Effects of Low Dose Sympathetic Inhibition on Glomerulosclerosis and Albuminuria in Subtotally Nephrectomized Rats

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Abstract. A potential role of the sympathetic nervous system in progression of renal failure has received little attention. This study examined whether nonhypotensive doses of moxonidine, an agent that reduces sympathetic activity, affects glomerulosclerosis, urine albumin excretion, and indices of renal handling of norepinephrine (NE) in subtotally nephrectomized (SNX) rats. Sprague Dawley rats were SNX or sham-operated (control). SNX rats were either left untreated or treated with moxonidine in a dose (1.5 mg/kg body wt per d) that did not modify telemetrically monitored 24-h BP. Glomerular and renal morphology were evaluated by quantitative histology, immunohistochemistry, and in situ hybridization. Urine albumin excretion rate was analyzed by enzyme-linked immunosorbent assay, and kidney angiotensin II and NE content were measured using HPLC,3 H-NE uptake, and release. Body and kidney weight and BP were not significantly different between SNX with or without moxonidine. The glomerulosclerosis index was significantly lower in moxonidine-treated (0.88 ± 0.09) compared with untreated (1.55 ± 0.28) SNX rats, as was the index of vascular damage (0.32 ± 0.14 versus 0.67 ± 0.16). The number of proliferating cell nuclear antigen-positive glomerular and tubular cells per area was significantly higher in untreated SNX rats than in controls and moxonidine-treated SNX rats. The same was true for urine albumin excretion rate. Renal angiotensin II tissue concentration was not affected by moxonidine. In untreated SNX rats, renal nerve stimulation and exogenous NE induced an increase in isolated kidney perfusion pressure (102 ± 21 versus 63 ± 8 mmHg). Renal endogenous NE content was significantly lower in SNX rats than in controls (86 ± 14 versus 140 ± 17 pg/mg wet weight). Cortical uptake of [3 H]-NE was not different, but cortical NE release was significantly higher in SNX rats than in controls. Reduced function of presynaptic inhibitory α-adrenoceptors is unlikely because an α2-adrenoceptor antagonist increased NE release. At subantihypertensive doses, moxonidine ameliorates renal structural and functional damage in SNX animals, possibly through central inhibition of efferent sympathetic nerve traffic. In kidneys of SNX rats, indirect evidence was found for increased activity of a reduced number of nerve fibers.

It is widely acknowledged that after renal injury, progressive loss of renal function is perpetuated by maladaptive changes in the kidney (1). The role of the renin-angiotensin system in the progression of renal failure has been thoroughly investigated (2). In contrast, a potential role of overactivity of the sympathetic nervous system has not been investigated in detail. This is surprising because renal chemoreceptors and mechanoreceptors are apparently stimulated in damaged kidneys (3). The central nervous sympathetic activity is increased by afferent signals emanating from the lesioned kidney (4). Increased efferent sympathetic nerve traffic is known to contribute to hypertension of animals (5) and of patients with renal disease (6,7), but a potentially injurious BP-independent effect of sympathetic overactivity on progression of renal failure has not been documented thus far. We made the chance observation that in stroke-prone spontaneously hypertensive rats (SHR-SP), administration of moxonidine in a dose that failed to lower systemic BP was associated with strikingly less glomerulosclerosis (8).
The present controlled study was designed: (1) to confirm this observation in another model of renal failure, i.e., the subtotal nephrectomized rat (SNX); and (2) to evaluate potential pathomechanisms. The sympathoceptor agent moxonidine was administered in a dose that did not lower systemic BP. End points measured included morphologic indices of glomerular, vascular, and tubulointerstitial injury; urinary albumin excretion; and intrarenal content, uptake, and release of norepinephrine (NE).

Materials and Methods

Animals

Male 200-g Sprague Dawley rats were housed in single cages at constant room temperature (20°C) and humidity (75%) under a controlled light/dark cycle. The rats were fed a diet containing 40 g of protein and 0.6 g NaCl/100 g body wt (Altromin, Lage/Lippe, Germany). After a 3-d adaptation period, the animals were randomly allotted to three groups (study 1):

- Group 1: Sham-operated control group (n = 5) (control)
- Group 2: Subtotally nephrectomized group: no treatment (n = 12) (SNX)
- Group 3: Subtotally nephrectomized group: moxonidine 1.5 mg/kg per d (n = 10) (SNX + M)

Group 1 was sham-operated and left untreated, whereas groups 2 and 3 underwent two-step subtotal nephrectomy with removal of 75% (controlled by weighing) of renal cortical tissue of the right kidney (9). Forty-eight hours after the second operation, treatment was started in group 3. Moxonidine was given in food pellets to deliver a daily dose of 1.5 mg/kg body wt. In a pilot study of 4 wk duration, this dose was selected because it caused only very modest lowering of BP in control animals (systolic BP lowering: 1 mg/kg: −0.33%; 2 mg/kg: −2.49%) and no BP lowering in SNX animals. Water and food consumption were measured to control drug intake. Twenty-four-hour BP was measured in four animals per group over the entire 12-wk period using telemetry (see below).

