The renal endothelin system in the Prague hypertensive rat, a new model of spontaneous hypertension

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ABSTRACT

In a new model of spontaneous hypertension, namely the Prague hypertensive rat (PHR), hypertension is transferred with a kidney transplanted from the PHR to its normotensive counterpart (PNR) by an as yet unknown mechanism. One candidate may be endothelin (ET), since this potent vasoconstrictor affects vascular tone, renal haemodynamics and renal excretory function, and all members of this peptide family are located within the kidney and act in an autocrine/paracrine fashion. In the present study we investigated, in the renal tissue of PHRs and PNRs: (1) preproET-1 and preproET-3 mRNAs as well as ET-1 and ET-3 peptide distribution, (2) endothelin-converting enzyme (ECE)-1 mRNA expression, and (3) ET receptors and their characteristics in membranes of glomeruli and papillae. In addition, plasma ET concentration and urinary ET excretion were determined. Quantitative measurements by competitive reverse transcription–polymerase chain reaction revealed ET-1 mRNA levels in the renal cortex from PHRs and PNRs of $1.09 \pm 0.13$ and $1.29 \pm 0.18$ amol/μg of total RNA respectively, and in red medulla of $2.72 \pm 0.82$ and $3.30 \pm 0.68$ amol/μg respectively. In contrast, renal papilla from PHRs showed significantly lower levels of preproET-1 mRNA (1.81 ± 0.64 amol/μg of total RNA, compared with 4.25 ± 0.82 amol/μg in PNRs; each $n = 5$; $P < 0.05$). The ET-1 peptide concentration in papillary tissue was also significantly lower in PHRs than in PNRs (120.2 ± 30.8 and 491.3 ± 53.4 fmol/mg of protein respectively; $n = 5$; $P < 0.01$), whereas it was similar in cortex and medulla from PHRs and PNRs. The preproET-3 mRNA content in renal tissue was much lower than that of preproET-1 mRNA. It was significantly higher in red medulla from PHRs compared with that from PNRs (0.25 ± 0.05 and 0.13 ± 0.02 amol/μg of total RNA respectively; $P < 0.05$), but was similar in papillae of PHRs and PNRs (0.04 ± 0.02 and 0.05 ± 0.01 amol/μg respectively; $n = 5$). Cortical preproET-3 mRNA was at the lower limit of detection. Similarly, the ET-3 peptide concentration was slightly but significantly higher in the red medulla of PHRs compared with PNRs (15.4 ± 2.0 and 8.8 ± 0.8 fmol/mg of protein respectively; $n = 5$; $P < 0.05$), whereas no differences in ET-3 peptide concentration were found in papillae from PHRs and PNRs. ECE-1 mRNA levels were similar in the renal cortex, red medulla and papillae from PHRs and PNRs, ranging between 0.34 ± 0.03 and 0.56 ± 0.12 amol/μg of total RNA. Of the total ET receptors in glomerular membranes, 39% were ET$_A$ receptors, whereas papillary membranes contained exclusively ET$_B$ receptors. PHRs and PNRs showed similar $B_{max}$ and $K_d$ values for ET-1 in renal glomerular membranes ($B_{max}$, 6.5 ± 1.3 and 4.9 ± 1.2 pmol/mg of protein respectively; $K_d$, 0.69 ± 0.10 and 0.56 ± 0.10 nM respectively) and papillary membranes ($B_{max}$, 9.7 ± 1.1 and 11.3 ± 1.6 pmol/mg of protein respectively; $K_d$, 0.30 ± 0.04 and 0.42 ± 0.07 nM respectively).

Key words: genetic hypertension, Prague hypertensive rat, renal endothelin system.

Abbreviations: AVP, [Arg$^b$]vasopressin (arginine-vasopressin); BP, blood pressure; ET, endothelin; ECE, endothelin-converting enzyme; ir-, immunoreactive; PHR/PNR, Prague hypertensive and normotensive rat respectively; RT-PCR, reverse transcription–PCR; SHR, spontaneously hypertensive rat; WKY rat, Wistar–Kyoto rat.

