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Impaired response of the denervated kidney to endothelin receptor blockade in normotensive and spontaneously hypertensive rats

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Impaired response of the denervated kidney to endothelin receptor blockade in normotensive and spontaneously hypertensive rats.

Background. As yet, there are only limited data available on the exact role of endothelin (ET) acting through endothelin-A (ET_A) receptors in renal sodium and water regulation and the potential functional implications of an interaction of the renal ET system with renal nerves in normotensive and spontaneously hypertensive rats.

Methods. Experiments were carried out in 64 male conscious spontaneously hypertensive rats and in 56 normotensive Wistar-Kyoto (WKY) rats. Bilateral renal denervation (BRD) was performed in 32 spontaneously hypertensive rats and 28 WKY rats 7 days before the experiments. The ET_A receptor antagonist, BQ-123 (16.4 nmol/kg.min intravenously) or the endothelin-B (ET_B) receptor antagonist, BQ-788 (25 nmol/kg.min intravenously) were infused at a rate of 25 μL/min for 50 minutes.

Results. Renal papillary ET-1 concentration in intact spontaneously hypertensive rats was 67.8% lower than in intact WKY rats (154 ± 40 fmol/mg protein vs. 478 ± 62 fmol/mg protein, $P < 0.01$). BRD decreased papillary ET-1 by 73.5% in WKY rats to 127 ± 19 fmol/mg protein ($P < 0.001$), but had no effect in spontaneously hypertensive rats (122 ± 37 fmol/mg protein). BRD, BQ-123, or BQ-788 did not affect glomerular filtration rate (GFR) or renal blood flow (RBF) in any of the groups. In intact WKY, BQ-123 decreased urine flow rate (V) from 4.65 ± 0.44 μL/min.100 g body weight to 2.44 ± 0.35 μL/min.100 g body weight ($P < 0.01$), urinary excretion of sodium ($U_{Na}V$) from 238.2 ± 27.4 to 100.2 ± 17.0 ($P < 0.01$) and potassium (U_KV) from 532.1 ± 62.6 nmol/min.100 g body weight to 243.0 ± 34.2 nmol/min.100 g body weight ($P < 0.001$), whereas BQ-788 decreased only V and $U_{Na}V$. In renal denervated WKY, BQ-123 or BQ-788 did not alter V, $U_{Na}V$, or U_KV. In intact spontaneously hypertensive rats BQ-123 but not BQ-788 decreased V from 3.94 ± 0.48 μL/min.100 g body weight to 2.55 ± 0.44 μL/min.100 g body weight ($P < 0.05$). In renal denervated spontaneously hypertensive rats neither BQ-123 nor BQ-788 affected V, $U_{Na}V$, or U_KV.

Conclusion. An interaction between ET and renal nerves is involved in the control of renal function. Moreover, renal nerves participate in the regulation of ET-1 production within the kidney. Finally, decreased synthesis of ET-1 in the renal papilla of spontaneously hypertensive rats may contribute to development and/or maintenance of hypertension due to modulation of renal excretory function.

Key words: bilateral renal denervation, BQ-123, BQ-788, normotensive WKY rats, spontaneously hypertensive rats, renal excretory function.

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Numerous studies have provided strong evidence for a close relationship between hypertension and impaired renal function. Thus, it was clearly demonstrated that the kidney of spontaneously (i.e., genetically) hypertensive rats plays a key role in the maintenance of hypertension [1].

Kidneys are known to be richly innervated by the sympathetic nervous system and renal nerves produce alterations in renal hemodynamics, tubular reabsorption, and renin secretion that importantly contribute to renal adaptation to physiologic and pathologic stimuli [2]. Thus, stimulation of efferent renal nerves alters renal hemodynamics, enhances tubular fluid and electrolyte absorption throughout the nephron with decreased water and sodium excretion, and increases renin secretion. With respect to their possible role in hypertension it has been demonstrated that renal nerve activity of young spontaneously hypertensive rats is augmented and contributes to the development of hypertension by enhancing renal salt retention [3]. Moreover, kidney denervation in spontaneously hypertensive rats during the prehypertensive stage delays and attenuates the development of hypertension [4]. Thus, it is likely that increased renal nerve activity is involved in the pathogenesis of hypertension.

It is now well established that endothelin (ET) is distributed in a variety of organs and tissues including the kidney [5], where it can influence renal hemodynamics and tubular function [6]. In recent years it has become apparent that ET-1 plays a major role in salt-dependent hypertension by modulating renal salt and water excretion [7]. Thus, there is accumulating evidence suggesting that the ET system plays an important role in the development and maintenance of hypertension in animal models

such as the deoxycorticosterone acetate (DOCA)-salt-induced hypertensive rat [8] and the Dahl salt-sensitive rat [7]. In contrast to these models it remains unclear whether the renal ET system and to what extent it participates in the pathogenesis of hypertension in spontaneously hypertensive rats. In particular, it is unclear whether an interaction of the renal ET system with renal nerves is of significance for the regulation of renal excretory function during the development of spontaneous hypertension. Especially the mode of this interaction with respect to the intrarenal ET-1 distribution as well as its relative contribution to the development and/or maintenance of spontaneous hypertension remain largely unknown.

