CHEMICAL RENAL MEDULLECTOMY AND EXPERIMENTAL HYPERTENSION

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Summary
The possible vasodepressor role of the renal medulla was studied by chemical medullectomy (i.v. bromoethylamine hydrobromide) in rats. A significant increase in blood pressure (BP) was observed 10 weeks after injection which related to the increase in urinary volume and decrease in urinary prostaglandin E₂ (PGE₂) in medullectomised rats, but not to plasma renin concentration (PRC). Creatinine clearance was unchanged. The differences between control and medullary damaged rats were maintained over a wide range of sodium intakes although the patterns of response were similar in the two groups. The increase in BP observed following renal medullectomy is likely to be secondary to a reduction in interstitial cell function.

Introduction
Studies have indicated that the renal medulla may have an antihypertensive role in rats with experimental hypertension [1, 2]. Vasodepressor properties of the renal medulla appear to originate in the renal medullary interstitial cells which contain prostaglandins, particularly E₂ [3], and antihypertensive lipids [1].

Bromoethylamine hydrobromide selectively ablates the renal medulla [4] and it has been reported that this compound exacerbates experimental renovascular hypertension [5] and reduces the fall in blood pressure after unclipping in two-kidney one clip hypertension in the rat [2].

Accordingly, we have studied the effects of chemical renal medullectomy produced with bromoethylamine, on blood pressure, urinary prostaglandin E₂ (PGE₂) and plasma renin concentration (PRC) in normal rats, together with the effects of altering sodium intake in these animals.

Methods
White female Wistar rats were used and maintained on standard rat chow except where stated. Chemical renal medullectomy was performed by a single intravenous
injection of bromoethylamine hydrobromide (200mg/kg BW, Sigma, UK) in saline. Control rats were injected with an equivalent volume of 0.9 per cent saline alone. All animals were housed individually throughout the study. Two separate groups of rats were studied.

a) Acute effect of renal medullary damage on sodium balance

Rats were housed in metabolic cages and after a two day run-in period were either injected with bromoethylamine or saline and balances continued for seven days. PRC was measured before and after injection. Cumulative sodium balance was calculated as previously described [6]. Direct blood pressure (BP) was measured within one week of completion of the balances [2].

b) Responses to changes in sodium intake in rats with established renal medullary damage

Rats injected with bromoethylamine or saline alone two weeks previously were allocated randomly to either normal, low sodium (edosol and deionised water) or high salt (standard diet and one per cent saline to drink) diet for a period of two weeks. Dietary regimes were then changed so that all rats received the three diets in rotation, each for a two-week period. At the end of each dietary period a 24-hour urine sample was collected. This was cooled immediately and stored at −70°C and used for the estimation of volume, sodium content, PGE₂ [7] and creatinine excretion. At the same time a plasma sample was taken for PRC and creatinine estimation [2]. At the end of this study rats were returned to a normal diet and two weeks later direct blood pressure measured [2].

Statistical analysis

Results are expressed as mean ± SEM. Comparisons between and within groups were by unpaired and paired Student t-test respectively. PRC was logarithmically transformed before analysis.

Results

a) Acute effect of renal medullary damage on sodium balance (Table I)

<table>
<thead>
<tr>
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<th>Bromoethylamine (n = 13)</th>
<th>Control group (n = 8)</th>
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<tbody>
<tr>
<td></td>
<td>−1</td>
<td>7</td>
</tr>
<tr>
<td>U volume (ml)</td>
<td>12.2 ± 1.7</td>
<td>24.9 ± 2.4*</td>
</tr>
<tr>
<td>Body weight (g)</td>
<td>183 ± 5</td>
<td>174 ± 5*</td>
</tr>
<tr>
<td>Cumulative sodium balance (mmol)</td>
<td>+0.07 ± 0.07</td>
<td>−0.06 ± 0.41</td>
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* Difference from value on day −1, p<0.05
In all 13 rats that received bromoethylamine there was an immediate diuresis. Urine volume increased to over 20ml/day in the first 24 hours following the injection. During the next six days urine volume remained elevated and was significantly greater than baseline volume on day 7 ($p<0.01$). Urine volume was unchanged in the control group.

