Renal adaptation to dietary sodium restriction and loading in rats treated neonatally with enalapril

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1Department of Physiology, Institute of Physiology and Pharmacology, Göteborg University, S-413 90 Göteborg, Sweden; 2Department of Internal Medicine, University of Iowa College of Medicine, Iowa City, Iowa 52242–0001; and 3Department of Pathology, Karolinska Hospital, Stockholm, Sweden

Guron, Gregor, Annika Nilsson, Gerald F. DiBona, Birgitta Sundelin, Nicoletta Nitescu, and Peter Friberg. Renal adaptation to dietary sodium restriction and loading in rats treated neonatally with enalapril. Am. J. Physiol. 273 (Regulatory Integrative Comp. Physiol. 42): R1421–R1429, 1997.—Neonatal treatment of rats with angiotensin-converting enzyme inhibitors or the angiotensin II type 1 receptor antagonist losartan induces irreversible renal histological abnormalities, mainly papillary atrophy, in association with an impairment in urinary concentrating ability. In the present study, sodium and potassium balance were assessed during high and low sodium intake and dietary potassium restriction in adult Wistar rats treated neonatally with enalapril (10 mg·kg⁻¹·day⁻¹) from 3 to 24 days of age. During balance studies, neonatally enalapril-treated rats showed 1) normal adaptation to dietary sodium restriction, 2) sodium retention during dietary sodium loading, and 3) a transient, modest, renal potassium wastage during dietary potassium restriction. Renal clearance determinations under pentobarbital anesthesia showed elevated fractional excretions of sodium and potassium and osmolar clearance without changes in glomerular filtration rate or effective renal plasma flow in enalapril-treated compared with vehicle-treated rats. Thus, in addition to the impaired urinary concentrating ability, adult rats treated neonatally with enalapril demonstrated alterations in renal sodium and potassium handling, which may be related to the prevailing papillary atrophy.

angiotensin-converting enzyme inhibitor; renal development; renal medulla

IT HAS BEEN PREVIOUSLY SHOWN that neonatal blockade of the renin-angiotensin system (RAS) during the first 3 wk of life in rats (8, 12, 13) and pigs (7) induces a persistent defect in renal urinary concentrating ability associated with irreversible renal structural changes, mainly characterized by papillary atrophy, and chronic interstitial inflammation and fibrosis. These abnormalities in renal structure and function were induced by neonatal administration of either different angiotensin-converting enzyme (ACE) inhibitors or the angiotensin II (ANG II) type 1 (AT₁) receptor antagonist losartan, but not the ANG II type 2 (AT₂) receptor antagonist PD-123319 (8). Thus a specific lack of AT₁ receptor stimulation for a brief period of time postnatally impaired normal renal development in the rat, a finding that has been confirmed by other investigators (28). Additional evidence indicating an important role for the RAS in the development and maintenance of normal renal integrity has recently been obtained in transgenic mice deficient in ACE (5, 19) or angiotensinogen (18, 20) that developed alterations in renal histology similar to those observed in pharmacological studies in the neonatal rat (8, 12, 13). In accordance with these findings, all components of the RAS are expressed in the kidney and are developmentally regulated in a tissue-specific manner with increased gene transcription and elevated renal ANG II content occurring perinatally compared with the adult (9, 29). Although exactly how ANG II is involved in normal renal development remains to be elucidated, Tufro-McReddie et al. (28) have demonstrated that neonatal AT₁ receptor antagonism in the rat inhibits renal growth, impairs renal vascular development, and delays nephron maturation. Niimura et al. (20) observed a marked downregulation of platelet-derived growth factor A mRNA in the hypoplastic papilla of transgenic mice deficient in angiotensinogen, suggesting a role for ANG II in the maturational growth of the papilla by regulation of other growth factors.

