Influence of captopril on fluid and electrolyte balances and adrenocortical responses during sodium deprivation in the rat

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SUMMARY

An angiotensin I-converting enzyme inhibitor (captopril) was given by gastric lavage at a dose of 30 mg/kg body weight per day to Long-Evans rats for a 13-day period during which they received a sodium-deficient diet. This regime was preceded by a 3-day period during which measurements were made on the animals on a sodium-replete dietary intake. Control sodium-deprived rats showed increased plasma renin activities, increased peripheral aldosterone concentrations and reduced urinary sodium excretion; they maintained positive sodium balance and the zona glomerulosa of the adrenal cortex hypertrophied. Captopril-treated sodium-deprived rats failed to reduce urinary sodium excretion sufficiently and entered a period of marked and sustained negative sodium balance. Peripheral aldosterone concentrations after 12 days of sodium deprivation in the presence of captopril treatment were similar to those of sodium-replete rats. The adrenocortical zona glomerulosa of the captopril-treated rats did not increase in size and regressive changes were noted.

INTRODUCTION

In mammals, dietary sodium restriction is accompanied by a series of renal, cardiovascular, neural and endocrine events that together maintain long-term body fluid homeostasis in the face of incipient depletion of the total body reserves of sodium. Activation of the renin-angiotensin system is one of the earliest detectable endocrine changes and is arguably the primary one responsible for the complex of reactions that involves blood-pressure homeostasis, renal sodium retention, an increased aldosterone secretion, urinary dilution and shifts in internal body fluid distributions (Laragh & Sealey, 1973).

The aims of the present investigations were to examine the responses of rats to the imposition of a sodium-deficient diet in the presence of blockade of the renin-angiotensin system. Captopril, an inhibitor of angiotensin I-converting enzyme (Antonaccio & Cushman, 1981), was given to rats from the beginning of a sodium-restricted regime and the adrenocortical responses were determined together with measurements of the overall balances of fluid and electrolytes.

MATERIALS AND METHODS

Animals

Male Long-Evans rats, weighing 250–300 g, were bred in departmental colonies. They were maintained at 26°C in a lighting regime of 12h light:12h darkness (lights on 07.00–19.00 h) and a standard diet (RHM Agriculture, Poole, Dorset) and tap water were available ad libitum.
Water and electrolyte balances and adrenocortical function

Daily (24-h) food and water intakes and urinary excretory rates were measured in individual rats held in all-glass metabolism cages (Metabowl; Jencons, Hemel Hempstead). Animals were acclimatized to the cages for 7 days before measurements were begun (Balment, Chester Jones, Henderson & Oliver, 1976).

For the first 3 days after adaptation, rats were provided with tap water to drink and the normal diet containing 63 and 173 mmol sodium and potassium respectively per kg food. On day 4 a sodium-deficient diet was introduced (Tekland Test Diets, Madison, Wisconsin, U.S.A.) which contained 3 and 233 mmol sodium and potassium respectively per kg food. On the same day, control rats were given 1 ml tap water per os and the experimental rats 1 ml tap water containing captopril (SQ 14,225; Squibb Pharmaceuticals) at a dose of 30 mg/kg body weight. These treatments continued for 12 days with the drug or vehicle being administered at 16.00 h each day. Thus all animals were studied for 3 days on a normal dietary intake of sodium (days 1–3); one group was given captopril plus sodium-restricted diet (days 4–16) and one group the vehicle plus sodium-restricted diet (days 4–16).

On days 1, 3, 6, 9, 13 and 16, between 10.00 and 11.00 h, animals were lightly anaesthetized with ether and 1 ml blood was collected by cardiac puncture. Blood was collected within 90–120 s after removal from the cage.

Further results were obtained from rats grouped in cages studied under the same treatment regimes. Other control values were gathered from age-matched rats maintained on the normal diet.

Analyses

Plasma and urinary sodium and potassium concentrations were determined by flame-emission spectrophotometry (Pye Unicam SP 1950), chloride concentrations by electro-metric titration (Aminco Titrator; American Instruments, Silversprings, Maryland, U.S.A.) and total osmolarities by freezing-point depression (Knauer Osmometer, Berlin).

