Effects of Renal Papillary-Medullary Lesion on the Antihypertensive Effect of Furosemide and Development of Salt-Sensitive Hypertension in Dahl-S Rats

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ABSTRACT
To test the hypothesis that the long-term antihypertensive action of furosemide is mediated by a renomedullary vasodepressor substance, we measured mean arterial pressure (MAP) by radiotelemetry in Dahl-S rats with either intact or bromoethylamine-induced (BEA, 100 mg/kg i.p.) lesion of the renal papilla and medulla. Seven days of recovery after BEA administration, the rats diet was changed from 1 to 4% NaCl, and during days 8 to 31, rats were randomized to daily treatment with placebo or furosemide (50 mg/kg p.o.). Then furosemide treatment was stopped and the rat food was changed to 1% NaCl diet. After a 10-day wash-out period, renal function was measured. BEA produced a rapid (within min) and sustained increase in MAP which was accelerated during 4% NaCl diet. Furosemide prevented 4% NaCl-induced hypertension in both rats with intact kidneys and in rats with BEA-induced renal papillary-medullary lesion. A significant decrease in renal plasma flow (-34%) and glomerular filtration rate (-40%) was observed in all BEA-treated rats independent of previous furosemide treatment. In response to an i.v. load of isotonic saline (10% body weight), rats with renal papillary-medullary lesion had an impaired ability to excrete sodium. Histological examination showed that BEA-treated rats had severe lesions of the renal papilla and medulla, with light-to-moderate changes in the renal cortex. It is concluded that the antihypertensive effect of furosemide is not mediated by a renomedullary vasodepressor substance. The accelerated NaCl-sensitive hypertension in rats with BEA-induced renal papillary-medullary lesion is related to an impaired ability to excrete excess NaCl.

Diuretics have been the cornerstone in the treatment of hypertension for decades, but the exact mechanism(s) by which these drugs lower arterial blood pressure is still unknown. Several explanations have been proposed that include a direct smooth muscle relaxing effect, a vasodilatory effect resulting from changes in water and ion composition of the arteriolar wall, decreased secretion of an ouabain-like substance and indirect effects mediated by release of vasodilatory hormones (Canton et al., 1992).

Gerkens and coworkers performed a series of experiments in which they demonstrated that i.v. furosemide inhibits vasoconstrictor responses to sympathetic nerve stimulation in the in situ blood perfused mesenteric artery. Furthermore, the vasodilatory response to furosemide was blocked by bilateral nephrectomy, chemical renal medullectomy with BEA, and by indomethacin (Gerkens and Smith, 1984; Armsworth et al., 1986; Gerkens et al., 1987). To examine the role of the endothelium for furosemide-induced vasodilatation, the same investigators performed an experiment using periarterial electrical stimulation of sympathetic nerves of a tail artery that was cross-perfused ex vivo with blood from an anesthetized donor rat. These studies showed that sympathetic nerve-mediated vasoconstriction was attenuated by i.v. administration of furosemide in the donor rat, and that this response was abolished after removal of the endothelium from the tail-artery segment (Gerkens, 1987, a and b; Gerkens et al., 1988). Because prostaglandin-mediated vasodilation is endothelium-independent, these observations generated the hypothesis that furosemide stimulates renal prostaglandin synthesis that in turn causes release of a renomedullary hormone which produces endothelium-dependent vasodilatation (Gerkens 1987c). In the renal papilla and medulla, lipid-laden RIC are considered to be the source of the yet chemically unidentified renomedullary vasodilator.

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ABBREVIATIONS: BEA, bromoethylamine; C, renal clearance; Dahl-S rats, Dahl salt-sensitive rats; ERPF, effective renal plasma flow; FE, fractional excretion; GFR, glomerular filtration rate; MAP, mean arterial pressure; RIC, renal interstitial cells; TEA, 14C-tetraethylammonium.
substance called medullipin (Muirhead 1991). Thus, lipid fractions extracted from the renal medulla have been found to decrease arterial pressure, promote diuresis and natriuresis, and inhibit sympathetic nerve activity (Muirhead et al., 1983; Karlström et al., 1988, 1989). On basis of these results, Gerkens (1987c) proposed that the acute venodilatory effect and possibly the long-term antihypertensive effect of furosemide may be mediated by medullipin.

