Renal hemodynamics in renal artery stenosis and angiotensin II-dependent hypertension

Pathophysiological and diagnostic aspects

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ABSTRACT

Patients with renovascular hypertension have a poor renal and cardiovascular prognosis. To improve the care of these patients, we need to increase knowledge about the pathophysiological mechanisms involved. Thus, the aims of these studies were to examine: (1) renal hemodynamics and renal blood flow autoregulation (RBFA) in an experimental model of chronic angiotensin (Ang) II-dependent hypertension and the role of superoxide and endothelin (ET)-1; (2) the diagnostic value of intrarenal velocimetric color duplex sonography (CDS) indices in patients with suspected renal artery stenosis (RAS); and (3) biomarkers of oxidative stress (oxs), and ET-1, in hypertensive patients with atherosclerotic RAS (ARAS) and the effect of renal angioplasty.

In chronically Ang II-infused rats, high-NaCl intake (AngII-HNa) resulted in a marked impairment in the myogenic response (MR) of dynamic RBFA. This abnormality was not seen in sham rats on a high-NaCl diet and was significantly more pronounced than in Ang II-infused rats on a normal-NaCl diet. Chronic treatment with tempol, a superoxide dismutase mimetic, attenuated the abnormality in dynamic RBFA in AngII-HNa, whereas acute treatment with ET_A and/or ET_B receptor antagonists had no effect on this abnormality. In AngII-HNa, ET_A antagonism reduced arterial pressure (AP) and specifically increased outer medullary perfusion. These effects were attenuated or abolished by co-administration of ET_B receptor antagonist.

In a retrospective cohort of patients undergoing renal angiography for suspected RAS, acceleration indices of CDS; maximal systolic acceleration (ACCmax) and maximal acceleration index (AImax= ACCmax/peak systolic velocity) provided comparable, good diagnostic accuracy in detecting a hemodynamically significant RAS even in patients with markedly reduced kidney function, in contrast to pulsatility index which correlated significantly to age, renal function and pulse pressure, but not the degree of RAS.

In a prospective cohort of patients undergoing renal angiography for suspected ARAS (significant ARAS; n=83, and non-RAS; n=59) baseline (prior to angiography) inflammatory, but not oxs, biomarkers were significantly elevated in group ARAS vs. both group non-RAS and healthy matched controls (n=20). Plasma ET-1 at baseline was significantly increased in group ARAS vs. healthy controls and was significantly reduced compared to baseline 4 weeks after angioplasty. Angioplasty had no significant effects on AP, biomarkers of oxs, inflammation or serum creatinine.

In conclusion, in a rat model of AngII-dependent hypertension, high-NaCl intake produced a marked impairment in the MR of dynamic RBFA. Tempol attenuated this abnormality, whereas ET-1 receptor antagonists did not, indicating a role for superoxide in the impaired autoregulatory response. In the same animal model, acute ET_A antagonism reduced AP and selectively increased outer medullary perfusion. Our results suggest that selective ET_A antagonists are more effective than combined ET_{A+B} antagonists in this model. Acceleration indices ACCmax and AImax are superior to pulsatility index, and provide a similar, good, diagnostic accuracy in detecting a hemodynamically significant RAS, even in patients with markedly reduced kidney function. Biomarkers of inflammation, but not oxs, are elevated in patients with ARAS. Angioplasty did not decrease inflammatory biomarkers but reduced plasma levels of ET-1 4 weeks after intervention.

Key words: angiotensin II, autoregulation of renal blood flow, color duplex sonography, endothelin-1, oxidative stress, renal artery stenosis, renal hemodynamics, renovascular hypertension,

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LIST OF ORIGINAL PAPERS

The thesis is based on the following publications and manuscripts, which will be referred to in the text by their Roman numerals:

I. Aso Saeed, Göran Bergström, Karin Zachrisson, Gregor Guron, Elzbieta Nowakowska-Fortuna, Ellen Fredriksen, Lars Lönn, Gert Jensen, Hans Herlitz.
Accuracy of colour duplex sonography for the diagnosis of renal artery stenosis*.
Journal of Hypertens. 2009:27(8):1690-6. doi:10.1097/HJH.0b013e32832c417d

II. Aso Saeed, Gerald F. DiBona, Niels Marcussen, Gregor Guron.
High NaCl intake impairs dynamic autoregulation of renal blood flow in angiotensin II infused rats†.
American journal of physiology Regulatory, integrative and comparative physiology.2010 Nov;299(5):R1142-9. doi:10.1152/ajpregu.00326.2010

III. Aso Saeed, Hans Herlitz, Elzbieta Nowakowska-Fortuna, Ulf Nilsson, Alaa Alhadad, Gert Jensen, Ingrid Mattiasson, Bengt Lindblad, Anders Gottsäter, and Gregor Guron.
Oxidative stress and endothelin-1 in atherosclerotic renal artery stenosis and effects of renal angioplasty‡.
(In press, Kidney and Blood Pressure Research)

IV. Aso Saeed, Gerald F. DiBona, and Gregor Guron.
Effects of endothelin receptor antagonists on renal hemodynamics in angiotensin II-infused rats on high NaCl intake.
(Submitted)

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ABBREVIATIONS

ACCmax	maximal systolic acceleration
ACE	angiotensin converting enzyme
ACEI	angiotensin converting enzyme inhibitor
AImax	maximal acceleration index
Ang II	angiotensin II
AP	arterial blood pressure
ARAS	atherosclerotic renal artery stenosis
ARB	angiotensin receptor blocker
ASA	acetylsalicylic acid
AT1	angiotensin II type 1 receptor
AT2	angiotensin II type 2 receptor
CD	collecting duct
CDS	color duplex sonography
CKD	chronic kidney disease
CLDF	cortical laser-Doppler flux
CpO_2	cortical pO_2
CTA	computed tomography angiography
DBP	diastolic blood pressure
DSA	digital subtraction angiography
DTPA	diethylenetriaminepenta-acetic acid
EDV	end diastolic velocity
eGFR	estimated glomerular filtration rate
ESV	early systolic velocity
ET	endothelin
FF	filtration fraction
FMD	fibromuscular dysplasia
GFR	glomerular filtration rate
LD	laser-Doppler
LDL-BDC	baseline conjugated dienes in isolated LDL-cholesterol
MAG3	mercaptoacetyltriglycine
MAP	mean arterial pressure
MAPG	mean arterial blood pressure gradient
MR	myogenic response
MRA	magnetic resonance angiography
MV	mean velocity
NADPH	nicotinamide adenine dinucleotide phosphate
NO	nitric oxide
NOS	nitric oxide synthase
$O_2^{\bullet -}$	superoxide anion
OM	outer medulla

OMLDF	outer medullary laser-Doppler flux
$OMpO_2$	outer medullary pO_2
$ONOO^-$	peroxynitrite
oxs	oxidative stress
PC	protein carbonyls
PI	pulsatility index
PRA	peripheral plasma renin activity
PSD	power spectral density
PSV	peak systolic velocity
PTRA	percutaneous transluminal renal angioplasty
PU	perfusion units
RAAS	renin-angiotensin-aldosterone system
RAS	renal artery stenosis
RBC	red blood cell
RBF	renal blood flow
RBFA	renal blood flow autoregulation
RI	resistive index
ROS	reactive oxygen species
RVH	renovascular hypertension
RVR	renal vascular resistance
SBP	systolic blood pressure
SOD	superoxide dismutase
TAOC	total antioxidant capacity
Tempol	4- hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl
TF	transfer function
TGF	tubuloglomerular feedback
UA	uric acid
WBC	blood leukocyte count
VSMCs	vascular smooth muscle cells

INTRODUCTION

General background

The kidney and hypertension

Hypertension may have its origin in the kidney. However, hypertension, if not treated effectively, can cause kidney damage resulting in a vicious circle wherein kidney damage leads to exaggerated hypertension which in turn leads to further progression of renal disease. Evidence from experimental renal cross-transplantation studies indicates an important role for the kidney in the pathogenesis of hypertension. Renal grafts from genetically hypertensive rats increased arterial blood pressure (AP) in normotensive recipients, whereas renal grafts from genetically normotensive rats lowered AP in hypertensive recipients, suggesting that "blood pressure goes with the kidney" [1].

As early as in 1836, Richard Bright reported the first potential association between hypertension and renal disease when he associated autopsy findings of kidney disease and cardiac hypertrophy to an increased peripheral resistance [2]. In 1898 Tigerstedt and Bergman discovered that extract from the renal cortex of rabbits caused a marked increase in AP when injected intravenously (i.v.) to normotensive rabbits [3]. They hypothesized that the renal cortical tissue extract contained a hypertensive factor and hence named it renin [3]. The first successful experimental model of arterial hypertension caused by manipulation of the kidney was developed in 1934 when Goldblatt et al. showed that clamping of renal arteries in dogs produced a reproducible and persistent rise in AP [4]. Clamping other large arteries as splenic or femoral arteries had no effect on AP, indicating that hypertension resulted specifically from renal ischemia caused by renal artery stenosis (RAS) [4]. In 1938, Leadbetter and Burkland reported the first successful treatment of hypertension by nephrectomy in a patient with RAS [5]. Treatment of RAS changed with the introduction of surgical revascularization in 1954 [6] and later on, in 1978, with the introduction of percutaneous transluminal renal angioplasty (PTRA) [7].

Renovascular hypertension

Renovascular hypertension (RVH), defined as hypertension secondary to RAS, is a relatively common disease, constituting approximately 1–5% of patients with hypertension [8, 9]. However, the prevalence may be considerably higher in selected groups of patients in which the diagnosis is suspected [10, 11]. In patients with generalized atherosclerosis, RAS may lead to

aggravated hypertension, decreased glomerular filtration rate (GFR) and eventually to end-stage renal disease [12]. In addition, atherosclerotic RAS (ARAS) is associated with increased cardiovascular morbidity and mortality [13, 14]. Endovascular treatment by PTRA, with or without stenting, is a commonly used treatment of RAS in selected patients. However, despite improvement of vessel patency, it is at present uncertain if PTRA improves renal and cardiovascular outcomes in patients with ARAS [15, 16].

The renal circulation - anatomy and physiology

The kidneys play an essential role in maintaining a stable internal milieu for optimal cellular function (*homeostasis*) through the excretion of metabolic waste products and adjustment of urinary excretion of water and electrolytes. To achieve this homeostatic function, a high proportion of cardiac output (20-25%) passes through the renal circulation producing about 180 liters of glomerular filtrate (primary urine) per day. In the tubular system the reabsorption of water and electrolytes is adjusted to match the prevailing needs while waste products are retained in the urine and excreted. Almost all (≈ 99%) of the filtered water and sodium is normally reabsorbed in the tubules.

The kidneys receive their blood supply from the main renal arteries which arise from the abdominal aorta. Before reaching the hilum of the kidney, the renal artery generally divides into anterior and posterior branches which in turn give rise to four or five segmental arteries. The segmental arteries divide into interlobar arteries, which progress towards the cortex. At the junction between the cortex and medulla, interlobar arteries change course and become arcuate arteries that run in parallel to the kidney surface (Figure 1). These, in turn, give rise to interlobular arteries which radiate into the cortex and divide into afferent arterioles supplying blood to the glomeruli (Figure 1).

Each afferent arteriole supplies blood to a glomerulus, a tuft of capillaries attached to the mesangium and enclosed in Bowman's capsule. Glomeruli are drained by efferent arterioles that in the cortex give rise to the peritubular capillary plexus [17] (Figure 2). Efferent arterioles from juxtamedullary glomeruli form vasa recta capillaries that form long hair-pin loops that turn in the medulla [17] (Figure 2). Thus, the renal circulation consists of two capillary beds connected in series by the efferent arteriole. The first in the series is the glomerular capillary bed which is the site of filtration and formation of primary urine and the second is the peritubular capillary bed which transports reabsorbed water and solutes back to the systemic circulation.

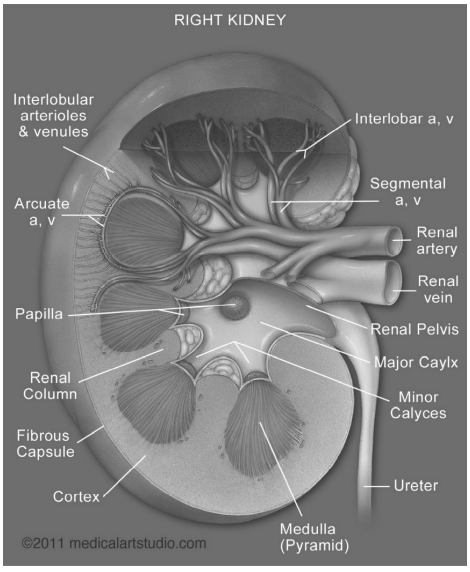

Figure 1. Schematic illustration of kidney blood supply. Reprinted with permission, copyright ©medicalartstudio.com.

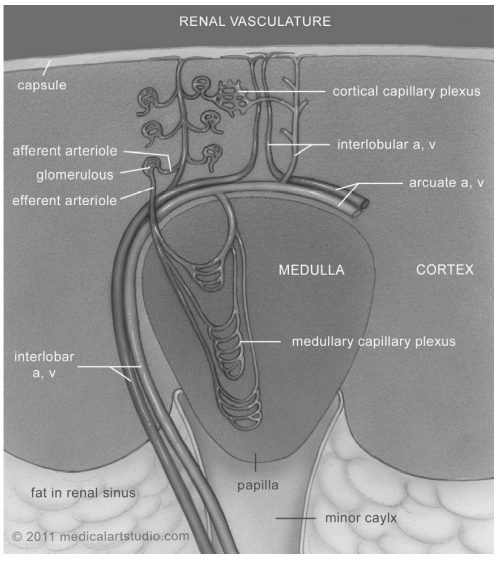

Figure 2. Schematic illustration of renal microcirculation. Reprinted with permission, copyright ©medicalartstudio.com.

Total renal blood flow (RBF) in a healthy adult is approximately 1.2 L/min which corresponds to about 20-25% of cardiac output. The high RBF is required to maintain a high GFR and effective excretion of waste products. Consequently, oxygen delivery to the kidneys is very high and renal oxygen extraction low. However, there is a marked regional difference in blood flow distribution in the kidney [18]. About 90% of total RBF is distributed to the cortex where the partial pressure of oxygen (pO_2) is high (≈ 50 mmHg), whereas approximately 10% of RBF goes to the medulla where the pO_2 is low (≈ 10-20 mmHg). In addition, oxygen consumption in the outer medulla (OM) is high due to active transport of sodium in the thick ascending loop of Henle making the OM vulnerable to ischemic injury [18]. However, low local blood flow in the medulla also plays important physiological roles in preventing washout of the medullary hyperosmotic gradient which is necessary for effective water reabsorption and urine concentration [17, 18].

Regulation of renal blood flow

Many factors are involved in the regulation of RBF. These factors can be classified into three main categories: a) systemic neurohumoral factors comprising renal sympathetic nerves, the endocrine renin-angiotensin-aldosterone system (RAAS), vasopressin, and the family of natriuretic peptides, b) paracrine and autocrine regulators including the intrarenal renin-angiotensin system, nitric oxide (NO), endothelin (ET), and prostaglandins and c) the intrinsic capacity of the kidney in regulating its own blood flow in response to altered perfusion pressure, i.e. autoregulation [17, 19].

Cortical blood flow is efficiently autoregulated [19]. Despite wide variations in AP, RBF and GFR remain relatively stable (Figure 3). Autoregulation of RBF is mediated mainly by two mechanisms: the myogenic response (MR) and the tubuloglomerular feedback mechanism (TGF) [19]. The MR is an intrinsic property of vascular smooth muscle cells (VSMCs) to contract, or dilate, in response to alterations in wall stress caused by changes in AP [19]. The TGF is a mechanism specific to the kidney which regulates RBF and GFR by sensing NaCl load at the macula densa in the early distal tubule and by transmitting this information to the afferent arteriole. Thus, increased AP leads to increased NaCl delivery to the macula densa which eventually leads to vasoconstriction of the afferent arteriole and a downregulation of RBF and GFR. In contrast, reduced AP leads to the opposite effects in an effort to maintain RBF and GFR [19].

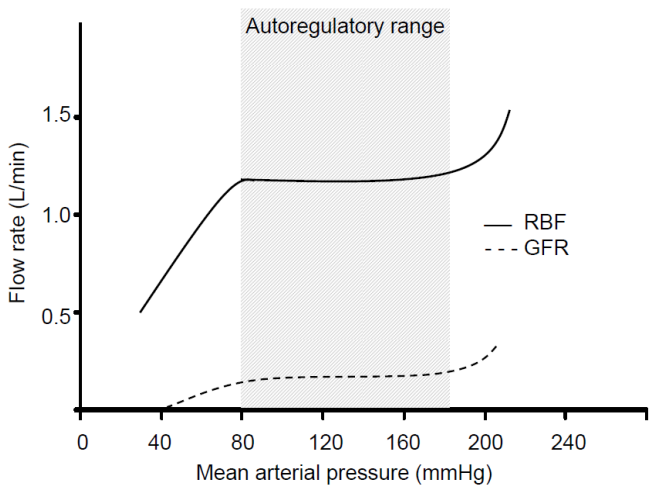

Figure 3. Schematic illustration of autoregulation of renal blood flow (RBF) and glomerular filtration rate (GFR). In the autoregulatory range (mean arterial pressure ~ 80-180 mmHg), RBF and GFR stay relatively constant despite changes in arterial blood pressure. *(Modified from Renal physiology. In Johnson RJ, Feehally J (eds): Comprehensive Clinical Nephrology, 2nd ed. London, Mosby 2003).*

In contrast to cortical blood flow that is efficiently autoregulated, autoregulation of medullary blood flow is uncertain [19]. It has been suggested that the renal medulla has no or a limited autoregulatory capacity [20]. However, these findings have been disputed in subsequent studies [19, 21, 22]. Yet, the autoregulatory range of the medulla may be narrower than that of the cortex [21, 22].

Epidemiology of renal artery stenosis

RAS can be caused by a variety of lesions. In Western populations atherosclerosis and fibromuscular dysplasia (FMD) are the main two causes of RAS [23, 24]. Atherosclerosis accounts for about 90% of all cases of RAS. These lesions are commonly ostial and are in many cases extensions of atheromatous aortic plaques that involve the proximal 1-2 cm of the renal artery [23, 24]. Patients with ARAS are typically over the age of 50 years and males are more commonly affected than females. ARAS is usually a manifestation of generalized atherosclerosis and hence these patients frequently have coronary artery disease (about 20 %) and peripheral vascular disease (about 35 %) [23, 24].

FMD accounts for about 10% of all cases of RAS. Medial fibroplasia is the most common subtype of FMD (75–80%) [23, 24]. The right renal artery is more commonly affected and the disease is most prevalent in 25- to 50- year old females [23, 24]. FMD may involve other major arteries, commonly internal carotid arteries, and less often the vertebral, iliac, subclavian, visceral and coronary arteries. The etiology of FMD is unknown although a number of factors have been suggested, including: a) genetic predisposition, b) hormonal influence, in view of the predominance in females, c) mechanical factors, such as stretching and trauma to the blood vessel wall and d) ischemia of the vascular wall due to fibrotic occlusion of the vasa vasorum [23, 24].

Pathophysiology of hypertension in renal artery stenosis

Luminal narrowing of the renal artery is not in itself sufficient to increase AP unless a "critical" degree of stenosis develops that reduces renal perfusion pressure below the range of autoregulation [25]. Experimental hemodynamic studies indicate that measurable changes in blood flow or perfusion pressure does not develop until the cross-sectional area of the stenotic lesion has been reduced by at least 75% [25]. Reduction of renal perfusion results in activation of the RAAS which is one of the most important counterregulatory pathways directed toward

restoring renal perfusion and GFR. However, the role of the RAAS in causing elevated AP in RAS depends on whether or not a contralateral, non-stenotic kidney is present [25, 26].

Unilateral RAS

In unilateral RAS reduced renal perfusion pressure distal to the stenosis, and decreased NaCl delivery to the macula densa result in release of renin from juxtaglomerular cells [25, 26]. Renin catalyzes the cleavage of angiotensinogen to angiotensin I which is subsequently converted to angiotensin II (Ang II) by the action of angiotensin converting enzyme (ACE). The biological actions of Ang II are multiple and mediated via two distinct receptors, denoted Ang II type 1 (AT1) and type 2 (AT2) with fundamentally opposite effects. Ang II, via AT1 receptor, elicits a potent vasoconstrictor action, enhances sodium and water reabsorption, and increases AP in an attempt to restore renal perfusion [25, 26]. However, the non-stenotic contralateral kidney responds to the elevated AP by excreting more sodium and water (pressure-natriuresis). Thus, renal perfusion remains low in the stenotic kidney resulting in sustained release of renin and activation of the RAAS. Hypertension in this situation is considered Ang II-dependent [25, 26] (Figure 4).

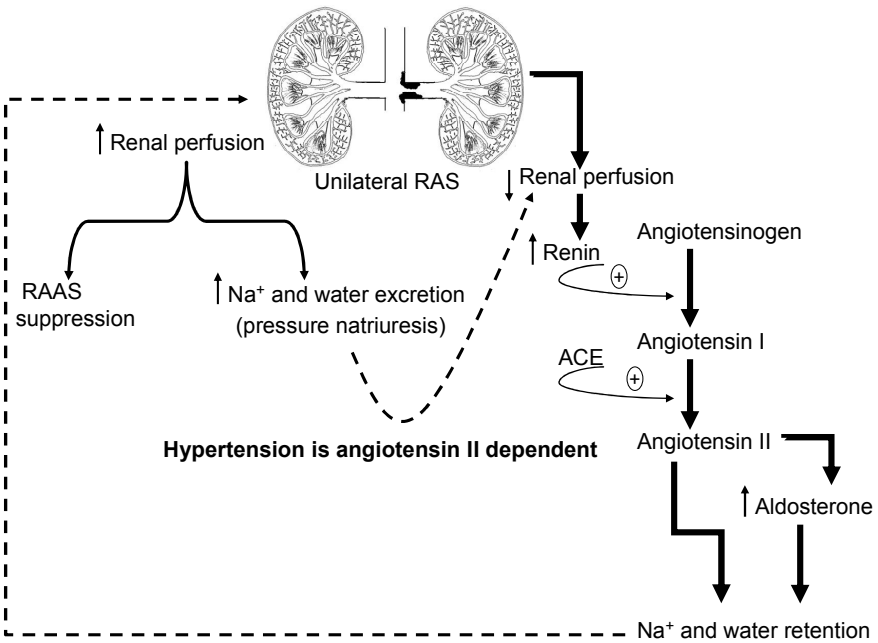

Figure 4. Schematic illustration of the pathogenesis of renovascular hypertension in unilateral renal artery stenosis. *(Modified from Renovascular Hypertension. In Johnson RJ, Feehally J (eds): Comprehensive Clinical Nephrology, 2nd ed. London, Mosby 2003).*

Bilateral RAS or stenosis in a solitary kidney

In this situation the entire renal mass has reduced perfusion and there is no non-stenotic kidney to excrete sodium in response to increased AP. Thus, sodium retention and the expansion of extracellular fluid volume persist. This eventually leads to restoration of renal perfusion and suppression of RAAS. Hypertension in this condition is typically not Ang II-dependent [25, 26] (Figure 5).

