Chemical Renal Medullectomy
Effect on Urinary Prostaglandin E₂ and Plasma Renin in Response to Variations in Sodium Intake and in Relation to Blood Pressure


SUMMARY We have studied the possible vasodepressor role of the renal medulla by chemical medullectomy. Bromoethylamine hydrobromide (200 mg/kg) was injected to induce selective renal medullary necrosis in rats. The acute effects on sodium balance and long-term effects on blood pressure, plasma renin concentration (PRC) and urinary prostaglandin E₂ (PGE₂) were studied and compared with saline injected controls. There was an immediate and sustained increase in urine volume of low osmolality. Direct blood pressure in conscious free-moving animals was higher at 2 and 10 weeks after injection in medullary-damaged rats, although this was only significant at 10 weeks (136 ± 3.3 vs 118 ± 4.5 mm Hg, p < 0.01). An initial negative sodium balance returned to normal by 7 days and rats with established medullary damage tolerated a wide range of sodium intakes. Although there was no evidence of sodium retention on the normal diet, with very high sodium loads some sodium retention was apparent since PRC was suppressed and body weight increased. Plasma creatinine and creatinine clearance were normal. PRC in rats with medullary damage was unchanged on normal diet and rose to similar levels as in control rats on low sodium intake. Urinary PGE₂ was markedly reduced (148 ± 54 vs 536 ± 71 ng/day, p < 0.01) in medullary-damaged rats, consistent with the renal medulla being the major source of urinary PGE₂. High salt intake increased urinary PGE₂ in normal and proportionally in medullary damaged rats, whereas on a low sodium intake, urinary PGE₂ was not different from that on the normal diet in either group. Direct blood pressure was positively correlated with urine volume (r = 0.60) and negatively with urinary PGE₂ (r = −0.43). Higher blood pressures were not related to PRC or the presence of renal cortical damage. These results are consistent with the theory that higher blood pressure in medullary-damaged rats is due to a reduction in renal medullary interstitial cell function. Chemical renal medullectomy induced by bromoethylamine hydrobromide may provide a useful model for unraveling the role of the renal medulla in blood pressure control. (Hypertension 5: 951-957, 1983)

KEY WORDS • renal medullectomy • blood pressure • renin • prostaglandin E₂

Both hypertensive and antihypertensive functions have been attributed to the kidney.1-5 The renin-angiotensin system and sodium balance have been most extensively studied, but even in renovascular hypertension, changes in these variables are probably insufficient to explain completely the involvement of the kidney in blood pressure elevation.6 7 Although an antihypertensive function other than sodium excretion was suggested even in the experiments performed by Goldblatt and coworkers,8 such a mechanism has remained controversial.

The studies of Muirhead and other workers3-5 have stimulated interest in the vasodepressor properties of the renal medulla. This effect appears to originate in the renal medullary interstitial cells, which are known to contain prostaglandins, particularly E₂,9 10 and antihypertensive lipids,1 all with potential hypotensive actions.5 11 Alterations in the granularity and lipid droplet content of these cells can be demonstrated in hypertension.12-15 Similarly, renal medullary transplants lower blood pressure in some experimental models.15-17 These alterations have also been related to the blood pressure response to changes in salt intake in certain experimental conditions.14

The physiological consequences of a compound that selectively ablates the renal medulla are therefore of considerable interest. Ethyleneimine18 and the parent compound, bromoethylamine hydrobromide,19 are two such compounds. Previous reports suggest bromoeth-
ylamine exacerbates experimental renovascular hypertension and reduces the fall in blood pressure after unclipping in two-kidney one clip hypertension in the rat. We have accordingly studied the effects of chemical renal medullectomy produced with bromoethylamine on blood pressure and have further investigated potential mechanisms by measuring urinary prostaglandin E₂, and plasma renin in response to changes in sodium intake.

Methods

White, female Wistar rats were used throughout and maintained on a standard diet containing 0.107 mmol sodium and 0.180 mmol potassium per gram, and tap water to drink ad libitum except where stated.

