Urinary acidification and net acid excretion in adult rats treated neonatally with enalapril

GREGOR GURON,1 NIELS MARCUSSEN,2 AND PETER FRIBERG1
1Department of Physiology, Institute of Physiology and Pharmacology, Göteborg University, S413–90 Göteborg, Sweden; and 2Department of Pathology, Århus Kommune Hospital, Århus University, DK-8000 Århus C, Denmark

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Neonatal blockade of the renin-angiotensin system in rats induces irreversible renal histological abnormalities, including papillary atrophy and an impaired urinary concentrating ability. The aim was to investigate urinary acidification and net acid excretion in adult Wistar rats treated neonatally with enalapril (10 mg·kg−1·day−1) or vehicle from 5 to 24 days of age. Analyses were performed in both metabolic balance studies and renal clearance experiments performed under pentobarbital sodium anesthesia. There were no differences between groups in urine pH or urinary excretion rates of bicarbonate, titratable acid, or ammonium, neither during control conditions nor after chronic NH4Cl loading (assessed of bicarbonate, titratable acid, or ammonium, neither during control conditions nor after chronic NH4Cl loading). Analyses were performed in both metabolic balance studies and renal clearance experiments performed under pentobarbital sodium anesthesia. There were no differences between groups in urine pH or urinary excretion rates of bicarbonate, titratable acid, or ammonium, neither during control conditions nor after chronic NH4Cl loading. Analyses were performed in both metabolic balance studies and renal clearance experiments performed under pentobarbital sodium anesthesia.

We have previously shown that neonatal ACE inhibition or AT1 receptor antagonism during the first 3 wk of life in rats induces irreversible renal histopathological changes, mainly characterized by papillary atrophy, vascular alterations, and chronic interstitial inflammation and fibrosis (10, 13–15). These histological abnormalities were associated with an impairment in urinary concentrating ability of renal origin (13, 15), sodium retention during dietary sodium loading (14), and a modest renal potassium wastage during dietary potassium restriction (14), defects that may be attributed to the papillary atrophy. In addition to its role in the final regulation of sodium, potassium, and water excretion, the inner medullary collecting duct is an important site of urinary acidification and net acid secretion (12). In vivo experiments using micropuncture (7) and microcatheterization (4, 12) techniques, as well as in vitro microperfusion studies (32), have uniformly demonstrated significant urinary acidification and/or net acid secretion along the inner medullary collecting duct in rats.

Accordingly, the aim of the present study was to assess urinary acidification and renal net acid excretion in neonatally enalapril-treated rats with papillary damage. Analyses were performed in both metabolic balance studies and renal clearance experiments under pentobarbital sodium anesthesia.

METHODS

General Procedures

Thirty-one male Wistar rats (Möllegaard Breeding Center, Ejby, Denmark) were used. Pregnant rats were carefully observed near end gestation for determination of the exact date of birth. Sex was determined in 4-day-old pups, and litters containing only males were transported to our facilities. Weight-matched male pups were divided into groups receiving daily intraperitoneal injections from 5 to 24 days of age with either enalapril maleate (10 mg/kg; Merck Sharp & Dohme, Sollentuna, Sweden) (n = 17) or isotonic saline vehicle (n = 14) in equivalent volumes of 10 ml/kg. After the neonatal treatment period, rats were left untreated until 9 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 regimen) and kept in rooms with a controlled temperature of 24°C and a 12:12-h light-dark cycle (6 PM–6 AM) throughout the study. All experiments were approved by the regional ethics committee in Göteborg.

Protocol

Group A. The experimental protocol for group A (enalapril, n = 9; vehicle, n = 7) consisted of the following: 1) an assessment of baseline fluid handling and urinary concen-
trating ability when rats were 9 wk of age, thereby matching the total NH4Cl intake in the studies and was to compensate for the increased fluid intake.

