Beneficial Effects of Calcimimetics on Progression of Renal Failure and Cardiovascular Risk Factors

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Abstract. In renal failure, parathyroid hormone (PTH) is not only involved in the genesis of disturbed calcium/phosphate metabolism and ostitis fibrosa; it is also a permissive factor in the genesis of hypertension, cardiovascular damage, and dyslipidemia. The allosteric activator of the calcium sensing receptor NPSR-568 (R-568) has been shown to reduce the serum intact PTH (iPTH) concentration in uremic rats. It was the purpose of this study in subtotally nephrectomized (SNX) rats to compare pharmacologic abrogation of secondary hyperparathyroidism by R-568 with parathyroidectomy (PTX). The effects on progression of renal failure, BP, and lipid and structural parameters of kidney and heart were studied. Four groups of male SD-rats were studied: (1) sham-operated + vehicle-treated rats (controls); (2) SNX + vehicle-treated rats (SNX); (3) parathyroidectomized SNX + vehicle-treated rats (SNX+PTX); and (4) SNX + calcimimetic R-568 –treated rats (SNX+R-568). R-568 (50 μmol/kg per d) was administered by gavage. Eight weeks after SNX, serum creatinine concentration, urinary albumin excretion, BP, and serum LDL-cholesterol concentration were significantly lower in both R-568 –treated and parathyroidectomized SNX compared with vehicle-treated SNX. In addition, structural abnormalities of the kidney (glomerulosclerosis, tubulointerstitial changes) and the heart (interstitial fibrosis, capillary length density, arteriolar wall thickness) were significantly less pronounced than in vehicletreated SNX. It is concluded that in experimental renal failure abrogation of hyperparathyroidism by administration of a calcimimetic or PTX similarly attenuates progression of renal failure. Furthermore, it interferes with the development of cardiovascular risk factors and cardiac remodeling.

Secondary hyperparathyroidism (sHPT) is a known complication of chronic renal failure. Elevated concentrations of parathyroid hormone (PTH) play a role not only in the pathogenesis of renal bone disease (1,2), but also in the development of cardiovascular risk factors such as disturbed lipid metabolism (3,4), glucose intolerance (5), and hypertension (6–8). Parathyroidectomy (PTX) attenuates progression of renal failure in subtotally nephrectomized rats (SNX) on a high protein (9) or high phosphate (10,11) diet. sHPT is also known to play an important role in the development of structural abnormalities of the heart in renal failure, including left ventricular hypertrophy, interstitial fibrosis, and arteriolar wall thickening of the heart (7,12–14).

Allosteric activators of the calcium sensing receptor, e.g., NPSR-568 (R-568), reduce PTH secretion in rats or patients with primary and secondary hyperparathyroidism (15–20). There is no information on whether calcimimetics also affect abnormalities of uremia other than calcemia, phosphatemia (21), PTH concentrations (22), and skeletal abnormalities (17). Therefore, it was the purpose of this study to compare the effects of the calcimimetic R-568 and of parathyroidectomy on progression of renal failure, BP, lipid parameters, and structure of kidney and heart.

Materials and Methods

Animals

Male Sprague-Dawley (SD) rats weighing 180 to 200 g 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 high-protein diet containing 40% protein, 0.6% NaCl, 0.75% phosphate, and 0.9% calcium (Altromin Co., Lage/Lippe, Germany).

Experimental Groups

After a 3-d adaptation period, the animals were randomly allotted to four groups (study 1):

- Control (n = 7): sham-operated (sham-op) control animals treated with vehicle
- SNX (n = 12): Subtotally nephrectomized animals treated with vehicle
- SNX+PTX (n = 11): Subtotally nephrectomized and parathyroidectomized animals treated with vehicle
- SNX+R-568 (n = 10): Subtotally nephrectomized animals treated with NPS R-568 50 μmol/kg per d
A pair-feeding protocol was used throughout the experiment. For subtotal nephrectomy, the right kidney was removed in a first session under anesthesia with ketamine (100 mg/kg body wt) and xylazine (2 mg/kg body wt). The weight of the right kidney was measured directly after excision. At the time of the first operation, SNX + PTX animals underwent PTX using microsurgical techniques and were subsequently given 5% calcium gluconate in the drinking water throughout the study to prevent development of hypocalcemia (3,4). Rats were given water ad libitum throughout the experiment. Seven days after uninephrectomy, cortical tissue of the hypertrophied remnant left kidney was removed, so that the amount removed corresponded to 75% of the weight of the previously excised right kidney. Care was taken to remove the tissue preferentially from the upper and lower pole without damaging large arteries. In sham-operated animals, the kidneys were decapsulated in two consecutive sessions.