In a separate experiment (study 2), animals (n = 9 to 10 per group) were placed in individual metabolic cages, and 24-h urine collections were performed to measure urine volume and albumin excretion. The kidneys of this repeat experiment were perfused with ice-cold NaCl and used for immunohistology and in situ hybridization (9,10).

Telemetric BP Measurements

Mean arterial pressure, systolic BP, diastolic BP, heart rate (derived from the peak systolic BP signal, min⁻¹), and motor activity of animals (U/10 min) were measured by 24-h telemetry (Data Sciences, St. Paul, MN) (11).

Urinary Albumin Measurements

Urinary albumin was measured using the microplate technique and a rabbit anti-rat albumin peroxidase conjugate (9).

Tissue Preparation

After 12 wk, blood samples were taken and the experiment was terminated by retrograde perfusion fixation via the abdominal aorta (9,12). The kidneys were processed and investigated as described below (9,12–14).

Immunohistochemistry

For staining of the proliferating cell nuclear antigen (PCNA), an anti-PCNA antibody (Immunotech 1510, Marseille, France) was used in a dilution of 1:150 as described previously in detail (9,10). The sections were examined using light microscopy at a magnification of ×400. The number of PCNA-positive glomerular cells was counted per glomerular area in 50 systematically subsampled glomeruli (9). The number of tubular cells per mm² tubulointerstitial area was counted on 50 systematically subsampled fields (0.1681 mm²), which were distributed over all cortical zones.

In Situ Hybridization

To study the effect of moxonidine treatment on transforming growth factor-β, renin, and endothelin-1 (ET-1) mRNA expression, nonradioactive in situ hybridization was performed as described previously in detail (9). For renin, the sense primer was 5′-ACCAT-GAAGGGGTCTCTCTC-3′; the antisense primer was 5′-CTGTCGATACTGCTCCTCA-3′, resulting in a PCR fragment of 296 bp. For ET-1, the sense primer was 5′-TGGCCTTTCAAGGAGTCCT-3′; the antisense primer was 5′-GCTGACAGAAATTCCAGC-3′, resulting in a PCR fragment of 339 bp.

Indices of Renal Damage

Gomerulosclerosis (as parameter of progression of renal failure in 100 systematically subsampled glomeruli per animal), tubulointerstitial changes (tubular atrophy, dilation, casts, interstitial inflammation, and fibrosis), and vascular damage (wall thickening, lumen obliteration, fibrinoid necrosis) were determined in a blinded manner, using a semiquantitative scoring system (9). The resulting index in each animal was expressed as a mean of all scores obtained.

Glomerular Geometry

Paraffin sections were examined by light microscopy, using hematoxylin and eosin and periodic acid-Schiff stains, at various magnifications. After determination of kidney weight and volume, the volume densities of cortex, medulla, and glomeruli were determined using a Zeiss eyepiece (magnifications: ×100 and ×400; Integrationsplatte II; Zeiss, Oberkochen, Germany) and the point counting method (Pe = Aα = Vα, 9,12–14). Glomerular number per area (Nv) was counted and total glomerular volume (Vglom) was calculated by multiplying volume density of glomeruli and cortex volume (Vvglom × Vc). Glomerular number per volume (Nv) was determined using the following equation (13):

\[ N_v = k / (S \times V^{1.5}) / (V_v^{0.5}) \]

where k = 1.03 and S = 1.382. The total glomerular number (Nvglom) was calculated by multiplication of glomerular number per volume (Nv) and cortex volume (Vc). Mean glomerular volume (v) was then derived from total glomerular volume and total number of glomeruli per kidney (Vglom/Nvglom).

