

Activity and functional significance of the renal kallikrein-kinin-system in polycystic kidney disease of the rat

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Activity and functional significance of the renal kallikrein-kinin-system in polycystic kidney disease of the rat.

Background. The kallikrein-kinin-system is a complex multi-enzymatic system that has been implicated in the control of systemic blood pressure, glomerular filtration rate, and proteinuria. The present study investigated its functional role in rat polycystic kidney disease (PKD), which is characterized by progressive renal failure and proteinuria in the absence of systemic hypertension and stimulated renin-angiotensin-system.

Methods. Kallikrein and bradykinin levels were measured in plasma and urine of rats with polycystic kidneys and compared to non-affected controls (SD) and rats with reduced renal mass. The functional relevance of the kallikrein-kinin system (KKS) was assessed by the effects of a short-term treatment with either a selective bradykinin (BK) B1-receptor antagonist (des-Arg9-[Leu8]-BK), a B2-receptor antagonist (HOE 140), an angiotensin converting enzyme inhibitor (ramipril), or an angiotensin II-receptor blocker (HR 720) on systemic and renal parameters.

Results. Urine levels of kallikrein were increased threefold in 9-month-old PKD, and BK excretion was increased tenfold in 3-month and 30-fold in 9-month-old PKD compared to age-matched SD rats. Blood pressure in 9-month-old PKD rats was decreased to the same degree by ramipril and HR 720. In contrast, only ramipril and HOE 140 significantly reduced proteinuria and albuminuria, independent from creatinine clearance. This effect was accompanied by an increased excretion of bradykinin. The B1 receptor antagonist had no influence on functional renal parameters.

Conclusions. The present study demonstrates an age-dependent activation of the renal KKS in rats with polycystic kidney disease. The bradykinin B2-receptor is involved in the pathogenesis of proteinuria, independent from systemic blood pressure or creatinine clearance. The antiproteinuric effect of ramipril in this model is angiotensin II-independent and related to its influence on the renal KKS.

Key words: bradykinin, PKD, reduced renal mass, HR 720, HOE 140, des-Arg9-[Leu8]-bradykinin, ramipril, renin-angiotensin system, ADPKD.

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Autosomal dominant polycystic kidney disease (ADPKD) is one of the most common genetic disorders worldwide, with a frequency of 1 in 1000 in the general population. It is the cause of approximately 10% of end-stage renal failure in patients undergoing chronic renal replacement therapy [1]. Morphologically it is characterized by the formation of multiple cysts and considerable enlargement of both kidneys. The clinical course is characterized by progressive loss of renal function in 50% to 75% of affected patients becoming manifest generally during the fourth to fifth decade of life. Although the genes responsible for most ADPKD cases have been identified [2, 3], the high degree of variability in the clinical course of patients is not well understood.

Knowledge of factors that modulate the course of renal disease in these patients would therefore be of prognostic value, and their identification could help establish therapeutical strategies aimed at delaying the progression of renal failure in subjects with ADPKD. Importantly, hypertension and proteinuria have been implicated in a more severe disease progression in ADPKD patients [4–6]. This situation is, however, not unique to ADPKD, but is observed in a large number of kidney diseases. Lowering of arterial blood pressure and/or proteinuria therefore remains a primary therapeutical goal. In most of these diseases, the renin-angiotensin-system (RAS) plays a seminal role with respect to arterial hypertension, regulation of glomerular filtration rate (GFR), development of proteinuria, and progression of chronic renal failure. This concept has been demonstrated by the nephroprotective effect of pharmacological blockade of the RAS in diabetic nephropathy [7], chronic allograft nephropathy [8], and remnant kidney model [9], to name only a few.

Biological effects of the RAS are not only directly exerted by stimulation of angiotensin receptors, but are also the result of a complex interplay with a number of other neurohumoral systems. One of them is the kallikrein-kinin-system (KKS). Comparable to the RAS, it

is composed of a series of potent vasoactive and proinflammatory molecules. An involvement in inflammatory processes has been demonstrated both in vitro and in vivo [10, 11]. The KKS also is activated in the advanced stage of diabetic nephropathy in streptozotocin (STZ)-treated rats, a condition characterized by proteinuria and decreased GFR [12]. Given the pronounced tubulointerstitial inflammation, the development of progressive renal failure and proteinuria, and the high incidence of arterial hypertension, the KKS may be activated in patients with ADPKD. Thus far, however, this has not been systematically investigated.