Twenty-four hours after the second operation, treatment was started in each group. NPS R-568 was dissolved in 10% aqueous cyclodextrin (2-hydroxypropyl-β-cyclodextrin; Sigma-Aldrich Chemie GmbH, Steinheim, Germany) and was administered daily by gavage (50 μmol/kg per d) between 8:00 a.m. and 10:00 a.m. Systolic BP was measured by tail cuff plethysmography at 2-wk intervals. The rats were weighed and placed in a metabolic cage for the collection of a 24-h urine sample. A blood sample was taken from the subclavian artery at week 4. One control rat did not complete the study (injured during gavage).

In a separate ancillary experiment (study 2), the same protocol was used to study animals (n = 5 to 6 per group) 2 wk after surgery using immunohistologic techniques.

### Measurements

Blood was obtained 2 h after the administration of the calcimetric. Blood (taken at week 4 and at the end of the experiment, i.e., week 8) and urine samples (taken at the indicated time points) were measured using standard laboratory methods with an automated multiparametric analyzer (Autoanalyzer; Hitachi, Japan). Serum PTH was determined planimetrically using a semiautomatic image analyzing system (Videooplan, Kontron Co., Eching, Germany). Wall-to-lumen ratio was calculated by dividing wall thickness and lumen diameter (27,28).

### Results

#### Animal Data

**Body and Organ Weights.** At the end of the experiment, body weight was lower in all SNX groups (SNX, SNX + PTX, SNX + R-568) as compared with pair-fed sham-op controls, but it was not significantly different between the SNX groups. The weights of the (remnant) left kidney, heart, and left ventricle were significantly higher in vehicle-treated SNX rats compared with controls as well as with SNX + intervention, either PTX, or R-568 treatment, respectively (Table 1).

**PTH Concentration.** PTH concentrations increased progressively with time in vehicle-treated SNX (at 4 wk, 270 ± 151; at 8 wk, 817 ± 899 pg/ml). No PTH was detectable in PTX rats. The efficacy of R-568 in suppressing PTH secretion is documented by the very low PTH levels in SNX + R-568 animals (at week 4, 11.8 ± 21.7; at week 8, 59.3 ± 65.6 pg/ml) (Figure 1). There was no significant difference of serum alkaline phosphatase concentration between the different groups.

### Immunohistochemistry of the Kidney

For staining of the proliferating cell nuclear antigen (PCNA), an anti-PCNA antibody (Immunotech 1510; Marseille, France) was used at a dilution of 1:150 as described previously in detail (23). The sections were examined using light microscopy at a magnification of X400. The number of PCNA-positive glomerular cells was counted per glomerular area in 50 systematically subsampled glomeruli (23). The number of tubular cells per mm² of tubulointerstitial area was counted on 50 systematically subsampled fields (0.1681 mm²) randomly sampled from all cortical zones.

### Quantitative Stereology of the Heart

All investigations were performed in a blinded fashion. Eight random samples of differently orientated left ventricular section per animal were embedded in epon araldite. Semithin sections were cut, stained with methylene blue and basic fuchsin, and investigated using the orientator method (26–28). The length density (Lv) of capillaries, i.e., the length of capillaries per unit tissue volume, and the volume density (Vv) of cardiac capillaries, i.e., the volume of capillaries per unit volume of myocardial tissue, were measured in eight systematically subsampled areas per section. The length density of myocardial capillaries (Lv) was determined using the equation Lv = 2QA, where QA is area density (for example, the number of capillary transects per area of myocardial reference tissue) (24,27,28).

Volume density (Vv) of capillaries, interstitial tissue, and myocytes was obtained using the point-counting method (27–29) according to the equation Pp = Vv, where Pp is point density. Intercapillary distance, defined as the distance between the centers of two adjacent intramyocardial capillaries, was calculated according to a modification of the formula of Henquell and Honig (27,29).