Measurement of Intrarenal Angiotensin (AngI and AngII)

Frozen tissue samples (0.4 to 1.2 g) were homogenized in 20 ml of ice-cold 0.1 mol/HCl/80% ethanol as described previously for cardiac tissue (15) with 125I-labeled angiotensin I (AngI), added before tissue homogenization, as an internal standard. Homogenates were centrifuged (20,000 × g, 10 min, 4°C), and ethanol in the supernatant was evaporated (constant air flow); the remainder was diluted (20 ml in 1% orthophosphoric acid) and centrifuged (20,000 × g). The super-
natant was diluted (equal volume of 1% orthophosphoric acid) and concentrated on SepPak cartridges (C18+; Waters, Milford, MA). Preparation of SepPak extracts for HPLC and the HPLC procedure were performed as described (15). Ang I and Ang II in the HPLC fractions were measured by RIA (16). The average recovery was 70% (results corrected for incomplete recovery).

**NE Content, Uptake, and Release**

**Experimental Design.** After decapitation of rats under general anesthesia, kidneys were removed (control: 1.5 ± 0.2 g wet weight [gww]; SNX: 1.8 ± 0.1 gww) and 0.3-mm slices of renal cortex were prepared (control: 24.8 ± 3.0 mg; SNX: 24.8 ± 1.8 mg) (17). Slices were incubated with [3H]-NE (0.5 μmol/L, 71.7 Ci/mmol, 60 min) in Krebs-Henseleit solution bubbled continuously with carbogen (95% O2, 5% CO2). Slices were transferred into superfusion chambers between two platinum electrodes and superfused in parallel with Krebs-Henseleit solution (2 ml/min, 37°C, 4.75 ml/min per g tissue) (18). The perfusion solution was continuously gassed with carbogen (5% CO2, 95% O2) and passed through a 0.8-μm filter before reaching the kidneys. Bipolar platinum electrodes were placed around the renal arteries to stimulate the renal nerves. Perfusion pressure was monitored continuously (Statham P23 Db pressure transducer; Gould, Oxnard, CA). After preparation, renal nerve stimulation (RNS) was performed (5 Hz, 30 s, 1 ms, 40 V) to test the viability of the preparation, followed by a stabilization period (70 min) and two periods of RNS separated by an interval of 10 min (S1, 1 Hz; S2, 5 Hz). Thirty minutes after S2, two doses of NE (0.1 and 0.4 μmol/L) were added to the perfusion line (0.158 μl/min) at an interval of 20 min until the pressor responses reached a maximum. For determination of the RNS-induced release of NE, 10 1-min fractions were collected 5 min before each stimulation. Samples were collected in vials (167 μl of 1 mol/L HCl, 13.3 μl of 0.067 mol/L ethylenediaminetetra-acetic acid, and 3.3 μl of 1 mol/L Na2SO4).

**Determination of Endogenous NE**
The NE in the isolated kidney samples was extracted (adsorption onto alumina, elution with HClO4). After isolation of NE from cortex (extraction from 100 mg of cortex with 3 ml of HClO4), NE content was determined by reversed-phase HPLC detection (19).

**Statistical Analyses**
Data are given as mean ± SD. Kruskal-Wallis test or ANOVA was used for analysis of variance, followed by Duncan multiple range test to determine whether intergroup differences were significant. The zero hypothesis was rejected at a level of P < 0.05.

**Results**

**Pilot Study for Dose Finding**
Based on a 4-wk pilot study in control rats, a nonhypotensive dose of 1.5 mg/kg moxonidine was chosen for the experiment with SNX rats.

**Animal Data**
Weight of left (residual) kidney and 24-h BP were higher after SNX, but they were not significantly different between untreated and moxonidine-treated SNX rats. Serum urea concentrations were significantly higher in both SNX groups compared with controls, and hemoglobin tended to be lower (Table 1).

<table>
<thead>
<tr>
<th>Table 1. Animal dataa</th>
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<tr>
<td>Group</td>
</tr>
<tr>
<td>Control</td>
</tr>
<tr>
<td>(n = 5)</td>
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<tr>
<td>Untreated SNX</td>
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<tr>
<td>(n = 12)</td>
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<tr>
<td>SNX + M</td>
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<tr>
<td>(n = 10)</td>
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<tr>
<td>ANOVA</td>
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a SNX, subtotal nephrectomy; M, moxonidine.
b By 24-h telemetry in four randomly selected animals per group.
c Intact or residual.
d P < 0.05 versus SNX.
e P < 0.05 versus control.
Morphologic Investigations

The indices of glomerulosclerosis and vascular damage were significantly lower in moxonidine-treated rats compared with untreated SNX rats (Table 2). Figure 1C shows the glomerulosclerosis values of the individual animals. The differences were significant, as there was no overlap between the groups. Figure 1, A and B, gives representative examples of glomerulosclerosis in untreated (Panel A) and moxonidine-treated SNX rats (Panel B).

