The effects of captopril on blood pressure, urinary water and electrolyte excretion and drinking behaviour in Brattleboro rats

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Summary

1. The effects of the orally active converting enzyme inhibitor, captopril (SQ 14225), on blood pressures and intakes and urine outputs of water and electrolytes were studied in rats with hereditary hypothalamic diabetes insipidus (Brattleboro strain) and in Long Evans rats (parent strain).

2. Captopril given in the drinking water (30 mg day⁻¹ kg⁻¹) caused an increase in fluid intake and urine output in both strains of rat; the difference between intake and measured output did not change.

3. Captopril caused a significant natriuresis when given to animals in the non-steady state but did not significantly affect the urinary electrolyte excretion of animals in a steady state; in the latter group, however, captopril caused a significant reduction in food intake. Hence, under both conditions, captopril caused a reduction in sodium balance.

4. Systolic blood pressures were reduced by captopril (given in the drinking water) in Long Evans rats and in Brattleboro rats; there was no accompanying change in heart rate.

5. Bolus administration of captopril (30 mg day⁻¹ kg⁻¹) either intragastrically or subcutaneously did not change the fluid intakes or outputs in either strain of rat.

6. In a separate experiment, rats were given the choice to drink water or a captopril solution. The results showed that the increased fluid intake in response to captopril was not due to a liking for the taste of the solution.

7. The dipsogenic response to captopril may have been due to the fall in blood pressure which occurred, leading to renin release and a peripheral build-up of angiotensin I, which was converted into angiotensin II in the central nervous system. The possibility that the same dose given as a bolus may have inhibited central, as well as peripheral, converting enzyme activity is discussed.

Key words: Brattleboro rats, captopril, drinking, electrolytes.

Introduction

In rats lacking the ability to synthesize vasopressin (Brattleboro strain) there are several indices of increased activation of the renin-angiotensin (ANG) system including elevated plasma renin activity [1], plasma renin concentration (2), renin substrate levels [2] and plasma ANG II concentrations [3]. Henderson et al. [4, 5] showed that oral administration of the converting enzyme inhibitor, captopril (N-2-methyl-3-mercaptopropanoyl-1-proline, SQ 14225), reduced water intake in Brattleboro rats, and suggested that, to some extent, the polydipsia which normally occurs in these animals was due to the dipsogenic effect of the high circulating ANG II levels. However, Elfont & Fitzsimons [6, 7] demonstrated an increase in water intake in response to captopril under experimental conditions when circulating ANG II would be expected to be high; this latter observation is more consistent with the increase in water intake which Mann et al. [8] reported to occur in Brattleboro rats in response to captopril.

It is unclear whether any effect of captopril on fluid intake is direct or indirect. There are at least three ways in which captopril could indirectly...
promote drinking: (i) as a result of renal electrolyte losses leading to volume depletion [9-11]; (ii) by inhibiting vasopressin secretion [12] and thus causing volume depletion; (iii) by causing hypotension (unassociated with any volume depletion [13]). In the present work we have studied the effects of captopril on intakes and outputs of fluid and electrolytes and on blood pressures in Brattleboro rats and in Long Evans rats (parent strain). In the first experiment we followed the protocol of Henderson et al. [4, 5] with animals being housed under similar conditions for the same periods of time, but analysis of our data at the end of that experiment revealed that the animals were not in a steady state, and, furthermore, the results were completely opposite to those of Henderson et al. [4, 5]. One difference between our experiment and the one which we were trying to follow [4, 5] was that our animals received a diet containing 0.26 mmol of sodium/g, whereas in the experiments of Henderson et al. [5] the diet contained only 0.067 mmol of sodium/g. Since the sodium status can markedly influence the prevailing renin-ANG II activity and the adrenal [14] and vascular [15] ANG II receptor sensitivity, we repeated the experiment, but on animals which had become fully acclimatized to the metabolism cages and with a diet closer in sodium content to that used by Henderson et al. [4, 5].

In the first two experiments, captopril was administered in the drinking water and it seemed feasible that the drinking response which we observed may have been due to the taste of the solution. We therefore performed separate experiments to determine whether the rats showed any preference for captopril when given the choice and whether any drinking response occurred when the same dose of the drug was given by a different route.

**Methods**

Male homozygous Brattleboro rats (body weight 260-320 g) and aged matched Long Evans rats (body weight 270-360 g) were used throughout this study.