In the kidney both endothelin-A (ET_A) and endothelin-B (ET_B) receptors mediate the renal effects of ET-1 [6]. Recent evidence suggests that the effects of ET-1 on water and sodium excretion are mediated essentially through ET_B receptors [9, 10]. So far, only few studies have investigated the effects of ET_A receptor inhibition on renal excretory function and these studies do not provide unequivocal data to conclude that ET_A receptors also contribute to the diuretic or natriuretic actions of ET-1.

Therefore, the present study was performed first to investigate the role of the renal ET system and especially the involvement of ET_A receptors in the regulation of renal excretory function in normotensive and spontaneously hypertensive rats. For this purpose, the selective ET_A receptor antagonist BQ-123 was used to determine the effects of ET_A receptor blockade on renal sodium and water handling and its effects were compared with those of ET_B receptor blockade with BQ-788.

Second, in the present study we determined the influence of renal nerves on the ET-1 content in renal cortex and papilla of normotensive and spontaneously hypertensive rats. Third, in order to determine the significance of an interaction between renal nerves and the renal ET system in the regulation of renal excretory function and renal hemodynamics, the selective ET_A and ET_B receptor antagonists, BQ-123 and BQ-788, were applied to conscious intact as well as to renal denervated normotensive and spontaneously hypertensive rats.

METHODS

Conscious, male spontaneously hypertensive rats, and Wistar-Kyoto (WKY) rats, weighing between 280 to 320 g were used. They were kept in individual cages for a minimum of 1 week before experiments were started. The rats were given a normal rat chow and tap water. The study was performed following approval by the state animal protection board at the Bezirksregierung Cologne, Federal Republic of Germany, as well as in accordance with institutional care and use of laboratory animals guidelines, Medical University, Sofia, Bulgaria. For surgical preparation, the rats were anesthetized with pentobarbital sodium (Narcoren) 40 mg/kg body weight given intraperitoneally. In part of the rats, both kidneys were denervated prior to vascular and bladder catheterization. Bilateral renal denervation (BRD) was performed retroperitoneally after flank incision. After careful dissection of renal vessels, all visible nerve fibers were removed. Thereafter, the renal vessels were coated with 10% phenol in absolute alcohol. One week was allowed for the rats to recover. Sham-operated WKY and spontaneously hypertensive rats were subjected only to flank incisions, but renal nerves were left intact. For urine collection, a modified polyethylene catheter was placed into the urinary bladder through a suprapubic incision, fixed by sutures to adjacent muscle, subcutaneous tissue and skin, and exteriorized at the back of the neck. For drug and solute infusion and for blood pressure measurement, catheters were placed into the femoral vein and into the femoral artery, respectively, and were tunnelled to the back of the neck and then exteriorized. The femoral vein catheter was connected to an infusion pump set to deliver 0.9% NaCl solution at a rate of 25 μL/min for the duration of the surgical preparation. To avoid clotting of catheters in the freely moving animals, the femoral artery and vein catheters were flushed with heparin in 0.9% sterile saline. The experiments were performed 1 day after catheter implantation in conscious normotensive and hypertensive rats without and with BRD placed in individual restraining plastic cages. The selective ET_A receptor antagonist BQ-123 and the selective ET_B receptor antagonist BQ-788 (Calbiochem) were applied through the femoral vein cannula. Urine was collected into preweighed tubes during a first 40-minute clearance period before administration of ET receptor blockers or vehicle. Ten minutes after the start of the infusion of ET receptor blockers or vehicle, urine was collected again during a second 40-minute clearance period. At the end of the experiment, under pentobarbital anesthesia, 40 mg/kg body weight, blood was drawn by cardiac puncture and the kidneys were removed and rapidly dissected on an ice-cold glass plate to obtain cortical and papillary tissues which were then frozen immediately in liquid nitrogen and stored at –70°C.

Spontaneously hypertensive rats and WKY rats were divided into six experimental groups undergoing different treatments. The first group of intact rats, 11 WKY rats and 13 spontaneously hypertensive rats, and the second group of rats with BRD, 11 WKY rats and 13 spontaneously hypertensive rats, received vehicle (0.9% sodium chloride) at a pump rate of 25 μL/min.

