Sympathetic hyperactivity in patients with chronic kidney disease

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Sympathetic hyperactivity in patients with chronic kidney disease

Verhoogde sympathische zenuwactiviteit bij patiënten met chronische nierziekte
(met een samenvatting in het Nederlands)

Sympathische Überaktivität bei Patienten mit chronischer Nierenerkrankung
(mit einer Zusammenfassung in deutscher Sprache)

Proefschrift

ter verkrijging van de graad van doctor aan de Universiteit Utrecht op gezag van de rector magnificus, prof. dr. W.H. Gispen, ingevolge het besluit van het college voor promoties in het openbaar te verdedigen op dinsdag 15 mei 2007 des middags te 4.15 uur

door
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geboren op 24 juli 1964 te Oldenburg, Duitsland

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Voor André, Daniël, Isha en Joël

Contents

Chapter 1

General introduction

Worldwide the number of patients with chronic kidney disease (CKD) is increasing. In these patients hypertension is common. Its prevalence varies between 30% and 100% depending on the target population, cause of renal disease and level of renal functioning [1]. Traditionally hypertension has been viewed as largely volume-dependent. More than three decades ago, Kim et al [2] showed that hypertensive and normotensive hemodialysis patients differ in peripheral vascular resistance and not in cardiac output. Importantly, after bilateral nephrectomy blood pressure was reduced by a decrease in peripheral resistance and not in cardiac output. This provided direct evidence that the diseased kidneys were somehow involved in the genesis of increased vascular resistance and therefore hypertension in CKD. There is now evidence that CKD is often characterized by an activated sympathetic nervous system (SNS). This may contribute to the pathogenesis of renal hypertension, but it may also adversely affect prognosis independently of its effect on blood pressure.

Sympathetic nervous system

The SNS is a part of the autonomic nervous system. It plays an important role in several regulatory systems. Together with the antagonizing part, the parasympathetic nervous system, it helps to control the visceral functions of the body, for example the arterial pressure, body temperature, gastrointestinal motility and secretion, bronchial activity and many other functions. Some activities are controlled entirely and others only partly. Usually, the sympathetic activity causes the excitatory effects and the parasympathetic activity the inhibitory effects.

Sympathetic nervous activity and blood pressure regulation

Several mechanisms are responsible for the regulation of blood pressure. The arterial pressure is regulated by feedback control systems. The vascular resistance is influenced by the activity of sympathetic vasomotor nerves, circulating vasoactive hormones and local factors like endothelial factors. The SNS plays an important role in the short term and the long term blood pressure

regulation. Therefore it interacts with other regulatory systems and hormones [3].

The sympathetic nervous system is composed of the vasomotor control centre located in the central nervous system and the peripheral afferent and efferent sympathetic nerves. The central part is regulated by complex mechanisms. In 1988 it was reported for the first time that there are angiotensin II sensitive receptors in the vasomotor control centre, which are located in the rostral ventrolateral medulla (RVML) [4] [5]. Tonically active neurons in the RVLM play an important role in maintaining the sympathetic activity and by this the resting arterial pressure. Microinjection of angiotensin II in the RVML increases the arterial pressure [6] and prior injection of a selective angiotensin receptor blocker in the same area prevented sympathoexitatory response. [7]. The sympathetic outflow from the RVML is also under the influence of the baroreceptor reflex [8], the nucleus tractus solitarius (NTS) [9] and the area postrema, located in the brainstem. Circulating angiotensin II stimulates the area postrema and counteracts the inhibitory effects of the NTS which is followed by an increase of the sympathetic outflow [10] [11].

Central sympathetic activity seems also to be under influence of the renal afferent nerves. In unilateral renal ischemia the sympathetic activity rises. De-afferention of the ischemic kidney prevents vasoconstriction in the contralateral kidney and blood pressure rise [12].

Sympathetic activity and kidney disease

Renal ischemia can lead to sympathetic activation. During renal ischemia, adenosine is released. This adenosine evokes an increase in afferent renal nerve traffic, as can be shown during adenosine infusion in the renal artery of uninephrectomized dogs [13] [14]. In rats, induction of renal artery stenosis [12], partial renal ablation by arterial ligation [15] or intrarenal phenol injection [16] cause excitation of the renal afferent nerves, which results in neurogenic hypertension. Even a small injury in one kidney caused by intrarenal injection of phenol, which does not affect the glomerular filtration rate (GFR), leads to hypertension in association with an increased central sympathetic activity [16].

In these animal models, renal denervation results in a reduction or total prevention of hypertension. Additionally, in the phenol hypertension model, nephrectomy of the injured kidney several weeks after the induction of renal damage results in normalization of blood pressure [15]. Thus, renal injury in experimental conditions can lead to sympathetic hyperactivity and hypertension and this hyperactivity is associated with activation of renal afferent nerves. The signal from the diseased kidneys goes through the afferent renal nerves to the central nervous system. It seemed that the kidney ischemia plays a crucial role in the pathogenesis of sympathetic hyperactivity. So in patients with renal artery stenosis and/or renal disease there is not only an effect by means of the RAAS, but also via the CNS.

Sympathetic activity and the progression of kidney disease

Kidney disease results in a progressive deterioration of functioning of the kidney. It is well-known that hypertension causes glomerulosclerosis. Furthermore, moxonidine, alpha and beta blockers (partially) prevent glomerular injury in experimental models, and this effect is independent of the blood pressure [17] [18]. Experimental studies showed that catecholamines have a proliferative effect on smooth muscle and on adventitial fibroblasts [19] [20]. In the animal model, this proliferative effect was mediated by adrenoceptors [20]. So, there is (mainly) experimental evidence indicating that sympathetic activity is involved in progression of kidney failure.

Sympathetic nervous activity – cardiovascular risk factor

Sympathetic activity is related to left ventricular hypertrophy (LVH). Indexes of left ventricular mass correlate with plasma noradrenaline levels [21]. The presence of LVH is associated with higher muscle sympathetic nerve activity [22], and cardiac noradrenaline spill over [23]. Chronic renal failure (CRF) patients also show a positive relation between noradrenaline and the risk of having LVH [24]. Both CRF patients not yet on dialysis and those on dialysis

often have LVH, and in end-stage renal disease (ESRD) patients LVH is associated with poor a prognosis [25] [26] [27] [28].

Additionally, sympathetic activity contributes to the development of other forms of organ damage independent of its effect on blood pressure (reviewed in[29], [30]). It is associated with heart failure, arrhythmias, and, in experimental conditions, with atherogenesis [29], [30]. Plasma noradrenaline was an independent predictor for all-cause mortality and cardiovascular events in hemodialysis patients without overt heart failure [31]. It is likely that sympathetic activation is associated with an even greater cardiovascular risk in renal patients with heart failure.

Measurement of sympathetic activity

There are several methods to measure sympathetic activity. Every method has his its' advantages and disadvantages. In our studies we used the microneurographic method to measure the muscle sympathetic nerve activity. We will briefly describe the various methods.

The activity of the sympathetic nervous system can be derived indirectly from sympathetic effector responses, for instance, blood pressure or heart rate. This method is non invasive and easy to apply. On the other hand it is very nonspecific, because effectors may also be influenced by mechanical, chemical, and hormonal stimuli, with the adverse effect a limited reproducibility within individuals. [32]. Also the absent relationship between specific hemodynamic responses and stimuli is not helpful [33],[32].

Urine noradrenaline

Measurement of noradrenaline excretion in urine is obsolete. The 24 hour excretion of noradrenaline and adrenaline with their precursors and metabolites gives no information on acute effects of adrenergic stimuli. Furthermore the excretion is strongly related to the kidney function and it is not possible to differentiate between the plasma and the renal sympathetic nerve excretion [34].

Plasma noradrenaline

Measurement of urinary output of catecholamines was replaced by measurement of plasma noradrenaline. But this method has several limitations as well. Catecholamines appearing into the plasma are the net result of discharge, reuptake, metabolism, and clearance. As a consequence its measurement is not suitable as a marker for activity. The measurement is also influenced by environmental factors. Furthermore it is only a fraction of the catecholamines secreted from the nerve terminals. Finally, the intralaboratory as well as interlaboratory reproducibility may be limited because of complexity of the laboratory techniques [35] [36] [37].

Noradrenaline spillover method

Another method is the noradrenaline spill-over rate measurement. In this radiotracer technique triated noradrenaline is infused. With organ specific blood sampling it is possible to determine organ-specific noradrenaline spill-over and the total body spill-over. The invasive method and the radioactive impact for patients are disadvantages of this method. [38]

Muscle sympathetic nerve activity (MSNA)

Several of these limitations can be overcome by the use of sympathetic nerve recordings. True sympathetic nerve activity can be assessed by the microneurographic technique, which was developed by Vallbo et al [39]. The intraneural recording is made with a tungsten microelectrode with a shaft of 0.2 mm and a tip of a few micrometers placed in a peripheral nerve, generally the peroneal or radial nerve (muscle sympathetic nerve activity) (MSNA). This is a postsynaptic sympathetic nerve activity. With this method it is possible to evaluate continuously the adrenergic activity to skeletal muscle circulation. This method is therefore suitable for investigating the sympathetic nerve activity in relation to blood pressure issues. Usually, nerve recordings cause minimal discomfort and negligible, transient after-effects, when studies are done by an experienced technician. However, the technique is not suitable for routine use, because it is laborious, time-consuming, and technically difficult [38].

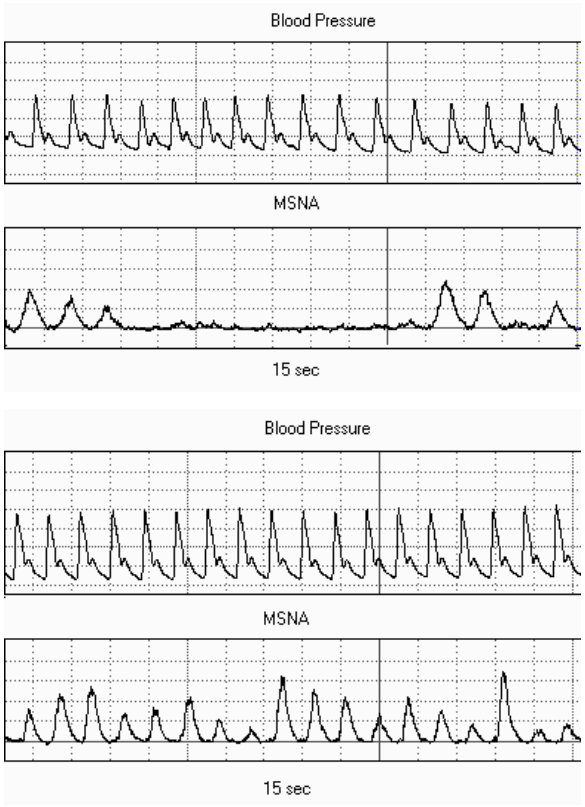

Figure 1

Typical examples of microneurographic assessment of sympathetic activity in the peroneal nerve. The upper tracing is a recording of a normal person and the lower tracing of a patient with chronic kidney disease.

Each peak represents a spontaneous burst of sympathetic nerve discharge. The rate of sympathetic nerve discharge in the patient is much higher than in the age-matched healthy control.

Blood pressure curves (finger blood pressure) in normal person and chronic kidney disease patient are shown as well.

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Chapter 2

Outline of the thesis

After very early studies by Cyon, Ludwig and Pawlov (reviewed in [1]), sympathetic activity has been a very important target in research in cardiovascular medicine. In 1970 increased catecholamine levels in CKD patients were reported. In the eighties of the previous century, it was shown that the sympathetic activity was increased in kidney disease patients [2],[3], [4]. It was suggested that higher sympathetic activity contributes to the hypertension. Converse et al reported in 1992 [5] on sympathetic activity in dialysis patients assessed by microneurographic technique. The SNS in hemodialysis patients who had undergone bilateral nephrectomy was comparable to controls, but in dialysis patients with native kidneys still present, sympathetic activity was high [5]. Ligtenberg et al showed that blood pressure and SNS in chronic renal failure patients was lowered by ACE inhibition (ACEi). A calcium antagonist decreased the blood pressure, but increased sympathetic activity [6]. Subsequent studies showed that angiotensin II blockage by angiotensin receptor blockers (ARB) decreased SNS comparable with ACEi [7].
This thesis contains studies on sympathetic nerve activity on patients with kidney disease on pharmacological modulation in an effort to normalize SNS.

The questions addressed in the studies presented in this thesis were:

First study (chapter 3)
To evaluate SNS activity in patients with parenchymal renal disease and to determine the influence of volume status and age. Secondly: to investigate the SNS activity after diminishing functional kidney tissue by unilateral nephrectomy for kidney donation.

Second study (chapter 4)
To evaluate the effect of the ARB eprosartan on the SNS activity and secondly to evaluate the effect of the addition of the centrally acting sympatholytic agent moxonidine to eprosartan treatment.

Third study (chapter 5)

To assess the efficacy of ACEi and ARB treatment to normalize SNS activity.

Fourth study (chapter 6)

To investigate the relationship between SNS activity and clinical and cardiovascular outcome during follow up.

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Chapter 3

Sympathetic nerve activity is inappropriately increased in chronic renal disease.

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Abstract

Background. We addressed the hypothesis that in hypertensive patients with renal parenchymal disease sympathetic activity is “inappropriately” elevated and that this overactivity is a feature of renal disease and not of a reduced number of nephrons per se.

Methods. Fifty seven patients with renal disease (various causes, no diabetes, all on antihypertensive medication) were studied, age range 18-62, creatinine clearance 10-114 ml/min/1.73 m2. Antihypertensives were stopped, but diuretics were allowed to prevent overhydration. Matched controls were also studied. The effect of changes in fluid status was examined in 7 patients, while on and after stopping diuretics, and in 8 controls, while on low and high sodium diet. Seven kidney donors were studied before and after unilateral nephrectomy. Sympathetic activity was quantified as muscle sympathetic nerve activity (MSNA) in the peroneal nerve.

Results. Mean arterial pressure (MAP), MSNA and plasma renin activity (PRA) were higher in patients than in controls, respectively, 115±12 and 88±11 mmHg, 31±15 and 18±10 bursts/min and 500 (20-6940) and 220 (40-980) fmol/l/sec ($p<0.01$ for all items). Extracellular fluid volume (ECV, bromide distribution) did not differ.

Seven patients were studied again after stopping diuretics. MSNA decreased from 34±18 to 19±18 bursts/min ($p<0.01$). Eight healthy subjects were studied during low and high sodium diet. MSNA was 26±12 and 13±7 bursts/min ($p<0.01$). The curves relating ECV to MSNA were parallel in the two groups, but shifted to a higher level of MSNA in the patients. In the kidney donors creatinine clearance reduced with 25%, but MSNA was identical before and after donation.

Conclusion. We conclude that in hypertensive patients with renal parenchymal disease sympathetic activity is inappropriately high for the volume status and that reduction of nephron number in itself does not influence sympathetic activity.

Introduction

Hypertension is common in chronic renal disease. Its prevalence varies between 30 and 100% depending on the target population, cause of renal disease and level of renal function [1,2]. Volume overload and activation of the renin system have long been recognized as main pathogenetic players.

There is some indication that sympathetic nervous activity is also involved. Experimental data have indicated that the presence of parenchymal injury results in neurogenic hypertension [3]. Muscle sympathetic nerve activity (MSNA) is increased in both dialysis and predialysis patients, whereas bilaterally nephrectomized patients have normal MSNA identical to controls [4-6]. Recently, we reported that MSNA is increased in hypertensive patients with polycystic kidney disease and still normal kidney function [7]. Thus the available data indicate that renal parenchymal changes can cause sympathetic hyperactivity. Sympathetic activity increases with age [7-9], and is feedback-regulated by baroreflex control and volume status 10. In chronic renal disease patients volume status may vary substantially. Therefore, it is critical that this should be taken into account when assessing sympathetic activity in individual patients.

The overall hypothesis for the present studies was that sympathetic activity is “inappropriately” increased in patients with renal parenchymal disease. In view of the above mentioned, the specific aims were (1) to establish that sympathetic activity is increased in relation to the volume status of these patients and (2) to establish that sympathetic hyperactivity is a feature of chronic renal disease and not of reduced number of nephrons but intact parenchymal structure.

Chapter 3

Methods

Subjects

Patients with hypertension (i.e. using antihypertensive drugs and/or blood pressure (BP) > 140/90 mmHg when off medication) and with stable chronic renal disease (creatinine clearance > 10 ml/min, and during last three months) could enter the study. Patients with clinically manifest heart disease (congestive heart failure, ischemic heart disease or atrial fibrillation), diabetes or patients who were on steroids were excluded. Renal disease was diagnosed by ultrasound studies (in case of polycystic kidney disease), intravenous urograms or kidney biopsy. Healthy controls were matched for age, gender and body mass index (BMI). Kidney donors were studied before and after unilateral nephrectomy. The kidney donors had normal blood pressure without medication, normal plasma creatinine (< 110 μmol/L) and no proteinuria.

Fifty-seven patients ((polycystic kidney disease (n=31), IgA nephropathy (n=5), obstructive uropathy disease (n=5), chronic pyelonephritis (n=2), nephrosclerosis (n=3), focal sclerosis (n=1), mesangial proliferative glomerulonephritis (n=1), interstitial nephritis (n=2), analgesic nephropathy (n=2), Alport syndrome (n=2) or unknown (n=3)), 57 controls and seven kidney donors were studied.

