25D, is an important stimulator of PTH secretion (Lopez-Hilker et al. 1990), but the extracellular Ca^{2+} significantly contributes to the regulation of plasma PTH levels as well (Drüeke 2001, Silver et al. 2002).

The control of PTH gene transcription by 1,25D is mediated by the vitamin D receptor, a protein with high affinity and specificity for the vitamin D hormone (Slatopolsky et al. 1999). The vitamin D receptor expression in the parathyroid glands of CRF patients seems to be markedly reduced (Korkor 1987) and similar results have been reported in experimental uremia as well (Merke et al. 1987). As renal failure progresses, there is a progressive decrease in the number of vitamin D receptors in the parathyroid glands, which makes the parathyroid glands more resistant to 1,25D. Therefore, 1,25D is suggested to be an important regulator of parathyroid cell growth, and in renal failure low levels of 1,25D may allow parathyroid cells to proliferate (Slatopolsky et al. 1999). In experimental animal models of renal failure 1,25D administration has suppressed parathyroid hyperplasia, perhaps through changes in serum calcium (Szabo et al. 1989) and the direct effect on the parathyroid gland mentioned above.

There is evidence for an intrinsic abnormality of the parathyroid glands in uremia that leads to a disordered calcium-regulated PTH secretion and insensitivity to the suppressive effect of calcium on PTH secretion (Brown et al. 1982). The parathyroid glands express a calcium-sensing mechanism via a specific calcium receptor (Brown et al. 1993, Silver et al. 2002), that enables the PTH secretion react to, for instance, hypocalcemia within 1-3 minutes (Slatopolsky et al. 1999). Calcium can also regulate PTH gene transcription (Okazaki et al. 1991) and cell proliferation (Silver et al. 2002). In addition to the impaired control of parathyroid function by calcium in CRF, the frequently observed decrease in dietary calcium intake and the impairment of intestinal calcium absorption due to low 1,25D also contribute to the development of hyperparathyroidism via a tendency towards hypocalcaemia (Drüeke 2001). Oral calcium supplements are used early in CRF to avoid calcium deficiency and control the development of SH (Fournier et al. 1996, Drüeke 2001). Moreover, oral calcium administration reduces hyperphosphataemia by binding phosphate in the intestine, which further helps to manage SH (Drüeke 2001).

Some reports have suggested that high-dose calcium supplements result in an uncontrolled intestinal absorption of unbound calcium and its potential deposition in soft tissues via an increase in Ca x P product (Drüeke 2001). An association has been published between the prescribed dose of oral calcium carbonate and arterial wall stiffness (Guérin et al. 2000) and another study reported an increase in coronary artery calcification in young dialysis patients that were given twice as much calcium-containing phosphate binders compared to the controls (Goodman et al. 2000). This risk of inducing extra-skeletal calcifications seems to be further enhanced by vitamin D (Drüeke 2001). Moreover, high PTH and phosphate levels predispose to ectopic calcifications (Slatopolsky et al. 2001). Excess of PTH is also associated with elevated blood pressure, and it may directly influence the function of arterial smooth muscle (Rostand and Drüeke 1999). Recently, raised PTH and phosphate levels emerged as cardiovascular mortality markers in a 6-year prospective study on Caucasian hemodialysis patients (Marco et al. 2003). Therefore, disturbed calcium-phosphate balance seems to contribute to the cardiovascular pathology in RF (Slatopolsky et al. 2001), and treatment of hyperphosphataemia and decrease of the Ca x P product are cornerstones in the management of advanced stages of SH (Locatelli et al. 2002).

Figure 4. Schematic representation of the factors involved in the pathogenesis of SH.



AIMS OF THE PRESENT STUDY

The objective of the present series of investigations was to examine the control of arterial tone in NO deficiency, NaCl-hypertension and renal failure. The effects of high calcium intake in NO-deficient hypertension and the influences of calcium supplementation and vitamin D-induced hypercalcemia in NaCl-hypertension on rat conduit artery function were studied. Furthermore, the changes in the tone of resistance arteries were evaluated in renal failure following the treatment of SH by high calcium diet.

The detailed aims were:

1. To examine the influence of high calcium diet on the control of arterial tone in NO deficient Wistar rats.

2. To investigate the effects of increased calcium intake and hypercalcemia induced by oral 1-α-OH vitamin D_3 in NaCl-hypertensive WKY rats.

3. To study the effects of renal failure on the function of the vascular endothelium and arterial smooth muscle in WKY rats.

4. To study the effects of renal failure on the function of the endothelium and smooth muscle of resistance arteries in WKY rats.

5. To examine the influence of the treatment of SH by increased calcium intake on the tone of resistance arteries in Sprague-Dawley rats subjected to 5/6 nephrectomy.

MATERIALS AND METHODS

1 Experimental animals

Normotensive male Wistar and Sprague-Dawley rats were obtained from the colony of the Medical School at the University of Tampere (I, V), whereas WKY rats were obtained from Møllegaard's Breeding Centre, Ejby, Denmark (II) and WKY rats also from M&B A/S, Ry, Denmark (III, IV). The rats were housed two (III, IV, V) or four (I, II) to a cage in a standard animal laboratory room (temperature +22ºC, 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 (III, IV, V).

2 Diets and drug treatments

All animals in studies III and IV, and those on control diet in study I received standard laboratory food pellets containing 1.1% calcium, 0.7% sodium chloride and 0.2% magnesium (Ewos, Södertälje, Sweden). The calcium supplementation in study I was accomplished by adding $CaCO_3$ after which the chow contained 3.0% calcium. In study II the control diet contained 6% sodium chloride and 1% calcium, the calcium supplemented chow contained 6% sodium chloride and 3% calcium, and the vitamin D chow contained 6% sodium chloride, 1% calcium and 1OH-D_3 (Etalpha®; Lövens, Ballerup, Denmark; vitamin D precursor which is hydroxylated to active 1,25$(OH)_2D_3$ in the liver) 21-27 µg per kg of chow, i.e. the daily average dose was 1.2 µg per kg of rat. In study V the control chow contained 0.3% calcium, whereas the high calcium diet contained 3.0% calcium. The extra calcium was supplied as the carbonate salt, and otherwise the chows were practically identical (AnalyCen, Lindköping, Sweden).

All the rats were freely provided with tap water excluding the L-NAME-treated animals in study I that received L-NAME (20 mg/kg/day) in bottled drinking fluid. The daily prepared solutions were kept in light-proof bottles. In order to obtain the desired daily L-NAME dose, the concentration in drinking water was adjusted according to 24 h fluid consumption measurements.

3 Blood pressure measurements

The systolic blood pressures of conscious rats restrained in plastic holders were measured indirectly by the tail cuff method at +28ºC. All measurements were performed with an IITC Inc. Model 129 Blood Pressure Meter (Woodland Hills, California, USA) equipped with a

photoelectric pulse detector. The blood pressure or each rat was obtained by averaging three reliable recordings.

4 Urine collection and measurement of fluid intake and food consumption

Urine was collected for 24 h individually in metabolic cages where animals had free access to food and water (III, IV, V). Urine volumes were measured and samples stored at -20ºC. The consumption of drinking fluid was measured by weighing the bottles after a 24 h period. Food consumption was monitored during periods in special metabolic cages.