Renal Clearance Experiments in Anesthetized Rats

General procedures. Glomerular filtration rate (GFR) was measured by the urinary clearance of 51Cr-labeled EDTA (Amersham Laboratories, Buckinghamshire, UK). Rats were anesthetized with pentobarbital sodium (60 mg/kg ip) and tracheotomized with a polyethylene catheter (PE-240), and 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) and pentobarbital sodium (12 mg·kg⁻¹·h⁻¹ intra-arterially) dissolved in isotonic saline, yielding a total infusion rate of 7 ml·kg⁻¹·h⁻¹. A 45-min equilibration period was allowed before the start of clearance measurements. Urine was collected in preweighed vials under mineral oil, and arterial blood was sampled anaerobically (0.3 ml) at the midpoint of each collection period. Urine was kept under mineral oil, handled anaerobically, and promptly analyzed for pH, TA, and P CO2. Urine was also stored at −20°C and analyzed within 2 wk for osmolality and the concentration of sodium, potassium, and ammonium. Mean arterial blood pressure (MAP) and heart rate were recorded continuously with Statham pressure transducers connected to a Grass polygraph.

Chronic NH4Cl loading and Na2SO4 infusion. Rats were NH4Cl loaded, identically to the procedure during the metabolic balance study, for 5 days before experimentation. After two baseline 40-min clearance measurements, an infusion of 4% Na2SO4 (12 ml·kg⁻¹·h⁻¹ iv) was initiated, as previously described (2, 29). After 40 min of equilibration, two consecutive 15-min clearance periods were carried out during the Na2SO4 infusion. Results are presented as the average for clearance measurements before and after infusing Na2SO4.

Baseline renal function, tubular bicarbonate reabsorption, and U-B P CO2 in alkaline urine. Rats consumed ordinary chow and tap water before experimentation. After two baseline 40-min clearance measurements, an infusion of 0.9 M NaHCO3 was initiated and the infusion rate elevated in a stepwise fashion from 4 to 26 ml·kg⁻¹·h⁻¹·h⁻¹, producing plasma bicarbonate concentrations from −20 to 65 mmol/l. After each increase in infusion rate, a 15-min equilibration period was performed before clearance measurements began. On each rat, 7–10 clearance periods were performed. At least three clearance periods were carried out per rat when urine pH exceeded 7.8 for the analyses of U-B P CO2 in highly alkaline urine (3).

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 using an arbitrary scale where 0 is normal, 1 is mild, 2 is moderate, and 3 is severe change, similar to what has been described previously (13). Assessments were made by an investigator blind to the treatment group.

Analytic Methods

Osmolality, sodium and potassium concentrations, and radioactivity were measured as previously described (13). Urine ammonium was analyzed enzymatically using glutamate dehydrogenase (18) (Sigma Chemical, St. Louis, MO). TA was assessed by the amount of 0.01 M NaOH used to titrate 1 ml of urine to the arterial pH (during metabolic balance studies no blood was sampled and urine was titrated to a pH of 7.40). Arterial pH, P CO2, and urine P CO2 were analyzed with an ABL 510 blood-gas analyzer (Radiometer, Copenhagen, Denmark). Urine pH was analyzed with a 691 pH meter (Metrohm, Herisau, Switzerland).

Calculations

Standard equations for clearance calculations were used. Fractional excretion rates of sodium (FE Na, %), potassium (FE K, %), and water (FE H2O, %) were estimated as the ratio of their respective clearances to that of 51Cr-EDTA, taken as GFR, × 100. The bicarbonate concentration in plasma and urine was calculated from the Henderson-Hasselbalch equation as previously described (21). A p K a value of 6.1 was used.
for blood. The pK used for urine was calculated as $6.33 - 0.5(U_{Na} + U_{K})^{0.5}$, where $U_{Na}$ and $U_{K}$ are the urine concentrations of sodium and potassium, expressed in moles per liter. The solubility constants for CO$_2$ used in calculations were 0.0301 and 0.0309 in blood and urine, respectively. The maximal rate of tubular bicarbonate reabsorption was calculated using a nonlinear regression model. Net acid excretion was calculated as the sum of urinary TA and ammonium excretion minus urinary bicarbonate excretion.

Statistics

Data in text and tables are presented as means ± SE. Results from metabolic balance studies were analyzed by analysis of variance. Cross-sectional data were analyzed using unpaired and paired $t$-tests for statistical analysis of data between and within groups, respectively. The Mann-Whitney nonparametric test was used on renal histopathological parameters. A $P < 0.05$ was considered statistically significant. Analyses were performed using software Statview 4.1 (Abacus Concepts) and Systat 5.2 (Systat) for Macintosh.