The (cy/+) rat is an animal model for ADPKD that shares many clinical and histological features with human ADPKD [13]. It provides a suitable tool to investigate the activity of the RAS and the KKS and their contribution to functional renal changes observed during the progression of renal failure. Their respective roles in regulation of blood pressure and proteinuria were investigated in the present study by short-term application of an inhibitor of the angiotensin-converting enzyme (ACE), ramipril, a highly potent angiotensin II receptor type 1 antagonist, HR 720, a selective BK-1 receptor antagonist (des-Arg9-[Leu8]-BK), and a selective bradykinin B2-receptor antagonist, HOE 140.

METHODS

Animals

Male homozygous unaffected (SD) and heterozygous affected (PKD) Han:SPRD rats inbred to the 20th generation in animal laboratories of the Center for Medical Research at the University Medical Center Mannheim, Germany, were used in the experiments. Animals were included in the study at the age of three and nine months. Heterozygous rats were detected by manual palpation of the polycystic kidneys. All experiments were performed in conscious animals. Environmental conditions were kept constant: temperature 20°C, air humidity 65% and a day/night cycle of 12 hours each. All rats were allowed free access to tap water and to a standard laboratory rat chow containing 19% protein (Altromin 1324®, Lage, Germany). All animal experiments were conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals.

Experimental protocol 1: KKS and ADPKD

Five groups of animals were studied: group 1 (3-month-old PKD; $N = 15$), group 2 (3-month-old SD; $N = 9$), group 3 (9-month-old PKD; $N = 26$), group 4 (9-month-old SD; $N = 18$), and group 5 (9-month-old SD with renal mass reduction; $N = 8$). Renal ablation was performed as previously described [14]. Briefly, in 6-month-old SD rats, the left kidney was exteriorized through a dorsal approach, and the upper and lower poles were surgically

resected. Two weeks later, uninephrectomy of the right kidney was performed. Animals were included in the experiments at the age of 9 months.

Rats were housed in metabolic cages twice for 24 hours. During the first 24 hours urine was collected in conventional tubes for determination of volume, creatinine, sodium, protein, prekallikrein and kallikrein. A blood sample was obtained for determination of creatinine, sodium, plasma renin activity, prekallikrein and kallikrein. Blood pressure was measured by tail plethysmography, for which all animals had been previously trained. After 24 hours of rest the animals were again housed in metabolic cages for 24 hours. Urine for determination of bradykinin was collected in tubes containing pure ethanol to arrest kinin formation and degradation. Volume of ethanol in the collection tube was chosen such that the concentration of urine at the end of the collection period did not exceed 30% of total volume.

Experimental protocol 2: Effects of ramipril, HR 720, HOE 140, and des-Arg9-[Leu8]-BK on renal function in PKD

Three- and nine-month old PKD and SD were treated for four days as follows: ramipril (2 mg/kg/day orally; $N = 10$, each group), HR 720 (10 mg/kg/day orally; $N = 10$, each group), HOE 140 (200 µg SC injection twice daily; $N = 10$, each group), or des-Arg9-[Leu8]-bradykinin (250 µg SC injection twice daily; $N = 10$, each group) in random order, with a two-week pause between each four-day-treatment period. Drugs dissolved in drinking water were given to the rats every day during the study period. Before and after the treatment period, blood samples were obtained for determination of creatinine, sodium and plasma renin activity. Urine was collected for determination of creatinine, sodium and protein. In addition, urinary excretion of prekallikrein, kallikrein, and bradykinin was determined before and after administration of each drug in 9-month-old PKD rats. Systolic blood pressure was measured by tail plethysmography.

Measurement of sodium, creatinine and protein in serum and urine

Concentration of sodium in serum and urine was determined by use of a flame photometer (FLM-3; Radiometer, Copenhagen, Denmark). Concentrations of creatinine in serum and urine were measured by standardized colorimetric method (Hitachi Autoanalyser; Boehringer Mannheim, Mannheim, Germany) and are expressed as mg/dL. Urinary protein concentration was determined following the method of Coomassie after centrifugation of urine samples. Values for creatinine clearance and fractional sodium excretion were calculated using standard formulas.

