Renal Endothelin System in Polycystic Kidney Disease

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Abstract. Polycystic kidney disease (PKD) is characterized by interstitial fibrosis and formation of renal cysts. Interestingly, interstitial fibrosis and renal cyst formation were also seen in human endothelin-1 (ET-1) transgenic mice. This study, therefore, analyzes the tissue distribution of ET-1, the tissue concentrations of ET-1, as well as the expression of ET receptor subtypes in the kidneys of a rat model of PKD: Han:SPRD rats. Six-week-old heterozygous (cy/+), as well as 6-mo-old heterozygous (cy/cy) Han:SPRD rats and the corresponding age-matched Sprague Dawley littermates (SD) (+/+), were analyzed. Furthermore, the acute effects of the mixed (A/B) endothelin receptor antagonist bosentan on hemodynamic and renal function were investigated in 6-mo-old, conscious, chronically instrumented (cy/cy) rats. The kidneys of affected rats showed significantly elevated tissue levels of ET-1 compared with age-matched controls (3.5 ± 0.3-fold in young cy/cy rats, P < 0.01; 1.4 ± 0.2-fold in young cy/+ rats, P < 0.01; 6.2 ± 0.4-fold in old cy/+ rats, P < 0.001) due to a highly increased ET-1 synthesis within the epithelial cells of the cysts. Analyzing tissue sections from patients with typical autosomal dominant PKD demonstrated a high ET-1 expression within the epithelial cells of the cysts as well. Scatchard analysis revealed a markedly decreased ET_A, ET_B receptor density in all groups of affected rats. The acute blockade of both endothelin receptor subtypes using bosentan in 6-mo-old heterozygous PKD rats led to a significant decrease in mean arterial BP (MAP) (−19.7 ± 2.1 mmHg, P < 0.05) and GFR (−41 ± 5%, P < 0.005). Renal blood flow (RBF) was significantly increased (+2.1 ± 0.5 ml/min, P < 0.05) after bosentan, whereas bosentan had no effect on MAP, GFR, and RBF in age-matched controls. These data show that the paracrine renal endothelin system is activated in PKD and participates in the regulation of MAP, GFR, RBF, and possibly contributes to renal cyst formation and fibrosis.

Autosomal dominant polycystic kidney disease (ADPKD), thought to be the most common hereditary kidney disease in humans, affects approximately 1 in 1000 live births. This disease accounts for up to 10% of all patients requiring renal replacement therapy. Cysts arise from renal tubular segments as focal areas of dilation. They progressively enlarge with age and may separate from the nephron of origin.

The Han:SPRD rat strain develops a form of progressive gender-dependent disease that appears similar in many respects to that seen in ADPKD in humans. ADPKD in humans as well as in Han:SPRD rats is characterized by structural alterations of the kidneys such as thickening of the tubular basement membrane, interstitial fibrosis, and formation of cysts, leading to end-stage kidney disease (1). The rat PKD gene was mapped on the rat chromosome 5, a quantitative trait locus controlling PKD, kidney mass, and plasma urea concentrations. The homology region is likely to reside on human chromosome 8. The gene responsible for PKD in Han:SPRD cy/+ rat is neither the PKD1 gene, localized on the short arm of human chromosome 16 encoding a high molecular weight protein of approximately 500,000 kD named polycystin, nor the PKD2 gene, localized on human chromosome 4 (2). Despite these recent advances in the determination of the genetic basis of PKD in humans and rats, little is known about the cell biology and underlying mechanisms that contribute to cyst formation in genetically or chemically induced animal models with renal cysts. Altered composition of the extracellular matrix (3) is thought to be implicated in cystogenesis. Interstitial fibrosis, glomerulosclerosis, and cyst formation were also seen in human endothelin-1 (ET-1) transgenic mice (4). In addition, the renal endothelin system seems to play a major role in renal disorders such as lupus nephritis, impaired renal function after 5/6 nephrectomy, and acute renal failure (reviewed in references 5 through 7). Thus, an activated paracrine renal endothelin system may play an important role in the pathogenesis of PKD.