RESULTS

Fluid Handling and Urinary Concentrating Ability

Water intake and urine flow rate ($V$) were elevated (water intake: $129 ± 4 vs. 115 ± 5$ ml·kg$^{-1}$·24 h$^{-1}$, $P < 0.05$; $V$: $51 ± 3 vs. 28 ± 2$ ml·kg$^{-1}$·24 h$^{-1}$, $P < 0.05$) and $U_{osm}$ reduced ($1,020 ± 34 vs. 1,639 ± 124$ mosmol/kg, $P < 0.05$) in neonatally enalapril-treated rats with free access to tap water. Although $U_{osm}$ rose in both groups after 24 h of water deprivation, $U_{osm_{max}}$ was markedly reduced in enalapril- compared with vehicle-treated rats ($2,112 ± 70 vs. 2,944 ± 90$ mosmol/kg, $P < 0.05$).

Metabolic Balance Study During Chronic NH$_4$Cl Loading

There were no differences between neonatally enalapril- and vehicle-treated rats in urinary excretion rates of TA, ammonium, or total acid (sum of TA and ammonium) throughout the study period (Fig. 1). Urine pH in neonatally enalapril-treated rats was transiently greater than that of vehicle-treated rats on days 2, 3, and 4 of NH$_4$Cl loading, but did not differ from vehicle on the last day of experimentation ($5.63 ± 0.03$ vs. $5.55 ± 0.03$, in neonatally enalapril- and vehicle-treated rats, respectively). The dietary intake of NH$_4$Cl was similar in the groups during NH$_4$Cl loading ($37 ± 1$ vs. $36 ± 1$ mmol·kg$^{-1}$·24 h$^{-1}$, in neonatally enalapril- and vehicle-treated rats, respectively). Water intake and $V$ were elevated and $U_{osm}$ reduced in neonatally enalapril- compared with vehicle-treated rats throughout the study period (data not shown). Although arterial acid-base parameters were not monitored during the metabolic balance study, a similar degree of metabolic acidosis was demonstrated in the two groups on the 5th day of NH$_4$Cl loading (Table 1).

Renal Function and Acid Excretion in NH$_4$Cl-Loaded Rats

Arterial pH and plasma bicarbonate concentrations were similar in neonatally enalapril- and vehicle-treated rats throughout clearance experiments (Table 2). There were no significant differences between groups in urine pH or urinary excretion rates of TA, ammonium, bicarbonate, or net acid, neither before nor after the infusion of Na$_2$SO$_4$ (Table 2). In addition, both groups showed an increase in urinary excretion rates of TA, ammonium, and net acid in response to Na$_2$SO$_4$ administration (Table 2). There was no difference between neonatally enalapril- and vehicle-treated rats in

Fig. 1. Results from metabolic balance study during NH$_4$Cl loading in 10-wk-old rats treated neonatally from 5 to 24 days of age with enalapril (10 mg·kg$^{-1}$·day$^{-1}$, n = 9) or isotonic saline vehicle (n = 7). NH$_4$Cl loading was performed for 5 days (see METHODS). $U_{NH_4V}$, urinary ammonium excretion; $U_{TAV}$, urinary titratable acid excretion; $U_{pH}$, urine pH. Values are means ± SE. *$P < 0.05$. 

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GFR, FE_{Na}, or FE_{K}, neither during baseline nor after infusing Na_{2}SO_{4} (Table 2). Administration of Na_{2}SO_{4} produced marked elevations in V, FE_{Na}, and FE_{K}, and a reduction in the plasma potassium concentration in both groups (Table 2).

**Baseline Renal Function and Acid Excretion**

V and FE_{Na} were elevated and U_{osm} was reduced in neonatally enalapril-treated rats (Table 3). GFR, MAP, FE_{K}, and plasma sodium and potassium concentrations did not differ between groups (Table 3). There was no difference between groups in arterial acid-base parameters, urine pH, or urinary excretion rates of TA, ammonium, bicarbonate, or net acid, during baseline conditions (Table 4).