Measurement of albumin in urine

Urinary albumin was measured using a commercially available kit (Nephrat; Exocell, Inc., Philadelphia, PA, USA). Urine samples were added to wells bearing anti-rat-albumin on the solid phase. After 20 minutes at room temperature rat-albumin-HRP conjugate was added (without washing), and a 45 minute incubation was completed. Wells were washed sixfold with phosphate-buffered saline (PBS; Gibco BRL, Paisley, Scotland, UK)/0.05% Tween 20 (Sigma, St. Louis, MO, USA), and bound conjugate was analyzed using TMB (3,3',5,5'-tetramethylbenzidine) substrate solution. The chromogenic reaction was stopped with diluted sulfuric acid, and color intensity determined using a microplate reader set at 450 nm.

Measurement of plasmin renin activity

Plasma renin activity was determined by radioimmunoassay as described by Fyhrquist et al [15]. Plasma renin values are expressed as ng angiotensin-1 (Ang-1)/mL/h.

Measurement of prekallikrein and kallikrein in urine

Activity of prekallikrein and kallikrein was determined following the method described by Amundsen et al [16]. The chromogenic peptide substrate H-D-Val-Leu-Arg-p-nitroanilide (S-2266; Haemochrom Diagnostika, Essen, Germany) was used in this assay, and extinction was measured photometrically at 405 nm. Each sample was compared to a negative control sample in which kallikrein was inhibited by the addition of aprotinine (100 KIU/mL). Activity of kallikrein in urine was derived from extinction difference between the two samples and expressed as units per liter (U/L). One unit is the amount of kallikrein that converts 1 μ mol of substrate under standardized conditions.

Measurement of prekallikrein and kallikrein in plasma

Enzymatic activity of plasma prekallikrein and kallikrein was determined with the amidolytic assay described by Gallimore and Friberger [17]. In this assay the chromogenic substrate H-D-Pro-Phe-Arg-p-nitroanilide (S2302; Haemochrom Diagnostika, Essen, Germany) was used.

Measurement of bradykinin in urine

After ethanol extraction and high-pressure liquid chromatography (HPLC) separation (ODP 50; Asahi-Pak) the concentration of bradykinin was measured using the radioimmunoassay developed in our laboratories [18]. Highly sensitive and specific antisera were raised against BK. 125 I-BK(Tyr⁸) was used as tracer. DesArg⁹-BK displayed a cross-reactivity of 24%. All other natural kinin derivatives showed a cross-reactivity of less than 1%.

Drugs

Ramipril, HR 720, and HOE 140 were a generous gift from Professor B.A. Schölkens (Hoechst AG, Frankfurt/Main, Germany). Des-Arg⁹-[Leu⁸]-bradykinin was purchased from Sigma-Aldrich (Deisenhofen, Germany).

Data calculation and statistical evaluation

All data are expressed as means \pm SEM. Differences between groups were assessed by one-way analysis of variance (ANOVA) when appropriate. Two-tailed paired *t* test with Bonferroni's correction for adjustment for multiple testing was used after ANOVA for further pair-wise comparison of the groups. Effects of treatment regimens were assessed by the Student *t* test for paired values. Statistical significance was defined as $P < 0.05$.

RESULTS

KKS and ADPKD

Creatinine clearance was lower in 3-month-old PKD compared to 3-month-old SD ($P < 0.05$) and lower in 9-month-old PKD and 9-month-old SD with renal ablation compared to 9-month-old SD ($P < 0.05$, respectively), whereas there was no difference between 9-month-old PKD and rats with remnant kidney. Creatinine clearance was reduced in 9-month-old SD in comparison to the young SD ($P < 0.05$), as was the clearance in old PKD in comparison to young PKD rats ($P < 0.05$; Table 1).

Fractional sodium excretion did not differ between 3-month-old SD and PKD, but was significantly higher in 9-month-old PKD compared to age-matched SD ($P < 0.05$). SD with reduced renal mass showed a significant higher fractional sodium excretion than 9-month-old PKD animals ($P < 0.05$; Table 1).

Plasma renin activity did not differ between 3-month-old SD and PKD, whereas it was suppressed in 9-month-old PKD compared to both 9-month-old SD and rats with reduced renal mass ($P < 0.01$, respectively). Proteinuria did not differ between young SD and PKD, but was significantly increased to the same level in both 9-month-old PKD and rats with reduced renal mass in comparison to 9-month-old SD ($P < 0.01$, respectively). Albuminuria was significantly increased in PKD and nephrectomized rats compared to age-matched SD ($P < 0.05$, respectively).