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INTRODUCTION

Experimental and clinical observations suggest that the kidney plays a crucial role in the development and/or maintenance of primary hypertension [1]. In several rat models of spontaneous hypertension, it was shown that high blood pressure (BP) is transferred with the kidney from a hypertensive to a normotensive control rat [2–6]. In a new model of spontaneous hypertension, the Prague hypertensive rat (PHR) and the corresponding normotensive rat (PNR), bred from the same parent pair of Wistar rats, hypertension was also transmitted with transplantation of the ‘hypertensive’ kidney. This was thought to be related to the renal synthesis of a hypertensinogenic factor or to the lack of a BP-lowering factor [2]. One such candidate could be the renal endothelin (ET) system, of which all components are located within the kidney.

ET, the most potent endogenous vasoconstrictor so far discovered, affects renal haemodynamics and excretory function independent of the extrarenal ET system. Thus, in an autocrine/paracrine fashion, ET-1 acts on the one hand at the preglomerular and glomerular level, e.g. it affects renin secretion, including modulation of tubuloglomerular feedback [8], and lowers renal blood flow, thus reducing salt and water excretion (for a review, see [7]). On the other hand, by its tubular effects, e.g. antagonism of the action of [Arg8]vasopressin (AVP; arginine-vasopressin) [9,10], it inhibits renal water (and salt) absorption at the level of the inner medullary collecting duct [11–13]. Thus the renal ET system may contribute to body fluid and BP regulation, and thereby also to the pathogenesis and/or maintenance of hypertension.

The difficulty, however, in assessing the physiological and pathological role of the ET system arises from the diversity of peptide isoforms [14], their metabolism [e.g. by endothelin converting-enzyme (ECE) activity] [15], their various receptors (e.g. ETa and ETb receptors) (for a review, see [16]) and their autocrine and/or paracrine action [17]. In the present study we investigated, therefore, in PHRs and PNRs: (1) preproET-1, preproET-3 and ECE-1 mRNA expression in the renal cortex, red medulla and papilla using competitive reverse transcription–PCR (RT-PCR) assays; (2) the contents of immunoreactive (ir-) ET-1 and ET-3 peptides in these tissues; and (3) the characteristics of the ET receptors in the glomeruli and papillae. Finally, we also determined plasma ET concentrations and urinary ET excretion. This approach was chosen in order to elucidate the potential contribution of the renal ET system as a mechanism for the development and/or maintenance of kidney-dependent spontaneous hypertension.

MATERIALS AND METHODS

Animals and experimental protocol

Studies were performed using male PNRs and PHRs aged 12–13 weeks, which were fed on a regular rat chow diet (Altromin®) and had free access to drinking water. Body weight was slightly but insignificantly lower in PHRs (222 ± 6 g) than in PNRs (248 ± 25 g). For adaptation, the animals were placed for 5 days in metabolic cages, and 24 h urine was collected on the sixth day. Under pentobarbital anaesthesia (50 mg/kg, intraperitoneal), blood was drawn by aortic puncture, and the kidneys were removed, weighed and rapidly dissected to obtain cortical, medullary and papillary tissues, which were weighed, frozen immediately in liquid nitrogen and then stored at –70 °C.

Determination of ir-ET-1 and ir-ET-3

Tissues were homogenized at 4 °C (on ice) in distilled water containing 100 kallikrein-inhibitory units/ml aprotinin using a 2 ml glass homogenizer (Kontes, Vineland, NJ, U.S.A.). The homogenates were then centrifuged at 600 g for 10 min. The supernatants were extracted using Sep-Pak C18 cartridges with 70% (v/v) methanol. The ET-1 concentration was determined by ELISA (Endothelin-1 ELISA-system; Amersham Buchler, Braunschweig, Germany) and ET-3 was measured by...
RIA (ET-3 RIA; Peninsula, Belmont, CA, U.S.A.). We have found previously that determination of ET-1 levels in unextracted urine and supernatants of isolated glomeruli and papillae by ELISA resulted in values almost identical to those obtained by ET-1/2 RIA [18]. For protein determination, the pellets were resuspended in 5 ml of 1% (w/v) SDS for bicinchoninic acid protein assay (Pierce, Rockford, IL, U.S.A.) according to the instructions of the manufacturer. Concentrations of ET-1/2 in extracted plasma and of ET-1 in urine were determined by RIA and ELISA respectively, as described previously [12,18].