We have previously demonstrated that a major functional defect in adult neonatally enalapril-treated rats is a marked impairment in urinary concentrating ability of renal origin that may be explained by the papillary atrophy (13). Apart from its important role in renal water reabsorption, the inner medullary collecting duct is the site at which the final adjustments of sodium and potassium excretion occur (27). Thus the hypothesis of the present study was that renal sodium and potassium handling may be impaired in neonatally enalapril-treated rats with papillary atrophy. To test this hypothesis, we assessed the adaptations in urinary sodium and potassium excretion in response to alterations in dietary sodium and potassium intake. During dietary sodium-loading experiments, when the drinking fluid was switched to isotonic saline, neonatally enalapril-treated rats showed a pronounced increase in fluid intake compared with controls. Measurements of NaCl preference and plasma osmolality (P₂₉₅) in rats drinking isotonic saline were performed to elucidate mechanisms underlying this behavioral alteration in enalapril-treated rats.

METHODS

General Procedures

Time-mated female Wistar rats (Charles River UK, Margate, Kent, UK) were transported to our facilities on their 16th day of pregnancy and were carefully observed for determination of day of delivery. Sex was determined in 2-day-old pups, and males were included in the study. Weight-
matched male pups were divided into groups receiving daily intraperitoneal injections from 3 to 24 days of age with either enalapril maleate (10 mg/kg; Merck, Sharp, and Dohme, Solventuna, Sweden) (n = 22) or isotonic saline vehicle (n = 22) in equivalent volumes of 10 ml/kg. After the neonatal treatment period, rats were left untreated and observed until 8 wk of age, at which time metabolic balance experiments were begun. Rats had free access to normal rat chow and tap water (when they were not subjected to any experimental dietary regime during metabolic balance studies) and were kept in rooms with a controlled temperature of 24°C and a 12:12-h dark-light cycle (6 PM–6 AM) throughout the study. Dietary potassium restriction. Rats were offered a low-potassium diet (K ÷ 0.3 mmol/kg; Na ÷ 1.14 mmol/kg; Analyce) during metabolic balance studies) and were begun. Rats had free access to normal rat chow and tap water. Thereafter rats were given a low-sodium diet (Na ÷ 120 mmol/kg; K ÷ 153 mmol/kg) and tap water. Thereafter rats were given a low-sodium diet (Na ÷ 6 mmol/kg; K ÷ 100 mmol/kg; Analyce) for 26 days, during which time sodium restriction and sodium-loading experiments were carried out. For the initial 4 days, sodium intake was normalized by adding NaCl to distilled water as drinking fluid (enalapril, 31 mM NaCl; vehicle, 45 mM NaCl). Enalapril-treated rats were provided with a lower NaCl concentration to compensate for their elevated fluid intake, thereby equalizing sodium intake in the two groups. Subsequently, one-half of the enalapril-treated and vehicle-treated rats was given distilled water to drink (sodium restriction), and the other one-half had their drinking fluid switched to isotonic saline (154 mM NaCl) (sodium loading) for 6 days. This was followed by 6 days of normal sodium intake before a similar 6-day period of dietary sodium restriction and sodium loading was performed in a crossover fashion. After the last dietary intervention, all rats were allowed a normal sodium intake for 4 days, thus completing the 26-day balance study.

Dietary potassium restriction. During dietary potassium restriction, rats were offered a low-potassium diet (K ÷ 0.3 mmol/kg; Na ÷ 1.14 mmol/kg; Analyce) throughout the 10-day experimental period. Similar to the sodium balance studies, a normal dietary potassium intake during the control period was achieved by adding KCl to distilled water as drinking fluid (enalapril, 65 mM KCl; saline vehicle, 100 mM KCl). Normal potassium intake was maintained for 4 days, and then the drinking fluid was switched to distilled water for the subsequent 6 days (potassium restriction).