Sodium and potassium were measured in aqueous extracts of the diets. Plasma renin activities were measured indirectly by radioimmunoassay of angiotensin I generated during a 16-h incubation of thawed plasma (Stockigt, Collins & Biglieri, 1971; Balment, Henderson & Oliver, 1975) and were expressed as ng equivalents of angiotensin I/ml plasma per 16 h.

Plasma corticosterone and aldosterone concentrations were measured by radio-immunoassays (Kime, 1977; Milne, 1981 respectively).

Statistics

Grouped data were compared by Student's t-test and data from the metabolism cage studies by a paired t-test (see Balment et al. 1976).

Histology of the adrenal gland

At the end of the experiments adrenal glands were excised, fixed in Bouin's fluid, sectioned at 6 µm and stained with haematoxylin and eosin. Radial measurements of zonal widths were taken from 16 sections of each gland.

RESULTS

Fluid and electrolyte balances

On the normal laboratory diet (days 1–3) the control group of rats had a sodium intake and urinary excretion (in µmol/100 g body weight per 24 h) of 513.6 ± 19.10 (S.E.M.) and 335.6 ± 39.8 respectively. In the group of rats that eventually received captopril the sodium intake was 504.7 ± 32.43 and the excretory rate 299.5 ± 21.6. There were no differences in the fluid and electrolyte values between the two groups at this stage (Text-fig. 1; Tables 1–3).
Text-fig. 1. Effects of captopril introduced at the onset of a sodium-deficient diet on (a) sodium excretion, (b) water intake, (c) urine production and (d) water balance of rats. Daily sodium excretory rates were studied for an initial 3-day period (normal diet; days 1–3). On day 4 a sodium-deficient diet was instituted (low sodium diet) and from days 4 to 16 inclusive some animals received tap water (○···○) and others captopril (●—●) per os each day. Points are mean values for five control and seven captopril-treated rats. In (a) hatched lines give average sodium intakes on normal and low sodium diets. In (d) water balance is defined as (intake/urinary output) × 100%.
Table 1. Effects of captopril on urinary composition and electrolyte excretory patterns during sodium deprivation. Day 1 gives the values before introduction of the sodium-deficient diet to untreated groups of rats and day 16 the values observed in rats treated with captopril (captopril-treated; n = 7) or its vehicle (control; n = 5) 12 days after introduction of the sodium-deficient regime. See text for further details. Values are means ± S.E.M. *.

<table>
<thead>
<tr>
<th>Day 1 (sodium-replete)</th>
<th>Day 16 (sodium-deprived)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Eventual control rats</strong></td>
<td><strong>Rats eventually treated with captopril</strong></td>
</tr>
<tr>
<td>Osmolarity (mosmol/l)</td>
<td>1542 ± 313</td>
</tr>
<tr>
<td>Solute excretion (mosmol/100 g body wt per 24 h)</td>
<td>8.55 ± 1.13</td>
</tr>
<tr>
<td>Sodium concentration (mmol/l)</td>
<td>75.75 ± 17.6</td>
</tr>
<tr>
<td>Potassium concentrations (mmol/l)</td>
<td>170.7 ± 36.6</td>
</tr>
<tr>
<td>Potassium excretion (µmol/100 g body wt per 24 h)</td>
<td>103.4 ± 38.1</td>
</tr>
<tr>
<td>Chloride concentration (mmol/l)</td>
<td>98.0 ± 21.8</td>
</tr>
<tr>
<td>Chloride excretion (µmol/100 g body wt per 24 h)</td>
<td>499.8 ± 88.5</td>
</tr>
</tbody>
</table>

* P < 0.001 compared with value on day 1; † P < 0.001 compared with control value on day 16 (Student’s t-test).