Preparation of total RNA

Dissected renal tissues were carefully homogenized in 1 ml of RNA lysis buffer (4 M guanidinium thiocyanate, 1% mercaptoethanol) using a 1.5 ml glass homogenizer (Kontes). Total cell RNA was obtained using the RNaseasy Total RNA kit (Qiagen, Hilden, Germany) according to the instructions of the manufacturer. After spectrophotometric determination of RNA concentration, the integrity of the RNA was checked by denaturing agarose-gel electrophoresis [19]. RNA was stored at −70 °C until reverse transcription was performed.

DNase treatment and reverse transcription of RNA

To obtain cDNA samples free of genomic DNA, DNase treatment was carried out with aliquots of 1 μg of total RNA in a mixture of 5 μl of 5× first-strand buffer (Gibco BRL, Gaithersburg, MD, U.S.A.), 0.5 unit of DNase (Boehringer Mannheim, Mannheim, Germany) and 20 units of RNAsin (Promega, Madison, WI, U.S.A.), adjusted by addition of RNase-free water to a total reaction volume of 19.75 μl for each sample. After incubation at 37 °C for 10 min and inactivation of DNase by incubation at 65 °C for 5 min, 0.5 mM of each dNTP (Boehringer Mannheim), 0.5 μg of oligo(dT)$_{12-18}$ primer (Gibco BRL) and 100 units of SuperScript II reverse transcriptase (Gibco BRL) were added to the reaction mixture for cDNA synthesis [20], to give a final reaction volume of 25 μl. Reverse transcription was carried out in a DNA thermocycler (PE 2400; Perkin Elmer) at 45 °C for 45 min and then at 52 °C for 30 min, and was stopped by heating for 10 min at 100 °C. The cDNA samples obtained were stored at −20 °C until amplification by PCR.

PCR amplification of β-actin cDNA

To confirm the uniformity of RNA recovery and the efficiency of cDNA synthesis, β-actin cDNA was amplified for each sample as a relative control. Amplification of β-actin cDNA was carried out in 25 cycles in a 25 μl reaction volume and under standard PCR conditions with 1 μl of reverse transcription product as template using β-actin-specific primers (sequences as published in [21]). To ensure that PCR amplification had completed the exponential state before reaching the plateau phase, care was taken to perform no more amplification cycles than necessary. The PCR product of length 350 bp was run in a 2.5% (w/v) agarose gel stained with ethidium bromide (0.5 μg/ml). The intensities of the β-actin bands were compared densitometrically using the GelDoc 1000 Video Gel Documentation System with Molecular Analyst Software (Bio-Rad). Only cDNA samples generating similar amounts of β-actin product were used for further quantification. To minimize the possibility of artificially induced differences in amounts of cDNA, caused by slight deviations in reverse transcription or β-actin PCR efficiencies, the total number of samples from one tissue each from a PHR and a PNR, which were intended for comparison, were prepared together in one reverse transcription and β-actin run.

PCR amplification of preproET-1 cDNA

PCR amplification of preproET-1 cDNA was performed in a 25 μl reaction volume with 0.38 unit of Taq polymerase (Boehringer Mannheim) and corresponding PCR buffer (final concentrations: 50 mM Tris/HCl, 500 mM KCl, pH 8.3), 2.0 mM MgCl$_2$, 0.2 mM of each dNTP and 15 pmol of each primer (sequences as published in [21]), using 1 μl of reverse transcription product as template. The thermal cycler program was begun with a melting step (3 min at 94 °C), followed by 30–35 cycles at 94 °C for 45 s, 54 °C for 45 s and 72 °C for 30 s in the first cycle, with an increment of 3 s in each subsequent cycle. Final extension was at 72 °C for 10 min. PCR products of length 231 bp were run in a 2.0% (w/v) agarose gel.

PCR amplification of preproET-3 cDNA

PCR amplification of preproET-3 cDNA was performed in a 25 μl reaction volume with 0.6 unit of Taq polymerase and corresponding PCR buffer (composition as above), 1.5 mM MgCl$_2$, 0.2 mM of each dNTP and 15 pmol of each primer (sequences as published in [22]), using 1 μl of reverse transcription product as template. The thermal cycler program had an initial step at 94 °C for 3 min, followed by 30–40 cycles at 94 °C for 20 s, 57 °C for 20 s and 72 °C for 40 s in the first cycle, with an increment of 3 s in each subsequent cycle. Final extension was at 72 °C for 10 min. PCR products of length 383 bp were run in a 2.5% (w/v) agarose gel.