Therefore, to examine whether the long-term antihypertensive effect of furosemide is mediated by release of a renomedullary vasodpressor substance, we compared the antihypertensive effect of chronic furosemide treatment in groups of Dahl-S rats with either intact renal medulla or BEA-induced renal papillary-medullary lesion. We chose this model of genetic salt-sensitive hypertension, because furosemide is an effective antihypertensive agent in this strain (Garthoff et al., 1984). Furthermore, studies on isolated RIC from Dahl-S rats have demonstrated that these cells produce a marked antihypertensive response after implantation in Dahl-S rats fed a 8% NaCl diet (Pitcock et al., 1985). Thus, if furosemide decreases arterial pressure by stimulating release of a renomedullary vasodilatory hormone from the RIC, the Dahl-S rats should be a sensitive model to examine the role of this mechanism.

Methods

Animals

Nineteen 7 to 9 wk old inbred Dahl-S rats of the John Rapp strain (280–290 g), were purchased from Harlan Sprague-Dawley Inc., Indianapolis, IN. Rats were housed in a temperature (22–24°C) and moisture (45–70%) controlled room with a 12-hr light-dark cycle (light-on at 8.00 A.M. and light-off at 8.00 P.M.). Rats were fed a standard rat diet containing 1% NaCl (Altromin no. 1314, Altromin International, Lake, Germany) and tap water ad libitum. After implantation of telemetric blood pressure transducers the rats were housed individually.

Experimental Groups

The experimental design is summarized in table 1. All animals were fed a 1% NaCl diet during days 0 to 7, a 4% NaCl diet during days 8 to 31 and a 1% NaCl diet during days 32 to 41.

Group 1 (control) (n = 5): Placebo-treated animals with intact renal medulla. Rats received a single i.p. injection of vehicle (isotonic saline) on day 2 and they were treated with per oral vehicle (isotonic saline, pH = 9.0) once daily from day 8 through day 31.

Group 2 (FUR) (n = 5): Furosemide-treated animals with intact renal medulla. Rats received a single i.p. injection of vehicle (isotonic saline) on day 2 and they were treated with per oral furosemide, 50 mg/kg once daily from day 8 through day 31.

Group 3 (BEA) (n = 5): Placebo-treated animals with renal papillary-medullary lesion. Rats received a single injection of BEA, 100 mg/kg i.p. on day 2 and they were treated with per oral vehicle (isotonic saline, pH = 9.0) once daily from day 8 through day 31.

Group 4 (BEA + FUR) (n = 4): Furosemide-treated animals with renal medullary-papillary lesion. Rats received a single injection of BEA, 100 mg/kg i.p. on day 2 and they were treated with per oral furosemide, 50 mg/kg once daily from day 8 through day 31.

Surgical Procedures

Implantation of telemetric blood pressure transducers. A total of 10 to 14 days before start of experiments, rats were anesthetized with halothane-N₂O and prepared for surgery with aseptic technique. The abdominal aorta was exposed through a midline incision and carefully isolated using sterile cotton-tipped applicators. The aorta was ligated shortly at a level below the renal arteries and a small hole was made using a 21G needle through which the tip of the catheter was inserted. The catheter was fixed to the vessel and the surrounding tissue with a polyester disk and tissue-glue (HIS-TOACRYL, B. Braun Melsungen AG, Melsungen, Germany). The body of the transmitter was fixed with a silk suture to the inside of the abdominal wall. Rats were given buprenorphin, 0.2 mg/kg (ANORFIN, A/S GEA, Copenhagen, Denmark) to minimize postsurgical pain, and prophylactic ampicillin, 1.25 mg/kg (Nycomed Pharma, Oslo, Norway).

Instrumentation for Renal Function Study. Eight days before clearance experiments (on day 33), a medical grade tygon catheter was implanted into the right jugular vein during halothane-N₂O anesthesia. The catheter was filled with 50% dextrose with 100 I.U. heparin/ml and 5000 I.U. streptokinase/ml and sealed with a nylon pin. It was exteriorized through the neck and fixed with a s.c. polyester disc which was glued to the catheter (Petersen et al., 1991).