5 Blood and heart samples

The rats were anaesthetised by the intraperitoneal administration of urethane (1.3g/kg) and the carotid arteries were cannulated. Blood samples were drawn into chilled tubes on ice containing 2.7 mM ethylenediaminetetraacetic acid (I, II), and into tubes and glass capillaries containing heparin (III, IV, V), after which the samples were centrifuged, and the plasma stored at -70ºC until analysis. After exsanguination, the thoracic and abdominal cavities of the animals were opened, the hearts (I, III, IV, V) and the kidneys (V) removed and weighed. The tissue samples were frozen in liquid nitrogen and stored at -70ºC until analyses.

6 Biochemical determinations

6.1 Nitrite and nitrate

To measure nitrite and nitrate (Nox) concentrations in plasma and urine (III), vanadium chloride in HCl was used to convert NOx to NO, which was quantified by the ozone-chemiluminescence method (Braman and Hendrix 1989). The samples were first treated with ethanol at -20ºC 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 95ºC, and NO formed under these reducing conditions was measured by the NOA 280 analyser (Sievers Instruments Inc. Boulder, Colorado, USA) using sodium nitrate as the standard.

6.2. Sodium, potassium, calcium, magnesium, urea nitrogen, phosphate, creatinine, haemoglobin, PTH, $1,25(OH)_2D_3$ and proteins

Plasma sodium, potassium (II, III, IV, V) and magnesium (II) concentrations were measured by potentiometric direct dry chemistry, urea nitrogen (III, IV, V) by colorimetric enzymatic dry chemistry, and phosphate (III, IV, V) by colorimetric end-point dry chemistry (Vitros 950

analyzer, Johnson & Johnson Clinical Diagnostics, Rochester, New York, USA). Creatinine (III, IV, V) was determined by the kinetic colorimetric assay according to Jaffe, and plasma proteins (V) were measured by colorimetric measurement according to Biuret (Cobas Integra analyzer, F. Hoffman-La Roche Ltd, Diagnostics Division, Basel, Switzerland). PTH (V) levels were measured by an immunoradiometric assay specific for intact rat PTH (Catalog #50-2000, Immunotopics, San Clemente, California, USA), and vitamin D (V) by radioassay designed for the quantitative determination of $1,25(OH)_2D_3$ (competitive protein-binding assay, Catalog #40-6041, Nichols Institute Diagnostics, San Juan Capistrano, California, USA). Ionised calcium (II, III, IV, V) was measured by an ion selective electrode (Ciba Corning 634 Ca^{2+}/pH Analyzer, Ciba Corning Diagnostics, Sudbury, UK). Haemoglobin (III, IV, V) was determined by photometric analysis using Technicon cyanide free haemoglobin reagent (Technicon H*2^{TM}, Technicon Instruments Corporation, Tarrytown, New York, USA).

7 Mesenteric arterial responses *in vitro*

7.1. Arterial preparations and organ bath solutions

The superior mesenteric arteries (I, II, III) were carefully cleaned of adherent connective tissue, excised, and placed on a Petri dish containing physiological salt solution (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_2PO_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 we 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 physiological salt solution described above. The small second (IV) or third order (V) branches from the mesenteric arterial bed were carefully excised under a dissecting microscope (Nikon, Japan) and mounted over two 40 μm wires in a small organ bath chamber (volume 5 ml) containing physiological salt solution. The endothelium were left intact or removed by perfusing air through the vascular lumen. The preparations were aerated with 95% O_2 and 5 % CO_2 at +37ºC, 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^+ (20-125mM), NaCl was replaced with KCl on an equimolar basis. In Ca^{2+}-free solutions, $CaCl_2$ was omitted without substitution.

7.2. Arterial contractile and relaxation responses

In studies I, II and III the arterial rings were initially equilibrated for 1 h at +37ºC with a resting preload 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 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. In studies IV and V a Mulvany multimyograph Model 610A (J.P. Trading, Aarhus, Denmark) was employed for studies with vascular preparations. In this system the isometric micromyographs consist of two jaws, one of which is connected to a length displacement device and the other to a force transducer linked to a computer with Myodaq software (J.P. Trading). The small arterial rings were placed over two thin wires, each of which was attached to one of the myograph jaws. Normalisation of the vascular preparations was then performed so that the internal diameter of the vessel was set at 90 % of that obtained when exposed to an intraluminal pressure of 100 mmHg in the relaxed state (Mulvany and Halpern 1977). The presence of intact endothelium in the vascular preparations was confirmed by a clear relaxation to 1 μM ACh in NA-precontracted rings, and the absence of endothelium by the complete lack of this response.

Agonist-induced contractions. The contractions of the endothelium-intact preparations to NA were studied in the absence (I, II, III, IV, V) and presence of L-NAME (0.1 mM) (I, III), and in the presence of diclofenac (3 μM) (II) or diclofenac plus L-NAME (I, II, III). In study III, the contractions to NA were also elicited in the presence of L-Arginine (1 mM). The contractions elicited by ET-1 were investigated in the endothelium-denuted preparations in study V.

Depolarization-induced contractions. The concentration-response curves of the endothelium-denuded rings to KCl were determined in the absence (II, III, IV, V) and presence of L-NAME (0.1 mM) (I).

Ca^{2+} contractions. The contractile responses of the endothelium-denuded rings to cumulative addition of Ca^{2+} to the organ bath chamber after precontraction with KCl (125 mM) in Ca^{2+}-free buffer in the presence of L-NAME (0.1 mM) (I) or phentolamine (1 μM) and atenolol (10 μM) (III) were studied. Thereafter, the effect of nifedipine (0.5 nM) on these responses was examined (III).

Endothelium-dependent relaxations to ACh and adenosine 5'-diphosphate (ADP). Mesenteric arterial relaxations were studied in response to ADP (II) and ACh (II, III, IV, V) in rings precontracted with NA (1 μM in II, III; 3 μM in IV and 5 μM in V). The ACh-induced relaxations after NA-precontraction were also elicited in the presence of diclofenac

(II), L-NAME (I, III, IV, V), diclofenac and L-NAME (I, II, III, IV, V), diclofenac, L-NAME and tetraethylammonium (1 mM) (II), diclofenac, L-NAME, and apamin (50 nM) plus charybdotoxin (0.1 μM) (III, IV, V). The responses to ACh were further studied in the presence of SOD (50 U/ml) (III); L-NAME and SOD (I); SOD plus catalase (100 U/ml) (III) and SOD, L-NAME and catalase (I). The ACh-induced relaxations were also examined in the presence of L- arginine (1 mM) (III). Furthermore, the relaxations to ACh and ADP were investigated in rings precontracted with KCl (50 mM) in the absence and presence of L-NAME (II), and the relaxations to ACh after KCl precontraction in the presence of L-NAME and diclofenac (I).

Endothelium-independent relaxations to sodium nitroprusside (SNP), isoprenaline, cromakalim, levcromakalim and 11,12-epoxyeicosatrienoic acid (EET). The relaxation responses of NA-precontracted (I, II, III, IV, V) and KCl-precontracted (I, II) endothelium-denuded rings to SNP were examined. The vasorelaxations elicited by isoprenaline and cromakalim (I, II, III, IV) or levcromakalim (V) were studied in endothelium-denuded rings precontracted with NA (I, II, III, IV, V). Moreover, responses to isoprenaline were also studied in rings precontracted with KCl (II). In study I, the endothelium-independent responses to SNP, isoprenaline and cromakalim were studied in the presence of L-NAME when testing vessels from L-NAME-treated animals. In study V, the relaxation responses to EET were examined after precontraction with NA.