PCR amplification of ECE-1 cDNA

PCR amplification of ECE-1 cDNA was performed in a 25 μl volume containing PCR buffer (composition as above), 2.0 mM MgCl$_2$, 0.4 mM of each dNTP, 30 pmol of each primer (sequences as published in [22]), 0.6 unit of Taq polymerase and 1 μl of the reverse transcription-product as a target template.
reaction product. The thermal cycler program had an initial step at 94 °C for 3 min, followed by 25–28 cycles of 94 °C for 20 s, 54 °C for 20 s and 72 °C for 40 s in the first cycle, with an increment of 3 s in each subsequent cycle. Final extension was at 72 °C for 10 min. PCR products of length 529 bp were run in a 2.0% (w/v) agarose gel.

**Competitive PCR for quantification of cDNAs for preproET-1, ECE-1 and preproET-3**

For quantification of each ‘target’ cDNA (preproET-1, ECE-1 and preproET-3) by competitive PCR, specific competitor ‘mimics’ possessing the same primer sites, similar G/C content and similar length to the corresponding amplification fragment of the target cDNA were constructed. Therefore the mimics are supposed to have amplification characteristics similar to those of their corresponding target cDNA [23]. The different mimics for preproET-1, ECE-1 and preproET-3 were prepared by PCR amplification of a DNA fragment with neutral amplification characteristics (BamHI/EcoRI fragment of v-erbB cDNA; Clontech, Palo Alto, CA, U.S.A.) using oligonucleotide primers composed of a sequence complementary to the neutral DNA fragment in the 3’ direction and the sequences of the primers used for amplification of the related target cDNA in the 5’ direction. The primer sequences were: 5’-ATGGATTATTTCCTCCGTATGAGATGATTC-CC-3’ (sense) and 5’-GGGAGTTGACCCAGATGAAAGTATTGATTCTGGACCAGCATC-3’ (antisense) for the ET-1 mimic; 5’-CTGTTATACAGGGAGATGAAA-3’ (sense) and 5’-GTGCCACACAAAAACTACAGGAGATGAAAATGTTGTAGATT-3’ (antisense) for the ECE-1 mimic; and 5’-GCACITTCGTTCACCTTATATAAGTGTAT-ACAGGGAGATGAAA-3’ (sense) and 5’-CAGAAAGCAAAGCATCGTTACATCTGCAAAGGC-3’ (antisense) for the ET-3 mimic. The amplification products were separated by agarose-gel electrophoresis and the band representing the competitor DNA was isolated. After elution, the competitor DNA was re-amplified using the target-specific primers and electrophoretic separation, and elution was repeated. After spectrophotometric determination of the DNA concentration at 260 nm, serial dilutions of the competitor DNA were prepared.

To quantify the target cDNA, several reaction mixtures were prepared for each determination. To each mixture was added 1 μl of sample with an unknown amount of target cDNA and 1 μl of a dilution series of competitor DNA of known concentration as an internal standard, for co-amplification. PCR conditions were as described above for preproET-1, ECE-1 or preproET-3 amplification. The amplification products were separated in an agarose gel stained with ethidium bromide (Figure 1).

Band intensities were scanned and analysed densitometrically. At low concentrations of competitor DNA (in relation to the amount of target cDNA in the sample), the band specific for the target cDNA was dominant, whereas at high concentrations of competitor DNA the band representing the competitor was dominant. At the equivalence point, both bands had the same intensity, i.e., the same amounts of target cDNA and competitor DNA were synthesized during PCR, it can be concluded that the concentration of the target cDNA in the sample was same as the concentration of competitor added. Since the efficiency of reverse transcription is
estimated to be of the order of 35–40% [24,25], the values were divided by 0.35 in the calculation of the underlying mRNA concentration. Moreover, a correction factor of 2 was included in the calculation to compensate for the discrepancy of the target cDNA being single-stranded in the first cycle, while the competitor DNA is double-stranded from the beginning of the PCR amplification.