Table 2. Effects of captopril on plasma solute, sodium and potassium concentrations of rats during sodium deprivation. Blood samples were collected by cardiac puncture from untreated rats fed a normal diet and subsequently 6 and 12 days after introduction of the sodium-deficient diet, during which time they were treated with either captopril (captopril-treated) or its vehicle (control). Further details are given in the text. Values are means ± S.E.M.; group A, n = 5; group B, n = 7

<table>
<thead>
<tr>
<th>Sodium status and treatment</th>
<th>Osmolarity (mosmol/l)</th>
<th>Sodium (mmol/l)</th>
<th>Potassium (mmol/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium-replete A Eventual control rats</td>
<td>300.0 ± 4.3</td>
<td>138.8 ± 7.1</td>
<td>4.35 ± 0.25</td>
</tr>
<tr>
<td>B Rats eventually treated with captopril</td>
<td>303.3 ± 6.1</td>
<td>148.0 ± 3.8</td>
<td>4.78 ± 0.21</td>
</tr>
<tr>
<td>Sodium restricted for 6 days A Control</td>
<td>311.0 ± 10.5</td>
<td>143.5 ± 10.4</td>
<td>4.89 ± 0.27</td>
</tr>
<tr>
<td>B Captopril-treated</td>
<td>320.6 ± 8.0</td>
<td>143.7 ± 4.2</td>
<td>4.63 ± 0.10</td>
</tr>
<tr>
<td>Sodium restricted for 16 days A Control</td>
<td>273.0 ± 3.4*</td>
<td>148.8 ± 3.9</td>
<td>4.65 ± 0.22</td>
</tr>
<tr>
<td>B Captopril-treated</td>
<td>270.6 ± 4.3*</td>
<td>142.0 ± 1.2</td>
<td>4.48 ± 0.20</td>
</tr>
</tbody>
</table>

* P < 0.01 compared with sodium-replete values (Student’s t-test).

Introduction of the sodium-deficient diet reduced the intakes of sodium to 20.12 ± 0.72 and 23.59 ± 0.70 µmol/100 g body weight per 24 h in the control and captopril-treated rats respectively. Rats given the tap water per os each day had significantly reduced rates of sodium excretion (4.32 ± 0.7 µmol/100 g body weight per 24 h). Captopril introduced at the same time as the sodium-deficient regime produced striking effects on sodium excretory...
Table 3. Effects of captopril on plasma aldosterone and corticosterone concentrations and plasma renin activity of rats during sodium deprivation. Blood samples were collected by cardiac puncture from untreated rats fed a normal diet (sodium-replete) and subsequently 6 and 12 days after introduction of the sodium-deficient diet, during which time they were treated with either captopril or its vehicle (control).

<table>
<thead>
<tr>
<th>Sodium status and treatment</th>
<th>Corticosterone (nmol/1 plasma)</th>
<th>Aldosterone (nmol/1 plasma)</th>
<th>Plasma renin activity (ng equiv. angiotensin I/ml per 16-h incubation)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sodium-replete</td>
<td>168.4 ± 32.9</td>
<td>0.95 ± 0.07</td>
<td>89.4 ± 13.1</td>
</tr>
<tr>
<td>Control</td>
<td>182.9 ± 27.2</td>
<td>2.13 ± 0.66*</td>
<td>435 ± 100*</td>
</tr>
<tr>
<td>Sodium-deprived 6 days</td>
<td>168.4 ± 32.9</td>
<td>0.95 ± 0.07</td>
<td>89.4 ± 13.1</td>
</tr>
<tr>
<td>Control sodium-deprived 12 days</td>
<td>73.4 ± 10.6*</td>
<td>3.85 ± 0.76*</td>
<td>315.6 ± 34.6*</td>
</tr>
<tr>
<td>Sodium-deprived + captopril 6 days</td>
<td>155.1 ± 57.1</td>
<td>0.97 ± 0.19†</td>
<td>248 ± 64*</td>
</tr>
<tr>
<td>Sodium-deprived + captopril 12 days</td>
<td>124.7 ± 34.2</td>
<td>1.30 ± 0.46†</td>
<td>202.5 ± 34.3*</td>
</tr>
</tbody>
</table>

* P < 0.01 compared with sodium-replete values; † P < 0.001 compared with equivalent sodium-deprived rats (Student's t-test).

patterns. Animals entered a marked and sustained state of negative sodium balance (Text-fig. 1a) and although the excretory rate of 32.9 ± 7.0 μmol/100 g body weight per 24 h was reduced compared with the 3-day control dietary period (see above), the value was some eightfold greater than that seen in the control sodium-restricted rats. In other words, captopril significantly impaired the renal conservation of sodium in the face of a restricted intake.