8 Morphological studies

In study II the rat aortas were fixed in 4 % formaldehyde, embedded in paraffin, and a 5 μm transverse section was cut and stained with hematoxylin and eosin. Fibrosis, inflammation, calcification and wall thickening were scored. The apoptosis of aortic smooth muscle cells was measured by the in situ end-labelling technique (ApopTaq-kit, Oncor Inc., Gaithersburg, Maryland, USA). Prostates from castrated and non-castrated rats were used as controls in apoptosis staining, and the results were scored in a blinded fashion using an Olympus BX50 microscope (Olympus, Tokyo, Japan). One hundred cells were counted from 10 fields on each slide (at 200x magnification) and results expressed as percentage of apoptotic cells.

In study IV the small vascular rings from the second or third order branches of the rat mesenteric artery were mounted on the Mulvany myograph Model 610**.** The myograph together with the Myodaq software determine and record the lumen diameter of each preparation during the standard normalisation process which sets internal diameter of the vessel at 90 % of that obtained when the intraluminal pressure is set at 100 mmHg. In study V the morphology of small arteries was examined with a pressure myograph (Living Systems Instrumentation Inc., Burlington, Vermont, USA), and the development of myogenic tone was inhibited by Ca^{2+}-free solution containing 30 mmol/l EDTA (Suo et al. 2002).

9 Compounds

The following drugs and chemicals were used: ACh chloride, apamin, catalase, charybdotoxin, cromakalim, 11,12-epoxyeicosatrienoic acid, isoprenaline hydrochloride, NA bitartrate, L-NAME hydrochloride, SOD, tetraethylammonium chloride (Sigma Chemical Co., St. Louis, Missouri, USA), levcromakalim (SmithKline Beecham AB, West Sussex, U.K.), atenolol (Leiras Pharmaceutical, Turku, Finland), ketamine (Parke-Davis Scandinavia AB, Solna, Sweden), cefuroxim, diazepam, nifedipine (Orion Pharma Ltd., Espoo, Finland), metronidazole (B. Braun AG, Melsungen, Germany), buprenorphine (Reckitt & Colman, Hull, U.K.), SNP, NA hydrogentartrate (Fluka Chemie AG, Buchs SG, Switzerland), diclofenac, phentolamine (Voltaren® injection solution, Ciba-Geigy, Basle, Switzerland), sodium salt of adenosine 5'-diphosphate (Boehringer Mannheim GmbH, Germany), and 1OH-D_3 (Etalpha®, Lövens, Ballerup, Denmark). 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 cromakalim, levcromakalim and nifedipine (in 50 % ethanol), and EET (in 99% ethanol). Drinking fluids containing L-NAME hydrochloride were made by dissolving the compound in tap water. All solutions were freshly prepared before use and protected from light. The chemicals used in the preparation of physiological salt solution were of highest grade available and obtained from E. Merck AG (Darmstadt, Germany).

10 Analysis of results

The statistical analysis was performed using one-way or two-way analysis of variance (ANOVA) supported by Bonferroni test or by two-tailed t-test when carrying out pairwise comparisons between the study groups. ANOVA for repeated measurements was applied for data consisting of repeated observations at successive time points. Spearman's correlation coefficient was used in the correlation analyses. All results were expressed as mean ± SEM. The data were analysed with BMDP Statistical Software version PC90 (Los Angeles, California, USA).

Table 1. Summary of the experimental design of the studies on arterial reactivity.

Study	Treatment Rats Vessel	E+ relaxations (precontraction)	E- relaxations (precontraction)	Contractions
Experimental hypertension				
I	L-NAME hypertensive Wistar rats Mesenteric artery	ACh (NA) + L-NAME and diclofenac ACh(KCl) ACh (NA) + SOD + catalase	Isoprenaline (NA) Cromakalim (NA) Nitroprusside (NA and KCl)	NA + L-NAME and diclofenac KCl Calcium
II	NaCl hypertensive WKY rats Mesenteric artery	ACh (NA) + diclofenac + L-NAME + TEA ACh(KCl) + L-NAME ADP (NA) ADP (KCl) + L-NAME	Isoprenaline (NA and KCl) Cromakalim (NA) Nitroprusside (NA and KCl)	NA + diclofenac + L-NAME KCl
Renal failure				
III	5/6 nephrectomized WKY rats Mesenteric artery	ACh (NA) + L-NAME + diclofenac + apamin and charybdotoxin ACh (NA) + SOD + catalase ACh (NA) + L-Arginine	Isoprenaline (NA) Cromakalim (NA) Nitroprusside (NA)	NA + L-NAME + diclofenac KCl Calcium + nifedipine
IV	5/6 nephrectomized WKY rats Small mesenteric artery	ACh (NA) + L-NAME + diclofenac + apamin and charybdotoxin	Isoprenaline (NA) Cromakalim (NA) Nitroprusside (NA)	NA KCl
V	5/6 nephrectomized Sprague-Dawley rats Small mesenteric artery	ACh (NA) + L-NAME + diclofenac + apamin and charybdotoxin	Isoprenaline (NA) Cromakalim (NA) Nitroprusside (NA) EET (NA)	NA KCl ET-1

ACh, acetylcholine; ADP, adenosine 5'diphosphate; E+, endothelium-dependent; E-, endothelium-independent; EET, 11,12-epoxyeicosatrienoic acid; ET-1, endothelin-1; L-NAME, N_G-nitro-L-arginine methyl ester; NA, noradrenaline; SOD, superoxide dismutase; TEA, tetraethylammonium.

RESULTS

1 Blood pressure, arterial morphology and apoptosis, heart weight, total renal mass, drinking fluid and urine volumes

Blood pressure. The systolic blood pressures of untreated Wistar (I) and WKY (II) rats were 139 mmHg and 154 mmHg, respectively, when measured at the end of the follow up periods. The long-term administration of L-NAME resulted in the elevation of blood pressure up to 198 mmHg in Wistar rats (I) and the NaCl-diet elevated the blood pressure to 184 mmHg (II), whereas calcium supplementation clearly attenuated (I) or completely prevented (II) the increase in blood pressure, while 1OH-D_3-treatment also attenuated the development of NaCl-hypertension (II). Concomitant calcium supplementation during the 1OH-D_3-treatment did not affect the blood pressure in NaCl-hypertension (II). The CRF in WKY (III, IV) rats was not associated with elevation of blood pressure, nor did the high calcium diet affect the blood pressure in CRF (V). When analysed by two-way ANOVA, a small but significant increase in blood pressure was uncovered in Sprague-Dawley (V) rats with renal failure when compared with sham-operated controls (i.e. both NTX groups compared with both sham-operated groups).

Arterial morphology and apoptosis. No differences were observed in the smooth muscle apoptosis in the aortic wall in study II. Signs of mild fibrosis with some perivascular lymphocytes were observed in 20% and 27% of the aortas in the Control and Calcium groups of study II, respectively, while calcifications in the adventitia or media with inflammatory cells were observed in 50% and 57% of the aortic sections from the 1OH-D_3 and 1OH-D_3+Calcium groups in study II, respectively. The CRF was not associated with alterations in mesenteric arterial morphology (unpublished observation in study III). The lumen diameters of the small mesenteric arteries in studies IV and V were corresponding between the groups, and no difference in the wall thickness or in the cross-sectional area was detected in study V. However, the wall to lumen ratio of isolated perfused third order mesenteric artery brances in study V was increased in the NTX and NTX+calcium groups when compared with the Sham group.