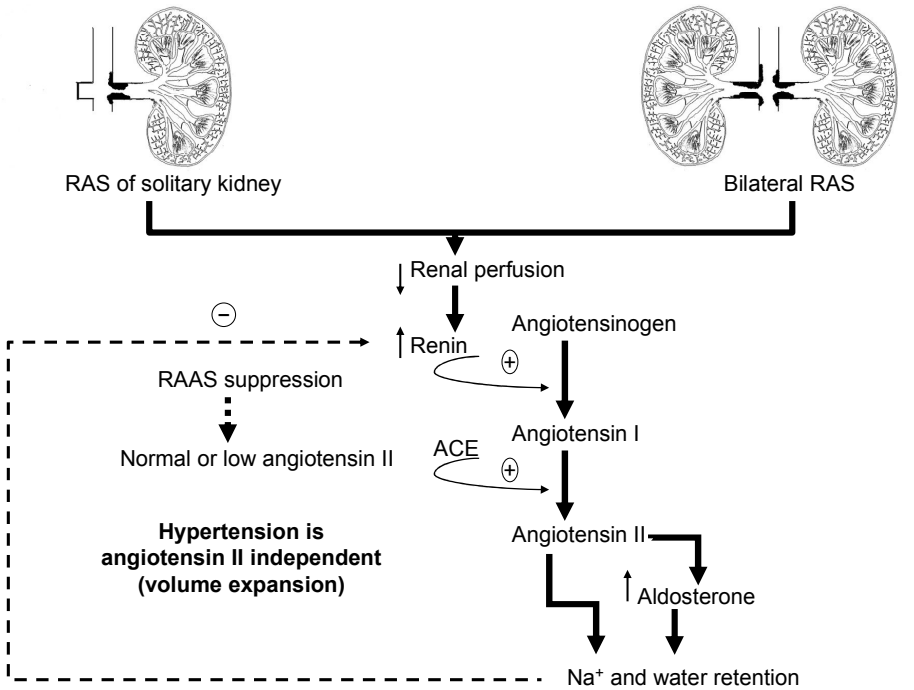

Figure 5. Schematic illustration of the pathogenesis of renovascular hypertension in bilateral renal artery stenosis or stenosis in a solitary kidney. *(Modified from Renovascular Hypertension. In Johnson RJ, Feehally J (eds): Comprehensive Clinical Nephrology, 2nd ed. London, Mosby 2003).*

Additional mechanisms in renovascular hypertension

Evidence from experimental studies suggests that additional mediators are involved in the long-term hypertensive effect of RAS. These comprise oxidative stress (oxs), ET-1, sympathetic nerve activity, and inflammatory factors and cytokines that through different pathophysiological pathways maintain and/or exacerbate hypertension and promote end-organ damage [25, 26].

Oxidative stress

Aerobic metabolism results in generation of highly reactive chemical entities known as "oxidants" or "reactive oxygen species" (ROS) [27, 28]. These include both free radicals and non-radical species. Free radicals are chemical species with one or more unpaired electrons in the outer orbital, yielding the radical unstable and highly reactive. Free radicals of importance in biological systems are e.g. the superoxide anion ($O_2^{\bullet -}$), hydroxyl radical ($^{\bullet}OH$) and nitric oxide ($NO^{\bullet}$) [27, 28]. Non-radical species, e.g. hydrogen peroxide (H_2O_2) and peroxynitrite ($ONOO^-$), are more stable and less reactive, but can easily be converted to radicals [27, 28]. Under physiological conditions deleterious effects of ROS are counteracted by a variety of antioxidant defense mechanisms such as the enzymes superoxide dismutase (SOD), catalase, and glutathione peroxidase. Thus, oxidative stress is defined as a state of imbalance between the production of oxidants, and native antioxidant defense mechanisms, in favor of the oxidants [27, 28].

In the arterial vasculature $O_2^{\bullet -}$, via redox-sensitive processes, can induce pro-inflammatory gene transcription, oxidative tissue injury, and rapidly react with NO resulting in diminished NO bioavailability, endothelial dysfunction and vasoconstriction [28, 29]. Compelling evidence indicates that Ang II via AT1 receptors can induce oxs by stimulating the production of $O_2^{\bullet -}$ through activation of nicotinamide adenine dinucleotide phosphate (NADPH) oxidase [28, 30]. Increased $O_2^{\bullet -}$ generation and oxs by Ang II has been demonstrated in a large number of studies in experimental models of RVH [28, 30, 31]. In addition, treatment with SOD mimetics, and other antioxidants, has been shown to reduce AP and to diminish end-organ damage in these models [28, 32, 33]. However, the involvement of oxs in RVH in humans is less consistent and only a few small studies have addressed this issue [34, 35]. In these studies, biomarkers of oxs were elevated in patients with RVH compared to patients with essential hypertension and/or healthy controls. In addition, angioplasty of the stenotic lesion was associated with reduced levels of oxs biomarkers [34, 35].

The endothelin system

The ET family comprises three 21 amino acid peptides (ET-1, ET-2 and ET-3), with a variety of biological functions including modulation of vascular resistance, sodium homeostasis and AP regulation. ET-1 is formed within the cells by cleavage of the large precursor, prepro-ET-1, to Big ET-1, which is inactive. Big ET-1 is then converted to biologically active ET-1 by an ET converting enzyme. Once produced, ET-1 exerts its biological actions through two major receptor subtypes; ET_A and ET_B. The vascular effects of

ET-1 are influenced by the distribution and relative abundance of ET_A and ET_B receptors. Activation of ET_A and ET_B receptors on VSMCs mediates vasoconstriction whereas activation of endothelial cell ET_B receptors produces vasodilatation which is mainly caused by activation of nitric oxide synthase (NOS) and increased production of NO [36-38].

In the kidney, ET-1 is produced by most tubular segments and regulates sodium and water reabsorption predominantly through ET_B receptors in the thick ascending limb and collecting duct (CD) [39]. In these tubular segments ET_B receptor activation has been shown to inhibit sodium and water reabsorption by suppression of Na-K-ATPase and epithelial sodium channel activity [39] . Interestingly, the highest levels of ET-1 and ET-1 receptors in the body are found in the renal medulla [40] where ET-1 is mainly synthesized by CD cells and acts in an autocrine and paracrine manner [36]. However, ET-1 also modulates medullary blood flow [41, 42] and may inhibit tubular sodium reabsorption by causing medullary vasodilatation.

The ET system has been implicated in the pathophysiology of different forms of hypertension. ET_B receptor antagonism has been shown to increase AP, reduce medullary blood flow, and to blunt the pressure-natriuresis relationship in rats on a high NaCl intake [43]. In addition, rats deficient in ET_B receptors [44] and mice with collecting duct-specific deletion of ET-1 [45], ET_B receptors [46], or ET_A and ET_B receptors [47], develop hypertension that is exaggerated by a high NaCl intake. Taken together, ET-1 in the renal medulla is involved in long-term AP regulation, particularly during high NaCl intake and exerts antihypertensive effects primarily via ET_B receptors by promoting urinary sodium and water excretion.

Ang II has been show to promote intrarenal ET-1 synthesis [48, 49] and hence ET-1 may contribute to hypertension in Ang II-dependent forms of hypertension. The antihypertensive effect of ET_A receptor antagonists has been shown to be more prominent in Ang II-infused rats on a high NaCl diet compared to those on a normal NaCl diet [50]. In line with these results ET_A receptor antagonists lower AP also in other salt-sensitive models of hypertension [51, 52]. However, the role of ET-1 in RVH in humans is less clear.

Renal impairment in renovascular hypertension

Ischemic nephropathy

The mechanisms causing impaired renal function in patients with RVH are multiple, complex and not fully understood. The term “ischemic nephropathy” has been commonly used to describe renal injury beyond a stenotic lesion in the renal artery [25, 53]. The histological picture in ischemic nephropathy is rather unspecific and includes arteriolar nephrosclerosis, collapsed glomeruli and interstitial fibrosis [53]. In addition to hypoxia *per se*, injury of the

stenotic kidney may also be caused by the activation of vasopressor systems such as the RAAS and ET-1. Ang II and ET-1 may in turn increase oxidative stress which can trigger redox-sensitive gene transcription that ultimately leads to tissue injury [25, 53].

Interestingly, in contrast to in ARAS, FMD rarely results in impaired renal function despite similar degrees of stenosis. These observations suggest an important role for the burden of atherosclerotic disease and comorbidity in determining the consequence of RAS on kidney integrity [53]. In addition, pre-existing essential hypertension, seen in many patients with ARAS, may also contribute to renal injury in this patient group [53].

Autoregulation of renal blood flow and hypertensive renal injury

Hypertension is a common cause of kidney injury and end-stage renal disease but also accelerates loss of kidney function in patients with chronic kidney disease (CKD) regardless of the underlying etiology [54]. However, the risk of hypertensive renal injury is variable and the pathophysiological mechanisms are complex and incompletely understood [55-59]. Notably, the deleterious effect on kidney function is observed with even mild-to-moderate AP elevations in CKD patients, indicating an enhanced vulnerability to hypertensive renal damage. Normally, increases in systemic AP are prevented from being transmitted to the renal microvasculature by autoregulatory vasoconstriction of the preglomerular arterioles such that RBF and glomerular hydrostatic pressure are maintained constant.

As mentioned above, renal blood flow autoregulation (RBFA) is maintained by two major mechanisms, the MR and the TGF [19]. An abnormality in any of these two mechanisms may therefore make the kidney prone to hypertensive renal damage [57]. Importantly, even in the absence of severe hypertension, renal damage can still develop if there is an enhanced transmission of systemic AP to the renal microvasculature, which might be the case if renal autoregulatory behavior is impaired. Impaired RBFA in genetic rat models has been suggested as a possible explanation for the vulnerability to hypertensive renal injury seen in these models [60, 61]. In addition, the importance of autoregulatory capacity as a determinant of the susceptibility to progressive pressure-induced renal injury in CKD has been demonstrated in 5/6 nephrectomized (5/6 NTX) rats treated with calcium channel blockers (CCBs) that impair the MR [62]. In these studies CCBs reduced the AP threshold, and increased the slope of the relationship between AP and glomerulosclerosis, such that greater glomerular damage was observed at any given AP elevation as compared with untreated animals. Thus, a potential impairment in RBFA in patients with RVH would make the non-stenotic kidney in unilateral RAS susceptible to pressure-induced renal injury and this could be an important cause of

renal failure in these patients. Hence, in the present studies we wanted to examine RBFA in an animal model of RVH characterized by elevated circulating Ang II levels.

Clinical clues to renovascular hypertension

The typical clues to suggest the diagnosis RVH include: (a) abrupt early (age <30 years) or late (age >50 years) onset of hypertension suggestive of FMD or ARAS respectively, (b) resistant, accelerated or malignant hypertension, (c) hypertension associated with reduced renal function or worsening of renal function during treatment with ACE inhibitor, Ang II receptor antagonist or renin inhibitor, (d) unexplained asymmetry in renal size, or (e) recurrent pulmonary edema associated with hypertension [23].

Diagnostic tests for renal artery stenosis

Digital subtraction angiography

Digital subtraction angiography (DSA) is the gold standard technique for the diagnosis of RAS. In addition to morphological assessment of the stenosis, intra-arterial measurement of the pressure gradient over the stenosis has also been used to evaluate the degree of the stenosis [63]. However, there is as yet no consensus regarding the level of trans-stenotic pressure gradient that indicates a hemodynamically significant RAS. A trans-stenotic mean AP gradient (MAPG) cut-off value of 10 mmHg has previously been shown to be able to predict those patients with RVH responding to angioplasty with reduced AP or a reduced need for antihypertensive drugs [64]. Although DSA is the gold standard for the diagnosis of RAS, it is an expensive invasive procedure associated with risks [65]. Thus, several less invasive screening methods have been used for the evaluation of RAS**.**

Magnetic resonance angiography

Gadolinium contrast-enhanced magnetic resonance angiography (MRA) is widely used as screening test with a high sensitivity (around 95%) and specificity (around 90%) for detecting proximal RAS, but may overestimate the degree of the stenosis [25, 66]. In addition, MRA has considerably lower sensitivity in detecting stenotic lesions in the middle and distal segments of the renal artery caused by e.g. FMD due to inadequate visualization of these segments [25, 66]. Another drawback is the risk of nephrogenic systemic fibrosis caused by gadolinium-based contrast agents in patients with severe renal failure.

Computed tomography angiography

The diagnostic accuracy of computed tomography angiography (CTA) in detecting RAS is similar to that of MRA [25, 66]. Limitations include the risk of contrast nephropathy in patients with reduced renal function and inadequate visualization of the distal segments of renal artery.

ACE inhibitor renography

A technetium-labeled agent such as diethylenetriaminepenta-acetic acid (DTPA) which is excreted by glomerular filtration, or mercaptoacetyltriglycine (MAG3) that is excreted by glomerular filtration and proximal tubular secretion, is injected i.v. and renal uptake of the isotope is recorded by a gamma camera. Time-activity curves provide information on total and single-kidney GFR and RBF. The test is preceded by administration of an ACE inhibitor (ACEI), usually oral captopril 25-50 mg one hour before the procedure. The underlying principle of the test is the assumption that in the stenotic kidney Ang II–induced vasoconstriction of the efferent arteriole is reduced by captopril resulting in a selective reduction of GFR and an exaggerated difference in GFR between the stenotic and non-stenotic kidney [65, 66]. A positive ACEI scan (delayed peak uptake of the isotope >11 minutes, decreased relative uptake, increased cortical retention of the isotope or slower washout of the isotope in the stenotic kidney) is usually followed by a non-ACEI scan. If the abnormalities were more prominent during ACEI, a significant RAS is considered to be present. The sensitivity and specificity of ACEI renography is in the range of 85%, but varies widely in different series [65, 66]. In addition, the test has been shown to be of value in identifying patients with clinical improvement (better AP control) after revascularization with a positive predictive value varying between 51% and 100% (mean, 85%) [66]. However, ACEI renography is less reliable in patients with significant renal parenchymal disease and renal insufficiency or bilateral RAS [65, 66].

Color duplex sonography

Color duplex sonography (CDS) is an inexpensive non-invasive screening test for RAS. It provides both an anatomical evaluation and a hemodynamic assessment of the renal vessels [66, 67]. Two main approaches are used to detect RAS with CDS:

1. Direct visualization of the main renal artery and assessment of renal blood flow velocity (proximal criteria): The commonly used criteria to diagnose significant RAS are: (a) increased peak systolic velocity in the stenotic renal artery (commonly used cut-off is 1.8-2.0

m/s), (b) a renal-to-aortic ratio of peak systolic velocity >3.5 and (c) absence of Doppler signal despite visualization of the renal artery indicates occlusion [66, 67]. The main limitations of this approach include technical difficulties associated with direct scanning of the main renal artery such as inability to identify the renal artery due to overlying fat or bowel gas, inability to obtain optimal scan angle, and difficulty to identify accessory arteries [66].

2. Analysis of the intrarenal Doppler waveforms (distal criteria): Parenchymal arteries (segmental or interlobar arteries) in different regions of the kidney are scanned systematically. The basis of this analysis is that the intrarenal blood flow, downstream to a hemodynamically significant RAS, should become damped with a reduced acceleration of flow during systole. This phenomenon has been called the ‘‘tardus-parvus’’ [66, 67]. Many distal quantitative criteria have been used for detection of significant RAS: (*a*) early systolic acceleration <3.0-4.7 m/s^2, (*b*) acceleration time >0.05-0.08 seconds, and (*c*) differences between kidneys in resistive index (RI) >5% or in pulsatility index (PI) >0.12-0.20 with reduced values in the stenotic kidney [66, 67].

The sensitivity and specificity of CDS in experienced centers is around 80 to 95% [66, 67]. Although assessment of intrarenal Doppler waveforms has overcome the abovementioned technical difficulties associated with direct scanning of the main renal artery, there are still considerable variations in diagnostic criteria and accuracy [68].

Furthermore, intrarenal RI may be useful as a determinant of renal functional outcome after revascularization. Radermacher et al. [69] showed that RI ≥80 served as a negative predictor for revascularization outcome [69]. However, these results were not confirmed in other studies [70, 71].

Plasma renin analysis

Peripheral plasma renin activity

Measurement of peripheral plasma renin activity (PRA) has low diagnostic value as only 50-80% of patients with RVH have elevated PRA [72]. Administration of oral captopril 25-50 mg one hour before the measurement has improved the diagnostic value of the test with a sensitivity and specificity ranging from 75-100% and 60-95%, respectively [73]. The practical usefulness of the test is limited by the need to discontinue antihypertensive agents that interfere with renin activity [73].

Renal vein renin

In unilateral RAS, renal vein renin levels from the stenotic kidney should be elevated, while corresponding levels of renin from the contralateral non-stenotic kidney should be suppressed. Thus, comparison of renal vein renin can be used to predict potential improvement after revascularization [72, 73]. However, studies of renal vein renin are now generally not used because of the high cost and complexity of the procedure and the poor specificity [72, 73].

Management of renovascular hypertension

Treatment options in patients with RVH comprise mainly medical treatment and renal revascularization either surgically or by PTRA with or without stenting [23, 25].

Medical treatment

Aggressive pharmacological control of AP is essential for all patients with RVH independent of the decision to perform renal revascularization. Although different classes of antihypertensive drugs can be used in patients with RVH, treatment with ACEI or angiotensin receptor blocker (ARB) is preferable, since the RAAS plays an important role in the pathogenesis of hypertension. The main concern in using ACEI or ARB in patients with RVH is the potential of these agents to cause a significant reduction in GFR, mainly in patients with bilateral RAS or stenosis of a solitary kidney. However, this may be an indication to consider revascularization [23, 25].

Renal revascularization

Endovascular renal revascularization by PTRA with or without stenting has replaced surgery since the overall outcomes with successful PTRA are generally comparable to those with surgery and associated with a much lesser risk. In addition, stent placement in patients with ARAS has been shown to be associated with better technical success and patency and lower restenosis rate. However, surgical interventions are still necessary in complicated cases e.g. in renal artery aneurysm [23, 25, 74].

Treatment of fibromuscular dysplasia of the renal arteries

PTRA is the treatment of choice in most patients with renal FMD, although some distally located complicated lesions may necessitate surgery as they can not be approached by

PTRA. The results of PTRA are good in FMD, with cure or improvement of hypertension in a high percentage (about 75%) of patients [75].

Treatment of atherosclerotic renal artery stenosis

As ARAS is part of generalized vascular disease, there is a wide agreement that multiple medical interventions comprising tight control of AP by antihypertensive medications, cholesterol lowering therapy by statins, use of antiplatelet agents, and lifestyle modification by cessation of smoking, reduced dietary intake of salt and increased exercise constitute keystones of treatment regardless of renal revascularization [23]. However, in contrast to FMD, it is unclear if restoring vessels patency by PTRA improves outcomes in patients with ARAS [23].

Medical versus endovascular therapy in atherosclerotic renal artery stenosis

Several randomized studies have compared medical therapy with endovascular therapy in the treatment of ARAS:

- The Dutch Renal Artery Stenosis Intervention Cooperative (DRASTIC) study [76]: 106 patients with ARAS (≥50% stenosis) and diastolic AP > 95 mmHg despite treatment with at least two antihypertensive agents were randomized to either medical therapy or PTRA. There were no significant differences between groups in AP at 3 or 12 months (i.e. the primary outcome measures) [76]. The average number of antihypertensive drugs taken by the PTRA group (1.9) was significantly lower than the number taken by the medical therapy group (2.4) at 12 months. Estimated creatinine clearance according to the formula of Cockcroft and Gault [77] was higher in the PTRA group at 3 months, but was similar between groups at 12 months . The study has been criticized due to the enrolment of patients with <70% stenosis [78, 79]. In addition, after 3 months 22 of the 50 patients randomized to medical therapy crossed over to the PTRA group as their AP became difficult to control [78, 79].
- The Essai Multicentrique Medicaments versus Angioplastie (EMMA) trial [80]: 49 patients with unilateral ARAS were randomly assigned to PTRA or medical therapy. Mean ambulatory AP at termination of the study (after 6 months) did not differ between control (141/84 mm Hg) and PTRA (140/81 mm Hg) groups [80]. However, the number of patients requiring at least two antihypertensive medications was 8 of 23 in the PTRA group compared to 22 of 25 with medical therapy [80]. This study has been criticized as the study size was too small and as there was a high rate (27%) of

crossover from the medical to the PTRA group [80]. In addition, many patients failed to reach the goal diastolic AP of < 95 mmHg [80].

- The Newcastle Renal Artery Stenosis Collaborative (NRASC) trial [81]: 55 patients with ARAS (at least 50% stenosis by DSA) and a diastolic AP of ≥95 mmHg despite treatment with at least two antihypertensive drugs were randomly assigned to either medical therapy or PTRA [81]. A significant reduction in AP was observed in patients with bilateral ARAS randomized to PTRA (-34/-11 mmHg) vs. medical therapy (-8 /-1 mmHg; p=0.02) at follow-up (range 3-54 months). However, in patients with unilateral ARAS no significant difference in AP was observed between groups [81]. In addition, no significant differences in serum creatinine levels or major cardiovascular outcomes were found. PTRA resulted in some serious or potentially serious complications, bleeding at the arterial site being the most frequent [81]. Similar to other studies, the NRASC trial has been criticized because no patient underwent stenting and as patients with probably no hemodynamically significant RAS (patients with 50% stenosis) were included [81]. In addition, the goal AP was not stated and the number of patients were small making interpretations difficult [81].
- The Stent placement Atherosclerosis Renal artery (STAR) trial [82]: 140 patients with Cockcroft-Gault estimated creatinine clearance <80 mL/min (mean 46 mL/min) and ARAS of at least 50% were randomized to PTRA with stenting plus optimal medical treatment (antihypertensive drugs + statin+ antiplatelet therapy) or optimal medical treatment alone. The primary end point was a 20% or greater decrease in estimated creatinine clearance [82]. In the intention-to-treat analysis there was no difference between groups in reaching the primary end point (16% in the stent placement group and 22% in the medication group; hazard ratio, 0.73 and 95% CI, 0.33 to 1.61) [82]. PTRA with stenting was associated with serious complications including 2 procedure-related deaths (3%). The STAR trial has been criticized mainly due to enrolment of mild cases with RAS as at least 33% of patients assigned to stenting had 50-70% stenosis [78, 82]. In addition, 12 of the 64 patients who were randomized to stenting had <50% stenosis and hence did not undergo stenting [78, 82].
- The Angioplasty and Stenting for Renal Artery Lesions (ASTRAL) trial [16]: 806 patients with ARAS were randomly assigned to either PTRA with stenting combined with medical treatment (antihypertensive drugs + statin+ antiplatelet) or medical treatment alone. The primary outcome was the rate of decline in renal function, calculated as the mean slope of the reciprocal of the serum creatinine level over time.