We therefore analyzed the expression of ET-1 by immunohistochemistry, measured tissue concentrations of ET-1, and analyzed the expression of endothelin receptor subtypes by Scatchard analysis in the kidneys of male Han:SPRD rats compared with age-matched controls. In addition, the acute
effect of bosentan, a combined ET$_A$/ET$_B$ receptor antagonist (8), on renal blood flow (RBF), GFR, heart rate (HR), and BP was analyzed in conscious chronically instrumented PKD rats. Furthermore, we analyzed tissue sections from patients with typical ADPKD and could demonstrate a very high ET-1 expression within the epithelial cells of the cysts.

Materials and Methods

Male 6-wk-old heterozygous (cy/+) and homozygous (cy/cy), as well as 6-mo-old heterozygous (cy/+) Han:SPRD rats (9) and the corresponding age-matched Sprague Dawley rats (SD) (+/+ ) were analyzed. The animals (a generous gift from Dr. N. Gretz, Klinikum Mannheim, Mannheim, Germany) were fed a commercial diet (Altromin®; Altromin, Lange, Germany) and given water ad libitum. All animal experiments were conducted in accordance with local institutional guidelines for the care and use of laboratory animals. [125I]-ET-1 (2000 Ci/mmol) was obtained from DuPont (Hannover, Germany). Unlabeled ET-1 was from Peninsula Laboratories (Frankfurt, Germany). The mixed (A/B) endothelin receptor antagonist bosentan (Ro 47-0203, 4-tert-butyll-N-[6-(2-hydroxy-ethoxy)]=S-(2-methoxy-phenoxyl)-2,2'-bispypyridimine-4-yl]-benzenesulfonyamide) was a generous gift from Dr. Martine Clozel (Pharma Division, F. Hoffmann-La Roche Ltd., Basel, Switzerland). The selective endothelin receptor ligands (BQ 123 and BQ 3020) were from California Peptides, Inc. (Napa, CA). The polyclonal rabbit anti-ET-1 antibody was from Peninsula Laboratories. Unless otherwise stated, all reagents were of analytical grade and were purchased from Merck (Darmstadt, Germany), Boehringer-Mannheim (Mannheim, Germany), or Sigma (Munich, Germany).

Phenotypic Determination

The carrier status of each animal was established by determination of the kidney weight/body weight ratio, typical kidney histology, and serum and urine urea concentrations as recently described by Biborou et al. (2).

ET-1 Radioimmunoassay

Tissue Preparation. Kidney tissue-immunoreactive endothelin levels were measured as recently described (10). After thawing, kidney samples were suspended in 2 ml ⋅ g$^{-1}$ wet weight of 0.2 M CH$_3$COOH, 0.5 M NaCl, disrupted using a polytron and subsequently homogenized. The homogenate was centrifuged at 4°C for 15 min at 25,000 × g. The supernatant was retained for ET-1 RIA and the pellet was discarded.

Radioimmunoassay. Immunoreactive ET-1 was extracted from tissue supernatant using Amprep® 500 mg C2 columns (Amersham). One-milliliter samples were acidified with 0.25 ml of 2 M HCl, centrifuged at 10,000 × g for 5 min at room temperature, and loaded onto the columns. The columns were washed with 5 ml of water + 0.1% trifluoroacetic acid, and the adsorbed peptide was eluted with 2 ml of 80% methanol in water + 0.1% trifluoroacetic acid. The eluents were collected in polypropylene tubes and dried under a stream of nitrogen.

The probes were reconstituted in 250 μl of assay buffer (0.02 M borate buffer, pH 7.4, containing 0.1% sodium azide), and 2 × 100 μl were taken for analysis in a commercial ET-1 [125I]RIA kit (ET-1,2 (high sensitivity)) [125I]assay system, Amersham). Separation of the antibody-bound fraction was effected by magnetic separation using the Amerlex-M Separator (Amersham). This assay reacts 100% with ET-1 and cross-reacts 189% with Big ET-1. Cross-reactivity with ET-3 was less than 0.001.

