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Regulatory Peptides 124 (2005) 127–135

REGULATORY PEPTIDES

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Altered renal response to acute volume expansion in transgenic rats harboring the human tissue kallikrein gene

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Received 28 February 2004; accepted 2 July 2004
Available online 13 August 2004

Abstract

The renal response to acute volume expansion was investigated in transgenic (TGR) rats harboring the human tissue kallikrein gene. After a primer injection of 0.9% NaCl (3 ml/100 g, i.v), Sprague–Dawley (SD) or TGR rats received a continuous infusion of 0.9% NaCl (15 μl/100 g/min, i.a.) through a catheter placed into the carotid artery. Acute volume expansion was produced by a second injection of 0.9% NaCl (2 ml/100 g, i.v.) 65 min after the first injection. Plasma vasopressin (AVP) and atrial natriuretic peptide (ANP) concentration was measured before and within 10 min of volume expansion. TGR animals presented a blunted response to acute volume expansion evidenced by an attenuated increase in total and fractional water and sodium excretion. Before or after volume expansion, plasma AVP and ANP did not differ between SD and TGR. Pre-treatment with the BK-B_2 antagonist HOE-140 (7.5 μg/100 g. i.a) partially improved the renal response of TGRs and severely blunted the response in SD rats. These data show that TGR (hKLK1) rats have an impaired renal response to acute volume expansion that can not be accounted for by changes in the release of AVP or ANP.

Keywords: Kinins; Transgenic rats; Human genes

1. Introduction

The kallikrein–kinin system (KKS) plays an important role in the regulation of renal and cardiovascular functions. Kinins are liberated from kininogen through the action of kallikreins and interact with receptors, B_1 and B_2. In addition to eliciting vasodilatation and natriuresis, kinins also elicit smooth muscle contraction, nociception, and other inflammatory responses [1].

All the components of the KKS are expressed within the kidney [2,3], establishing a paracrine system capable of controlling local nephron functions. This peptide system is also involved in the regulation of hydroelectrolyte balance through the interactions with others peptides such as, vasopressin (AVP) [4] and atrial natriuretic peptide (ANP) [5].

To analyze the functions of this system, transgenic techniques have been employed either to overexpress or ablate pertinent genes. This approach has led to numerous new hyper- and hypotensive animal models and has substantially increased the understanding of the physiological role of the KKS [6]. For instance, both kinin receptors, B_1 and B_2, have been inactivated in mice by gene-targeting technologies [7,8]. Mice lacking B_2, which mediates most of the effects of the KKS, develop increased blood pressure [9,10]. In contrast, mice and rats that overexpress either this

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0167-0115/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2004.07.008

receptor subtype or the human tissue kallikrein gene (hKLK1) manifest hypotension [11–13]. In addition, transient expression of hKLK1 in hypertensive rats by somatic gene transfer elicited similar responses [14–17], which supports the fact that kinins are important physiological regulators of mammalian blood pressure.

However, the role of the KKS in renal function and its interaction with other peptides was only poorly investigated in transgenic animal models yet. So, in this study, we used a novel transgenic animal model expressing human tissue kallikrein [TGR (hKLK1)] [13] to further understand the role of the KKS in renal function regulation and the mechanisms involved in its effects.

2. Methods

2.1. Experimental protocols

TGR rats were produced using a 5.6-kb DNA fragment containing the entire hKLK1 gene under the control of the mouse metallothionein (mMT1) promoter (see Ref. [13], for details).

Male TGR (hKLK1) and Hannover Sprague–Dawley (SD) rats, 350–450 g, were used. The animals were kept in a temperature-controlled room on a 14/10-h light/dark cycle with free access to standard chow and tap water.

Forty-eight hours before arterial and venous catheter implantation, the rats were housed in metabolic cages (Rochester, New York) with free access to chow and water. TGR (hKLK1) and age-matched SD rats were anaesthetized with ether and their femoral and carotid artery and jugular vein were cannulated.

Acute volume expansion was performed as previously described [18]. Twenty-four hours after cannulation, the rats received an intravenous (IV) injection of 0.9% NaCl (3 ml/100 g) followed by a continuous infusion of 0.9% NaCl (15 $\mu l \times 100\ g^{-1} \times min^{-1}$) into the descending aorta through the carotid catheter. After a control period of 30 min, a volume expansion (expansion or experimental period) was performed by intravenous injection of 0.9% NaCl (2 ml/100 g over 1 min). After 30 min (recovery period), an additional urine sample was collected (recovery). Pulsatile and mean arterial pressures were continuously recorded by a pressure transducer connected to data-acquisition systems (BIOPAC, Santa Barbara, Ca, USA).