Heart weight and total renal mass. The heart-body weight ratios were comparable in L-NAME hypertensive and normotensive Wistar rats while calcium supplementation was without significant effect on the relative heart weights (I) In CRF the heart-body weight ratios remained comparable in WKY (III, IV) and Sprague-Dawley (V) rats and high calcium intake did not influence the relative heart weights in CRF (V). The total renal mass following the subtotal nephrectomy and the development of CRF was approximately 70% in the nephrectomized animals compared with sham-operated controls in study V.

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 CRF when compared with the control animals (III, IV, V).

2 Plasma sodium, potassium, calcium, 1,25(OH)$_2$D$_3$, magnesium, urea nitrogen, creatinine, PTH, phosphate, proteins, haemoglobin and NOx

1OH-D$_3$-administration increased plasma total Ca^{2+} concentrations, while plasma Na^+, K^+ and Mg^{2+} were similar in all study groups in study II. In rats with CRF, the plasma creatinine and urea nitrogen values were increased, while plasma sodium, haemoglobin and calcium concentrations were decreased when compared with control WKY rats (III, IV). Renal failure did not influence plasma potassium, phosphate, pH (III, IV) or NOx (III). In study V, CRF elevated the plasma urea nitrogen, creatinine, PTH and phosphate concentrations and decreased plasma ionized calcium, 1,25(OH)$_2$D$_3$, proteins and haemoglobin. The high calcium intake significantly lowered the plasma PTH and phosphate concentrations and elevated the ionized calcium (V).

3 Control of arterial tone *in vitro*

3.1. Arterial tone in L-NAME and NaCl hypertension and the influence of calcium supplementation and vitamin D-induced hypercalceamia

3.1.1 Arterial contractile responses

Both in the presence of L-NAME and in the presence of L-NAME and diclofenac, the vascular rings of the study groups showed comparable sensitivity to NA (I). In the presence of L-NAME, the rings of the control and L-NAME groups showed similar contractile sensitivity to KCl, whereas the sensitivity was somewhat higher in the calcium supplemented groups (I). Arterial sensitivity to the addition of Ca^{2+} during depolarization with 125 mM KCl was similar in the calcium and control groups but higher in the calcium group when compared with the L-NAME and calcium+L-NAME groups (I).

In study II, maximal wall tension and sensitivity to NA, in the absence and presence of diclofenac and L-NAME, was corresponding in the control and low-NaCl groups, and the high Ca^{2+} diet was without significant effects on the NA-elicited contractions. In contrast, the 1OH-D$_3$ treatment markedly decreased maximal wall tension in response NA without changing the sensitivity (II). In the vascular rings of both 1OH-D$_3$-treated groups lower maximal wall tension response to NA was also observed after pretreatment with L-NAME and diclofenac, while sensitivity remained comparable in all groups. Maximal wall tension in response to KCl was also reduced in both of the 1OH-D$_3$-treated groups when compared with

the other groups (II), yet the sensitivity to KCl was comparable in all the study groups (II).

3.1.2 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 controls (I). 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, calcium supplementation clearly improved the vasodilatations to SNP, isoprenaline and cromakalim in L-NAME-treated animals (I). Even the control rats exhibited improved vasorelaxation to SNP after precontraction with KCl and to isoprenaline after NA-precontraction following calcium supplementation (I).

The high NaCl intake impaired the vasodilatations to SNP, isoprenaline and cromakalim in NA-precontracted arterial rings when compared with the low-NaCl group (II). High calcium diet enhanced these responses, and the relaxations in the calcium group were not different from those in the low-NaCl group (II). The chronic 1OH-D_3-induced hypercalcemia, without or with calcium intake, had no effects on the endothelium- independent relaxations, except for the response to 0.33 μM cromakalim was reduced in both 1OH-D_3-treated groups when compared with the control rats (II).

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 significantly improved by calcium supplementation in both of these groups (I). The addition of diclofenac to the organ bath chamber improved the relaxations to ACh in the L-NAME and control groups but not in the calcium supplemented groups (I). Diclofenac also abolished the difference in the ACh response between the control and the calcium supplemented groups, whereas the relaxations still remained impaired in the L-NAME group when compared with the others (I). The responses to ACh were almost completely abolished in all groups when induced in KCl-precontracted rings in the presence of L-NAME and diclofenac (I). When L-NAME and SOD were added to the organ bath, the relaxations to ACh were enhanced in the L-NAME group, but the responses remained impaired when compared with the other groups (I). In addition, SOD augmented the relaxation to ACh in the control group, whereby the difference in response to ACh between the control group and the calcium supplemented groups was abrogated (I). The further addition of catalase had no effect on the relaxation to ACh in any of the study groups.

In study II, the relaxations induced by ACh and ADP in NA-precontracted arterial rings were impaired in the control group when compared with the low-NaCl group, while these

responses were improved by calcium supplementation so that they did not differ from those of the low-NaCl group. The 1OH-D_3-induced chronic hypercalcemia, without or with concomitant calcium supplementation, had no influence on the endothelium-dependent vasorelaxation in NaCl-hypertensive rats (II). The addition of diclofenac had no significant effect on the relaxations to ACh, but the addition of L-NAME reduced the responses to ACh, yet clear relaxations were still observed in the calcium and low-NaCl groups (II). The addition of tetraethylammonium reduced the L-NAME and diclofenac-resistant relaxation to higher concentrations of ACh by 30-40 % in the low-NaCl and calcium groups, and almost completely abolished the response to ACh in the control, 1OH-D_3 and 1OH-D_3+calcium groups (II). When the relaxations to ACh and ADP were elicited after precontractions with KCl, the responses were lesser than after precontractions with NA, and the relaxations were more pronounced in the calcium and low-NaCl groups than in the other groups. The responses to ACh and ADP in KCl-precontracted rings were abolished in the presence of L-NAME.

3.2 Effects of renal failure on the control of macro- and microvessel tone

3.2.1 Arterial contractile responses

Vasoconstrictor responses. CRF did not alter the arterial contractile sensitivity to NA or KCl in large mesenteric arterial rings (III) nor in the resistance arterial rings (IV, V). The maximal contractions to NA in the absence (III, IV, V) and presence (III) of L-NAME were also comparable between renal failure and control rats both in conduit vessels (III) and in resistance arteries (IV, V). However, in the presence of L-NAME and diclofenac the maximal contractile force generation induced by NA was higher in the conduit arterial rings of 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 (III).

3.2.2 Arterial relaxation responses

Endothelium-independent relaxations. CRF did not modulate arterial relaxations to SNP in large mesenteric arteries (III) or small arteries (IV). However, the relaxations to isoprenaline and cromakalim were impaired in large arteries of rats with CRF (III). The vasorelaxation to isoprenaline was also attenuated in the resistance vessels of CRF rats but the relaxation responses to cromakalim remained similar between the study groups (IV).

Endothelium-dependent relaxations. The relaxations induced by higher concentrations of ACh (1-10μM) in NA-precontracted conduit arterial rings were impaired in the renal failure rats when compared with the control rats (III). 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 (III). Diclofenac was without significant effects on ACh-induced relaxations in both groups (III). The addition of apamin and charybdotoxin further reduced the relaxations to ACh in the control rats but not in the renal failure rats, thereby abolishing the difference in the remaining relaxation to ACh (III).