Secondary outcomes were AP, the time to renal and major cardiovascular events, and mortality [16]. There were no significant differences between groups in the estimated slope of decline in renal function or in secondary outcomes [16]. However, serious complications associated with revascularization occurred in 23 patients. An important limitation of the ASTRAL trial was the exclusion of patients who were considered having a high likelihood of requiring revascularization within 6 months [16, 78]. In addition, according to the protocol, patients were eligible to participate only "if the patient's doctor was uncertain that the patient would definitely have a worthwhile clinical benefit from revascularization" [16] . Furthermore, the authors did not define which degree of stenosis that was considered hemodynamically significant and hence many patients with very mild ARAS may have been included [16, 78].

Conclusion regarding treatment of patients with atherosclerotic renal artery stenosis

Results from available studies suggest that there is no rational for revascularization of ARAS without strong clinical indications that the procedure will provide a benefit. This conclusion is based mainly on the ASTRAL trial, the largest randomized trial performed as yet on this patient group. The other studies have been small, underpowered and should be interpreted with great caution. The Cardiovascular Outcomes in Renal Atherosclerotic Lesions (CORAL) trial [83] is an ongoing multicenter randomized controlled trial comparing revascularization with medical therapy in 1080 patients with resistant hypertension and angiographically defined ARAS of at least 60% with a ≥20 mmHg systolic trans-stenotic pressure gradient or a stenosis >80% without pressure gradient [83]. This study will provide further answers regarding the potential clinical benefits of revascularization of ARAS as more severe and clinically relevant cases will be included.

AIMS

The overall aim of this work is to improve the care of patients with renovascular hypertension. To achieve this we need to increase our knowledge about the pathophysiological mechanisms and abnormalities in renal hemodynamics that are involved in the initiation, and maintenance of renovascular hypertension.

The specific aims were:

- to evaluate the diagnostic value of intrarenal velocimetric color duplex sonography indices in the screening and diagnosis of renal artery stenosis and the influence of non-renal systemic factors on these indices

- to examine biomarkers of oxidative stress, and endothelin-1 in hypertensive patients with atherosclerotic renal artery stenosis and to evaluate the effect of endovascular revascularization on these variables

- to investigate renal hemodynamics and renal blood flow autoregulation in an experimental model of chronic angiotensin II dependent hypertension and to evaluate the roles of high dietary salt intake, superoxide and endothelin-1

MATERIAL AND METHODS

A combination of experimental animal studies (II, IV) and clinical investigations in patients were performed (I, III). All animal experiments were approved by the regional ethics committee in Gothenburg, Sweden. In addition, the Ethics Committees of the Universities of Gothenburg and Lund approved of the clinical studies and all participants gave their written consent.

Experimental animal studies (II, IV)

Animals

Male Sprague-Dawley rats (Harlan, Horst, The Netherlands) weighing ~300 g were used. Rats had free access to rat chow and tap water throughout and were kept in rooms with a controlled temperature of 24–26°C and a 12:12-h dark-light cycle.

Protocols

Chronically Ang II-infused rats on normal or high NaCl diet and effects of tempol (II)

Rats received Ang II (250 ng/kg/min, s.c.) or isotonic saline vehicle (sham) via osmotic minipumps (Alzet model 2002) for 14 days, after which acute experiments were performed. Rats were either on a normal (NNa, 0.4% NaCl) or high (HNa, 8% NaCl) NaCl diet (Lantmännen, Sweden), creating the following groups: *1*) sham NNa (n = 10); *2*) sham HNa (n = 9); *3*) Ang II NNa (n = 9); and (4) Ang II HNa (n = 8) (Figure 6).

In separate groups, the membrane-permeable superoxide dismutase mimetic 4-hydroxy-2,2,6,6-tetramethylpiperidine-1-oxyl (tempol) was administered in the drinking water (1 mM) throughout the 14-day period: *5*) sham NNa+tempol (n = 8); *6*) sham HNa+tempol (n = 10); *7*) Ang II NNa+tempol (n = 10); and *8*) Ang II HNa+tempol (n = 10) (Figure 6). Tempol administered by drinking water in this concentration has been demonstrated to significantly reduce AP and markers of oxidative stress in several hypertensive rat models [84-86] including in Ang II HNa [87].

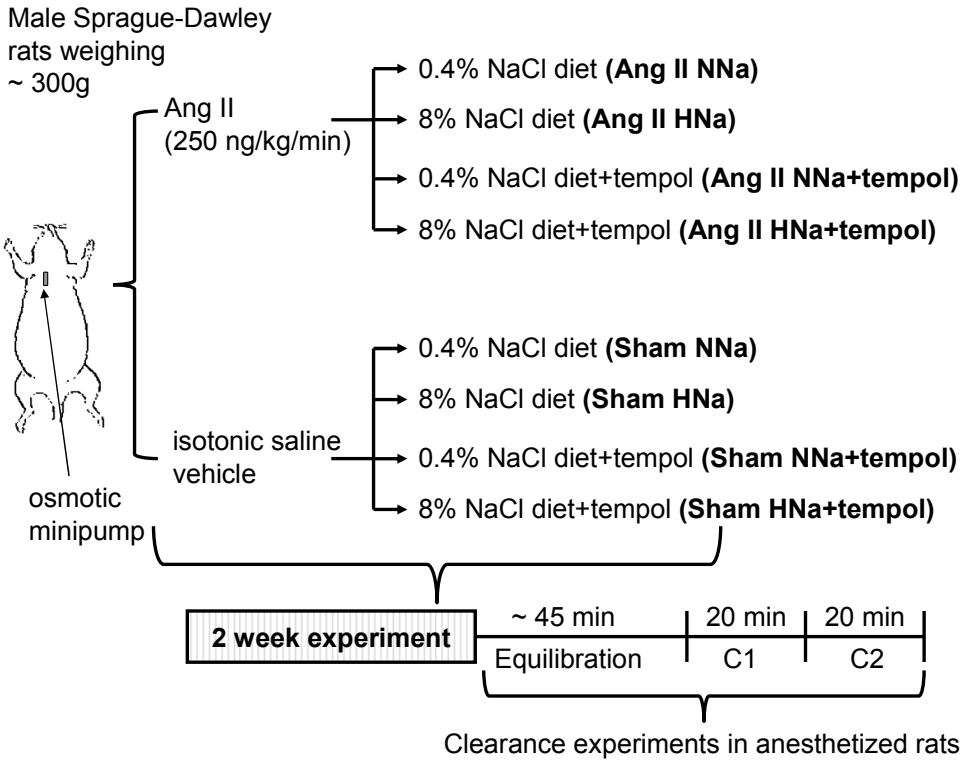

Figure 6. Experimental protocol, study II.

Chronically Ang II-infused rats on high NaCl diet and effects of endothelin receptor antagonists (IV)

Rats received Ang II (250 ng/kg/min, s.c.) via osmotic minipumps (Alzet model 2002) and a high NaCl (8% NaCl) diet (Lantmännen, Sweden) for 14 days after which acute renal clearance experiments were performed. After two 20-minute baseline clearance periods, rats were infused i.v. with either: (a) isotonic saline vehicle (AngII HNa-vehicle, n=8); (b) the ET_A receptor antagonist BQ-123 (30 nmol/kg/min, AngII HNa-BQ123, n=9); (c) the ET_B receptor antagonist BQ-788 (30 nmol/kg/min, AngII HNa-BQ788, n=10); or (d) BQ-123 + BQ-788 (both in doses of 30 nmol/kg/min, AngII HNa-BQ123+BQ788, n=9).

Based on previous studies these doses were expected to result in steady state plasma concentrations that completely block responses to both endogenous and exogenous (0.3 nmol/kg, i.v. bolus) ET-1 via ET_A and ET_B receptors [88-90]. Drugs and vehicle saline were infused in equivalent volumes of 4 ml/kg/h throughout six consecutive 20-minute clearance periods (Figure 7).

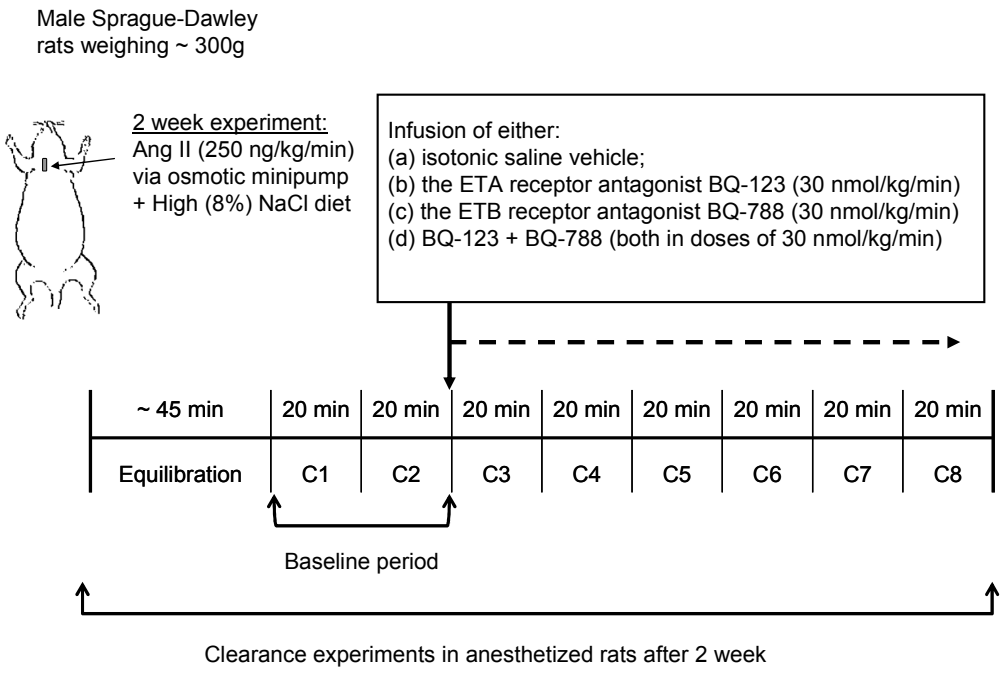

Figure 7. Experimental protocol, study IV.

Renal clearance experiments in anesthetized rats (II, IV)

General procedures

Rats were anesthetized with thiobutabarbital (Inactin, 120 mg/kg i.p.), placed on a heating table and tracheotomized to facilitate spontaneous breathing. A polyethylene catheter (PE50) inserted via the femoral artery was connected to a pressure transducer (Smiths Medical, Kirchseeon, Germany) for monitoring of AP [pulsatile and mean (MAP)] and heart rate (HR) using a data acquisition program (Biopac MP 150, Biopac Systems, Santa Barbara, CA, USA). Infusions of saline and drugs were administered through a femoral vein catheter. The left kidney was exposed by a flank incision and immobilized in a plastic cup and embedded in cotton wool soaked in warm (37° C) saline. The surface of the kidney was covered with warm (37° C) paraffin oil. The left ureter was catheterized (PE25) for urine collection. Rectal and kidney temperatures were kept at 37° C. After completion of the surgical preparations, a 40-45 minute equilibration period was allowed before renal clearance measurements started (Figure 8).

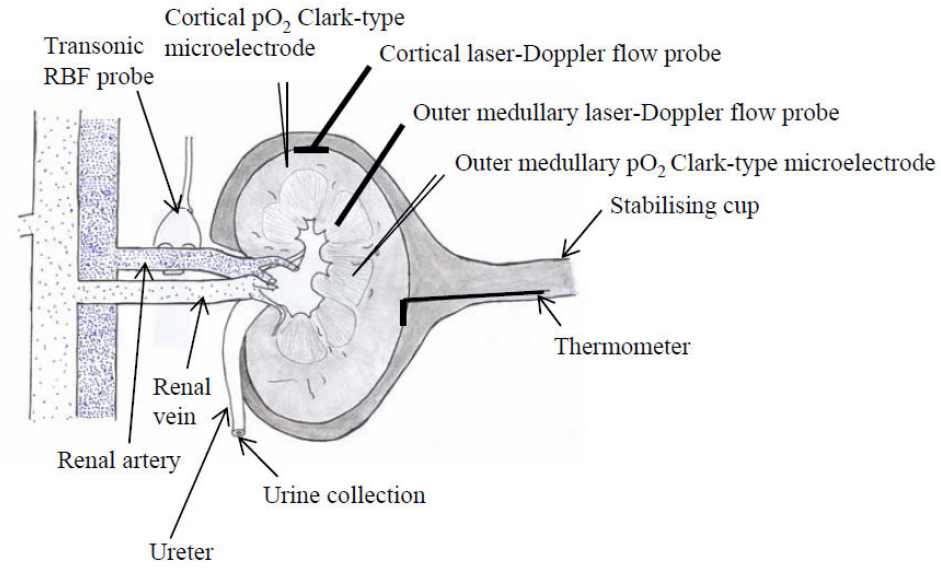

Figure 8. Experimental set-up for intrarenal hemodynamics and oxygen tension registrations *(Reprinted with permission from Nitescu N: Studies on pathophysiological mechanisms in experimental models of acute renal failure, University of Gothenburg, Sweden, 2007).*

Clearance measurements (II, IV)

GFR was determined by measuring renal ^{51}Cr-EDTA clearance (^{51}Cr-ethylenediamine tetraacetic acid, Amersham Laboratories, Buckinghamshire, UK). A bolus dose of 10 µCi/kg ^{51}Cr-EDTA, injected i.v. at the start of the equilibration period, was followed by an infusion of 15µCi/kg/h throughout. Urine was collected during each clearance period into pre-weighed vials. Blood was sampled in heparin coated tubes at the start and completion of each 20-minute clearance period. Plasma was obtained by centrifuging at 2000 rpm for 5 minutes. Values of plasma and urine radioactivity were used to calculate GFR. Drawn blood samples (0.3 ml) were replaced by equivalent volumes of 4 % bovine serum albumin in isotonic saline. Rats were infused with a total volume of 10 ml/kg/h of isotonic saline throughout.

Renal blood flow measurements (II, IV)

A perivascular ultrasonic transit-time flow probe (0.7 VB, T206, Transonic Systems Inc., Ithaca, NY, USA) was placed around the left renal artery for measurement of RBF. Perivascular transit-time probes measure blood flow by emitting a plane wave of ultrasound that crosses the blood vessel in both the upstream and downstream directions. During the upstream cycle, the ultrasonic wave travels against flow and total transit time is increased by a flow-dependent amount. On the other hand, the sound wave travels with flow during the downstream cycle and hence, total transit time is decreased by the same flow-dependent amount (Figure 9). The difference between the integrated transit-times is a measure of volume per minute. The ultrasound transit-time method has been shown to provide reliable and accurate measurements of RBF in anesthetized rats [91]. Renal vascular resistance (RVR) was calculated as MAP (mmHg)/RBF (ml/min/g kidney weight [KW]), and filtration fraction (FF) was calculated as GFR/RBF as hematocrit data were not available for calculation of renal plasma flow.

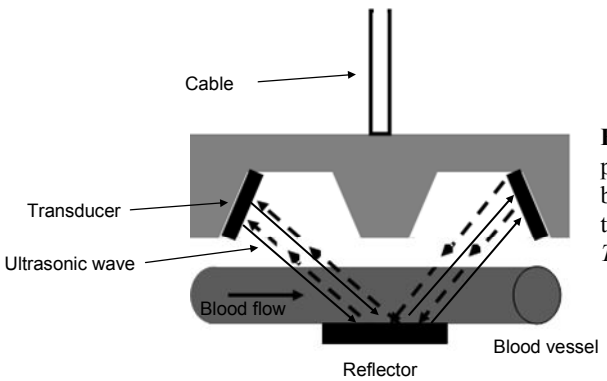

Figure 9. Schematic illustration of the principle for measurement of renal blood flow by a perivascular ultrasonic transit-time flow probe *(Modified from Transonic Systems Inc).*

Intrarenal perfusion measurements with laser-Doppler technique (IV)

Renal cortical (CLDF) and outer medullary (OMLDF) laser-Doppler fluxes were measured by laser-Doppler (LD) flowmetry (PF5000; Perimed, Stockholm, Sweden). Cortical perfusion was measured by a fiber optic LD probe (diameter 1.0 mm, model 407; Perimed) applied on the kidney surface. Outer medullary perfusion was measured by a needle probe (diameter 0.45 mm, model 411; Perimed) at a depth of 3.5 mm into the kidney. Placement of LD probes was performed by micromanipulators, and correct localization was verified by dissecting the kidney at the end of each experiment. The positioning of probes was accurate in all experiments and no data were discarded.

Measurement of intrarenal perfusion with LD technique is based on the emission of laser light from a fiber-optic probe focused on a small volume of tissue. The light is then scattered and partly absorbed by the tissue being studied. Moving red blood cells (RBCs) in the tissue vasculature reflect the light with a shift in frequency (Doppler shift) that is proportional to RBCs velocity and number in the sample volume, while light hitting static objects is unchanged. The reflected light with different frequencies is picked up by the receiver part of the probe, converted into an electronic signal and analyzed (Figure 10). Perfusion is the product of the velocity and the concentration of moving RBC signals within the measured volume and expressed in perfusion units (PU), which are arbitrary.

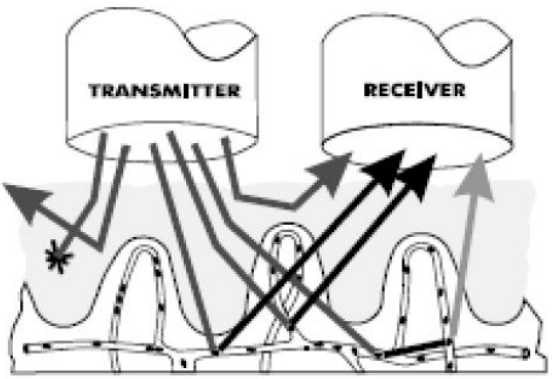

Figure 10. The principle for laser-Doppler measurements *(Reprinted with permission from Perimed).*

The LD technique has widely been used to estimate relative changes in regional tissue blood flow [92-94]. However, data are influenced by the biophysical properties of the investigated tissue, e.g. local hematocrit, structure and density of the capillary beds, and light absorption and scattering. In addition, the LD device provides perfusion measurements within a small tissue volume (0.3-0.5 mm^3), and this could be a limitation as microvascular perfusion is heterogeneous [92-94]. Nevertheless, renal LD data have previously been shown to be highly correlated to regional RBF measured by other methods [92-94]. The main advantage of the LD technique is that it enables continuous measurements of rapid changes in regional tissue perfusion. However, LD measurements can not provide absolute perfusion values and data are expressed in PU which are arbitrary.

Renal oxygen tension measurements (IV)

Renal oxygen tension was measured using Clark-type microelectrodes (outer tip diameter, 10 μm; OX 10; Unisense, Aarhus, Denmark) inserted at depths of 1.0 mm in the cortex and 3.5 mm in the outer medulla as described [95]. Electrodes were polarized at -0.800 V. Oxygen diffuses through the silicone membrane at the tip of the microsensor and is subsequently reduced at the gold cathode surface. The reduction of oxygen gives rise to a small current (<0.50 picoampere) that is linearly related to the pO_2 around the electrode tip [96]. The current is measured by a picoamperemeter. Prior to each experiment electrodes were calibrated in isotonic saline saturated with N_2 and air at 37° C. Placement of oxygen microelectrodes was performed by micromanipulators, and correct localization was verified by dissecting the kidney at the end of each experiment. The positioning of probes was accurate in all experiments and no data were discarded.

Transfer function analysis (II, IV)

Data used to examine the dynamic relationship between AP and RBF, i.e. dynamic autoregulation of RBF, were sampled at 62.5 Hz yielding 75,000 data points for each 20 minute period (150,000 data points for the two 20 minute periods). Processing of AP and RBF data was performed offline using previously developed software routines written for Matlab 7.14 (The MathWorks Inc., Natick, MA, USA). After subtracting the mean value from the data files, they were digitally low-pass filtered (3.0 Hz cut-off frequency, finite-impulse response, order 50) and then resampled to a rate of 6.25 Hz. These 6.25 Hz data files were split into blocks of 2048 data points, yielding a frequency discrimination of 0.003 Hz. Power spectral density (PSD) of AP and RBF was calculated, as described [97, 98]. The transfer function (TF) spectra were calculated from AP (input) and RBF (output). The TF gain was taken as the quotient of the cross spectrum of input and output divided by the power spectrum of the input [97, 98]. Coherence is a frequency domain estimate of a linear correlation (i.e. squared coherence, akin to coefficient of determination) between two signals indicating the degree to which the variance in one signal can be explained by a linear operation on the other signal [97, 98]. The coherence spectra were calculated from AP (input) and RBF (output). The coherence function was taken as the quotient of the square of the cross spectrum of input and output divided by the product of the power spectral densities of AP and RBF [97, 98]. These algorithms involved a Hanning window with 50% overlap of the blocks (12 blocks in the two 20 minute recording periods). To permit comparison among rats, the TF gain (magnitude) values over the frequency range have been normalized to the mean value of the renal vascular

conductance for the entire data set. After conversion of the normalized TF gain values into decibels (20 log [gain]) a mean spectrum was calculated from the consecutive spectra in each rat, and these were subsequently averaged for all rats.

The TF gain corresponds to the ratio of the amplitude of normalized fluctuations in RBF divided by those of AP. In the presence of RBFA, fluctuations of RBF are attenuated vs. those of AP causing the TF gain to be negative. Thus, positive TF gain values indicate impaired RBFA [19, 99]. Phase and coherence spectra were similarly calculated and averaged. Data over the range of frequencies for the MR (0.08-0.18 Hz) and the TGF mechanism (0.03-0.06 Hz) were analyzed [19, 99]. The slope of gain reduction in the frequency range of the MR was determined by least squares fitting of the linear region of gain reduction and the phase peak was estimated as the average phase value within the same frequency interval. In addition, to assess the contribution of the MR to RBFA, mean gain values in the frequency range of 0.06 -0.09 Hz were used to minimize corruption by TGF (<0.06 Hz) and by myogenic transients (>0.09 Hz)[61]. To determine the threshold for coherence above which it exceeds zero with a certain significance level, we used the method described by Koopmans [100] which depends on the total number of samples, the total number of blocks, and the nature of the tapering window. In this study with large sample numbers, coherence values > 0.1 are significantly different from zero at $P < 0.001$.