**Experiment 1: effects of captopril on fluid and electrolyte balance**

**Part A.** Rats (n = 6 in each group) were housed individually in glass metabolism cages (Metabowl, Jencons) with free access to food (Heygates 41B; sodium content 0.26 mmol/g; potassium content 0.26 mmol/g) and tap water. The animals were allowed 7 days to adapt to the metabolism cages before any measurements were made (cf. [4, 5]). Daily measurements of food intake (± 0.1 g), water intake (± 1.0 ml), urine output (± 0.1 ml) and faecal output (± 0.1 g) were then made for 3 days before drug administration, for 6 days whilst captopril was present in the drinking water, and for 3 days after the last drug administration. The concentration of captopril was adjusted according to intake such that each rat received approximately 30 mg day⁻¹ kg⁻¹; the actual intake was noted. The urine and faecal samples were frozen and stored for analysis at the end of the experiment.

**Part B.** Seven Brattleboro rats and nine Long Evans rats were acclimatized to individual metabolism cages for 4 weeks before the experiment began, with free access to food (Labsure 41B: sodium 0.09 mmol/g; potassium 0.14 mmol/g) and tap water. Thereafter daily measurements of food intake, water intake, urine output and faecal output were made for 3 days before captopril, for 6 days whilst captopril was present in the drinking water (intake approximately 30 mg day⁻¹ kg⁻¹) and for 5 days after the last drug administration. The urine and faecal samples were frozen and stored for analysis at the end of the experiment.

**Urine analysis:** the sodium and potassium concentrations of the urine were measured by flame photometry (EEL model 150).

**Faecal analysis:** weighed samples of the faeces were dried to constant weight in an oven at 80°C and the water content was determined from the weight loss. The dried samples were then dissolved in concentrated nitric acid and the sodium and potassium concentrations of the supernatant were measured by flame photometry.

**Food analysis:** the water and electrolyte contents of the diets were measured by the same procedure as outlined above for the faeces.

**Experiment 2: effect of captopril on systolic blood pressure and heart rate**

This experiment was performed on the same rats as were used in experiment 1 (Part B). Systolic blood pressure was measured daily in conscious rats by the tail-cuff method; heart rate was derived from the microphone output of the blood pressure recorder (W & W Electronics, 8005). At least four consecutive cycles (inflation/deflation rate 250 mmHg/min) were performed on each animal and the mean of the last three readings was taken as the systolic blood pressure. Measurements were made for 10 days before captopril was given and the values recorded on the last 3 days were used as the baseline. As before (experiment 1), captopril
was administered in the drinking water at the concentration required to achieve an average intake of 30 mg day\(^{-1}\) kg\(^{-1}\). Systolic blood pressures were measured during the captopril treatment and on the 4 days after the last drug administration. Fluid intakes and urine outputs were measured throughout the experiment. In addition, the weight loss incurred during each heating period before blood pressure measurement (20 min at 32°C) was taken as body fluid loss and added to the urine output.

Experiment 3: influence of the route of administration on the effects of captopril on water intake and urine output

Rats were allowed 3 weeks to adapt to the metabolism cages before the experiments began. Thereafter daily measurements of water intake and urine output were made for 3 days before captopril, for 6 days during treatment with captopril, and for 3 days after the last dose of captopril. The drug was administered by two different routes.

Group 1 rats (five Brattleboro rats, six Long Evans rats) were given captopril by gavage (3 mg/ml in a volume of 10 ml/kg; daily dose 30 mg/kg).

Group 2 rats (five Brattleboro rats, six Long Evans rats) were given captopril by subcutaneous injection (30 mg/ml in a volume of 1 ml/kg; daily dose 30 mg/kg).

Experiment 4: voluntary intake of captopril

Six Brattleboro rats and six Long Evans rats were housed individually in standard laboratory cages equipped with two, valved drinking bottles, one on the right-hand side and one on the left-hand side of the cage. On each day the intake from each bottle was recorded and the position of the bottles was reversed. Measurements were made for 12 days with water present in both bottles and the values recorded on the last 6 days were used as the baseline. On the next 6 days, a captopril solution was present in one of the drinking bottles at the same concentration as that given to the Brattleboro rats (0.04 mg/ml) and Long Evans rats (0.46 mg/ml) in the previous experiments. The same bottle contained the captopril solution throughout the 6 days but the side of the cage on which it was presented alternated. On the last 3 days of the experiment measurements were made again with water present in both bottles.

Data analysis

In all experiments, mean values for each animal on the 3 days before captopril and on the last 3 days of the captopril administration have been calculated and any changes tested for statistical significance by Student’s paired \(t\)-test.

Results

Experiment 1: effects of captopril on fluid and electrolyte balance

Part A. (a) Intakes and outputs of fluid. Captopril caused significant increases in water intake and urine output in both groups of rats (Fig. 1). On the 3 days before captopril administration, the average daily water intake of the Brattleboro rats was 286 ± 2 ml and urine output was 267 ± 21 ml; on the last 3 days of captopril treatment water intake was 363 ± 21 ml (0.01 > \(P\) > 0.001; \(n = 6\)) and urine output was 343 ± 21 ml (0.01 > \(P\) > 0.001; \(n = 6\)). Faecal water output did not significantly change during the captopril treatment (Fig. 1); the difference between the intake and measured output remained constant (before captopril, 16 ± 1 ml; during captopril, 15 ± 2 ml).