Renal Clearance Experiments in Anesthetized Rats

Glomerular filtration rate (GFR) and effective renal plasma flow (ERPF) were measured by the urinary clearances of 51Cr-labeled EDTA (Amersham Laboratories, Buckinghamshire, UK) and 125I-labeled hippuran (Institutt for Energiteknikk, Kjeller, Norway), respectively. Rats were anesthetized with pentobarbital sodium (60 mg/kg ip) and tracheotomized with a polyethylene catheter (PE-240), and their body temperature was maintained at 38°C throughout the experiment. The left jugular vein and carotid artery were catheterized with PE-50 tubing. The urinary bladder was catheterized through a midline abdominal incision with a PE-160 catheter. Throughout the experiment, rats were infused with 51Cr-EDTA (20 µCi · kg ÷1 · h ÷1 iv), 125I-hippuran (10 µCi · kg ÷1 · h ÷1 iv), and pentobarbital sodium (12 mg · kg ÷1 · h ÷1 ia) dissolved in isotonic saline, yielding a total infusion rate of 10 ml · kg ÷1 · h ÷1. After 45 min of equilibration, two consecutive 20-min urine collection periods with mid-point arterial blood sampling were performed. Urine was collected in preweighed vials, and urine density was assumed to be 1,000 g/ml urine. Urine and plasma were analyzed for radioactivity (Packard 3-channel scintillation counter, model 5019; Packard, Amana, IA), osmolality, and sodium and potassium concentrations. Mean arterial blood pressure (MAP) and heart rate were recorded continuously with Statham pressure transducers connected to a Grass polygraph. Filtration fraction was calculated as (GFR/ERPF) · 100, and renal vascular resistance was calculated as MAP/ERPF. Solute-free water reabsorption (T(H2)O) was calculated as osmolar clearance (Cosm ÷ V · Cosem = Urine osmolality (Uosm)/plasma osmolality (Posm) · urine flow rate (V)). Fractional excretions of sodium (FENa) and potassium (FElK) were estimated as the ratio of their respective clearances to that of 51Cr-EDTA, taken as GFR, 100. Data are presented as mean values for the two clearance periods.

Kidney Weight and Histology

After renal clearance experiments, kidneys were rapidly excised, decapsulated, and weighed. Left kidneys were dried for 24 h at 100°C and reweighed for dry weight. After weighing, right kidneys were immediately immersion fixed in 4% formaldehyde and processed for semiquantitative histological analysis by light microscopy with use of an arbitrary scale where 0 = normal, 1 = mild, 2 = moderate, and 3 = severe changes, as described previously (12).

NaCl Preference

Rats were kept individually in cages with free access to normal, pelleted rat chow and drinking fluid throughout the experiment. Rats were provided with two identical bottles per
cage. Initially, baseline measurements of fluid intake were performed for 3 days, during which time both bottles contained tap water. Subsequently tap water was replaced by isotonic saline in one of the bottles for 3 consecutive days, thereby providing rats with a free choice of drinking fluid.

Plasma Sodium and Osmolality During Sodium Loading

Rats were kept in individual cages with free access to normal pelleted rat chow and drinking fluid throughout the experiment. After 2 days with tap water as drinking fluid, conscious rats had tail blood sampled. Subsequently the drinking fluid was switched to isotonic saline. After the rats drank isotonic saline for 3 days, another tail blood sample was taken. Plasma sodium, potassium, and osmolality were measured on tail blood samples.

Skeletal Muscle Potassium

Rats consuming a normal diet were anesthetized with pentobarbital sodium (60 mg/kg ip), and aortic blood was collected. A skeletal muscle biopsy was taken from the right quadriceps muscle and carefully deaneded of fat for measurement of skeletal muscle potassium content. Skeletal muscle dry weight was measured after 24 h in 100°C. Dry muscle tissue was subsequently homogenized in 37% hydrochloric acid, and potassium was measured in the homogenate.

Statistics

Data in text, Tables 1–3, and Figs. 1–3 and 5 are means ± SE. Analysis of variance (ANOVA) for repeated measurements was used in the balance studies followed by Scheffé's post hoc test. Student's unpaired and paired two-tailed t-tests were used for statistical analysis of data between and within groups when appropriate. Wilcoxon's rank sum test was used for statistical analysis of data between groups when appropriate. Student's unpaired and paired two-tailed -tests were used for statistical analysis of data between and within groups. Analysis of variance (ANOVA) for repeated measurements was used for statistical analysis of data between and within groups. The Student's t-test was used for statistical analysis of data between groups.