The tubulointerstitial damage index tended to be lower in moxonidine-treated animals, but this did not reach statistical significance. The total number of glomeruli per kidney was lower and the mean glomerular volume was higher after SNX, but values were not significantly different between the two SNX groups.

Table 2. Renal morphologya

<table>
<thead>
<tr>
<th>Group</th>
<th>Indices of Renal Damage</th>
<th>Glomerular Geometry</th>
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<tbody>
<tr>
<td></td>
<td>Glomerulosclerosis Index</td>
<td>Tubulointerstitial Index</td>
</tr>
<tr>
<td>Control (n = 5)</td>
<td>0.07 ± 0.01b</td>
<td>0.07 ± 0.03</td>
</tr>
<tr>
<td>Untreated SNX (n = 12)</td>
<td>1.55 ± 0.28c</td>
<td>1.50 ± 0.52</td>
</tr>
<tr>
<td>SNX + M (n = 10)</td>
<td>0.88 ± 0.09b</td>
<td>1.25 ± 0.13</td>
</tr>
<tr>
<td>ANOVA</td>
<td>P &lt; 0.05</td>
<td>NS</td>
</tr>
</tbody>
</table>

a Abbreviations as in Table 1.
b P < 0.05 versus SNX.
c P < 0.05 versus control.

Urinary Albumin Excretion Rate

Urinary albumin excretion rate at weeks 4, 8, and 12, respectively, was significantly higher in untreated SNX rats (Table 3). From week 8 onward, values were significantly correspondingly higher in moxonidine-treated compared with untreated SNX rats. Figure 4 shows the individual animal values after 12 wk.

AngII Levels in the Kidney

Renal AngII concentrations per unit wet weight (ww) were significantly lower in SNX rats, both untreated and moxonidine-treated, compared with controls. There was no significant difference of AngI or AngII:AngI ratio between moxonidine-treated and untreated SNX rats (data not shown) (Figure 5).

Sympathetic Innervation of Isolated Kidneys of SNX

To test the integrity of the renal sympathetic system after SNX, the effects of RNS and NE on renal vascular resistance were tested in isolated kidneys. After RNS (1 and 5 Hz), pressor responses were 12 ± 1 and 63 ± 8 mmHg, respectively. NE (0.1 and 0.4 μmol/L) infusion induced pressor responses of 30 ± 6 and 102 ± 21 mmHg. RNS (1 and 5 Hz) induced an NE release of 210 ± 65 and 1470 ± 195 pg/gww, respectively (Figure 6).

[3H]-NE Release and its Modulation by α2-Adrenoceptors

In the renal cortex, endogenous NE content was reduced after SNX (Figure 7A), but uptake of [3H]-NE was similar in both groups (Figure 7B). The stimulation-induced fractional release of [3H]-NE from cortex slices was greater in SNX rats than in controls (Figure 8A). The α2-adrenoceptor antagonist rauwolfscine significantly increased [3H]-NE release in both groups. The increase was slightly greater in SNX rats than controls (Figure 8B).

Discussion

The main finding of the present study is the observation that despite unchanged systemic BP, administration of the sympatholytic agent moxonidine was clearly associated with lower morphologic indices of vascular and glomerular injury in the kidney remnant of SNX rats. This was accompanied by a correspondingly lower urinary albumin excretion. Additional

In Situ Hybridization

As depicted in Figure 3, a qualitative assessment of transforming growth factor-β mRNA expression showed markedly higher expression in untreated SNX rats (Panel A) compared with moxonidine-treated (Panel B), sham-operated (Panel D), and sense controls (Panel C). The figure shows representative examples. Qualitatively, expression of renin and ET-1 mRNA was not different between untreated and moxonidine-treated SNX rats (data not shown).
measurements of NE turnover indicated increased NE uptake in the presence of diminished NE content, consistent with the notion of increased single nerve fiber activity in the kidney. Taken together, these observations support the hypothesis that sympathetic activity is an important BP-independent codeterminant of morphologic and functional changes of the kidney in chronic renal failure.