**Tubular Bicarbonate Reabsorption**

There was no difference between neonatally enalapril- and vehicle-treated rats in tubular bicarbonate reabsorption at any level of plasma bicarbonate concentration (Fig. 2). The maximal rate of tubular bicarbonate reabsorption was 35.1 ± 1.9 vs. 33.4 ± 1.7 mmol/l GFR in neonatally enalapril- and vehicle-treated rats, respectively.

**U-B PCO_{2} in Alkaline Urine**

The maximal U-B PCO_{2} gradient in highly alkaline urine during NaHCO_{3} infusion and at comparable urine bicarbonate concentrations did not differ between groups (32 ± 2 vs. 33 ± 3 mmHg, enalapril- and vehicle-treated rats, respectively; Fig. 3).

**Kidney Weight and Histology**

Renal histological abnormalities in neonatally enalapril-treated rats were qualitatively similar to those described in more detail previously (13, 14) and mainly consisted of significant degrees of papillary atrophy (1.1 ± 0.2 vs. 0.0 ± 0.0 arbitrary units, P < 0.05), interstitial inflammation and fibrosis, tubular atrophy, and concentric wall thickening comprising both the intima and media of interlobular arteries (data not shown). In addition to a reduction in the size of the papilla, papillae of neonatally enalapril-treated rats showed a markedly distorted structural architecture, an increase in the proportion of interstitial area associated with inflammatory and fibrotic changes, and dilated collecting ducts. There were no differences between groups in wet or dry kidney weights (data not shown).

**DISCUSSION**

The main finding of the present study was that adult neonatally enalapril-treated rats showed an intact urinary acidification and renal net acid excretion despite exhibiting renal histopathological abnormalities, which comprised the inner medulla and were associated with a urine concentration defect. This finding indicates that neonatal ACE inhibition has a differentiated effect on long-term tubular function in which urinary concentration is impaired but tubular acidification and acid excretion are preserved.

In the present study, neonatal enalapril treatment induced irreversible renal histological changes, which were qualitatively similar to those previously reported after neonatal ACE inhibition or AT_{1} receptor antagonism in the rat (10, 13–15, 24, 25, 31), underlining the importance of an intact RAS for the development and maintenance of a normal renal morphology. In accordance with previous studies (10, 13–15), the main functional abnormality in adult rats treated neonatally with enalapril was an impairment in urinary concentrating ability. We have previously determined the pathophysiological mechanisms underlying the impairment of renal concentrating ability in these rats.
Table 3. Baseline renal function and hemodynamics in anesthetized rats

<table>
<thead>
<tr>
<th>GFR, ml·min⁻¹·g kidney wt⁻¹</th>
<th>MAP, mmHg</th>
<th>V̇, µl·min⁻¹·g kidney wt⁻¹</th>
<th>Uosm, mmol/kg</th>
<th>PNa, mmol/l</th>
<th>PK, mmol/l</th>
<th>FEH₂O, %</th>
<th>FEx, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle 1.14 ± 0.07</td>
<td>122 ± 5</td>
<td>4.5 ± 0.7</td>
<td>1,917 ± 107</td>
<td>141 ± 1</td>
<td>3.4 ± 0.1</td>
<td>0.41 ± 0.11</td>
<td>26 ± 3</td>
</tr>
<tr>
<td>Enalapril 1.10 ± 0.08</td>
<td>125 ± 4</td>
<td>12.3 ± 3.1*</td>
<td>1,009 ± 147*</td>
<td>145 ± 3</td>
<td>3.4 ± 0.1</td>
<td>1.02 ± 0.25*</td>
<td>30 ± 2</td>
</tr>
</tbody>
</table>

Values are means ± SE. Renal function and hemodynamics in 14- to 15-wk-old, pentobarbital sodium-anesthetized rats, treated neonatally with enalapril (10 mg·kg⁻¹·day⁻¹, n = 8) or isotonic saline vehicle (n = 7) from 5 to 24 days of age. MAP, mean arterial pressure; Uosm, urine osmolality. *P < 0.05.