2.1.1. Protocol 1. Effect of acute volume expansion on the renal function variables in TGR and SD rats

Renal function variables were determined in TGR rats (hKLK1) (n=9) and in age-matched SD (n=6) rats.

2.1.2. Protocol 2. Effect of blockade of BK B_2 receptors on renal function variables

In order to evaluate the involvement of BK B_2 receptors on the renal response to acute volume expansion, an in bolus i.v. injection of HOE-140 (7.5 μg/100 g b.w.) was performed 10 min before the beginning of the expansion period (TGR, n=5 and SD, n=5).

2.1.3. Protocol 3. Effect of acute volume expansion on plasma ANP and AVP concentration

To investigate the role of AVP and ANP in the renal response to volume expansion in TGR rats, additional groups of TGR (n=5) and SD (n=5) rats were used. The plasma levels of AVP and ANP were determined in blood samples collected 10 min after acute volume expansion.

2.2. General procedures

2.2.1. Blood sampling

Blood samples (1 ml) for the measurements of serum osmolality, sodium, potassium and creatinine concentration were collected into heparinized tubes and were centrifuged at 2000×g for 10 min. Plasma samples were used. Blood sample for ANP and AVP measurements (1 ml) were collected in tubes containing a mixture of peptidase inhibitors (1 mM p-OH Hg-benzoate, 10 μl; 30 mM O-phenantroline, 50 μl; 1 mM PMSF, 10 μl; 0.2 M EDTA, 50 μl; 1 mM Pepstain, 20 μl).

Plasma samples for AVP were extracted using activated (99% MeOH/0.1% TFA) C18 Bond-Elut Columns (Varian, Harbor City, CA, USA). After sample application, the columns were washed with 20 ml of 0.1% TFA. The peptides were eluted with 3 ml of 99% MeOH/0.1% TFA.

Plasma samples for ANP were extracted using activated (acetonitrile and 0.2% ammonium acetate) Sep-Pak Cartridges (Waters, Milford, Massachusetts, USA). After sample application, the columns were washed with 5 ml of 0.2% ammonium acetate and the peptides were eluted with 3 ml of 60% acetonitrile.

After evaporation, the samples were stored at −20 °C until analysis.

2.2.2. Urine samples

After collection, urine samples were centrifuged at 3000×g for 5 min (room temperature) for measurement of urine osmolality, sodium, potassium and creatinine concentration.

2.3. Analytical procedures

Sodium and potassium concentrations were measured by flame photometry (Celm FC-180, USA). Serum and urine osmolality were measured by a freezing-point osmometer (μOsmette, Natick, MA, USA). Creatinine clearance measurements were performed using a Kit that minimizes the interference of endogenous chromogens (Labtest, cat. 35E, MG, Brazil). Imumunoreactive plasma levels of AVP and ANP were measured by radioimmunoassay (RIA) using previously described techniques [19,20].

2.4. Binding of bradykinin and ANP to kidney slices

2.4.1. Bradykinin

Kidneys of SD and TGR (hKLK1) rats were snap-frozen in liquid nitrogen. Sections (14 μm) were serially cut starting from the central area of the kidney, mounted on 1% gelatinized slides and dried at 4 °C before the binding assay. Experiments were performed in duplicate or quadruplicate. All slices were pre-incubated in a 10 mM Na-phosphate buffer, pH 7.4, containing 120 mM NaCl, 5 mM $MgCl_2$, 0.2% BSA and 0.005% bacitracin for 10 min. The sections were then incubated in the same buffer containing 100 μM PMSF, 100 μM indometacin and 500 μM *o*-phenanthroline, and for slices used to determine nonspecific binding (NSB), with 10^{-6} M cold bradykinin. Total binding was determined by incubation of the sections with 10 mM Na-phosphate buffer, pH 7.4 complemented with the same enzyme inhibitors as above and 0.5 nM ^{125}I-[Tyr^0-bradykinin] for 60 min at room temperature. Tyr^0-Bradykinin was labelled with ^{125}I by the chloramine T method and purified by HPLC, as described [21]. The binding reaction was stopped by washing the slices four times (1 min each) in 250 ml of ice-cold Tris–HCl 50 mM, pH 7.4. After drying at room temperature, the slices were exposed to autoradiographic film (Kodak Biomax MS) for 2 to 3 days at −80 °C.