Protocol

The institutional committee for studies in humans approved the protocol. All subjects gave their written informed consent. Patients were studied when taken off antihypertensive medication for at least two weeks. Diuretics were continued to maintain normovolemia in 26 of the 57 patients, but were not taken on the day of the study. Vitamin D supplements, phosphate binders and/or HMG-CoA-reductase inhibitors were continued as well.

A subgroup of seven patients was studied twice, when clinically normovolemic (i.e. no clinical signs of fluid overload) and when taken off diuretics for > 7 days. During both study sessions patients were without angiotensin converting

enzyme (ACE) inhibition or angiotensin II antagonists. An additional group of eight controls was studied twice as well, while on high salt diet (regular diet + salt supplements: 200 mmol/day for five days) and while on low salt diet (30 mmol/day for five days, and frusemide, 20 mg bid, during the first two days). The order of the investigations regarding changes in volume status was randomized. Fluid status was assessed by extra cellular volume (ECV) measurements (see below).
Seven living kidney donors were investigated shortly before (2 to 12 weeks) and after (12 to 58 weeks) unilateral nephrectomy.

All subjects underwent an identical set of measurements, in supine position in a quiet room with an ambient temperature of 22-24°C. The protocol, which was described in more detail previously [6,7], included measurement of: supine BP, heart rate, MSNA, baroreflex sensitivity, ECV and plasma renin activity (PRA). The day prior to the MSNA measurement, subjects collected 24h urine.

BP and heart rate were measured in a recumbent position with a standard mercury sphygmomanometer; means of three measurements are presented. During the baroreceptor sensitivity assessments, BP and heart rate were recorded continuously by finger plethysmography[11]. MSNA was recorded with a unipolar tungsten microelectrode placed in a muscle nerve fascicle of the peroneal nerve [6,7,12]. Success rate of obtaining an adequate neural signal was approximately 85%. The interbeat intervals were measured from the ECG. From the interbeat interval the heart rate was computed. An intravenous cannula for infusion and blood sample collection was inserted into an antecubital vein. After instrumentation the subjects rested for 20 minutes. Baseline measurements for blood pressure, heart rate and MSNA were obtained, blood was sampled for PRA and bromide. Next, the arterial baroreflex sensitivity was assessed as the response of MSNA and of heart rate to changes in BP induced by subsequent continuous infusion of sodium nitroprusside and phenylephrine [6,7]. Bromide distribution volume was used an index of ECV [13]. Plasma bromide levels ranged between 1 and 3 mmol/L, which was well

below the therapeutic and toxic levels. Normalization for lean body mass allows comparison between males and females [14]. The normal range in our laboratory is 273 to 334 ml per kilogram of lean body mass. PRA was measured by RIA 15.

Data analysis

Data are mean ± SD, unless indicated otherwise. MSNA was expressed as the number of bursts of sympathetic activity per minute or as the number of bursts per 100 heart beats to correct for differences in heart rate. Intra-observer and inter-observer reproducibility were 4.5±0.5 % and 6.2±0.7 %. Baroreflex sensitivity is quantified as described elsewhere [6,7].

Statistical analysis

PRA was analyzed after logarithmic transformation. Baseline characteristics of patients and controls were compared by Student‘s unpaired t-test. Only independent variables were included in the regression analysis. Analysis of covariance was used to calculate differences between slopes when appropriate. Pearson’s correlation coefficients were calculated followed by stepwise linear regression when appropriate. P <0.05 was considered to be statistically significant.

Results

All patients were on antihypertensive drugs (always including an ACE-inhibitor or angiotensin II antagonist), in 26 cases combined with diuretics. Their office BP (in sitting position using mercury sphygmomanometer) was 137/84 mmHg (±11/8). After stopping the antihypertensive medication, BP increased in all patients but no patient became severely hypertensive (diastolic BP > 120 mmHg).

Patients and control subjects were matched for age, gender and BMI. Creatinine clearance in patients was lower and ranged from 10 - 118 ml/min. Despite the fact that ECV did not differ between the two groups, BP, MSNA and PRA were markedly higher in the patients (all <0.001; *Table 1*). BP did not

correlate with MSNA. Baroreceptor sensitivity was not different between patients and control subjects (*Table 1*).

Table 1.

Baseline characteristics of chronic renal disease patients and controls.

	Patients (n=57)	Controls (n=57)
Age (years)	43 ± 11	38 ± 14
Gender (M/F)	37/20	35/22
Systolic arterial pressure (mmHg)	159 ± 19	123 ± 15***
Diastolic arterial pressure (mmHg)	93 ± 9	70 ± 10***
Mean arterial pressure (mmHg)	115 ± 12	88 ± 11***
Heart rate (beats/min)	66 ± 11	63 ± 9
Plasma renin activity (fmol/l/sec)	500 (20-6940)	220 (40-980)***
Creatinine clearance (ml/min/1.73 m2)	52 ± 32	96 ± 15***
MSNA (bursts/min)	31 ± 15	18 ± 10***
MSNA (bursts/100 bpm)	47 ± 23	31 ± 15***
ECV (ml/kg LBM)	321 ± 38	305 ± 27
Baroreceptor sensitivity		
for MSNA (bursts/min/mmHg)	-2.2 ± 1.5	-2.1 ± 0.6
for heart rate (beats/min/mmHg)	-1.2 ± 1.2	-1.1 ± 0.6

Values are mean ± SD, except plasma renin activity (median(range)).

MSNA = muscle sympathetic nerve activity,

ECV = extracellular volume

*** p<0.001 controls compared to patients.

In patients correlations existed between age and creatinine clearance (r= -0.59; p<0.001) and age and MSNA (r=0.62; p<0.001), between creatinine clearance and MAP (r= -0.31; p<0.05) and between MSNA and log PRA (r=0.29; p<0.05). In control subjects age correlated with creatinine clearance (r= -0.65; p<0.001), MSNA (r=0.69; p<0.001) and log PRA (r= -0.36; p<0.05) and a correlation existed between MAP and log PRA (r= -0.44; p<0.05). Multiple regression analysis revealed age and PRA as predictive for MSNA in patients (MSNA= -9.3

+ 0.86 x age + 0.004 x PRA; r2=0.48; p<0.001). In control subjects age predicted MSNA (MSNA= 2.6 + 0.39 x age; r2=0.37; p<0.001). The regression line for age and MSNA was steeper in patients compared to control subjects (p<0.01).

Effects of changes in fluid status.

Seven patients (diagnosis: nephrosclerosis (n=2), polycystic kidney disease (n=2), Alport disease (n=2) and chronic pyelonephritis (n=1); age: 49±7 year; 4 males; creatinine clearance: 39±18 ml/min) were examined twice, while clinically normovolemic and after stopping diuretic therapy. After stopping the diuretics body weight increased with 1.6±0.6 kg. ECV and BP increased, and MSNA and PRA were significantly suppressed in the hypervolemic compared with the normovolemic condition (*Table 2*, *Figure 1*).

Table 2.

Characteristics of patients (n=7) in normovolemic and hypervolemic state

	No diuretics	With diuretics
Systolic arterial pressure (mmHg)	166 ± 23	156 ± 18**
Diastolic arterial pressure (mmHg)	96 ± 8	93 ± 9
Mean arterial pressure (mmHg)	120 ± 12	115 ± 10*
Heart rate (beats/min)	62 ± 8	64 ± 10
Plasma renin activity (fmol/l/sec)	260 (20-300)	430 (370-3480)**
MSNA (bursts/min)	19 ±18	34 ± 18**
MSNA (bursts/100 bpm)	30 ± 26	57 ± 21**
ECV (ml/kg LBM)	343 ± 20	314 ± 21
Weight (kg)	78.7 ± 11.6	77.1 ± 12.1***
Baroreceptor sensitivity		
for MSNA (bursts/min/mmHg)	-2.2 ± 1.4	-2.6 ± 0.8
for heart rate (beats/min/mmHg)	-0.8 ± 0.9	-1.3 ± 0.6

Values are mean±SD, except plasma renin activity :median(range).

MSNA= muscle sympathetic nerve activity;

ECV= extracellular volume; *p<0.05, **p<0.01, ***p<0.001

Eight controls (age: 34±15 year; 4 women) were measured while on a low sodium diet and high sodium diet. Dietary compliance was good, evidenced by the urinary sodium output. These manipulations resulted in changes in body weight comparable to those in the patients. Again an increase in ECV coincided with a suppression of MSNA and PRA (*Table 3*, *Figure 1*).

Table 3.
Characteristics of controls (n=8) during low and high sodium diet

	High salt	High salt
Systolic arterial pressure (mmHg)	125±8	119±12
Diastolic arterial pressure (mmHg)	76±5	72±7
Mean arterial pressure (mmHg)	93±5	87±7*
Heart rate (beats/min)	58±5	60±5
Plasma renin activity (fmol/l/sec)	175(20-330)	790(530-2260)**
Urine sodium (mmol/24 h)	327±64	29±23***
MSNA (bursts/min)	13±7	26±12**
MSNA (bursts/100 bpm)	23±11	42±18***
ECV (ml/kg LBM)	328±30	292±30*
Weight (kg)	72.9±8.3	71.1±8.3***
Baroreceptor sensitivity		
for MSNA (bursts/min/mmHg)	-2.0±0.7	-2.4±0.6
for heart rate (beats/min/mmHg)	-0.8±0.3	-1.3±0.4

Values are mean±SD, except plasma renin activity :median(range).
MSNA= muscle sympathetic nerve activity; ECV= extracellular volume;
*p<0.05, **p<0.01, ***p<0.001

Plots of MSNA versus ECV in patients and control subjects showed parallel curves, but for patients it was shifted to a higher level of sympathetic activity. The MSNA levels corresponding to the low and high ECV were significantly higher than in controls ($p < 0.01$ and $p<0.05$ respectively; *figure 1*). Plots of PRA

versus ECV revealed a similar pattern. Baroreceptor sensitivity was not different between the different conditions, or between patients and control subjects.

Kidney donors.

Creatinine clearance decreased, but BP, PRA and MSNA remained unchanged (*Table 4*).

Table 4.

Characteristics of kidney donors (n=7) before and after nephrectomy

	before	after
Systolic arterial pressure (mmHg)	132 ± 12	138 ± 6
Diastolic arterial pressure (mmHg)	74 ± 6	74 ± 8
Mean arterial pressure (mmHg)	91 ± 7	94 ± 7
Heart rate (beats/min)	62 ± 9	62 ± 11
Plasma renin activity (fmol/l/sec)	180 (90-990)	140 (20-530)
Creatinine clearance (ml/min/1.73m2)	107 ± 15	78 ± 12**
MSNA (bursts/min)	22 ± 10	23 ± 10
MSNA (bursts/100 bpm)	35 ± 14	37 ± 12
ECV (ml/kg LBM)	328 ± 30	292 ± 30*
Weight (kg)	72.9 ± 8.3	71.1 ± 8.3***
Baroreceptor sensitivity		
for MSNA (bursts/min/mmHg)	-2.0 ± 0.8	-2.6 ± 1.0
for heart rate (beats/min/mmHg)	-0.8 ± 0.7	-1.2 ± 0.5

Values are mean±SD, except plasma renin activity :median(range);

MSNA= muscle sympathetic nerve activity;

** p< 0.01 compared to before nephrectomy

Discussion

To our knowledge this is the first study that shows that the relation between short-term changes in sympathetic activity, quantified as MSNA, and volume parallels that in healthy subjects, but is shifted to a higher level of MSNA. The data give further support to our previous findings that MSNA, which represents

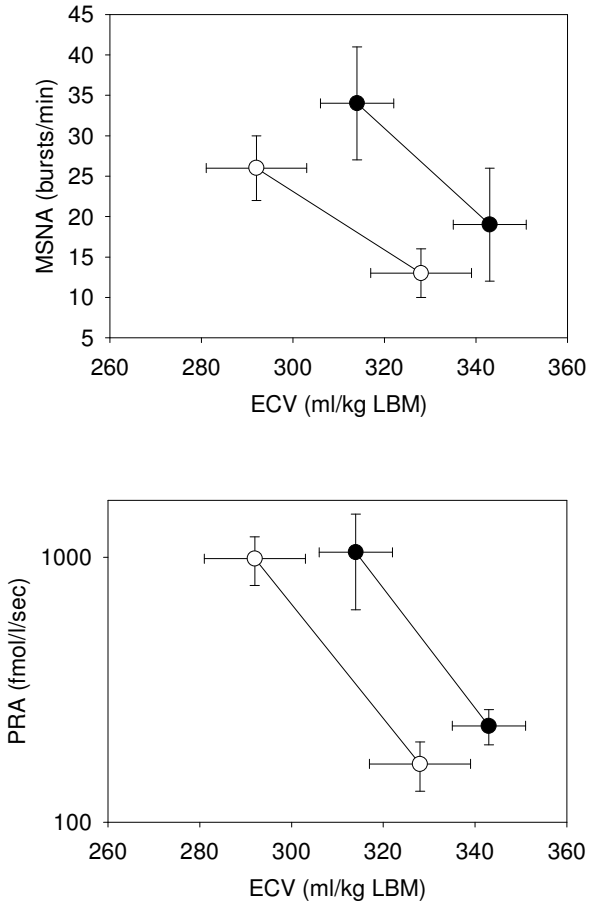

Figure 1a and 1b.
Plots of ECV and MSNA (1a) and ECV and PRA (1b) in chronic renal failure patients (filled circles) in normovolemic and hypervolemic condition and in healthy volunteers (open circles) during low and high sodium diet.

the sympathetic activity to the resistance vessels, is increased in normovolemic hypertensive patients with renal parenchymal disease studied in steady state [5,7]. MSNA does not change significantly after unilateral nephrectomy in healthy kidney donors.

Fluid overload is considered to play a major role in the pathogenesis of nephrogenic hypertension. It has long been recognized that the renin-angiotensin system is “inappropriately” activated in relation to the fluid status [16]. This contributes to the hypertension, and explains why hypertension may persist even after correction of the fluid overload. The role of the sympathetic nervous system in renal hypertension is less clear. Whereas the renin system is especially involved in the long-term regulation of BP, the sympathetic nervous system is particularly relevant for the short-term regulation. This is apparent from the quick baroreflex-mediated changes in sympathetic activity, confirmed also in the present study. Arterial baroreceptor sensitivity in patients, that is the ability of the sympathetic nervous system to respond to acute changes in BP - was not different from control subjects. Changes in volume status have a reciprocal effect on plasma noradrenaline [17] and, as is shown in our study, on MSNA. MSNA is also suppressed in subjects with mineralocorticoid excess 18. However in clinical situations with sustained low BP, such as heart failure, resting sympathetic activity is elevated [19]. A recent study showed that MSNA remained consistently elevated over eight weeks in hypertensive subjects switched to a low salt diet [20]. Clearly, changes in volume status and BP have a sustained effect on sympathetic activity.

The present study shows that resting sympathetic activity is, on average, elevated in hypertensive patients with renal parenchymal disease compared with control subjects. The physiological increase with age is still present 8,9. Although the number of studied subjects was limited, the present data clearly show that changes in the volume status induced comparable changes in sympathetic activity as observed in the healthy subjects, however, at a higher level of sympathetic activity. Therefore, we conclude that in hypertensive patients with renal parenchymal disease sympathetic activity is inappropriately high for the volume status, similar as has been described for the activity of the renin angiotensin system [16].

Animal experiments have shown that intrarenal ischemia by arterial constriction [21] or by intrarenal phenol injection can cause sympathetic activation by stimulation of renal afferents [4]. That indeed the diseased kidney induces the

sympathetic activation is confirmed by the observation that sympathetic activity is not increased in patients who underwent bilateral nephrectomy because of hypertensive end-stage renal disease [5]. Our hypothesis is that renal parenchymal disease, by causing local or diffuse compromised perfusion, leads to stimulation of both the renin angiotensin system and the sympathetic nervous system. This suggests that not the renal failure itself, but the renal structural changes are crucial. Indeed, we have shown that hypertensive subjects with adult polycystic kidney disease show increased MSNA before developing loss of GFR [7]. In the present study, we found no independent correlation between the severity of renal failure and sympathetic activity. We also observed that unilateral nephrectomy does not increase MSNA. Even though this was based on a few subjects, it appears that a major reduction in the number of functioning nephrons without damage to the residual parenchyma will by it self not induce significant sympathetic activation.

The relation between PRA and MSNA may indicate a cause-effect relation or a common origin. Angiotensin II is able to increase central sympathetic output [22]. Renal ischemia will stimulate the renin-angiotensin system. Indeed, the patients in this study had increased PRA. Previously, we demonstrated that an ACE-inhibitor and an angiotensin II receptor antagonist reduce sympathetic overactivity, strongly suggesting that angiotensin II contributes importantly in the pathogenesis of the sympathetic hyperactivity [5,23].

The present study was limited to hypertensive patients with renal parenchymal disease, starting from the idea that in these patients that renin activity is increased [16]. Sympathetic activity is apparently also high in these patients. We have shown that MSNA is normal in patients with normotensive polycystic kidney disease [7]. It remains unclear whether sympathetic activity is lower or normal in normotensive patients with other types of renal disease. Furthermore, many of the present subjects had polycystic kidney disease, a condition associated with pronounced parenchymal structural changes. The numbers of patients with other conditions are not large enough to allow separate evaluation of the data.