Intravenous treatment with the ET_A receptor antagonist BQ-123, 16.4 nmol/kg.min at an infusion rate of 25 μL/min for 50 minutes, was performed in the third group of intact rats, 11 WKY rats and 13 spontaneously hypertensive

rats, and in the fourth group of rats with BRD, 11 WKY rats and 13 spontaneously hypertensive rats. Intravenous treatment with the ET_B receptor antagonist BQ-788, 25 nmol/kg.min at an infusion rate of 25 μL/min for 50 minutes, was performed in the fifth group of intact rats, six WKY rats and six spontaneously hypertensive rats, and in the sixth group of rats with BRD, six WKY rats and six spontaneously hypertensive rats. Doses of BQ-123 [11] and BQ-788 [12] used in the present study, were previously shown not to affect arterial blood pressure.

For time control experiments urine was collected over a time period of 90 minutes in both intact WKY rats ($N = 10$) and spontaneously hypertensive rats ($N = 10$) as well as renal-denervated WKY rats ($N = 9$) and spontaneously hypertensive rats ($N = 9$).

Arterial blood pressure was monitored in all groups using a Gould/Statham P23ID pressure transducer connected to a catheter in the femoral artery. After analogue-to-digital conversion by a data acquisition system Biopac MP100WS, systolic blood pressure, diastolic blood pressure, and mean arterial pressure (MAP) were estimated. These variables were stored online on a computer disc and continued analysis by Biopac AcqKnowledge software was made. Urine volume was assessed gravimetrically to estimate urine flow rate (V).

Hematocrit was obtained from all blood samples. Plasma and urine concentrations of sodium and potassium were determined by flame photometry (Corning 410C). Osmolality was determined by using a vapor pressure osmometer (Vescor 5500A). On the basis of these data urinary excretion rates of sodium ($U_{Na}V$) and potassium (U_KV) as well as urinary osmolyte excretion ($U_{osm}V$) were calculated. The effectiveness of renal denervation was assessed by determination of renal tissue norepinephrine content [2]. For this purpose, catecholamines were concentrated and purified by selective adsorption onto aluminum oxide, and after that fluorescent norepinephrine derivatives were formed and determined spectrofluorometrically [13, 14]. Renal denervation resulted in reduction of norepinephrine content from an average of 5.48 ± 0.31 in each 10 WKY rats and spontaneously hypertensive rats to 0.24 ± 0.12 nmol/g renal tissue in each nine renal-denervated WKY rats and spontaneously hypertensive rats ($P < 0.001$).

Plasma and urine concentrations of inulin were determined by the anthrone method [15]. Glomerular filtration rate (GFR) was estimated by the clearance of inulin. Urinary and plasma concentrations of para-aminohippurate (PAH) were determined by the modified method of Smith et al [16]. Effective renal plasma flow (ERPF) was estimated from the clearance of PAH and renal blood flow (RBF) was calculated as ERPF/1-hematocrit.

In six rats of each group, plasma and urine as well as tissue concentrations of ET-1 were determined by enzyme-linked immunosorbent assay (ELISA) (Endothelin-1 ELISASystem) (Amersham Buchler, Braunschweig, Germany) as previously described [17]. For ET-1 determination in renal cortex and papilla, tissues were homogenized on ice at 4°C in distilled water containing 100 kallikrein-inhibitory units/mL of aprotinin using a 2 mL glass homogenizer (Kontes, Vineland). The homogenates were then centrifuged at 600*g* for 10 minutes. The supernatants were extracted using Sep-Pak C18 cartridges with 70% (vol/vol) methanol and ET-1 concentration was determined by ELISA. For protein determination, the pellets were resuspended in 5 mL of 1% (wt/vol) sodium dodecyl sulfate (SDS) for bicinchoninic acid protein assay (Pierce, Rockford, IL, USA) according to the instructions of the manufacturer. All chemicals used were purchased from Sigma Co. (Diesenhofen, Germany).

Statistical analysis was performed using Student *t* test, Wilcoxon test for unpaired data, or one-way analysis of variance (ANOVA) (Tadpole computer program, Elsevier Publishers, Amsterdam, The Netherlands) when appropriate. Results are presented as mean ± SEM. Differences at a level of $P < 0.05$ were considered statistically significant.

RESULTS

Throughout the time control experiments, spontaneously hypertensive rats and WKY rats with innervated or denervated kidneys showed stable arterial blood pressure and renal excretory function. Values from time control experiments were not different from control periods in either the intact or the BRD experimental groups of rats.