Systolic blood pressure did not significantly differ between the four groups of SD and PKD (although there was a trend toward higher values in older rats), but was elevated in rats with renal ablation ($P < 0.05$, compared to 9-month-old SD and PKD; Table 1).

Plasma levels for prekallikrein and kallikrein did not differ between the four groups of SD and PKD, but were significantly higher in SD with remnant kidney ($P <$

Table 1. Creatinine clearance, fractional sodium excretion, plasma renin activity, proteinuria, albuminuria, and systolic blood pressure in SD, PKD, and SD with reduced renal mass

	3 months		9 months		5/6 Nx N = 8
	SD N = 9	PKD N = 15	SD N = 18	PKD N = 26	
Creatinine clearance <i>mL/min/100 g</i>	0.65 ± 0.04	0.54 ± 0.02 ^{ac}	0.51 ± 0.01	0.42 ± 0.02 ^{ac}	0.43 ± 0.02 ^e
Fractional sodium excretion %	0.34 ± 0.01	0.41 ± 0.02	0.24 ± 0.02	0.85 ± 0.02 ^c	1.08 ± 0.1 ^{eg}
Plasma renin activity <i>ng Ang I/mL/h</i>	4.04 ± 0.47	3.97 ± 0.36	4.03 ± 0.28	2.47 ± 0.23 ^d	4.18 ± 0.54 ^g
Proteinuria <i>mg/24 h</i>	22 ± 2	24 ± 2	29 ± 3	175 ± 16 ^{hd}	183 ± 25 ^f
Albuminuria <i>mg/24 h</i>	1.35 ± 0.25	3.80 ± 0.56 ^c	2.68 ± 0.44	16.46 ± 1.28 ^{hd}	14.50 ± 1.92 ^f
Systolic blood pressure <i>mm Hg</i>	134 ± 8	139 ± 4	144 ± 6	149 ± 4	168 ± 7 ^{eg}

Values for creatinine clearance (mL/min/100 g), fractional sodium excretion (%), plasma renin activity (ng Ang I/mL/h), proteinuria (mg/24 h), albuminuria (mg/24 h), and systolic blood pressure (mm Hg) in 3- and 9-month-old SD and PKD and 9-month-old 5/6 nephrectomized SD. Data are presented as means ± SEM; N = number of animals.

^{a,b}P < 0.05, 0.01; 9-month-old SD and PKD compared to 3-month-old SD and PKD, respectively

^{c,d}P < 0.05, 0.01; 3- and 9-month-old PKD compared to age-matched SD

^{e,f}P < 0.05, 0.01; 5/6 Nx compared to 9-month-old SD

^{g,h}P < 0.05, 0.01; 5/6 Nx compared to 9-month-old PKD

Table 2. Values for prekallikrein, kallikrein, and bradykinin in SD, PKD and 5/6 Nx

	3 months		9 months		5/6 Nx N = 8
	SD N = 9	PKD N = 15	SD N = 18	PKD N = 26	
Plasma prekallikrein <i>U/mL</i>	2.27 ± 0.15	2.05 ± 0.30	2.51 ± 0.15	2.55 ± 0.28	3.45 ± 0.27 ^{cd}
Plasma kallikrein <i>U/L</i>	243 ± 8	238 ± 10	250 ± 10	219 ± 10	354 ± 17 ^{cd}
Urine prekallikrein <i>mU/24 h</i>	1216 ± 152	1464 ± 74	506 ± 85 ^a	713 ± 88 ^a	315 ± 42 ^d
Urine kallikrein <i>mU/24 h</i>	218 ± 26	266 ± 23	185 ± 30	687 ± 88 ^c	173 ± 26 ^d
Urine bradykinin <i>ng/24 h</i>	2.0 ± 0.2	15.2 ± 3.6 ^b	5.8 ± 1.1	131.0 ± 24.0 ^b	51.7 ± 19.8 ^c

Values for plasma prekallikrein (U/mL), plasma kallikrein (U/L), urine prekallikrein (mU/24 h), urine kallikrein (mU/24 h), and urine bradykinin (ng/24 h) in 3- and 9-month-old SD and PKD and 9-month-old 5/6 nephrectomized SD. Data are presented as means ± SEM. N = number of animals.