In conclusion, the present large study shows definitely that sympathetic activity is increased in hypertensive patients with renal parenchymal disease. Sympathetic activity is inappropriately increased for the volume status. It is not increased in subjects with a reduced number of nephrons in the absence of renal parenchynal disease. Recent evidence indicates that sympathetic activity is associated with mortality and cardiovascular outcomes in patients with chronic renal failure [24]. Although not specifically addressed by this study, we suggest that normalization of sympathetic overactivity should be considered a specific goal of treatment of hypertensive patients with renal parenchymal disease.

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Chapter 4

Moxonidine normalizes sympathetic hyperactivity in patients with eprosartan-treated chronic renal failure

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Abstract

Enalapril and losartan reduce but not normalize sympathetic hyperactivity in patients with hypertensive chronic renal failure (CRF). This study assessed the effect of chronic eprosartan on BP and sympathetic activity, and assessed the effect of moxonidine during chronic eprosartan treatment. In 11 stable patients with CRF (creatinine clearance 47 ± 10 ml/min), muscle sympathetic nerve activity (MSNA; peroneal nerve), BP, and baroreceptor sensitivity were measured in the absence of antihypertensive drugs (except diuretics) during chronic eprosartan therapy (600 mg for 6 wk) and in 9 patients after moxonidine (0.2 mg for 6 wk) was added. Normovolemia was controlled by diuretics and confirmed by extracellular fluid volume measurements. BP, heart rate, and MSNA were higher in patients than in 22 controls. During eprosartan therapy, mean arterial pressure (111 ± 9 to 98 ± 7 mmHg, $P < 0.001$), heart rate (71 ± 10 to 65 ± 8 bpm, $P < 0.001$), and MSNA (35 ± 10 to 27 ± 8 bursts/min, $P < 0.001$) decreased. After the addition of moxonidine ($n = 9$), a further reduction of mean arterial pressure to 89 ± 7 mmHg ($P < 0.05$) and of MSNA to 20 ± 10 bursts/min ($P < 0.05$) occurred. Sympathetic activity in patients with CRF can be normalized, and angiotensin II–independent sympathetic hyperactivity contributes to the pathogenesis of renal hypertension. Sympathetic hyperactivity is associated with poor cardiovascular outcomes, implying that reduction might be beneficial to the patients. The addition of moxonidine to angiotensin II antagonist treatment might be appropriate.

Introduction

Chronic renal failure (CRF) is often characterized by the presence of sympathetic hyperactivity [1–3]. This contributes to the pathogenesis of renal hypertension. It is also associated with cardiovascular morbidity and mortality independently of its effect on BP [4]. These data suggest that reducing sympathetic hyperactivity might be beneficial. We have shown previously that both the angiotensine-converting enzyme ACE inhibitor enalapril and the angiotensine II (AngII) antagonist losartan when quantified by measuring muscle sympathetic nerve activity (MSNA) reduce sympathetic hyperactivity, but do not normalize it [5,6]. These studies indicate that AngII - mediated mechanisms are important in the pathogenesis of renal hypertension. The data also suggest that an additional intervention is needed to normalize sympathetic activity.

Eprosartan is an AngII antagonist with a chemical structure distinct from losartan and most other AngII antagonists. Some but not all experimental evidence suggests that eprosartan has a higher affinity to presynaptic Ang receptors than other AngII antagonists, thereby more effectively reducing sympathetic activity [7,8]. In the present study, we evaluated in patients with CRF the effects of eprosartan on BP and MSNA. Second, we hypothesized that if sympathetic activity is not normalized during chronic eprosartan, addition of a centrally acting sympatholytic agent with a mode of action different from AngII antagonists would further decrease sympathetic activity.

Materials and Methods

Subjects

Eleven hypertensive stable patients with CRF (two women) participated (Table 1). Their renal diagnoses were as follows: polycystic kidney disease (n = 4), urological disorders (n = 3), IgA nephropathy (n = 1), Alport disease (n = 1), and unknown (n = 2). Patients with clinically manifest heart disease (congestive heart failure, coronary heart disease, or atrial fibrillation) or diabetes mellitus, as well as patients known to have had adverse reactions to ACE inhibitors or AngII

antagonists or who were receiving drugs known to influence sympathetic activity, were excluded. The patients were selected because they had a stable creatinine clearance between 30 and 70 ml/min and were hypertensive (sitting office BP >145/90 mmHg, repeated measurements) after stopping all antihypertensive medications,. They were normovolemic judged clinically by the absence of edema and confirmed with volume measurements. To control this normovolemic state, six patients were maintained on therapy with diuretics throughout the study. The data of the patients were compared with data obtained in a historical group of matched healthy subjects (*Table 1*).

We obtained adequate MSNA measurements in 11 patients twice (baseline and during chronic eprosartan therapy) and in nine patients three times (baseline, during chronic eprosartan therapy, and during chronic therapy with eprosartan combined with moxonidine). The two cases of failure were caused by an inability to find an adequate MSNA signal and by refusal of the patient to undergo the third study.

Protocol

All patients provided informed consent to participate in the study, which was approved by the institutional committee for studies on humans. They were all receiving chronic antihypertensive treatment, which included an ACE inhibitor or an AngII antagonist. Patients were studied on three occasions: when taken off antihypertensive medication for more than 2 wk, during chronic (6 wk) eprosartan therapy (600 mg, once daily) or during chronic (6 wk) therapy with eprosartan combined with 0.2 mg of moxonidine (600 mg, both once daily). Vitamin D supplements, phosphate binders, and/or hydroxymethylglutaryl (HMG)–coenzyme A reductase inhibitors were continued.

The subjects underwent an identical set of measurements while they were in the supine position in a quiet room with an ambient temperature of 22 to 24°C. All study sessions were performed in the morning between 2 and 5 h after drug intake. These measurements included supine BP, heart rate, MSNA, baroreflex sensitivity, extracellular fluid volume (ECFV), and plasma renin activity (PRA). BP was measured in a recumbent position by an automatic oscillometric device (Accutorr Plus; Datascope Corp, Paramus, NJ). Means of three measurements

are presented. During the baroreflex sensitivity assessments, BP was recorded continuously by finger plethysmography (Finapres; Datex-Ohmeda, Louisville, CO). The Finapres device is especially suitable for analysis of changes in BP during short-term interventions [5,6]. MSNA was recorded with a unipolar tungsten microelectrode placed in a muscle nerve fascicle of the peroneal nerve by means of the technique of Vallbo et al. [9], and described by us previously [5,6,10,11]. The correct position of the electrode was evaluated by means of a Valsalva maneuver: the patient was asked to blow into a mouthpiece of an aeroid manometer to 40 mmHg for 15 seconds while BP, heart rate, and MSNA are continuously recorded. The BP overshoot after the restart of breathing is associated with a short pause in neural activity. The neural signal during the BP overshoot is considered to be the background noise. This procedure was done at the beginning and at the end of the study session. Success rate of obtaining an adequate neural signal approximately 85%. The heartbeat intervals were measured from the electrocardiogram. The sample frequency is 200 Hz. An intravenous cannula for infusion and blood sample collection was inserted into an antecubital vein.

After instrumentation, the subjects rested for 20 min. Baseline measurements for BP, heart rate, and MSNA were obtained, blood was sampled for measurement of PRA and bromide, and bromide was injected intravenously for measurement of the ECFV. Next, baroreflex sensitivity was assessed as the response of MSNA and of heart rate to changes in BP induced by subsequent continuous infusion of sodium nitroprusside and phenylephrine. Sodium nitroprusside (333 µg/ml in glucose 5%) was infused starting at a rate of 33 µg/min and individually increased (in 3-min steps) to obtain a reduction of mean arterial pressure of at least 12 mmHg. After a second 20-min rest period, a continuous infusion of phenylephrine (333 µg/ml in saline 0.9%) was started at a rate of 33 µg/min and individually increased (in 3-min steps), to increase mean arterial pressure by at least 12 mmHg. The nerve activity was monitored online (Poly 5; Inspectors Research Systems, Amsterdam, The Netherlands) and stored on disk for offline analysis.

Laboratory Analyses

Bromide distribution volume, as an index of extracellular fluid volume, was calculated from plasma bromide concentration in blood samples obtained at 90, 120, and 150 min after injection of 4 g of sodium bromide. Plasma bromide was measured colorimetrically at 440 nm by the gold bromide technique and corrected for plasma bromide before injection. The distribution volume was corrected for bromide penetration into erythrocytes for plasma water content and for the Donnan equilibrium effect and expressed as ml/kg lean body mass [12]. Plasma bromide levels range between 1 and 3 mmol/L, which is well below the therapeutic and toxic level [12]. Lean body mass, estimated from weight and height, is the most suitable index for normalization of body fluid volumes in humans and allows comparison between men and women [13]. The normal range in our laboratory is 273 to 334 ml/kg of lean body weight. PRA was measured by RIA [14].

Effects of eprosartan in hypertensive chronic renal failure patients.

Characteristic	Patients		Controls
	Baseline	Eprosartan	
No. (male)	11 (9)		22 (18)
Age (yr)	45 ± 14		45 ± 14
BMI (kg/m^2)	26.2 ± 2.4	26.3 ± 2.2	25.2 ± 2.3
Plasma creatinine (µmol/L)	217 ± 40[a]	219 ± 47[a]	92 ± 15
Creatinine clearance (ml/min)	47 ± 10[a]	48 ± 13[a]	105 ± 18
Systolic arterial pressure (mmHg)	147 ± 12[a]	130 ± 10[b]	126 ± 15
Diastolic arterial pressure (mmHg)	94 ± 8[a]	82 ± 6[b]	70 ± 8
Mean arterial pressure (mmHg)	111 ± 9[a]	98 ± 7[b]	88 ± 9
Heart rate (beats/min)	71 ± 10[a]	65 ± 8[c]	62 ± 10
log Plasma renin activity (fmol/L/sec)	2.64 ± 0.17[a]	3.11 ± 0.38[ab]	2.34 ± 0.18
MSNA (burst/min)	35 ± 10[a]	27 ± 8[b]	20 ± 11
MSNA (burst/100 bpm)	46 ± 13[a]	41 ± 9[d]	31 ± 16
Baroreceptor sensivity			
for MSNA (bursts/min per mmHg)	1.55 ± 0.95	1.71 ± 0.8	
for heart rate (beats /min per mmHg)	0.71 ± 0.43	0.92 ± 0.53	

Values are mean ± SD. Arterial blood pressures represent values obtained in supine position. BMI, body mass index; MSNA, muscle sympathetic nerve acitivity.

[a] $p < 0.001$compared with controls; [b] $p<0.001$ compared with baseline;

[c] $p=0.02$ compared with baseline; [d] $p=0.05$ compared with baseline

Data Analyses

Data are given as mean ± SD, unless indicated otherwise. MSNA was expressed as the number of bursts of sympathetic activity per minute or as the number of bursts per 100 heart beats to correct for differences in heart rate. Intraobserver and interobserver reproducibility are, respectively, 4.5% ± 0.5% and 6.2% ± 0.7% [6]. During the sodium nitroprusside and phenylephrine infusion, MSNA was counted for 1 min during each infusion step. The results of the continuous registration of BP and heart rate were averaged per minute. Baroreflex sensitivity was expressed as changes in MSNA and heart rate versus BP. It was calculated for each subject by least-squares analysis of the linear part of the baroreflex curves that included the baseline value and expressed as the number of bursts per minute per millimeter of mercury and the number of beats per minute per millimeter of mercury, respectively.

Statistical Analyses

PRA was analyzed after logarithmic transformation. Baseline characteristics of patients and controls were compared by unpaired t test. Differences between different occasions of patients were examined by repeated-measure ANOVAs. If variance reached statistical significance, the means were analyzed by Student-Newman-Keuls test in parametric variables and Kruskal-Wallis ANOVA on ranks in nonparametric variables. A P value of 0.05 was considered to be statistically significant.

Results

When untreated, BP, heart rate, PRA, and MSNA in the patients were clearly higher than in controls (*Table 1*). The ECFV was within normal limits (patients 313 ± 22, controls 303 ± 28 ml/kg lean body mass [LBM]). Six weeks of treatment with eprosartan reduced mean arterial pressure by 12% ± 6% ($P < 0.001$) and MSNA by 23% ± 11% ($P < 0.001$) (*Table 1*). Heart rate decreased, and, as expected, PRA increased. Six weeks after the addition of moxonidine ($n = 9$) to the eprosartan treatment, BP and MSNA were further decreased; mean arterial pressure decreased from 98 ± 7 to 89 ± 7 mmHg ($P < 0.05$) and MSNA from 26 ± 9 to 20 ± 8 ($P < 0.001$). Mean arterial pressure and MSNA became

identical to controls (88 ± 10 mmHg and 20 ± 10 bursts/min, respectively) (*Figure 1*). Heart rate (from 65 ± 9 to 66 ± 8 bpm) and PRA remained unchanged after the addition of moxonidine. Baroreceptor sensitivity did not change (baseline, during eprosartan therapy, and during the combination eprosartan and moxonidine therapy: MSNA, 1.55 ± 0.95, 1.71 ± 0.80, and 1.71 ± 0.85 bursts/min mmHg; heart rate, 0.71 ± 0.43, 0.92 ± 0.53, and 0.71 ± 0.43 beats/min mmHg; (*Figure 2*). Throughout the study period, ECFV and plasma creatinine levels did not change.

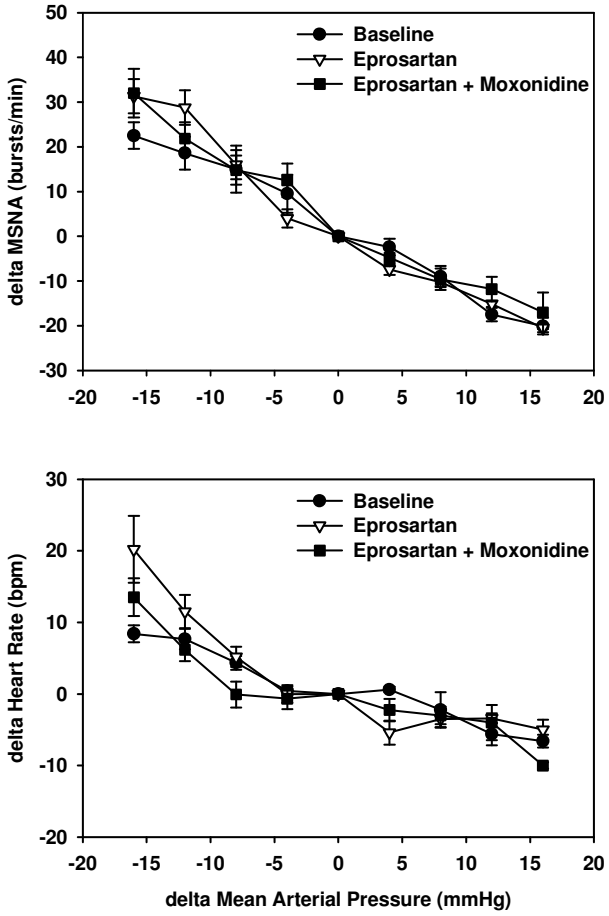

Figure 2. Baroreflex response to changes in mean arterial pressure (MAP, mmHg) in patients with chronic renal failure without antihypertensive treatment, during chronic eprosartan and chronic eprosartan combined with moxonidine treatment. Changes in MAP are plotted against changes in muscle sympathetic nerve activity (MSNA) and heart rate. Differences in slopes were NS.

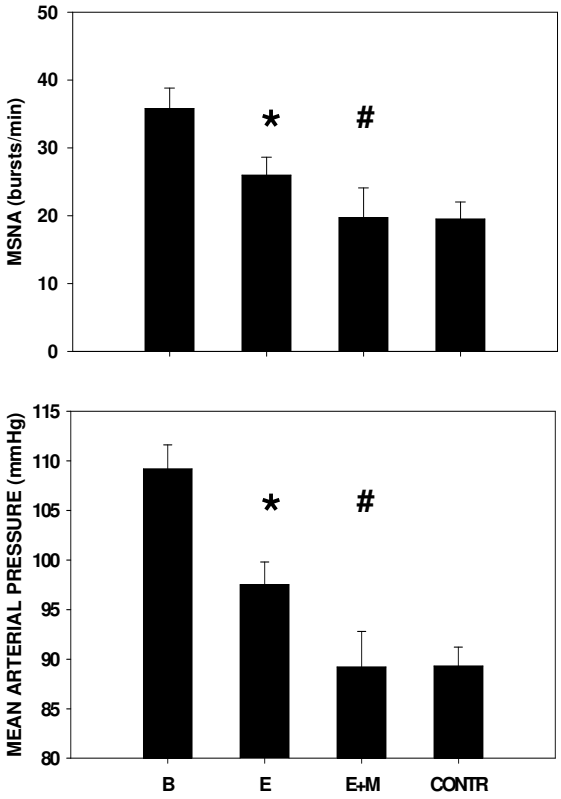

Figure 1. (a) Mean arterial pressure (mmHg) in patients with chronic renal failure (n = 9) when untreated (B = baseline), during chronic eprosartan (E) and chronic E combined with moxonidine (E + M) treatment. Controls (CONTR) are healthy age-matched persons (n = 22). Data are means ± SD. *P < 0.05 baseline versus E. #P < 0.05 E versus E + M. (b) Muscle sympathetic nerve activity (MSNA, burst/min) in patients with chronic renal failure (n = 9) when untreated (B = baseline) during chronic E and chronic E + M treatment. Controls (CONTR) are healthy age-matched persons (n = 22).). Data are means ± SD. *P < 0.05 baseline versus E. #P < 0.05 E versus E + M.