Urinary sodium concentrations fell in both groups when placed on the sodium-deficient diet but the overall urinary dilution of this ion was much less in the captopril-treated than in the control rats (Table 1). Table 1 also shows that urinary osmolarities and urinary potassium and chloride concentrations declined after introduction of the sodium-restricted diet, and in all cases the reductions were greater in the captopril-treated than in the control rats. Total solute excretory rates were similar in the two groups, but captopril-treated rats excreted less potassium and chloride than control animals on the sodium-deficient regime.

Sodium restriction alone resulted in a gradual increase in daily urine volume: in control rats from 4.47 ± 0.73 to 11.26 ± 2.43 ml/100 g body weight per day and in captopril-treated from a control value of 4.91 ± 0.50 to 30.41 ± 3.21 after 6 days of sodium restriction. The percentage of the fluid intake excreted increased from 34.86 ± 5.03 to 71.33 ± 3.41% in control rats and from 40.61 ± 2.83 to 80.52 ± 4.43% in captopril-treated rats 6 days after instituting the sodium-deficient regime (Text-fig. 1d). There were, however, differences in the extent of these changes when captopril-treated and control rats were compared. In the former, urinary volumes increased immediately (from 4.91 ± 0.50 to 15.08 ± 3.17 ml/100 g body weight per day on day 5) and there was a subsequent increase in drinking to reach a maximum of 38.04 ± 4.35 on day 11 (Text-fig. 1b). Throughout the period of sodium restriction, drinking and urinary production rates were much greater in the captopril-treated than in the control rats (Text-fig. 1b, c). Full details of these temporal changes have been given (McKeever, 1982).

Plasma concentrations of sodium and potassium were not significantly altered by sodium deprivation either separately or combined with captopril treatment, although after 16 days of sodium restriction both control and captopril-treated rats had lower plasma osmolarities (Table 2).
Endocrine changes

Plasma renin activities increased in all rats placed on the sodium-restricted diet, with the increase being on average slightly greater in the control than in the captopril-treated animals. Plasma corticosterone concentrations did not vary either with the introduction of the sodium-deficient diet combined or otherwise with captopril, at least for the first 6 days (experimental days 4–9) (Table 3). After 12 days of sodium deprivation the corticosterone concentrations were lower in the control than in the captopril-treated rats.

Captopril markedly inhibited the adrenocortical aldosterone response to sodium restriction. Peripheral aldosterone concentrations showed on average a fourfold increase in the control animals, from about 0·95 to 3·83 nmol/1 plasma. In the captopril-treated sodium-deprived rats the rises in mean concentrations after 6 and 12 days were not statistically significant (Table 3).

The normal increase in the width of the adrenocortical zona glomerulosa seen in the control sodium-deprived rats was not apparent in sodium-deprived captopril-treated animals (Plate; Table 4). Indeed in one captopril-treated rat a zona glomerulosa could not be identified. In this particular animal the peripheral aldosterone was 0·83 nmol/1 plasma.

Table 4. Dimensions of the adrenocortical zona glomerulosa of rats on a normal sodium diet, on a sodium-deficient diet and on a sodium-deficient diet but treated with captopril. Values are means ± S.E.M. of the number of sections examined (see text). Numbers of animals are given in parentheses.