To assess the effect of filtering the original signal at either 30 Hz or 100 Hz (filter setting options on Transonic TS420 flowmeter module), we made two consecutive recording periods in a single rat with 30 Hz filter/62.5 Hz sampling followed by 100 Hz filter/62.5 Hz sampling. Each of these data sets was subjected to the same processing and analysis as described above. The results showed that over the frequency range of interest (0.01-1.0 Hz) there were no significant differences in PSD for AP and RBF or in TF gain, phase or coherence in the TGF (0.03-0.06 Hz) or MR (0.08-0.18 Hz) frequency ranges.

Generally, a number of different techniques can be applied to assess RBFA. The analysis is based on measurements of RBF in response to changes in renal perfusion pressure. A common approach is to measure steady-state autoregulatory responses using pressure steps. Renal perfusion pressure is manipulated upward and downward in steps and RBF is allowed to stabilize at each level. This method can be applied both to whole kidney investigations and analyses of isolated nephron or arterial segments [19, 99]. An alternative approach is to analyze dynamic RBFA which means that the influence of spontaneous AP fluctuations on RBF is analyzed. Dynamic analysis of RBFA provides a more physiological assessment of the autoregulatory capacity compared to steady-state analysis. In addition, by using TF analyses

in the frequency domain different components contributing to the autoregulatory response (e.g. the MR and TGF mechanisms) can be assessed separately and simultaneously [19, 99]. However, in contrast to steady-state approaches wherein autoregulatory responses to a wide range of perfusion pressure can be measured, assessment of dynamic RBFA by TF analyses is applicable only for the range of spontaneous fluctuations in AP [19].

Kidney histology (II)

At the end of the experiments rats were sacrificed by an overdose of pentobarbital sodium and kidneys were excised and weighed. Kidneys were immersion fixed in paraformaldehyde and prepared for histological analyses using routine techniques. Transverse sections through the hilar area were prepared (3 μm thick) and stained with hematoxylin and eosin, periodic acid-Schiff (PAS) and elastin-vanGieson's. Histopathological changes were scored semiquantitatively (0-3) by an investigator (N.M.) blinded to treatment group. The score 0 was used when no pathologic changes were present, 1 when few of the structures showed changes and the changes were mild, 2 when moderate changes were present, and 3 when severe changes were present in the structures under investigation. For glomerular parameters, the percentage of pathologically altered glomeruli was estimated. Cortical arteries (i.e. interlobar, arcuate and interlobular arteries) and arterioles (i.e. afferent and efferent arterioles) were scored separately.

Clinical studies (I, III)

Participants

Color duplex sonography in renal artery stenosis (I)

This study was a retrospective analysis of all consecutively studied patients with suspected RAS who were evaluated by renal angiography at the Nephrology section at Sahlgrenska University Hospital during 2002–2007. The suspicion of RAS was based on the following criteria: hypertension [resistant, accelerated, malignant, or with elevation of serum creatinine during treatment with ACEI or ARBs], hypertension accompanied by a progressive increase in serum creatinine levels, or recurrent pulmonary edema without overt left ventricular dysfunction. In order for patients to be included, an examination with CDS had

had to be performed in less than 4 months (mean 34 days) prior to renal angiography. A total of 169 patients fulfilled these criteria.

Oxidative stress and endothelin-1 in atherosclerotic renal artery stenosis (III)

Between 2003 and 2008, all patients at the Nephrology section, Sahlgrenska University Hospital, Gothenburg, and the Department of Vascular Diseases at Skåne University Hospital, Malmö, Sweden, undergoing renal angiography for suspected RAS were considered for this study. Indications for angiography were clinical suspicion of RAS (see above) together with a positive screening test for RAS by duplex ultrasonography or by CTA or MRA (≥50% diameter stenosis). To avoid pharmacological interference with the RAAS, patients in whom treatment with ACEI, ARBs or aldosterone receptor antagonists were clearly indicated (e.g. patients with congestive heart failure or diabetic nephropathy), were excluded (for exclusion criteria see Table 1). In remaining patients treated with RAAS inhibitors, who were included in the study, these agents were replaced by other antihypertensive drugs two weeks prior to renal angiography. Hence, included patients were not on any RAAS inhibiting drugs during the study period starting from two weeks prior to baseline measurements. In addition, only patients with unilateral ARAS were included and individuals with RAS of other etiology, or with either bilateral RAS or stenosis of a solitary kidney, were excluded. Twenty age-matched healthy subjects without any medications were recruited from the database of the Gothenburg MONICA study [101] and served as controls.

Table 1. Exclusion criteria, study III

Renal size < 7.5 cm at the stenotic side
Age >80 years
Pregnancy or nursing
CKD stage 5 (eGFR <15 ml/min/1.73m^2)
Congestive heart failure
RAS of other etiology than atherosclerosis
Bilateral RAS or RAS of a solitary kidney
Urinary albumin excretion >1 g/day
Diabetes mellitus with urinary albumin excretion >0.3 g/day
Contraindication for renal angiography/angioplasty (e.g. serious contrast allergy)
Other forms of secondary hypertension
Malignant disease
Treatment with immune modulating drugs, e.g. cyclosporine or oral steroids

CKD, chronic kidney disease; eGFR, estimated glomerular filtration rate according to the 4-variable equation from the Modification of Diet in Renal Disease (MDRD) study; RAS, renal artery stenosis.

Protocol and measurements

Study I

Patients were admitted to the hospital at least 1 day before renal angiography. Systolic and diastolic BPs (SBP and DBP) were measured in the non-dominant arm, in a sitting position, after 5 minutes rest on the morning of renal angiography. Estimated glomerular filtration rate (eGFR) was calculated according to the 4-variable equation from the Modification of Diet in Renal Disease Study (MDRD) formula [102].

Study III

Patients were subjected to baseline measurements one day before angiography (Figure 11). A significant RAS was defined as a lesion with a trans-stenotic MAPG >10 mmHg, or a >50% diameter stenosis on angiography in those cases in which the MAPG was not measured because of technical difficulties due to high-grade stenosis and luminal occlusion during the procedure. Accordingly, 83 patients had significant RAS and underwent PTRA, whereas 59 individuals had no significant RAS and were therefore only subjected to diagnostic procedure (Figure 11).

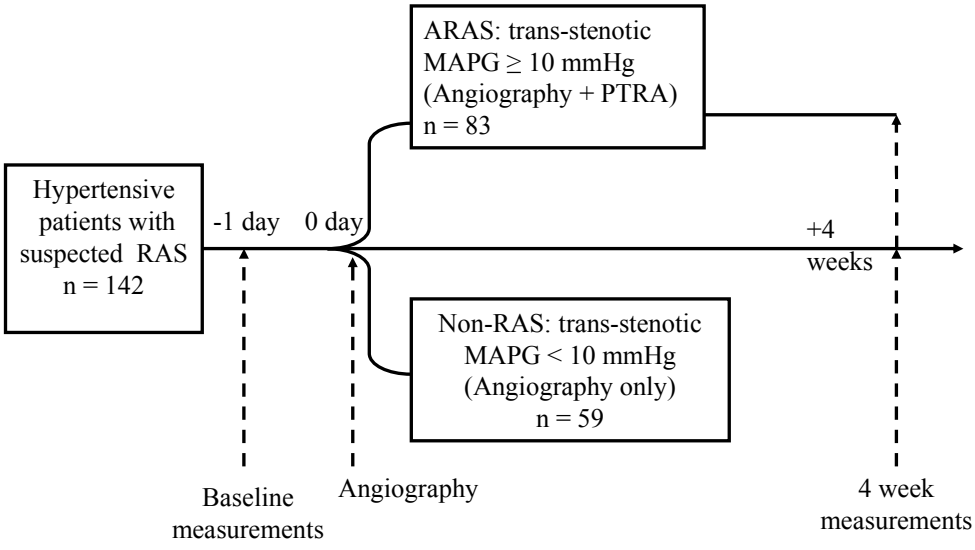

Figure 11. Protocol and measurements, study III.

Systolic and diastolic BP were measured after 5 minutes rest in the sitting position immediately before and one day and 4 weeks after renal angiography. Routine laboratory analyses and biomarkers were measured immediately before renal angiography in all patients. In patients that were subjected to PTRA (n=83) analyses were repeated 4 weeks after intervention. Estimated GFR was calculated according to the 4-variable equation from the MDRD Study [102].

All patients with suspected RAS had been on treatment with a HMG-CoA reductase inhibitor (i.e. statin) for at least 2 weeks at the time of baseline measurements. The majority of patients was already on statin therapy at the time of study inclusion and continued with the same dosage throughout. In remaining patients treatment with simvastatin was started at the time of study inclusion in a daily dose of 20 mg that was maintained throughout. In addition, most patients in both hypertensive groups (90% in group ARAS and 74% in non-RAS) were already on low-dose acetylsalicylic acid (ASA) at this time-point, while treatment with ASA was started 1-2 days before PTRA among those patients who were not already on this treatment. Treatments with statins and ASA were maintained unaltered in patients with ARAS throughout the study period. Healthy controls (n=20) were only studied at one time-point and data were compared with baseline values from hypertensive groups.

Color duplex sonography (I, III)

Color duplex sonography was carried out using Acuson Sequoia 512 with a V4 Transducer (Acuson Corp., Mountain View, California, USA) equipment by experienced technicians with the patient in the lateral decubitus position. After B-scanning for determination of kidney size, blood flow velocities were localized within interlobar arteries. Blood flow velocity spectra were registered for at least 4–8 s with the patient holding breath at the end of a normal expiration. During the examination, at least three measurements in different interlobar arteries covering the upper pole, the midportion, and the lower pole of each kidney were registered, and an average value was calculated. Antihypertensive medication was continued except for ACEI and ARBs, which were withheld 2 weeks prior to the investigation.

Doppler indices (I)

Velocimetric indices from the analysis of Doppler waveforms are described in (Figure 12). In cases with two kidneys, a side-to-side difference in PI, delta (Δ) PI, was calculated and

used for analysis. PI and maximal acceleration of blood flow during the early systolic phase (ACC_{max}) were estimated in every patient directly at the time of examination, whereas maximal acceleration index (AI_{max}) was calculated retrospectively by going back to stored Doppler data.

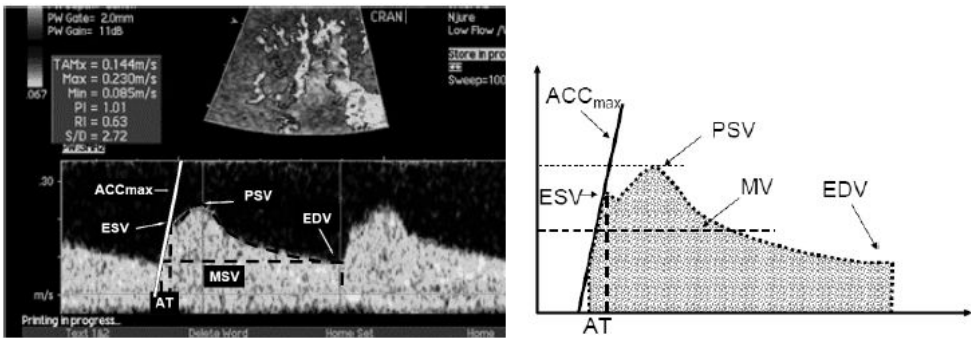

Figure 12. Velocimetric Doppler indices. Representative Doppler waves from an interlobar artery of a non-stenotic kidney (left panel) and a schematic presentation (right panel). ACCmax denotes maximal systolic acceleration; AImax, maximal acceleration index; EDV, end diastolic velocity; ESV, early systolic velocity; MV, mean velocity; PSV, peak systolic velocity.

PI = PSV-EDV/MV

ACCmax = the visually judged maximum derivative of the early systolic upstroke

AImax = ACCmax/PSV

Renal angiography and angioplasty (I, III)

Digital subtraction angiography was used for evaluating renal arteries. Percutaneous transluminal renal interventions were performed in two angiosuites using Philips ViewForum (Integris Release 3.1 or GE Advantx, Waukesha, Wisconsin, USA). A 6 French (F) vascular sheath was placed in the femoral artery after which 3000–5000 IU of heparin was administered. Through a guiding catheter (Vista Brite tip 7F 55 cm RDC), the target renal artery was selectively engaged using a multifunctional probing Catheter 3F (Boston Scientific, Natick, Massachusetts, USA) over a 0.14 stabilizer guide-wire (Cordis, Buenos Aires, Argentina) or a 4F catheter (Cordis). Routinely, a 4F end-hole catheter was used for measuring AP in the aorta and distal to the stenotic lesion enabling estimation of the MAPG. A significant RAS was defined as a lesion with a trans-stenotic MAPG $\geq$10 mmHg, or an at

least 50% diameter stenosis on angiography in those cases in which the MAPG was not measured mainly because of technical difficulties due to high-grade stenosis and luminal occlusion during the procedure. The morphological degree of the stenosis was estimated manually in all cases. Indications for stent placement were angioplasty failure (elastic recoil or flow-limiting dissection resulting in >30% residual luminal narrowing, absence of antegrade flow, or significant residual MAPG), or restenosis. Of patients treated with PTRA, 39 (47%) received stents in study III.

Plasma endothelin-1 and biomarkers of oxidative stress (III)

Blood was sampled and kept on ice until centrifuged (2000 rpm for 10 min at 4° C). Plasma was snap-frozen in liquid nitrogen and stored at -80° C until analyzed. Collected urine was similarly stored at -80° C until analyzed.

Plasma concentrations of ET-1 were measured by a RIA kit (Nichols Institute Diagnostics, San Juan Capistrano, CA, USA). The detection limit for ET-1 was 0.25 pg/ml, the intraassay coefficien of variation (CV) based on pooled samples was 11.3%, and the interassay CV was 22%.

Plasma levels of baseline conjugated dienes in isolated LDL-cholesterol (LDL-BDC) were estimated by the method of Ahotupa et al. [103]. In brief, serum LDL-cholesterol (C) was isolated by precipitation with buffered heparin. Lipids were extracted from LDL-C samples by chloroform-methanol, dried under nitrogen, then redissolved in cyclohexane and analyzed spectrophotometrically at 234 nm (Perkin-Elmer Lambda 2 spectrometer). Intra- and interassay CV were 9.6% and 10.9% respectively. The levels of LDL-BDC were corrected for serum LDL-C (LDL-BDC:LDL-C) to express the LDL-C oxidation degree.
Plasma total antioxidant capacity (TAOC) was determined by trolox equivalent antioxidant capacity assay according to Miller et al. with some modification [104]. In brief, potassium peroxodisulfate was used to induce the oxidation of 2, 20-azinobis-(3-ethylbenzthiazoline-6-sulfonate) (ABTS) [Aldrich, Deisenhofen, Germany] to form the stable radical cation ($ABTS^+$). The cation $ABTS^+$ was measured photometrically at 734 nm. Antioxidants present in the added plasma caused a reduction in absorption proportional to their concentration. Results are expressed in mmol/L of trolox equivalent.

Plasma protein carbonyls (PC) were measured by a colorimetric assay as described by Reznick et al.[105]using a commercially available kit (Cayman Chemicals, Ann Arbor, MI, USA). The intra- and interassay CVs were 4.7% and 8.5% respectively.

Urinary 8-iso-PGF2α was measured in spot urine samples by an 8-isoprostane EIA Kit (Cayman Chemicals, Ann Arbor, MI, USA). Urinary creatinine (U-Cr) was measured by standard enzymatic laboratory methods and the levels of U-8-iso-PGF2α were corrected for U-Cr values. Results are expressed in pg/mg of creatinine. The intra- and interassay CVs were 18.6%, and 29.3% respectively.

Statistical analysis

All values are means±SE if not otherwise stated. Analyses were performed using one-way analysis of variance (ANOVA). Normality was tested with the Shapiro-Wilk test and equality of variances was assessed by Levene's test. If data were not normally distributed or had unequal variances, Kruskal-Wallis one-way ANOVA on ranks was used. Unpaired t-test or Mann–Whitney U test was used when appropriate. Bonferroni corrections were made for multiple comparisons between groups. The sensitivity and specificity, as well as the ideal cut-off limits for Doppler indices in study I, were determined with receiver operating characteristic (ROC) analysis. The Pearson correlation (Spearman correlation when data did not meet assumption about normality) coefficient was used to evaluate correlations.
A P value <0.05 was considered statistically significant.
The statistical software SPSS 17.0 (SPSS Inc., Chicago, Illinois, USA) was used. To assess drug effects in study IV, the area under/over the curve (AUC, AOC) for the intervention period was calculated by the trapezoidal formula using the statistical program Prism version 5 (GraphPad Software Inc., San Diego, CA).

REVIEW OF RESULTS

Experimental studies in rats (II, IV)

Effects of high-NaCl intake and chronic ANG II infusion on renal hemodynamics and kidney function (II)

Effects of high-NaCl diet (Table 2): Group sham HNa showed elevated RBF and reduced FF compared with sham rats on a normal NaCl diet. However, there was no significant difference between groups in MAP.

Effects of Ang II and high-NaCl diet (Table 2): In group Ang II NNa, MAP and RVR were increased compared with sham NNa. Similarly, MAP and RVR were significantly elevated and RBF reduced, in group Ang II HNa compared with both sham NNa and sham HNa. In addition, GFR and FF were significantly elevated in Ang II HNa versus (vs.) sham HNa Furthermore, RVR and FF were elevated in Ang II HNa vs. Ang II NNa.

Effects of tempol in hypertensive groups (Table 2): In Ang II NNa+tempol, GFR was reduced compared with Ang II NNa, but tempol had no significant effects on any of the other variables. In Ang II HNa+tempol, MAP, GFR, and FF were reduced vs. Ang II HNa.

Effects of high-NaCl intake and chronic ANG II infusion on dynamic RBFA (II)

Effects of high-NaCl diet (Table 3, Figure 13): In sham rats high-NaCl intake had no statistically significant effects on dynamic RBFA.

Effects of Ang II (Table 3, Figure 13): In Ang II NNa gain values reflecting the regulatory action of the MR (0.06-0.09 Hz) were not significantly different from those in Sham NNa. In the frequency range of the MR (0.08-018 Hz), the slope of gain reduction and the associated local maximum in phase were reduced in Ang II NNa compared to Sham NNa suggesting attenuation in the MR. In the TGF frequency range, gain values in Ang II NNa were comparable to those in Sham NNa.

Table 2. Kidney function and renal hemodynamics

	Sham NNa (n=10)	Sham HNa (n=9)	Ang II NNa (n=9)	Ang II NNa + Tempol (n=10)	Ang II HNa (n=8)	Ang II HNa + Tempol (n=10)
Body weight (g)	300±15	318±9	283±10	297±11	281±16	267±9[f]
MAP (mmHg)	118±3	120±3	146±3[a]	149±5[e]	156±4[a, b]	139±5[d, f]
LVW (g/kg BW)	2.18±0.05	2.48±0.04[c]	2.81±0.09[a]	2.72±0.07[e]	2.87±0.09[a, b]	2.81±0.09[f]
HR (bpm)	379±6	359±6	387±6	376±13	392±10[b]	339±10[d]
GFR (ml/min/g KW)	1.15±0.06	1.00±0.08	1.28±0.08	0.96±0.07[d]	1.40±0.04[b]	0.85±0.04[d]
RBF (ml/min/g KW)	7.9±0.3	9.1±0.3[c]	7.1±0.4	6.3±0.5[e]	6.0±0.3[a, b]	5.0±0.3[f]
RVR (mmHg/[ml/min/g KW])	15.1±0.6	13.2±0.5	20.9±1.2 [c]	24.9±2.5[e]	26.4±1.6[c]	29.5±3.0[f]
FF (GFR/RBF, %)	14.7±1.0	10.7±0.8[a]	18.2±1.2	16.0±1.7	23.5±0.7[c]	17.6±1.2[d, f]
UV (µl/min/g KW)	4.36±0.74	5.69±0.91	8.30±2.31	16.25±5.29[e]	32.80±5.85[c]	23.02±5.90[f]
$U_{Na}V$ (µmol/min)	0.62±0.15	2.13±0.42[c]	0.46±0.09	1.30±0.49	6.08±0.88 [c]	4.23±1.19
FE_{Na} (%)	0.36±0.09	1.42±0.19[c]	0.26±0.05	0.94±0.30	3.07±0.47[c]	3.45±0.90

Data are means ±SE of two 20-min clearance periods in thiobutabarbital anesthetized rats (see METHODS). NNa, normal NaCl; HNa, high NaCl; MAP, mean arterial pressure; LVW, left ventricular weight; BW, body weight; GFR, glomerular filtration rate; KW, kidney weight; RBF, renal blood flow; RVR, renal vascular resistance; FF, filtration fraction; UV, urine flow rate; FE_{Na}, fractional urinary excretion of Na; $U_{Na}V$, urinary Na excretion. [a]$P < 0.05$ vs. sham NNa, [b]$P < 0.05$ vs. sham HNa, [c]$P < 0.05$ vs. all other groups excluding groups with tempol, [d]$P < 0.05$ vs. corresponding Ang II group without tempol, [e]$P < 0.05$ vs. sham NNa, and [f]$P < 0.05$ vs. sham HNa.

Effects of Ang II and high-NaCl diet (Table 3, Figure 13): In the frequency range of the MR (0.08-018 Hz), the normal transition in gain from positive to negative values (seen in both Sham groups) did not occur in Ang II HNa, and the corresponding local maximum in phase was missing, indicating an impaired MR. Gain values in the frequency range of 0.06-0.09 Hz were significantly elevated in Ang II HNa compared to Sham NNa, Sham HNa and Ang II NNa. In the TGF frequency range (0.03-0.06 Hz) gain values in Ang II HNa were significantly elevated compared to Sham NNa and Sham HNa.

Effects of tempol (Table 3, Figure 14): Tempol had no statistically significant effects on dynamic RBFA in sham groups (data not shown) or in Ang II NNa+tempol. However, in Ang II HNa+tempol, there was a clear transition in gain from positive to negative values in the frequency range of the MR and this was associated with a local maximum in phase indicating an active MR. Consequently, gain values were significantly reduced in Ang II HNa+tempol compared to Ang II HNa in the frequency range of both the MR (0.06-0.09 Hz) and the TGF (0.03-0.06 Hz). Nevertheless, TF analysis variables remained significantly different in Ang II HNa+tempol compared to Sham HNa in the frequency range of both the MR and the TGF indicating that tempol did not normalize RBFA.

Kidney histology (Table 4)

There were no statistically significant differences between groups in tubulointerstitial changes or glomerular abnormalities (data not shown). There was significant thickening of intimal and medial layers, and hyaline deposition/necrosis, of cortical arteries in both Ang II NNa and Ang II HNa compared to Sham NNa. In addition, cortical arterioles (i.e. afferent and efferent arterioles) showed significant hyperplasia and hyalinosis compared to sham. However, the magnitude of arterial and arteriolar changes in Ang II NNa and Ang II HNa were not significantly different, and tempol had no significant effects on vascular abnormalities. Vascular changes in hypertensive animals were generally mild and showed a focal distribution.