The relaxations induced by ACh in endothelium-intact NA-precontracted mesenteric small arterial rings were similar in the renal failure rats when compared with the control rats (IV). In the presence of L-NAME the relaxations to ACh were reduced, but the responses remained comparable between the study groups (IV). Diclofenac was without effect on the ACh-induced relaxations (IV). In contrast, the further addition of apamin and charybdotoxin clearly reduced the relaxations in both study groups (IV), but still no significant difference was observed in the small arterial reactivity between the renal failure and control groups (IV).

3.3 Influence of phosphate binding by high calcium diet on the control of microvessel tone in renal failure

3.3.1 Arterial contractile responses

Vasoconstrictor responses. CRF did not alter the arterial contractile sensitivity to NA or KCl in the resistance arterial rings. The maximal contractions to NA were also comparable between renal failure and control rats. The resistance arteries of the renal failure rats on high calcium diet exhibited somewhat higher sensitivity to KCl than the arteries from the control group. The sensitivity and maximal wall tension induced by ET-1 remained comparable between all study groups in the resistance arterial rings.

3.3.2 Arterial relaxation responses

Endothelium-independent relaxations. CRF did not modulate arterial relaxations to SNP or isoprenaline in the small arteries. The resistance vessels of CRF rats were less sensitive to levcromakalim and EET, while these impairments in endothelium-independent vasorelaxation were normalized by high calcium intake. *Endothelium-dependent relaxations.* The relaxations induced by ACh in NA-precontracted small artery preparations were impaired in the NTX group, whereas the groups on the high calcium diet did not differ from the sham-operated control animals. In the presence of L-NAME, the relaxations to ACh were diminished in all groups, but the responses remained less marked in the NTX group when compared with the others. Further addition of diclofenac was without effects on the responses to ACh. The addition of apamin and charybdotoxin clearly reduced the relaxations in all study groups, and the difference in the remaining response to ACh was abolished. The reduction in relaxation by apamin and charybdotoxin was smaller in the NTX group when compared to other groups.

Table 2. Summary of the alterations in arterial relaxations in hypertensive rats after high calcium intake and vitamin D -induced hypercalcemia compared with untreated hypertensive controls.

Variable	NO deficiency		NaCl Hypertension			
		$+Ca^{2+}$		$+Ca^{2+}$	$+1OH\text{-}D_3$	$+Ca^{2+}+1OH\text{-}D_3$
E+ relaxations (precontraction)						
ADP (NA and KCl)			↓	↑	↔	↔
Acetylcholine (NA)			↓	↑	↔	↔
+ L-NAME	↓	↑				
+ diclofenac			↓	↑	↔	↔
+ L-NAME and diclofenac	↓	↑	↓	↑	↔	↔
+ L-NAME, diclofenac, TEA			↓	↑	↔	↔
+ L-NAME and SOD	↓	↑				
Acetylcholine (KCl)			↓	↑	↔	↔
+ diclofenac and L-NAME	↔	↔				
E- relaxations (precontraction)						
Nitroprusside (NA)	↓	↑	↓	↑	↔	↔
Nitroprusside (KCl)	↓	↑	↓	↑	↔	↔
Isoprenaline (NA)	↓	↑	↓	↑	↔	↔
Isoprenaline (KCl)	↔	↔	↔	↔	↔	↔
Cromakalim (NA)	↓	↑	↓	↑	↓	↓

ADP, adenosine 5'diphosphate; E+, endothelium-dependent; E-, endothelium-independent; L-NAME, N_G-nitro-L-arginine methyl ester; NA, noradrenaline; SOD, superoxide dismutase; TEA, tetraethylammonium. ↑, ↓ and ↔ indicate an increase, reduction and no change when compared with the corresponding control group, respectively.

Table 3. Summary of the alterations in arterial relaxations in renal failure rats when compared with sham-operated controls and the effect of high calcium intake.

Variable	**Renal failure 6 weeks**		**Renal failure 12 weeks**	
	large artery	small artery	small artery	small artery **+Ca^{2+}**
E+ relaxations (precontraction)				
Acetylcholine (NA)	↓	↔	↓	↑
+ L-NAME	↓	↔	↓	↑
+ L-NAME and diclofenac	↓	↔	↓	↑
+ L-NAME, diclofenac, AP and CHBD	↔	↔	↔	↔
+ SOD	↓			
E- relaxations (precontraction)				
EET (NA)			↓	↑
Nitroprusside (NA)	↔	↔	↔	↔
Isoprenaline (NA)	↓	↓	↔	↔
Cromakalim (NA)	↓	↔	↓*	↑*

ADP, adenosine 5'diphosphate; AP, apamin; CHBD, charybdotoxin; E+, endothelium-dependent; E-, endothelium-independent; EET, 11,12-epoxyeicosatrienoic acid; ↑, ↓ and ↔ indicate an increase, reduction and no change when compared with the corresponding control group, respectively. * levcromakalim.

DISCUSSION

The present investigation examined the effects of dietary calcium on conduit artery responses in L-NAME and NaCl hypertension as well as the effect of vitamin D induced hypercalcaemia on conduit artery responses in NaCl hypertension. The influence of CRF on the tone of large and small mesenteric arteries was evaluated. Furthermore, the effect of treating SH by dietary calcium on the resistance vessel tone was studied.

1 Experimental models of the study

The two models of experimental hypertension employed were chronic inhibition of NOS (Baylis et al. 1992) and NaCl-induced hypertension. NO deficiency presents an interesting model of hypertension, since the endothelial production of NO is essential for the maintanence 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 (Ribeiro et al. 1992), which reached its maximum within four weeks, whereas calcium supplementation reduced the elevation of blood pressure significantly. Moreover, dietary NaCl administration also resulted in elevation of blood pressure, which was completely prevented by calcium supplementation. The 1OH-D_3-treatment also attenuated the development of NaCl-hypertension, and although this could be in part due to the reduced arterial constrictor responses the impaired growth of the 1OH-D_3-treated rats may have caused the decrease in blood pressure. The significant impairment in growth of 1OH-D_3-treated animals may have even been a toxemic effect but this remains unclear since the vitamin D levels in plasma were not measured. There are previous reports of chronic vitamin D-induced hypercalcaemia impairing growth in rats (Bukoski et al 1993). The third experimental model in this study was the subtotal (5/6) nephrectomy in rats, which did not result in elevation of blood pressure in WKY but a small increase was observed in Sprague-Dawley rats.

2 Cardiovascular remodelling and morphology in experimental hypertension and renal failure

Cardiac hypertrophy is the primary chronic compensatory mechanism to increased haemodynamic overload in hypertension (Mosterd et al. 1999). Therefore, L-NAME administration and the consequent NOS-inhibition -induced hypertension would be expected to cause cardiac hypertrophy. However, the heart weight-body weight ratios did not differ

between L-NAME hypertensive and normotensive control rats. This finding agrees with reports, which suggest that this is explained by the negative metabolic effects of L-NAME on protein synthesis (Arnal et al. 1993, Bartunek et al. 2000) and the subsequent inhibition of cardiovascular growth processes (Li et al. 1996, Banting et al. 1997). Calcium supplementation had no effect on the relative heart weights in L-NAME hypertension when compared with the control rats. Since $1,25(OH)_2D_3$ and intracellular Ca^{2+} are known regulators of apoptosis (Van den Bemd et al. 2000), we examined whether there would be alterations in NaCl hypertension following the treatments with $1,25(OH)_2D_3$ and Ca^{2+}. However, no differences were found in the apoptosis of aortic smooth muscle cells, but approximately half of the aortic cross sections showed signs of calcifications after the $1,25(OH)_2D_3$ treatment. This may have played a role in the reduced vasoconstrictor responses of mesenteric arteries after $1,25(OH)_2D_3$-induced hypercalcemia in NaCl hypertension.