Scatchard Analysis

Membrane Preparation. Membranes were prepared according to Nambi et al. (11). The rats were sacrificed and the kidneys were immediately frozen with liquid nitrogen and stored at −80°C until further analysis. Approximately 150 mg of kidney tissue was homogenized at 4°C in 10 ml of 20 mmol/L NaHCO$_3$, using a motor-driven pestle homogenizer. The homogenate was centrifuged at 4°C for 15 min at 1000 × g. The supernatant was decanted and centrifuged at 4°C for 30 min at 40,000 × g. The pellet, consisting of crude plasma membranes, was resuspended in assay buffer (1 mg/ml bacitracin, 100 mM Tris-HCl, 5 mM MgCl$_2$, and 0.1% bovine serum albumin [BSA], pH 7.4) at a protein concentration of 200 μg/ml.

Binding Assay for ET$_A$ and ET$_B$. Binding studies were performed as described previously (12). To analyze the expression of endothelin receptor subtypes (ET$_A$, ET$_B$) in the kidney, binding assays were performed in the presence or absence of the subtype-specific endothelin receptor ligands BQ123 (3 μM) and/or BQ3020 (5). The assay buffer for binding studies contained 1 mg ⋅ ml$^{-1}$ bacitracin, 100 mM Tris-HCl, 5 mM MgCl$_2$, and 0.1% BSA, pH 7.4, in a total volume of 150 μl. The [125I]-ET-1 tracer concentration was kept constant at 40,000 cpm/tube, whereas the concentration of unlabeled ET-1 was increased from 0 to 25 nM (competition studies with “cold saturation”). Samples from crude plasma membranes were used at a concentration of 0.53 mg of protein ⋅ ml$^{-1}$. Binding studies were performed at room temperature for 120 min. Nonspecific binding was assessed in the presence of excess ET-1 (5 μM). After adding 1 ml of cold binding buffer, free and receptor-bound radioactivity was separated by centrifugation at 30,000 × g (4°C) for 15 min, and the pellets thus obtained were washed two additional times with 1 ml of cold binding buffer. [125I] was counted in a Packard gamma counter (78% counting efficiency for [125I]).

Immunohistochemistry

Immunohistochemistry for the detection of ET-1 in the kidney was performed with minor modifications, as recently described by Schäfer et al. (3) and Bachmann and Ramasubbu (13), using a polyclonal rabbit anti-ET-1 antibody. Briefly, for antibody incubation, 5-μm thick cryostat sections were mounted on poly-L-lysine-coated glass slides. Polyclonal rabbit anti-ET-1 antibody (Peninsula Laboratories) was applied at dilutions of 1:50 in phosphate-buffered saline containing 1% BSA. Detection of the bound antibody was performed using a biotinylated second antibody and streptavidin-Texas red (Amersham Buchler, Braunschweig, Germany), according to the manufacturer’s instructions. Control experiments were performed omitting the first antibody and using phosphate-buffered saline instead.

For immunohistochemistry, male 6-wk-old heterozygous (cy/+) and homozygous (cy/cy), as well as 6-mo-old heterozygous (cy/+) Han:SPRD rats and the corresponding age-matched Sprague Dawley rats (SD) (+/+ ) were analyzed. In a second set of experiments, we analyzed kidney sections from patients with typical PKD compared with normal human kidney.

Measurement of GFR, Mean Arterial BP, HR, and RBF in Conscious, Chronically Instrumented Rats

Surgical Procedures. One week before the acute experiments, the rats were anesthetized with ether, and femoral artery and vein catheters were implanted, as recently described (14). Three days before starting the experiments, flow probes (1RB with implantable
connector, Transsonic Systems, Inc., Ithaca, NY) were chronically implanted around the left renal artery. The left kidney was exposed by a retroperitoneal access. The renal artery was carefully dissected. The flow probe was then placed around the artery and, after the best signal had been achieved, the probe was fixed in proper position using a small envelope of Merocel Op-Wipe® (Merocel Corp., Mystic, CN) covering the probe and the artery at the point of reflector attachment. To improve signal conductance, the envelope was filled with ultrasound gel. All catheters and cables were led subcutaneously to the rat’s neck (14).