RESULTS

Metabolic Balance Studies

Fluid handling. During the initial 4-day control period on normal sodium intake, enalapril-treated rats showed an increase in daily fluid intake (157 ± 6 vs. 124 ± 10 ml·kg⁻¹·day⁻¹, P < 0.05) and urine volume (97 ± 4 vs. 74 ± 9 ml·kg⁻¹·day⁻¹, P < 0.05) and a reduction in Uosm (609 ± 31 vs. 821 ± 83 mosmol/kg, P < 0.05). These alterations in fluid handling persisted throughout all metabolic balance studies, although they varied in magnitude depending on the composition of drinking fluid.

Sodium restriction. During dietary sodium restriction, sodium intake was similar in both groups (0.35 mmol·kg⁻¹·day⁻¹; when not related to body weight, this equaled a sodium intake of 0.11 mmol/day) (Fig. 1, A and B). There was no difference between groups in daily or cumulative sodium balance throughout the experiment (Fig. 1, C and D). In both groups, daily sodium balance was negative only on the first day (day 5) of dietary sodium restriction (Fig. 1C). There was no difference between groups in body weight gain or potassium balance throughout the experiment.

Sodium loading. There was no difference between groups in sodium intake or balance during the initial 4-day control period (Fig. 2, A–D). Neonatally enalapril-treated rats retained more sodium than vehicle-treated rats during the period of sodium loading (P < 0.05; Fig. 2, C and D) and displayed an increase in cumulative sodium balance after 2 days of high sodium intake that persisted throughout the study, even after normalization of sodium intake (P < 0.05, Fig. 2D). During sodium loading, sodium intake averaged 34.7 ± 2.7 and 19.9 ± 2.3 mmol·kg⁻¹·day⁻¹ in enalapril-treated and vehicle-treated rats, respectively (P < 0.05, Fig. 2, A and B). The elevated sodium intake in enalapril-treated rats resulted from a significant increase in fluid intake when the drinking fluid was switched to isotonic saline (53 ± 11 vs. 5 ± 1% increase in fluid intake compared with baseline in enalapril-treated and vehicle-treated rats, respectively; P < 0.05). During the 6-day period of high sodium intake, there was no difference between groups in body weight gain (22 ± 1 vs. 25 ± 2 g in enalapril-treated and vehicle-treated rats, respectively) or potassium balance.

Potassium restriction. During the control period, potassium intake and balance were similar in both groups (Fig. 3, A–C). During potassium restriction, potassium intake reached very low values (0.01 mmol·kg⁻¹·day⁻¹ in both groups). Enalapril-treated rats showed a significantly more negative potassium balance than controls, transiently, from days 5 to 9 (P < 0.05, Fig. 3C). Cumulative potassium balance for the period of potassium restriction was more negative in enalapril-treated rats (−5.54 ± 0.48 vs. −3.42 ± 0.20 mmol/kg in enalapril-treated and vehicle-treated rats, respectively; P < 0.05). There was no difference between groups in urinary potassium concentration during potassium restriction, and values reached 3.6 ± 0.4 and 3.8 ± 0.3 mM in enalapril-treated and vehicle-treated rats, respectively, on the last day of experimentation.

Renal Function and Hemodynamics

Renal clearance experiments did not reveal any significant differences between groups in either GFR or ERPF. (Table 1). V, FE, FEK, and Ceq were elevated and Uosm was reduced in neonatally enalapril-treated rats (P < 0.05, Table 2). There was no difference between groups in plasma sodium (PNa) or potassium (PK) concentrations (PNa, 137 ± 1 vs. 136 ± 1 mM in enalapril-treated and vehicle-treated rats, respectively; PK, 3.8 ± 0.1 mM in both groups). Neither wet nor dry kidney weight (KW) differed between groups (KWwet, 2.74 ± 0.10 vs. 2.94 ± 0.12 g; KWdry, 0.58 ± 0.02 vs. 0.61 ± 0.02 g in enalapril-treated and vehicle-treated rats, respectively).