2.4.2. Atrial natriuretic peptide

Autoradiography methods were performed as previously described [22]. The frozen kidneys were mounted on cryostat chucks, and 10-μm-thick sections were prepared. The sections were thaw-mounted on pre-cleaned, gelatin-coated slides and stored at −80 °C until used. ANP receptor autoradiography was performed as described previously [23,24]. Briefly, the kidney sections were pre-incubated for 15 min at room temperature in 50 mM Tris–HCl buffer (pH 7.4) containing 0.1% polyethyleneimine to reduce non-specific binding. The sections were then incubated at room temperature for 60 min with either [^{125}I] ANP alone or with 10^{-6} M of unlabeled ANP, in 50 mM Tris–HCl buffer (pH 7.4) containing 150 mM NaCl, 5 mM $MgCl_2$, 40 μg/ml Bacitracin (Sigma, St. Louis, MO), and 0.5% BSA. At the end of the incubation period, the slides were rinsed four times (2 min each) with Tris–HCl buffer, pH 7.4, at 4 °C. This was followed by a wash in distilled water, after which the slides were dried under a stream of cold air. The dried tissue sections were placed in a phosphor-sensitive cassette for 48 h, after which the images were scanned, visualized and quantified using a PhosphorImager (Fuji, Japan). Binding in the presence of 10^{-6} M ANP was considered nonspecific.

2.5. Statistical analysis

Results are expressed as mean±S.E.M. Data were analyzed (GraphPad Prism 3.0 Software) using ANOVA followed by Dunnet test to compare the three consecutive experimental periods within the same group. When appropriate, the data obtained in two consecutive periods (control and expansion) were compared using paired Student's *t*-test. For the comparison between the results obtained in TGR rats and SD rats, the non-paired Student's *t*-test was used. The level of significance was set at $p<0.05$.

3. Results

3.1. Renal response to acute volume expansion in TGR (hKLK1) rats

The urine flow of TGR rats was significantly lower in the control period (0.048±0.010 ml/min) in comparison with SD rats (0.100±0.015 ml/min) (Fig. 1A). Acute volume expansion increased this parameter in TGR and SD rats (0.093±0.017 and 0.289±0.015 ml/min, respectively); however, the increase in urine flow was significantly smaller in the TGR group. The lower urine flow of TGR rats was associated with a larger water reabsorption ($T^c_{H_2O}$) in comparison with SD rats (Table 1). Accordingly, the fractional excretion of water (FE_{H_2O}) in the TGR group was significantly smaller than in the SD group in all periods. More importantly, the fractional water excretion did not change significantly in TGR rats following acute volume expansion (control=0.040±0.012, expansion=0.055±0.010, recovery=0.056±0.011), in contrast with the increase observed in SD rats (Fig. 1B). Creatinine clearance (C_{cr}) did not change in the experimental period in both groups but showed statistical difference between the groups in the recovery period (1.52±0.37 ml/min in TGR vs. 0.67±0.10 ml/min in SD rats, $p<0.05$, Table 1).

As observed for urine flow, sodium excretion in TGR was lower than in SD rats in the control period (Fig. 1C). Acute volume expansion significantly increased the sodium excretion in TGR (control=3.66±1.05 μEq/min to expansion=7.21±1.22 μEq/min) and in SD rats (control=8.68±1.44 to expansion=23.48±1.53 μEq/min). However, the increase in sodium excretion was significantly smaller in the TGR group (Fig. 1C). Fractional excretion of sodium (FE_{Na}) of the TGR group showed a progressive increase reaching statistical significance in the recovery period (control=0.017±0.005, expansion=0.029±0.005, recovery=0.058±0.011). However, this increase was smaller than that observed in SD rats (control=0.054±0.008, expansion=0.123±0.021, recovery=0.106±0.016) (Fig. 1D).

Urine osmolality of the TGR rats was significantly higher than that of SD rats in all periods. A very high value of urine osmolality (>1500 mosM/kg H_2O) was observed in two of the rats of the TGR group contributing to higher values in this group. In both groups, urine osmolality decreased after acute volume expansion (Table 1). No