Table 4. Baseline renal net acid excretion in anesthetized rats

<table>
<thead>
<tr>
<th>Arterial pH</th>
<th>Pco₂, mmHg</th>
<th>Plasma HCO₃, mmol/l</th>
<th>Urine pH</th>
<th>U₄₇V/GFR, mmol/l</th>
<th>U₄₇₃V/GFR, mmol/l</th>
<th>U₄₇₃CO₂V/GFR, mmol/l</th>
<th>NAE/GFR, mmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle</td>
<td>7.44 ± 0.01</td>
<td>38 ± 1</td>
<td>25.1 ± 0.4</td>
<td>5.99 ± 0.11</td>
<td>0.11 ± 0.03</td>
<td>0.21 ± 0.03</td>
<td>0.005 ± 0.002</td>
</tr>
<tr>
<td>Enalapril</td>
<td>7.45 ± 0.01</td>
<td>37 ± 1</td>
<td>25.4 ± 0.5</td>
<td>6.15 ± 0.11</td>
<td>0.12 ± 0.02</td>
<td>0.22 ± 0.02</td>
<td>0.024 ± 0.009</td>
</tr>
</tbody>
</table>

Values are means ± SE. Renal net acid excretion in 14- to 15-wk-old, pentobarbital sodium-anesthetized rats, treated neonatally with enalapril (10 mg·kg⁻¹·day⁻¹, n = 8) or isotonic saline vehicle (n = 7) from 5 to 24 days of age.
and acid secretion in the papilla can be compensated for by remaining tubular structures, the impaired ammonium excretion in these rats could be explained by the nephrectomy and the prevailing reduction in GFR.

The present study was not designed to elucidate mechanisms that could be involved in the induction and maintenance of a compensatory increase in urinary acidification and acid excretion in undamaged nephron segments outside of the injured papilla. Still, one might speculate that a small reduction in blood pH in neonatally enalapril-treated rats, which we were unable to detect with conventional methods, could enhance the synthesis or activity of H^+·ATPase and/or H^+·K^+·ATPase in intercalated cells along the collecting duct proximal to the papillary damage, thereby upregulating distal hydrogen ion secretion. Moreover, acidemia could stimulate ammonium synthesis from glutamine in proximal tubular cells and increase the buffer delivery to undamaged acidifying sites in the collecting duct. Clearly, additional studies are needed to resolve these issues.

In conclusion, in concert with previous studies neonatal ACE inhibition in the rat produced irreversible abnormalities in renal morphology, including papillary atrophy and an impairment in urinary concentrating ability. However, despite papillary damage, urinary acidification and renal net acid excretion were intact in adult, neonatally enalapril-treated rats. Thus neonatal enalapril treatment produces a differentiated abnormality in tubular function in which urine concentration is impaired but urinary acidification and net acid excretion are intact.

Perspectives

The use of ACE inhibitors by pregnant women is known to cause renal tubular dysplasia and anuria in the neonate (30), although the pathogenetic mechanisms have not been determined. In addition to regulating perinatal renal function and hemodynamics (22), recent studies in a number of species (8, 10, 13–16, 19, 20, 24, 25, 27, 31), indicate that ANG II may also be essential for normal renal morphogenesis. Nephrogenesis is completed at about gestational week 36 in humans (23), but continues into the second postnatal week in the rat (26). Thus the neonatal rat provides a suitable experimental model for analyzing the effects of pharmacological blockade of the RAS on immature kidneys with ongoing nephrogenesis. We have previously demonstrated that a main characteristic of adult rats treated neonatally with ACE inhibitors or AT1 receptor antagonists is papillary atrophy in association with an impaired urinary concentrating ability (10, 13–15). Thus an intact RAS may be of particular importance for the development and/or maintenance of a normal inner medullary morphology and function. The present study extends our knowledge of the long-term effects of neonatal ACE inhibition on tubular function and indicates that an intact RAS, although essential for the formation of a structurally intact papilla, is not mandatory for the development of normal urinary acidification and net acid excretion.
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Address for reprint requests: G. Guron, Dept. of Physiology, Institute of Physiology and Pharmacology, Göteborg Univ., Medicinargatan 11, S-413 90 Göteborg, Sweden.

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