^aP < 0.05 compared to 3-month-old SD or PKD

^bP < 0.05 compared to age-matched SD

^cP < 0.05; 5/6 Nx compared to 9-month-old SD

^dP < 0.05; 5/6 Nx compared to 9-month-old PKD

^eP = 0.06 compared to 9-month-old SD

0.05, compared to 9-month-old SD and PKD; Table 2). Urinary excretion of prekallikrein was reduced in 9-month-old SD and PKD in comparison to young controls ($P < 0.05$, respectively), whereas it did not differ between SD and PKD. Urinary prekallikrein was further reduced in rats with reduced renal mass when compared to old PKD ($P < 0.05$). Urine excretion of kallikrein was markedly elevated in old PKD compared to both young PKD and old SD ($P < 0.05$, respectively), whereas excretion in partially nephrectomized rats did not differ from 9-month-old SD (Table 2).

Urinary bradykinin excretion was increased tenfold in 3-month-old PKD and 30-fold in 9-month-old PKD compared to age-matched SD ($P < 0.05$, respectively). In remnant kidney there was a trend toward higher bradykinin levels in comparison to old SD ($P = 0.06$).

Chromatographic separation by HPLC of the urinary bradykinin fraction in 9-month-old PKD revealed a second activity peak. This peak could be identified as des-Arg9-bradykinin, or T-kinin. Its concentration made up 13% of total bradykinin concentration in old PKD. T-kinin could not be demonstrated by HPLC in any of the other investigated groups (data not shown).

Effect of ramipril, HR 720, HOE 140, and des-Arg9-[Leu8]-BK on renal function in PKD

No influence on creatinine clearance, fractional sodium excretion, and urine volume was found in the different treatment groups (Table 3; data only shown for creatinine clearance).

Plasma renin activity was markedly increased by ramipril and HR 720 in all groups ($P < 0.05$). Although this increase was numerically higher in ramipril-treated rats, it did not statistically differ from animals treated with HR 720. Bradykinin-receptor blockade had no influence on PRA (Table 4).

Systolic blood pressure was not influenced in 3-month-old SD and PKD (Fig. 1), whereas a significant decrease was observed in 9-month-old PKD and 9-month-old SD treated with ramipril and HR 720 ($P < 0.05$). HOE 140 and des-Arg9-BK had no effect on systolic blood pressure.

Ramipril and HOE 140 significantly reduced proteinuria in 3- and 9-month-old PKD, whereas ramipril and HOE 140 had no influence on proteinuria in 3- and 9-month-old SD. HR 720 and des-Arg9-BK had no statistically significant effect on the proteinuria in all groups

Table 3. Creatinine clearance before and after treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg s.c. HOE 140, or 2 × 250 µg des-Arg9-[Leu8]-BK for four days

	Ramipril		HR 720		HOE 140		Des-Arg9-BK	
	Before	After	Before	After	Before	After	Before	After
SD 3 N = 10	0.71 ± 0.03	0.63 ± 0.02	0.74 ± 0.03	0.68 ± 0.03	0.64 ± 0.04	0.59 ± 0.02	0.69 ± 0.06	0.68 ± 0.05
PKD 3 N = 10	0.59 ± 0.02	0.63 ± 0.02	0.58 ± 0.04	0.60 ± 0.02	0.59 ± 0.03	0.61 ± 0.03	0.57 ± 0.04	0.58 ± 0.05
SD 9 N = 10	0.52 ± 0.05	0.50 ± 0.04	0.52 ± 0.05	0.53 ± 0.04	0.49 ± 0.03	0.50 ± 0.05	0.55 ± 0.06	0.56 ± 0.05
PKD 9 N = 10	0.45 ± 0.04	0.41 ± 0.04	0.46 ± 0.04	0.36 ± 0.04	0.43 ± 0.4	0.37 ± 0.03	0.41 ± 0.05	0.38 ± 0.04

Values for creatinine clearance (mL/min/100 g) before and after treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg s.c. HOE 140, or 2 × 250 µg des-Arg9-[Leu8]-BK for four days. Data are presented as means ± SEM. N = number of animals.