Chemical renal medullectomy was produced by 2-bromoethylamine hydrobromide (200 mg/kg body weight; Sigma UK, London, England), made up as a 10% solution (wt/vol) in 0.9% saline and injected intravenously in rats under ether anesthesia. Control animals were injected with an equivalent volume of 0.9% saline alone. The quantity of sodium injected was 0.04–0.06 mmol per animal. Animals were housed individually throughout the study. Two separate groups of rats were studied to determine: 1) the acute effect of bromoethylamine on sodium balance; and 2) the effect of changes in sodium intake on urinary composition and prostaglandin E₂ and plasma renin in rats with established renal medullectomy.

Acute Effect of Renal Medullectomy on Sodium Balance

Rats were placed in metabolic cages (Jencons Metabowl, Jencons Scientific Ltd., Leighton Buzzard, England) and after 2 to 3 days of acclimatization, balances were started. Following a 2-day run-in period, rats were given a single intravenous injection of either bromoethylamine (200 mg/kg) in saline or saline alone. The balances were continued for a further 7 days. A total of eight rats were injected with saline and 25 with bromoethylamine. Before the injection and at the end of the balance, a sample of blood was taken for plasma renin concentration under light ether anesthesia. Plasma renin concentration, creatinine, and osmolality were measured on the blood sample.

At the end of the study, all animals were returned to a normal diet, and 2 weeks later direct blood pressure measured. All animals were killed at the end of the studies, and postmortem examination was carried out with particular attention to the kidneys.

Laboratory Techniques

Blood Pressure

Direct blood pressure was measured by cannulation of the carotid artery under ether anesthesia. The catheter was exteriorized between the scapulae, and connected to a Statham P23 gb transducer and Grass recorder. The readings were made 4 to 6 hours after cannulation when the animals were fully conscious and freely moving, and expressed as mean blood pressure (diastolic + 1/3rd pulse pressure).

Urinary Prostaglandin E₂ (PGE₂)

Urine samples (ng/d) were stored at −70°C until assayed. PGE₂ was measured by radioimmunoassay following silicic acid chromatography. Four ml of water and 800 dpm of ³H-PGE₂ (Amersham International, Amersham, UK; 160 Ci/mmol) were added to 1 ml of urine at 0°C. The sample was acidified to pH 3.5 with 1 M hydrochloric acid and extracted twice with 5 ml of ethyl acetate. The pooled solvent layers were evaporated to dryness and residue redissolved in 0.2 ml of benzene/ethyl acetate/methanol (60/40:10) and 0.6 ml of benzene/ethyl acetate (60/40) then added. Silicic acid columns (0.5 g, 100–300 mesh, Sigma UK) were prepared fresh for each sample. The silicic acid was added as a slurry in benzene/ethyl acetate (60/40) to the columns. The sample was then added, followed by 5 ml of benzene/ethyl acetate (60/40) and the eluate discarded (PG A and B). The PGEs were then eluted with 20 ml benzene/ethyl acetate/methanol (60:40:2) and collected, dried under air, and redis-

Changes in Sodium Intake in Rats with Established Renal Medullectomy

The survivors from groups of rats injected with either bromoethylamine in saline (n = 13) or saline alone (n = 10) 2 weeks previously were randomly allocated to either normal, low sodium, or high salt diet for a period of 2 weeks. Dietary regimes were then changed so that all rats received the three diets in rotation, each for a 2-week period. The normal diet was as above. Low sodium diet consisted of Edosol (sodium content 0.013 mmol/g; potassium 0.175 mmol/g; Cow and Gate Foods, Trowbridge, UK) and deionized water to drink. High salt diet was the standard diet with 1% saline to drink. At the end of each 2-week dietary period, animals were housed individually in metabolic cages and a 24-hour urine sample collected. This was immediately cooled and stored at −70°C and used for estimation of volume, osmolality, sodium and potassium content, prostaglandin E₂, and creatinine excretion. On completion of each diet period, a sample of blood was taken from the tail under light ether anesthesia. Plasma renin concentration, creatinine, and osmolality were measured on the blood sample.