Renal Histology

Rats treated neonatally with enalapril showed renal histological abnormalities qualitatively identical to those described in greater detail previously (8, 12, 13). In brief, enalapril-treated rats showed a significant...
degree of papillary atrophy (2.8 ± 0.0 vs. 0.0 ± 0.0 arbitrary units; P < 0.05), chronic interstitial inflammation (0.8 ± 0.1 vs. 0.0 ± 0.0 arbitrary units; P < 0.05), and renal vascular changes (0.8 ± 0.1 vs. 0.0 ± 0.0 arbitrary units; P < 0.05) (Fig. 4). The papillary atrophy score of 2.8 in enalapril-treated rats corresponded to an almost complete loss of the renal papilla (Fig. 4). As previously described (12), renal vascular changes were limited to interlobular arteries and consisted of thickening of both the intima and media.

NaCl Preference

There was no difference between groups in daily isotonic saline or osmolar intake when rats were given a free choice of drinking fluid (Table 3). However, enalapril-treated rats showed an approximately fourfold increase in tap water intake and a reduced isotonic saline preference compared with vehicle-treated rats (Table 3).

P_Na During Sodium Loading

When drinking tap water, neonatally enalapril-treated rats showed a 2.4-fold increase in fluid intake compared with vehicle-treated rats, whereas P_Na and P_osm (299 ± 1 vs. 297 ± 1 mosmol/kg in enalapril-treated and vehicle-treated rats, respectively) were similar in both groups (Fig. 5). Fluid intake increased in both groups when rats drank isotonic saline, although to a larger extent in enalapril-treated rats (Fig. 5). On experimental day 5, fluid intake had increased 154 ± 9 vs. 36 ± 16% compared with baseline intake in enalapril-treated and vehicle-treated rats, respectively (P < 0.05, Fig. 5). P_Na and P_osm (309 ± 4 mosmol/kg in enalapril-treated rats on experimental day 5) rose significantly in enalapril-treated rats drinking isotonic saline but remained unaltered in vehicle-treated rats (Fig. 5).

Skeletal Muscle Potassium

Skeletal muscle potassium content in rats fed rat chow with a normal potassium content before experimentation was slightly reduced in neonatally enalapril-treated rats (421 ± 8 vs. 450 ± 7 mmol/kg dry weight in enalapril-treated and vehicle-treated rats, respectively; P < 0.05). There was no difference between groups in P_K (3.7 ± 0.2 vs. 3.8 ± 0.2 mM in enalapril-treated and vehicle-treated rats, respectively).
DISCUSSION

The main findings of the present study were that adult rats treated neonatally with enalapril showed 1) a preserved capacity to conserve sodium in response to dietary sodium restriction and 2) sodium retention during dietary sodium loading. In addition, enalapril-treated rats demonstrated a slight reduction in skeletal muscle potassium content when they consumed a normal diet and modest, transient potassium wastage during dietary potassium restriction. Furthermore, assessment of renal function in anesthetized rats revealed an elevated $F_E_{Na}$, $F_E_K$, and $C_{osm}$ without changes in GFR or ERPF in enalapril-treated rats. Neonatally enalapril-treated rats developed marked renal morphological abnormalities, mainly characterized by a nearly complete atrophy of the papilla, confirming our previous findings (8, 12, 13). This result was associated with an impairment in urinary concentrating capacity, as evidenced by an increase in urine volume and a reduction in $U_{osm}$. In a recent study (13), we demonstrated marked reductions in maximal $U_{osm}$ and tubular free water reabsorption in neonatally enalapril-treated rats after administration of supramaximal doses of 1-desamino-8-D-arginine vasopressin, indicating that the impairment in urinary concentrating ability is of renal origin and is presumably a consequence of papillary atrophy. We previously found slight reductions in GFR and ERPF in similarly enalapril-treated rats (12), in contrast to the results of the present study. Although the apparent reason for this difference remains obscure, it may be because Wistar rats from different breeding stocks were used in the two studies.