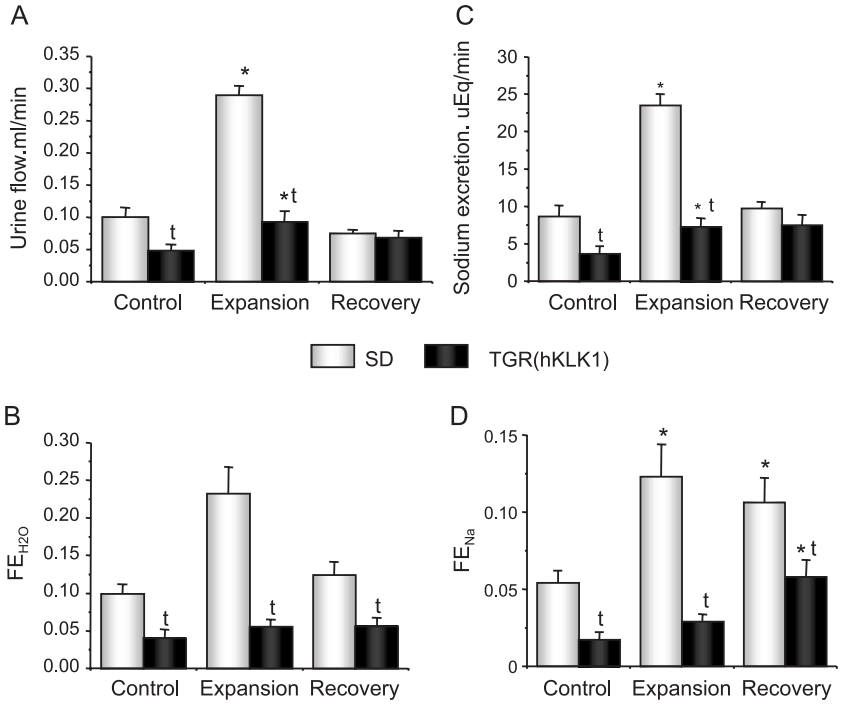

Fig. 1. Effect of acute volume expansion in TGR (hKLK1) and Sprague–Dawley rats. Urine flow, sodium excretion, fractional H_2O excretion (FE_{H_2O}) and fractional sodium excretion were determined in TGR (n=5) and SD (n=6) using samples collected at the end of three consecutive periods of 30 min: control, acute volume expansion (0.9% NaCl, 2 ml/100 g i.v.) and recovery period. *p<0.05, compared to the control period; $^t p$<0.05, SD vs. TGR.

significant differences in potassium excretion, osmolar clearance, plasma osmolality and sodium urinary concentration were observed between TGR and SD rats (Table 1). On the other hand, urinary potassium concentration was significantly higher in TGR while plasma ion concentration (sodium and potassium) was significantly lower in the transgenic animals (Table 1). No significant changes in MAP were observed after acute volume expansion in TGR or SD rats (control values 118±6.3 in TGR and 118±4.2 in SD, respectively).

In order to verify if the differences found for water and sodium excretion were due to changes in plasma AVP and/or ANP concentration, these hormones were measured in plasma samples taken 10 min after acute volume expansion. As shown in Fig. 2, plasma AVP levels after acute volume expansion did not differ between SD (1.16±0.47 pg/ml) and

Table 1
Effects of the acute volume expansion on the renal function on TGR (hKLK1) and Sprague–Dawley rats

	Sprague–Dawley			TGR (hKLK1)		
	Control	Expansion	Recovery	Control	Expansion	Recovery
$T^c_{H_2O}$ (ml/min)	−0.015±0.005	+0.049±0.019*	−0.027±0.004	−0.070±0.019**	−0.084±0.033**	−0.080±0.028
Creatinine clearance (ml/min)	1.03±0.12	1.43±025	0.67±0.10*	2.17±0.70	1.77±0.22	1.52±0.37
Osmolar clearance (ml/min)	0.115±0.018	0.240±0.022*	0.102±0.008	0.118±0.024	0.177±0.040	0.148±0.036
Urine osmolality (mosM/kg H_2O)	305.92±12.81	222.25±25.35*	348.50±9.52*	925.40±220.68**	624.7±130.57*,**	588.89±78.48**
Plasma osmolality (mosM/kg H_2O)	269.92±10.12	264.08±8.53	258.25±7.78	294.63±9.56	289.11±8.44	283.56±8.39
Potassium excretion (μEq/min)	3.31±0.33	4.01±0.48	1.46±0.14*	3.37±0.99	3.38±0.84	3.15±0.84
Sodium urine concentration (mEq/l)	86.50±3.69	82.50±7.48	128.50±5.00*	75.06±16.06	84.89±12.38	106.72±12.62*
Potassium urine concentration (mEq/l)	34.63±2.43	13.88±1.59*	19.35±0.89*	90.89±26.80	52.72±18.52*	45.30±9.64*,**
Sodium plasma concentration (mEq/l)	159.10±2.83	154.56±2.66*	150.02±2.88*	143.36±2.36**	145.42±2.46**	147.47±3.32
Potassium plasma concentration (mEq/l)	3.52±0.23	3.31±0.13	3.10±0.11*	2.28±0.25	2.28±0.24**	2.28±0.24**

Data are mean±S.E. Sprague–Dawley, n=6; TGR (hKLK1), n=9.
* p<0.05, compared to the control period.
** p<0.05, SD vs. TGR.