Wall thickness and lumen diameter of intracardiac arteries were determined planimetrically using a semiautomatic image analyzing system (Videooplan, Kontron Co., Eching, Germany). Wall-to-lumen ratio was calculated by dividing wall thickness and lumen diameter (27,28).
they were significantly lower in SNX+PTX and SNX+R568 than in vehicle-treated SNX (Figure 1).

At the end of the 8-wk experiment, serum phospate was significantly higher in SNX compared with controls. It was significantly higher in SNX+PTX compared with SNX, but it was not significantly different between SNX+R-568 and SNX (Figure 1).

After 8 wk, urinary calcium excretion (mg/d) was 0.31 ± 0.06 in controls, 0.83 ± 0.09 in SNX treated with vehicle, 0.86 ± 0.09 in SNX+PTX and 0.71 ± 0.11 in SNX+R-568. The values in all SNX groups were significantly higher than in controls ($P < 0.01$), but there were no significant differences among the SNX groups.

**Lipid Parameters.** At the end of the experiment, total cholesterol, HDL cholesterol, and LDL cholesterol concentrations were significantly higher in SNX compared with controls. The LDL-cholesterol concentration was significantly lower in SNX+PTX and SNX+R-568 than in SNX. There was no significant difference in LDL-cholesterol concentration between SNX+PTX and SNX+R-568 (Table 2).

**Blood Pressure.** Systolic BP (SBP) was significantly higher in SNX rats compared with control rats as early as 2 wk after SNX and increased progressively with time thereafter. SBP was significantly lower in SNX+PTX and SNX+R-568 compared with vehicle-treated SNX and was not significantly different from controls (Figure 2).

**Renal Function.** Serum creatinine concentrations (Figure 1) 4 wk after SNX were significantly higher in vehicle-treated SNX compared with controls. It was significantly higher in SNX+PTX compared with SNX, but it was not significantly different between SNX+R-568 and SNX (Figure 1).
SNX compared with controls. The concentration increased subsequently more markedly in SNX than in SNX+PTX or SNX+R-568, respectively.

**Urinary Albumin Excretion Rate.** As early as 2 wk after SNX, urinary albumin excretion rate (UAE) was significantly higher in vehicle-treated SNX compared with controls. At the end of the experiment, UAE was markedly higher in SNX compared with controls; despite a similar initial increase of UAE at 2 wk, the final UAE was significantly lower in SNX+PTX and SNX+R-568 compared with vehicle-treated SNX (Table 3).

<table>
<thead>
<tr>
<th>Group</th>
<th>2 wk</th>
<th>4 wk</th>
<th>6 wk</th>
<th>8 wk</th>
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<tbody>
<tr>
<td>Control (n = 6)</td>
<td>0.63 ± 0.54</td>
<td>1.40 ± 0.76</td>
<td>2.89 ± 3.01</td>
<td>1.78 ± 0.70</td>
</tr>
<tr>
<td>SNX (n = 12)</td>
<td>4.89 ± 2.59</td>
<td>34.0 ± 21.5a</td>
<td>74.5 ± 36.4a</td>
<td>124.0 ± 78.7a</td>
</tr>
<tr>
<td>SNX+PTX (n = 11)</td>
<td>1.75 ± 1.08</td>
<td>3.25 ± 4.66b</td>
<td>9.76 ± 13.6b</td>
<td>19.3 ± 21.4b</td>
</tr>
<tr>
<td>SNX+R-568 (n = 9)</td>
<td>4.88 ± 6.72</td>
<td>20.5 ± 21.5</td>
<td>26.2 ± 25.3b</td>
<td>28.6 ± 19.2b</td>
</tr>
</tbody>
</table>

**ANOVAP = 0.054 P = 0.0001 P < 0.0001 P < 0.0001**

*Table 3. Urinary albumin excretion (mg/24 h) at different time points of the experiment*

**Structural Abnormalities of the Kidney**

In vehicle-treated SNX rats, the glomerulosclerosis index (GSI) and the tubulointerstitial damage index (TID) were significantly higher than in control rats (Figure 3). Both indices were significantly lower but did not reach control values in SNX+PTX and SNX+R-568 rats, respectively.