Experimental Protocol

On day 0, base-line arterial blood pressure was monitored for 24 hr. Based on average 24-hr MAP, rats were stratified into four groups. On day 2, rats were either given a single i.p. injection of BEA (100 mg/kg in a solution made isotonic with NaCl; Sigma Chemical Company, St. Louis, MO) or sham-injection with isotonic saline, 10 ml/kg. To assure that BEA-induced renal papillary-medullary lesion was complete before onset of furosemide treatment, animals were allowed a 1-wk observation period on 1% NaCl diet after BEA/vehicle injection (Murray et al., 1972). During day 8 to 31, all animals were fed a 4% NaCl diet and treated with per oral furosemide (50 mg/kg; Dumex A/S, Copenhagen, Denmark) or sham-injection with isotonic saline, 10 ml/kg. To assure that BEA-induced renal papillary-medullary lesion was complete before onset of furosemide treatment, animals were allowed a 1-wk observation period on 1% NaCl diet after BEA/vehicle injection. During day 8 to 31, all animals were fed a 4% NaCl diet and treated with per oral furosemide (50 mg/kg; Dumex A/S, Copenhagen, Denmark) or sham-injection with isotonic saline, 10 ml/kg. On day 32, daily administration of furosemide and placebo was stopped and the diet was changed back to a 1% NaCl diet. On day 33, a catheter was implanted into the right jugular vein and 1 wk later (on day 41) renal clearance experiments were performed.

Renal clearance experiment. Two days before the clearance experiment, the 1% NaCl standard diet was added 4 mmol lithium citrate per kg that produced a plasma lithium concentration of 0.30 to 0.40 mmol/liter on the day of the renal clearance study. With this dose of lithium given with the diet, lithium has no detectable effects on renal tubular function (Shalni and Thomsen, 1989; Leyssac et al., 1994). On the day of the experiment, the animals were transferred to

<table>
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<th>TABLE 1</th>
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<tr>
<td>Group</td>
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<tr>
<td>1. Control</td>
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<td>2. FUR</td>
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<td>3. BEA</td>
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<td>4. BEA + FUR</td>
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a metabolic cage (Techniplast model 1700; Ugo Basile, Milan, Italy) and an i.v. infusion with 150 mM glucose added 3H-inulin (1.5 mCi/ml; Amersham, Buckinghamshire, UK; batch no. 135; specific activity 3.1 Ci/mmol), TEA bromide (0.5 mCi/ml; New England Nuclear, Boston, MA; lot no. 2967-044; specific activity 5 mCi/mmol), and LiCl (3.3 mmol/ml) was started with a loading dose of 3 ml over 15 min followed by 3 ml/hr. After a 1.5-hr equilibration period, the bladder was emptied by applying a light pressure on the abdominal wall over the urinary bladder and a capillary blood sample of 250 μl was collected from the tail tip. Urine was collected over a 3-hr period and at the end of the clearance period, the bladder was emptied and a second blood sample was drawn. The urinary collector was carefully rinsed with distilled water to optimize recovery of electrolytes and clearance markers.

Intravenous saline load. At the end of the 3-hr clearance period, rats were given an i.v. load of isotonic NaCl (10% body weight over 30 min) and urine was collected for another 2 hr. Then the urinary bladder was emptied again and the collector was rinsed with distilled water.

Evaluation of histological renal changes. After the saline loading experiment, rats were anesthetized with halothane-N2O and the kidneys were removed, divided in two with a longitudinal cut through the papilla, and fixed by immersion in 4% formaldehyde in phosphate buffer, pH = 7.2. Tissue was embedded in paraffin and 3- to 4-μm thick sections were stained with periodic-acid Schiff, hematoxylin-eosin and Masson-trichrome. Morphological changes in the renal medulla and cortex were graded blindly by a pathologist using a modification of the criteria for acute changes described by Davies and Tange, (1992). In the medulla the following grading of chronic changes was used: grade 0: no changes. Grade 1: narrowing of the tip of the papilla with slight disappearance of RIC. Grade 2: narrowing of the inner half of the papilla with disappearance of the majority of RIC. Grade 3: disappearance of the entire papilla with only a few RIC left in the nonpapillary part of the inner medullary zone. Grade 4: missing papilla and narrowing of the nonpapillary part of the inner medulla with no discernible RIC. Cortical changes was graded as follows: grade 0: no changes. Grade 1: tubular dilatation with minimal loss of brush border. Grade 2: disperse minor changes with atrophy and dilation of tubules. Grade 3: multifocal changes with moderately severe dilatation and atrophy. Grade 4: widespread changes with many larger foci and continuous areas with atrophic and severely dilated tubules.