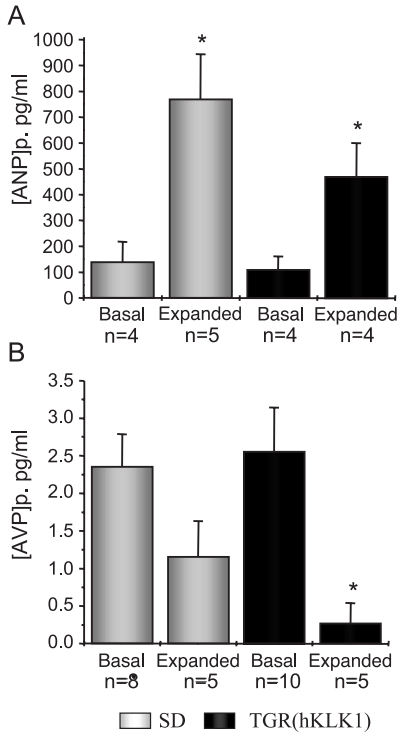

Fig. 2. Effect of acute volume expansion on atrial natriuretic peptide (A) and vasopressin (B) plasma concentration in TGR (hKLK1) and Sprague–Dawley rats. Plasma concentration of the peptides was determined using samples collected ten min after acute volume expansion (0.9% NaCl, 2 ml/100 g). *p<0.05, basal vs. expanded.

TGR (0.27±0.27 pg/ml) rats. A similar finding was obtained for plasma ANP. Although a smaller concentration of ANP was observed in TGR (TGR=470.09±129.09 pg/ml vs. SD=769.61±173.63 pg/ml), the difference did not reach statistical significance. Basal values of AVP and ANP measured in additional groups of animals were not statistically different, either (Fig. 2).

3.2. Effect of HOE-140 administration in TGR (hKLK1) rats submitted to acute volume expansion

Treatment with HOE-140 partially reversed the blunted response of TGR rats to acute volume expansion. A significant increase of fractional excretion of water after volume expansion was observed in HOE-140-treated TGR rats (control=0.028±0.002, expansion=0.097±0.014) (Fig. 3B). This increase, absent in non-treated TGR rats, did not result in significant differences in urine flow between treated and non-treated groups due to the lower values of GFR in the treated group (Table 2). Similar data were obtained for sodium excretion (Fig. 3D).

No significant differences in osmolar clearance, urine and plasma osmolality, potassium excretion, potassium urine concentration and sodium plasma concentration were observed between treated or non-treated TGR animals (Table 2). HOE treatment normalized the lower plasma potassium concentration observed in TGR (Tables 1 and 2).

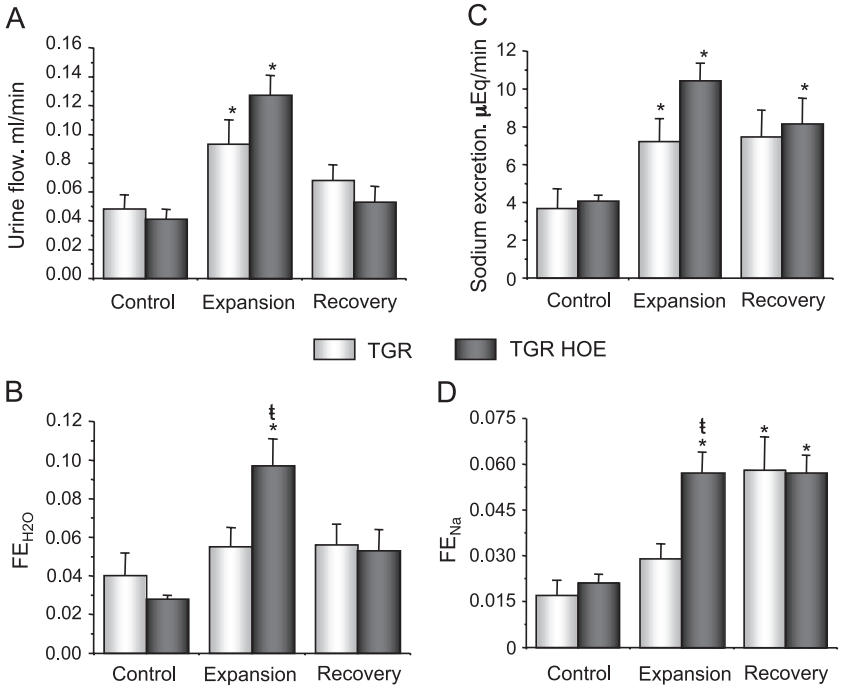

Fig. 3. Effect of HOE-140 administration in TGR (hKLK1) rats submitted to acute volume expansion. Urine flow, sodium excretion, fractional H_2O excretion (FE_{H_2O}) and fractional sodium excretion were determined in control TGR (n=9) and HOE-treated TGR (n=5) using samples collected at the end of three consecutive periods of 30 min: control, acute volume expansion (0.9% NaCl, 2 ml/100 g i.v.) and recovery period. TGR rats received HOE intra-arterial injection (7.5 µg/100 g) 10 min before the volume expansion. *p<0.05, compared to the control period; ^{t}p<0.05, control vs. HOE-treated rats.