Systolic and diastolic blood pressure and MAP were significantly higher in spontaneously hypertensive rats than in WKY rats ($P < 0.001$). BRD did not significantly change blood pressure, GFR or RBF in WKY rats or spontaneously hypertensive rats (Table 1). ET_A receptor blockade with intravenous BQ-123, 16.4 nmol/kg.min, did not change systolic or diastolic blood pressure or MAP in intact or renal denervated WKY rats. BQ-123 administration in intact and renal denervated spontaneously hypertensive rats decreased systolic blood pressure only slightly ($P < 0.05$), while MAP or diastolic blood pressure did not change. BQ-123 infusion did not result in changes in GFR or RBF in intact and renal denervated WKY rats or spontaneously hypertensive rats (Table 1). Hematocrit did not change in any of the groups investigated.

Renal denervation in WKY rats was followed by an increase in urine flow rate from 4.65 ± 0.44 μL/min.100 g body weight to 6.34 ± 0.59 μL/min.100 g body weight ($P < 0.05$) and a decrease in urine osmolality ($P < 0.05$) but had no effect on renal excretory function in spontaneously hypertensive rats (Table 1) (Fig. 1).

Table 1. Blood pressure, RBF (μL/min.100 g body weight), GFR (μL/min.100 g body weight), urine osmolality, and osmolyte excretion in conscious intact and renal denervated Wistar-Kyoto WKY rats as well as intact and renal denervated spontaneously hypertensive rats (SHR) during intravenous BQ-123 infusion (16.4 nmol/kg/min)

	WKY	WKY + BQ-123	WKYD	WKYD + BQ-123
Number	11	11	11	11
Systolic blood pressure	143 ± 3	137 ± 3	137 ± 3	136 ± 2
Diastolic blood pressure	82 ± 3	78 ± 2	83 ± 3	81 ± 2
MAP	103 ± 2	98 ± 2	104 ± 3	101 ± 2
RBF	3989 ± 372	3973 ± 414	4520 ± 475	4814 ± 458
GFR	805 ± 27	928 ± 89	861 ± 32	871 ± 105
U_{osm}	941 ± 49	1124 ± 55[a]	805 ± 42[a]	949 ± 95
$U_{osm}V$	4218 ± 301	2250 ± 248[a]	4792 ± 358	3844 ± 306[b]
	SHR	SHR + BQ-123	SHRD	SHRD + BQ-123
Number	13	13	13	13
Systolic blood pressure	183 ± 3[a]	176 ± 2[c]	185 ± 2	177 ± 2[d]
Diastolic blood pressure	110 ± 2[a]	109 ± 1	109 ± 2	109 ± 2
MAP	139 ± 2[a]	134 ± 2	137 ± 3	134 ± 2
RBF	4256 ± 295	4050 ± 409	4077 ± 383	4024 ± 316
GFR	815 ± 139	832 ± 80	957 ± 92	953 ± 55
U_{osm}	986 ± 68	954 ± 96	955 ± 65	995 ± 51
$U_{osm}V$	3432 ± 246	2876 ± 256	4744 ± 316	5383 ± 364

Abbreviations are: WKYD, renal denervated WKY rats; SHRD, renal denervated SHR; MAP, mean arterial pressure, RBF, renal blood flow; GFR, glomerular filtration rate; $U_{osm,}$ urine osmolality, mOsm/kg H_2O; $U_{osm}V$, osmolyte excretion, nOsm/min.100 g body weight.

Statistically significant difference ($P < 0.05$) as compared to WKY ([a]); to WKY + BQ-123 ([b]); to SHR ([c]); and to SHRD ([d]).

In intact WKY rats BQ-123 decreased urine flow rate to 2.44 ± 0.35 μL/min.100 g body weight ($P < 0.01$) and increased urine osmolality ($P < 0.05$). Sodium and potassium excretion also decreased from 238 ± 27 to 100 ± 17 ($P < 0.01$) and from 532 ± 63 nmol/min.100 g body weight to 243 ± 34 nmol/min.100 g body weight ($P < 0.05$), respectively, resulting in a decrease of urinary osmolyte excretion ($P < 0.05$). In renal denervated WKY rats BQ-123 did not significantly alter neither urine flow rate nor urinary sodium, potassium, or osmolyte excretion. In intact spontaneously hypertensive rats BQ-123 also led to a decrease in urine flow rate from 3.94 ± 0.48 μL/min.100 g body weight to 2.55 ± 0.44 μL/min.100 g body weight ($P < 0.05$) but was not accompanied by significant changes in urinary sodium, potassium, or osmolyte excretion. In renal denervated spontaneously hypertensive rats BQ-123 did not change any of the parameters investigated (Fig. 1) (Table 1).

Similar to the effect of BQ-123, administration of BQ-788 decreased urine flow rate and sodium excretion in intact WKY rats ($P < 0.001$) (Fig. 1). Urine osmolality increased from 940 ± 49 mOsm/kg H_2O to 1469 ± 249 mOsm/kg H_2O ($P < 0.05$). In renal denervated WKY rats and in intact and in renal denervated spontaneously hypertensive rats BQ-788 was not accompanied by significant changes in urine flow rate, urinary sodium, potassium, or osmolyte excretion (Fig. 1).