**Circulatory Measurements.** Mean arterial BP (MAP) and HR were measured via the arterial line with a Statham pressure transducer P23Db and a Gould pressure processor coupled to a Gould Brush 2600 recorder. RBF was measured via the chronically implanted flow probe with a transit time flowmeter (T106, Transsonic Systems, Inc.) and continuously recorded on a Gould Brush 2600 recorder. The Transsonic flowmeter system determines absolute volume flow (15). The flow probes were precalibrated, and measured absolute blood flow with an accuracy of ±2%.

**Measurement of GFR.** GFR was measured using the inulin single-shot method (16). The single-shot clearance was evaluated by displaying a two-compartment model with resolution of the plasma inulin concentrations into two monoeponential functions. The rats received an intravenous bolus injection of 150 mg of inulin (Inustest®). Blood samples for determination of serum inulin concentrations were drawn at 0, 15, 30, 90, 135, and 180 min after injection. Inulin was measured by a modified β-fructosidase method (17). GFR is expressed as ml/min per 100 g body wt.

**Effects of the Mixed (A/B) Endothelin Receptor Antagonist Bosentan on MAP, HR, and RBF.** To examine the endogenous endothelin system in PKD rats, the effect of bosentan on resting MAP, HR, and RBF in 6-mo-old heterozygous (cy/+) Han:SPRD (PKD) rats and the corresponding age-matched Sprague Dawley littersmates (SD) was analyzed. The rats were divided into four groups: Group 1 (SD; n = 6) and group 2 (PKD; n = 7) received cumulative intravenous bolus injections of bosentan (10 mg/kg) every 15 min up to a total load of 100 mg/kg. Group 3 (SD; n = 6) and group 4 (PKD; n = 6) received intravenous bolus injections of vehicle.

**Effects of the Mixed (A/B) Endothelin Receptor Antagonist Bosentan on GFR.** To analyze the endogenous renal endothelin system in PKD rats, we tested the effect of bosentan on GFR in 6-mo-old heterozygous (cy+) Han:SPRD (PKD) rats and the corresponding age-matched Sprague Dawley littersmates (SD). The rats were divided into four groups: Group 1 (SD; n = 6) and group 2 (PKD, n = 6) received a single injection of 100 mg/kg bosentan, and group 3 (SD, n = 7) and group 4 (PKD, n = 7) received a single injection of placebo, followed by an injection of 150 mg of inulin 5 min later. At 0, 15, 30, 90, 135, and 180 min after injection of inulin, blood samples (200 μl) were taken from the arterial catheter for determination of serum inulin concentrations.

**Effects of Exogenously Applied ET-1 on BP and RBF.** In contrast to the above-described set of experiments analyzing the impact of the endogenous renal endothelin system in PKD rats, the following experiments were performed to analyze the effect of exogenously added ET-1 on BP and RBF. We tested the hemodynamic responses to increasing doses of ET-1 (20, 50, 100, and 200 ng of ET-1, intravenously) in 6-mo-old heterozygous (cy+/+) Han:SPRD (PKD) rats and corresponding age-matched Sprague Dawley littersmates. To avoid possible tachyphylaxis after repeated injections of ET-1, the rats received in independent experiments intravenous bolus injections of ET-1.

**Statistical Analyses**

The unpaired t test was used for determination of statistical difference of group means. ANOVA followed by t test was used if appropriate. Results were considered statistically significant at a value of P < 0.05.

**Results**

In 6-wk-old as well as in 6-mo-old rats with PKD, the kidneys of affected rats showed significantly elevated tissue concentrations of ET-1 compared with age-matched littermates. In 6-wk-old PKD rats, we observed a gene–dose effect, because tissue ET-1 concentrations were much higher in homozygous (cy/cy) Han:SPRD rats compared with heterozygous (cy/+ ) Han:SPRD rats (Figure 1).