Table 4. Plasma renin activity before and after treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg s.c. HOE 140, or 2 × 250 µg des-Arg9-[Leu8]-BK for four days

	Ramipril		HR 720		HOE 140		Des-Arg9-BK	
	Before	After	Before	After	Before	After	Before	After
SD 3 N = 10	4.1 ± 0.8	107 ± 24 ^a	4.1 ± 0.7	10.1 ± 1.5 ^a	4.2 ± 0.8	4.8 ± 0.3	3.6 ± 0.9	5.2 ± 1.4
PKD 3 N = 10	3.9 ± 0.5	53 ± 10.8 ^a	4.1 ± 0.4	15.8 ± 1.7 ^b	3.8 ± 0.6	3.2 ± 0.3	3.4 ± 0.7	3.6 ± 0.5
SD 9 N = 10	4.1 ± 0.4	131 ± 15 ^b	4.5 ± 0.5	13.0 ± 1.6 ^a	4.2 ± 0.4	5.9 ± 0.9	3.9 ± 0.6	4.2 ± 0.5
PKD 9 N = 10	2.5 ± 0.3	43.0 ± 7.1 ^b	2.5 ± 0.3	8.3 ± 1.6 ^a	2.3 ± 0.4	1.8 ± 0.6	2.1 ± 0.2	2.6 ± 0.7

Plasma renin activity (PRA; ng Ang 1/mL/h) before and after treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg s.c. HOE 140, or 2 × 250 µg des-Arg9-[Leu8]-BK for four days. Data are presented as means ± SEM. N = number of animals.

^aP < 0.05, ^b0.01, treatment effect compared to baseline value

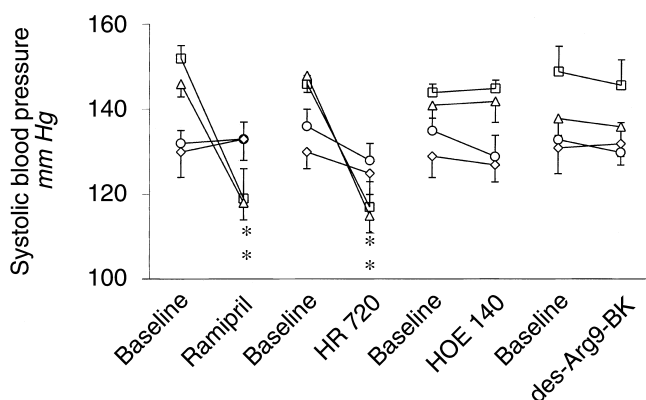


Fig. 1. Effect of treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg SC HOE 140, or des-Arg9-BK for four days on systolic blood pressure in 3- and 9-month-old non-affected controls (SD) and polycystic kidney disease (PKD) rats (N = 10, each group). Symbols are: (□) 9-month-old PKD; (△) 9-month-old SD; (○) 3-month-old PKD; (◇) 3-month-old SD. Systolic blood pressure was significantly reduced in 9-month-old PKD and SD after treatment with ramipril and HR 720 (*P < 0.05). Data are presented as means ± SEM.

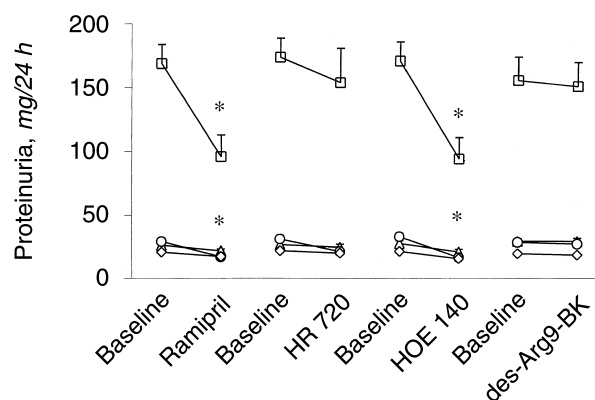


Fig. 2. Effects of treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg SC HOE 140, or des-Arg9-BK for four days on proteinuria in 3- and 9-month-old SD and PKD rats (N = 10, each group). Symbols are: (□) 9-month-old PKD; (△) 9-month-old SD; (○) 3-month-old PKD; (◇) 3-month-old SD. Proteinuria was significantly reduced in 3- and 9-month-old PKD after treatment with ramipril and HOE 140 (*P < 0.05). Data are presented as means ± SEM.