Table 3. Characteristics of the transfer function between arterial pressure and renal blood flow

	Sham NNa (N = 10)	Sham HNa (N = 9)	AngII NNa (N = 9)	Ang II NNa + Tempol (N = 10)	AngII HNa (N = 8)	AngII HNa + Tempol (N = 10)
Gain 0.03-0.06 Hz (dB)	-3.45±0.63	-5.84±0.65	-2.68±0.98	-2.42±0.66	-0.02±0.50[a, b]	-2.87±1.04[d, f]
Gain 0.06-0.09 Hz (dB)	-3.17±0.50	-5.55±0.92	-2.56±1.24	-1.80±0.68	1.26±0.50[c]	-2.22±1.03[d, f]
Gain slope 0.08-0.18Hz (dB/decade)	18.60±1.68	18.62±2.05	9.22±3.05[a]	8.45±1.88[e]	2.33±1.11[a, b]	8.30±2.92[f]
Phase 0.08-0.18 Hz (rad)	0.58±0.37	0.61±0.06	0.41±0.07[a]	0.40±0.06[e]	0.18±0.03[c]	0.39±0.10[f]

Data are means±SE. Gain values in the frequency range 0.03–0.06 Hz correspond to the TGF mechanism and values in the range 0.06–0.09 Hz reflect the regulatory action of the MR (see METHODS). [a] $P < 0.05$ vs. Sham NNa, [b] $P < 0.05$ vs. Sham HNa, [c] $P < 0.05$ vs. all other groups excluding groups with tempol, [d] $P < 0.05$ vs. corresponding Ang II group without tempol, [e] $P < 0.05$ vs. Sham NNa, and [f] $P < 0.05$ vs. Sham HNa.

Table 4. Arterial and arteriolar changes in kidney cortex

	Sham NNa	Ang II NNa	Ang II HNa	Ang II NNa + Tempol	AngII HNa + Tempol
Arteriolar hyperplasia	0	1.25± 0.16*	0.88±0.30*	0.63±0.18	0.50±0.19
Arteriolar hyalinosis	0	0.38±0.18	1.00±0.13*	0.50±0.27	0.62±0.33
Thickening of the intimal layer of arteries	0	0.13±0.13	0.13±0.13	0.38±0.18	0.13±0.13
Thickening of the media layer of arteries	0.12±0.13	1.50±0.27*	1.63±0.18*	1.38±0.38	1.25±0.25
Hyaline deposition/ necrosis of arteries	0	1.00±0.27*	1.50±0.27*	1.38±0.42	0.88±0.30

Semiquantitative assessment of cortical arteries and arterioles (i.e., afferent and efferent arterioles) was performed by using a scale from 0 to 3 (see METHODS). Kruskal-Wallis test was used to compare the groups: Sham NNa, Ang II NNa, and Ang II HNa. Mann-Whitney *U*-test was used to compare group Ang II NNa vs. Ang II NNa+Tempol and group Ang II HNa vs. Ang II HNa+Tempol. Values are means±SE. *$P < 0.05$ vs. Sham NNa.

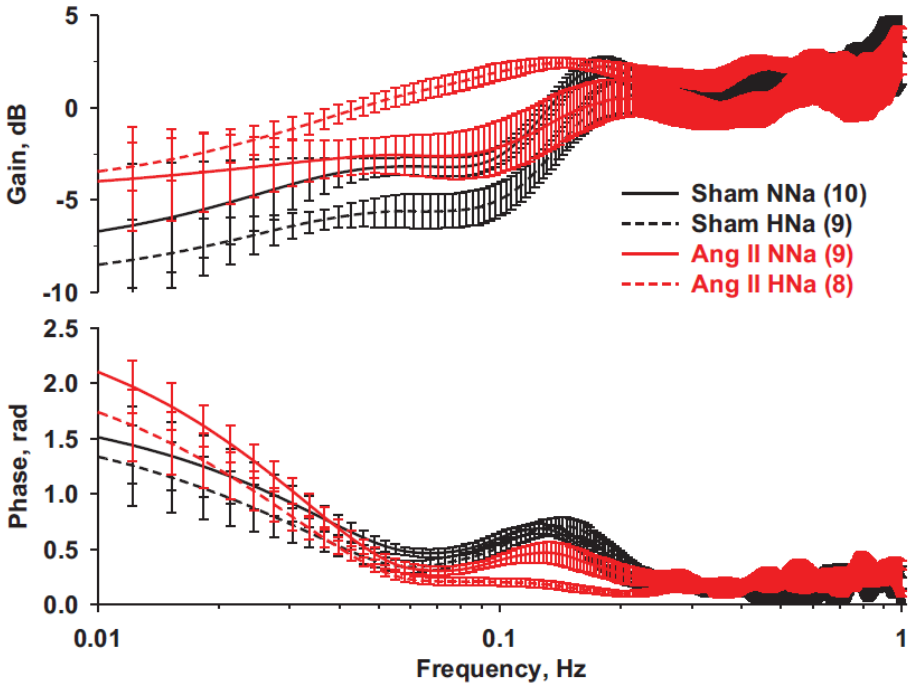

Figure 13. Transfer function gain (top) and phase (bottom) in anesthetized Sprague-Dawley rats after 14 days of ANG II (250 ng/kg/min s.c.) or saline vehicle (sham) infusion. Rats were on either a NNa or HNa diet (see METHODS). In the frequency range of the myogenic response (MR; 0.08 – 018 Hz), gain values showed a positive-to-negative transition in groups sham NNa, sham HNa, and Ang II NNa. The positive-to-negative transition in gain in these groups was associated with the expected local maximum in phase, indicating active MR. In Ang II HNa, the positive-to-negative transition in gain in the frequency range of the MR remained above 0 dB, and the local maximum in phase was missing, indicating impaired MR. Values are means±SE.

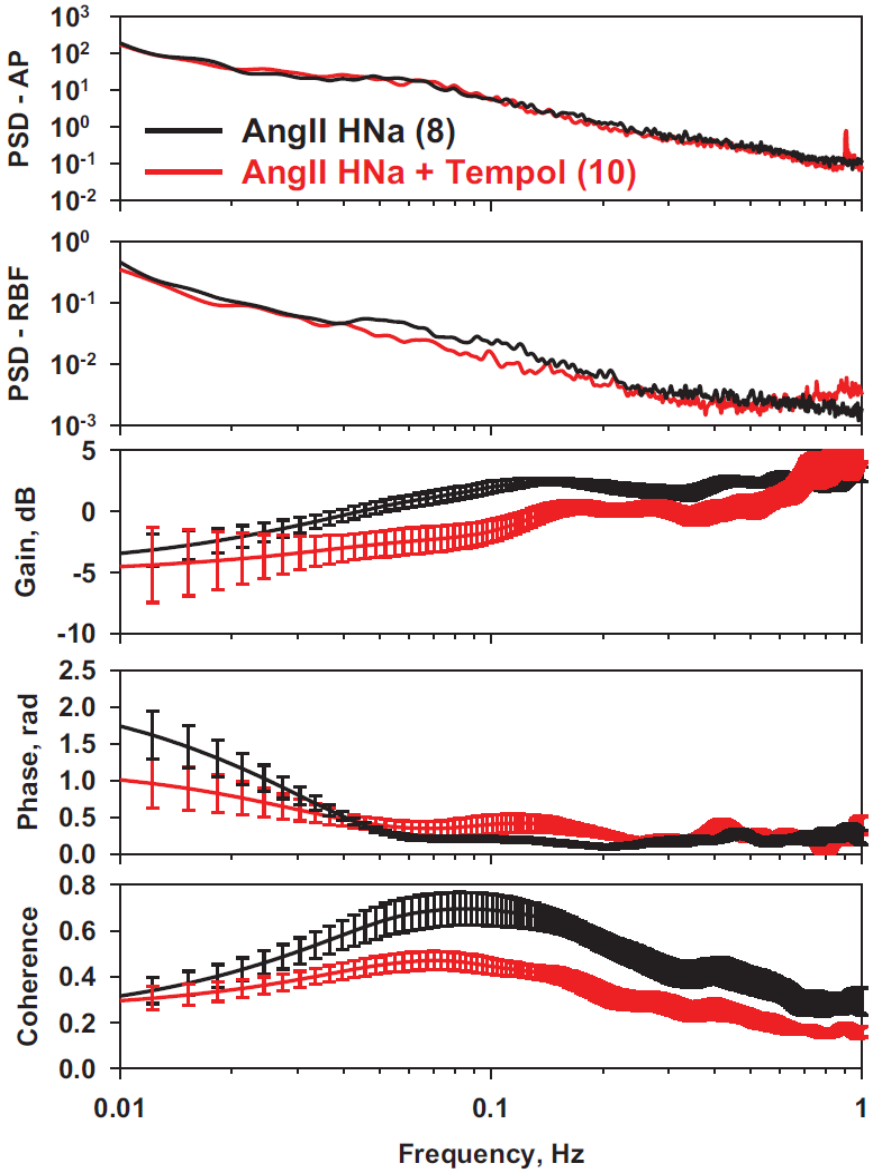

Figure 14. Power spectral density (PSD) for arterial pressure (AP) and renal blood flow (RBF), and transfer function gain, phase, and coherence in anesthetized Sprague-Dawley rats after 14 days of Ang II-infusion (250 ng/kg/min s.c.) with or without tempol in drinking water (1 mM). Rats were on a HNa diet (see METHODS). Contrary to in And II HNa, there was, in group Ang II HNa+tempol, a clear transition in gain from positive to negative values in the frequency range of the MR (0.08 – 018 Hz), and this was associated with a local maximum in phase, indicating an active MR. Values are means ±SE.

Effects of endothelin receptor antagonists on renal hemodynamics in Ang II-infused rats on high NaCl intake (IV)

Renal hemodynamics and function – baseline data

At baseline, prior to drug administration, there were no significant differences between groups in MAP, RBF, RVR or GFR (Table 5). There were small but statistically significant differences between groups in FF, OMLDF, CpO_2, urine flow rate and urinary sodium excretion (Table 5).

Renal hemodynamics and function - effects of ET receptor antagonists

Effects of BQ-123

BQ-123 reduced MAP vs. vehicle ($p<0.05$, Figure 15a). In addition, group AngII HNa-BQ123 showed a progressive increase in OMLDF vs. AngII HNa-vehicle ($p<0.05$) whereas RBF or CLDF were not significantly affected (Figure 16). During the last clearance period OMLDF had increased by 29±8 % vs. baseline in AngII HNa-BQ123. Combined treatment with BQ-123 and BQ-788 abolished the increase in OMLDF produced by BQ-123 alone ($p<0.05$, AngII HNa-BQ123+BQ788 vs. AngII HNa-BQ123, Figure 16c).

Effects of BQ-788

BQ-788 caused a continuous decrease in RBF ($p<0.05$ vs. vehicle) and during the final clearance period RBF had decreased by 29±3 % vs. baseline (Figure 16a). BQ-788 had no statistically significant effects on MAP or GFR and hence RVR and FF increased significantly vs. vehicle ($p<0.05$, Figures 15). Combined treatment with BQ-123 and BQ-788 abolished the effects of BQ-788 alone (Figures 15 and 16).

Effects of endothelin receptor antagonists on dynamic RBFA (IV)

In general, baseline (prior to drug administration) transfer function variables of dynamic RBFA were consistent with our previous data in group Ang II HNa in study II. Endothelin receptor antagonists did not produce any changes in investigated transfer function variables that were significantly different from responses in group AngII HNa-vehicle (data are not shown).

Table 5. Renal hemodynamics and function at baseline, prior to drug administration

	AngII HNa-vehicle (n=8)	*AngII HNa-BQ123 (n=9)*	*AngII HNa-BQ788 (n=10)*	*AngII HNa-BQ123+BQ788 (n=9)*	*ANOVA*
Body weight (g)	281±16	261±11	263±6	262±8	ns
MAP (mmHg)	156±4	146±4	144±5	146±2	ns
HR (bpm)	392±10	373±12	374±9	393±6	ns
GFR (ml/min/g KW)	1.40±0.04	1.22±0.10	1.10±0.08	1.19±0.05	ns
RBF (ml/min/g KW)	6.0±0.3	6.1±0.3	5.8±0.3	6.5±0.3	ns
RVR (mmHg/[ml/min/g KW])	26.4±1.6	24.4±1.4	25.6±1.5	22.9±1.1	ns
FF (GFR/RBF, %)	23.5±0.7	20.4±1.7	19.3±1.1*	18.6±0.9*	p<0.05
CLDF (PU)	670±28	643±20	626±24	686±22	ns
OMLDF (PU)	146±5	133±7	110±9*	129±8	p<0.05
CpO_2 (mmHg)	51.9±0.8	53.2±0.8	50.2±1.0*	53.8±0.7‡	p<0.05
$OMpO_2$ (mmHg)	32.1±0.6	31.1±0.8	29.7±0.7	30.3±0.7	ns
UV (µl/min/g KW)	32.8±5.8	45.5±11.4	39.1±12.8	13.5±2.3*†‡	p<0.05
$U_{Na}V$ (µmol/min/g KW)	6.27±0.95	4.67±0.83	6.31±1.82	2.17±0.44†‡	p<0.05
FE_{Na} (%)	3.07±0.47	3.89±0.92	4.84±1.31	1.77±0.34†‡	p<0.05

Data are means±SE of two baseline 20-minute clearance periods prior to drug administration in thiobutabarbital anesthetized rats (see Methods). MAP, mean arterial pressure; HR, heart rate; GFR, glomerular filtration rate; KW, kidney weight; RBF, renal blood flow; RVR, renal vascular resistance; FF, filtration fraction; CLDF, cortical laser-Doppler flux; OMLDF, outer medullary laser-Doppler flux; PU, perfusion units; CpO_2, cortical pO_2; $OMpO_2$, outer medullary pO_2; UV, urine flow rate; FE_{Na}, fractional urinary sodium excretion, $U_{Na}V$, urinary sodium excretion. * $P < 0.05$ vs. AngII HNa-Vehicle, † $P < 0.05$ vs. AngII HNa-BQ123 and ‡ $P < 0.05$ vs. AngII HNa-BQ788.

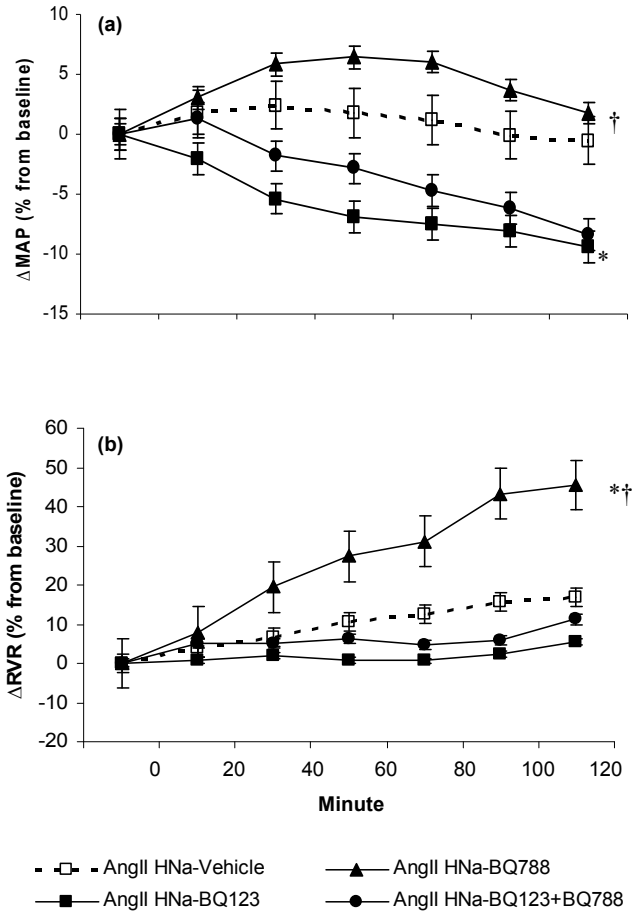

Figure 15. Changes in (a) mean arterial pressure (MAP) and (b) renal vascular resistance (RVR) in response to intravenous BQ123, BQ788, BQ123 + BQ788 or vehicle isotonic saline in hypertensive Sprague-Dawley rats subjected to chronic Ang II-infusion and high NaCl (HNa) diet. Drug administration was started at time zero (see Methods). Values are means±SE. The area under/over the curve was used for comparisons between groups. A P value <0.05 was considered statistically significant. * P <0.05 vs. AngII HNa-Vehicle, † P <0.05 AngII HNa-BQ788 vs. AngII HNa-BQ123+BQ788.

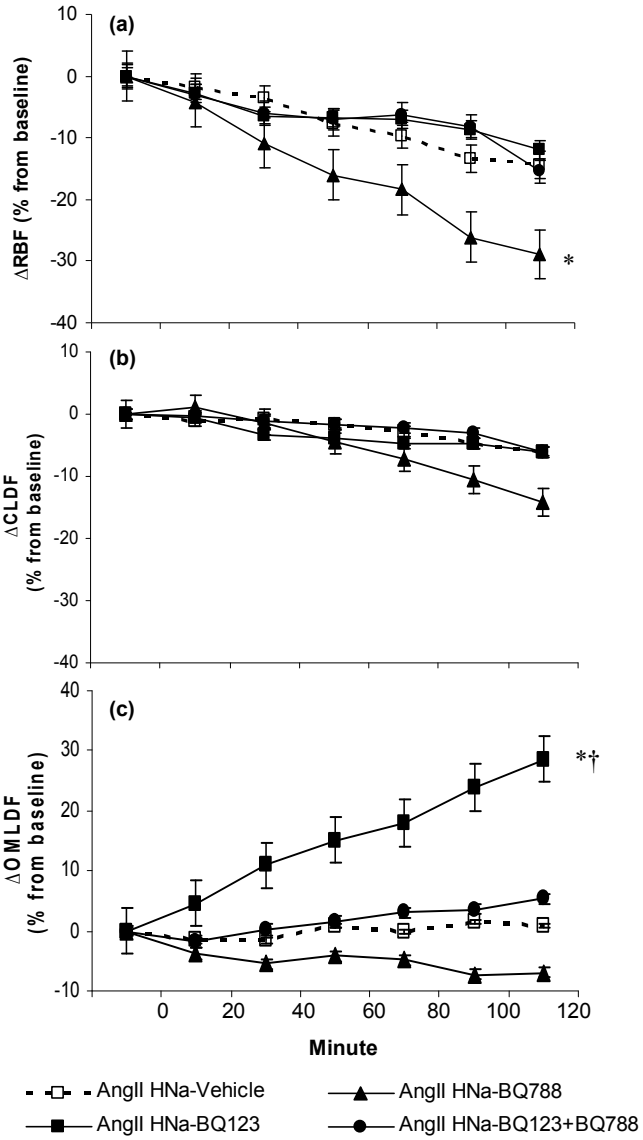

Figure 16. Changes in (a) renal blood flow (RBF) and renal (b) cortical (CLDF) and (c) outer medullary (OMLDF) laser-Doppler fluxes in response to intravenous BQ123, BQ788, BQ123 + BQ788 or vehicle isotonic saline in hypertensive Sprague-Dawley rats subjected to chronic Ang II-infusion and high NaCl (HNa) diet. Drug administration was started at time zero (see Methods). Values are means±SE. The area under/over the curve was used for comparisons between groups. A P value <0.05 was considered statistically significant. * P <0.05 vs. AngII HNa-Vehicle, † P<0.05 AngII HNa-BQ123 vs. AngII HNa-BQ123+BQ788.

Clinical studies (I, III)

Color duplex sonography in renal artery stenosis (I)

Diagnostic value of velocimetric Doppler indices

The distribution of patients and kidneys included in the study is shown in figure 17. In the 136 patients (exclusion of patients with solitary kidney and those with bilateral RAS), there was a good and comparable diagnostic accuracy of ΔPI, and of both ACC_{max} and AI_{max}, for the detection of a significant unilateral RAS (Table 6).

When taking all kidneys into account, the diagnostic accuracy of ACC_{max} and AI_{max} were better than that of PI, especially in patients with eGFR less than 30 ml/min per 1.73m^2 (Table 6). There was no significant difference in the AUC showing sensitivity for detection of a significant RAS versus 1-specificity for different ACC_{max} and AI_{max} cut-off values (Figure 18).

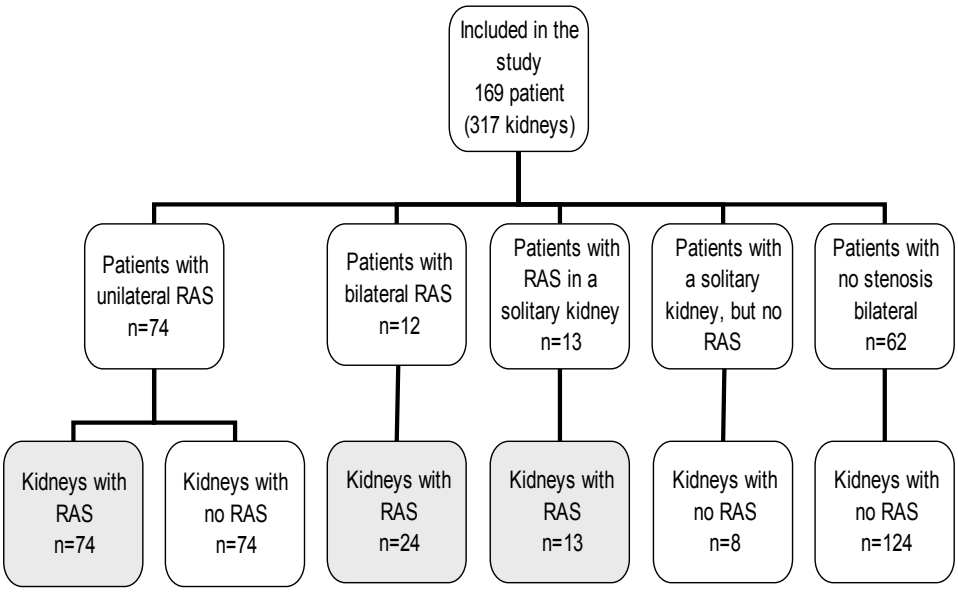

Figure 17. Distribution of patients and kidneys included in the study. For description of included patients see 'Methods'. RAS, renal artery stenosis.