In an ancillary experiment (experiment 2), the number of PCNA-positive cells per glomerulus as well as the number of PCNA-positive cells per mm² of tubulointerstitial area were significantly higher 2 wk after SNX compared with controls. PCNA-positive cells as an index of cell proliferation were significantly lower in SNX+PTX and SNX+R-568 than in vehicle-treated SNX rats (Figure 3).

**Structural Abnormalities of the Heart**

The lumen diameter of the small intramyocardial arteries was similar in all groups, but there was a significant difference of arterial wall thickness (Table 4). It was significantly higher in vehicle-treated SNX compared with control rats. Arterial wall thickness was significantly lower in SNX+PTX and SNX+R-568, respectively, compared with vehicle-treated SNX.

The volume density of interstitial tissue (excluding capillaries) was significantly higher in vehicle-treated SNX compared with controls (Table 5). It was significantly lower in SNX+PTX and strikingly lower in SNX+R-568 compared with vehicle-treated SNX.

Finally, capillary length density was significantly lower in vehicle-treated SNX compared with controls (Table 6). This reduction was completely prevented in SNX+PTX and

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**ANOVAP = 0.054 P = 0.0001 P < 0.0001 P < 0.0001**

*Table 2. Lipid parameters at the end of the experiment (week 8)*

<table>
<thead>
<tr>
<th>Group</th>
<th>Tchol (mg/dl)</th>
<th>HDL-chol (mg/dl)</th>
<th>LDL-chol (mg/dl)</th>
<th>TG (mg/dl)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 6)</td>
<td>87.4 ± 22.1</td>
<td>59.3 ± 16.8</td>
<td>12.6 ± 4.2</td>
<td>64.6 ± 25.5</td>
</tr>
<tr>
<td>SNX (n = 12)</td>
<td>173 ± 68.5b</td>
<td>113.3 ± 33.2b</td>
<td>49.0 ± 27.1b</td>
<td>61.7 ± 43.6</td>
</tr>
<tr>
<td>SNX+PTX (n = 11)</td>
<td>123 ± 22.3</td>
<td>88.1 ± 15.9</td>
<td>27.9 ± 6.9c</td>
<td>36.3 ± 12.9</td>
</tr>
<tr>
<td>SNX+R-568 (n = 9)</td>
<td>125 ± 27.5</td>
<td>90.2 ± 16.6</td>
<td>24.7 ± 7.0d</td>
<td>40.6 ± 12.4</td>
</tr>
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</table>

*P = 0.001 P = 0.0002 P = 0.0001 P = 0.07*
The values of intercapillary distance varied inversely with the capillary length density.

**Discussion**

The present study on the calcimimetic agent R-568 in subtotal nephrectomized rats was designed to evaluate the effect of R-568 on progression of renal failure and cardiovascular changes. It yielded three salient results. First, R-568 indeed attenuated the rate of progression as indicated by measurements of serum-creatinine, creatinine clearance (data not shown; an admittedly poor index of GFR), albumin excretion as well as indices of glomerulosclerosis, tubulointerstitial damage, and proliferating cells number in the glomeruli and the tubulointerstitium. Second, we made the serendipitous observation that R-568 had also a beneficial effect on cardiac structure, i.e., less interstitial fibrosis as well as less thickening of the wall of intermyocardial arteries and less diminution of capillary density as indices of less pronounced microvessel disease. Third, lower SBP values as well as less pronounced dyslipidemia were noted in R-568–treated SNX rats.

Several aspects of the methodology require comment. We deliberately used a model of fast progression first by surgical removal of 70% of renal cortical mass and second by administration of a high-protein diet.

Because calcium sensing receptors occur in numerous organs and are virtually ubiquitous, we considered that evaluating only the effect of R-568 would not permit to distinguish between an intrinsic pharmacologic effect of the calcimimetic on the one hand and abrogation of hyperparathyroidism on the other hand. As a control, we therefore included a group of SNX–R-568, respectively. The values of intercapillary distance varied inversely with the capillary length density.