Membrane preparation
Membranes from renal glomeruli and papillae were prepared as previously described [26]. In brief, renal glomeruli were isolated by graded sieving, whereas papillae were minced with a razor blade. All tissues were centrifuged for 5 min at 3000 g, and the pellets were then resuspended in 0.250 mol/l sucrose and homogenized with two bursts of 30 s each in a Polytron homogenizer. After centrifugation for 5 min at 1000 g, the resulting supernatants were centrifuged at 5000 g for 60 min. The membrane pellets were kept frozen at −70 °C until binding assays were performed. Membranes obtained from individual animals were used for binding assays.

Receptor binding studies
Membranes were suspended in assay buffer (50 mmol/l Tris/HCl, pH 7.4, 135 mmol/l NaCl, 2 mmol/l N-acetyl-D,L-methionine, 1.1 mmol/l MgCl₂, 0.5 mmol/l PMSF, 0.01 mmol/l aprotinin). After adjusting membrane suspensions to a protein concentration of 2.5 ml, BSA was added to a final concentration of 0.5% (w/v). Membrane suspension (400 ml) was incubated in duplicate for 3 h at 21 °C with 50 ml of 125I-ET-1 (Amersham) (approx. 15 fmol; specific radioactivity 1900 Ci/mmol) and 50 ml of various concentrations of unlabelled ET-1 (0.01–10 nmol/l).

For discrimination of ETₐ receptors from total ET receptors, the specific ETₐ receptor blocker BQ-123 (Peninsula) was employed. Membranes were preincubated with BQ-123 (1 μmol/l) for 30 min at 21 °C, and kinetic studies were performed as described above. Dose–response curves for the effects of BQ-123 were established by incubating membranes with a constant amount of labelled 125I-ET-1 (10000 c.p.m.) and concentrations of BQ-123 between 0.01 nmol/l and 10 μmol/l. The reaction was stopped by dilution with 2.0 ml of ice-cold assay buffer and rapid filtration through 0.33% polyethyleneimine-treated Whatman GF/C filters. After rinsing three times with 2.0 ml of assay buffer, filters were dried and radioactivity was counted in a Kontron γ-radiation counter with 80% efficiency. Non-specific binding was defined as filter-associated radioactivity in the presence of 100 nmol/l ET-1, and represented approx. 5% of total radioactivity.

The protein concentration of membrane preparations was measured with a Bio-Rad protein microassay based on the method of Bradford [27].

Data analysis
Binding data were analysed by linear regression of Scatchard analysis using the EBDA/LIGAND [28] computer program (Elsevier-Biosoft, Cambridge, U.K.). Statistical analysis of the results was performed with Student’s t test for unpaired or paired data, or, if necessary, by analysis of variance (Origin; Micrcal Software, Northampton, MA, U.S.A.). Data are presented as means ± S.E.M.

RESULTS
BP, kidney weight, and plasma and urinary ET
Systolic BP was 208±15 mmHg in PHRs and 121±5 mmHg in PNRs (P < 0.01). Kidney weight was significantly higher in PHRs than in PNRs (866±14 and 739±20 mg/100 g body weight respectively; P < 0.01). The plasma ET-1/2 concentration (PHRs, 10.4±1.3 fmol/ml; PNRs, 12.2±1.2 fmol/ml) and urinary ET excretion (PHRs, 3.1±0.3 pmol/24 h; PNRs, 3.0±0.2 pmol/24 h) did not differ significantly between hypertensive and normotensive rats.

PreproET-1 mRNA and ir-ET-1 in renal tissues
PreproET-1 mRNA was detectable in all renal tissues examined (n = 5 in each group) (Figure 2A). The expression of preproET-1 mRNA in PHRs and PNRs was similar in both cortex (1.09±0.13 and 1.29±0.18 amol/μg of total RNA respectively) and medulla (2.72±0.82 and 3.30±0.68 amol/μg respectively), whereas a markedly lower preproET-1 mRNA concentration was found in the papilla of PHRs (1.81±0.64 amol/μg of total RNA) compared with PNRs (4.25±0.82 amol/μg).

The concentrations of ir-ET-1 in suspensions of renal tissues are shown in Figure 2(B). In agreement with the distribution of preproET-1 mRNA, the ir-ET-1 content was lower in the cortex (PNRs, 4.0±0.7 fmol/mg of protein; PHRs, 3.1±0.5 fmol/mg) than in the medulla (PNRs, 28.3±9.3 fmol/mg; PHRs, 25.6±9.6 fmol/mg). The highest amount of ir-ET-1 was detected in the papillary tissue of PHRs (1.81±0.64 amol/μg of total RNA) compared with PNRs (4.25±0.82 amol/μg).