At the end of the study, all animals were returned to a normal diet, and 2 weeks later direct blood pressure measured. All animals were killed at the end of the studies, and postmortem examination was carried out with particular attention to the kidneys.
solved in 1 ml ethanol. Aliquots were used to estimate recovery (78.5±1.0%, n=25) and after drying down, the residue was redisolved in assay buffer for radioimmunoassay of PGE2. The antibody used was purchased from the Institut Pasteur, Paris, and cross reactivity with PGs A, B, and F was <0.3% and with PGE1, 11%.

**Urine Electrolytes**

Sodium and potassium concentrations (mmol) were determined by flame photometry.

**Urine and Plasma Osmolality**

Urine and plasma osmolality (mosmol/kg) was measured with an osmometer (Advanced Instruments, Needham Heights, Massachusetts).

**Plasma Renin Concentration**

Samples of tail vein blood (0.5–1 ml) were collected on ice in EDTA (50 μl of a saturated solution). Plasma was separated by centrifugation at 4°C and stored at −20°C until assayed. A 100 μl aliquot of plasma was incubated with 400 μl of nephrectomized rat plasma, as substrate, containing phenyl methyl sulphonyl fluoride to inhibit angiotensinases at pH 6.5. Angiotensin I (ng angiotensin 1/ml/hr) generated at 37°C was measured by radioimmunoassay.

**Plasma and Urine Creatinine**

Plasma and urine creatinine were measured by the Jaffe colorimetric method without deproteinization using a test kit (Boehringer Mannheim GmbH Diagnostica, Mannheim, West Germany). Creatinine clearance was calculated when the animals had completed 2 weeks of the normal diet.

**Statistics**

Results are expressed as means ± SEMS. Comparisons between and within groups were by unpaired and paired Student t tests respectively. PRCs were logarithmically transformed before analysis as PRC is not normally distributed.

**Results**

**Immediate Effect of Bromoethylamine on Sodium Balance**

Twelve of 25 rats given bromoethylamine died within 7 days of the injection. Analysis has been carried out on the 13 survivors that completed the whole 7 days of the sodium balance study and the eight saline-injected control rats.

Urine volume increased to over 20 ml/day in all bromoethylamine-injected rats in the first 24 hours following the injection and remained elevated during the next 6 days (table 1 and fig. 1, baseline = 12 ±...
1.7 ml; day 7 = 25 ± 2.4 ml, p < 0.01). Urine volume remained unchanged in the control group.

Sodium balance was negative in the 24 hours following the injection of bromoethylamine (−0.65 ± 0.12 mmol/day). This was due to reduced sodium intake (0.99 ± 0.07 mmol vs 1.98 ± 0.09 mmol on Days 1 and −1 respectively, p < 0.01), while sodium losses remained unchanged (1.64 ± 0.14 mmol and 1.97 ± 0.10 mmol, respectively, p > 0.1). During the next 6 days, sodium balance was corrected and the cumulative sodium balance for the 7 days after injection of bromoethylamine was −0.12 ± 0.37 mmol. In contrast, sodium intake (1.70 ± 0.10 and 1.72 ± 0.07 mmol on Days 1 and −1 respectively, p > 0.5) and losses (1.55 ± 0.18 and 1.69 ± 0.11 mmol respectively, p > 0.1) in the control group were unchanged in the first 24 hours after injection. Although sodium retention occurred in the first 4 days after the injection, by Day 7, cumulative sodium balance was not significantly different (+0.20 ± 0.24 mmol) and was not significantly different from that in bromoethylamine-injected group (p > 0.5).

At 1 week, body weight had fallen in the bromoethylamine group (183 ± 5 to 174 ± 5 g, p < 0.01) but was unchanged in the control group (188 ± 2 to 189 ± 5 g, p > 0.5). Plasma renin concentration (PRC) on Day 7 was slightly higher in the bromoethylamine group (167 ± 29 ng Al/ml/hr vs 121 ± 41 in controls, p < 0.01). Blood pressure was unchanged in the control group (188 ± 2 to 189 ± 5 mmHg, p > 0.1). Urine sodium excretion, as expected, rose in both groups but the increase was significantly greater in the medullectomized group (p < 0.01). Urine potassium excretion was unchanged in the medullectomized group, but fell in the control group. Urine sodium excretion, as expected, rose in both groups but the increase was significantly greater in the medullectomized group (p < 0.01). Urine potassium excretion was unchanged in the control group (p > 0.1). Plasma renin concentration (PRC) on Day 7 was 2.75 ± 0.45* in the control group whereas it fell but this was only significant for the medullectomized group in which body weight increased significantly, being unchanged in the control group.