Telemetric Blood Pressure Recording

Arterial blood pressure was recorded using a telemetric blood pressure recording system consisting of a transducer (model TA11PA-C40), a receiver (model RLA1020) and a consolidation matrix (model BCM100) connected to an IBM compatible PC with a Dataquest IV plug-in card and the Dataquest IV data acquisition software system (Data Sciences, St. Paul, MN) (Brockway et al., 1991). Heart rate, systolic, diastolic and mean arterial pressure were monitored for 24 hr every second day throughout the study. The sample scan period was 1.5 min and the sample duration was 5 sec.

BEA and Furosemide Doses

Doses of BEA and furosemide were selected based on a series of preliminary experiments in male Wistar rats. The lowest dose of BEA, which produced consistent renal papillary-medullary lesion was 100 mg/kg body weight. At higher doses, BEA produced moderate to severe lesions in the outer medulla and cortex as well.

The selected dose of furosemide, 50 mg/kg p.o., produced an initial natriuresis compared to placebo in rats fed a 4% NaCl diet (Na excretion during the first 4 h: 1.1 ± 0.1 vs. 0.6 ± 0.2 mmol/100 g body weight; P < .05) whereas 24 hr sodium excretion was unchanged (4.7 ± 0.2 vs. 4.7 ± 0.4 mmol/100 g body weight; n = 4).

Analyses

Urine volume was measured gravimetrically. Sodium, potassium and lithium were determined by atomic absorption spectrophotometry, using a Perkin-Elmer model 2380 atomic absorption spectrophotometer (Allerød, Denmark). 3H-inulin and 4C-TEA were determined by dual-label scintillation counting in a Tri-Carb liquid scintillation analyzer (model 2250 CA, Packard Instruments, Greve, Denmark). Quench correction was performed by automatic efficiency control using the transformed Spectral Index of External standard as quench-indication parameter.

Calculations and Statistics

Renal clearance was calculated by the standard formula C = UV/P. Inulin clearance was used as an estimate of GFR, TEA-clearance was used as an estimate of ERPF (Petersen and Christensen, 1987; Rasmussen et al., 1990; Petersen and DiBona, 1992) and lithium-clearance was used as an indicator of proximal tubular handling of Na and water (Shalmi and Thomsen 1989; Leyssac et al., 1994). FE was calculated as C/GEFR.

Overall statistical comparisons were performed by ANOVA for one-way classified data and by ANOVA for repeated measurements for two-way classified data. Individual comparisons were performed by Student's t test for paired or unpaired data with Bonferroni's correction of the level of significance (Godfrey, 1985). Differences were considered statistically significant at the 0.05 level. All data are expressed as means ± S.E.M.

Results

Telemetric arterial blood pressure (figs. 1 and 2). Base-line 24 hr MAP were similar in the four groups (overall average: 115 ± 1 mm Hg). BEA produced an immediate and sustained increase in MAP. Thus, MAP was significantly higher in BEA-treated animals already 10 min postinjection (138 ± 1 vs. 131 ± 2 mm Hg, P < .05; fig. 1). After 24 hr, MAP was 136 ± 2 mm Hg in BEA-treated rats and 112 ± 2 mm Hg in vehicle-treated animals (P < .001). The difference in MAP between BEA and vehicle-treated animals was maximal 4 days after injection (145 ± 2 vs. 117 ± 1 mm Hg, P < .001; fig. 2). During the last day of recording before change from 1 to 4% NaCl diet, MAP were similar in both BEA-treated groups (BEA: 139 ± 4 mm Hg; BEA+Fur: 133 ± 3 mm Hg). MAP were similar and unchanged in vehicle-treated groups during day 0 to 7.