Table 2
Effects HOE-140 administration on the renal function of TGR [h(KLK1)] rats submitted to acute volume expansion

	TGR (hKLK1)			HOE-140 7.5 μg/100 g		
	Control	Expansion	Recovery	Control	Expansion	Recovery
$T^c_{H_2O}$ (ml/min)	−0.070±0.019	−0.084±0.033	−0.080±0.028	−0.045±0.012	−0.023±0.026	−0.068±0.018*
Creatinine clearance (ml/min)	2.17±0.70	1.77±0.22	1.52±0.37	1.53±0.26	1.36±0.10	1.02±0.13
Osmolar clearance (ml/min)	0.118±0.024	0.177±0.040	0.148±0.036	0.087±0.015	0.150±0.023*	0.122±0.022*
Urine osmolality (mosM/kg H_2O)	925.40±220.68	624.7±130.57*	588.89±78.48	563.20±73.16	325.50±55.36*	659.24±110.40
Plasma osmolality (mosM/kg H_2O)	294.63±9.56	289.11±8.44	283.56±8.39	263.33±5.66	262.00±6.52	260.67±7.49
Potassium excretion (μEq/min)	3.37±0.99	3.38±0.84	3.15±0.84	2.42±0.41	3.13±0.54	2.41±0.30
Sodium urine concentration (mEq/l)	75.06±16.06	84.89±12.38	106.72±12.62*	106.00±16.51	87.27±12.51	168.50±26.36*,**
Potassium urine concentration (mEq/l)	90.89±26.80	52.72±18.52*	45.30±9.64*	59.00±8.32	26.20±5.44*	53.80±11.38
Sodium plasma concentration (mEq/l)	143.36±2.36	145.42±2.46	147.47±3.32	138.17±3.37	138.83±3.02	139.50±2.69
Potassium plasma concentration (mEq/l)	2.28±0.252	.28±0.24	2.28±0.24	3.42±0.42	3.25±0.37**	3.08±0.33

Data are mean±S.E. TGR (hKLK1) control, n=9; HOE-treated, n=5.
* p<0.05, compared to the control period.
** p<0.05, control vs. HOE-treated.

3.3. Effect of HOE-140 administration in Sprague–Dawley rats submitted to an acute volume expansion

In sharp contrast with the finding in TGR, HOE-140 treatment produced marked changes in the renal response to acute volume expansion in SD rats. There was a significant attenuation of the increase in urine flow, FE_{H_2O}, sodium excretion and FE_{Na} observed after volume expansion (Fig. 4).

Water reabsorption ($T^c_{H_2O}$), creatinine clearance, urine osmolality, potasssium excretion and sodium and potassium urine concentration were significantly higher in SD rats treated with HOE-140 than in control SD rats (Table 3).

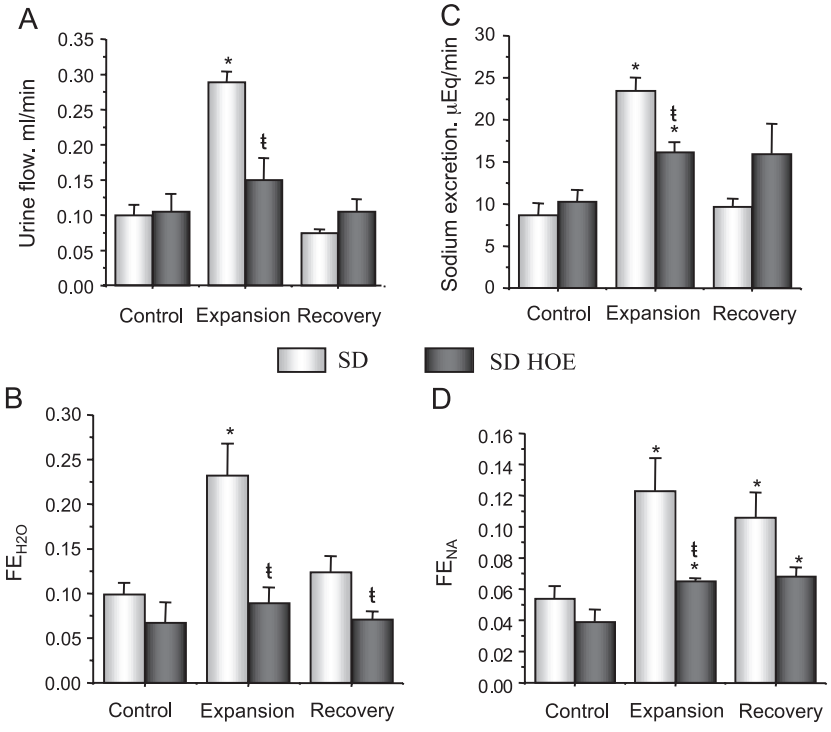

Fig. 4. Effect of HOE-140 administration in Sprague–Dawley rats submitted to acute volume expansion. Urine flow, sodium excretion, fractional H_2O excretion (FE_{H_2O}) and fractional sodium excretion were determined in control SD (n=6) and HOE-treated SD (n=5) using samples collected at the end of three consecutive periods of 30 min: control, acute volume expansion (0.9% NaCl, 2 ml/100 g i.v.) and recovery period. SD rats received HOE intra-arterial injection (7.5 μg/100 g) 10 min before the volume expansion. *p<0.05, compared to the control period; $^t p$<0.05, control vs. HOE-treated rats.