PreproET-3 mRNA and ir-ET-3 in renal tissues
In the renal cortex, preproET-3 mRNA was almost undetectable in both PHRs and PNRs. In contrast, significant amounts of preproET-3 mRNA were found in the renal medulla, and these were significantly greater in PHRs than in PNRs (0.25±0.05 and 0.13±0.02 amol/μg of total RNA respectively; P < 0.05) (Figure 3A). The
levels of preproET-3 mRNA in the papillary tissue of PHRs and PNRs were similar (0.040 ± 0.015 and 0.052 ± 0.013 amol/μg respectively).

Similar to preproET-3 mRNA, the lowest levels of ir-ET-3 were found in the renal cortex, with no difference between PHRs and PNRs (6.5 ± 1.2 and 7.7 ± 2.2 fmol/mg of protein respectively) (Figure 3B). Again, as was found for preproET-3 mRNA, the concentration of ir-ET-3 peptide in medullary tissue was slightly but significantly higher in PHRs than in PNRs (15.4 ± 2.0 and 8.8 ± 0.8 fmol/mg respectively; \( P < 0.05 \)), whereas levels were similar in the papillae of PHRs and PNRs (30.7 ± 7.1 and 41.7 ± 3.0 fmol/mg respectively; \( n = 5 \) in each group).

**ECE-1 mRNA in renal tissues**

In all organs and tissues examined, ECE-1 mRNA was detectable, with no differences between PNRs and PHRs. In the renal cortex of PHRs and PNRs the concentrations were 0.34 ± 0.03 and 0.36 ± 0.04 amol/μg of total RNA respectively, in medullary tissue 0.40 ± 0.05 and 0.56 ± 0.12 amol/μg respectively, and in papillary tissue 0.45 ± 0.11 and 0.44 ± 0.01 amol/μg respectively.

**Characterization of ET receptors in renal glomeruli and papillae**

As shown in Table 1, \( B_{\text{max}} \) for ET-1 was higher in papillary than in glomerular membranes. The binding affinity for ET-1, expressed as \( K_d \), was slightly lower in renal glomerular membranes than in papillary mem-

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<th>ET-1 (pmol/mg protein)</th>
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<td>Rats</td>
<td>Glomeruli</td>
<td>Papillae</td>
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<td>PNRs</td>
<td>4.9±1.2</td>
<td>11.3±1.6</td>
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<td>PHRs</td>
<td>6.5±1.3</td>
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Using the ET\(_A\) receptor antagonist BQ-123, ET\(_A\) receptors were calculated to account for approx. 39% of total ET receptors in glomerular membranes, with no significant difference between PHRs and PNRs. In contrast, binding of ET was unaffected by BQ-123 in papillary membranes, indicating the presence of ET\(_B\) receptors only. Again, no difference between PHRs and PNRs was noted.

**DISCUSSION**

In our new model of spontaneous hypertension, i.e. the PHR and its normotensive counterpart (the PNR) with complete graft histocompatibility, high BP was shown to be transferred with the kidney transplanted from the hypertensive to the normotensive animal [2,29]. This
agrees with data obtained in other spontaneously hypertensive rats (SHRs) [1]. Since the glomerular filtration rate and Na and K excretion were not different between PHRs and PNRs at 12 weeks of age [29], the intrinsic mechanisms of the ‘hypertensive’ kidney in producing a high BP are still unknown, but a renal hypertensogenic factor or the lack of a BP-lowering factor were postulated [2]. One such candidate could be the renal ET system, all the components of which are located in the kidney, where it has important haemodynamic and tubular effects, e.g. antagonism of the action of AVP [10], which may contribute to body fluid regulation. In this respect, it is of interest that PHRs were found to have increased plasma and blood volumes [29].

In order to elucidate a potential role for the renal ET system in the hypertension of PHRs, in the present study we investigated plasma and urinary ET levels in PHRs and PNRs, as well as preproET-1, preproET-3 and ECE-1 mRNA expression, ET-1 and ET-3 peptide distribution, and ET receptor characteristics in renal tissues from PHRs and PNRs. We found no difference in plasma ir-ET-1/2 levels between PHRs and PNRs, thus excluding a systemic role of circulating ET.