Response to Changes in Sodium Intakes in Rats with Established Renal Medullectomy (Tables 2 and 3)

Animals were studied between 2 and 8 weeks after injection (10 controls and 13 rats injected with bromoethylamine).

Normal Diet

Urine volume was increased, osmolality decreased, and daily sodium excretion higher in medullectomized rats, but potassium excretion was similar in the two groups. Urinary prostaglandin E2 was significantly reduced in the medullectomized group (p < 0.005).

Serum creatinine and creatinine clearance, plasma renin concentration and osmolality, and body weight were not significantly different in medullectomized rats.

High Salt Diet (Normal Diet with 1% Saline to Drink)

Urinary volume increased significantly in both groups after 2 weeks of high sodium intake, although it only exceeded 20 ml/day in two of 10 control rats, and the difference between the groups persisted. Urine osmolality was unchanged in the medullectomized group but fell in the control group. Urine sodium excretion, as expected, rose in both groups but the increase was significantly greater in the medullectomized group (p < 0.01). Urine potassium excretion was unchanged.

Urinary PGE2 increased on a high salt intake in proportion to the baseline value, the difference between the groups was therefore maintained (table 3).

### Table 2. Urine Composition in Chemically Medullectomized and Control Rats on Normal (ND), High Salt (HS), and Low Salt (LS) Diets

<table>
<thead>
<tr>
<th>Urine Composition</th>
<th>Bromoethylamine group (n = 13)</th>
<th>Control group (n = 10)</th>
<th>Diff</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Urine volume (ml)</strong></td>
<td>NS</td>
<td>ND 31.0 ± 2.4</td>
<td>7.8 ± 1.8</td>
</tr>
<tr>
<td><strong>Sodium (mmol/day)</strong></td>
<td>ND 1.68 ± 0.11</td>
<td>1.33 ± 0.09</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td><strong>Potassium (mmol/day)</strong></td>
<td>ND 2.31 ± 0.08</td>
<td>2.20 ± 0.14</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Osmolality (mosm/kg)</strong></td>
<td>ND 625 ± 34</td>
<td>1740 ± 88</td>
<td>p &gt; 0.01</td>
</tr>
<tr>
<td><strong>PGE2 (ng/day)</strong></td>
<td>ND 148 ± 54</td>
<td>736 ± 71</td>
<td>p &gt; 0.05</td>
</tr>
<tr>
<td><strong>Creatinine (mg/dl)</strong></td>
<td>ND 927 ± 219</td>
<td>366 ± 56</td>
<td>p &gt; 0.01</td>
</tr>
</tbody>
</table>

Abbreviations as in table 2. Values are means ± SEM.

*Difference from value on normal diet, p < 0.05. Values are means ± SEM.

### Table 3. Plasma Renin Concentration (PRC), Plasma Osmolality, Body Weight, Plasma Creatinine, and Creatinine Clearance in Chemically Medullectomized and Control Rats

<table>
<thead>
<tr>
<th>PRC (ng Al/ml/hr)</th>
<th>Bromoethylamine group (n = 13)</th>
<th>Control group (n = 10)</th>
<th>Difference</th>
</tr>
</thead>
<tbody>
<tr>
<td>NS</td>
<td>HS 34 ± 11</td>
<td>63 ± 10</td>
<td>p &lt; 0.05</td>
</tr>
<tr>
<td>Low</td>
<td>LS 239 ± 55</td>
<td>234 ± 31</td>
<td>NS</td>
</tr>
</tbody>
</table>

| Osmolality (mosmol/kg) | HS 308 ± 2.6 | 302 ± 4 | NS |
|-----------------------| LS 310 ± 2.6 | 300 ± 5 | NS |

| Body weight (g) | HS 233 ± 2.8 | 207 ± 2 | p < 0.01 |
|-----------------| LS 213 ± 7 | 202 ± 6 | NS |

| Creatinine (µmol/liter) | HS 60 ± 2 | 55 ± 2 | NS |
|-------------------------| LS 60 ± 2 | 55 ± 2 | NS |

| Creatinine clearance (ml/min) | HS 0.90 ± 0.05 | 0.75 ± 0.07 | NS |

Abbreviations as in table 2. Values are means ± SEM.