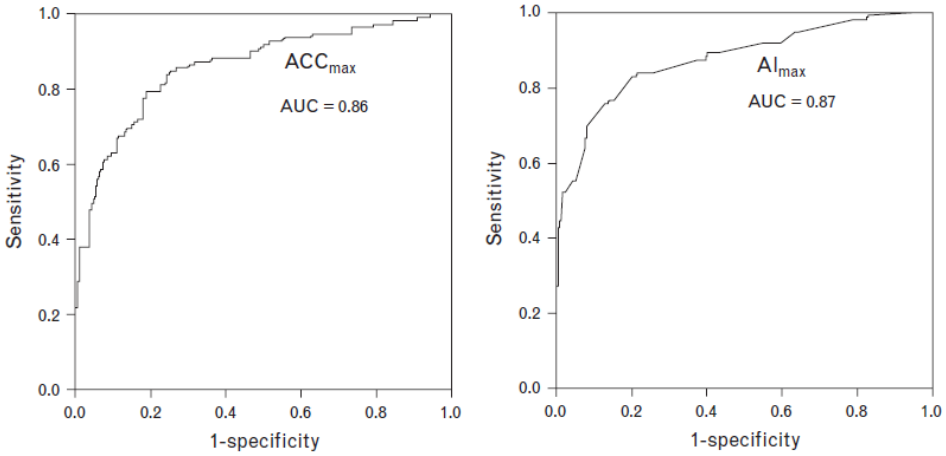

Figure 18. Receiver operating characteristic curves (ROC) for ACCmax and AImax. ROC curves showing sensitivity for detection of a significant RAS vs. 1-specificity for different cut-off values for maximal systolic acceleration (ACCmax, left) and maximal acceleration index (AImax, right). Included in the analysis are all examined kidneys (n =317).

Table 6. Sensitivity, specificity and predictive values of velocimetric indices for the diagnosis of renal artery stenosis

	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
In 136 patients (exclusion of patients with solitary kidney and those with bilateral RAS)				
ACCmax	84	76	55	93
AImax	80	79	59	92
PI	69	71	46	88
ΔPI	78	85	86	76
In all kidneys (n = 317)				
ACCmax	85	75	65	90
AImax	83	79	67	90
PI	60	72	54	77
In kidneys of patients with eGFR>30 ml/min per 1.73m^2 (n = 265)				
ACCmax	84	75	64	90
AImax	85	77	66	91
PI	64	69	52	78
In kidneys of patients with eGFR<30 ml/min per 1.73m^2 (n = 52)				
ACCmax	90	73	65	92
AImax	74	88	78	86
PI	37	88	64	71

Values were calculated for the ideal cut-off limits established with the receiver operating characteristic (ROC) curve. Best cut-off: ACCmax ≤ 3.80 m/s^2, AImax ≤ 15.0 s^{-1}, PI <1.1 (analyzed in 317 kidneys) and delta (Δ)PI > 0.20. ΔPI were only analyzed on patients with unilateral RAS (n = 136), excluding patients with a solitary kidney and those with bilateral RAS. ACCmax, maximal systolic acceleration; AImax, maximal acceleration index; eGFR, estimated glomerular filtration rate based on the MDRD formula; PI, pulsatility index.

Correlation between velocimetric Doppler indices and the degree of RAS (Table 7)

In kidneys with significant RAS, the transstenotic MAPG showed a significant negative correlation to ACC_{max} and AI_{max}. There was also a negative, though not significant, correlation to PI.

Table 7. Analyses of correlations

	ACC_{max}	AI_{max}	PI
In kidneys with significant RAS ($n=111$)			
Age (year)	$r=-0.05$ ($P=0.58$)	$r=0.20$ ($P=0.04$)	$r=0.54$ ($P<0.001$)
S-Creatinine (µmol/l)	$r=-0.10$ ($P=0.29$)	$r=-0.11$ ($P=0.23$)	$r=0.28$ ($P<0.01$)
eGFR (ml/min per 1.73 m^2)	$r=0.06$ ($P=0.56$)	$r=-0.15$ ($P=0.12$)	$r=-0.40$ ($P<0.001$)
Pulse pressure (mmHg)	$r=0.10$ ($P=0.28$)	$r=-0.10$ ($P=0.32$)	$r=0.52$ ($P<0.001$)
Transstenotic MAPG (mmHg)	$r=-0.26$ ($P=0.02$)	$r=-0.29$ ($P=0.01$)	$r=-0.22$ ($P=0.054$)
In kidneys without significant RAS ($n=206$)			
Age (year)	$r=-0.004$ ($P=0.953$)	$r=-0.02$ ($P=0.82$)	$r=0.54$ ($P<0.001$)
S-Creatinine (µmol/l)	$r=-0.09$ ($P=0.19$)	$r=0.17$ ($P=0.01$)	$r=0.21$ ($P<0.01$)
eGFR (ml/min per 1.73 m^2)	$r=0.08$ ($P=0.15$)	$r=-0.12$ ($P=0.10$)	$r=-0.33$ ($P<0.001$)
Pulse pressure (mmHg)	$r=0.26$ ($P<0.001$)	$r=0.21$ ($P=0.003$)	$r=0.57$ ($P<0.001$)

Pearson correlation coefficients between renal Doppler indices and clinical variables. ACCmax, maximal systolic acceleration; AImax, maximal acceleration index; eGFR, estimated glomerular filtration rate based on the MDRD formula; MAPG, mean arterial pressure gradient; PI, pulsatility index; RAS, renal artery stenosis; r, Pearson correlation coefficient.

Influence of non-renal systemic factors on velocimetric Doppler indices (Table 7)

Pulse pressure correlated significantly to both ACC_{max} and AI_{max} in non-stenotic kidneys. However, these correlations were not significant in stenotic kidneys. In contrast to ACC_{max} and AI_{max}, PI showed a significant correlation to patient age, serum creatinine levels, eGFR, and pulse pressure in both stenotic and non-stenotic kidneys.

Oxidative stress and endothelin-1 in atherosclerotic renal artery stenosis (III)

Baseline characteristics and biomarkers prior to angiography (Table 8)

Systolic BP, DBP and serum creatinine levels were elevated, and eGFR reduced, in both hypertensive groups (ARAS and non-RAS) compared to healthy controls.

Plasma TAOC was significantly elevated in groups ARAS and non-RAS compared to healthy controls. However, there were no statistically significant differences between groups in oxs markers plasma PC, LDL-BDC:LDL-C or in urinary 8-iso-PGF2α.

Patients with ARAS showed increased levels of high sensitive C-reactive protein (hs-CRP) and blood leukocyte count (WBC) compared to healthy controls and group non-RAS.

Plasma levels of ET-1 were significantly increased in groups ARAS and non-RAS versus healthy controls. However, there was no significant difference in plasma ET-1 concentrations between group ARAS and non-RAS.

Effects of PTRA on functional variables in patients with ARAS (Table 8)

Four weeks after PTRA, SBP had decreased from 163±24 mmHg to 159±22 mmHg ($p=0.053$) whereas DBP remained largely unchanged. The number of antihypertensive drugs used was not significantly affected. Serum creatinine levels and eGFR were also not significantly affected by PTRA.

Effects of PTRA on biomarkers in patients with ARAS (Table 8)

Plasma ET-1 and serum levels of uric acid (UA) were significantly reduced four weeks after PTRA. There was no significant correlation between changes in plasma ET-1 or UA and those in SBP following PTRA. Changes in plasma levels of ET-1 four weeks after PTRA correlated only to changes in serum UA concentrations ($r = 0.32$, $p<0.05$). There were no statistically significant effects of PTRA on any of the other biomarkers.

Table 8. Blood pressure, kidney function and biomarkers at baseline and 4 weeks after PTRA

	Healthy controls (n=20)	Non-RAS baseline (n=59)	ARAS baseline (n=83)	ARAS 4 weeks Post-PTRA (n= 83)
SBP (mmHg)	116±10	153±22*	163±24*†	159±22
DBP (mmHg)	74±7	89±11*	87±11*	86±11
Antihypertensive drugs (n)	0	2.5±1.1*	2.6±0.9*	2.4±1.1
S-Creatinine (µmol/L)	77±14	102±36*	121±52*	118±44
eGFR (ml/min/1.73m²)	78±13	64±23*	56±22*	57±21
S-Albumin (g/L)	39±3	38±4	37±4	39±3§
tU-Albumin (mg/24hrs)	7±4	63±138*	91±160*	115±250
S-Uric acid (µmol/L)	267±40	370±122*	392±101*	366±92§
Fasting B-glucose (mmol/L)	4.7±0.9	5.9±1.3*	6.7±2.6*	6.5±3.6
P-ET-1 (pg/ml)	0.54±0.24	1.08±0.52*	1.29±0.71*	1.04±0.45§
S-Aldosterone (nmol/L)	0.55±0.21	0.51±0.49	0.57±0.72	0.54±0.97
PRA (ng/ml/h)	1.23±0.65	1.23±1.44	3.67±6.41†	2.33±2.78
P-Ang II (pg/ml)	11.9±4.6	11.4±7.2	18.2±18.3†	16.0±17.9
U-8-iso-PGF2α (pg/mg creatinine)	884±755	648±511	657±533	615±419
P-TAOC (mmol/L)	4.1±0.7	4.6±0.8*	4.6±0.9*	4.6±0.8
P-LDL-BDC:LDL-C (µmol/mmol)	12.5±4.0	15.4±7.1	14.6±5.3	16.1±5.8
P-PC (nmol/mg)	0.24±0.06	0.27±0.08	0.28±0.08	0.27±0.07
hs-CRP (mg/L)	1.9±1.6	4.2±7.5	5.6±6.3*†	6.4±8.5
B-WBC ($x10^9$/L)	6.3±2.1	6.9±1.8	7.8±1.9*†	7.9±2.1

Data are means ± SD. * denotes p< 0.05 vs. healthy controls, † denotes $P < 0.05$ vs. non-RAS and § denotes $P < 0.05$ ARAS post-PTRA vs. ARAS baseline. PTRA; percutaneous transluminal renal angioplasty; RAS, renal artery stenosis; ARAS, atherosclerotic RAS; SBP, systolic blood pressure; DBP, diastolic blood pressure; eGFR, estimated glomerular filtration rate according to the 4-variable equation from the Modification of Diet in Renal Disease (MDRD) study; tU-Albumin, total urinary albumin excretion; P-ET-1, plasma endothelin-1; PRA, plasma renin activity; P-Ang II, plasma angiotensin II; U-8-iso-PGF2α, urinary 8-iso-prostaglandin F2 alpha; P-TAOC, plasma total antioxidant capacity; P-LDL-BDC:LDL-C, plasma baseline conjugated dienes in isolated low density lipoprotein: isolated low density lipoprotein-cholesterol ratio; P-PC, plasma protein carbonyls; hs-CRP, high sensitivity C-reactive protein; B-WBC, blood leucocyte count.

DISCUSSION

Experimental models of Ang II-dependent hypertension (II, IV)

The 2-kidney, 1 clip (2K1C) model is a commonly used model of RVH that bears similarities to patients with unilateral RAS. Still, there are obvious differences to the clinical situation. In the clinic, RAS develops gradually over a long period of time and is mainly caused by atherosclerosis. However, 2K1C hypertension is usually induced in healthy animals without prevailing vascular disease and the onset of renal hypoperfusion is immediate following clip placement. It is well established that increased release of renin from the stenotic kidney results in elevated circulating Ang II levels, at least during the early phase, and Ang II-dependent hypertension in the 2K1C model [26]. By substituting renin release with a chronic infusion of Ang II, chronic Ang II-infused hypertensive animal models have been used to mimic 2K1C hypertension and RVH [106, 107]. Chronic Ang II infusion produces a more reproducible hypertension than 2K1C in rats and major surgery can be avoided. The dose of Ang II infused determines the rate of onset, and magnitude, of hypertension. Several investigators have used low, sub-pressor, doses of Ang II that do not increase AP immediately, instead AP increases gradually over time. Chronic infusion of sub-pressor doses of Ang II has been proposed as a model of primary hypertension in humans. In the present studies (II, IV), rats received a dose of Ang II (250 ng/kg/min, s.c. via osmotic minipumps for 14 days) that gives rise to a reproducible and gradual increase in AP that is evident already after 24 h [108]. The Ang II dose used in our studies is higher compared to most, but not all, sub-pressor doses used by others [107-109]. Hence, our model of Ang II-dependent hypertension resembles hypertensive patients with unilateral RAS and elevated circulating Ang II levels and does not reflect the situation in primary hypertension.

High-NaCl intake impairs renal blood flow autoregulation in Ang II-infused rats (II)

Effect of chronic Ang II

In the frequency range of the MR, TF analysis showed a reduced slope of gain reduction, and a diminished phase peak, in Ang II NNa compared to Sham NNa indicating an impaired MR. This result differs from a previous study in which dynamic RBFA was assessed in conscious dogs infused with Ang II to produce plasma Ang II levels within the physiological range[110]. These investigators found that Ang II did not affect RBFA and the relative contribution of the MR and TGF components [110]. The discrepancy between studies could be explained by differences in the experimental protocols. For instance, in our study

animals were anesthetized, hypertensive and infused with much higher Ang II concentrations and for a longer duration. However, impaired RBFA has been demonstrated in several studies in chronically Ang II-infused hypertensive animals using the in vitro blood-perfused juxtamedullary nephron model in which responses of afferent arterioles to step-wise changes in perfusion pressure were analyzed [107, 111, 112]. In contrast to these in vitro investigations, our study presents data on dynamic autoregulation of whole kidney RBF during spontaneous fluctuations in AP in intact Ang II hypertensive animals. We also extend previous findings by suggesting that chronic Ang II infusion selectively affects the MR component of RBFA.

Effect of chronic Ang II and high-NaCl intake

The major finding was the much more pronounced impairment in dynamic RBFA in Ang II HNa compared to Ang II NNa. This abnormality in Ang II HNa was characterized by an almost complete absence of the MR. This synergistic effect of Ang II and high NaCl intake on dynamic RBFA represents a novel finding. One could hypothesize that the impairment in RBFA, mainly in group Ang II HNa, would make kidneys vulnerable primarily to reductions in AP, and not to glomerular hypertension, as RVR was markedly elevated and RBF reduced in these rats. However, despite the reduction in RBF, GFR tended to be elevated in Ang II HNa and FF was significantly increased versus the other groups. These results indicate that the increase in resistance predominantly occurred at efferent arterioles and that glomerular capillary pressure was maintained or elevated despite reductions in RBF. In this situation it is reasonable to speculate that the aforementioned abnormalities in RBFA in Ang II HNa could eventually cause pressure-induced glomerular injury. Notably, we were unable to observe significant differences between groups in glomerular histology after 2 weeks. However, it is possible that glomerular abnormalities could have been detected in hypertensive animals with longer study duration. In line with our results, Elmarakby et al. [58] showed that autoregulatory responses of afferent arterioles to step-wise increases in perfusion pressure were blunted in Ang II-infused rats on high dietary NaCl [58]. However, in that study no comparison was made to Ang II-infused animals on a normal NaCl intake. Interestingly, Zhao et al. [113] using the in vitro blood-perfused juxtamedullary nephron preparation, showed that afferent arteriolar vasodilator responses to acetylcholine and sodium nitroprusside were significantly more attenuated in Ang II-infused rats on a high NaCl diet compared to those on a normal NaCl diet [113]. Although RBFA was not assessed, these results demonstrate that the combined influence of Ang II and high NaCl intake produces more pronounced abnormalities in afferent arterioles, the main site of RBFA, than either stimulus alone.

Effect of tempol

Tempol attenuated, but did not normalize, the abnormal MR in Ang II HNa indicating an important role for $O_2^{\cdot-}$ in the impairment of autoregulatory response specifically under the circumstance of both elevated Ang II and high NaCl intake. In addition, tempol reduced AP only in Ang II HNa. In line with our results previous studies in Ang II-infused animals have demonstrated that high NaCl intake further increased $O_2^{\cdot-}$ generation and lipid peroxidation compared to animals on a normal NaCl intake [114, 115]. The mechanisms by which tempol attenuated abnormalities in RBFA in Ang II HNa remain to be elucidated. One might speculate that the AP reduction, per se, could play a role as indicated by Inscho et al. in Ang II-infused rats on a normal NaCl diet [116]. However, in the present study, tempol clearly did not return AP to normal, suggesting that other, pressure-independent mechanisms might be involved. In addition, tempol did not significantly affect renovascular histological changes and there was no correlation between arterial histopathology and autoregulatory capacity in hypertensive animals. Taken together, these findings indicate that abnormalities in dynamic RBFA could not be explained by structural alterations of the renal vasculature. Interestingly, Sharma et al. [117] showed that transforming growth factor (TGF)-β1 markedly attenuated RBFA in rats using the blood-perfused juxtamedullary nephron technique and that this was associated with increased arteriolar $O_2^{\cdot-}$ production [117]. In addition, pretreatment with tempol prevented the impairment in RBFA induced by TGF-β1 indicating an important role for $O_2^{\cdot-}$ [117] similar to the finding in our study.

Effects of endothelin receptor antagonists on renal hemodynamics in chronic Ang II-infused rats on a high NaCl diet (IV)

Effects of ET_A receptor antagonist BQ-123

BQ-123 reduced MAP indicating a role for ET_A receptors in this model of hypertension. This result supports previous findings with ET_A receptor antagonists in similar models [50, 118]. Interestingly, combined ET_A and ET_B receptor antagonism did not decrease MAP significantly suggesting that at least some of the effect of BQ-123 was mediated through ET_B receptors. Hence, selective ET_A receptor antagonists seem to be more effective antihypertensive agents than combined ET_A and ET_B receptor antagonists in this model.

Interestingly, selective ET_A receptor antagonism with BQ-123 specifically increased OM perfusion by approximately 30 % in spite of reducing MAP. This finding is potentially important as renal medullary vasodilatation and increased medullary blood flow has been shown to exert antihypertensive effects by increasing urinary sodium and water excretion and

by facilitating pressure-natriuresis [20]. However, although BQ-123 reduced MAP in our study this was not associated with increased urinary sodium excretion suggesting that the acute antihypertensive effect was mediated through other mechanisms.

Combined ET_A and ET_B receptor antagonism abolished the increase in OM perfusion produced by BQ-123 alone, indicating that this effect of BQ-123 was dependent on stimulation of ET_B receptors. Previous results demonstrate that there is a complex interaction between ET_A and ET_B receptors in the regulation of renal medullary blood flow. Systemic infusion of ET-1 has been shown to selectively increase medullary perfusion via ET_B receptors [41, 42, 119]. In addition, Vassileva et al. [43] showed that medullary vasodilation produced by Big ET-1, through ET_B receptors, was more prominent in rats on high NaCl intake compared to animals on normal NaCl intake. However, ET-1 has also been shown to cause renal medullary vasoconstriction via ET_A receptors when infused directly into the medulla [120]. In addition, Silldorff et al. [121] demonstrated that ET-1 constricted isolated descending vasa recta in vitro and that this effect could be blocked by ET_A antagonists. Considering that OM vasodilation by BQ-123 seemed to be mediated through ET_B receptors in the present study one might have anticipated that selective ET_B antagonism with BQ-788 should have reduced OM perfusion. However, although BQ-788 tended to reduce OM perfusion this decrease did not reach statistical significance.

Our interpretation is that ET-1 has the potential to cause both vasodilation and vasoconstriction in the renal medulla depending on the prevailing balance between ET_B and ET_A receptor stimulation. Presumably, the vasodilatory influence of endogenous ET-1 through ET_B receptors required concomitant removal of ET_A-mediated vasoconstriction in the present study. Taken together, we hypothesize that the increase in OM perfusion caused by BQ-123, which was dependent on ET_B receptor stimulation, could potentially have beneficial effects in this model either by exerting antihypertensive effects or by protecting the medulla from hypoxic injury. However, these hypothetical effects clearly need to be investigated further. In support of a antihypertensive role of ET_B receptors, rats deficient in ET_B receptors [44] and mice with collecting duct-specific deletion of ET-1[45], ET_B receptors[46], or ET_A and ET_B receptors[47], develop salt-sensitive hypertension. In addition, rats chronically treated with ET_B receptor antagonist[43], and mice with collecting duct-specific deletion of ET-1[122] show a blunted pressure-natriuresis relationship.

Effects of ET_B receptor antagonist BQ-788

Selective ET_B receptor antagonism with BQ-788 caused a significant increase in RVR and a marked decrease in RBF of approximately 30%. However, combined ET_A and ET_B receptor antagonism fully prevented these changes indicating that renal vasoconstriction was completely dependent on ET_A receptor stimulation. These results are in agreement with those previously reported in normotensive animals [88, 119, 123]. Still, it may seem contradictory that selective ET_A antagonism with BQ-123 alone did not have significant effects on RBF or RVR. A possible explanation is that ET_B antagonism increased plasma levels of ET-1 as ET_B receptors play an important role in clearance of ET-1 from the circulation [124]. Thus, increased plasma levels of ET-1 produced by ET_B antagonism could have caused an exaggerated renal vasoconstrictor response through ET_A receptors. Interestingly, selective ET_B receptor antagonism with BQ-788 had no significant effect on GFR despite reducing RBF and hence FF increased. These results suggest that ET_B receptor antagonism predominantly increased RVR by constriction of efferent arterioles. Also this effect was abolished by combined ET_A and ET_B receptor antagonism indicating that the increase in efferent arteriolar resistance was mainly caused by ET_A receptor stimulation. In accord with our findings, Inscho et al. [125] have demonstrated that ET_A receptor blockade prevents ET-1-mediated vasoconstriction of efferent arterioles.

Effects of ET receptor antagonists on renal blood flow autoregulation

As discussed above, dynamic RBFA is impaired in Ang II-infused rats, and exaggerated by a high NaCl intake. In addition, these abnormalities were attenuated by the superoxide dismutase mimetic tempol [126]. As ET-1 has been shown to stimulate NADPH oxidase activity and superoxide production [127, 128] we speculated that ET receptor antagonists might attenuate abnormalities in RBFA in this model. However, acute administration of ET receptor antagonists did not have any significant effects on dynamic RBFA, clearly suggesting that ET-1 is not involved in these defects in autoregulatory behavior.

Clinical perspectives from experimental animal studies (II, IV)

Our results suggest that a high NaCl intake in Ang II-dependent forms of hypertension could increase the susceptibility to hypertensive glomerular injury by impairing RBFA. For instance, in patients with RVH and unilateral RAS the avoidance of a high NaCl intake might protect the non-stenotic kidney from progressive glomerular injury and the development of renal failure. Alternatively, pharmacological agents that target $O_2^{\cdot-}$ may exert renoprotective

effects by attenuating abnormalities in RBFA in the same situation. It remains to be investigated whether the effect of high NaCl intake on RBFA in Ang II-infused rats is specific for this model or relevant also in other hypertensive states.

In addition, selective ET_A receptor antagonism reduces AP and at the same time increases OM perfusion in a hypertensive model of elevated Ang II and high NaCl intake. The implications of the increase in medullary perfusion in this situation need to be examined further in future studies. Still, the increase in medullary perfusion could potentially have beneficial effects in the non-stenotic kidney of patients with hypertension and unilateral RAS by exerting antihypertensive effects or by protecting the medulla from hypoxic injury. However, ET-1 antagonists are unlikely to exert renoprotective effects by improving autoregulatory behavior.