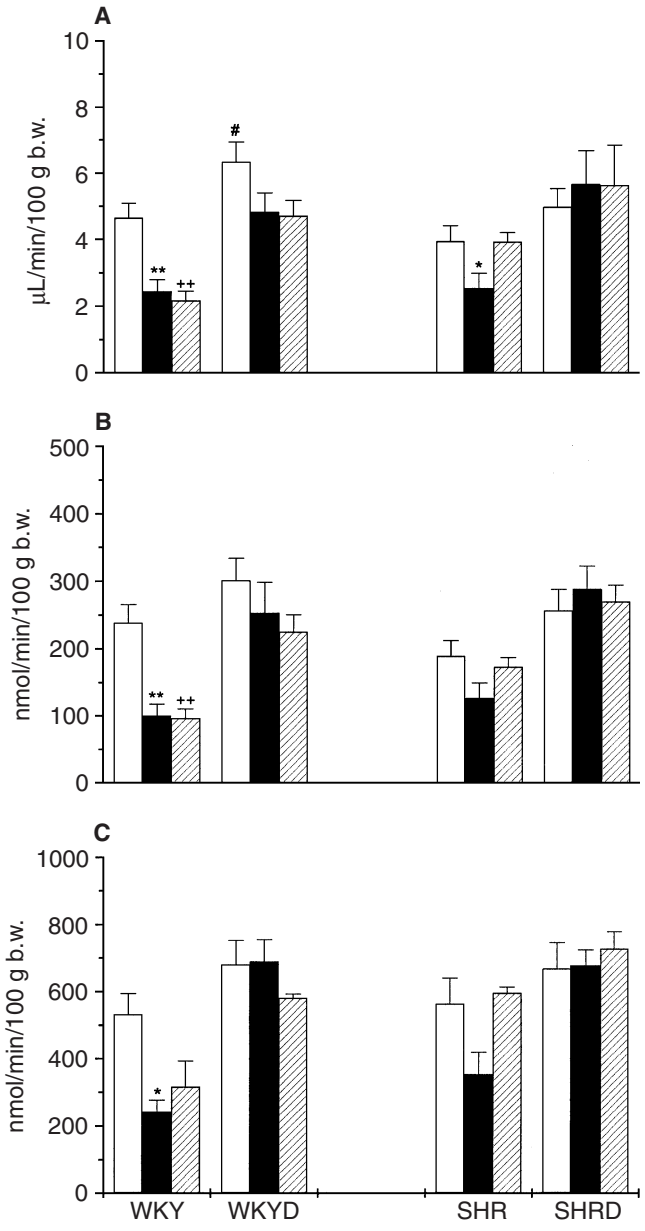

Fig. 1. Urine flow rate (*A*) and sodium (*B*) and potassium (*C*) excretion in intact Wistar-Kyoto rats (WKY), spontaneously hypertensive rats (SHR) and in denervated WKY rats (WKYD) and denervated SHR (SHRD). Symbols are: (□) control; (■) intravenous infusion of 16.4 nmol/kg/min BQ-123; (▨) intravenous infusion of 25 nmol/kg/min BQ-788. $^{*}P < 0.05$; $^{**}P < 0.01$, BQ-123 vs. control; $^{++}P < 0.001$, BQ-788 vs. control; $^{\#}P < 0.05$, WKYD vs. WKY.

Plasma ET-1 concentrations in intact WKY rats and spontaneously hypertensive rats were 1.05 ± 0.10 fmol/mL and 0.90 ± 0.11 fmol/mL and were not altered after renal denervation (1.01 ± 0.10 fmol/mL and 1.02 ± 0.11 fmol/mL, respectively). After BQ-123 administration plasma ET-1 level in intact WKY rats was