Table 3
Effects HOE-140 administration over renal function of Sprague–Dawley rats submitted to acute volume expansion

	Sprague–Dawley			HOE-140 7.5 μg/100 g		
	Control	Expansion	Recovery	Control	Expansion	Recovery
$T^c_{H_2O}$ (ml/min)	−0.015±0.005	+0.049±0.019*	−0.027±0.004	−0.120±0.043**	−0.047±0.032	−0.079±0.008**
Creatinine clearance (ml/min)	1.03±0.12	1.43±025	0.67±0.10*	1.93±0.28**	1.68±0.13	1.54±0.27**
Osmolar clearance (ml/min)	0.115±0.018	0.240±0.022*	0.102±0.008	0.225±0.050	0.197±0.034	0.184±0.022
Urine osmolality (mosM/kg H_2O)	305.92±12.81	222.25±25.35*	348.50±9.52*	669.25±129.13	397.00±69.83*,**	499.50±36.54**
Plasma osmolality (mosM/kg H_2O)	269.92±10.12	264.08±8.53	258.25±7.78	272.43±4.18	272.10±3.13	271.77±2.09
Potassium excretion (μEq/min)	3.31±0.33	4.01±0.48	1.46±0.14*	5.19±1.27	3.75±0.80	3.24±0.45**
Sodium urine concentration (mEq/l)	86.50±3.69	82.50±7.48	128.50±5.00*	118.50±22.45	125.50±21.84**	149.25±24.11
Potassium urine concentration (mEq/l)	34.63±2.43	13.88±1.59*	19.35±0.89*	55.25±10.85	25.75±3.15*,**	32.00±2.41*,**
Sodium plasma Concentration (mEq/l)	159.10±2.83	154.56±2.66*	150.02±2.88*	150.88±0.40**	148.13±0.77*	145.38±0.76*
Potassium plasma concentration (mEq/l)	3.52±0.23	3.31±0.13	3.10±0.11*	3.42±0.16	3.45±0.17	3.48±0.20

Data are mean±S.E. Sprague–Dawley Control, n=6; HOE-treated, n=5.
* p<0.05, compared to the control period.
** p<0.05, control vs. HOE-treated.

3.4. Binding of bradykinin and ANP

Binding of [^{125}I] ANP and [^{125}I]-Tyr^0-Bradykinin to kidney sections of SD and TGR was determined by radioautography. As shown in Fig. 5A, the binding of labeled ANP in kidney slices of TGR (hKLK1) was preserved and even increased. As observed for ANP, ^{125}I-Tyr^0-Bradykinin binding was not altered in kidney slices of TGR as compared to SD rats (Fig. 5B).

4. Discussion

The kallikrein–kinin system is believed to play an important role in regulating renal function [1,25,26]. Contrary to what would be expected considering previous observations [25,26], transgenic rats harboring the tissue human kallikrein gene presented a blunted renal response to acute volume expansion. Equally important, TGR (hKLK1) presented a significant decrease in sodium and water