Sodium balance became negative in the 24 hours following bromoethylamine ($-0.65 \pm 0.12$mmol/day) but corrected over the next six days, so that the cumulative sodium balance for the seven days after injection was $-0.12 \pm 0.4$ mmol. In contrast, the control group retained sodium for the first four days after injection but by day seven cumulative sodium balance ($+0.2 \pm 0.24$mmol) was not significantly different from baseline or from the bromoethylamine group ($p>0.5$). At seven days the bromoethylamine group had a significant fall in body weight ($p<0.01$, Table I) and PRC was slightly higher (167 ± 29ng AI/ml/hr) than in the control group (121 ± 41, $p>0.1$).

b) Responses to changes in sodium intake in rats with established medullary damage (Figure 1)

i) Normal diet: Urine volume was increased ($p<0.01$) and daily excretion was higher ($p<0.05$), whereas urinary PGE$_2$ was significantly reduced ($p<0.01$) in medullectomised rats compared to controls. PRC, plasma osmolality and body weight were not significantly different. Serum creatinine was the same in both groups (medullectomised = 60 ± 2, control = 55 ± 2μmol/L) with creatinine clearance slightly higher in the medullectomised group (0.9 ± 0.05 vs 0.8 ± 0.07 ml/min).

ii) High salt: Urine volume increased significantly in both groups after two weeks although the difference between the two groups was maintained. Urine sodium excretion rose in both groups but the increase was significantly greater in the medullectomised group ($p<0.01$). Urinary PGE$_2$ increased on a high salt intake in proportion to the baseline value, thus maintaining the difference between the two groups. PRC fell but this was only significant for the medullectomised group. Body weight increased significantly in the latter.

iii) Low salt: Urine sodium was reduced to very low levels and PRC was elevated to a similar degree in both groups. Urine volume in the medullectomised group was similar to that when on a normal diet but was higher in the control group. Urinary PGE$_2$ was similar to that on a normal diet in both groups.

Direct blood pressure

When measured on day 14 after injection in rats in study (a), BP was higher in medullectomised animals compared to controls ($130 \pm 3.6$mmHg, vs $123 \pm 4.2$, $p>0.1$). On week 10 after injection this increase in BP was significant: study (b) ($136 \pm 3.3$ vs $118 \pm 4.5$mmHg, $p<0.01$). At 10 weeks there was a positive correlation between direct BP and daily urine volume ($r = 0.6$, $p<0.01$) and a negative correlation with urinary PGE$_2$ ($r = -0.43$, $p<0.05$), but no significant correlation with PRC ($r = 0.01$).
Figure 1. Daily urine volume, sodium and PGE$_2$ excretion and PRC in control (hatched columns, n = 10) and renal medullary damaged rats (open columns, n = 13) on normal, high salt and low salt diets. (Mean ± SEM, *p<0.05 for differences within each group, †p<0.05 and ‡p<0.01 for differences between control and medullectomised rats)
Discussion

A single injection of bromoethylamine is a reproducible method of inducing renal medullary necrosis, resulting in a high urine volume of low osmolality as seen in this and earlier studies [4, 5]. Histology confirmed destruction of the renal papilla with minimal or no damage to the renal cortex and urine volume increased in the first 24 hours and this persisted for at least 10 weeks. Despite the continuing high volume of urine the initial negative sodium balance was rapidly returned to normal. Further evidence for unimpaired renal sodium conservation in medullary damaged rats was the low urinary sodium excretion on the low salt diet, which was similar to the control group. However, there was some evidence of greater sodium retention in the medullectomised group on the high salt diet, i.e. reduced PRC and increased body weight, although the administration of salt in the drinking water resulted in a significantly greater sodium load in this group.

This study demonstrates that following damage to the renal medulla there is a marked reduction in urinary PGE$_2$ consistent with the medulla being a major source of this material in urine. A high salt diet increased urinary PGE$_2$ in normal rats and to a lesser extent in medullectomised rats, whereas a low sodium diet produced no change. This would be consistent with a role for PGE$_2$ in the excretion of a sodium load although in medulla-damaged rats, despite substantial reductions in PGE$_2$, sodium excretion could be modulated within wide limits. There may, however, be a role for the remaining PGE$_2$ production which probably originates from the outer medulla or cortex [8].

Direct blood pressure was significantly higher at 10 weeks in medullectomised rats compared to saline injected controls and this was positively correlated with urine volume and negatively correlated within urinary PGE$_2$. This suggests that the increase in blood pressure could be related to the degree of medullary damage. There was no relationship with PRC and no evidence of sodium retention on the normal diet. Interestingly PRC changes on different sodium intakes were appropriate in the medulla-damaged rats. Although the rise in blood pressure may follow interference with a medullary vasoactive mechanism, the fall in urinary PGE$_2$ does not necessarily implicate the prostaglandin system as this may only be acting as a marker of medullary interstitial cell damage.

The results presented here document some of the biochemical sequelae of medullectomy following an injection of bromoethylamine and confirm the elevation of blood pressure produced by this technique.

References

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