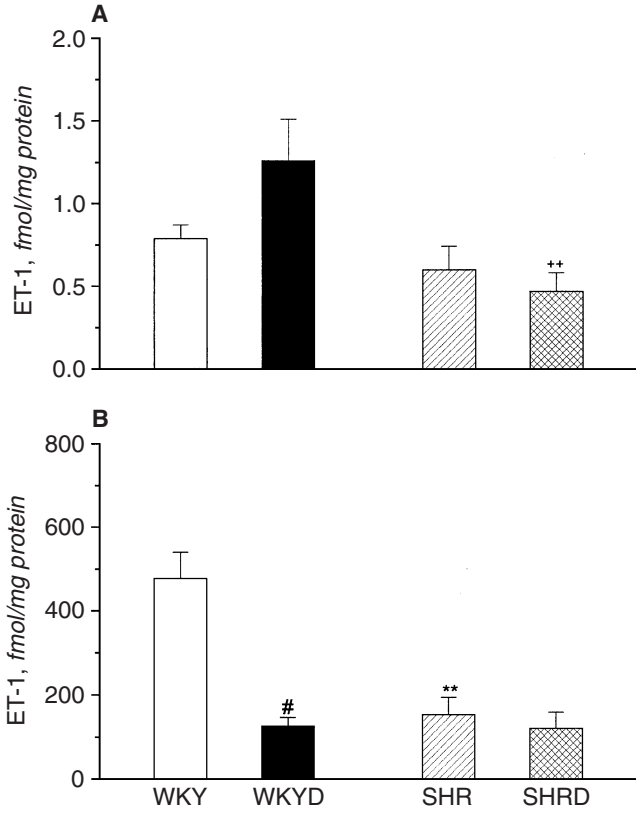

Fig. 2. Tissue endothelin-1 (ET-1) content in renal cortex (*A*) and papilla (*B*) in intact Wistar-Kyoto rats (WKY), spontaneously hypertensive rats (SHR), and in denervated WKY rats (WKYD) and denervated SHR (SHRD) (each $N = 6$). $^{\#}P < 0.001$ WKYD vs. WKY rats; $^{**}P < 0.01$ SHR vs. WKY rats; $^{++}P < 0.01$ SHRD vs. WKYD.

2.06 ± 0.03 fmol/mL, in renal denervated WKY rats 2.16 ± 0.24 fmol/mL, in intact spontaneously hypertensive rats 2.40 ± 0.35 fmol/mL. and in renal denervated spontaneously hypertensive rats 1.90 ± 0.38 fmol/mL, respectively ($P < 0.05$).

Renal cortical ET-1 concentration was low and did not differ between intact spontaneously hypertensive rats and WKY rats (0.60 ± 0.14 fmol/mg protein and 0.79 ± 0.08 fmol/mg protein). In renal denervated spontaneously hypertensive rats it was lower than in renal denervated WKY rats (0.47 ± 0.11 fmol/mg protein vs. 1.26 ± 0.25 fmol/mg protein; $P < 0.01$) (Fig. 2).

Renal papillary ET-1 concentration in intact spontaneously hypertensive rats was 67.8% lower than in intact normotensive WKY rats (154 ± 40 fmol/mg protein vs. 478 ± 62 fmol/mg protein, $P < 0.01$). BRD decreased renal papillary ET-1 concentration by 73.5% in WKY rats to 127 ± 19 fmol/mg protein ($P < 0.001$), but had no effect in spontaneously hypertensive rats (122 ± 37 fmol/mg protein) (Fig. 2).

Urinary ET-1 excretion in intact WKY rats and spontaneously hypertensive rats was 379 ± 35 fmol/24 hours and 34 ± 12 fmol/24 hours, respectively ($P < 0.001$). Renal denervation decreased urinary ET-1 excretion in WKY rats to 84 ± 13 fmol/24 hours ($P < 0.001$) but had no effect in spontaneously hypertensive rats (41 ± 11 fmol/24 hours).

DISCUSSION

In the present study we found plasma ET-1 levels, which were similar to those which have been reported previously in normotensive and spontaneously hypertensive rats [6]. However, since ET-1 is known to act in a paracrine/autocrine fashion, its plasma concentration is not indicative of its local action and/or production. Therefore, determination of tissue ET-1 content in the kidney should provide a better estimate of local changes in peptide production.

Several studies have provided evidence that implicates locally produced ET-1 in the renal medulla and papilla in the development of hypertension [17, 18]. In the present study we found high ET-1 concentrations in the renal papilla as compared to renal cortical tissue in both normotensive (WKY) and hypertensive rats. More important, however, is the finding that the ET-1 content in the renal papilla of our spontaneously hypertensive rats was significantly lower than that of their WKY counterparts. These data confirm our previous observation of significantly lower levels of ET-1 peptide and of preproET-1 mRNA expression in the renal papilla of genetically hypertensive rats [Prague hypertensive (Wistar) rats] than in renal papillary tissue of their normotensive controls (Prague normotensive rats) [17]. As in this previous study the present study again failed to demonstrate a difference in ET-1 content in renal cortical tissue between spontaneously hypertensive rats and WKY rats.

Similar alterations in tissue medullary ET-1 content were reported by Sasser, Pollock, and Pollock [19] in the chronic angiotensin II infusion model of hypertension. High salt diet, or angiotensin II infusion, reduced inner medullary immunoreactive ET content compared with control values but increased the renal cortical and outer medullary ET content and urinary ET excretion. Thus, chronic elevation in angiotensin levels and sodium intake produce a differential effect on immunoreactive ET levels within the kidney [19]. Therefore, renal tissue ET levels can be regulated in a very site-specific manner.