Immunohistochemical analysis of 6-wk-old and 6-mo-old rats (Figure 2) with PKD revealed that the increased tissue ET-1 concentrations as described above are due to ET-1 expression mainly within the epithelial cells of the cysts (Figure 2). A weaker specific signal was obtained within the interstitial renal tissue, blood vessels, and glomeruli of homozygous (cy/ cy) and heterozygous (cy/+ ) Han:SPRD rats.

However, there are some differences with respect to the ET-1 fluorescence pattern of ET-1 in 6-wk-old homozygous and 6-mo-old heterozygous Han:SPRD rats. ET-1 staining of the epithelial cells of the cysts is stronger in 6-mo-old heterozygous PKD rats compared with 6-wk-old homozygous PKD rats. ET-1 staining of the interstitial tissue, on the other hand, appears stronger in homozygous PDK rats compared with heterozygous PKD rats. Control rats (6-wk-old as well as 6-mo-old littersmates) had a very low renal ET-1 tissue immunoreactivity near the detection limit of the method used in this study (Figure 2C).

Interestingly, a similar pattern of ET-1 immunoreactivity within the kidney was seen in kidney sections from patients with ADPKD (Figure 3). Kidneys were removed in these patients due to the size of the polycystic kidneys. We analyzed sections from four different patients (38 to 56 yr old; three men, one woman) with classic ADPKD compared with normal

**Figure 1.** Bar graph of renal tissue endothelin-1 (ET-1) concentrations in 6-wk-old and 6-mo-old rats with polycystic kidney disease (PKD) and the corresponding age-matched littersmates are shown. Data are means ± SEM.
Figure 2. (A) Kidney section from a 6-mo-old male heterozygous PKD rat (cy+/) (Hematoxylin and eosin [H&E] staining). (B) Immunohistochemical staining using an ET-1 antibody showing a highly increased ET-1 expression within the epithelial cells of renal cysts in the kidneys of a 6-mo-old male heterozygous PKD rat. (C) The corresponding age-matched littermate also stained with an ET-1 antibody showed only a very weak fluorescence signal within the tubules, blood vessels, and glomeruli. (D) Kidney section from a 6-wk-old male homozygous PKD rat (cy/cy) (H&E staining). (E and F) Immunohistochemical staining using an ET-1 antibody showing an increased ET-1 expression within the epithelial cells of renal cysts but also of the interstitial tissue in the kidneys of a 6-wk-old male homozygous PKD rat. ET-1 staining of the epithelial cells of the cysts is stronger in 6-mo-old heterozygous PKD rats (B) compared with 6-wk-old homozygous PKD rats (E), whereas staining of the interstitial tissue appears stronger in homozygous PKD rats (E and F) compared with heterozygous PKD rats (B).

human kidney tissue from nephrectomies due to kidney cancer. We always detect a very high ET-1 immunoreactivity in the epithelial cells of the renal cysts of ADPKD patients. Again, interstitial tissue also showed a specific ET-1 fluorescence signal. Normal kidney tissue from the nephrectomies due to kidney cancer showed only a very weak signal.
Scatchard analysis, on the other hand, revealed a markedly decreased $E_T$, as well as $E_B$ receptor density ($B_{max}$), in 6-wk-old and 6-mo-old affected PKD rats (Figure 4). In this case, an inverse gene-dose effect was observed. The receptor density was much more reduced in homozygous (cy/cy) Han:SPRD rats compared with heterozygous (cy/+) Han:SPRD rats (Figure 4).

The binding affinity of the $E_T$ and the $E_B$ receptor was slightly reduced in 6-wk-old PKD rats compared with control rats (Table 1), whereas the binding affinity in 6-mo-old PKD rats was only reduced for the $E_B$ receptor compared with controls (Table 1). ET-1 tissue concentrations and the expression of endothelin receptor subtypes in 6-mo-old homozygous (cy/cy) Han:SPRD rats could not be determined, because homozygous (cy/cy) Han:SPRD rats usually died at the age of approximately 10 wk due to end-stage renal disease.