(Fig. 2). Similarly, albuminuria was significantly lowered by ramipril and HOE 140 only in the 3- and 9-month-old PKD rats (Fig. 3).

Ramipril had no influence on urinary excretion of pre- and kallikrein, whereas bradykinin excretion was markedly increased (Table 5). HR 720 had no effect on these parameters. Selective bradykinin-B2 receptor blockade increased excretion of pre- and kallikrein as well as of bradykinin, whereas B1 receptor blockade decreased excretion of all these molecules.

DISCUSSION

The present study demonstrates an age-dependent increase in the activity of the renal KKS in the Han:SPRD rat model of ADPKD, which paralleled the development of progressive renal failure.

In addition to a marked increase in urinary excretion of kallikrein and bradykinin, we showed that 9-month-old PKD rats excrete T-kinin (desArg9-bradykinin). This inflammatory peptide was first isolated in rat plasma [19, 20], where it is cleaved from its biosynthetic precursor

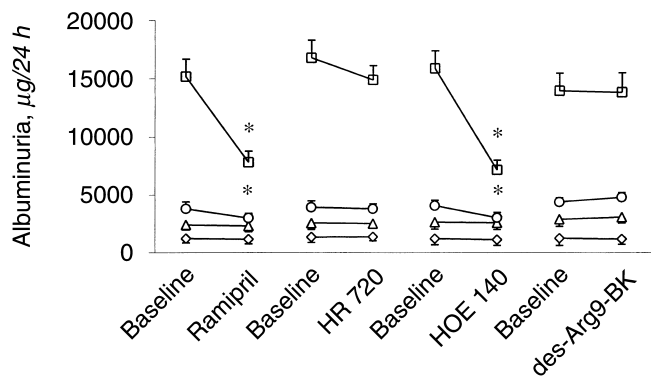


Fig. 3. Effect of treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg SC HOE 140, or des-Arg9-BK for four days on albuminuria in 3- and 9-month-old SD and PKD rats ($N = 10$, each group). Symbols are: (□) 9-month-old PKD; (△) 9-month-old SD; (○) 3-month-old PKD; (◇) 3-month-old SD. Albuminuria was significantly reduced in 3- and 9-month-old PKD after treatment with ramipril and HOE 140 (* $P < 0.05$). Data are presented as means ± SEM.

T-kininogen. Urinary kinins in Sprague-Dawley rats have been identified as bradykinin, whereas kallidin, arginyl-BK, or T-kinin could not be detected [21]. Our study is, to our knowledge, the first demonstration of a urinary excretion of T-kinin in rats.

Previous experimental studies have reported a reduced excretion of kallikrein in acute renal failure [22], anti-glomerular basement membrane nephritis and aminonucleoside nephrosis [22], unilateral nephrectomy [23], and diabetic nephropathy [12]. No kallikrein inhibitors could be found in the urine of diseased animals, and it was concluded that tubular damage and/or destruction of kallikrein-producing interstitial cells were the most likely cause of decreased urinary kallikrein excretion. Compared to rats with polycystic kidney disease, animals subjected to renal mass reduction in our study excreted significantly less kallikrein and also less bradykinin. Because creatinine clearance and proteinuria were virtually identical in these animals, our findings strongly suggest disease-specific differences in the activity of the renal kallikrein-kinin-system.

The treatment of polycystic kidney rats with drugs that interfere at different levels with the RAS and the KKS provides important new insights in the pathophysiology of PKD. Both ACE inhibitors and angiotensin II-type 1 receptor antagonists markedly reduced systolic blood pressure to the same degree in 9-month-old PKD rats, whereas bradykinin B1- and B2-receptor antagonists had no effect. Systemic blood pressure regulation in PKD therefore depends on the action of angiotensin II, despite a suppressed plasma renin activity in these animals [13]. The KKS obviously does not play a role in blood pressure regulation in polycystic kidney disease rats.

In contrast, proteinuria and albuminuria were signifi-

cantly decreased by treatment with ramipril and HOE 140. Angiotensin II receptor-blockade had no effect on protein excretion, despite an equal suppression of the RAS, as indicated by a significant increase of PRA and a blood pressure reduction equal to ramipril. Thus, an angiotensin II-independent pathway is responsible for the proteinuria in PKD rats.