<table>
<thead>
<tr>
<th>Group</th>
<th>Total adrenocortical width (µm)</th>
<th>Width of zona glomerulosa (µm)</th>
<th>Zona glomerulosa (% whole cortex)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Experimental group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control, normal diet (8)</td>
<td>929 ± 29</td>
<td>50 ± 2</td>
<td>5·38</td>
</tr>
<tr>
<td>Sodium-deficient diet (12 days) (5)</td>
<td>1025 ± 34</td>
<td>71 ± 3*</td>
<td>6·95</td>
</tr>
<tr>
<td>Sodium-deficient diet with captopril (12 days) (7)</td>
<td>1021 ± 36</td>
<td>38 ± 10†</td>
<td>3·71</td>
</tr>
</tbody>
</table>

*P < 0·001 compared with animals on a normal diet; †P < 0·01 compared with animals on a sodium-deficient diet (Student’s t-test).

Discussion

The renin-angiotensin system plays a key role in the adaptational events that allow mammals to maintain positive sodium balances when dietary sodium is restricted (Laragh & Sealey, 1973; Brunner & Gavras, 1980). In the present experiments, the control animals placed on the sodium-deficient regime gave the usual responses to sodium deprivation with increased plasma renin activities, increased peripheral aldosterone concentrations, reduced renal sodium excretion and overall urinary dilution (Spielman & Davis, 1974; Kenyon, Mosley, Hargreaves, Balment & Henderson, 1978; Hall, Guyton, Smith & Coleman, 1979; Tarjan, Spät, Balla & Szekely, 1980). These consequences of reduced dietary sodium were significantly modified by simultaneous treatment with captopril. The angiotensin I-converting enzyme inhibitor was given from the day of initiating the sodium restriction with the aim of preventing even an initial response to incipient depletion. Such a protocol differs from others previously employed in which animals have been allowed to adapt to sodium restriction for perhaps several days before beginning inhibition of converting enzyme.

Sodium deprivation produced the expected increase in plasma renin activity in the control and experimental rats, but rather surprisingly the captopril-treated animals did not
have significantly greater activities than the control rats, although the rate of increase was more rapid (McKeever, 1982). These effects may suggest that maximal renin releasing mechanisms were induced by sodium deficiency and inhibition of angiotensin I-converting enzyme failed to produce further increments.

The captopril-treated rats on a restricted sodium intake did not adjust their sodium excretion sufficiently to attain a positive sodium balance and their peripheral aldosterone response was much blunted compared with the control sodium-restricted animals. The attenuated aldosterone response produced by captopril confirms the findings of Aguilera & Catt (1978) in the sodium-deficient rat, although in sodium-replete animals considerably higher doses of captopril failed to alter peripheral aldosterone concentrations (Kokubu, Ueda, Ono, Kawabe, Hayashi & Kan, 1980). In the present studies the relative hypoaldosteronism of captopril-treated animals was associated with structural differences in the adrenal cortex; in particular the zona glomerulosa failed to hypertrophy in the face of sodium depletion in the presence of inhibition of angiotensin I-converting enzyme. In control sodium-deprived rats, an increase in the width of the zona glomerulosa was apparent, while in the captopril-treated animals normal or regressed outer zones were seen, with an intermediate zone between the zonae fasciculata and glomerulosa being more apparent. Using another angiotensin I-converting enzyme inhibitor (teprotide, SQ 20,881), Weaver, Nickerson, Molteni, Solliday & Albertson (1981) found few changes in adrenocortical morphology of sodium-replete rats, although they did note ‘... possible reduction in numbers of cells of the zona glomerulosa ...’ and a clearer delineation between the fasciculata and glomerulosa; peripheral aldosterone concentrations were unchanged in teprotide-treated rats compared with controls. In Brattleboro rats with hypothalamic diabetes insipidus captopril produced regressive adrenocortical changes (McKeever, Kenyon, Oliver & Henderson, 1980) and in these studies the potassium wasting state of such rats (Möhring, Möhring, Dauda & Haack, 1974; Kenyon, Hargreaves & Henderson, 1978) was ameliorated by the converting enzyme inhibitor. Brattleboro rats with diabetes insipidus display reduced levels of aldosterone (Milne, Balment, Henderson, Mosley & Chester Jones, 1982) and this relative hypoaldosterone state was further suppressed by captopril (Henderson, McKeever & Kenyon, 1979).