Anesthetized neonatally enalapril-treated rats on normal dietary sodium intake before experimentation showed an increase in $F_E_{Na}$ in agreement with our previous findings (12) and with observations made in rats with papillary necrosis induced by bromoethylamide hydrobromide (BEA) (1). The elevated $F_E_{Na}$ in enalapril-treated rats with papillary atrophy may be because of a markedly impaired sodium reabsorption in the papillary collecting duct, a loss of functioning juxtamedullary nephrons with a consequent increase in single-nephron GFR and filtered sodium load in remaining nephrons, or both. Studies in rats with BEA-induced papillary necrosis have shown a specific reduction in the percentage of filtering juxtamedullary nephrons.
nephrons (25), implying that this might also be the case in kidneys with papillary atrophy resulting from neonatal ACE inhibition.

During dietary sodium restriction, several effector systems, including the renal sympathetic nerves (2) and the RAS-aldosterone system (14, 16), are activated to maintain sodium balance. Previous work has shown that an intact RAS is required for normal renal sodium conservation in this setting (16). In the present study, adult rats treated neonatally with enalapril demonstrated a preserved capacity to conserve sodium during dietary sodium restriction, suggesting the existence of a functionally intact RAS. Furthermore, this finding implies that the activation of sodium-conserving effector systems will be able to compensate for an abolished sodium reabsorption in the papillary collecting duct by enhancing sodium reabsorption in more proximal nephron segments.

During sodium loading, neonatally enalapril-treated rats retained sodium, leading to an increase in cumulative sodium balance that persisted after normalization of dietary sodium intake. Because there was no difference between groups in sodium balance when rats were on a normal sodium intake, this sodium retention did not result from a preexisting sodium deficit but rather an impairment in urinary sodium excretion. It is noteworthy that enalapril-treated rats showed an elevated sodium intake compared with controls during dietary sodium loading, which could partially explain the difference in sodium balance between groups. However, even after initiating a very high sodium intake, normal rats rapidly increase urinary sodium excretion within 24 h.

**Table 1. Renal hemodynamics**

<table>
<thead>
<tr>
<th>Group</th>
<th>BW, g</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>GFR, ml·min⁻¹·g KW⁻¹</th>
<th>ERPF, ml·min⁻¹·g KW⁻¹</th>
<th>FF, %</th>
<th>RVR, mmHg/(ml·min⁻¹·g KW⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>460 ± 14</td>
<td>126 ± 5</td>
<td>422 ± 11</td>
<td>0.98 ± 0.08</td>
<td>3.44 ± 0.38</td>
<td>29.2 ± 1.2</td>
<td>43 ± 5</td>
</tr>
<tr>
<td>Enalapril</td>
<td>465 ± 7</td>
<td>120 ± 3</td>
<td>393 ± 15</td>
<td>0.99 ± 0.05</td>
<td>3.29 ± 0.18</td>
<td>30.3 ± 1.0</td>
<td>39 ± 2</td>
</tr>
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</table>

Values are means ± SE; n = 12 rats in each group. Renal clearance data during steady-state conditions in pentobarbital sodium-anesthetized 15-wk-old Wistar rats treated neonatally from 3 to 24 days of age with enalapril (10 mg·kg⁻¹·day⁻¹) or isotonic saline vehicle. BW, body weight; MAP, mean arterial blood pressure; HR, heart rate; GFR, glomerular filtration rate; ERPF, effective renal plasma flow; FF, filtration fraction; RVR, renal vascular resistance; KW, kidney weight.
to match the level of sodium intake and thereby achieve a new level of sodium balance (11, 15). This was clearly not the case in enalapril-treated rats, which showed a more positive daily sodium balance than controls throughout the 6-day period of sodium loading.