Discussion

To our knowledge, this is the first study to indicate that sympathetic hyperactivity in patients with CRF chronically treated with an AngII antagonist is further reduced and normalized after the addition of moxonidine. This study adds new information to the knowledge of the pathogenesis of the sympathetic hyperactivity in renal hypertension. It confirms earlier data that AngII-mediated *(Table 1)*. Effects of eprosartan in hypertensive patients with chronic renal failure

mechanisms are important, but the study suggests that AngII-independent mechanisms are also involved.

AngII can stimulate sympathetic activity on various levels. It increases central sympathetic outflow, and facilitates ganglionic transmission and synaptic noradrenaline release by stimulation of presynaptic receptors. Previously, we have demonstrated in hypertensive patients with CRF that both enalapril and losartan therapy reduce but do not normalize MSNA [5,6]. These results underscore the relevance of AngII in the pathogenesis of renal hypertension, and they indicate that both agents pass the blood-brain barrier. The present study confirms that hypertensive patients with CRF exhibit sympathetic hyperactivity. Heart rate is also increased, suggesting that not only MSNA, being the centrally originated sympathetic activity to the resistance vasculature, but also the sympathetic activity affecting heart rate is increased.

The chemical structure of eprosartan is distinct from that of most other AngII antagonists [15]. It passes the blood-brain barrier in experimental models [16]. Studies comparing AngII antagonists in this respect are not available. Some experimental studies suggest that eprosartan has a higher sympatho-inhibiting potential than other AngII antagonists [7,8]. The study presented here shows a 23% ± 11% reduction in MSNA by eprosartan, which is comparable to the MSNA reduction of approximately 20% during losartan or enalapril [6)] Also, heart rate decreased significantly, giving support to the notion that cardiac sympathetic activity is decreased as well. This study indicates that in humans, eprosartan passes the blood-brain barrier. It suggests that its capacity to reduce MSNA is comparable to that of losartan and enalapril. The dose-response curve of eprosartan in essential hypertension with respect to the effect on BP is flat above 600 mg, suggesting that the present dosage is optimal. We cannot exclude the possibility that a higher dosage would have a more pronounced sympatho-inhibiting effect.

Moxonidine is a high affinity agonist of imidazoline I1 receptors, which are located in the rostral ventrolateral medulla (RVLM) of the brainstem. Stimulation of these receptors reduces central sympathetic outflow, and subsequently BP, by a decrease in peripheral vascular resistance [17,18]. After oral ingestion, peak concentration are achieved within 1 h. The plasma half-life is 2 h, and this

is extended in kidney failure [19]. The duration of the antihypertensive effect is much longer than the plasma half-life suggests, which implies retention in the central nervous system. The sympatho-inhibitory effect of moxonidine seems to be mediated almost entirely by its effect on these brainstem receptors. It has low affinity to central nervous system alpha2 adrenoceptors, explaining why it is associated with fewer side effects than the centrally acting compound clonidine [17,18]. Its antihypertensive efficacy is well established in essential hypertension; it is comparable to most other antihypertensive agents [17]. In the present study addition of moxonidine to chronic treatment with an AngII antagonist resulted in a 9 ± 3 mmHg reduction of mean arterial pressure. This effect is comparable to the approximately 10 mmHg reduction with this dose during chronic treatment in patients with essential hypertension [20]. This study was not specifically designed to monitor side effects, but it confirms an earlier study in patients with CRF in which addition of moxonidine to treatment with an ACE inhibitor or an AngII antagonist was well tolerated and resulted in a decrease in BP and a slower decline in GFR than calcium antagonist treated patients [21]. We found a 24% ± 11% further reduction of MSNA. In patients with essential hypertension, a 19% reduction was seen when patients received monotherapy with an identical dose [20]. In that study, doubling the dose provided no additional effect on MSNA. The addition of moxonidine had no influence on baroreceptor sensitivity—that is, the capacity to increase or decrease MSNA or heart rate to buffer short-term BP fluctuations. In that respect, moxonidine does not differ from clonidine, which also does not affect baroreceptor sensitivity, when assessed by identical methodology [22,23].

The rostral ventrolateral medulla (RVLM) of the brainstem contains both imidazoline I1 and AngII receptors. Microinjection of AngII into the RVLM increases BP and peripheral sympathetic activity, and these effects are blocked by AngII antagonists [24,25]. Microinjection of moxonidine into the RVLM also reduces BP by a reduction of sympathetic activity. Ligands for AngII receptors and imidazoline receptors have structural similarities. However, experimentally, moxonidine has no cross-reactivity with AngII receptors [26)] making it unlikely that effects of moxonidine in this study are merely explained by a more complete blockade of the AngII receptors. We cannot exclude the possibility

that the effect of moxonidine is a nonspecific effect of BP reduction. We have not studied the effect of the addition of other antihypertensive agents to AngII antagonist treatment.

The study presented here and our previous studies indicate that in renal hypertension, blocking AngII-mediated mechanisms only partially reduces BP and sympathetic hyperactivity. The study shows that after eliminating the effects of the enhanced renin-angiotensin system, central mechanisms sensible to imidazoline I1 receptor agonism are still involved in maintaining increased BP. Whether these central mechanisms are identical to those in essential hypertension or whether they are specific to the uremic environment is unknown. We have recently shown that increasing the frequency of hemodialysis from three to six times weekly results in a decrease in MSNA, without effect on PRA [27]. The MSNA returned to its initial level after the patients returned after 6 mo to the three-times-a-week regimen. This finding points to mechanism(s) independent of the renin-angiotensin system in the pathogenesis of sympathetic hyperactivity. Also, in patients with heart failure who receive chronic treatment with an ACE inhibitor or AngII antagonist, MSNA was normalized only after the addition of clonidine [22].

The present data may have important implications for the treatment of patients with CRF. There is substantial evidence that sympathetic hyperactivity should be considered as a cardiovascular risk factor [1–3]. Sympathetic activity is associated with all-cause mortality and poor cardiovascular outcomes in patients with CRF, suggesting that treatment of sympathetic hyperactivity could be beneficial. ACE inhibitors appear to be particularly effective in reducing left ventricular hypertrophy in patients with CRF [28–30]. A retrospective analysis in hemodialysis patients receiving ACE inhibitors, independent of its effect on BP, found that it seems to be associated with a dramatic reduction in mortality [31]. In patients with CRF not on dialysis, ACE inhibitor therapy also improved survival rates independent of its effect on BP [32]. A recent study shows that in dialysis patients with dilated cardiomyopathy, addition of carvedilol reduces morbidity and mortality to the standard therapy regimen including ACE inhibitors as compared with placebo. Carvedilol reduces cardiac sympathetic activity, possibly by its effect on prejunctional beta2-adrenergic receptors [33,34]. These

data suggest that in selected patients, addition of an additional sympatholytic agent may improve prognosis. Indeed, ACE inhibitors and AngII antagonists have now been accepted by Guidelines Committees both in Europe and the United States as first-choice treatment in patients with CRF, combined with diuretics or ultrafiltration to obtain normovolemia [35,36]. Both Committees recognized that in patients with CRF, a third agent is often necessary to obtain normotension. A sympatholytic agent such as moxonidine might be an appropriate choice. Whether this addition indeed improves clinical outcomes in patients with CRF remains to be established.

In conclusion, this study shows that the combination of an AngII antagonist with the centrally acting sympatholytic agent moxonidine normalizes both BP and MSNA in hypertensive normovolemic patients with CRF. The data indicate that apart from the enhanced activity of the renin-angiotensin system, AngII-independent mechanisms are also involved in the pathogenesis of renal hypertension.

Acknowledgments

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Chapter 5

Sympathetic hyperactivity in hypertensive chronic kidney disease patients is reduced during standard treatment.

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Abstract

Standard treatment in chronic kidney disease (CKD) patients includes an angiotensin converting enzyme inhibitor (ACEi) or angiotensin II receptor blocker (ARB). CKD is often characterized by sympathetic hyperactivity. This study investigates the prevalence of sympathetic hyperactivity (quantified by assessment of muscle sympathetic nerve activity, MSNA) in a sizable group of patients with CKD and assessed whether chronic ACEi or ARB normalizes increased MSNA.

In 74 CKD patients (creatinine clearance 54 ± 31 ml/min) MSNA, blood pressure and plasma renin activity (PRA) were measured in absence of antihypertensive drugs except of diuretics. In a subgroup of 31 patients another set of measurements were obtained after at least 6 weeks enalapril (10mg orally), losartan (100mg orally) or eprosartan (600mg orally).

Patients as compared with controls (n=82) had higher mean arterial pressure (MAP, 113 ± 13 and 89 ± 7 mmHg), age-adjusted MSNA (31 ± 9 and 19 ± 7 bursts/min) and log plasma renin activity (log PRA) logPRA (2.67± 036 and 2.40± 0.32 fmol/L/sec) (all $p<0.001$). In 82% of the patients age-adjusted MSNA was higher than the mean of controls. During ACEi or ARB therapy (n=31) MAP (115 ± 11 to100 ± 9 mmHg) and age-adjusted MSNA (31 ± 8 to 23 ± 7 bursts/min) decreased (both $p<0.01$), but were still higher than in controls (both $p< 0.01$). Multiple regression analysis identified age and PRA as predictive for MSNA.

In conclusion, sympathetic hyperactivity occurs in a substantial proportion of hypertensive CKD patients. Angiotensin-converting enzyme inhibitor (ACEi) or angiotensin II receptor (ARB) treatment reduce, but not normalize MSNA.

Introduction

Angiotensin-converting enzyme inhibition (ACEi) and AngII receptor blockade (ARB) are well accepted as the cornerstones of the treatment of CKD patients, because they may help to prevent the progression of kidney failure. Chronic kidney disease (CKD) is often characterized by the presence of sympathetic hyperactivity. This may be important because of its effect on cardiovascular function and structure [1-4]. Previously we have shown that these agents reduce sympathetic outflow hyperactivity [5-7]. Because sympathetic hyperactivity might affect clinical outcome, it seems important to know its prevalence and to what extent it is normalized by ACEi and ARB. Therefore the aims of the present study were, firstly: to assess the prevalence of sympathetic hyperactivity by quantifying MSNA in a sizable group of hypertensive CKD patients in comparison with controls and to establish factors that predict sympathetic activity, and secondly: to assess the efficacy of ACEi and ARB to fully normalise sympathetic activity.

Methods

Subjects

Consecutive patients with hypertension (i.e. using antihypertensive drugs and/or blood pressure >145/90 mmHg when off medication) with stable chronic kidney disease (CKD) could enter the study. CKD was defined as a condition with persistent proteinuria and/or decreased glomerular filtration rate and/or anatomical abnormalities (in the case of polycystic kidney disease). Patients with clinically manifest heart failure, diabetics and patients on drugs influencing

sympathetic activity, such as betablockers and immunosuppressive agents were excluded.

In 74 patients with various renal diagnoses (polycystic kidney disease (41), IgA nephropathy (7), focal segmental glomerulosclerosis (2), nephrosclerosis (3) chronic glomerulonephritis (2), Alport disease (1), chronic tubulointerstial nephritis (2), analgesic nephropathy (2), obstructive uropathy (2), reflux nephropathy (3), chronic pyelonephritis (1) and chronic kidney disease of unknown cause (8)) we obtained a MSNA measurement when they were off antihypertensive medication. Renal diagnosis was made on clinical criteria and confirmed by ultrasound, other radiological procedures and/or kidney biopsy when appropriate.

Control subjects (n=82) had normal kidney function, normal blood pressure and were not on any medication.

Protocol

All of the subjects gave informed consent to participate in the study, which was approved by the institutional committee for studies on humans.

All patients were on chronic antihypertensive treatment before the study, which in all cases included an ACEi or an ARB. Patients were studied at baseline when taken off antihypertensive medication for more than two weeks. None of the patients were on other medication known to affect sympathetic activity, such as centrally acting agents. Diuretics were continued to maintain normovolemia, which was quantified by assessment of extracellular fluid volume (ECV).

A subgroup of 31 patients was studied twice, i.e. in untreated condition and during chronic treatment (at least six weeks) with ACEi or ARB. Ten patients were studied on chronic enalapril 10 mg o.d., 10 patients on losartan 100 mg o.d. and 11 patients were on eprosartan 600 mg o.d.. Controls were studied once. The order of studies was randomized, that is 15 patients were firstly studied while on chronic medication and then taken off medication and studied again, and 16 patients were first taken off medication and then studied, re-instituted on medication and studied for the second time.

The subjects underwent an identical set of measurements, in supine position in a quiet room with an ambient temperature of 22-24°C. All study sessions were done in the morning between 2 and 5 hours after drug intake. These measurements included supine blood pressure, heart rate, muscle sympathetic nerve activity (MSNA) and plasma renin activity (PRA). Blood pressure was measured in a recumbent position by an automatic oscillometric device (Accurtorr Plus, Datascope Corp, Paramus, NJ, USA). Means of three measurements are presented. MSNA was recorded with a unipolar tungsten microelectrode placed in a muscle nerve fascicle of the peroneal nerve using the technique of Wallin et al. [8] and described by us previously [6]. The correct position of the electrode is evaluated by means of a Valsalva maneuver: the patient is asked to blow into a mouthpiece of an aeroid manometer to 40 mmHg for 15s, while blood pressure (Finapress, Ohmeda, Engelwood, CO, USA), heart rate (ECG) and MSNA are continuously recorded. The sample frequency of all signals is 200 Hz. The blood pressure overshoot after the restart of breathing is associated with a short pause in neural activity. The neural signal during the blood pressure overshoot is considered to be the background noise, that is signal without electrical activity. This procedure is done at the beginning and at the end of the study session. Success rate of obtaining an adequate neural signal is approximately 85%. The heart beat intervals were measured from the ECG. After instrumentation the subjects rested for 20 minutes. Baseline measurements for blood pressure, heart rate and MSNA were obtained during approximately 15 minutes; blood was sampled for measurement of PRA.

The nerve activity was monitored on-line (software: Poly 5, Inspectors Research Systems, Amsterdam, The Netherlands) and stored on disk for off-line analysis. ECV was quantified by the bromide distribution volume as described previously and normalized for lean body mass [9,10]. The normal range in our laboratory is 273 to 334 ml/kg lean body mass (mean ±SD are 305±28 ml/kg). PRA was measured by radioimmunoassay (RIA) [11].

Data analysis

Data are mean ± SD, unless indicated otherwise. MSNA was expressed as the number of bursts of sympathetic activity per min or as the number of bursts per 100 heartbeats to correct for differences in heart rate. The best description of the relationship between age and MSNA was obtained with a quadratic regression.

Statistical methods

PRA was analyzed after logarithmic transformation. Baseline parameters analysis was performed with **S**tudent's unpaired t test between patients and healthy controls. Pearson correlations coefficients were calculated followed by stepwise linear regression when significant correlations were found. Only independent variables were included in regression analyses. The Z test was used to compare the adjusted MSNA above the mean between groups.

Statistical significance was defined as $P < 0.05$. All the analyses were performed with the statistical package SigmaStat 3.1 (Systat Software Inc).

Results

The patient characteristics are summarized in the Table. Patients were older than controls. As expected, creatinine clearance (Cockcroft-Gault method) was lower, whereas blood pressure, MSNA and PRA were higher in patients than in healthy controls. Also, MSNA expressed per 100 heart beats was higher, 47±19 versus 28±15 bursts/100 heart beats.

Multiple regression analysis in patients revealed age and PRA as predictive for MSNA (MSNA = -21.1 + 0.71 *x* age + 7.74 *x* log PRA; r^2=0.45, p<0.001). In controls only age was predictive for MSNA (MSNA = 3.94 + 0.33 *x* age; r^2 = 0.30, p=0.002). Figure 1 shows the relation between age and MSNA in control subjects and patients.

Age adjusted MSNA was in 82% of patients higher than the mean in controls, 31±9 versus 19±7 bursts/min (figure 1). PRA was higher in patients than in controls. ECV in patients was 321±33 ml/kg lean body mass.

Table. Baseline characteristics of chronic kidney disease patients and healthy controls

	Patients (n=74)	**Controls (n=82)**
Age (yr)	44 ± 12	34 ± 14*
Gender (M/F)	54 / 20	59 / 23
Body mass index (kg/m^2)	25.5 ± 3.3	24.0 ± 2.8
Creatinine clearance (ml/min per 1.73m^2)	54 ± 31	96 ± 13
Muscle sympathetic nerve activity (bursts/min)	31 ± 11	17 ± 9*
Systolic arterial pressure (mmHg)	155 ± 19	125 ± 15*
Diastolic arterial pressure (mmHg)	92 ± 11	71 ± 9*
Mean arterial pressure (mmHg)	113 ± 13	89 ± 10*
Heart rate (bpm)	65 ± 10	63 ± 9
Plasma renin activity (fmol/L/sec)	430 (20-3480)	280 (40-980)*
log Plasma renin activity (fmol/L/sec)	2.67 ± 0.36	2.40 ± 0.32*

Values are mean ± SD, except plasma renin activity [median(range)].
Arterial blood pressure represents values obtained in supine position.
* $p < 0.001$ controls versus patients.