When rats were fed a 4% NaCl diet (days 8–31), MAP increased significantly in both the control and the BEA group. However, the increase in MAP was significantly greater in BEA than control rats (ΔMAP: +47 ± 9 vs. +27 ± 4 mm Hg; P < .001). MAP was significantly lower in both
furosemide-treated groups compared with the respective placebo-treated groups when fed a 4% NaCl diet.

After cessation of furosemide treatment and reduction of dietary NaCl content from 4 to 1% (day 32–40), MAP decreased significantly in group BEA (MAP = -23 ± 6) whereas it did not change significantly in group BEA + FUR (MAP = +8 ± 6). Thus, on day 40, MAP was similar in the two BEA-treated groups. In the FUR group, MAP decreased slightly during the wash-out period (days 32–40) (MAP = -4 ± 1, P < .05), whereas no significant changes were observed in the control group (MAP = -5 ± 3). MAP remained lower in group FUR than in the control group on days 32 to 40 (P < .01).

Body weight and water intake (figs. 3 and 4). Body weights were similar in the four groups before BEA injection (346 ± 3 g) (fig. 3). Administration of BEA caused a 10% body weight loss. BEA animals had a lower body weight compared to animals with intact renal medulla during day 2 to 41. Furosemide did not affect body weight in either normal rats or in rats with renal papillary-medullary damage.

Daily water intake was significantly greater in BEA-animals than in animals with intact renal medulla throughout the study (fig. 4). Furosemide did not affect water intake in either BEA treated or in animals with intact renal medulla.

Renal function studies (table 2). On the day of the renal clearance-experiment (day 41), MAP and all renal parameters were similar in the two BEA-treated groups as well as in the two groups with intact renal medulla, respectively. Therefore, data from animals with intact renal medulla were pooled and compared to pooled data from rats with BEA-induced renal papillary-medullary lesion. BEA-treated rats had a 40% decrease in GFR (P < .01) and a 54% decrease in ERPF (P < .05), and thus the filtration fraction (GFR/ERPF) was significantly reduced in these animals (P < .001). Fractional excretions of lithium (FE_{Li}) and sodium (FE_{Na}) were similar. However, during the first 2 hr after an i.v. load of isotonic NaCl (10% body weight), rats with intact renal medulla excreted significantly more of the NaCl load than BEA-treated rats (41.0 ± 2.3% vs. 28.9 ± 3.2% (P < .01)).

Renal histology (Table 3; Fig. 5). Kidney weight and kidney-to-body weight ratio were significantly increased in BEA-treated rats (table 2). Representative light micrographs from control and BEA-treated rats are shown in figure 5. Results from light microscopic semiquantitative examination of kidneys from all four groups are summarized in table 3. Kidneys from control animals were largely normal and only age-related changes were observed. In control animals, RIC were observed throughout the renal papilla and medulla with the highest density at the tip of the renal papilla. Most BEA-treated animals (eight/nine) showed moderate to severe pathological changes in the renal medulla and some rats (five/nine) also had moderate pathological changes in the renal cortex. All BEA-treated animals had a decreased number of RIC in the inner medulla and the majority (seven/nine) had a severe decrease in RIC due to pronounced narrowing of the papilla and medulla.

Discussion

The major new finding of this study is that the antihypertensive effect of furosemide is preserved in Dahl-S rats without intact renal papilla and medulla. Morphological exami-
nation of kidneys from BEA-treated animals showed extensive renal papillary-medullary lesions with elimination of the majority of RIC. These findings argue against the hypothesis that the antihypertensive effect of furosemide is mediated by a renodvalaur vasodilator substance released from the RIC. Furthermore, BEA produced an immediate and sustained increase in MAP in rats fed a 1% NaCl diet and the hypertension induced by 4% NaCl diet was accelerated in Dahl-S rats with renal papillary-medullary lesion.