Several points must be considered when interpreting the results. The number of animals used for telemetric BP measurements for the entire 12-wk period was limited for logistical reasons. β-error calculation shows that the protocol (four animals per group) had a 90% chance of detecting a 15% BP difference (α = 0.05). For the morphologic studies, only five control animals were used since large numbers of control rats

Figure 1. Glomerulosclerosis. (A and B) Representative glomeruli of untreated (A) and moxonidine-treated (B) subtotally nephrectomized (SNX) rats with renal failure of 12 wk duration. Periodic acid-Schiff-stained paraffin section. Magnification: ×250. Note expansion of mesangial matrix, capillary dilation or occlusion, and synechia of the capillary tuft with Bowman’s capsule in untreated SNX (A) compared with the normal morphology of the glomerulus in a moxonidine-treated SNX rat (B). (C) Individual values and means of glomerulosclerosis index. Note no overlap between untreated SNX and SNX + moxonidine.
Moxonidine was administered to lower central (20) and potentially peripheral (21) renal sympathetic activity. We acknowledge, however, that moxonidine may also have interacted with imidazoline (I)2 receptors on renal cells (22). Injection of moxonidine into the renal artery or administration in the isolated perfused kidney preparation caused natriuresis (23). Because alternative modes to block sympathetic activity were not used in the present study, the conclusion that moxonidine acted via inhibiting sympathetic tone is plausible, but remains inferential. The dose of moxonidine administered to the rats was high compared with the therapeutic dose in humans. Because alternative modes to block sympathetic activity were not used in the present study, the conclusion that moxonidine acted via inhibiting sympathetic tone is plausible, but remains inferential. The dose of moxonidine administered to the rats was high compared with the therapeutic dose in humans. However, it is in line with other animal experiments. In a pilot study, we had verified that this dose did not cause marked systemic BP lowering. This was confirmed by 24-h telemetric BP measurements, so we can exclude confounding changes in systemic BP by moxonidine. We acknowledge, however, that we cannot exclude changes in intrarenal resistances, and thus intrarenal hemodynamic effects.

The study protocol did not address the issue of whether the presumed effects of the sympathetic nervous system were due to direct or indirect actions of catecholamines. For instance, sympathetic stimulation activates the renin system via β-adrenergic mechanisms (24). Catecholamines and the renin system interact in the kidney also by other mechanisms (25), and the two systems may mutually reinforce each other (26). Nevertheless, the measurements of AngII in residual renal tissue argue against an effect of moxonidine on the intrarenal renin system, but this conclusion must be made cautiously in view of the known compartmentation of renal AngII (27).

Direct effects of catecholamines that may potentially be relevant for renal damage include proproliferative effects mediated via the β-adrenoreceptor (28) that have been documented in tubular epithelial cells, or influences of catecholamines on the function of podocytes, key cells in the genesis of glomerular injury (29). Podocytes express α1- and β2-adrenoreceptors (30), and stimulation of α1-adrenoreceptor increases [Ca2+]i, while stimulation of β2-adrenoreceptor induces depolarization via opening of cAMP-dependent Cl− conductance. Apart from changes in glomerular hydraulic pressure gradient (31), sympathetic nerve stimulation and NE application decrease the glomerular diameter (32), and also Ke, possibly via contraction of podocyte foot processes (33). In a previous study (34), we compared the effect of the angiotensin-converting enzyme inhibitor ramipril and a high dose of moxonidine on glomerular morphology in SNX rats. Although glomerulosclerosis was similarly lowered by both treatments, only ramipril had beneficial effects on podocyte morphology. Apparently, the agents had different effects on glomerular cells.

The mechanisms by which pharmacologic reduction of sympathetic activity attenuates progression are unclear. Apart from direct consequences in altered renal hemodynamics, indirect effects, e.g., via reduction in proteinuria (35), must be considered.

Glomerular hyperfiltration may be important for progression of renal failure. Mühlbauer et al. (36) noted that amino acid-induced glomerular hyperfiltration was completely abolished by bilateral renal denervation, suggesting a role of the sympathetic nervous system. Microneurographic studies clearly provided evidence for increased sympathetic nerve activity in patients with terminal and preterminal renal failure (6,26). This has also been confirmed in experimental studies (5,6). Campese and Kogosov documented that the rise in BP after subtotal nephrectomy can be partly prevented by dorsal rhizotomy, i.e., section of the dorsal roots with interruption of excitatory afferent signals (5). Turnover rates of NE in the posterior and lateral hypothalamic region were increased in rats with renal failure (37). Renal afferent nerves project to the hypothalamus running through the dorsolateral aspect of the medulla. Factors potentially involved in the activation of the sympathetic nervous system are complex and may include changes in nitric oxide, ET-1, AngII (26,38,39), and leptin (40). The present observation that NE content and turnover are
changed in residual renal tissue is fully consistent with the concept of increased efferent sympathetic nerve traffic.