Measurement of GFR, MAP, HR, and RBF in conscious, chronically instrumented rats was performed only in the 6-mo-old rats, because the methods used in our study are not suitable for very small animals such as 6-wk-old rats. Measurement of MAP, HR, and RBF was started 90 min before the functional experiments were performed and was completed 90 min after the last injection of bosentan. Mean basal MAP before the functional experiments were begun was 118.4 ± 9.3 mmHg in Han:SPRD (cy/+ ) rats and 109.8 ± 8.7 mmHg in the corresponding littermates. The basal MAP differences between Han:SPRD (cy/+ ) rats and the corresponding controls were not significant. The acute blockade of both endothelin receptor subtypes using bosentan (10 mg/kg intravenously every 15 min for 2.5 h up to a total load of 100 mg/kg) in 6-mo-old heterozygous PKD rats led to a significant decrease in MAP (Figure 5). No significant effect of bosentan on BP was seen in age-matched Sprague Dawley littermates, and no effect of bosentan was seen in the control group (Figure 5). RBF,
Table 1. ET<sub>A</sub> and ET<sub>B</sub> receptor binding affinities (K<sub>d</sub>) in the kidneys of 6-wk-old and 6-mo-old Han:SPRD and corresponding control rats<sup>a</sup>

<table>
<thead>
<tr>
<th>Category</th>
<th>6-wk-old</th>
<th>6-mo-old</th>
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<tr>
<td></td>
<td>Control SD</td>
<td>Heterozygous PKD</td>
</tr>
<tr>
<td>ET&lt;sub&gt;A&lt;/sub&gt;-receptor binding affinity (nmol/L)</td>
<td>0.24 ± 0.08</td>
<td>0.60 ± 0.01&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>ET&lt;sub&gt;B&lt;/sub&gt;-receptor binding affinity (nmol/L)</td>
<td>0.31 ± 0.01</td>
<td>0.68 ± 0.02&lt;sup&gt;b&lt;/sup&gt;</td>
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<sup>a</sup> Values are mean ± SEM. SD, Sprague Dawley; PKD, polycystic kidney disease.

<sup>b</sup> P < 0.01 compared with age-matched control rats (Sprague Dawley littermates).

However, was significantly increased in 6-mo-old heterozygous PKD rats. HR was not affected by bosentan in PKD or in Sprague Dawley rats (data not shown).

GFR was markedly reduced in 6-mo-old heterozygous PKD rats compared with control rats. A single bolus injection of bosentan led to a further significant (P < 0.005) decrease of GFR in PKD rats only (0.42 ± 0.06 ml/min per 100 g body wt in PKD rats treated with placebo and 0.29 ± 0.06 ml/min per 100 g body wt in PKD rats treated with bosentan) (Figure 5).

Intravenous bolus injections of 20, 50, 100, and 200 ng of ET-1 produced a dose-dependent biphasic blood pressure response: An initial, short-lasting depressor effect was followed by a long-lasting BP elevation. The changes in BP were accompanied by reciprocal alterations in HR. The observed effects of ET-1-induced alterations of the pressor as well as the depressor response were dose-dependent, and qualitatively and quantitatively similar in 6-mo-old rats with PKD and corresponding controls. RBF showed a dose-dependent monophasic response pattern to exogenously applied ET-1. RBF decreased to a similar extent in the Han:SPRD (cy/+) rats and the corresponding controls (data not shown).

Discussion

The paracrine endothelin system is highly activated in the kidneys of Han:SPRD-PKD rats. Renal tissue concentrations of ET-1 were 3.5 ± 0.3-fold increased in young cy/cy rats and even more in old cy/+ rats (6.2 ± 0.4-fold). This finding is probably due to a highly increased ET-1 synthesis within the epithelial cells of the cysts in Han:SPRD-PKD rats, as shown by immunohistochemistry. Patients with ADPKD are also characterized by a highly increased ET-1 synthesis within the epithelial cells of the kidney cysts.