The efficacy of BEA-induced renal papillary-medullary lesion was confirmed by histological evaluation. Light microscopic examination showed that BEA-treated animals had severe lesions of the papilla and inner medulla where most of the RIC are located (Osvaldo and Latta, 1966; Lemley and Kriz, 1991). In agreement with previous studies (Axelsen, 1973; Gobe and Axelsen, 1982; Bach Kriz, 1991). In agreement with previous studies (Axelsen, the RIC are located (Osvaldo and Latta, 1966; Lemley and

### TABLE 3

Data from renal clearance and saline loading experiment on day 41 (1 wk after withdrawal of furosemide and 4% NaCl diet)

<table>
<thead>
<tr>
<th></th>
<th>- BEA (Group 1+2)</th>
<th>+ BEA (Group 3+4)</th>
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<tbody>
<tr>
<td>MAP (mm Hg)</td>
<td>139 ± 4</td>
<td>161 ± 4</td>
</tr>
<tr>
<td>GFR (µl/min/g kidney weight)</td>
<td>839 ± 61</td>
<td>506 ± 68</td>
</tr>
<tr>
<td>ERPF (µl/min/g kidney weight)</td>
<td>2880 ± 198</td>
<td>1995 ± 241</td>
</tr>
<tr>
<td>FF (%)</td>
<td>29.5 ± 0.5</td>
<td>25.1 ± 0.9</td>
</tr>
<tr>
<td>FE, (%)</td>
<td>17.8 ± 0.7</td>
<td>21.1 ± 1.6</td>
</tr>
<tr>
<td>FEK (%)</td>
<td>0.29 ± 0.04</td>
<td>0.34 ± 0.05</td>
</tr>
<tr>
<td>Excretion of an acute i.v. sodium load (% in 2h)</td>
<td>41.0 ± 2.3</td>
<td>28.9 ± 3.2</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>445 ± 6</td>
<td>417 ± 8</td>
</tr>
<tr>
<td>Kidney weight (g)</td>
<td>3.27 ± 0.08</td>
<td>3.78 ± 0.16</td>
</tr>
<tr>
<td>Kidney: body weight (%)</td>
<td>0.74 ± 0.01</td>
<td>0.91 ± 0.04</td>
</tr>
</tbody>
</table>

|           | (n = 10)         | (n = 9)          |

**Mean ± S.E.M.**

*P < .01.

*P < .05.

**TABLE 3**

Morphological changes in the renal medulla and cortex in the four groups

<table>
<thead>
<tr>
<th>Grade of Medullary Changes</th>
<th>0</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>Mean ± S.E.M.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (n = 5)</td>
<td>4</td>
<td>1</td>
<td>0.2 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FUR (n = 5)</td>
<td>5</td>
<td>0.0 ± 0.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEA (n = 5)</td>
<td>1</td>
<td>4</td>
<td>2.8 ± 0.2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BEA + FUR (n = 4)</td>
<td>1</td>
<td>3</td>
<td>3.3 ± 0.8</td>
<td></td>
<td></td>
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</tr>
</tbody>
</table>

**Grade of cortical changes**

| Control (n = 5)            | 1 | 3 | 1.0 ± 0.3 |
| FUR (n = 5)                | 1 | 4 | 0.8 ± 0.2 |
| BEA (n = 5)                | 2 | 3 | 2.6 ± 0.2 |
| BEA + FUR (n = 4)          | 1 | 2 | 2.3 ± 0.5 |

**Medullary changes:** grade 0: no changes. Grade 1: narrowing of the tip of the papilla with slight disappearance of RIC. Grade 2: narrowing of the inner half of the papilla with disappearance of the majority of RIC. Grade 3: disappearance of the entire papilla with only a few RIC left in the nonpapillary part of the inner medullary zone. Grade 4: missing papilla and narrowing of the nonpapillary part of the inner medulla with no discernible RIC. **Cortical changes:** grade 0: no changes. Grade 1: tubular dilatation with minimal loss of brush border. Grade 2: discrete minor changes with atrophy and dilatation of tubules. Grade 3: multifocal changes with moderately severe dilatation and atrophy. Grade 4: widespread changes with many larger foci and continuous areas with atrophic and severely dilated tubules.
with BEA being concentrated in the renal medullary interstitium, we speculate that BEA produces generalized sympathoexcitation via stimulation of afferent renal nerves (Barajas et al., 1992; Petersen and DiBona 1995). Several other groups have reported that BEA produces hypertension in the rat, but all previous observations have been performed days to weeks after BEA administration (Susić et al., 1983; Taverner et al., 1983, 1984, 1985; Russell et al., 1986; Dawson and Wallace, 1990). Thus, whereas Na and fluid retention may be involved in the late development of BEA-induced hypertension (see below), the mechanism(s) responsible for the immediate pressor response reported in this study is unknown.