In 31 patients a second set of measurements was done during treatment with an ACEi or an AngII receptor blocker. The reductions in blood pressure (enalapril 15±9%, losartan 15±6% and eprosartan 12±6%) and in MSNA (enalapril 20±8%, losartan 22±8% and eprosartan 23±11%) during the three treatments did not differ. Therefore in further analysis the data were taken as one group. Blood pressure (MAP 115±11 mmHg) and age adjusted MSNA (31±8 bursts/min), gender distribution (22 males) and renal diagnosis ((polycystic kidney disease (13), IgA nephropathy (4), focal segmental glomerulosclerosis (1), Alport disease (1), chronic tubulointerstial nephritis (2), obstructive uropathy (2), reflux nephropathy (3) and chronic kidney disease of unknown cause (5)) were identical to the whole group indicating that this was a representative subgroup. In this group age-adjusted MSNA was in 90% of

untreated patients higher than the mean in controls (figure 2).

Age-adjusted MSNA and MAP were reduced during treatment from 31±8 to 23±7 bursts/min and from 115±11 to 100±9 mmHg (both p< 0.01). Age adjusted MSNA was still higher in 71% of patients than the mean of controls (p<0.01) (figure 2). Heart rate was reduced during eprosartan (from 71±10 to 65±8 beats/min, p<0.05), but remained unchanged during enalapril and losartan.

The change in MSNA correlated with the MSNA in untreated condition (r=0.48, r=0.0063), but showed no relation with change in blood pressure or baseline PRA. MSNA did not correlate with ECV.

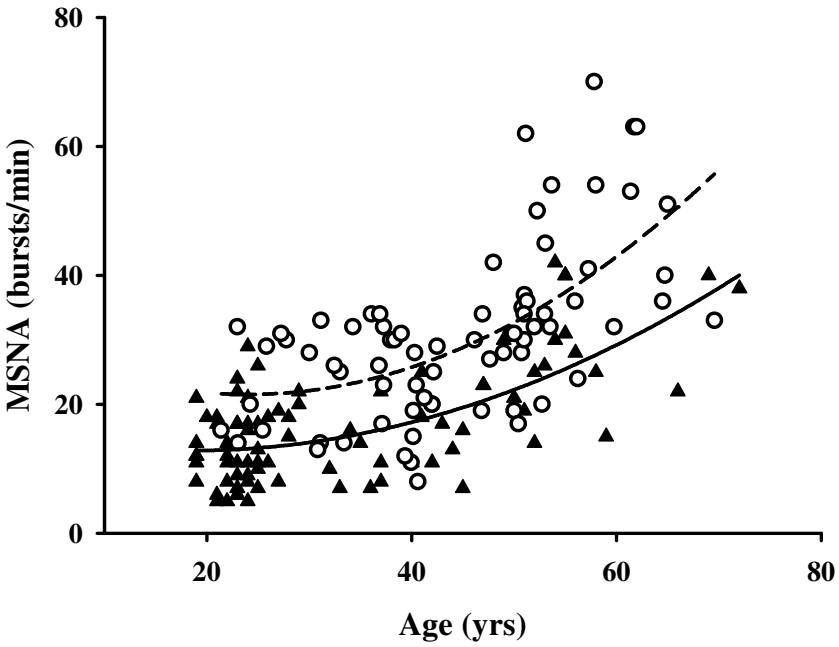

Figure 1

Relation between age and muscle sympathetic nerve activity (MSNA) for patients (when taken off antihypertensive medication) (○, dashed line; r=0.61, p<0.01) and controls (▲, continuous line; r=0.67, p<0.01)

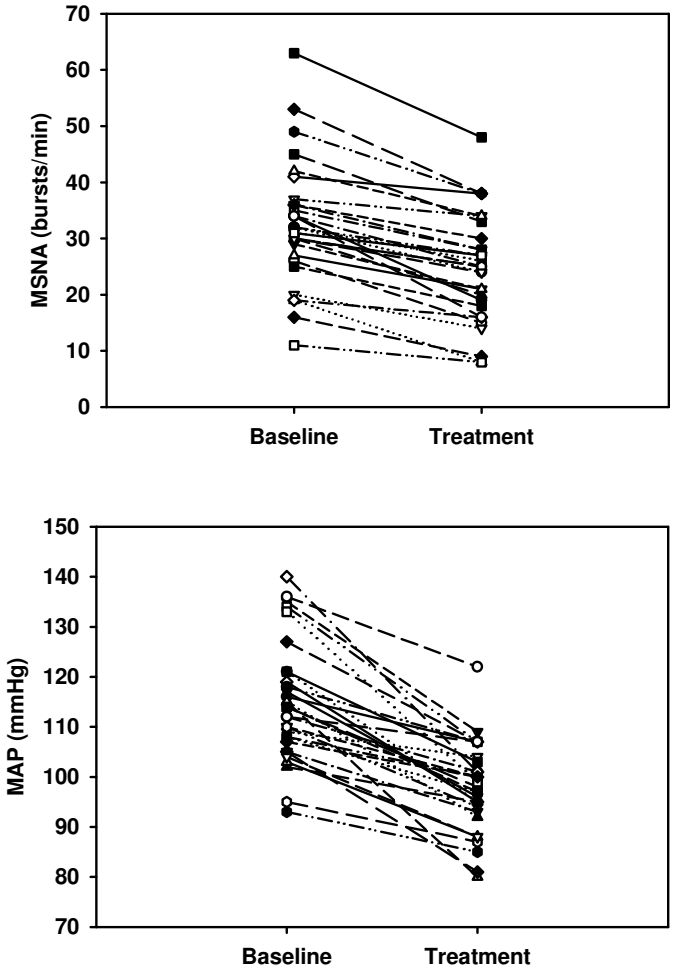

Figure 2

Individual results of muscle sympathetic nerve activity (MSNA) and mean arterial pressure (MAP) in chronic kidney disease patients (n=31) when taken off antihypertensive medication (Baseline) and during chronic treatment with an ACE inhibitor or and angiotensin II receptor blocker (Treatment).

Discussion

To the best of our knowledge, our database represents the largest available set of data on MSNA assessments in CKD patients. All measurements in patients as well as in healthy controls were done using an identical protocol. This

present study shows that sympathetic activity quantified by assessment of MSNA is increased in a substantial proportion of hypertensive CKD patients. In patients, MSNA correlates with PRA and is reduced by ACEi and ARB, suggesting that in CKD patients the activated renin-angiotensin system contributes to the pathogenesis of sympathetic hyperactivity or that the hyperactivities of the two systems share a common origin. The data indicate that chronic treatment with an ACEi or ARB does not normalize MSNA, suggesting that also other mechanisms are involved. However, we can not exclude that higher dosages of ACEi or ARB or the combination of these agents would have resulted in more profound suppression.

Three decades ago it was already shown that in CKD patients the sympathetic nervous system is activated[12-14]. Converse et al. were the first to show that MSNA, which is the centrally originated sympathetic activity directed towards the resistance vasculature is increased in hemodialysis patients[15]. The present study indicates that in a substantial proportion of hypertensive CKD patients SNA is increased. In fact, in more than 80% of patients MSNA is higher than the mean of healthy controls.

Our hypothesis is that renal ischemia is critical in the pathogenesis. The presence of sympathetic hyperactivity is not related to kidney function. Experimental studies have indicated that only minimal kidney damage without affecting function, results in hypertension of central origin[16]. The idea that kidney damage and not function is critical is strengthened by our previous findings that patients with hypertensive polycystic kidney disease with normal kidney function have increased MSNA[17] and that MSNA does not change after unilateral nephrectomy for transplantation purpose [5].

Both ACEi and ARB treatment reduced MSNA by 20-25%. In the dosage used in the present study, the effects of the various treatments did not differ. This could indicate two pathophysiologic mechanisms. Firstly, the findings may be interpreted as a support for the well established facts that AngII stimulates sympathetic activity on various levels. It increases central sympathetic outflow (which can be detected by MSNA), facilitates ganglionic transmission and

synaptic noradrenaline release by stimulation of presynaptic receptors. Secondly, the relation of the hyperactivities of the renin and sympathetic system could point at a common origin, i.e. kidney ischemia. The fact that MSNA is not normalized suggests that other mechanisms are involved as well. In this study it is not investigated whether these agents also block sympathetic activity on a peripheral level (which is not detected by MSNA measurements) and might show differences in this respect. Finally, the absence of a relation between the decrease in MSNA and blood pressure shows that part of the action of the renin system on sympathetic activity does not result in an effect on blood pressure.
An important feature of the present study is that subjects were studied when clinically normovolemic, which was evidenced by assessment of ECV. Most patients had an ECV within the normal range or only slightly increased. Previously, we have shown that hypervolemia suppresses sympathetic activity**,** parallel to PRA [5]. This indicates that normal sympathetic activity in the presence of hypervolemia should be considered abnormal.

In the population of hypertensive CKD patients of this study more than 80% had a MSNA above the mean of controls. There is substantial evidence that sympathetic hyperactivity is detrimental to the patients. The consequences of the sympathetic hyperactivity are multiple and include the pathogenesis of functional and structural cardiovascular abnormalities. It contributes to the hypertension. The finding that heart rate did not increase despite the substantial blood pressure reduction, indicates that baroreceptor set point was set on a lower level [5-7]. In fact, heart rate slightly decreased during eprosartan, whereas it remained unchanged during enalapril and losartan, which might indicate that, despite identical effects on MSNA, agents differently affect inotropic sympathetic activity.
Furthermore, there is substantial evidence that sympathetic activity also affects cardiovascular prognosis without its effect on blood pressure [1-4]. ACE inhibition or AngII receptor blocker treatment are the cornerstones of treatment of CKD patients. A recent study shows that in dialysis patients with dilated cardiomyopathy addition of carvedilol to the standard therapy regimen, which included an ACE inhibitor or AngII receptor blocker, reduces cardiovascular

morbidity and mortality as compared with placebo [18]. Another study in CKD patients, who were almost all on an ACE inhibitor or an AngII receptor blocker, suggests that the addition of moxonidine may help to slow down kidney failure progression [19]. Also in heart failure, a condition characterized by high activity of the renin and sympathetic system, the addition of a beta-blocker to standard therapy improves prognosis. It remains to be established, but it seems worth studying, whether adding a sympatholytic agent, such as moxonidine or a beta blocker, to standard treatment reduces cardiovascular risk in CKD patients. Recently, we have shown that such combination results in normalization of sympathetic hyperactivity in chronic kidney disease patients [7].

This study is limited in the sense that we have not tested higher dosages of ACEi or ARB treatment or the combination of these two types of treatment. The fact that the three treatments result in identical reductions in both blood pressure and MSNA is compatible with the idea that the maximum effect is obtained. The study indicates that a sympatholytic agent, with a mechanism other than ACEi or ARB, is needed to fully normalize blood pressure and MSNA. Also in heart failure patients, who have activated renin and sympathetic systems, MSNA was only normalized after adding clonidine to chronic treatment with an ACEi or ARB [20].

In conclusion, sympathetic hyperactivity occurs in a substantial proportion of CKD patients, which in dialysis patients, is associated with increased cardiovascular risk [21,22]. Decreasing the activity of the renin-angiotensin system by ACE inhibition or ARB treatment reduces sympathetic activity, suggesting a cause-effect relationship between these 2 effects or a common origin**,** possibly kidney ischemia.

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Chapter 6

Relation between sympathetic hyperactivity and clinical outcome in chronic kidney disease patients during standard treatment

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Abstract

Sympathetic hyperactivity is common in patients with chronic kidney disease (CKD) and has been associated with target organ damage and adverse clinical outcome. Angiotensin converting enzyme inhibitors (ACEi) and angiotensin II receptor blockers (ARB) have been shown to reduce sympathetic activity in CKD patients. The objective of the present study was to investigate whether sympathetic hyperactivity was related to clinical outcome in CKD patients treated with ACEi or ARB. Muscle sympathetic nerve activity (MSNA) was measured by microneurography in 66 non diabetic patients (70% men) with CKD, median age 45 years (range 21-65) and mean glomerular filtration rate (GFR) 50 ± 28 mL/min/1.73m2 (± SD). Patients were followed up for a median of 78 months (range 6 - 123) and subsequent clinical events were recorded.
During follow-up, average blood pressure was 131±10 mmHg systolic and 83±5 mmHg diastolic. Twenty-one events (4 deaths and 17 atherosclerotic events) occurred in 16 patients. The MSNA among the group with events was 40±18 compared to 30±11 bursts/min in those with no events during follow-up (p=0.009). An increase of MSNA of 10 bursts/min was related to an increased risk of an event (HR=1.7, 95% CI 1.2 – 2.4). Age attenuated this relation (HR=1.4, 95% CI 0.9 – 2.2). In conclusion Sympathetic hyperactivity was related to the composite of all-cause mortality and atherosclerotic events in CKD patients, despite treatment with an ACEi or ARB. This effect was partly be explained by age. This analysis warrants further studies to investigate whether additional sympatholytic therapy may be beneficial in these patients.

Introduction

Sympathetic hyperactivity has been associated with cardiovascular target organ damage and adverse clinical outcome [1-3]. Independent of blood pressure, sympathetic activity correlated with left ventricular dimensions in different patient populations [4-6]. In addition, in patients with end-stage renal disease (ESRD), sympathetic hyperactivity was predictive for both cardiovascular events and all-cause mortality [3].

Sympathetic hyperactivity is very common in patients with chronic kidney disease (CKD) [7-9]. Most likely, renal ischaemia plays a critical role in the pathogenensis of sympathetic hyperactivity in these patients [10-13]. Renal ischaemia causes activation of the renin angiotensin system (RAS), with high plasma renin activity (PRA) and elevated levels of angiotensin II (AngII). Circulating AngII stimulates the sympathetic nervous system at different levels. AngII receptors in the brainstem directly stimulate central sympathetic outflow [14]. Furthermore, AngII causes peripheral sympathetic activation by means of AngII receptors in presynaptic nerve endings [15].

In previous studies in CKD patients, not yet on renal replacement therapy, we have shown that sympathetic hyperactivity, as assessed by muscle sympathetic nerve activity (MSNA), could be reduced by 20-25% after treatment with an angiotensin converting enzyme inhibitor (ACEi) or Ang II receptor antagonist (ARB) [9, 16-18]. ACEi and ARBs are well accepted by Guideline Committees as first choice therapy in CKD, as these drugs improve both cardiovascular and renal outcome. However, despite adequate treatment with these drugs, CKD patients still have an increased cardiovascular risk.

Possibly, sympathetic hyperactivity could in part be the drive of that increased risk. We hypothesize that sympathetic hyperactivity is related to adverse clinical outcome in CKD, despite treatment with ACEi or ARBs. The present study was performed to test this hypothesis in CKD patients that had sympathetic activity quantified in the past by MSNA measurement and were subsequently treated with an ACEi or ARB.

Methods

Subjects

The study cohort was comprised of all patients with CKD from the outpatient department of the University Medical Center Utrecht in the Netherlands, who had undergone a MSNA measurement during 1995 to 2003, because of participation in one of our previous studies into mechanisms of sympathetic activity [8, 16-18]. All patients had hypertension (i.e. using antihypertensive drugs and/or blood pressure >145/90 mmHg when off medication) with stable CKD. Patients with clinically manifest heart failure, diabetics and patients on drugs influencing sympathetic activity, such as β-blockers and immunosuppressive agents were excluded. Of the 72 participants, 6 patients were lost to follow-up (age 36 ± 4 years [±SD], MSNA 16 ± 6 bursts/min). Analyses were therefore restricted to 66 subjects, who were followed up for a median of 78 months (range 6 to 123 months), and had various primary renal diagnoses, including polycystic kidney disease (32), interstitial nephritis (6), glomerulonephritis (12), congenital (3), reflux nephropathy (3), vascular disease (2), other (1), and CKD of unknown etiology (7). All patients gave informed consent to participate in the study, which was approved by the local institutional review board for studies on humans.

Baseline measurements

All patients were studied when taken off antihypertensive medication for more than two weeks. Diuretics were continued to maintain normovolemia, which was quantified by assessment of extracellular fluid volume (ECV). The patients underwent an identical set of measurements, in supine position in a quiet room with an ambient temperature of 22-24°C. All study sessions were done in the morning between 2 and 5 hours after drug intake. These measurements included supine blood pressure, MSNA, heart rate, and PRA. Blood pressure was measured in a recumbent position by an automatic oscillometric device (Accurtorr Plus, Datascope Corp, Paramus, NJ, USA). Means of three measurements are presented. MSNA was recorded with a unipolar tungsten

microelectrode placed in a muscle nerve fascicle of the peroneal nerve using the technique of Wallin et al. [19] and described by us previously [17]. Heart beat intervals were measured from the ECG. After instrumentation the subjects rested for 20 minutes. Baseline measurements for blood pressure, MSNA and heart rate were obtained; blood was sampled for measurement of PRA. The nerve activity was monitored on-line (software: Poly 5, Inspectors Research Systems, Amsterdam, The Netherlands) and stored on disk for off-line analysis. ECV was quantified by the bromide distribution volume as described previously and normalized for lean body mass [20]. The normal range in our laboratory is 273 to 334 ml/kg lean body mass. Glomerular filtration rate (GFR) was estimated by the Cockroft-Gault equation and corrected for body surface area.