*Difference from value on normal diet, p < 0.05.
Low Salt Diet (Edosol and Deionized Water to Drink)

Urine sodium was reduced to very low levels in both groups after 2 weeks. Urine volume in the medullectomy group was similar to that when on a normal diet, but was slightly higher in the control group. Urine potassium excretion fell to similar levels in both groups. Urine osmolality was lower on low sodium intake than on either normal or high salt diets, but was still significantly lower in the medullectomy group.

Urinary PGE2 on low sodium intake was similar to that on a normal diet in both medullectomized and control rats (table 2). PRC was elevated to a similar degree in both groups. Plasma osmolality was unchanged, and body weights were the same as on normal diets.

Direct Blood Pressure

The direct blood pressure measured on Day 14 after injection in rats from Study A was higher in the bromoethylamine group, but this was not significant compared with controls (130 ± 3.6 vs 123 ± 4.2 mm Hg, p > 0.1). However, at the end of Week 10 after injection, when all rats in Study B had been back on a normal diet for at least 2 weeks, direct blood pressure was significantly elevated in the medullectomy group compared with controls (136 ± 3.3 vs 118 ± 4.5 mm Hg, p < 0.01).

At 10 weeks, there was a positive correlation between direct blood pressure and daily urine volume (r = 0.60, p < 0.01) and a negative correlation with urinary PGE2 excretion (r = -0.43, p < 0.05). Direct blood pressure was not related to PRC (r = 0.01).

Pathology

The kidneys in all animals were examined at the end of the study. In bromoethylamine-injected rats, all except two of 26 animals showed evidence of moderate or severe renal papillary necrosis, and seven showed minor degrees of cortical scarring. No abnormality was seen in the kidneys of control rats. At 10 weeks after injection, there was no difference in either the direct blood pressure (139 ± 6 and 134 ± 4 mm Hg, p > 0.5) or PRC (49 ± 20 and 88 ± 29 ng Al/ml/hr, p > 0.7) in those medullectomized animals with or without cortical scarring, respectively. No other pathological changes were noted at postmortem.

Discussion

A single injection of bromoethylamine hydrobromide is an effective and reproducible method of producing renal medullary necrosis. The effect of such chemicals on the renal medulla is immediate. Thus, clamping of the renal artery for 50 minutes after the injection prevents the development of medullary damage. Destruction of the renal concentrating mechanism results in a high urine volume of low osmolality, as shown in this and earlier studies. A similar effect can be produced by surgical renal papillectomy. The increase in urine volume was observed in the first 24 hours after injection and persisted for at least 10 weeks. Histology confirmed destruction of the renal papilla with minimal or no damage to the renal cortex with the doses used in this study.

Direct blood pressure in conscious, freely moving animals was higher in medullary damaged rats at 2 and 10 weeks after bromoethylamine, compared with saline injected controls. A similar effect on blood pressure in bromoethylamine-injected normal and two-kidney one clip hypertensive rats has been reported, and we have previously observed that the fall in blood pressure after reversal of two-kidney one clip hypertension was attenuated in bromoethylamine-pretreated rats. Direct blood pressure at 10 weeks after injection was positively correlated with urine volume and negatively correlated with urinary PGE2 excretion. This suggests that the increase in blood pressure was related to the degree of medullary damage. It is unlikely to be attributable to either cortical damage, which has been described as a late sequela of chemical medullectomy, or increased renin secretion.

Thus, there is no difference in blood pressure in rats with cortical damage compared to those without, and there was no relationship between blood pressure and PRC. Although infusion of renin-angiotensin antagonists would help to confirm this, previous work has shown that the blood pressure response to saralasin in this strain is directly related to PRC. Normal plasma renin levels are also against significant sodium retention.