In untreated Dahl-S rats, MAP increased as anticipated when the NaCl content in the rat diet was changed from 1 to 4%. However, the increase in MAP in response to 4% NaCl was markedly accelerated in Dahl-S rats with renal papillary-medullary lesion. Similar findings have been reported in normotensive Long-Evans rats on a high dietary NaCl intake. Thus, Long-Evans rats with renal papillary-medullary lesion developed hypertension while arterial pressure in Long-Evans rats with intact renal medulla was not salt-sensitive (Susić et al., 1983). With the accumulating evidence for a close relationship between increased medullary blood flow and pressure natriuresis, our findings are compatible with an impaired pressure-natriuresis relationship in the Dahl-S rat that deteriorates further when the renal medullary-papillary region is lesioned by BEA (Roman 1986; Cowley and Roman 1996). Thus, chronic administration of vasoconstrictor agents into the renal medulla (e.g., vasopressin type-1 agonists, nitric oxide synthase inhibitors) produce sodium retention and hypertension, and conversely, intramedullary administration of vasodepressor agents that increase renal medullary blood flow (e.g., atrial natriuretic peptide, converting enzyme inhibitors, calcium antagonists) causes natriuresis along with a reduction of arterial pressure in hypertensive rats (Mattson et al., 1994; Szczepanska-Sadowska et al., 1994; Garcia-Estan & Roman, 1990; Lu et al., 1992 and 1994). Based on these observations, the accelerated hypertension in BEA-treated animals is likely to be due to an impaired pressure-natriuresis function due to lesioning of the renal medulla.

Renal function studies at the end of the experiment showed that BEA-treated animals had a moderate reduction in ERPF and GFR which is consistent with what has been reported by others (Vanholder et al., 1981; Cuttino et al., 1981; Bing et al., 1983; Taverner et al., 1984 and 1985; Edmunds et al. 1990; Bergström et al., 1992). Dysfunction of the medullary collecting tubules was reflected in the 2-fold increase in daily water intake that is consistent with the marked polyuria that has been described in BEA-treated rats and dogs (Taverner et al., 1983 and 1984; Russell et al., 1986; Bergström et al., 1992; Szenasi et al., 1994). In addition, rats with BEA-induced renal papillary-medullary lesion had an impaired ability to excrete an acute i.v. load of isotonic saline (10% body weight). Thus, the more pronounced decrease in MAP in the BEA group than in the control animals upon change from 4 to 1% NaCl diet, suggest an increased salt-sensitivity in Dahl-S rats with renal papillary-medullary lesion that is reflected in an impaired ability to excrete an acute load of sodium. Our

**Fig. 5.** Renal papilla and medulla from control animals (A and C), and from rats that received a single injection of bromoethylamine (100 mg/kg i.p.) 41 day earlier (B and D). A, Normal renal medulla and papilla. B, Flattening and narrowing of the medulla with the rejected necrotic papilla in the pelvis (arrow). C, Numerous renomedullary interstitial cells of which the majority are arranged like the rungs of a ladder between vasa recta and thin loops of Henle (arrowheads). D, Interstitial edema and fibrosis with no discernible renomedullary interstitial cells, but with preserved structure of medullary collecting duct (asterisk). PAS, A and B bars: 400 μm; C and D bars: 20 μm.
data do not allow interpretation of the relative significance of reduced GFR or impaired renal medullary influence on sodium excretion for the increased salt-sensitivity in BEA-treated animals.

In conclusion, the antihypertensive effect of furosemide is preserved in Dahl-S rats with BEA-induced renal papillary-medullary lesion. This finding argues against the hypothesis that the antihypertensive effect of furosemide is mediated by a renomedullary vasodepressor substance. Hypertension induced by 4% NaCl diet in Dahl-S rats was accelerated in animals with renal papillary-medullary lesion. Because these animals had decreased ERPF, decreased GFR, and an impaired ability to excrete an acute i.v. load of isonicotic saline, these results suggest that the accelerated NaCl-induced hypertension in BEA-treated Dahl-S rats is due to an impaired ability to excrete excess NaCl. These results are compatible with an important role of the renal medulla in the long-term regulation of arterial pressure during alterations in dietary NaCl intake.

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References


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