Although no detailed analysis of intrarenal sodium handling was performed in the present study, the impairment in urinary sodium excretion in neonatally enalapril-treated rats may be a consequence of the pronounced papillary atrophy, because the renal medulla has been suggested to be important in maintaining sodium homeostasis (24). Previous studies have shown that the natriuretic response to acute extracellular fluid volume expansion with isotonic saline is associated with a shift in the distribution of intrarenal blood flow to the medulla, leading to an increase in papillary blood flow (6). These changes in medullary hemodynamics are accompanied by an increase in renal interstitial pressure (RIHP) (17, 22), which may inhibit sodium reabsorption in proximal tubules of both superficial and deep nephrons (10). In addition, an increase in medullary blood flow may contribute to the natriuretic response to volume expansion by inhibiting sodium reabsorption in the thin loop of Henle of juxtamedullary nephrons, secondary to washout of the medullary osmotic gradient (3). In a recent study (21) a blunted natriuresis in adult neonatally enalapril-treated rats was found after acute extracellular fluid volume expansion with isotonic saline, corroborating findings in the present study. The blunted natriuresis was accompanied by virtually no increase in RIHP, whereas control rats exhibited a 40% increase in RIHP during isotonic saline infusion, suggesting that an intact renal papilla and vasa recta vasculature may be crucial in the generation of an elevated RIHP in this setting. Furthermore, the chronic interstitial inflammatory changes may alter the functional characteristics of the interstitium and attenuate increases in RIHP. It should also be pointed out that neonatal ACE inhibition and the associated papillary injury may adversely affect autocrine-paracrine factors synthesized within the medulla, such as prostaglandin E2 and nitric oxide, which would contribute to the aforementioned sodium retention.

Neonatally enalapril-treated rats showed a pronounced increase in fluid intake when the drinking fluid was switched to isotonic saline during sodium-loading experiments, whereas this was not seen in control rats. Because the RAS is important in the regulation of NaCl appetite (4), we investigated whether this behavioral alteration was associated with a preference for NaCl in rats given a choice of isotonic saline or tap water as drinking fluid. However, enalapril-treated rats demonstrated a marked reduction in NaCl preference and a similar sodium intake as controls, suggesting that an increased sodium appetite was not primarily responsible for the high intake of isotonic saline during sodium-loading experiments. Subsequently, the increase in fluid intake in neonatally enalapril-treated rats drinking isotonic saline was found to be associated with elevations in PNa and Posm. This finding suggests that the increase in Posm may have been a primary afferent signal leading to thirst and increased fluid intake. The increase in Posm may in turn be explained.

Table 2. Renal electrolyte and water handling

<table>
<thead>
<tr>
<th>Group</th>
<th>V, μl·min⁻¹·g KW⁻¹</th>
<th>Uosm, mosmol/kg</th>
<th>Psm, mosmol/kg</th>
<th>FENa, %</th>
<th>FEk, %</th>
<th>Csm, μl·min⁻¹·g KW⁻¹</th>
<th>TH2O, μl·min⁻¹·g KW⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>3.4 ± 0.4</td>
<td>1,271 ± 96</td>
<td>302 ± 3</td>
<td>0.13 ± 0.02</td>
<td>15.1 ± 1.2</td>
<td>14.0 ± 1.6</td>
<td>10.6 ± 1.3</td>
</tr>
<tr>
<td>Enalapril</td>
<td>11.2 ± 1.2*</td>
<td>662 ± 59*</td>
<td>302 ± 3</td>
<td>0.27 ± 0.05*</td>
<td>28.8 ± 1.6*</td>
<td>23.2 ± 1.3*</td>
<td>12.2 ± 1.1</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 12 rats in each group. Renal clearance data during steady-state conditions in pentobarbital sodium-anesthetized 15-wk-old Wistar rats treated neonatally from 3 to 24 days of age with enalapril (10 mg·kg⁻¹·day⁻¹) or isotonic saline vehicle. V, urine flow rate; Uosm, urine osmolality; Psm, plasma osmolality; FENa and FEk, fractional excretion of Na and K, respectively; Csm, osmolar clearance; TH2O, solute-free water reabsorption. * P < 0.05.

Fig. 4. Representative kidney sections from adult rats treated neonatally from 3 to 24 days of age with isotonic saline vehicle (A) or enalapril (10 mg·kg⁻¹·day⁻¹) (B). Note the deranged renal architecture and papillary atrophy in neonatally enalapril-treated rat (B). Sections were stained with hematoxylin and eosin; magnification, ×4.
Fig. 5. Plasma sodium concentration (P Na, A) and fluid intake (B) in adult rats treated neonatally from 3 to 24 days of age with enalapril (10 mg·kg⁻¹·day⁻¹, n = 10) or isotonic saline vehicle (n = 10). Rats had free access to tap water on experimental days 1–2 and isotonic saline on experimental days 3–5 as drinking fluid. Tail blood was sampled on experimental days 2 and 5. Values are means ± SE. †P < 0.05 within group difference between baseline value during tap water intake and value when drinking isotonic saline, *P < 0.05 between groups.