Using competitive RT-PCR, our results show that, in control kidneys from PNRs, the amounts of preproET-1 mRNA are similar in the red medulla and papilla, but significantly lower in the renal cortex. No differences in preproET-1 mRNA content in the red medulla or cortex were found between PHRs and PNRs. In contrast, significantly lower amounts of preproET-1 mRNA and of ET-1 peptide were present in papillary tissue from PHRs compared with that from PNRs. These findings agree well with the observations of Hughes et al. [30] in SHRs, who found slightly lower preproET-1 mRNA expression in the renal cortex and outer medulla and significantly lower levels of preproET-1 mRNA in inner medullary (i.e. papillary) tissue from SHRs as compared to normotensive Wistar–Kyoto (WKY) rats. If it is assumed that the ET system in the renal cortex plays a role in renal haemodynamics [31], whereas that located in the red medulla and papilla is involved in the renal concentrating mechanism [10,12,31], our data, together with the normal glomerular filtration rate observed previously [29], suggest that ET in the renal papilla rather than that in the renal cortex plays a pathophysiological role in spontaneous experimental hypertension. Since ET-1 antagonizes the tubular effects of AVP by suppressing cAMP synthesis [9,10], decreased papillary ET-1 synthesis in our PHRs may result in increased water absorption from the inner medullary collecting duct.

The content of preproET-3 mRNA in the different parts of the kidney was 10–80 times lower than that of preproET-1 mRNA, and was almost undetectable in renal cortex. When compared with PNRs, PHRs had significantly higher amounts of preproET-3 mRNA and of ET-3 peptide in the red medulla. It is known that ET-3 binds predominantly to ET\(_B\) receptors, which are present in the medullary collecting duct [26], and the medullary vasculature may also possess predominantly ET\(_A\) receptors [32]. However, since the renal preproET-3 content is so much lower than that of preproET-1, and since we cannot differentiate from the present data between tubular epithelial and vascular effects, the finding of increased synthesis of ET-3 in red medulla from PHRs requires further study. Nevertheless, to our knowledge preproET-3 mRNA expression in the outer renal medulla of SHRs has not been described previously.

With respect to ECE-1 mRNA, we found similar amounts of ECE-1 amplification product in the different regions of the kidney. ECE-1, with its widespread expression in many tissues [33], is present on the surface and in intracellular compartments of endothelial and other cells (e.g. tubular epithelial cells), acting to convert inactive ‘big’-ET-1 into biologically active ET-1 [15]. The only other ECE isoenzyme presently known, ECE-2, may be the major ECE in neural tissues, and its expression is estimated to represent only 1–2% of all ECE mRNA [34]. Although in the present study we observed no differences in renal ECE-1 mRNA content between PHRs and PNRs, others have found greater amounts of ECE-1 mRNA and ECE-1 protein in isolated glomeruli of adult SHRs as compared with WKY rats [35]. The divergent findings may result from methodological differences (e.g. whole kidney cortex in our study compared with isolated glomeruli) or strain-related differences.

In our PHRs and PNRs, we found that, in isolated glomeruli, ET\(_A\) receptors represented 39% of total ET receptors, whereas papillary tissue contained exclusively ET\(_B\) receptors. A slightly higher binding capacity for ET was observed in the renal cortex from PHRs as compared with PNRs. In contrast with the findings of Gellai et al. [36] in SHRs, we found no significant differences in ET\(_A\) and ET\(_B\) receptor subtype distribution between PHRs and PNRs.

In summary, the lower amounts of preproET-1 mRNA and ET-1 peptide in renal papillary tissue from PHRs compared with PNRs suggest that ET-1, which affects tubular fluid absorption via modulation of AVP-induced cAMP synthesis at the level of the inner medullary collecting duct, contributes to the development and/or maintenance of high BP in PHRs, a new model of spontaneous hypertension. The differences between PHRs and PNRs in preproET-3 mRNA and ET-3 peptide content in the renal medulla may point to altered medullary perfusion in the hypertensive rat. However, further investigations, including studies in prehypertensive animals, are required in order to elucidate more precisely the potential role of the renal ET system in the pathophysiology of spontaneous hypertension in this rat model.
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