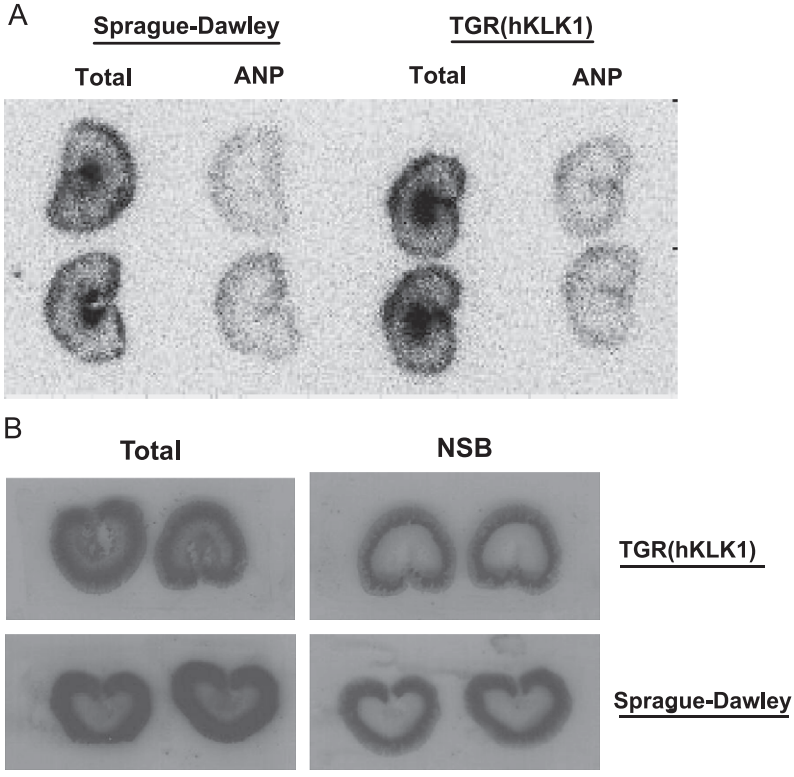

Fig. 5. Autoradiography of [^{125}I]-ANP and [^{125}I]-Tyr-Bradykinin to Sprague–Dawley and TGR (hKLK1) rat kidneys. Total binding and displacement with 10^{-6} M ANP (A) or with Bradykinin (B) are shown. NSB=nonspecific binding.

excretion in basal conditions. These changes were accompanied by decreased potassium excretion and increased urinary osmolality. However, TGR (hKLK1) presented an increased creatinine clearance indicating an increased GFR. These data suggest that the decreased urine volume in TGR is due to an increased water reabsorption (reduced $T^c_{H_2O}$) and decreased sodium excretion. These changes could not be attributed to an increase in plasma AVP or decreased plasma ANP suggesting that alterations in the responsiveness to these hormones may be present in these animals.

There are few reports in the literature dealing with possible interactions of kinins with ANP and AVP. Boric et al. [27] have reported that kinins can counteract the renal effects of ANP through an intra-renal B_2 receptor dependent mechanism. In disagreement with our data, Alfie et al. [28] described an enhanced AVP effect in bradykinin B_2 receptor null mutant mice. However, conflicting results have been reported in bradykinin B2 receptor knockout mice [29] raising concerns about conclusions on the role of kinins in kidney function based on observation derived from these animals. Our binding data for ANP in kidney slices do not support a decreased density of ANP binding in TGR (hKLK1), suggesting that other factors such as imbalances in ANP signaling are responsible for the changes observed. In this regard, it has been described that human kallikrein can hydrolyze ANP, generating inactive fragments [30–32]. Accordingly, a likely explanation for our findings could be the ANP degradation by tissue kallikrein in the heart, kidney or possibly other tissues expressing the transgene. The smaller ANP plasma levels after acute volume expansion in TGRs are in keeping with this interpretation. Concerning AVP, the apparently decreased responsiveness could be due to changes in signal transduction pathways, decreased receptor density or both. These possibilities deserve future studies to be confirmed.

The fact that no macroscopic changes in kidney structure could be detected in TGR (hKLK1) (data not shown) and the partial reversal of the functional changes by HOE-140 treatment makes it different from the fact that morphological alterations are contributing to the decreased renal function in TG rats. It has been recently reported that bradykinin B_2 receptor transgenic mice presented an enhanced renal function suggesting that our results could be due to downregulation of bradykinin B_2 receptors induced by elevated local kinin concentration [33]. However, our autoradiographic data do not support this possibility.

Marked differences in the response to HOE-140 were observed between SD and TG rats. Indeed, the effect of HOE-140 in the TGR was essentially opposite to that observed in SD rats. As expected, HOE-140 severely blunted the renal response to acute volume expansion in SD rats essentially suppressing the rise in water excretion and significantly decreasing the sodium excretion after volume overload. Conversely in TGR (hKLK1), pretreatment with HOE-140 partially reversed the impaired response to acute volume expansion especially if one considers the fractional sodium and water excretion. Interestingly, a similar finding was obtained for Angiotensin-(1–7) which decreased the renal response to volume expansion in SD and increased it in TGR (hKLK1) [18]. Although future studies are obviously needed to clarify these observations, the results obtained with HOE-140 clearly suggest an involvement of B2 receptor-related mechanism in the attenuated response of TGR (hKLK1) to acute volume expansion.

In summary, we showed that transgenic rats harboring the human tissue kallikrein gene have an impaired renal response to acute volume expansion, which cannot be ascribed to differences in blood pressure or GFR (higher in TGR) or expressive changes in plasma levels of hormones known to participate in this response, ANP and AVP.

Acknowledgments

We thank José Roberto Silva for his technical support and would like to acknowledge the Brazilian agencies, FINEP (PRONEX), CNPq, and CAPES for the financial support.

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