Clinical studies (I, III)

Good diagnostic accuracy of color duplex sonography acceleration indices in patients with suspected renal artery stenosis (I)

Early systolic pulse acceleration and delta pulsatility index

For the diagnosis of a unilateral RAS, the sensitivity and specificity for ΔPI and ACC_{max} were high in our study and comparable to those previously reported by Johansson et al. [10]. However, if we included patients with bilateral RAS (n = 12) the diagnostic accuracy of ΔPI was lower (sensitivity 67% and specificity 73%) for obvious reasons as PI is reduced in both kidneys in this situation. In our study, unlike in the study by Johansson and co-workers [10], patients with serum creatinine values exceeding 200 μmol/l were included, and 13 % of all patients had an eGFR below 30 ml/min per 1.73m^2. Thus, we could extend the findings by Johansson et al. by demonstrating a high diagnostic accuracy for ΔPI and ACC_{max} in the detection of unilateral RAS even in patients with markedly reduced GFR. Furthermore, in our study stricter criteria for the diagnosis of a significant RAS were applied and a trans-stenotic MAPG $\geq$10mmHg was required.

Pulsatility index

The absolute PI value demonstrated a very poor accuracy in diagnosing a significant RAS. Indeed, PI correlated significantly to age, eGFR, and pulse pressure in both stenotic and non-stenotic kidneys, whereas there was no significant correlation to the trans-stenotic MAPG. These results indicate that PI, like resistive index that is often used by other investigators [129], is not a reliable velocimetric index for the diagnosis of RAS.

Acceleration index AI_{max}

Acceleration indices ACC_{max} and AI_{max} showed high diagnostic values for detecting RAS in all kidneys regardless of unilateral or bilateral involvement. In addition, both ACC_{max} and AI_{max} showed a good diagnostic accuracy independent of GFR. Furthermore, there were no significant correlations between acceleration indices and age, or pulse pressure, in stenotic kidneys, whereas both ACC_{max} and AI_{max} were significantly correlated to the degree of stenosis as measured by the trans-stenotic MAPG. The results indicate that acceleration indices during early systole are mainly influenced by the trans-stenotic pressure gradient and reflect the hemodynamic consequences of the stenotic lesion. Taken together, these data suggest that ACC_{max} and AI_{max}, in contrast to PI, are reliable Doppler indices for the diagnosis of RAS even in patients with single kidneys, marked renal impairment and in elderly individuals with increased arterial stiffness. Our data confirm the findings by Bardelli et al. [130] by demonstrating a better diagnostic accuracy of indices estimating the acceleration of blood flow during the early systolic phase compared with PI for the diagnosis of RAS. In both studies, distal velocimetric indices were used, but in the study by Bardelli et al. [130], the ideal cut-off value for ACC_{max} was higher, probably reflecting different sampling sites of the renal arterial tree.

Importantly however, our data can only partially support the results by Bardelli et al. [130] demonstrating an improved diagnostic accuracy of early systolic acceleration indices if they were corrected for peak systolic velocity. In our study, ROC curves for ACC_{max} and AI_{max} had similar AUCs, and there was no significant difference between the correlation of ACC_{max} and AI_{max} to the trans-stenotic MAPG. One explanation for the discrepancy between studies could be that unlike in the Italian multicentre study, all patients in our cohort were examined in a single centre by a few experienced technicians in a strictly standardized manner. As AI_{max} was suggested to correct ACC_{max} for the absolute flow regimen and, hence, reduce the operator dependency, the potential disadvantage of ACC_{max} compared with AI_{max}

caused, for example, by scanning of different regions of the kidney was presumably eliminated under these strictly standardized circumstances in our single-centre study.

Considerations regarding the design of the study

It should be emphasized that our study was not optimally designed to assess the diagnostic accuracy of different velocimetric indices of CDS in RAS. The population was highly selected as patients with negative screening tests for RAS did not undergo further evaluation by renal angiography and hence, were not included in the study. Thus, to evaluate the diagnostic accuracy of the novel index AI_{max} we compared the predictive values of AI_{max} with those of ACC_{max}, as this index has previously been shown to have high predictive values for the detection of significant RAS at our centre [10]. Furthermore, we compared how these velocimetric Doppler indices correlated to the trans-stenotic MAPG.

Elevated inflammatory indices and plasma endothelin-1 but no increase in biomarkers of oxidative stress in atherosclerotic renal artery stenosis (III)

Biomarkers of oxidative stress

We found no difference in biomarkers of oxs between hypertensive patients with, or without, ARAS although inflammatory indices were elevated in individuals with ARAS. Our findings are different from those in two previous studies in which patients with ARAS showed increased levels of biomarkers of oxs compared to healthy controls and to patients with essential hypertension [34, 35]. Higashi et al. [34] found that urinary excretion of 8- hydroxy-2-deoxyguanosine (8-OHdG) and serum malondialdehyde-modified LDL (MDA-LDL) were elevated in 15 patients with RVH compared to matched healthy subjects. In addition, these biomarkers were significantly reduced following renal angioplasty. However, no antihypertensive agents were administered for at least two weeks before measurements and no patient received ASA or statins [34]. In the study by Minuz et al. [35] urinary 8-iso-PGF2α excretion was elevated in patients with RVH. As in our study all patients were on antihypertensive treatment and drugs interfering with the RAAS were not used. However, only five patients were on low-dose ASA and no patient received statin treatment [35]. In contrast, all patients (ARAS and non-RAS) in our study were treated with statins and low-dose ASA throughout the study period.

A growing body of evidence from both experimental [131] and human studies [132, 133] clearly indicate antioxidant effects of statins. In addition, ASA has been shown to possess

antioxidant properties [134]. Hence, the discrepancy between our findings and earlier results could at least in part be explained by differences in drug use between study populations. Another possible explanation for the discrepancy between results could be that different biomarkers of oxs were analyzed. Higashi et al. analyzed urinary 8-OHdG excretion which is a marker of oxidative DNA damage that occurs in the cell nucleus or mitochondria [34]. Thus, it is possible, at least in theory, that patients with ARAS could have had increased intracellular oxs leading to oxidative DNA damage that we were unable to detect with our biomarkers. In addition, protein oxidation was not assessed by Higashi et al. [34] whereas we examined protein oxidation by measuring PC. However, lipid peroxidation was evaluated both by Higashi et al. who measured serum levels of MDA-LDL [34] and by us in the present study (U-8-iso-PGF2α excretion). Both U-8-iso-PGF2α excretion and serum MDA-LDL are considered reliable markers of lipid peroxidation [135, 136]. For instance, treatment of patients with coronary atherosclerosis with statins has been shown to reduce both U-8-iso-PGF2α [132] and serum MDA-LDL [136]. Thus, different characteristics of these biomarkers of lipid peroxidation are less likely to explain the discrepant results in the present study compared to those by Higashi et al. It should also be acknowledged that the small sample size of the healthy control group in our study might have reduced the power in detecting differences between groups in biomarkers. Notably, we observed that plasma TAOC was significantly elevated in both hypertensive groups compared to healthy controls. This could at least partly be explained by the antioxidant effects of ASA and statins. In addition, although several studies have shown reduced antioxidant capacity in patients with atherosclerosis [137] adaptive elevations in antioxidant activity have also been reported [131, 138].

Inflammatory biomarkers

Patients with ARAS showed increased levels of hs-CRP and WBC compared to healthy controls and group non-RAS. Accumulating evidence demonstrates that statins exert anti-inflammatory actions [139, 140]. Still, patients with ARAS who were on statin treatment showed significantly increased levels of hs-CRP and WBC compared to both healthy controls and hypertensive patients without RAS. This finding clearly indicates that in our cohort of patients ARAS was associated with inflammation but not with increased oxs. Notably, PTRA had no significant effects on hs-CRP or WBC when analyzed 4 weeks after intervention. These results indicate that inflammation in ARAS patients was not caused by the stenotic

lesion of the renal artery *per se* but may have reflected the burden of generalized atherosclerotic disease.

Plasma endothelin-1

Endothelin-1, a powerful vasoconstrictor peptide, has been implicated in Ang II-mediated hypertension [50] and treatment with ET_A receptor antagonists has been shown to reduce AP and to attenuate cardiovascular remodeling in experimental models of Ang II-dependent hypertension [141]. Consistent with these findings, we found that plasma levels of ET-1 were significantly increased in both hypertensive groups versus healthy controls. In addition, PTRA significantly reduced plasma ET-1 concentrations in ARAS patients suggesting a beneficial effect of PTRA on vascular integrity. In line with our findings, Higashi et al. [34] showed impaired endothelium-dependent vasodilatation in patients with RVH prior to PTRA and an improved vasodilatory response following intervention. However, the functional consequences of reduced plasma levels of ET-1 following PTRA in our study need to be examined further. We also observed that changes in plasma levels of ET-1 in response to PTRA only correlated to changes in serum UA during the same time period, suggesting that reductions in ET-1 might have been mediated by decreased UA levels. Interestingly, UA has been implicated in endothelial dysfunction in hypertension [142] and has been shown to stimulate ET-1 gene expression [143]. The association between plasma levels of UA and ET-1 following PTRA is a novel observation that needs to be investigated further.

Serum uric acid

Evidence from previous studies shows strong associations between serum levels of UA and biomarkers of inflammation [144]. Consistent with these findings, we showed that baseline hs-CRP, which was significantly elevated in ARAS patients, only correlated to serum UA levels. However, the reduction in serum UA after PTRA was independent of inflammatory biomarkers as hs-CRP was not affected by intervention. In addition, the reduction in UA after PTRA did not correlate to changes in SBP or in serum creatinine levels. The mechanism by which PTRA reduced serum UA is unclear and needs to be elucidated. Interestingly, experimental studies have shown increased blood and renal tissue levels of UA precursors adenosine, inosine, and hypoxanthine after only a few minutes of renal ischemia [145, 146]. In addition, reperfusion for only 15 minutes resulted in a normalization of renal tissue levels of adenosine and inosine [145]. Thus, one could speculate that elevated serum UA in

patients with ARAS was caused by renal ischemia and that PTRA reduced levels of UA by restoring RBF.

CONCLUSIONS

- In Ang II-infused rats, an animal model of unilateral RAS, high dietary NaCl intake aggravates the impairment in dynamic RBFA and causes a markedly abnormal MR. This abnormality was attenuated by tempol, suggesting a pathogenetic role for $O_2^{\cdot-}$ in the impaired autoregulatory response
- In Ang II-infused rats on a high NaCl diet, ET_A antagonism with BQ-123 reduces AP and selectively increases OM perfusion without affecting total or cortical RBF. These effects were attenuated or abolished by combined ET_A and ET_B antagonism indicating an important role for ET_B receptors in mediating these effects. ET receptor antagonists did not affect dynamic RBFA.
- Acceleration Doppler indices ACC_{max} and AI_{max} provide good diagnostic accuracy in the detection of hemodynamically significant RAS even in patients with markedly reduced kidney function and have an advantage over PI. Although the diagnostic accuracy of AI_{max} was not superior to ACC_{max} in our single-centre study, this novel index may have benefits in multicentre studies.
- Patients with ARAS show no increase in oxidative stress despite elevated inflammatory indices. Notably, despite treatment with antihypertensive agents, ASA and statins, inflammation was still elevated in ARAS patients and was not reduced by PTRA. The effect of PTRA on biomarkers 4 weeks after intervention was differentiated and plasma levels of ET-1 and UA were reduced although inflammatory indices were unaffected.

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REFERENCES

1. Rettig R, Grisk O. The kidney as a determinant of genetic hypertension: evidence from renal transplantation studies. *Hypertension.* 2005;46(3):463-468.

2. Bright R. Tubular view of the morbid appearances in 100 cases connected with albuminous urine: With observations. *Guy's Hosp Rep.* 1836;1:380–400

3. Tigerstedt R, Bergman PG. Niere und Kreislauf. . *Skand Arch Physiol.* 1898;8:223–271.

4. Goldblatt H, Lynch J, Hanzal RF, Summerville WW. Studies on experimental hypertension, I: the production of persistent elevation of systolic blood pressure by means of renal ischemia. *J Exp Med.* 1934;59(3):347-379.

5. Leadbetter W, Burkland C. Hypertension in unilateral renal disease. . *J Urol* 1938.:611-626.

6. Freeman NE, Leeds FH, Elliott WG, Roland SI. Thromboendarterectomy for hypertension due to renal artery occlusion. *J Am Med Assoc.* 1954;156(11):1077-1079.

7. Gruntzig A, Kuhlmann U, Vetter W, Lutolf U, Meier B, Siegenthaler W. Treatment of renovascular hypertension with percutaneous transluminal dilatation of a renal-artery stenosis. *Lancet.* 1978;1(8068):801-802.

8. Hansen KJ, Edwards MS, Craven TE, Cherr GS, Jackson SA, Appel RG, Burke GL, Dean RH. Prevalence of renovascular disease in the elderly: a population-based study. *J Vasc Surg.* 2002;36(3):443-451.

9. Sawicki PT, Kaiser S, Heinemann L, Frenzel H, Berger M. Prevalence of renal artery stenosis in diabetes mellitus--an autopsy study. *J Intern Med.* 1991;229(6):489-492.

10. Johansson M, Jensen G, Aurell M, Friberg P, Herlitz H, Klingenstierna H, Volkmann R. Evaluation of duplex ultrasound and captopril renography for detection of renovascular hypertension. *Kidney Int.* 2000;58(2):774-782.

11. Zoccali C, Mallamaci F, Finocchiaro P. Atherosclerotic renal artery stenosis: epidemiology, cardiovascular outcomes, and clinical prediction rules. *J Am Soc Nephrol.* 2002;13 Suppl 3:S179-183.

12. Mailloux LU, Napolitano B, Bellucci AG, Vernace M, Wilkes BM, Mossey RT. Renal vascular disease causing end-stage renal disease, incidence, clinical correlates, and outcomes: a 20-year clinical experience. *Am J Kidney Dis.* 1994;24(4):622-629.

13. Conlon PJ, Little MA, Pieper K, Mark DB. Severity of renal vascular disease predicts mortality in patients undergoing coronary angiography. *Kidney Int.* 2001;60(4):1490-1497.

14. Johansson M, Herlitz H, Jensen G, Rundqvist B, Friberg P. Increased cardiovascular mortality in hypertensive patients with renal artery stenosis. Relation to sympathetic activation, renal function and treatment regimens. *J Hypertens.* 1999;17(12 Pt 1):1743-1750.

15. Karagiannis A, Tziomalos K, Anagnostis P, Gossios T, Athyros VG. Atherosclerotic renal artery stenosis: medical therapy alone or in combination with revascularization? *Angiology.* 2009;60(4):397-402.

16. The Astral I, Wheatley K, Ives N, Gray R, Kalra PA, Moss JG, Baigent C, Carr S, Chalmers N, Eadington D, Hamilton G, Lipkin G, Nicholson A, Scoble J. Revascularization versus medical therapy for renal-artery stenosis. *N Engl J Med.* 2009;361(20):1953-1962.

17. Pallone TL, Zhang Z, Rhinehart K. Physiology of the renal medullary microcirculation. *Am J Physiol Renal Physiol.* 2003;284(2):F253-266.

18. Mattson DL. Importance of the renal medullary circulation in the control of sodium excretion and blood pressure. *Am J Physiol Regul Integr Comp Physiol.* 2003;284(1):R13-27.

19. Cupples WA, Braam B. Assessment of renal autoregulation. *Am J Physiol Renal Physiol.* 2007;292(4):F1105-1123.

20. Cowley AW, Jr. Role of the renal medulla in volume and arterial pressure regulation. *Am J Physiol.* 1997;273(1 Pt 2):R1-15.

21. Cohen HJ, Marsh DJ, Kayser B. Autoregulation in vasa recta of the rat kidney. *Am J Physiol.* 1983;245(1):F32-40.

22. Mattson DL, Lu S, Roman RJ, Cowley AW, Jr. Relationship between renal perfusion pressure and blood flow in different regions of the kidney. *Am J Physiol.* 1993;264(3 Pt 2):R578-583.

23. Cheung CM, Hegarty J, Kalra PA. Dilemmas in the management of renal artery stenosis. *Br Med Bull.* 2005;73-74:35-55.

24. Safian RD, Textor SC. Renal-Artery Stenosis. *New England Journal of Medicine.* 2001;344(6):431-442.

25. Garovic VD, Textor SC. Renovascular hypertension and ischemic nephropathy. *Circulation.* 2005;112(9):1362-1374.

26. Navar LG, Ploth DW. Pathophysiology of renovascular hypertension. *Hypertension primer the essentials of high blood pressure.* 2007;4th edition(Chapter A51):162-165.

27. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: how should you do it and what do the results mean? *Br J Pharmacol.* 2004;142(2):231-255.

28. Paravicini TM, Touyz RM. NADPH oxidases, reactive oxygen species, and hypertension: clinical implications and therapeutic possibilities. *Diabetes Care.* 2008;31 Suppl 2:S170-180.

29. Reckelhoff JF, Romero JC. Role of oxidative stress in angiotensin-induced hypertension. *Am J Physiol Regul Integr Comp Physiol.* 2003;284(4):R893-912.

30. Rajagopalan S, Kurz S, Munzel T, Tarpey M, Freeman BA, Griendling KK, Harrison DG. Angiotensin II-mediated hypertension in the rat increases vascular superoxide production via membrane NADH/NADPH oxidase activation. Contribution to alterations of vasomotor tone. *J Clin Invest.* 1996;97(8):1916-1923.

31. Lerman LO, Nath KA, Rodriguez-Porcel M, Krier JD, Schwartz RS, Napoli C, Romero JC. Increased oxidative stress in experimental renovascular hypertension. *Hypertension.* 2001;37(2 Part 2):541-546.

32. Guron GS, Grimberg ES, Basu S, Herlitz H. Acute effects of the superoxide dismutase mimetic tempol on split kidney function in two-kidney one-clip hypertensive rats. *J Hypertens.* 2006;24(2):387-394.

33. Welch WJ, Mendonca M, Aslam S, Wilcox CS. Roles of oxidative stress and AT1 receptors in renal hemodynamics and oxygenation in the postclipped 2K,1C kidney. *Hypertension.* 2003;41(3 Pt 2):692-696.

34. Higashi Y, Sasaki S, Nakagawa K, Matsuura H, Oshima T, Chayama K. Endothelial function and oxidative stress in renovascular hypertension. *N Engl J Med.* 2002;346(25):1954-1962.

35. Minuz P, Patrignani P, Gaino S, Degan M, Menapace L, Tommasoli R, Seta F, Capone ML, Tacconelli S, Palatresi S, Bencini C, Del Vecchio C, Mansueto G, Arosio E, Santonastaso CL, Lechi A, Morganti A, Patrono C. Increased oxidative stress and platelet activation in patients with hypertension and renovascular disease. *Circulation.* 2002;106(22):2800-2805.

36. Kohan DE, Rossi NF, Inscho EW, Pollock DM. Regulation of blood pressure and salt homeostasis by endothelin. *Physiol Rev.* 2011; 91(1):1-77.

37. Just A, Olson AJ, Arendshorst WJ. Dual constrictor and dilator actions of ET(B) receptors in the rat renal microcirculation: interactions with ET(A) receptors. *Am J Physiol Renal Physiol.* 2004;286(4):F660-668.

38. Verhaar MC, Strachan FE, Newby DE, Cruden NL, Koomans HA, Rabelink TJ, Webb DJ. Endothelin-A receptor antagonist-mediated vasodilatation is attenuated by inhibition of nitric oxide synthesis and by endothelin-B receptor blockade. *Circulation.* 1998;97(8):752-756.

39. Kohan DE. Biology of endothelin receptors in the collecting duct. *Kidney Int.* 2009;76(5):481-486.

40. Kitamura K, Tanaka T, Kato J, Eto T, Tanaka K. Regional distribution of immunoreactive endothelin in porcine tissue: abundance in inner medulla of kidney. *Biochem Biophys Res Commun.* 1989;161(1):348-352.

41. Gurbanov K, Rubinstein I, Hoffman A, Abassi Z, Better OS, Winaver J. Differential regulation of renal regional blood flow by endothelin-1. *Am J Physiol.* 1996;271(6 Pt 2):F1166-1172.

42. Hercule HC, Oyekan AO. Role of NO and cytochrome P-450-derived eicosanoids in ET-1-induced changes in intrarenal hemodynamics in rats. *Am J Physiol Regul Integr Comp Physiol.* 2000;279(6):R2132-2141.

43. Vassileva I, Mountain C, Pollock DM. Functional role of ETB receptors in the renal medulla. *Hypertension.* 2003;41(6):1359-1363.

44. Gariepy CE, Ohuchi T, Williams SC, Richardson JA, Yanagisawa M. Salt-sensitive hypertension in endothelin-B receptor-deficient rats. *J Clin Invest.* 2000;105(7):925-933.

45. Ahn D, Ge Y, Stricklett PK, Gill P, Taylor D, Hughes AK, Yanagisawa M, Miller L, Nelson RD, Kohan DE. Collecting duct-specific knockout of endothelin-1 causes hypertension and sodium retention. *J Clin Invest.* 2004;114(4):504-511.

46. Ge Y, Bagnall A, Stricklett PK, Strait K, Webb DJ, Kotelevtsev Y, Kohan DE. Collecting duct-specific knockout of the endothelin B receptor causes hypertension and sodium retention. *Am J Physiol Renal Physiol.* 2006;291(6):F1274-1280.

47. Ge Y, Bagnall A, Stricklett PK, Webb D, Kotelevtsev Y, Kohan DE. Combined knockout of collecting duct endothelin A and B receptors causes hypertension and sodium retention. *Am J Physiol Renal Physiol.* 2008;295(6):F1635-1640.

48. Alexander BT, Cockrell KL, Rinewalt AN, Herrington JN, Granger JP. Enhanced renal expression of preproendothelin mRNA during chronic angiotensin II hypertension. *Am J Physiol Regul Integr Comp Physiol.* 2001;280(5):R1388-1392.

49. Sasser JM, Pollock JS, Pollock DM. Renal endothelin in chronic angiotensin II hypertension. *Am J Physiol Regul Integr Comp Physiol.* 2002;283(1):R243-248.

50. Ballew JR, Fink GD. Role of ET(A) receptors in experimental ANG II-induced hypertension in rats. *Am J Physiol Regul Integr Comp Physiol.* 2001;281(1):R150-154.

51. Allcock GH, Venema RC, Pollock DM. ETA receptor blockade attenuates the hypertension but not renal dysfunction in DOCA-salt rats. *Am J Physiol.* 1998;275(1 Pt 2):R245-252.

52. Ikeda T, Ohta H, Okada M, Kawai N, Nakao R, Siegl PK, Kobayashi T, Maeda S, Miyauchi T, Nishikibe M. Pathophysiological roles of endothelin-1 in Dahl salt-sensitive hypertension. *Hypertension.* 1999;34(3):514-519.

53. Textor SC. Ischemic nephropathy: where are we now? *J Am Soc Nephrol.* 2004;15(8):1974-1982.

54. Hsu CY, McCulloch CE, Darbinian J, Go AS, Iribarren C. Elevated blood pressure and risk of end-stage renal disease in subjects without baseline kidney disease. *Arch Intern Med.* 2005;165(8):923-928.