Patients with CRF have been 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 conduit arteries has even been seen in the absence of structural changes (Mourad et al. 1997). Consistent with this, experimental renal failure in our study was not associated with morphological changes in large mesenteric arteries. Moreover, no changes in cardiac weight were observed in renal failure rats compared with control animals. However, we found that the small arteries of rats that were investigated 12 weeks following the subtotal nephrectomy featured increased wall to lumen ratio, which was not affected by increased calcium intake. Since the cross-sectional area of arterial wall was not increased, the observed change in vascular morphology in CRF rats is compatible with eutrophic inward remodelling (Mulvany 1999). The vascular wall to lumen ratio exhibits the ability of the vessel to contract against intravascular pressure, while the cross-sectional area indicates the amount of material within the vascular wall, and provides information of vascular growth (Mulvany 1999). Therefore, the results concerning small arterial relaxation following increased calcium intake in renal failure indicated that high calcium diet improved vasorelaxation, although the structure of the resistance vessels was not corrected.

3 Influence of dietary calcium and vitamin D-induced hypercalcemia on arterial contractions in experimental hypertension and renal failure

There is a multitude of approaches that can be applied to study the vascular constrictor responses. The contractile force can be related to segment length or weight, media cross sectional area or lumen diameter, and thus the results depend on the experimental method (Mulvany et al. 1991, Arvola et al. 1993b, Bennet et al. 1996). Various approaches to report arterial contractions were also applied in different original communications of this study. In study I the contractile forces were expressed as the actual forces that were recorded (g),

whereas in studies II, III, IV and V the contractile forces were related to the arterial segment length and expressed as wall tensions (mN/mm). Thus, the numerical values obtained from the studies are incomparable as such. Moreover, the present literature does not reach a consensus regarding the method of the expression of arterial contractile forces.

In the present study, the maximal large artery contractile tension generated by NA or KCl was markedly reduced in the 1OH-D_3-treated groups in the absence and presence of NO synthase and COX inhibition, and also in endothelium-denuded preparations. Thus, alterations in the synthesis or release of NO and prostanoids did not explain these changes, yet the underlying abnormality may have located in the arterial smooth muscle following the 1,25$(OH)_2D_3$-treatment in NaCl-induced hypertension. Previously, contradictory results on the effects of vitamin D on arterial contractile properties in rats have been published, but some reports have described an enhanced contractile reponse to NA in isolated rat mesenteric arteries following the treatment with 1,25$(OH)_2D_3$ for short periods of 3-7 days (Hatton et al. 1994, Bian et al. 1996).

Chronic L-NAME hypertension did not affect the contractile responses of the isolated conduit arteries of Wistar rats, whereas calcium supplementation slightly increased the sensitivity of the arterial rings to KCl. However, the deviation was small. In CRF, the maximal contraction force of the large mesenteric arteries to KCl in endothelium-denuded rings and to NA in endothelium-intact rings were increased. The maximal wall tensions in the resistance arteries to NA, KCl and ET-1 were unaffected by renal failure. Morever, the high calcium intake in renal failure was without significant effect on these responses. Thus, the present results suggest that calcium supplementation does not have a significant effect on the arterial contractile responses, whereas 1,25$(OH)_2D_3$-treatment markedly reduces the maximal tension of the rat large mesenteric artery in NaCl-hypertension. This reduction in vascular contractility could be due to the observed vascular calcification following 1,25$(OH)_2D_3$-treatment.

In the experimental hypertension models of this study some differences in vasoconstrictor sensitivity following the present treatments were observed. Calcium supplementation slightly increased the vascular sensitivity to KCl in both the normotensive and L-NAME-hypertensive groups, and to Ca^{2+} in the normotensive group. In CRF, the large artery sensitivity to NA, KCl and Ca^{2+} was not altered, nor was the resistance vessel sensitivity to NA, KCl and ET-1. Taken together, no marked changes in the arterial sensitivity to constrictor agonists were recorded in the experimental models of the present study.

4 Influence of dietary calcium and vitamin D-induced hypercalcemia on arterial relaxation in experimental hypertension

ACh induces relaxation in arterial smooth muscle by releasing dilatory factors from the

vascular endothelium, the most prominent of these being NO, PGI_2 and EDHF (Busse and Fleming 1993). The relaxations of arterial rings to ACh were impaired in the L-NAME hypertensive and in NaCl hypertensive rats, and effectively improved by calcium supplementation. In contrast, the 1OH-D_3 treatment, without or with high calcium intake, did not have any influences on the endothelium-dependent relaxations in NaCl hypertension. The finding concerning the impaired ACh-induced relaxation in L-NAME hypertension is not new (Küng et al. 1995), but the influence of high calcium intake on arterial relaxations in L-NAME hypertension has not been previously reported. Since calcium supplementation also significantly reduced the elevated blood pressure of the L-NAME rats and completely prevented the NaCl-induced hypertension, the beneficial effect on the vasculature was at least partly mediated via the decrease in blood pressure. Although not directly determined in the present studies, high calcium intake may have also affected the natriuresis of the hypertensive animals. Previously, such an effect has been reported in numerous studies (Pörsti et al. 1991, Hatton and McCarron 1994, Butler et al. 1995). Moreover, the 1OH-D_3-treatment also attenuated the development of NaCl-hypertension.

The chemical antagonism between superoxide anions and NO is an important modulator of vascular tone. In addition, superoxide can inhibit the vascular synthesis of PGI_2 without affecting that of the vasoconstrictor thromboxane A_2 (Katusic 1996). Therefore, increased cardiovascular production of superoxide could contribute to the development of hypertension. In the present L-NAME study, the relaxations to ACh were examined after the addition of the superoxide anion scavenger SOD to the organ bath. The reduction of blood pressure by calcium supplementation may have reduced the production of superoxide in the arteries of L-NAME-treated rats, because the addition of SOD enhanced the relaxations to ACh in the L-NAME group but not in the calcium+L-NAME group. Moreover, SOD also enhanced the relaxations to ACh in the control group but was without effect in the calcium group, suggesting that calcium supplementation reduced the vascular production of superoxide also in the normotensive rats. The present indirectly detected result of reduced superoxide production following calcium supplementation has not been previously reported.

The attenuated vasorelaxation in hypertension could partly be explained by enhanced release of EDCFs (Lüscher and Vanhoutte 1986, Küng et al. 1995). Previously, increased production of vasoconstrictor prostanoids has been shown to potentially contribute to the impaired vasodilatation in L-NAME hypertensive rats (Küng et al. 1995). In the present study, the COX inhibitor diclofenac enhanced the relaxations to ACh in L-NAME hypertensive but not in NaCl hypertensive rats, suggesting that there is an imbalance in the production of vasocontstrictor and vasodilator prostanoids in the vessels of L-NAME hypertensive rats, which favours vasoconstriction. Calcium supplementation appeared to reduce the production of these factors in L-NAME rats, because in the responses to ACh after diclofenac no significant changes were detected in the supplemented groups. Furthermore,

diclofenac enchanced the relaxations to ACh in the control group but was without effect in the calcium group, suggesting that calcium supplementation decreased the release of vasoconstrictor prostanoids also in the control rats. In addition, decreased arterial superoxide production may also have contributed to the enhanced endothelium-mediated vasodilatation after diclofenac administration, because COX is a siginificant source of superoxide (Katusic 1996). In the present model of NaCl hypertension, the administration of diclofenac to the organ bath was without significant effect on the responses to ACh, whereby the role of COX-derived contractile substances seemed negligible in the endothelium-mediated relaxations.