In agreement with observations by others [20] we found that selective ET_A receptor blockade with BQ-123 did not affect blood pressure in normotensive WKY rats but moderately decreased systolic blood pressure in spontaneously hypertensive rats, indicating that the ET system does not contribute significantly to the maintenance of blood pressure in normotensive state but is involved to some degree in the regulation of high blood pressure in spontaneous hypertension. The relatively small, decrease in systolic blood pressure in the absence of changes in MAP or diastolic blood pressure is unlikely to affect

autoregulation of GFR. Accordingly, our data show no changes in GFR or in RBF both in intact and in renal denervated WKY and spontaneously hypertensive rats before and during ET_A receptor blockade.

Since the renal medulla in rats [21] and in humans [22] is rich in ET_B receptors and contains ET_A and ET_B receptors with an approximate ratio of 30:70, additional experiments were designed to determine the role of ET_B receptors in the response to denervation in spontaneously hypertensive rats and WKY rats. For this purpose BQ-788 was used to investigate the effects of ET_B receptor blockade on renal excretory function and its effects were compared with those of ET_A receptor blockade with BQ-123 in intact as well as renal denervated normotensive and hypertensive rats.

In the rat kidney a role of the ET_A receptor in the regulation of tubular reabsorption of sodium has been suggested [23]. In the present study BQ-123 infusion decreased urine flow rate in both WKY and spontaneously hypertensive rats, whereas BQ-788 infusion decreased urine flow rate only in normotensive rats. It appears, therefore, that ET acts also via ET_A receptors to affect tubular water transport in normotensive and in spontaneously hypertensive states. Moreover, our present results indicate that ET_A receptors, in addition to ET_B receptors as shown recently [9, 10], also mediate the actions of the endogenous ligand ET-1 to inhibit tubular sodium and potassium reabsorption in normotensive rats. In contrast to these results and to our surprise, spontaneously hypertensive rats revealed an impaired response of tubular sodium and potassium excretion to ET_A as well as ET_B receptor blockade. Referring to the ET-1 content in renal tissue that we observed we may conclude that the reduced content of ET-1 in the renal papilla of spontaneously hypertensive rats may contribute to the impaired natriuretic action of this peptide system in spontaneous hypertension. Thus, since ET-1 has diuretic and natriuretic properties when produced locally, the decreased renal production of ET-1 might be of relevance to the pathophysiology of hypertension.

With respect to the mechanisms of these diuretic and natriuretic actions of ET-1 it has been shown recently that administration of big ET-1 induced diuretic and natriuretic responses mediated mainly through stimulation of nitric oxide production via an ET_B receptor-coupled mechanism [10]. It was demonstrated that ET_B receptor deficiency resulted in large increases in blood pressure in female and, more pronounced, in male rats, especially during high salt intake and that increased nitric oxide synthase (NOS) in inner medullary collecting duct tissue protects against rises in blood pressure in female rats [24]. In addition, it was found that low-dose ET-1 inhibits renal epithelial sodium channels through ET_B receptor activation, whereas high-dose ET-1 increased renal epithelial sodium channel transport through activation of ET_A receptors [25]. Thus, from our present results we conclude that ET acting through renal tubular ET_A receptors also modulates renal sodium and potassium handling.

It is well recognized that renal nerves contribute substantially to the control of kidney function. Thus, renal nerve activity may affect renal hemodynamics, renin release, and sodium and water excretion [2]. The innervation of proximal and distal convoluted tubules, of the thick ascending limb of Henle's loop, as well as of cortical collecting tubules, is well established [26]. Moreover, the additional release of norepinephrine into the renal interstitium could be an effective route for the delivery of this neurotransmitter to its receptors located on the basolateral membrane of renal tubular epithelial cells throughout the entire length of the renal nephron [2]. Both α- [27] and β-adrenoceptors [28] are expressed in rat inner medullary collecting duct cells.

Acute as well as chronic renal denervation have been shown in micropuncture studies to decrease proximal tubule fluid reabsorption. This effect occurs independently of changes in GFR or RBF indicating that renal nerves directly modulate tubular transport processes [29, 30]. Accordingly, in the present study BRD did not affect blood pressure, GFR, or RBF in either normotensive or spontaneously hypertensive rats.

As expected, BRD resulted in a rise in urine flow rate with a reduction in urine osmolality, which was significant in normotensive WKY rats. It was suggested [31] that in the denervated kidney of conscious dogs a moderate impairment of urine concentration may result from decreased medullary tonicity since the medullary content of total solutes per kilogram wet tissue weight was found to be significantly lower in the denervated kidney than in the intact kidney. This mechanism may also underly the greater urine flow rate and lower urine osmolality in our renal denervated WKY rats.