It is not practical to carry out sodium balances for 10 weeks. However, a single injection of bromoethylamine produced an initial negative sodium balance, the result of reduced food intake, while urinary sodium losses continued at preinjection levels. Subsequently, sodium balance was restored despite continuing high urine volume. Low urinary sodium excretion on the second day after bromoethylamine injection was noted by Shimamura although at all other times it was slightly increased. In contrast, control rats receiving saline injections developed a positive sodium balance (maximum, 0.7 mmol on Day 4). Similar changes have previously been seen after anaesthesia and a minor surgical procedure and could not be attributed to the saline injection (< 0.06 mmol/rat). Cumulative sodium balance at 1 week in control rats was slightly positive (0.2 mmol) and in bromoethylamine-injected rats slightly negative (−0.12 mmol). The difference was not significant. Thereafter, urinary sodium was slightly greater in medullary-damaged rats than in controls while on a normal diet, presumably reflecting increased sodium ingestion to maintain balance. Significant sodium retention is therefore unlikely. This is supported by similar levels of PRC and body weights in the two groups of rats.

The maintenance of sodium balance despite the continuing high urine volume in medullary-damaged rats is likely to be due in part to increased tubular reabsorption of sodium. Reduced glomerular filtration due to dehydration or cortical damage is unlikely, as plasma creatinine was normal and creatinine clearance slightly...
higher in medullary-damaged rats. Previous workers have reported elevated BUN and plasma creatinine and reduced GFR in medullectomized rats. However, on high sodium intakes there was evidence of sodium retention in medullary-damaged rats, i.e., reduced PRC and increased body weight. This would be consistent with previous reports of reduced fractional sodium excretion following medullectomy. However, since the additional sodium was given in the drinking water, the sodium load to which medullary-damaged rats were subjected was far greater than that of the control rats (at least ×2). Therefore, no conclusions on the sodium excretory capacity of the medullary-damaged rats used in this study can be made. Renal sodium conservation was unimpaired, and on a low sodium diet urinary sodium was reduced to similar levels in both control and experimental groups.

The renal medulla is known to contain prostaglandin-synthesizing tissue, and urinary PGE_2 is thought to be derived from renal synthesis rather than the circulation. The present study has shown that damage to the renal medulla results in a marked reduction in urinary PGE_2 excretion consistent with the renal medulla being the major source of this material in the urine. PGE_2 may act as a local hormone in the kidney, and it has been implicated in the control of intrarenal hemodynamics, sodium excretion, and renin release.

High salt intake increased PGE_2 excretion in normal rats and to a lesser extent in medullary-damaged rats, whereas low sodium diet produced no change compared to normal diet. Previous reports on urinary PGE_2 and dietary sodium have been contradictory, possibly because of species differences and experimental conditions. The present results would be consistent with a role for PGE_2 in the excretion of a sodium load although in medullary-damaged rats, despite substantial reductions in PGE_2 excretion, sodium excretion could be modulated within wide extremes (see above). However, a role for the modest PGE_2 production remaining, which presumably originates from the outer medulla or cortex, cannot be excluded. PRC changes in medullary-damaged rats were appropriate on all dietary regimes. It is unlikely, therefore, that the reduction in PGE_2 reflects or bromoethyamine itself effects changes in cortical prostaglandin synthesis.

Chemical renal medullectomy with bromoethyamine resulted in a rise in blood pressure that was not attributable to increased renin secretion or sodium retention. The most likely explanation is interference with a medullary vasoactive mechanism. The reduction in urinary PGE_2 and the relationship between urinary PGE_2 and blood pressure does not necessarily implicate the prostaglandin system, as this may only be acting as a marker of medullary interstitial cell damage and therefore changes in antihypertensive lipids. In-deed, previous observations by us have indicated that medullectomy is more effective in attenuating the fall in blood pressure after reversal of two-kidney, one clip hypertension than infusions of indomethacin.

The results presented here document for the first time some of the biochemical sequelae of chemical medullectomy and confirm the elevation in blood pressure produced by this technique. While our data do not establish a causal relationship between these sequelae and hypertension, they do indicate that the renal medulla plays an important role in blood pressure control. Chemical medullectomy therefore provides an important tool for unraveling this role.

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