Endothelin System in PKD Compared with Other Models of Chronic Renal Failure with Involvement of the Renal Endothelin System

It is important to note that the increase in renal tissue ET-1 immunoreactivity is much higher in rats with PKD than in any other animal model of chronic kidney disease reported thus far (4–7,10,12). ET-1 transgenic mice and ET-2 transgenic rats,
for example, are characterized by the development of a pathologic renal phenotype (4,10). This pathology developed in spite of only slightly elevated tissue ET-1 or ET-2 concentrations. Impaired renal function in rats with liver cirrhosis (12) is also associated with a significant but mild increase in renal tissue ET-1 concentrations. However, the pathophysiologic relevance of these findings in cirrhotic rats is clearly demonstrated, because the blockade of the endothelin system in these cirrhotic animals with bosentan resulted in a decreased water excretion and increased formation of ascites (12). Thus, our data strongly suggest that the stronger activation of the renal endothelin system—compared with the above-mentioned pathophysiologic conditions—in PKD (Han:SPRD rats as well as humans) may play a major role in the pathogenesis of PKD.

The finding of an increased ET-1 tissue concentration in PKD is in agreement with a recent report (18) showing increased ET-1 mRNA expression in the kidneys of a mouse PKD model (ckp mice). The role of the renal endothelin system in human PKD is supported by the finding of an inverse relationship between ET-1 concentrations and sodium concentration in the cyst fluid of patients with ADPKD (19).

**Renal Endothelin System as a Cofactor in the Development of Renal Cysts**

Overexpression of the human ET-1 gene in the kidneys of male ET-1 transgenic NMRI mice promoted renal cyst growth (4). Nontransgenic NMRI mice develop only a small number of small renal cysts. Therefore, we propose that primary genetic alterations such as mutations in the PKD1 or PKD2 gene in humans or mutations within a yet unknown gene on rat chromosome 5, leading to the development of PKD in rats (2), might cause a downstream secondary activation of the renal endothelin system, thus promoting growth and formation of renal cysts. The hypothesis that ET-1 seems to be an important cofactor in the pathogenesis of renal cysts requiring additional primary genetic or environmental stimuli is supported by: (1) the finding that ET-2 transgenic rats (10) and their corresponding nontransgenic littermates (rats without genetic predisposition for renal cysts) did not develop renal cysts (B. Hocher, unpublished observation); and (2) the finding that patients with typical ADPKD are also characterized by a highly increased ET-1 synthesis within the epithelial cells of the cysts (Figure 3), as seen in PKD rats (Figures 1 and 2).

The finding of an activated renal endothelin system in patients with ADPKD is of clinical relevance, because the therapeutic strategies currently available are limited (e.g., lowering of BP and low protein diet).

Kidney cysts in PKD arise from renal tubular segments as focal areas of dilation. Tubular epithelial cells mainly express ET$_B$ receptors (10,12,20), and the ET$_B$ receptor is involved in the growth of tubular cells, as demonstrated recently by Ong et al. (21). Thus, ET$_B$ blockade may reduce cyst formation in PKD.

However, there have been no studies analyzing long-term effects of ET$_A$ or ET$_B$ receptor blockade in humans or in animal models of PKD that might prove the hypothesis that the renal endothelin system is a major progression factor of chronic renal failure in PKD, as suggested by the present study and the findings in ET-1 transgenic mice (4).

**Downregulation of Endothelin Receptors in Han:SPRD Rats**

The data presented in this study suggest that the highly increased ET-1 synthesis in Han:SPRD rats resulted in a reactive downregulation of the receptor density of both endothelin receptor subtypes. These results are in agreement with recent in vitro experiments showing a downregulation of endothelin receptors in response to increased autocrine production of ET-1 (22).