With respect to an interaction between the renal ET system and renal nerves our findings show for the first time that BRD decreases papillary ET concentration in WKY but not in spontaneously hypertensive rats, whose tissue ET concentration is already low as we reported earlier [17] and as was confirmed in the present study. In support of these changes in papillary ET-1 content, we found parallel changes in urinary ET-1 excretion, which thus probably reflects tissue ET-1 synthesis.

With respect to our finding of a reduced papillary ET-1 content in denervated kidneys of WKY rats it has been demonstrated that ET production in the renal medulla is modulated by ambient osmolality. Indeed, there is evidence to suggest that increasing the osmolality of the incubation medium with NaCl, but not urea, causes a reduction in ET-1 release in cultured inner medullary collecting duct cells, but had no effect on ET-1 production by rat endothelial or mesangial cells [32]. On the other hand, increased extracellular tonicity induced by betaine or urea

was shown in vitro to stimulate ET synthesis in collecting duct cells [33] as well as peptide production and mRNA expression of ET-1 in the inner medullary collecting duct [34]. From the results of the present in vivo study, it seems reasonable to speculate that decreased papillary ET-1 concentration in renal denervated WKY rats may have resulted from reduced medullary and papillary tonicity that occur after renal denervation. This effect may not become apparent in spontaneously hypertensive rats with their a priori low ET-1 content in the renal papilla and may also be obscured by well-known rightward shift of the pressure-diuresis and pressure-natriuresis curves in hypertension [35]. Although the precise molecular mechanisms responsible for the observed changes in renal papillary ET-1 concentration cannot be derived from the results of the current study, our data provide strong evidence for a potential role of an interaction of renal nerves with the renal ET system in modulating renal function in normotensive and hypertensive states.

The results from our current study are also consistent with a role for renal nerves in modulating renal excretory function with respect to ET_A and ET_B receptor blockade. In contrast to the antidiuretic and antinatriuretic effects of BQ-123 in intact WKY rats, neither BQ-123 nor BQ-788 administration resulted in changes of renal excretory function in renal denervated WKY rats or in intact or renal denervated spontaneously hypertensive rats whose papillary ET content is low as compared to the intact WKY rats.

In the kidney ET_A receptor mRNA and/or binding sites have been found not only in renal vessels as would be expected but also in inner medullary interstitial and collecting duct cells [6] as well as in the descending thin limb of Henle's loop of long-looped nephrons [36]. Descending thin limbs of long-looped nephrons are located in close proximity to the thick ascending limbs and thus, paracrine inhibition of sodium reabsorption could be the route by which ET-1 exerts its diuretic and natriuretic effects via ET_A receptors [36]. Moreover, there is strong evidence that ET-1 functions as an autocrine regulator of sodium and water reabsorption in the collecting duct and thereby plays an important role in body fluid and volume regulation [24]. Thus, it is well known that ET is an inhibitory modulator of the hydro-osmotic action of arginine-vasopressin (AVP) in renal collecting ducts by suppressing AVP-induced cyclic adenosine monophosphate (cAMP) accumulation in the inner medullary collecting duct [37, 38]. α_2-adrenoceptors, also capable of inhibiting the action of AVP, have been found in the papillary collecting duct [39] and it was demonstrated that α-adrenergic agonists directly inhibit AVP-mediated water permeability in the collecting tubule [40]. Futhermore, activation of α_2-adrenoceptors can suppress cAMP formation stimulated by vasopressin [27]. Thus, not only ET but also adrenergic stimulation are important determinants for the regulation of renal water excretion. Moreover, it was shown that the ET system contributes substantially to the ability of the kidney to control sodium excretion [24] possibly by inhibition of Na^+/K^+-ATPase activity in the inner medullary collecting duct and thick ascending limb of Henle's loop [6]. Since both renal nerves and ET affect cAMP synthesis, this intracellular mediator may serve as the mutual target for the action of the renal ET system and of renal nerves to modulate renal fluid and sodium excretion. Thus, a direct interaction between the renal ET system and renal nerves appears to be involved in the control of renal excretory function.

Taken together, the data from our present study provide for the first time substantial evidence that renal nerves selectively regulate the activity of the ET system in the rat kidney and that renal denervation results in a blunted functional response to ET_A and ET_B receptor blockade in WKY and spontaneously hypertensive rats. Moreover, our data show that spontaneously hypertensive rats reveal a blunted response of tubular handling of sodium and potassium to ET_A and ET_B receptor blockade suggesting an association of hypertension with an altered intrarenal ET system. Finally, decreased synthesis of ET-1 in the renal papilla of spontaneously hypertensive rats may contribute to enhanced salt and water reabsorption in the inner medullary collecting duct and may thereby promote the development and maintenance of hypertension in spontaneously hypertensive rats.

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