55. Susic D, Zhou X, Frohlich ED. Angiotensin blockade prevents salt-induced injury of the renal circulation in spontaneously hypertensive rats. *Am J Nephrol.* 2009;29(6):639-645.

56. Laursen JB, Rajagopalan S, Galis Z, Tarpey M, Freeman BA, Harrison DG. Role of superoxide in angiotensin II-induced but not catecholamine-induced hypertension. *Circulation.* 1997;95(3):588-593.

57. Loutzenhiser R, Griffin K, Williamson G, Bidani A. Renal autoregulation: new perspectives regarding the protective and regulatory roles of the underlying mechanisms. *Am J Physiol Regul Integr Comp Physiol.* 2006;290(5):R1153-1167.

58. Elmarakby AA, Quigley JE, Olearczyk JJ, Sridhar A, Cook AK, Inscho EW, Pollock DM, Imig JD. Chemokine receptor 2b inhibition provides renal protection in angiotensin II - salt hypertension. *Hypertension.* 2007;50(6):1069-1076.

59. Bigazzi R, Bianchi S, Baldari D, Sgherri G, Baldari G, Campese VM. Microalbuminuria in salt-sensitive patients. A marker for renal and cardiovascular risk factors. *Hypertension.* 1994;23(2):195-199.

60. Van Dokkum RP, Alonso-Galicia M, Provoost AP, Jacob HJ, Roman RJ. Impaired autoregulation of renal blood flow in the fawn-hooded rat. *Am J Physiol.* 1999;276(1 Pt 2):R189-196.

61. Wang X, Ajikobi DO, Salevsky FC, Cupples WA. Impaired myogenic autoregulation in kidneys of Brown Norway rats. *Am J Physiol Renal Physiol.* 2000;278(6):F962-969.

62. Griffin KA, Picken MM, Bidani AK. Deleterious effects of calcium channel blockade on pressure transmission and glomerular injury in rat remnant kidneys. *J Clin Invest.* 1995;96(2):793-800.

63. Martin LG, Rundback JH, Sacks D, Cardella JF, Rees CR, Matsumoto AH, Meranze SG, Schwartzberg MS, Silverstein MI, Lewis CA, Society of Interventional Radiology. Standards of Practice C. Quality improvement guidelines for angiography, angioplasty, and stent placement in the diagnosis and treatment of renal artery stenosis in adults. *J Vasc Interv Radiol.* 2002;13(11):1069-1083.

64. Alhadad A, Mattiasson I, Ivancev K, Lind M, Gottsäter A, Lindblad B. Does the pressure gradient in renal artery stenosis before and after percutaneous transluminal renal angioplasty predict initial and long-term outcome? *Journal of Renovascular Disease.* 2006;4:7-13.

65. Bernheim JW, Kent KC. Renal Artery Imaging and Physiologic Testing. *Rutherford RB. Vascular Surgery e-dition, 6th edition. Philadelphia, WB Saunders.* 2005;Section XIX(Chapter 128.):1773-1788.

66. Soulez G, Oliva VL, Turpin S, Lambert R, Nicolet V, Therasse E. Imaging of renovascular hypertension: respective values of renal scintigraphy, renal Doppler US, and MR angiography. *Radiographics.* 2000;20(5):1355-1368; discussion 1368-1372.

67. Zucchelli PC. Hypertension and atherosclerotic renal artery stenosis: diagnostic approach. *J Am Soc Nephrol.* 2002;13 Suppl 3:S184-186.

68. Williams GJ, Macaskill P, Chan SF, Karplus TE, Yung W, Hodson EM, Craig JC. Comparative accuracy of renal duplex sonographic parameters in the diagnosis of renal artery stenosis: paired and unpaired analysis. *AJR Am J Roentgenol.* 2007;188(3):798-811.

69. Radermacher J, Chavan A, Bleck J, Vitzthum A, Stoess B, Gebel MJ, Galanski M, Koch KM, Haller H. Use of Doppler ultrasonography to predict the outcome of therapy for renal-artery stenosis. *N Engl J Med.* 2001;344(6):410-417.

70. Garcia-Criado A, Gilabert R, Nicolau C, Real MI, Muntana X, Blasco J, Ganau S, Bru C. Value of Doppler sonography for predicting clinical outcome after renal artery revascularization in atherosclerotic renal artery stenosis. *J Ultrasound Med.* 2005;24(12):1641-1647.

71. Zeller T, Frank U, Muller C, Burgelin K, Sinn L, Bestehorn HP, Cook-Bruns N, Neumann FJ. Predictors of improved renal function after percutaneous stent-supported angioplasty of severe atherosclerotic ostial renal artery stenosis. *Circulation.* 2003;108(18):2244-2249.

72. Wilcox CS. Use of angiotensin-converting-enzyme inhibitors for diagnosing renovascular hypertension. *Kidney Int.* 1993;44(6):1379-1390.

73. Norman MK, Burton DR. Screening for renovascular hypertension. *Uptodate. www.uptodate.com.* 2010.

74. Leertouwer TC, Gussenhoven EJ, Bosch JL, van Jaarsveld BC, van Dijk LC, Deinum J, Man In 't Veld AJ. Stent placement for renal arterial stenosis: where do we stand? A meta-analysis. *Radiology.* 2000;216(1):78-85.

75. Olin JW. Recognizing and managing fibromuscular dysplasia. *Cleve Clin J Med.* 2007;74(4):273-274, 277-282.

76. van Jaarsveld BC, Krijnen P, Pieterman H, Derkx FHM, Deinum J, Postma CT, Dees A, Woittiez AJJ, Bartelink AKM, Man in `t Veld AJ, Schalekamp MADH. The Effect of Balloon Angioplasty on Hypertension in Atherosclerotic Renal-Artery Stenosis. *New England Journal of Medicine.* 2000;342(14):1007-1014.

77. Cockcroft DW, Gault MH. Prediction of creatinine clearance from serum creatinine. *Nephron.* 1976;16(1):31-41.

78. Weinberg MD, Olin JW. Stenting for atherosclerotic renal artery stenosis: one poorly designed trial after another. *Cleve Clin J Med.*77(3):164-171.

79. van Jaarsveld B, Krijnen P, Bartelink A, Dees A, Derkx F, Man in't Veld A, Schalekamp M. The Dutch Renal Artery Stenosis Intervention Cooperative (DRASTIC) Study: rationale, design and inclusion data. *J Hypertens Suppl.* 1998;16(6):S21-27.

80. Plouin PF, Chatellier G, Darne B, Raynaud A. Blood pressure outcome of angioplasty in atherosclerotic renal artery stenosis: a randomized trial. Essai Multicentrique Medicaments vs Angioplastie (EMMA) Study Group. *Hypertension.* 1998;31(3):823-829.

81. Webster J, Marshall F, Abdalla M, Dominiczak A, Edwards R, Isles CG, Loose H, Main J, Padfield P, Russell IT, Walker B, Watson M, Wilkinson R. Randomised comparison of percutaneous angioplasty vs continued medical therapy for hypertensive patients with atheromatous renal artery stenosis. Scottish and Newcastle Renal Artery Stenosis Collaborative Group. *J Hum Hypertens.* 1998;12(5):329-335.

82. Bax L, Woittiez AJ, Kouwenberg HJ, Mali WP, Buskens E, Beek FJ, Braam B, Huysmans FT, Schultze Kool LJ, Rutten MJ, Doorenbos CJ, Aarts JC, Rabelink TJ, Plouin PF, Raynaud A, van Montfrans GA, Reekers JA, van den Meiracker AH, Pattynama PM, van de Ven PJ, Vroegindeweij D, Kroon AA, de Haan MW, Postma CT, Beutler JJ. Stent placement in patients with atherosclerotic renal artery stenosis and impaired renal function: a randomized trial. *Ann Intern Med.* 2009;150(12):840-848, W150-841.

83. Murphy TP, Cooper CJ, Dworkin LD, Henrich WL, Rundback JH, Matsumoto AH, Jamerson KA, D'Agostino RB. The Cardiovascular Outcomes with Renal Atherosclerotic Lesions (CORAL) study: rationale and methods. *J Vasc Interv Radiol.* 2005;16(10):1295-1300.

84. Griendling KK, Ushio-Fukai M. Reactive oxygen species as mediators of angiotensin II signaling. *Regul Pept.* 2000;91(1-3):21-27.

85. Hisaki R, Fujita H, Saito F, Kushiro T. Tempol attenuates the development of hypertensive renal injury in Dahl salt-sensitive rats. *Am J Hypertens.* 2005;18(5 Pt 1):707-713.

86. Trolliet MR, Rudd MA, Loscalzo J. Oxidative stress and renal dysfunction in salt-sensitive hypertension. *Kidney Blood Press Res.* 2001;24(2):116-123.

87. Ogihara T, Asano T, Ando K, Chiba Y, Sakoda H, Anai M, Shojima N, Ono H, Onishi Y, Fujishiro M, Katagiri H, Fukushima Y, Kikuchi M, Noguchi N, Aburatani H, Komuro I, Fujita T. Angiotensin II-induced insulin resistance is associated with enhanced insulin signaling. *Hypertension.* 2002;40(6):872-879.

88. Matsuura T, Miura K, Ebara T, Yukimura T, Yamanaka S, Kim S, Iwao H. Renal vascular effects of the selective endothelin receptor antagonists in anaesthetized rats. *Br J Pharmacol.* 1997;122(1):81-86.

89. Ishikawa K, Ihara M, Noguchi K, Mase T, Mino N, Saeki T, Fukuroda T, Fukami T, Ozaki S, Nagase T, et al. Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. *Proc Natl Acad Sci U S A.* 1994;91(11):4892-4896.

90. Qiu C, Samsell L, Baylis C. Actions of endogenous endothelin on glomerular hemodynamics in the rat. *Am J Physiol.* 1995;269(2 Pt 2):R469-473.

91. Welch WJ, Deng X, Snellen H, Wilcox CS. Validation of miniature ultrasonic transit-time flow probes for measurement of renal blood flow in rats. *Am J Physiol.* 1995;268(1 Pt 2):F175-178.

92. Smits GJ, Roman RJ, Lombard JH. Evaluation of laser-Doppler flowmetry as a measure of tissue blood flow. *J Appl Physiol.* 1986;61(2):666-672.

93. Hansell P. Evaluation of methods for estimating renal medullary blood flow. *Ren Physiol Biochem.* 1992;15(5):217-230.

94. Edwards A, Silldforff EP, Pallone TL. The renal medullary microcirculation. *Front Biosci.* 2000;5:E36-52.

95. Liss P, Nygren A, Revsbech NP, Ulfendahl HR. Intrarenal oxygen tension measured by a modified clark electrode at normal and low blood pressure and after injection of x-ray contrast media. *Pflugers Arch.* 1997;434(6):705-711.

96. Gundersen JK, Ramsing NB, Glud RN. Predicting the signal of O2 microsensors from physical dimensions, temperature, salinity and O2 concentration *Limnology and Oceanography* 1998;43(8):1932-1937.

97. DiBona GF. Dynamic analysis of patterns of renal sympathetic nerve activity: implications for renal function. *Exp Physiol.* 2005;90(2):159-161.

98. DiBona GF, Sawin LL. Effect of endogenous angiotensin II on the frequency response of the renal vasculature. *Am J Physiol Renal Physiol.* 2004;287(6):F1171-1178.

99. Just A. Mechanisms of renal blood flow autoregulation: dynamics and contributions. *Am J Physiol Regul Integr Comp Physiol.* 2007;292(1):R1-17.

100. Koopmans LH. Preface to the Second Edition. *The Spectral Analysis of Time Series*. San Diego: Academic Press; 1995.

101. Wilhelmsen L, Johansson S, Rosengren A, Wallin I, Dotevall A, Lappas G. Risk factors for cardiovascular disease during the period 1985-1995 in Goteborg, Sweden. The GOT-MONICA Project. *J Intern Med.* 1997;242(3):199-211.

102. Levey AS, Coresh J, Greene T, Stevens LA, Zhang YL, Hendriksen S, Kusek JW, Van Lente F, Chronic Kidney Disease Epidemiology C. Using standardized serum creatinine values in the modification of diet in renal disease study equation for estimating glomerular filtration rate. *Ann Intern Med.* 2006;145(4):247-254.

103. Ahotupa M, Ruutu M, Mantyla E. Simple methods of quantifying oxidation products and antioxidant potential of low density lipoproteins. *Clin Biochem.* 1996;29(2):139-144.

104. Rice-Evans C, Miller NJ. Total antioxidant status in plasma and body fluids. *Methods Enzymol.* 1994;234:279-293.

105. Reznick AZ, Packer L. Oxidative damage to proteins: spectrophotometric method for carbonyl assay. *Methods Enzymol.* 1994;233:357-363.

106. Wang CT, Chin SY, Navar LG. Impairment of pressure-natriuresis and renal autoregulation in ANG II-infused hypertensive rats. *Am J Physiol Renal Physiol.* 2000;279(2):F319-325.

107. Inscho EW, Imig JD, Deichmann PC, Cook AK. Candesartan cilexetil protects against loss of autoregulatory efficiency in angiotensin II-infused rats. *J Am Soc Nephrol.* 1999;10 Suppl 11:S178-183.

108. Modlinger P, Chabrashvili T, Gill PS, Mendonca M, Harrison DG, Griendling KK, Li M, Raggio J, Wellstein A, Chen Y, Welch WJ, Wilcox CS. RNA silencing in vivo reveals role of p22phox in rat angiotensin slow pressor response. *Hypertension.* 2006;47(2):238-244.

109. Welch WJ, Blau J, Xie H, Chabrashvili T, Wilcox CS. Angiotensin-induced defects in renal oxygenation: role of oxidative stress. *Am J Physiol Heart Circ Physiol.* 2005;288(1):H22-28.

110. Just A, Ehmke H, Wittmann U, Kirchheim HR. Role of angiotensin II in dynamic renal blood flow autoregulation of the conscious dog. *J Physiol.* 2002;538(Pt 1):167-177.

111. Casellas D, Bouriquet N, Moore LC. Branching patterns and autoregulatory responses of juxtamedullary afferent arterioles. *Am J Physiol.* 1997;272(3 Pt 2):F416-421.

112. Zhao X, Cook AK, Field M, Edwards B, Zhang S, Zhang Z, Pollock JS, Imig JD, Inscho EW. Impaired Ca2+ signaling attenuates P2X receptor-mediated vasoconstriction of afferent arterioles in angiotensin II hypertension. *Hypertension.* 2005;46(3):562-568.

113. Zhao X, Pollock DM, Inscho EW, Zeldin DC, Imig JD. Decreased renal cytochrome P450 2C enzymes and impaired vasodilation are associated with angiotensin salt-sensitive hypertension. *Hypertension.* 2003;41(3 Pt 2):709-714.

114. Braga VA. Dietary salt enhances angiotensin-II-induced superoxide formation in the rostral ventrolateral medulla. *Auton Neurosci.*

115. Johansson ME, Bernberg E, Andersson IJ, Bie P, Skott O, Gan LM, Bergstrom G. High-salt diet combined with elevated angiotensin II accelerates atherosclerosis in apolipoprotein E-deficient mice. *J Hypertens.* 2009;27(1):41-47.

116. Inscho EW, Cook AK, Murzynowski JB, Imig JD. Elevated arterial pressure impairs autoregulation independently of AT(1) receptor activation. *J Hypertens.* 2004;22(4):811-818.

117. Sharma K, Cook A, Smith M, Valancius C, Inscho EW. TGF-beta impairs renal autoregulation via generation of ROS. *Am J Physiol Renal Physiol.* 2005;288(5):F1069-1077.

118. Boesen EI, Pollock JS, Pollock DM. Contrasting effects of intervention with ETA and ETB receptor antagonists in hypertension induced by angiotensin II and high-salt diet. *Can J Physiol Pharmacol.*88(8):802-807.

119. Evans RG, Madden AC, Oliver JJ, Lewis TV. Effects of ET(A) - and ET(B)-receptor antagonists on regional kidney blood flow, and responses to intravenous endothelin-1, in anaesthetized rabbits. *J Hypertens.* 2001;19(10):1789-1799.

120. Nakano D, Pollock DM. Contribution of endothelin A receptors in endothelin 1-dependent natriuresis in female rats. *Hypertension.* 2009;53(2):324-330.

121. Silldorff EP, Yang S, Pallone TL. Prostaglandin E2 abrogates endothelin-induced vasoconstriction in renal outer medullary descending vasa recta of the rat. *J Clin Invest.* 1995;95(6):2734-2740.

122. Schneider MP, Ge Y, Pollock DM, Pollock JS, Kohan DE. Collecting duct-derived endothelin regulates arterial pressure and Na excretion via nitric oxide. *Hypertension.* 2008;51(6):1605-1610.

123. Nitescu N, Grimberg E, Herlitz H, Guron G. Role of endothelin ET(A) and ET(B) receptor subtypes in the regulation of intrarenal blood flow and oxygen tension in rats. *Clin Exp Pharmacol Physiol.* 2008;35(10):1227-1232.

124. Fukuroda T, Fujikawa T, Ozaki S, Ishikawa K, Yano M, Nishikibe M. Clearance of circulating endothelin-1 by ETB receptors in rats. *Biochem Biophys Res Commun.* 1994;199(3):1461-1465.

125. Inscho EW, Imig JD, Cook AK, Pollock DM. ETA and ETB receptors differentially modulate afferent and efferent arteriolar responses to endothelin. *Br J Pharmacol.* 2005;146(7):1019-1026.

126. Saeed A, DiBona GF, Marcussen N, Guron G. High-NaCl intake impairs dynamic autoregulation of renal blood flow in ANG II-infused rats. *Am J Physiol Regul Integr Comp Physiol.* 2010;299(5):R1142-1149.

127. Elmarakby AA, Loomis ED, Pollock JS, Pollock DM. NADPH oxidase inhibition attenuates oxidative stress but not hypertension produced by chronic ET-1. *Hypertension.* 2005;45(2):283-287.

128. Li L, Fink GD, Watts SW, Northcott CA, Galligan JJ, Pagano PJ, Chen AF. Endothelin-1 increases vascular superoxide via endothelin(A)-NADPH oxidase pathway in low-renin hypertension. *Circulation.* 2003;107(7):1053-1058.

129. Bude RO, Rubin JM. Relationship between the resistive index and vascular compliance and resistance. *Radiology.* 1999;211(2):411-417.

130. Bardelli M, Veglio F, Arosio E, Cataliotti A, Valvo E, Morganti A, Italian Group for the Study of Renovascular H. New intrarenal echo-Doppler velocimetric indices for the diagnosis of renal artery stenosis. *Kidney Int.* 2006;69(3):580-587.

131. Ceylan A, Karasu C, Aktan F, Guven C, Can B, Ozansoy G. Effects of simvastatin treatment on oxidant/antioxidant state and ultrastructure of diabetic rat myocardium. *Gen Physiol Biophys.* 2003;22(4):535-547.

132. Cangemi R, Loffredo L, Carnevale R, Perri L, Patrizi MP, Sanguigni V, Pignatelli P, Violi F. Early decrease of oxidative stress by atorvastatin in hypercholesterolaemic patients: effect on circulating vitamin E. *Eur Heart J.* 2008;29(1):54-62.

133. Yilmaz MI, Baykal Y, Kilic M, Sonmez A, Bulucu F, Aydin A, Sayal A, Kocar IH. Effects of statins on oxidative stress. *Biol Trace Elem Res.* 2004;98(2):119-127.

134. Wu R, Lamontagne D, de Champlain J. Antioxidative properties of acetylsalicylic Acid on vascular tissues from normotensive and spontaneously hypertensive rats. *Circulation.* 2002;105(3):387-392.

135. Roberts LJ, Morrow JD. Measurement of F(2)-isoprostanes as an index of oxidative stress in vivo. *Free Radic Biol Med.* 2000;28(4):505-513.

136. Tani S, Watanabe I, Anazawa T, Kawamata H, Tachibana E, Furukawa K, Sato Y, Nagao K, Kanmatsuse K, Kushiro T, Surugadai Atherosclerosis Regression I. Effect of pravastatin on malondialdehyde-modified low-density lipoprotein levels and coronary plaque regression as determined by three-dimensional intravascular ultrasound. *Am J Cardiol.* 2005;96(8):1089-1094.

137. Demirbag R, Yilmaz R, Kunt AS, Gur M, Ulucay A, Unlu D. Relationship between plasma total antioxidant capacity and thoracic aortic intima-media thickness. *Echocardiography.* 2006;23(3):183-188.

138. Reinisch N, Kiechl S, Mayr C, Schratzberger P, Dunzendorfer S, Kahler CM, Buratti T, Willeit J, Wiedermann CJ. Association of high plasma antioxidant capacity with new lesion formation in carotid atherosclerosis: a prospective study. *Eur J Clin Invest.* 1998;28(10):787-792.

139. Ridker PM, Rifai N, Lowenthal SP. Rapid reduction in C-reactive protein with cerivastatin among 785 patients with primary hypercholesterolemia. *Circulation.* 2001;103(9):1191-1193.

140. Macin SM, Perna ER, Farias EF, Franciosi V, Cialzeta JR, Brizuela M, Medina F, Tajer C, Doval H, Badaracco R. Atorvastatin has an important acute anti-inflammatory effect in patients with acute coronary syndrome: results of a randomized, double-blind, placebo-controlled study. *Am Heart J.* 2005;149(3):451-457.

141. Moreau P, d'Uscio LV, Shaw S, Takase H, Barton M, Luscher TF. Angiotensin II increases tissue endothelin and induces vascular hypertrophy: reversal by ET(A)-receptor antagonist. *Circulation.* 1997;96(5):1593-1597.

142. Zoccali C, Maio R, Mallamaci F, Sesti G, Perticone F. Uric acid and endothelial dysfunction in essential hypertension. *J Am Soc Nephrol.* 2006;17(5):1466-1471.

143. Chao HH, Liu JC, Lin JW, Chen CH, Wu CH, Cheng TH. Uric acid stimulates endothelin-1 gene expression associated with NADPH oxidase in human aortic smooth muscle cells. *Acta Pharmacol Sin.* 2008;29(11):1301-1312.

144. Johnson RJ, Kang DH, Feig D, Kivlighn S, Kanellis J, Watanabe S, Tuttle KR, Rodriguez-Iturbe B, Herrera-Acosta J, Mazzali M. Is there a pathogenetic role for uric acid in hypertension and cardiovascular and renal disease? *Hypertension.* 2003;41(6):1183-1190.

145. Osswald H, Schmitz HJ, Kemper R. Tissue content of adenosine, inosine and hypoxanthine in the rat kidney after ischemia and postischemic recirculation. *Pflugers Arch.* 1977;371(1-2):45-49.

146. Miller WL, Thomas RA, Berne RM, Rubio R. Adenosine production in the ischemic kidney. *Circ Res.* 1978;43(3):390-397.