Follow-up

All medical records were reviewed up to August 31 2005 to retrieve information on mortality, vascular atherosclerotic events, and dates of renal transplant or initiation of chronic haemodialysis. In addition, laboratory parameters (creatinine, total cholesterol, HDL, LDL, and triglycerides), blood pressure measurements, and use of anti-hypertensive and lipid-lowering medication were collected from the records. In 7 cases a second hospital doctor or a general practitioner was contacted to complete the database. The remaining patients were visiting our out-patient department regularly. In contrast to the baseline blood pressure measurements, the blood pressure measurements during follow-up were sitting office blood pressure measurements in treated condition. The average blood pressure during follow up was calculated by the mean of all first available blood pressure measurements each calendar year.

End-point definitions

The primary outcome was defined as the composite of all-cause mortality and atherosclerotic vascular events. Atherosclerotic vascular events were defined as: ischaemic heart disease (cardiac arrest, myocardial infarction, instable angina pectoris, coronary artery bypass graft surgery, or angioplasty),

cerebrovascular disease (cerebrovascular accident, including transient ischaemic attack, or carotid artery surgery), and peripheral vascular disease (clinically evident claudication with/without radiological evidence of arterial disease, peripheral revascularization procedure, abdominal aortic aneurysm). Clipping or coiling of cerebral aneurysms in cystic kidney disease patients were not considered as vascular events. All endpoints were evaluated and agreed upon by an independent physician blinded for the results of the MSNA measurements.

Data analysis

All variables were reported as mean and standard deviation (SD) for normally distributed data or as the median and range for non-normally distributed data. Comparisons between groups were be made by Students t-tests, Chi-square tests, or Fisher exact tests where appropriate. Correlations between MSNA and patient characteristics were evaluated by Spearman correlation analysis. Parameters that correlated with MSNA ($p<0.10$) were included into a multivariate linear regression model using a stepwise backward elimination procedure.

Survival curves were estimated by the Kaplan-Meier method and compared with the logrank test. If a patient had experienced multiple events, the survival analysis was limited to the first event. The relation between MSNA (expressed as the number of bursts of sympathetic activity per minute) and the outcome measures was analysed by Cox-proportional hazard models. The crude hazard ratio and its 95% confidence interval of MSNA on the outcome was calculated. In addition, separate hazard ratios were presented after adjustment for each potential confounder and after multivariate adjustment.

Results

Baseline

The mean age of the patients was 45 ± 12 years and 70% was male (table 1). The mean glomerular filtration rate (GFR) was 50.4 ± 28.4 ml/min per 1.73m^2. This included 8 patients with stage I kidney disease, 15 patients with stage II,

Table 1:

Baseline patient characteristics. Patients with the highest 25% MSNA measurements as compared to the remainder of the study population.

	All patients	≤75th percentile MSNA	>75th percentile MSNA	P value#
Number of patients	66	51	15	
MSNA (bursts/min)	32.2 (±13)	26.5 (±7.1)	51.6 (±9.7)	**<0.001**
Age (years)	44.9 (±12)	41.3 (±10)	56.8 (±5.3)	**<0.001**
Female	20 (30%)	17 (33%)	3 (20%)	0.52
GFR (ml/min/1.73m2)	50.4 (±28.4)	55.7 (±28.8)	32.3 (±18.0)	**0.004**
BMI	25.6 (±3.5)	25.5 (±3.6)	26.0 (±3.1)	0.60
SBP *baseline* (mmHg)	157 (±19)	157 (±20)	157 (±15)	0.98
DBP *baseline* (mmHg)	94 (±10)	94 (±11)	93 (±8)	0.73
PRA (fmol/L/sec)	589 (±457)	537 (±425)	765 (±534)	0.12
logPRA (fmol/L/sec)	2.7 (±0.34)	2.6(±0.35)	2.8(±0.32)	0.13
Heart rate (bpm)	66 (±10)	65 (±10)	68 (±10)	0.36

GFR: glomerular filtration rate (estimated by Cockroft and Gault equation corrected for body surface area); BMI: body mass index; SBP *baseline*: systolic blood pressure at baseline (untreated); DBP *baseline*: diastolic blood pressure at baseline (untreated); PRA: plasma renin activity.
MSNA >75th percentile versus ≤75th percentile

26 patients with stage III, 10 patients with stage IV, and 7 patients with stage V kidney disease. MSNA correlated with age (r=0.63, p<0.001), GFR (r=-0.42, p<0.001), male gender (r=0.28, p=0.03), and body mass index (BMI, r=0.33, p=0.008), but not with blood pressure level and heartrate. A nonsignificant trend was observed in the correlation between MSNA and PRA (log transformed). In

a multivariate linear regression model age and logPRA were independent predictors of MSNA (MSNA = -22.8 + 8.2*logPRA + 0.73*age, r^2=0.46).

We categorized the patients into 2 groups based on the MSNA measurement and compared the patients with the highest 25% MSNA values (n=15) with the other 75% (n=51). The patients in the upper quartile were significantly older (56.8 ± 5.3 versus 41.3 ± 10, p<0.001), and had a lower baseline GFR (32.3 ±

Table 2:
Patient characteristics during follow up subdivided based on baseline MSNA measurements

	All patients	<75th percentile MSNA	>75th percentile MSNA	P value#
Number of patients	66	51	15	
Events	16 (24%)	9 (18%)	7 (47%)	**0.04**
SBP *follow-up* (mmHg)	131 (±10.7)	130 (±10)	133 (±12)	0.35
DBP *follow-up* (mmHg)	83 (±6)	83 (±6)	82 (±5)	0.56
Cholesterol (mmol/L)	5.0 (±0.7)	4.9 (±0.8)	5.3 (±0.6)	0.11
Statins	39 (59%)	31 (61%)	8 (53%)	0.61
ACEi	42(65%)	31(61%)	11(73%)	0.54
ARB	18(27%)	15(29%)	3(20%)	0.74
ACEi and ARB	61 (92%)	46 (90%)	15 (100%)	0.58
β-Blockers	11 (17%)	9 (18%)	2 (13%)	0.69
Diuretics	32 (49%)	25 (49%)	7 (47%)	0.87
CCB	3 (4.5%)	2 (3.9%)	1 (6.7%)	0.55

SBP *follow-up*: average systolic blood pressure during follow-up; DBP *follow-up*: average diastolic blood pressure during follow-up; Cholesterol: total serum cholesterol; ACEi: Angiotensin converting enzyme inhibitor; ARB: Angiotensin II receptor blocker; CCB: calcium channel blocker.
MSNA >75th percentile versus ≤75th percentile

18.0 versus 55.7 ± 28.8, p=0.004). The baseline patient characteristics are summarized in *table 1*. At baseline, three patients had a positive medical history for symptomatic vascular disease.

Follow-up

Twenty-one events occurred during the study period in 16 patients. Among these events were 4 deaths (causes: sepsis, pancreatitis, malignancy, sudden death), and 17 atherosclerotic vascular events: cardiac arrest (1), myocardial infarction (4), coronary artery bypass grafting (2), cerebral vascular accident (1), transient ischemic attack (4), intermittent claudication (3), femoral bypass surgery (1), and femoral endarterectomy (1). When only the first events were considered, forty-seven percent of these events occurred in the group of patients with a MSNA within the upper quartile range (p=0.04). Blood pressure, cholesterol, and use of statins during the study period was not different in patients with a high or low MSNA at baseline (*Table 2*). During the study period 65% of the patients was taking an ACEi and 27% an ARB. In total, ninety-two percent of the patients received an ACEi or an ARB. The 5 patients without ACEi or ARB therapy were younger (age 38 ± 14 years) and had lower MSNA values (26 ± 11 burst/min) than the other patients. Furthermore, β-blockers were prescribed in 17% of the patients, diuretics in 49%, and calcium channel blockers in 4.5%. The use of these drugs were equally distributed among the patients with and without an event. Renal replacement therapy was started in 23 patients (35%) of which 18 patients started with chronic intermittent dialysis and 5 patients received a kidney transplant. One patient developed diabetes mellitus during the study period.

MSNA and clinical outcome

The patients with an event had a baseline MSNA that was significantly higher, than those who remained free from a symptomatic event (p=0.009). Furthermore, these patients were older (51.1 ± 11 versus 42.9 ± 11, p=0.01), and had higher systolic and diastolic blood pressures during follow up (systolic

136 ± 10 versus 129 ± 11, p=0.03 and diastolic 86 ± 6 versus 82 ± 6, p=0.04). The Kaplan-Meier curves in figure 1 show that the cumulative incidence of events was higher in patients above the 75th percentile MSNA (logrank: p= 0.002).

In table 3, the results of Cox regression analysis are shown. Univariate Cox regression analysis demonstrated that MSNA as a continuous variable was significantly related to the composite endpoint (HR [per 10 bursts/min increase of MSNA] 1.7, 95% CI 1.2 – 2.4, p=0.003). In addition, age (HR [per year] 1.09, 95% CI 1.0 – 1.2, p=0.005) and average systolic blood pressure during follow-up (HR [per mmHg] 1.05, 95% CI 1.0 – 1.1, p=0.02) were also related to the outcome in univariate analysis. When both MSNA and age were included into the model, the HR of MSNA decreased to 1.4 (95% CI 0.9 – 2.2, p=0.16) and the HR of age decreased to 1.05 (95% CI 1.0 – 1.1, p=0.18). Average systolic blood pressure, sex, creatinine clearance and age not substantially attenuate the hazard ratio of MSNA. Exclusion of the 5 patients that did not receive an ACEi or an ARB during follow-up did not have an effect on the results (Table 3).

Table 3.

Relationship of baseline MSNA to the occurrence of clinical events (composite of cardiovascular events and mortality) in the total cohort (n=66) and when patients that did not receive ACEi or ARB were excluded (n=61).

	All patients	Patients with ACEi or ARB
Baseline MSNA	Hazard ratio (95% CI)	Hazard ratio (95% CI)
Crude#	1.7 (1.2 – 2.4)	1.8 (1.3 – 2.6)
Adjusted for single variables:		
Age	1.4 (0.9 – 2.2)	1.4 (0.8 – 2.3)
Female sex	1.7 (1.2 – 2.5)	1.8 (1.2 – 2.7)
GFR	1.9 (1.3 – 2.9)	2.1 (1.3 – 3.2)
SBP *follow-up*	1.6 (1.1 – 2.2)	1.7 (1.2 – 2.5)
Multivariate adjustment*	1.4 (0.9 – 2.4)	1.4 (0.8 – 2.5)

MSNA as continuous variable, hazard ratios per 10 bursts/min increment of MSNA.

* Adjusted for age, female sex, GFR, SBP *follow-up.*

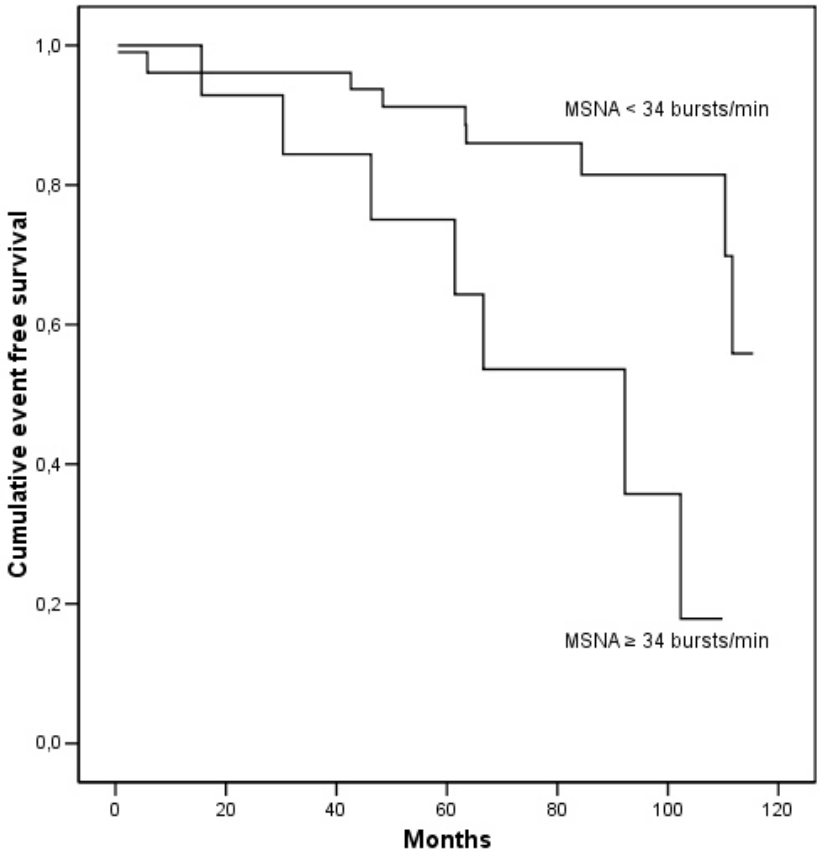

Figure 1:

Kaplan-Meier survival curves for the composite of all-cause mortality and vascular events in patients below and above the 75th percentile of baseline MSNA.

Logrank: p=0.002

Discussion

To the best of our knowlegde, this is the first study demonstrating a relation between sympathetic hyperactivity and clinical outcome in CKD patients not yet on dialysis. Importantly, this relationship existed despite of the fact that these patients were treated with an ACEi or ARB and blood pressure was reasonably

well controlled. The relationship was partially explained by the well known effect of age on sympathetic activity [3, 9, 21].

All patients were treated with the advised and commercially licensed dosages of ACEi or ARB, targeting adequate blood pressure control. However, there is increasing awareness that higher dosages of ACEi and/or ARBs are needed for complete blockade of the RAS [22, 23]. Experimental evidence has shown that the intrarenal RAS is compartmentalized from the systemic RAS [24]. Tissue ACE, and therefore renal RAS, is not inhibited by plasma concentrations of ACEi with currently used dosages. Thus, adequate dosages of ACEi or ARBs for blood pressure control may be inadequate for controlling sympathetic hyperactivity.

Of importance, there is accumulating clinical evidence that adding a second sympatholytic agent on top of standard treatment with an ACEi or an ARB, is beneficial in specific patient populations, independent of its effect on blood pressure [22, 23, 25-27]. In the COOPERATE study, 263 non-diabetic CKD patients were randomised between monotherapy with an ACEi, monotherapy with an ARB, and the combination of these drugs [26]. After follow-up, 11% of the patients on combination treatment reached the primary endpoint, ie. doubling of serum creatinine or commencing dialysis, whereas in the ACEi and ARB groups 23% of the patients reached the endpoint. These results were independent of blood pressure and could be explained by incomplete blockade of the RAS in the mono-therapy groups. In dialysis patients with congestive heart failure, all-cause and cardiovascular mortality was reduced when telmisartan was added on top of standard treatment, including ACEi or ARB and compared with placebo treatment [27]. Similarly, improved survival was observed with the 3rd generation β-blocker carvedilol in dialysis patients with dilated cardiomyopathy [25]. Interestingly, carvedilol has more pronounced sympatholytic effects than selective β-blockers such as metoprolol, probably by blocking peripheral prejunctional β-adrenergic receptors [28]. In another study, moxonidine, a central sympatholytic acting imidazoline agonist, was compared with nitrendipine in hypertensive chronic kidney disease patients [29]. After 6 months follow-up, blood pressure levels were comparable in both groups.

However, kidney function was decreased to a significantly lesser degree in the moxonidine group than in the control group. We have shown that addition of moxonidine to chronic treatment with an ARB resulted in full normalization of sympathetic activity in CKD patients [17]. Summarizing, it is conceivable that more intensive sympatholytic treatment by combining ACEi with ARBs, or by adding either a third generation β-blocker or a central acting sympatholytic agent on top of standard treatment, is superior in terms of clinical outcome. However, it is also possible that increasing dosages of ACEi or ARB monotherapy would have resulted in similar beneficial outcomes.

Finally, the present study is one of the very few series in which MSNA measurements were related to clinical outcome. Measurement of the MSNA is considered to be a very accurate technique for quantification of sympathetic nervous activity, especially because of the high intra-individual reproducibility. However, the technique is technically difficult and time-consuming and therefore not suitable for routine use in larger populations [30].

In conclusion, sympathetic hyperactivity was related to the composite of atherosclerotic events and all-cause mortality in CKD patients, despite of the fact that these patients were treated with an ACEi or ARB. This relation was partly explained by age and was independent of blood pressure. It is conceivable that additional sympatholytic therapy on top of standard treatment with an ACEi or an ARB may be beneficial in these patients. Results from clinical studies support this hypothesis. Our results warrant future studies investigating the role of additional sympatholytic therapy in the prevention of cardiovascular disease in CKD patients.

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Presentation history

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Summary and perspectives

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Sympathetic hyperactivity in chronic kidney disease: pathogenesis, clinical relevance, and treatment. Kidney Int. 2004 May;65(5):1568-76.

This thesis describes studies aimed to investigate the pathophysiological mechanisms involved in the pathogenesis of sympathetic hyperactivity in chronic kidney disease (CKD). Furthermore, we assessed the effect of sympathetic hyperactivity on long-term clinical outcome.

Chapter 1 is an introduction on the sympathetic nerve activity in CKD patients. Kidney disease is characterized by hypertension and increased cardiovascular morbidity and mortality.

There are intense interactions between the renin and the sympathetic system. Inappropriate renin secretion in relation to the state of sodium-volume balance has long been recognized [1]. Angiotensin II (Ang II) is a direct vasoconstrictor and it increases aldosteron production. There is clear evidence that Ang II enhances sympathetic activity, both at peripheral and central sites. It directly stimulates MSNA, which indicates central sympathetic activation [2]. On the other hand, sympathetic activation results in further activation of the renin-angiotensin system [3].