Table 3. NaCl preference

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Total Fluid Intake, ml·kg⁻¹·day⁻¹</th>
<th>Tap Water</th>
<th>Isotonic Saline</th>
<th>Osmolar Intake, mosmol·kg⁻¹·day⁻¹</th>
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<tr>
<td></td>
<td></td>
<td></td>
<td>ml·kg⁻¹·day⁻¹</td>
<td>%</td>
<td>ml·kg⁻¹·day⁻¹</td>
</tr>
<tr>
<td>Vehicle</td>
<td>5</td>
<td></td>
<td>123 ± 12</td>
<td>32 ± 5</td>
<td>91 ± 6</td>
</tr>
<tr>
<td>Enalapril</td>
<td>5</td>
<td></td>
<td>208 ± 17*</td>
<td>126 ± 8*</td>
<td>82 ± 13</td>
</tr>
</tbody>
</table>

Values are means ± SE for 5 rats in each group and are average values for 3 days. Fluid intake in 16-wk-old rats treated neonatally from 3 to 24 days of age with either saline vehicle or enalapril (10 mg·kg⁻¹·day⁻¹) and given a free choice of tap water and isotonic saline as drinking fluid. Rats were kept individually and were provided with 2 bottles, which contained tap water and isotonic saline, respectively. Intakes of tap water and isotonic saline are presented in absolute values and in percent of total fluid intake. *P < 0.05 between groups.

by the defect in renal water conservation (13) in association with the aforementioned impairment in urinary sodium excretion during sodium loading.

Neonatally enalapril-treated rats showed a moderate and transient potassium wasting during dietary potassium restriction, suggesting either enhanced tubular secretion or impaired tubular reabsorption of potassium mainly in the collecting duct (26). However, enalapril-treated rats were able to decrease the urinary potassium concentration to a value similar to that of controls, suggesting normal tubular potassium reabsorption. Thus the potassium wasting in enalapril-treated rats during dietary potassium restriction was primarily a consequence of the increase in urine volume secondary to the defect in renal water conservation. Although enalapril-treated rats did not show any alteration in potassium balance when on a normal potassium intake, skeletal muscle potassium content was slightly reduced and FEK was increased in anesthetized, hydropenic rats. The mechanisms underlying these changes need further investigation but may result from an increased potassium secretion in the distal nephron as a consequence of increases in distal flow rate and/or sodium delivery to these nephron segments.

In conclusion, in addition to an impaired urinary concentrating ability, neonatally enalapril-treated rats retained sodium when challenged with a dietary sodium load but showed a normal renal adaptation to a dietary sodium restriction. Furthermore, enalapril-treated rats showed a moderate and transient potassium wasting during potassium restriction. Although the mechanisms underlying these abnormalities in renal electrolyte handling remain to be determined, the pronounced papillary atrophy in neonatally enalapril-treated rats may provide a possible explanation.

Perspectives

The present study confirms previous studies in rodents (5, 8, 12, 13, 18–21, 28) in which a crucial role for ANG II in renal development has been demonstrated. In addition, this study extends our knowledge of the long-term physiological consequences of neonatal ACE inhibition in the rat and demonstrates alterations in renal sodium and potassium handling that may relate to the prevailing papillary atrophy. Importantly, we have recently been able to demonstrate a pivotal role of the RAS for normal renal development in the pig (7), suggesting that an intact RAS may also be required during renal development in humans. Indeed, although the pathogenetic mechanisms have not been determined, the use of ACE inhibitors during pregnancy has long been known to be associated with renal tubular dysplasia and anuria in the neonate (23). Thus the present study further emphasizes that ACE inhibitors or AT₁ receptor antagonists should not be used during pregnancy. Additional studies are needed to determine both the mechanism of ANG II involvement in normal renal development and the clinical implications of these findings.

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