In the L-NAME hypertension study, we had to recognize the phenomenom that the inhibitory effect of orally administered L-NAME on ACh-induced relaxations is known to decline during successive responses in isolated arterial preparations from L-NAME treated rats (Deng et al. 1993). We found that 100 μM L-NAME was needed in the organ bath to prevent this, and therefore the ACh-induced relaxations were performed in the presence of 100 μM L-NAME.

In the presence of diclofenac, the inhibition of NO synthase by L-NAME clearly reduced the responses to ACh in all groups of the NaCl-hypertension study, indicating important contribution of NO to the relaxations. However, the calcium and low-NaCl groups showed distinct L-NAME-resistant relaxations, which indicates that these remaining responses to ACh were mediated by endothelial products other than NO or COX.

The endothelium-dependent relaxations, which remain resistant to NOS and COX inhibition, are mediated by another vasoactive substance, EDHF (Cohen and Vanhoutte 1995). The chemical characteristics of EDHF remain unknown, but functionally this factor is a K^+ channel opener, the action of which can be inhibited by K^+ channel blockers or by depolarization of the cell membrane with high concentrations of K^+ (Adeagbo and Triggle 1993). In the L-NAME hypertension study, although all of the present groups showed distinct NOS- and COX inhibitor-resistant relaxations to ACh, the remaining responses in the L-NAME group were attenuated when compared with other groups, whereas the responses in the calcium+L-NAME group did not differ from control. Thus, calcium supplementation prevented the impairment of endothelium-dependent hyperpolarization in L-NAME treated Wistar rats. The precontraction of arterial rings with KCl almost abolished the remaining NOS- and COX inhibitor-resistant relaxatios to ACh, suggesting that these responses were indeed mediated by EDHF. Furthermore, the findings in the NaCl-hypertension study supported the view that calcium supplementation normalized the impaired endothelium-mediated relaxations by enhancing smooth muscle hyperpolarization. The 1OH-D_3-induced chronic hypercalcemia was without effects on the endothelium-dependent vasorelaxations in NaCl-hypertension. Decreased endothelium-dependent hyperpolarization has previously been observed in various forms of experimental hypertension (Fujii et al. 1992, Van de Voorde et al. 1992, Mäkynen et al. 1996), and the present results suggested that the same holds true in

L-NAME-induced hypertension as well.

Impaired endothelium-dependent hyperpolarization could result from decreased endothelial release of EDHF or from reduced sensitivity of smooth muscle to EDHF. The present results, whereby the relaxations induced by the K_{ATP} opener cromakalim were attenuated in L-NAME hypertension, suggest that the sensitivity of smooth muscle to hyperpolarizing factors was decreased. Furthermore, isoprenaline has been also been reported to hyperpolarize arterial smooth muscle via K_{ATP} and K_{Ca} (Randall and McCulloch 1995, Song and Simard 1995). Therefore, the finding, whereby relaxation to isoprenaline was impaired in L-NAME hypertensive rats, is in agreement with the view of reduced hyperpolarization of arterial smooth muscle in these animals. Moreover, enhanced hyperpolarization could also explain the improved vasorelaxation to isoprenaline and to cromakalim following calcium supplementation in NaCl hypertension as well. Because similar endothelium-independent changes in vasorelaxation were detected in both L-NAME and NaCl hypertension with or without increased calcium intake, they are likely to result from the long-term elevation of blood pressure and the antihypertensive effect of high calcium diet. In our study, the 1OH-D_3 treatment also attenuated the development of NaCl-induced hypertension, but this effect may have been explained by reduced arterial constrictor responses and the impaired growth of the rats. It is noteworthy, that the 1OH-D_3-induced hypercalcemia did not have any favourable influences on vasorelaxation in NaCl hypertension, but it prevented the changes induced by high calcium diet in the control of arterial tone. Therefore, the vascular effects of calcium supplementation may partially be mediated via the suppression of Ca^{2+} regulating hormones.

The arterial relaxations induced by the NO donor SNP have been found to be enhanced or to remain unaffected in L-NAME hypertensive rats (Bryant et al. 1995, Dowell et al. 1996). In our studies, the L-NAME- and the NaCl-hypertensive rats showed attenuated relaxations to SNP in both NA- and KCl-precontracted endothelium-denuded arterial rings, suggesting that the sensitivity of arterial smooth muscle to NO was decreased. Calcium supplementation normalized this abnormality in the calcium+L-NAME group. Previously, high calcium diet has been suggested to enhance sensitivity to exogenous NO in deoxycorticosterone-NaCl hypertension (Mäkynen et al. 1996) and in SHR as well (Tolvanen et al. 1998).

5 Arterial relaxation in renal failure

In rats with CRF induced by the subtotal renal artery ligation, the large mesenteric artery relaxation to ACh was attenuated, and although L-NAME diminished the relaxations, this effect was more pronounced in the renal failure rats than in controls. Therefore, endothelium-mediated relaxations in the renal failure WKY 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, suggesting that the products of the COX pathway were not playing a significant role in the responses to ACh in the conduit arteries of these rats.

The distinct NOS and COX inhibitor-resistant relaxations to ACh were more pronounced in the sham-operated control 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, so that the difference in the remaining relaxation to ACh between the groups was abolished. This suggests that decreased endothelium-dependent dilatation of large mesenteric arteries in the renal failure rats was associated with reduced relaxation via mechanism including the activation of K^+ channels and the subsequent hyperpolarization of arterial smooth muscle.

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 in the large mesenteric arteries induced by isoprenaline and cromakalim were impaired in WKY 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 as discussed above (Randall and McCullough 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.

In spite of the crucial role of small arteries in the regulation of peripheral vascular resistance, the present knowledge about resistance vessel function in CRF is scarce. Isolated subcutaneous resistance arteries from uremic patients with systolic hypertension have shown impaired endothelium-mediated relaxation (Morris et al. 2001), but no such impairment has been found in the skin microcirculation of normotensive patients with renal failure (Cupisti et al. 2000). Experimental renal failure has been reported to impair the vasorelaxation induced by hypoxia in rat resistance arteries (Liu et al. 1997a) whereas the endothelium-mediated relaxation to Ach in the presence of COX inhibition in vitro did not differ from control (Thuraisingham and Raine 1999). However, in these experimental studies, blood pressure was also elevated in the uraemic rats (Liu et al. 1997, Thuraisingham and Raine 1999).

The resistance arteries of the renal failure WKY rats showed similar responses to ACh in the absence and presence of L-NAME when compared with the control animals. Moreover, diclofenac was also without significant effect on the responses, but the further adding of apamin and charybdotoxin in the organ bath chamber markedly diminished the NOS and COX inhibitor-resistant relaxations that still remained comparable between the study groups. This suggests that the endothelium-dependent vasodilatation via the mechanisms that include the activation of K^+ channels and the subsequent hyperpolarization of arterial smooth muscle was preserved in the resistance arteries of the uraemic WKY rats.