Sympathetic activity can be derived indirectly from sympathetic effector responses, for instance, blood pressure or heart rate. However, this is very nonspecific, because effectors may also be influenced by mechanical, chemical, and hormonal stimuli. Plasma noradrenaline is the net result of discharge, re-uptake, metabolism, and clearance, and as a consequence is not suitable as a marker for activity. Several of these limitations can be overcome by the use of sympathetic nerve recordings. True sympathetic nerve activity can be assessed by the microneurographic technique, which was developed by Vallbo et al [4]. The intraneural recording is made by a microelectrode placed in a peripheral nerve, generally the peroneal or radial nerve. With this method it is possible to measure the muscle sympathetic nerve activity (MSNA). This is a postsynaptic sympathetic nerve activity. In our studies we have used this technique.

Chapter 2 summarizes the questions addressed in this thesis.

It has long been recognized that sympathetic activity increases with age [5] and is feedback-regulated by baroreflex control and volume status [6]. In CKD patients, volume status may vary substantially. Therefore, it is critical that this should be taken into account when assessing sympathetic activity in individual patients. The study in **chapter 3** was aimed to unravel the mechanisms affecting the sympathetic nervous system in CKD patients. We studied a large group of CKD patients with various renal parenchymal diseases when in normovolemic condition, which was evidenced by assessment of extracellular fluid volume (ECFV). On average MSNA was higher in normovolemic patients than in controls. Multiple regression analysis revealed age and plasma renin activity as significant predictors for MSNA. Additionally, both patients and controls were studied in two different volume states (i.e., in patients when on and off diuretics and in controls on high and low salt diet). The relation between changes in volume and MSNA in patients parallels that in healthy subjects, but is shifted to a higher level of MSNA. This relation is very similar to that for volume and plasma renin activity. This similarity suggests a cause-effect relation or a common origin. A second aim was to investigate the effect of unilateral nephrectomy for kidney donation. The study subjects were healthy people. After nephrectomy the creatinine clearance decreased by 25% but blood pressure and MSNA remained unchanged. These results support the idea that it is renal injury an not renal failure that is responsible for sympathetic hyperactivity.

Previous studies have demonstrated that treatment with angiotensin converting enzyme inhibitor (ACEi) and angiotensin II receptor blocker (ARB) decreased sympathetic nerve system (SNS) in CKD patients. There was no normalization. Central inhibition with methyldopa and clonidine lowered blood pressure and catecholaminen in CKD patients [7] [8]. These agents have many side effects. Another and newer central sympatholytic agent called moxonidine has few adverse effects during chronic use. [9]. In a small study moxonidine seemed to be safe in patients with impaired renal function. The study in **Chapter 4** describes the effect of treatment with the ARB eprosartan and the combined therapy with eprosartan and moxonidine in hypertensive CKD patients. In earlier

studies we showed that enalapril and losartan treatment equally reduce sympathetic activity in CKD patients. [10]. Some experimental studies suggest that eprosartan has a higher sympatho-inhibiting potential than other ARB [11] [12]. In the present study eprosartan reduced blood pressure and MSNA comparably to enalapril and losartan. There was no normalization of MSNA and blood pressure. Therefore we added moxonidine to the eprosartan therapy. With this combination, blood pressure and MSNA normalized to levels comparable to healthy controls.

With the results of the previous studies in mind, the question raised how often sympathetic activity is increased in hypertensive patients with CKD. The study in **chapter 5** contains a large data set of MSNA in CKD patients and healthy controls. All measurements were done with the same protocol. More than 80% of the hypertensive patients had a MSNA higher than the mean in healthy controls. Chronic ACEi or ARB therapy reduced the sympathetic activity but did not normalize it. The fact that these treatments both decrease the activities of the renin angiotensin system and the sympathetic activity, suggests a cause and effect relationship between these two systems or a common origin.
There are several possible pathophysiologic mechanisms through which kidney injury can result in increased activities of the renin and sympathetic system.

First: Inappropriate renin secretion in relation to the state of sodium-volume balance has long been recognized [1]. The renin output of the kidney is the sum of the production of all nephrons. Nephrons of the diseased kidneys are not affected equally and do not secrete equal amounts of renin. Severely affected nephrons, hypofilter, and show impaired sodium excretion and renin hypersecretion and less affected nephrons will adapt to the elevated blood pressure by hyperfiltering and suppressing renin secretion. Blood pressure will not be high enough to suppress renin production in all nephrons.
Second: Renal ischemia can lead to sympathetic activation. During renal ischemia, adenosine is released. This adenosine evokes an increase in afferent renal nerve traffic, as can be shown during adenosine infusion in the renal artery of uninephrectomized dogs [13]. In rats, induction of renal artery stenosis

[14], partial renal ablation by arterial ligation [15] or intrarenal phenol injection [16] cause excitation of the renal afferent nerves, which results in neurogenic hypertension. Even a small injury in one kidney which does not affect the glomerular filtration rate (GFR), leads to hypertension in association with an increased central sympathetic activity [17]. In these animal models, renal denervation results in a reduction or total prevention of hypertension. Additionally, in the phenol hypertension model, nephrectomy of the injured kidney several weeks after the induction of renal damage results in normalization of blood pressure [18]. Thus, renal injury can lead to sympathetic hyperactivity and hypertension which is associated with activation of renal afferent nerves. The signal from the diseased kidneys goes through the afferent renal nerves to the central nervous system.

And third: In animals, the sympathoexcitatory effect of nitric oxide inhibition has been clearly demonstrated during systemic administration of nitric oxide synthesis inhibitors and is greatly attenuated by sympathectomy or by renal denervation [19] [20]. Basal activity of central sympathetic activity is inhibited by central nitric oxide production [16]. Nitric oxide synthesis inhibition by l-arginine analog N-nitro-l-arginine methylester (L-NAME) results in an increase of central sympathetic activity. The overall conclusion of experimental studies is that nitric oxide has sympathoinhibitory and vagotonic effects with attenuation of cardiovascular end-organ responses, acting both on central and peripheral mechanisms (review in [21]). In vivo and ex vivo animal experiments have provided evidence that neuronal nitric oxide is a major component of the signal transduction pathway involved in the tonic restraint of central sympathetic outflow [22]. In rats with CRF some specific brain areas (i.e., posterior hypothalamic nuclei and the locus coeruleus), which are involved in blood pressure regulation by their effective sympathetic outflow, show a greater turnover rate of norepinephrine, than control rats [23].

In the phenol renal injury model neuronal nitric oxide synthase (nNOS)-mRNA expression in brain-areas involved in noradrenergic control of blood pressure is decreased as compared to controls. Intravenous administration of losartan

results in an increase of the abundance of nNOS-mRNA in these brain nuclei [24]. These studies suggest that stimulation of the central sympathetic nervous system activity by renal afferent impulses may be mediated by local activation of Ang II, which stimulates central sympathetic outflow by inhibition of NOS-mRNA abundance. The sympatho-inhibitory effect of intravenously administered losartan is mediated by blockage of local Ang II, resulting in an up-regulation of NOS-mRNA expression.

Based on the pathophysiological considerations outlined above, it seems logical that an ACEi or an ARB reduces MSNA. Indeed, enalapril, losartan and eprosartan decrease blood pressure and MSNA in CRF patients [25] [26] (Chapter 3). In the dosages used in our studies, the drugs are equally effective in both their blood pressure lowering and MSNA-inhibiting effect. This is in contrast to amlodipine, which reduces blood pressure but increases MSNA [25]. These studies provide evidence in humans that centrally located Ang II importantly contributes in the pathogenesis of this form of hypertension, thereby confirming the experimental data outlined above.

As described earlier in the general introduction sympathetic hyperactivity seems to be an important risk factor for cardiovascular (CV) events [27] [28]. Until now there are hardly any data on the long term effect of sympathetic hyperactivity on cardiovascular outcome in CKD patients. In **chapter 6** we study the relationship between MSNA and clinical outcome in CKD patients. CV morbidity and mortality were followed in patients who were on standard treatment with an ACEi or ARB for a median of 78 months. Despite of the positive effect of ACEi and ARB on blood pressure and MSNA, the sympathetic hyperactivity predicted poor clinical outcome. Part of this effect was explained by the effect of age on sympathetic activity. We showed that the patients in the highest quartile of MSNA had the poorest clinical outcome during follow up. This relation existed independently of the blood pressure level and despite of the fact that all patients were treated with an ACEi or an ARB and blood pressure was adequately controlled. These findings warrant detailed analysis of these patients and

subsequently the exploration of the possible benefit of additional treatment on clinical end points.

So, we are only partially successful in reducing CV morbidity and mortality in CKD patients.

A possible explanation is the incomplete blockage of RAS by ACEi. It was found that in ACEi treated rats angiotensin concentrations in renal interstitial fluid were higher than in plasma. [29]. Apparently, our standard dosages of ACEi and ARB were not sufficient to fully block the intrarenal renin system.

Future perspectives

In conclusion, this thesis presents relevant information on the pathophysiology of sympathetic hyperactivity and hypertension in CKD patients. Despite of the positive effect of ACEi and ARB on blood pressure and MSNA the risk for CV morbidity and mortality is still high in CKD patients. Sympathetic hyperactivity contributes to the hypertension. There is evidence that also independent of its effect on blood pressure, it is important in the pathogenesis of cardiovascular organ damage. ACE inhibitors and ARB reduce, but do not normalize, sympathetic activity, as assessed by MSNA.
It is known that hypertension is associated with increased CV risk. When there is also evidence of increased left ventricle mass (LVM), this risk substantially increases above and beyond the risk of hypertension alone. Increased LVM is often present and is identified to have a strong negative prognostic impact in patients with CKD [30] [31] [32] [33]

The prevalence of left ventricular hypertrophy (LVH) or increased LVM varies with the population and the method used. In hypertensive patients visiting a cardiology clinic 37% had elevated LV mass. [34]. In CKD patients, not yet on dialysis, LVH is present in up to 30% [31]. In dialysis patients LVH varies between 52% and 75% [35] [36] depending on the investigation method and the time patients were on dialysis. In a very recent study in end stage renal disease (ESRD) patients 28% had signs of myocardial fibrosis which was associated with LVH [37]. Until now such data does not exist for CKD patients not on dialysis but the morbidity is possibly high too because most of the CKD patients have hypertension.

The pathogenesis of LVH is multifactorial, including blood pressure, anemia, volume expansion and hyperparathyroidism. Sympathetic hyperactivity most likely contributes importantly. Several studies showed relations between sympathetic hyperactivity and the presence and/or degree of increased LVM [38] [39] [40] [41] [42]. Given the intense RAAS and sympathetic interactions, it is difficult to separate the effects of these systems. A recent study suggests that

the sympathetic hyperactivity more closely relates with LVM than the renin system [43]. Also in CKD patients, a relation between the risk of having LVH and sympathetic hyperactivity was reported [44]. High plasma noradrenaline level was predictive for increased mortality and CV morbidity and mortality [44]. Experimental evidence relates the sympathetic nervous system with altered cardiac structure [27]. In experimental animal models of cardiac hypertrophy, sympathetic hyperactivity parallels LVH [45]. Noradrenaline infusion into the systemic circulation, even when devoid of any pressor effect, is capable of increasing LV weight, myocyte cross-sectional area and nucleic acid synthesis from myocardial tissue [46] [47] [48].
In conclusion, there is a large set of both experimental and clinical evidence relating sympathetic overactivity and increased LV mass.

Treatment in CKD patients should be aimed at correcting the abnormalities, likely to contribute to the impaired prognosis. ACEi and ARB in presently recommended dosages reduce but do not normalize MSNA. After the addition of the centrally acting sympatholytic agent moxonidine both MSNA and blood pressure were normalized. ACEi and ARB combined with diuretics (or fluid ultrafiltration in case of dialysis patients) are now considered “standard” treatment. In ESRD patients ACEi treatment is particularly effective in reducing LVH [49] [50] [51] [52]. In CKD patients both on and not yet on hemodialysis, ACEi use is associated with reduced mortality [53] [54]. On the other hand, USRDS data suggest that the use of beta-blockers is associated with improved survival [55]. There is increasing evidence that efficacy of treatment can be improved with respect to effects on relevant clinical endpoints. In dialysis patients with dilated cardiomyopathy the addition of carvedilol to standard treatment (including an ACEi or ARB) reduces CV morbidity and mortality as compared to placebo [56]. The same group showed that in patients on haemodialysis with heart failure the addition of an ARB to ACEi treatment reduces CV mortality and morbidity [57].
The addition of moxonidine to standard treatment in CKD patients (mean creatinine clearance 28 ml/min) reduces kidney function loss more effectively than a calcium channel blocker [58].

So, there is evidence, that standard treatment with either an ACEi or an ARB can be improved, and that “intensified” treatment might improve prognosis.

Defining the “optimal” dosage of ACEi or ARB is more difficult than previously thought. Presently advised and commercially licensed maximal dosages are based on the blood pressure lowering potential of the agents. However, experimental evidence has shown that the intrarenal RAAS is compartmentalized from the systemic RAAS [29]. Tissue ACE, and therefore renal RAAS, is not inhibited by plasma concentrations of ACEi with currently used dosages. Comprehensive reviews of combining therapies are summarized elsewhere [59] [60]. Dual therapy, i.e. combining ACEi and ARB, usually results in more reduction in blood pressure and proteinuria [60]. In a recent study an ACEi and an ARB were adjusted to reach identical blood pressure values in all treatment (ACEi, ARB and combi) [61]. The risk of reaching an end point (i.e. ESRD or doubling of serum creatinine) was reduced during combination therapy as compared to the respective mono-therapies. No “official” guidelines exist with respect to the use of dual therapy. However, given the importance of the diseased kidney in the pathogenesis of the sympathetic hyperactivity, it is attractive to hypothesize that more “intense” inhibition of the RAAS will result in more effective inhibition of sympathetic activity and that this will translate in an improvement of relevant clinical endpoints.

So, we are only partially successful in reducing CV morbidity and mortality in CKD patients. Given the evidence outlined above, it seems that sympathetic hyperactivity is (considerably) involved in the pathogenesis of CV morbidity and mortality and that more effective inhibition of this sympathetic hyperactivity might result in a meaningful improvement of efficacy of treatment.

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Samenvatting in het Nederlands

Men schat dat in 2005 ongeveer 40.000 mensen in Nederland een nieraandoening hadden. Hiervan waren ruim 5500 patiënten afhankelijk van dialyse. Voor dialysepatiënten maar ook voor patiënten die (nog) niet dialyseren betekent dat vaak een grote impact op hun leven en een verminderde kwaliteit van leven. Nierziekte gaat in het algemeen gepaard met tal van complicaties.
Nierpatiënten hebben vaak een te hoge bloeddruk (hypertensie). Hypertensie is een risicofactor voor hart- en vaatziekten en de achteruitgang van de nierfunctie. Vroeger dacht men dat vooral het teveel aan zout en vocht in het lichaam bij deze patiënten de oorzaak was van de hypertensie. Uit onderzoek bleek dat de sympathische zenuwactiviteit een belangrijke rol speelt.
Onderwerp van dit proefschrift is de pathofysiologie van verhoogde sympathische activiteit bij nierpatiënten te onderzoeken door o.a. farmacologische interventies en het effect op de lange termijn prognose vast te stellen.

Hoofdstuk 1 is een inleiding tot het onderwerp. Patiënten met hypertensie hebben in het algemeen een verhoogde concentratie renine in het bloed. Deze stof is onderdeel van een regulatiesysteem. het renine angiotensine systeem (RAS). Renine wordt door speciale cellen in de nieren uitgescheiden. Dit gebeurt wanneer de bloeddruk of de doorstroming van de nieren daalt of de zoutconcentratie in het bloed te laag is. Ook een verhoogde sympathische activiteit zorgt voor een grotere afgifte van renine. Renine is een enzym dat wederom een stof splitst. Via een aantal stappen wordt uiteindelijk het eindproduct angiotensine II gevormd, een hormoon. Angiotensine II stimuleert de afgifte van aldosteron dat wederom ervoor zorgt dat minder zout en water door de nieren wordt uitgescheiden.
Angiotensine II heeft nog meer eigenschappen. Het leidt tot sterke samentrekking van bloedvaten (vasoconstrictie) waardoor de weerstand in de bloedvaten stijgt. Het gevolg hiervan in de nieren is een verminderde uitscheiding van water en zout in de urine. De verhoogde weerstand in de bloedvaten buiten de nieren heeft tot gevolg dat de bloeddruk stijgt. Verder

wordt aan angiotensine II de eigenschap toegeschreven dat het de groei van de vaatwandcellen bevordert wat de aanzet tot aderverkalking kan zijn.
Het renine, aldosteron en angiotensine II zorgen met wat andere stoffen en regelmechanismen dat de bloeddruk en de water- en zouthuishouding in evenwicht blijven. Het sympathische zenuwstelsel heeft invloed op angiotensine II. Tegelijkertijd heeft angiotensine II ook invloed op de sympathische zenuwactiviteit.
Het sympathische zenuwstelsel maakt deel uit van het onwillekeurige zenuwstelsel. Samen met het parasympathische zenuwstelsel zorgt dit systeem voor automatische aanpassingen van allerlei systemen en organen op veranderingen in het lichaam en veranderingen van buitenaf. De hartslag en de bloeddruk kunnen zo bijvoorbeeld automatisch en binnen enkele seconden aangepast worden. Als men vanuit rust gaat inspannen, bijvoorbeeld opstaat of gaat lopen gaat de hartslag omhoog. Ook de pupilreactie op licht en de toename van ademfrequentie, hartslag, afname van darmperistaltiek in een stresssituatie zijn hier voorbeelden van. Het sympathische systeem is een belangrijk regulatiesysteem. Het bestaat uit een centraal controlecentrum in de hersenen en uit perifere aanvoerende en afvoerende zenuwbanen.
De in de hersenen gelegen centra genereren de centrale sympathische activiteit. D.m.v. een complex regulatiesysteem worden hier alle signalen vanuit het lichaam verwerkt en worden de passende signalen (sympathische activiteit) uitgezonden. Uit onderzoek bij dieren is gebleken dat door een verhoogde angiotensine II concentratie bij deze centra de centrale sympathische activiteit toeneemt. In het bloed circulerend angiotensine II kan bij deze controlecentra in de hersenen komen.