Because we postulated that increased sympathetic activity was involved in progression of renal failure, it appeared important to provide at least indirect information on renal sympathetic nerves in the SNX kidney remnants. The above hypothesis presupposes that the renal nerves are intact (41). This point was tested using the isolated kidney preparation (18).

Stimulation of the renal nerves at 1 and 5 Hz caused marked NE release and frequency-dependent increases of vascular resistance, likely due to activation of \( \alpha_1 \)-adrenoceptors by neuronally released NE (42), although we cannot exclude that other sympathetic neurotransmitters such as neuropeptide Y (NPY) and ATP participate (43). The endogenous NE content of renal cortex was significantly lower in SNX rats than in controls, but the proportion of non-neuronal tissue is presumably increased in the kidney remnant. To characterize the renal sympathetic nerves, it was therefore relevant to investigate NE release. \([^3H]\)-NE uptake was similar in SNX rats and controls, presumably indicating the increased uptake capacity of SNX rats. The enhanced release of NE by the RNS is compatible with the notion that the renal sympathetic system is more active in SNX rats than in controls. NE release from sympathetic nerve endings is regulated via presynaptic modulatory mechanisms, *i.e.*, autoreceptor inhibition mediated by \( \alpha_2 \)-adrenoceptors (44,45). The \( \alpha_2 \)-adrenoceptor antagonist rauwolscine enhanced NE release in SNX kidney cortex, which indicates that NE activates presynaptic \( \alpha_2 \)-autoreceptors to inhibit its own release in this model of chronic renal failure. The effect of rauwolscine was even greater in SNX rats than in controls. Thus, less marked inhibition of NE release by \( \alpha_2 \)-adrenoceptors does not explain enhanced NE release in SNX. We ac-

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**Figure 3.** Nonradioactive in situ hybridization of transforming growth factor-\( \beta \)1 (TGF-\( \beta \)1) mRNA. Note higher TGF-\( \beta \)1 mRNA expression in untreated SNX rat (A) compared with moxonidine-treated SNX rat (B), sense controls (C), and sham-operated control animals (D).

**Table 3.** Urinary albumin excretion rate (study 2)*

<table>
<thead>
<tr>
<th>Group</th>
<th>4 Weeks after SNX</th>
<th>8 Weeks after SNX</th>
<th>12 Weeks after SNX</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (( n = 10 ))</td>
<td>5.19 ± 7.07</td>
<td>11.6 ± 11.4</td>
<td>16.6 ± 9.6</td>
</tr>
<tr>
<td>Untreated SNX (( n = 9 ))</td>
<td>44.5 ± 17.4(^b)</td>
<td>111 ± 30.6(^b)</td>
<td>210 ± 119(^b)</td>
</tr>
<tr>
<td>SNX + M (( n = 10 ))</td>
<td>39.7 ± 18.7(^b)</td>
<td>71.2 ± 37.0(^{b,c})</td>
<td>136 ± 57.2(^{b,c})</td>
</tr>
<tr>
<td>ANOVA</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
<td>( P &lt; 0.001 )</td>
</tr>
</tbody>
</table>

* Results are given as mg/24 h. Abbreviations as in Table 1.
\(^b\) \( P < 0.05 \) versus control.
\(^c\) \( P < 0.05 \) versus SNX.
knowledge that it may not be appropriate to assume that all sympathetic effects are mediated via catecholamines (see above). NPY concentrations are increased in renal failure (46), and NPY has been shown to act on the kidney (43,47).

Fragmentary clinical observations are consistent with an effect of sympathetic activity on progression of renal disease. In patients with type I diabetes and diabetic nephropathy, Weinrauch et al. (48) found that progression of renal dysfunction was predicted by the inability to vary the heart rate in response to the Valsalva maneuver. They concluded that unopposed elevation of sympathetic tone in the face of parasympathetic dysfunction was involved. Furthermore, preliminary studies in microalbuminuric patients with type 1 diabetes (unpublished data) show that despite no change in systemic BP by ambulatory BP measurement, low doses of moxonidine reduced urinary albumin excretion rate. Certainly, such clinical observations and the above experimental data do not allow a full assessment of the impact of sympathetic overactivity on progression in patients with renal disease. But our data suggest that the renin-angiotensin and the endothelin systems (49), as well as sympathetic overactivity, are apparently involved in the pathogenesis of structural and functional changes in the kidney.
in renal failure. This pathomechanism may provide a novel therapeutic window.

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