Furthermore, the binding constants of both endothelin receptor subtypes in the kidney of PKD rats are, with the exception of the ET$_A$ receptor in 6-mo-old PKD rats, approximately two times higher compared with Sprague Dawley littersmates. Thus far there are no reports showing that such a slight alteration of the binding affinity is of pathophysiologic relevance. Posttranslational structural alterations of endothelin receptors (phosphorylation or glycosylation) may explain these findings. N-glycosylation sites were identified in the outer cell domain of both endothelin receptor subtypes (23,24). In ckp mice with PKD, however, a yet unknown factor/mechanism increases both the expression of the ET-1 mRNA and the expression of the ET$_A$ and ET$_B$ receptor mRNA (18).

To analyze the pathophysiologic consequences of simultaneous upregulation of tissue ET-1 and downregulation of both endothelin receptor subtypes in Han:SPRD (cy/+) rats (Figures 1 and 4), we blocked the endogenous endothelin system using bosentan. These experiments demonstrate that the renal endothelin system in Han:SPRD (cy/+) rats is involved in the regulation of BP, GFR, and RBF despite the downregulation of endothelin receptors, because MAP, GFR, and RBF were significantly altered after acute blockade of both endothelin receptors in PKD rats only (Figure 5). In the corresponding Sprague Dawley littersmates, none of these parameters was significantly modified. In addition, the response to increasing doses of exogenously applied ET-1 was similar in PKD and control rats.

Both the blockade of the endogenous endothelin system using bosentan and the results after exogenous application of ET-1 indicate that the downregulation of endothelin receptors in PKD rats does not reduce or even abolish the biological effects of the high endogenous renal ET-1 concentrations and of exogenously applied ET-1 in PKD rats. Thus, postreceptor mechanisms are obviously counteracting the downregulation of the endothelin receptors in rats with PKD.

**Effects of Bosentan on BP, RBF, and GFR**

A major finding after acute blockade of the highly activated endogenous endothelin system in PKD rats using bosentan was the reduction of BP (−19.7 ± 2.1 mmHg), whereas the same dose of bosentan had no significant effect on MAP in Sprague Dawley littersmates (Figure 5). These data indicate that the paracrine endothelin system in Han:SPRD (cy/+) rats contributes substantially to the regulation of BP in PKD rats. The BP-lowering effect of the combined ET$_A$/ET$_B$ receptor antag-
onist in Han:SPRD (cy/+) rats is remarkable, because an activated endothelin system causes or contributes in general to structural alterations in cardiovascular (25) and kidney tissue (e.g., glomerulosclerosis and interstitial fibrosis), but does not affect BP as seen in ET-1 transgenic mice (4) and ET-2 transgenic rats (10). The much higher ET-1 tissue concentrations in PKD rats compared with the above-mentioned transgenic animal models of the endothelin system (4,10) may explain the additional hemodynamic effects of the endothelin system in PKD rats. Using immunohistologic techniques, we could demonstrate that the major sites of ET-1 expression in 6-mo-old PKD rats are the epithelial cells of the renal cysts. We propose that the ET-1 synthesized in the epithelial cells of the cysts migrates/diffuses in a passive manner to the interstitial tissue, blood vessels, and glomeruli, thus contributing to the regulation of BP and GFR.

GFR and RBF were also significantly altered after acute blockade of the renal endothelin system using bosentan in PKD rats. However, these alterations were less pronounced compared with the BP-lowering effect of bosentan in PKD rats. The reduction of MAP after a single injection of bosentan in PKD rats may explain the bosentan-induced reduction of GFR, probably by reducing BP in PKD rats below the setpoint of GFR autoregulation.

In conclusion, the present study shows that the paracrine renal endothelin system is activated in rats with PKD, may contribute to renal cyst formation and renal fibrosis, and is also involved in the regulation of BP, GFR, and RBF in PKD. The finding that patients with ADPKD are also characterized by a highly increased ET-1 synthesis within the epithelial cells of the kidney cysts suggests that the renal endothelin system might be involved in human ADPKD as well.

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