The sensitivity of resistance artery smooth muscle to NO was not altered in renal failure, since the vasorelaxations of endothelium-denuded rings to nitroprusside were similar in both study groups. Moreover, the endothelium-independent relaxations induced by cromakalim were also similar, indicating corresponding endothelium-independent hyperpolarization via the opening of K_{ATP}. However, the endothelium-independent small artery relaxations induced by the β-adrenoceptor agonist isoprenaline were impaired in the renal failure group. In the rat mesenteric resistance arteries, isoprenaline is proposed to induce relaxation by hyperpolarizing vascular smooth muscle via the opening of K_{ATP} (Fujii et al. 1999). The signalling cascade leading to vasorelaxation is suggested to be β-adrenoceptor/Gs protein/adenylate cyclase/K_{ATP} (Fujii et al. 1999). In our study, the vasorelaxation via K_{ATP} was not altered since the relaxations induced by the K_{ATP} opener cromakalim did not differ from controls. Therefore, the impaired resistance artery relaxation by isoprenaline in the uraemic rats could not be attributed to changes in the hyperpolarization mechanisms of arterial smooth muscle, but was probably inflicted by a defect at the level β-adrenoceptor/Gs protein/adenylate cyclase coupling step.

6 Influence of high calcium diet on resistance artery relaxation in more advanced renal failure

The present results showed a clear impairment in endothelium-dependent relaxations 12 weeks after surgical subtotal nephrectomy in Sprague-Dawley rats, while high calcium diet for 8 weeks normalized the response to ACh in NTX rats. Since the impaired response to ACh in the renal failure group was also observed in the presence of NOS and COX inhibition, but not in the presence of K_{Ca} blockade, these results imply that endothelium-dependent vasodilatation in experimental renal failure was reduced via a mechanism, which involved activation of K^+ channels in arterial smooth muscle. Reduced endothelium-dependent relaxation via K^+ channels could result from decreased endothelial release of EDHF, or reduced sensitivity of smooth muscle to EDHF. We found that the endothelium-independent relaxations to K_{ATP} opener levcromakalim and the K_{Ca} opener EET were reduced in the renal failure rats, while the high calcium intake also normalized these impairments. Therefore, the deficient vasodilatation at the level of smooth muscle K^+ channels was corrected by increased calcium intake in renal failure. The high calcium diet was without significant effect on blood pressure. The improved vasorelaxation did not correlate with plasma levels of creatinine and urea, but was associated with reduced plasma levels of PTH and phosphate, and elevated plasma levels of ionized calcium. These results support the view that alterations in calcium-phosphate balance contribute to the impairment of vasodilatation during deficient kidney function (Rostand and Drüeke 1999).

Changes in the plasma concentration of phosphate, calcium and PTH could affect blood

vessels in many ways. Elevated phosphate may influence metabolism of vascular smooth muscle, and induce phenotypic changes that predispose the vessel wall to calcification (Jono et al. 2000). A link between extracellular calcium and arterial tone is the calcium receptor located in pervascular nerves, the activation of which causes vasorelaxation via the release of a hyperpolarizing mediator (Mupanomunda et al. 1999). The link between PTH and vascular tone is complex. PTH excess may increase cytoplasmic Ca^{2+} or alter the production of endothelium-derived vasoactive factors (Rostand and Drüeke 1999). Acutely PTH causes vasodilatation, but subacute infusion of physiological doses of PTH raises blood pressure, since vascular desensitization to PTH takes place rapidly (Rostand and Drüeke 1999). Moreover, at the cellular level PTH is linked to increased production of 20-hydroxyeicosatetraenoic acid, an endogenous vasoconstrictor that acts in part by inhibiting the opening of K_{Ca} in smooth muscle (Roman et al. 2000).

In clinical renal failure, the importance of effective treatment of hyperphosphatemia in the prevention of cardiovascular complications is well recognized (Rostand and Drüeke 1999, Slatopolsky et al. 2001). The treatment of SH remains slightly controversial, since a 2-3-fold elevation in plasma PTH has been considered beneficial to bones of patients with impaired kidney function. However, the cardiovascular actions of moderate chronic elevations in PTH are not known in renal failure. Recently, it has been proposed that aggressive management of calcium and phosphorus disturbances at their earliest stage could prevent or attenuate the subsequent emergence of hyperparathyroidism and mitigate the associated risk of cardiovascular events (Drüeke and McCarron 2003). Therefore, these results warrant such clinical studies, where the vascular actions of long-term treatments of SH are elucidated in patients with moderate kidney failure.

SUMMARY AND CONCLUSIONS

The present study was designed to examine the effects of high calcium intake on blood pressure and arterial function in L-NAME- and NaCl-induced forms of experimental hypertension. Furthermore, the influence of vitamin D-induced hypercalcemia in NaCl hypertension was studied. The influence of CRF on the control of large and small artery tone was investigated and resistance artery relaxation was further evaluated following the treatment of SH by high calcium diet in CRF.

The major findings and conclusions are:

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

1.1. Chronic L-NAME hypertension was associated with the impairment of endothelium-dependent and –independent vasorelaxation. Concomitant long-term calcium supplementation clearly improved arterial dilatation in this form of NO deficiency and reduced the elevated blood pressure. The mechanisms underlying the augmented vasodilatation following calcium supplementation in this model of experimental hypertension may have included enhanced arterial hyperpolarization, increased sensitivity to NO in smooth muscle, decreased vascular production of superoxide and vasoconstrictor prostanoids.

1.2. Long-term calcium supplementation and 1OH-D_3-induced hypercalcemia differently influenced the control of arterial tone in NaCl hypertension. Increased calcium intake lowered blood pressure, and improved endothelium-dependent and –independent vasorelaxation, while vascular contractile properties remained unaffected. This is in good agreement with the findings in chronic NO deficiency, but the effect of high calcium intake on blood pressure was more pronounced in NaCl hypertension. The reduced blood pressure and improved arterial relaxation after calcium supplementation may have been explained by enhanced arterial hyperpolarization and increased sensitivity to NO in smooth muscle. Enhanced natriuresis following calcium supplementation may have played a role as well in the reduction of blood pressure, although this was not directly determined in either of the present experimental hypertension studies. The chronic 1OH-D_3-induced hypercalcemia also moderately lowered blood pressure in NaCl hypertension, which was probably explained by

reduced arterial contractile properties and impaired growth of the rats, which may have been an adverse effect of the 1OH-D_3. The 1OH-D_3 treatment also prevented all beneficial changes induced by high calcium diet in the control of arterial tone.

1.3. Rats with 6-week renal failure showed impaired endothelium-mediated relaxation of the large arteries in the absence and presence of NOS inhibition but not under conditions when hyperpolarization was blocked. In addition, endothelium-independent relaxations via the activation of β-adrenoceptors and the opening of K^+ channels were reduced. Therefore, impaired large artery relaxation also in experimental renal failure could be attributed to reduced vasodilation via potassium channels. The resistance arteries of renal failure rats featured impaired relaxations via the activation of β-adrenoceptors, but the endothelium-mediated small artery relaxations did not differ from control 6 weeks after the subtotal nephrectomy.

1.4. Experimental 12-week renal failure in subtotally nephrectomized rats resulted in modest elevation of blood pressure. CRF rats also showed impaired resistance artery relaxation via K^+ channels, which was normalized by increased calcium intake that also prevented the development of SH. The improved small artery relaxation was associated with reduced plasma levels of PTH and phosphate, and elevated plasma levels of ionized calcium. However, high calcium intake did not correct the eutrophic inward remodelling of the resistance arteries in rats with CRF.

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ORIGINAL COMMUNICATIONS