**Table 4. Effect of R-568 or PTX on small intramyocardial arteries in rats with subtotal nephrectomy**

<table>
<thead>
<tr>
<th></th>
<th>Wall to Lumen Ratio (×10⁻³)</th>
<th>Lumen Diameter (µm)</th>
<th>Wall Thickness (µm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 4)</td>
<td>71 ± 9</td>
<td>57.6 ± 11.0</td>
<td>3.98 ± 0.342</td>
</tr>
<tr>
<td>SNX (n = 7)</td>
<td>99 ± 14</td>
<td>58.8 ± 13.1</td>
<td>5.70 ± 0.645</td>
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<tr>
<td>SNX+PTX (n = 7)</td>
<td>74 ± 10</td>
<td>59.2 ± 1.1</td>
<td>4.42 ± 0.587</td>
</tr>
<tr>
<td>SNX+R-568 (n = 5)</td>
<td>66 ± 12a</td>
<td>61.1 ± 8.5</td>
<td>3.95 ± 0.301a</td>
</tr>
</tbody>
</table>

a $P < 0.001$ versus SNX.

b $P < 0.01$ versus SNX.
Table 5. Effect of R-568 or PTX on interstitial tissue in rats with subtotal nephrectomy

<table>
<thead>
<tr>
<th></th>
<th>Interstitial Volume Density (Vv)</th>
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<tbody>
<tr>
<td>Control (n = 4)</td>
<td>1.51 ± 0.31&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SNX (n = 7)</td>
<td>3.58 ± 0.87</td>
</tr>
<tr>
<td>SNX + PTX (n = 7)</td>
<td>2.33 ± 0.42&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>SNX + R-568 (n = 5)</td>
<td>1.98 ± 0.66&lt;sup&gt;b&lt;/sup&gt;</td>
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</table>

<sup>a</sup> P < 0.001 versus SNX.  
<sup>b</sup> P < 0.01 versus SNX.

parathyroidectomized SNX rats. Both R-568 and PTX lowered iPTH concentrations to virtually the same extent. In parallel, the results in PTX animals were practically identical with those obtained in the R-568–treated group. We conclude that the major, if not the only, factor explaining the effect of R-568 on nonclassical organs of PTH is the decrease in iPTH concentration. We acknowledge that the present experiment was not designed to evaluate whether some of the effects of R-568 were caused by an intrinsic action of the blockade of the calcium sensing receptor in tissues outside of the parathyroid. In parallel with low iPTH concentrations, higher phosphate and lower calcium concentrations were noted. This may also have influenced the results; however, there was no significant difference concerning these two parameters in the two intervention groups. This is of note because, independent of PTH and calcitriol, higher phosphate concentrations have a negative effect on structure and function of vascular smooth muscle cells (30) and cardiac fibroblasts (unpublished observation).

The main result of this study is the beneficial effect of R-568 on progression. Evaluation of this problem had been the primary purpose of this study. In a series of experiments, Bonjour and colleagues have shown that PTX prevented progression of chronic renal failure induced by a high-protein diet (9). PTX not only prevented the deterioration of renal function; it also improved survival as had previously been noted in veterinarian literature (31). PTX prevented the increase of the mass of the kidney remnant, i.e., renal hypertrophy, induced by high-protein diet in SNX rats, and the same prevention of renal hypertrophy was noted in uninephrectomized rats (32). In the latter case, this effect was related to increased insulin-like growth factor-1 (IGF-1) concentration. On the other hand, however, there are reports that PTX fails to improve renal function in humans (33), although this latter study comprised only three patients. Furthermore, an increase in serum creatinine and decrease in GFR is noted after PTX of transplanted patients. Such heterogeneity of findings is less surprising in view of the complex results that have recently been reported with respect to renal actions of PTH and PTHrp, respectively. The aminoterminal domain of PTHrp interacts with the receptor that is shared by PTH and PTHrp. Using immunohistochemistry and molecular techniques, the PTH/PTHrp receptor has been demonstrated in the kidney of uninephrectomized rats after a protein overload (34). The PTH/PTHrp receptor is particularly found in the glomerulus and in podocytes (35–38). Micropuncture studies in the rat showed that both PTH and cAMP cause a reduction in the glomerular ultrafiltration coefficient (Kf) (39). Conversely, PTX increases Kf. Further effects that may affect progression are the release of renin induced by PTH (40,41). PTH has vasodilatory effects on preglomerular vessels, while efferent arterioles are constricted, presumably secondary to renin release (42). To what extent PTH also has direct effect on renal cells in vivo is uncertain, but such actions of PTH and PTHrp have been demonstrated in cultured human mesangial cells (43). This observation is of interest in view of our observation that the calcimimetic as well as PTX influence renal cell proliferation. It is known that PTHrp is mitogenic for various renal cells (44). We cannot exclude that the lower SBP in our animals played a role in attenuating progression of renal failure, but such near normalization of BP had not been observed in a previous study (9) suggesting that the significant effect of low PTH is at least not fully explained by differences in BP. The calcium sensing receptor is expressed by various types of renal cells (45–47), but similar changes were seen in R-568 treated and PTX animals; it is therefore unlikely that a direct action of R-568 on the calcium sensing receptor played a role in the beneficial effect on progression observed in the present study. It is noteworthy that we observed no effects of R-568 or 5% dietary calcium supplement on BP in rats with normal renal function (unpublished data). The significantly lower number of PCNA-positive cells in the tubulointerstitium and the trend for fewer PCNA-positive cells in the glomeruli of SNX + PTX and SNX + R-568, respectively, suggest that the lesser weight of the remnant kidney reflects not only less fibrosis, but also less renal growth.