The role of the KKS with respect to proteinuria has been studied in several experiments. Infusion of kallikrein into the renal artery of dogs causes proteinuria, which can be reversed by the kallikrein inhibitor aprotinin [24]. Bradykinin and related kinins are potent stimulators of phospholipase A₂ and promote synthesis of arachidonic acid metabolites, including thromboxane A₂ [25], which has been implicated in the pathogenesis of proteinuria in nephrotic syndrome [26]. The results of the present study are consistent with experimental results in rat models with normal or suppressed plasma renin levels, where effects of ACE inhibition were related to the interference with the KKS and not to inhibition of angiotensin II production. Hutchison, Webster and Jaffa showed that aprotinin could prevent the reduction of albuminuria induced by an ACE inhibitor in passive Heymann nephritis [27]. The administration of phosphoramidon, which potentiates the kinin activity by inhibition of the neutral endopeptidase, reduced albuminuria to a similar extent as an ACE inhibitor. The angiotensin II receptor antagonist losartan, on the other hand, decreased systolic blood pressure without any change in albuminuria [28].

In the present study only HOE 140 decreased proteinuria and albuminuria to the same extent than the ACE inhibitor. This effect was independent from any influence on systemic blood pressure and glomerular filtration rate. Selective B1 receptor blockade had no effect on urinary protein excretion, demonstrating that the B2-receptor plays a seminal role in the pathogenesis of proteinuria and albuminuria in PKD rats. This hypothesis is further supported by the demonstration of T-kinin in urine of old PKD rats, given the fact that this molecule acts as an agonist at the B2 receptor [29, 30], where it is 200-times more active than at the B1 receptor.

Both HOE 140 and ramipril increased urinary excretion of bradykinin, suggesting that the antiproteinuric effect of these drugs is mediated via stimulation of the non-blocked B1 receptor. However, the mechanism of increased bradykinin excretion fundamentally differs between icatibant and ramipril. Inhibition of the ACE, which is identical to kininase II, decreases bradykinin degradation [31]. In contrast, no effect of ramipril on urinary excretion of prekallikrein and kallikrein was observed, which confirms previous studies in other animal models of chronic nephropathy [32, 33]. The increased kallikrein excretion observed after treatment with HOE 140 may be due to the inhibition of a recently described

Table 5. Urine prekallikrein, kallikrein, and bradykinin in 9-month-old PKD before and after treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg s.c. HOE 140, or 2 × 250 µg des-Arg9-[Leu8]-BK for four days

	Ramipril		HR 720		HOE 140		Des-Arg9-BK	
	Before	After	Before	After	Before	After	Before	After
Urine prekallikrein mU/24 h	790 ± 95	756 ± 68	835 ± 49	852 ± 73	745 ± 69	1228 ± 149 ^b	855 ± 94	614 ± 78 ^a
Urine kallikrein mU/24 h	685 ± 94	619 ± 87	711 ± 69	734 ± 96	776 ± 83	956 ± 89 ^a	749 ± 82	532 ± 68 ^a
Urine bradykinin ng/24 h	153 ± 19	397 ± 49 ^b	123 ± 16	136 ± 37	121 ± 27	289 ± 37 ^a	168 ± 23	111 ± 9 ^a

Urine prekallikrein (mU/24 h), kallikrein (mU/24 h), and bradykinin (ng/24 h) in 9-month-old PKD ($N = 6$, each treatment cycle) before and after treatment with 2 mg/kg/day ramipril, 10 mg/kg/day HR 720, 2 × 200 µg s.c. HOE 140, or 2 × 250 µg des-Arg9-[Leu8]-BK for four days. Data are presented as means ± SEM.

^a $P < 0.05$, ^b0.01; treatment effect compared to baseline value

B2 receptor-mediated negative feedback loop of renal kallikrein expression [34].

In summary, the present study demonstrates an age-dependent elevation of activity of the renal KKS in Han:SPRD rats, which parallels the progression of renal failure. The bradykinin B2-receptor is involved in the pathogenesis of proteinuria in rat polycystic kidney disease, independent from systemic blood pressure or creatinine clearance. The antiproteinuric effect of ramipril in this model is angiotensin II-independent and related to its influence on the renal KKS.

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