Bij ander onderzoek met dieren is gebleken dat de sympathische activiteit vanuit de nieren via de perifere zenuwbanen direct de hersenen tot een hogere centrale sympathicusactiviteit aan kan zetten. De nier zet waarschijnlijk door beschadigd nierweefsel aan tot verhoogde sympathische activiteit. Men schrijft dit enerzijds toe aan verhoogde angiotensine II spiegels en anderzijds aan zenuwimpulsen via directe zenuwbanen vanaf de nieren naar de hersenen. Dit laatste is gezien bij dierexperimenten waarbij deze zenuwbanen waren

doorgesneden. De sympathische activiteit was bij deze dieren niet verhoogd ondanks nierziekte.
De nier zelf blijkt door een verhoogde sympathische activiteit meer schade op te gaan lopen. Dit wordt toegeschreven aan verhoogde concentratie noradrenaline dat vrijkomt bij sympathische zenuwactiviteit.

De sympathische activiteit is bij nierpatiënten verhoogd t.o.v. controles. Bij patiënten met hartfalen wordt ook een verhoogde sympathische activiteit gevonden en geassocieerd met een slechtere prognose. Patiënten met een nierziekte hebben een veel grotere kans op het verkrijgen van hart en vaatziekten dan gezonde mensen. Voor hen is de verhoogde sympathische activiteit dus ongunstig.

In het laatste deel van de 'general introduction' worden de verschillende manieren uitgelegd hoe de sympathische activiteit te meten is. Bij de signaaloverdracht wordt de stof noradrenaline gebruikt die in principe in urine en bloed te meten is. Dit zijn echter geen secure meetmethoden. Bij de studies in dit proefschrift hebben wij gebruik gemaakt van de zogenaamde microneurografie waarbij de 'Muscle sympathetic nerve activity' (=MSNA) gemeten wordt. Dit is de sympathische activiteit die de spiercellen in de bloedvaten aanstuurt en verantwoordelijk is voor de actuele bloeddruk. Bij deze meetmethode wordt een zeer dunne naald (elektrode) in de peroneuszenuw ingebracht. Deze zenuw ligt dicht onder de huid aan de zijkant van de knie en bevat verschillende zenuwvezels waaronder ook sympathische zenuwvezels.
Microneurografie is geen eenvoudige onderzoeksmethode. Het plaatsen van de elektrode kan soms onaangename zenuwprikkeling veroorzaken. Deze methode is alleen geschikt voor onderzoeksdoeleinden. De resultaten zijn daarentegen betrouwbaar en goed reproduceerbaar.

In de eerste studie (chapter 3) hebben wij MSNA onderzocht in nierpatiënten en controles onder twee condities. Eerst zijn beide groepen onderzocht met normaal vochtgehalte in het lichaam en bij de tweede keer met een teveel aan vocht. MSNA was bij de patiënten hoger dan bij de controls. Bij beide groepen

steeg de MSNA in gelijke mate na het verhogen van het vochtgehalte in het lichaam. Bij deze studie onderzochten wij nog een groep gezonde nierdonoren bij wie de nierfunctie met 25% was verminderd. MSNA was voor en na de nierdonatie onveranderd. Deze studieresultaten wijzen erop dat MSNA in nierpatiënten onevenredig verhoogd is. Verder lijkt het erop dat ziek nierweefsel gerelateerd is aan een verhoogd MSNA en niet aan een verminderde hoeveelheid maar overigens gezond nierweefsel (nierdonoren).

In de tweede studie (chapter 4) wilden wij onderzoeken of het mogelijk was om de MSNA in nierpatiënten te normaliseren. De patiënten waren in eerste instantie behandeld met een middel dat het angiotensine II (Eprosartan) blokkeert. Dit was met soortgelijk onderzoeken eerder gedaan. Hierdoor werd de MSNA niet gelijk aan die van vergelijkbare controles. In onze studie hebben wij aan het eprosartan een centraal werkend medicijn (Moxonidine) tegen hoge bloeddruk toegevoegd. Moxonidine is een sympatholyticum, dat wil zeggen een middel dat sympathiscus activiteit vermindert. Met het toevoegen van moxonidine aan eprosartan werd er normalisatie van MSNA bereikt.

Microneurografie is zoals eerder vermeld geen eenvoudige onderzoeksmethode. Er zijn zover wij weten geen grote groepen nierpatiënten (niet dialyserend) onderzocht. In onze derde studie (chapter 5) hebben wij de MSNA van een grote groep van 74 patiënten geanalyseerd. Hieruit bleek dat in een substantieel deel van deze patiënten MSNA verhoogd was t.o.v. de gezonde controle personen. Verder waren de leeftijd en de renine activiteit voorspellend voor een verhoogde MSNA. Uit dit onderzoek bleek ook dat alleen een medicijn dat het angiotensine II of een voorstadium daarvan blokkeert niet voldoende is om MSNA te normaliseren. Dit zou kunnen bijdragen aan het hoge risico op hart en vaatziekten bij nierpatiënten.

Hierover ging ook ons volgende onderzoek. Tot nu bestaan er zover wij weten geen studies waarbij nierpatiënten met een aangetoond verhoogd MSNA voor een langere tijd zijn gevolgd op complicaties. In de vierde studie (chapter 6)

hebben wij een poging ondernomen een langere termijn analyse te maken. 66 nierpatiënten waren in mediaan 78 maanden gevolgd. Hieruit bleek dat patiënten met een hoger MSNA ook een hogere kans hadden op complicaties van hart en vaatziekten (bijv. hartinfarct, beroerte etc.) en overlijden. Die relatie bestond ondanks het feit dat de patiënten werden behandeld. De conclusie zou dus kunnen zijn dat de behandeling onvoldoende is.

Zusammenfassung in deutscher Sprache

Man schätzt, dass in den Niederlanden einer von 200 Erwachsenen (=60.000) eine verborgene Nierenschädigung hat. Ungefähr 40.000 Menschen sind bekennt mit einer Nierenbeschädigung. Gut 5.500 davon sind abhängig von der Dialyse. In Deutschland hat man vergleichbare Zahlen. Hier werden 45.000 Dialysepatienten behandelt. Patienten mit verminderten Nierenfunktion oder Nierenerkrankungen haben häufig eine eingeschränkte Lebensqualität. Sie haben ein größeres Risiko für Nebenwirkungen, sowie für Gefäßverkalkungen und Herzerkrankungen.

Nierenpatienten haben meistens einen hohen Blutdruck. Früher dachte man, dass der hohe Blutdruck durch eine Überwässerung des Körpers verursacht wird. Aufgrund von Forschungsergebnissen weiß man inzwischen, dass die sympathische Nervenaktivität eine wichtige Rolle darin spielt. Nierenpatienten besitzen meistens eine hohe sympathische Aktivität (Überaktivität).

Diese These enthält Studien über die Pathophysiologie und die Langzeitprognose bei Nierenpatienten mit Überaktivität von der sympathischen Nervenaktivität.

Das erste Kapitel ist eine Einleitung zum Thema. Nierenpatienten verzeichnen erhöhte Reninwerte im Blut. Renin ist ein Stoff, der durch die Nieren ausgeschieden wird, wenn die Durchblutung von den Nieren vermindert oder die Salzkonzentration im Blut zu niedrig ist. Renin löst eine Kaskade von Reaktionen aus. Letztendlich wird das Hormon Angiotensin II gebildet. Angiotensin II reguliert zusammen mit anderen Stoffen und Regelmechanismen den Salz- und Wasserhaushalt und den Blutdruck im Körper. Angiotensin II ist ein starker Gefäß-Verenger (Vasokonstriktion); auch hat es die Eigenschaft, die Zellen in den Blutgefäßwänden zum Wachstum zu stimulieren, was zur Aderverkalkung führen kann. Angiotensin II ist von großer Bedeutung. Zusammen mit oben erwähnten Stoffen und Regelmechanismen ist es verantwortlich für die Aufrechterhaltung des Blutdruckes und die Regelung des Wasser-Elektrolyt-Haushaltes. Angiotensin II in Übermaßen kann schädlich sein. Angiotensin II wird beeinflusst von der sympathischen Aktivität und gleichzeitig beeinflusst die sympathische Aktivität das Angiotensin II.

Der Sympathikus ist neben dem Parasympathikus ein Teil des vegetativen (=autonom) Nervensystems, Regulation erfolgt ohne bewusste Wahrnehmung.

Das vegetative Nervensystem steuert lebenswichtige Vorgänge. Der Sympathikus sorgt zum Beispiel dafür, dass das Herz schneller schlägt, wenn man in Bewegung kommt oder dass die Pupillen kleiner werden, wenn Licht ins Auge scheint.

Das sympathische Nervensystem besteht aus einem zentralen Kontrollzentrum im Gehirn, sowie peripheren Nervenbahnen in den Organen. Die Nieren haben ein großes Netzwerk von herausführenden und hinzuführenden sympathischen Fasern. In beiden Systemen werden sympathische Impulse empfangen und ausgesendet. Ein komplexes Regulationssystem im Gehirn verarbeitet einkommende Signale und sendet entsprechende sympathische Impulse zu den entsprechenden Organen. Tierexperimentelle Studien haben gezeigt, dass Angiotensin II im Gehirn die Ausströmung von sympathischen Impulsen steigert. Weiterhin hat man aus Experimenten schließen können, dass bei Nierenversagen die sympathische Nervenaktivität zunimmt. Hierdurch nimmt die Ausscheidung vom Angiotensin II zu und wird via direkter Nervenbahnen die zentrale sympathische Nervenaktivität erhöhen. Letzteres schließt man aus Tieruntersuchungen, bei denen Nieren geschädigt waren. Erhöhte sympathische Nervenaktivität wurde festgestellt, aber bei gleichzeitig durchgeschnittenen Nervenbahnen normalisierte sich die sympathische Nervenaktivität.

Aus Studien bei Patienten mit Herzbeschwerden wurde eine erhöhte sympathische Nervenaktivität bekannt. Patienten mit einer Überaktivität hatten eine schlechtere Prognose. Der sympathischen Überaktivität wird auch ein Progressionsfaktor für den fortschreitenden Verfall der Nierenfunktion zugeschrieben.

Man kann sympathische Aktivität indirekt messen durch den Gehalt von Noradrenalin im Blut oder durch ausgeschiedenes Noradrenalin in Urin. Diese Methoden sind allerdings nicht zuverlässig. Bei den Studien in diesem Buch haben wir die sympathische Nervenaktivität mit Microneurographie gemessen. Hierbei misst man die muskelsympathische Nervenaktivität (MSNA) mit einer dünnen Microelectrode in direkt unter der Haut liegenden Nervenbahnen. Diese Nervenaktivität steuert die Funktion der Muskelzellen in den Blutgefässen,

wodurch der Blutdruck reguliert wird. Diese Meßmethode ist kompliziert, hat sich aber als sehr zuverlässig erwiesen.

In der ersten Studie (Chapter 3) haben wir die MSNA bei Nierenpatienten und gesunden Freiwilligen, im normalen Zustand und bei Überwässerung, gemessen. In beiden Gruppen stieg die MSNA in gleichem Maße, war aber doch höher in der „Patientengruppe". Außerdem haben wir bei lebenden und gesunden Nierenspendern (eine Niere gespendet), nach der Operation, eine verminderte Nierenfunktion festgestellt, wobei jedoch die MSNA gleich geblieben war. Aus beiden Resultaten können wir schließen, dass beschädigtes Nierengewebe eine Erhöhung von MSNA verursacht, jedoch keine der Nierenfunktion.

In der zweiten Studie (Chapter 4) haben wir Nierenpatienten mit einem Medikament behandelt, das Angiotensin II blockiert. Hierdurch nahm MSNA ab, aber normalisierte sich nicht im Vergleich mit gesunden Freiwilligen. Nach der Zugabe eines weiteren Medikamentes, welches zentral sympathische Rezeptoren blockieren kann, normalisierte sich die MSNA.

In der dritten Studie (Chapter 5) haben wir bei einer größeren Anzahl Nierenpatienten MSNA analysiert. Da die Untersuchungsmethode kompliziert ist, gibt es bisher keine großen Studien von dieser Patientengruppe. Wir haben festgestellt, dass bei einem großen Teil die MSNA höher ist als bei gesunden Personen. Mit Hinblick darauf, dass eine hohe sympathische Nervenaktivität eine schlechte Prognose bei Herz- und Gefässerkrankungen haben kann, ist dieses ein wichtiges Resultat. Bisher ist bei diesen Nierenpatienten nicht untersucht worden, ob erhöhte sympathische Nervenaktivität die Langzeitprognose auf Herz und Gefässerkrankungen beeinflusst.

In der vierten Studie (Chapter 6) haben wir mit unseren Erkenntnissen weitere Untersuchungen durchgeführt. Hierbei wurden Patienten langzeitig medizinisch beobachtet, (in median 78 Monate). Es zeigte sich: je höher die MSNA, desto höher das Risiko für Patienten, kardiovaskuläre Komplikationen zu bekommen.

Aus unseren Studien dürfen wir folgern, dass wir die Entstehung von hohem Blutdruck bei Nierenpatienten besser verstehen. Die üblichen medikamentösen

Behandlungen sind nicht ausreichend, um die sympathische Nervenaktivität zu normalisieren. Nach neuen Möglichkeiten muss mit dem Ziel geforscht werden, das kardiovaskuläre Risiko bei Nierenpatienten zu vermindern.

List of publications

List of publications

Neumann J, Ligtenberg G, Klein IH, Blankestijn PJ
Pathogenesis and treatment of hypertension in polycystic kidney disease. Curr Opin Nephrol Hypertens. 2002 Sep;11(5):517-21

Klein IH, Ligtenberg G, **Neumann J**, Oey PL, Koomans HA, Blankestijn PJ
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Sympathetic hyperactivity in hypertensive chronic kidney disease patients is reduced during standard treatment. Hypertension 2007 Mar;49(3):506-10. Epub 2007 Jan 15.

E. Lars Penne **Jutta Neumann**, Inge H. Klein, P. Liam Oey, Michiel L. Bots, Peter J. Blankestijn
Relation between sympathetic hyperactivity and clinical outcome in chronic kidney disease patients during standard treatment. Submitted.

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Curriculum vitae

Jutta Neumann werd op 24 juli 1964 geboren te Oldenburg (Duitsland). In 1985 behaalde zij haar ‘Abitur’ aan het ‘Altes Gymnasium’ te Oldenburg. Wegens uitloting voor de studie Geneeskunde werd in hetzelfde jaar begonnen met studie scheikunde aan de Universiteit te Göttingen (Duitsland). In 1986 kreeg zij de mogelijkheid om de aan de Vrije Universiteit te Amsterdam te starten met de studie geneeskunde. In verband met het stichten van een gezin werd de studie voor een deel parttime gevolg. Het doctoraal werd behaald in 1993 en het artsexamen op 23 mei 1997. Hierna was zij 4 jaar werkzaam als AGNIO interne, cardiologie, longziekten, en als dialyse arts in diverse ziekenhuizen (Bovenij Ziekenhuis te Amsterdam, Rode Kruisziekenhuis te Beverwijk en het Medisch Centrum Haaglanden te Den Haag. Van 2001 tot 2004 was zij werkzaam als artsonderzoeker werkzaam bij de vakgroep Nefrologie van het Universitair Medisch Centrum Utrecht, alwaar het in dit proefschrift beschreven onderzoek is uitgevoerd. Op 1 maart 2005 is de auteur van dit proefschrift begonnen met de specialisatie tot huisarts aan de Vrije Universiteit te Amsterdam. Deze opleiding zal zij op 1 september van dit jaar voltooien.

List of abbreviations

ACE	angiotensin converting enzyme
ACEi	angiotensin converting enzyme inhibitor
Ang II	angiotensin II
ARB	angiotensin II receptor blocker
BMI	body mass index
BP	blood pressure
CV	cardiovascular
CKD	chronic kidney disease
CKF	chronic kidney failure
DBP	diastolic blood pressure
ECV	extracellular fluid volume
ESRD	en stage renal disease
GFR	glomerular filtration rate
HR	heart rate
LVH	ventricular hypertrophy
LV(M)	left ventricle (mass)
MAP	mean arterial pressure
MSNA	Muscle sympathetic nerve activity
PKD	polycystic kidney disease
PRA	plasma renin activity
SBP	systolic blood pressure
SNS	sympathetic nervous system
RAAS	renin angiotensin aldosteron system
RVL	rostral ventrolateral nucleus
RVML	rostral ventrolateral medulla