Previous experiments in our laboratory had shown that PTH was a permissive factor for the development of cardiac abnormalities in the renal ablation model such as left ventricular hypertrophy (48,49), while efferent arterioles are constricted, presumably secondary to renin release (42). To what extent PTH also has direct effect on renal cells in vivo is uncertain, but such actions of PTH and PTHrp have been demonstrated in cultured human mesangial cells (43). This observation is of interest in view of the complex results that have recently been reported with respect to renal actions of PTH and PTHrp, respectively. The aminoterminal domain of PTHrp interacts with the receptor that is shared by PTH and PTHrp. Using immunohistochemistry and molecular techniques, the PTH/PTHrp receptor has been demonstrated in the kidney of uninephrectomized rats after a protein overload (34). The PTH/PTHrp receptor is particularly found in the glomerulus and in podocytes (35–38). Micropuncture studies in the rat showed that both PTH and cAMP cause a reduction in the glomerular ultrafiltration coefficient (Kf) (39). Conversely, PTX increases Kf. Further effects that may affect progression are the release of renin induced by PTH (40,41). PTH has vasodilatory effects on preglomerular vessels, while efferent arterioles are constricted, presumably secondary to renin release (42). To what extent PTH also has direct effect on renal cells in vivo is uncertain, but such actions of PTH and PTHrp have been demonstrated in cultured human mesangial cells (43). This observation is of interest in view of our observation that the calcimimetic as well as PTX influence renal cell proliferation. It is known that PTHrp is mitogenic for various renal cells (44). We cannot exclude that the lower SBP in our animals played a role in attenuating progression of renal failure, but such near normalization of BP had not been observed in a previous study (9) suggesting that the significant effect of low PTH is at least not fully explained by differences in BP. The calcium sensing receptor is expressed by various types of renal cells (45–47), but similar changes were seen in R-568 treated and PTX animals; it is therefore unlikely that a direct action of R-568 on the calcium sensing receptor played a role in the beneficial effect on progression observed in the present study. It is noteworthy that we observed no effects of R-568 or 5% dietary calcium supplement on BP in rats with normal renal function (unpublished data). The significantly lower number of PCNA-positive cells in the tubulointerstitium and the trend for fewer PCNA-positive cells in the glomeruli of SNX + PTX and SNX + R-568, respectively, suggest that the lesser weight of the remnant kidney reflects not only less fibrosis, but also less renal growth.