The increased renal reabsorption of sodium necessary for animals placed on sodium-deficient diets reflects conjoint or separate actions of aldosterone and angiotensin (Johnson & Malvin, 1977; Hall, Guyton, Smith & Coleman, 1980a, b). The very considerable natriuretic state of the captopril-treated sodium-restricted rats rather surprisingly did not change plasma sodium concentrations, so that presumably a compensatory redistribution of the ion occurred to protect extracellular concentrations at the expense of depletion of total body reserves. The sodium loss was not associated with increased chloride excretion rendering a reduction in distal renal tubular sodium reabsorption likely in captopril-treated animals. It is, however, significant that in chronically sodium-depleted dogs given captopril, infusions of aldosterone failed to restore normal renal sodium regulation, whereas angiotensin infusion reversed all the effects of captopril (Hall et al. 1979; McCaa, 1979). Clearly there is considerable overlap in the actions of the renin-angiotensin system and aldosterone to promote renal regulation of sodium and potassium excretion during sodium deprivation, and the effects of captopril reported in the present study cannot distinguish clearly between them. The natriuresis possibly results from a lack of angiotensin, while the relatively greater potassium retention seen in the captopril-treated compared with the control sodium-restricted rats may result from the induced hypoaldosterone state.

During sodium restriction captopril increased the water turnover: In the control sodium-restricted group, small increases in both drinking and urine production rates occurred, whilst in the captopril group an increased urine production preceded a compensatory increase in the rate of drinking. The increased urine flow may have resulted from an acute fall in renal medullary interstitial sodium and chloride concentrations (Levens, Peach &
Carey, 1981) giving defective reabsorption of osmotically free water. In this context, a reduced release of vasopressin associated with falling plasma angiotensin levels (Share, 1979) may in part be responsible for the sometimes positive osmotically free water clearances seen in the captopril-treated group of rats. For example, during sodium restriction, control rats had a free water clearance of $-14-50 \text{ ml}/100 \text{ g body weight per 24 h}$ compared with $-22.19$ when fed the sodium-replete diet; on days 9 and 13 the captopril-treated animals gave positive values for osmotically free water clearance of, on average, $2.72$ and $0.68 \text{ ml}/100 \text{ g body weight per 24 h}$.

The nature of vasopressin–angiotensin interaction is, however, equivocal. Knepley & Meyer (1980) noted that angiotensins I, II and III are active in eliciting vasopressin release and that, in the presence of the kidneys, inhibition of angiotensin I-converting enzyme (by teprotide) increased plasma vasopressin, while no effect of the drug was seen in bilaterally nephrectomized rats. Furthermore, in sodium-deficient dogs, teprotide increased osmotically free water clearance in the presence of raised antidiuretic hormone induced by water deprivation or during water diuresis (Levens, Peach, Vaughan & Carey, 1981). The lack of angiotensin II in itself could therefore increase osmotically free water excretion, as seen in the present studies. The relative polydipsia when angiotensin II is depressed may also be due to other extracellular thirst mechanisms (Fitzsimons, 1972) responding to the increased renal water losses induced by captopril.

We thank Dr P. V. Piggott (Squibb Europe Ltd, Twickenham, Middlesex) and Dr Z. P. Horovitz (E. R. Squibb & Sons Inc., Princeton, New Jersey, U.S.A.) for the supply of SQ 14,225. We also thank Dr C. M. Milne and Mr David Hollingworth for their valuable assistance. These studies were in part supported by grants from SERC to I.W.H. A.M. held a SERC Studentship.

REFERENCES


DESCRIPTION OF PLATE

(Haematoxylin and eosin; × 360)
Effects of captopril on the histology of the adrenal cortex of sodium-deprived rats. Sections through the adrenal cortex of a rat on (Fig. 1) a normal sodium diet, (Fig. 2) a sodium-deficient diet for 12 days and (Fig. 3) a sodium-deficient diet for 12 days and treated with captopril for the period of sodium deprivation are shown. The bars show the width of the zona glomerulosa. See Table 4 for details.