Table 6. Effect of R-568 or PTX on capillaries in rats with subtotal nephrectomy

<table>
<thead>
<tr>
<th></th>
<th>Capillary Length Density (Lv mm/mm&lt;sup&gt;3&lt;/sup&gt;)</th>
<th>Capillary Area Density (Cap/mm&lt;sup&gt;2&lt;/sup&gt;)</th>
<th>Intercapillary Distance (m)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 4)</td>
<td>4003 ± 368&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2002 ± 184&lt;sup&gt;b&lt;/sup&gt;</td>
<td>17 ± 0.79&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SNX (n = 7)</td>
<td>2900 ± 647</td>
<td>1450 ± 323</td>
<td>20.3 ± 2.49</td>
</tr>
<tr>
<td>SNX + PTX (n = 7)</td>
<td>3637 ± 262&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1818 ± 131&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.8 ± 0.63&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>SNX + R-568 (n = 5)</td>
<td>3752 ± 247&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1876 ± 123&lt;sup&gt;a&lt;/sup&gt;</td>
<td>17.6 ± 0.57&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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</table>

<sup>a</sup> P < 0.05 versus SNX.  
<sup>b</sup> P < 0.01 versus SNX.
hypertrophy, interstitial fibrosis (12), or wall thickening of postcoronary arteries (13). PTH is significantly correlated to left ventricular mass in patients with essential hypertension (48) as well as in patients with renal failure (49). Experimental studies documented that PTH activates protein kinase C of cardiomyocytes, leading to hypertrophic growth and reexpression of fetal-type proteins (50). The present finding of less cardiac fibrosis and less wall thickening in R-568 treated and PTX+SNX, respectively, is perfectly in line with these observations. The similar effects R-568 and PTX suggest that these findings are indeed due to lower PTH concentration.

One might argue that the effect on cardiac structure was the result of less pronounced hypertension. This is unlikely, however, in view of the fact that in previous studies the development of cardiac structural abnormalities could clearly be dissociated from changes in BP (27,51). We acknowledge, however, that further experiments are necessary to formally exclude a confounding effect of higher BP.

The effect of PTH on BP is complex. It is important to keep in mind the possibility of species-related differences and of different short-term versus long-term BP effects of PTH. In the rat, PTH causes acute vasodilation and lowers BP (50), whereas infusion of human 1,34-PTH in healthy volunteers causes an acute modest increase in BP (6). It has been proposed that the acute BP-lowering effect in animals is superseded in the long run by an elevation of BP that results from cellular calcium loading. At least in humans, acute administration of PTH causes acute stimulation of sympathetic activity (52). Recent work in progress showed that PTH does have effects on sympathetic nerve activity in animals as well (53). Another PTH target with potential impact on BP is the endothelial cell. PTH was shown to activate NO production by single endothelial cells (54). In genetically hypertensive rats the BP increase after administration of the NO synthase inhibitor L-NAME is greater after PTX, suggesting less vasodilation. In patients with primary hyperparathyroidism, impaired flow-mediated vasodilation in the brachial artery is improved after PTX (55). A specific effect of PTH on vascular remodeling is suggested by the observation that PTH concentrations in renal patients are related to intima media thickness (56).

We also observed striking amelioration of dyslipidemia in R-568-treated or parathyroidectomized SNX rats. A beneficial effect of PTX on cholesterol levels had been observed by Shigematsu et al. (9) and numerous other authors (57–61), but the effect of PTH is probably independent of the presence or absence of renal failure as suggested by the observation of reversible hyperlipoproteinemia in patients with primary hyperparathyroidism (58). This was associated with a decrease in post-heparin LPL activity (60). The observation that administration of insulin corrected the disturbed metabolism of triglyceride-rich particles was interpreted to indicate that the effect of PTH is at least partially indirect, involving inhibition of the secretion of insulin or interference with the peripheral action of insulin (59). On the other hand, in vitro PTH decreased the activity of lipoprotein lipase in adipocytes without affecting LPL mRNA (62). In view of the strong evidence that dyslipidemia is an important risk factor in renal failure (61), our observation that dyslipidemia can be abrogated by R-568 is definitely of interest. One has to keep in mind, however, that there are important species differences of lipid metabolism between the rat and the human.

Calcimimetics are undoubtedly promising agents, with the potential to abrogate hyperparathyroidism (63), parathyroid hyperplasia (22,63), and bone disease (17,64) in renal failure. The present data further suggest that the benefit from calcimimetics may extend beyond classical target organs of PTH. The data further suggest that calcimimetics have important effects on progression as well as on cardiovascular risk factors such as hypertension and dyslipidemia. Demonstration that the same findings apply to humans will require further studies.

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