In the Long Evans rats, on the 3 days before captopril administration, average water intake was 24 ± 2.9 ml and urine output was 13.9 ± 1.3 ml; on the last 3 days of the captopril treatment, water intake was 37.4 ± 1.5 ml (0.02 > \(P\) > 0.01; \(n = 6\)) and urine output was 22.1 ± 0.9 ml (0.01 > \(P\) > 0.001; \(n = 6\)). Faecal water losses did not change significantly (Fig. 1); the difference between intake and measured outputs increased slightly but not significantly.

When the drug was withdrawn, the intakes and outputs of fluid did not return to their original levels in either group (Fig. 1), presumably because at the start of the experiment the animals were not in a steady state.

(b) Intakes and outputs of electrolytes. Because there was a gradual increase in food (and hence electrolyte) intake throughout the course of the experiment (Fig. 1), only the differences between intakes and measured outputs (urinary + faecal) have been analysed; these are termed the ‘balances’. The daily intakes and outputs of sodium and potassium are shown in Fig. 1.

In both groups of rats, captopril caused the sodium balance to become negative, output being greater than intake; this was due to an effect on urinary sodium excretion (Fig. 1). In the Brattleboro rats, sodium balance changed from +0.61 ±
FIG. 1. Effects of captopril on mean (±SEM) daily intakes (●) and urinary outputs (○) of water, sodium and potassium by Long Evans rats (n = 6) and Brattleboro rats (n = 6). The experiment was performed after 7 days of individual housing. Captopril was administered in the drinking water on the days included between the arrows and the concentration was adjusted to give a dose of about 30 mg day⁻¹ kg⁻¹; the actual doses achieved varied between 24 and 43 mg day⁻¹ kg⁻¹. Captopril caused a significant increase in water intake, urine output and urinary sodium excretion in both groups of rats (see the text for details). When the drug administration was stopped, the variables did not return to the levels they were at before treatment began, presumably because the animals were not in a steady state.
Gazopril and thirst

0.27 mmol/day before captopril to −0.90 ± 0.45 mmol/day (0.05 > P > 0.02) on the last 3 days of drug administration. In the Long Evans rats, sodium balance was +0.26 ± 0.37 mmol/day before captopril, and was −0.46 ± 0.22 mmol/day at the end of the captopril treatment.

There were no significant changes in the potassium balances of either group that could be attributed to captopril (Fig. 1; Brattleboro rats

![Graphs showing effects of captopril on mean (±SEM) daily intakes (•) and urinary outputs (○) of water, sodium and potassium by Long Evans rats (n = 9) and Brattleboro rats (n = 7). The experiment was performed after 4 weeks of individual housing. Captopril was administered in the drinking water on the days included between the arrows (intake varied between 28 and 34 mg day⁻¹ kg⁻¹). Captopril increased water intake and urine output and reduced food (and hence electrolyte) intake in both groups of rats.](image)

FIG. 2. Effects of captopril on mean (±SEM) daily intakes (•) and urinary outputs (○) of water, sodium and potassium by Long Evans rats (n = 9) and Brattleboro rats (n = 7). The experiment was performed after 4 weeks of individual housing. Captopril was administered in the drinking water on the days included between the arrows (intake varied between 28 and 34 mg day⁻¹ kg⁻¹). Captopril increased water intake and urine output and reduced food (and hence electrolyte) intake in both groups of rats.
before captopril, +0.84 ± 0.34 mmol/day; during captopril, +0.13 ± 0.41 mmol/day; Long Evans rats before captopril, +0.49 ± 0.24 mmol/day; during captopril, +0.48 ± 0.37 mmol/day).

(c) Body weight. During the 6 days of captopril treatment, body weight gain in the Brattleboro rats was +11 ± 2 g and in the Long Evans rats was +26 ± 3 g.

Part B. (a) Intakes and outputs of fluid. Faecal outputs of water and electrolytes are not included in this part of the experiment since there were no significant effects of captopril on these variables in part A. Thus the term 'balance' refers to the difference between intake and urinary output.

Captopril had similar effects on water intake and urine output to those described above (part A: compare Fig. 1 and Fig. 2). In the Brattleboro rats, water intake increased from 220 ± 14 ml/day to 267 ± 19 ml/day (P < 0.001; n = 7) and urine output increased from 207 ± 13 ml/day to 249 ± 18 ml/day (P < 0.001; n = 7). The balance became slightly more positive during captopril treatment but the change was not significant (before captopril, +13 ± 1 ml/day; during captopril, +17 ± 2 ml/day).

In the Long Evans rats, water intake increased from 20.8 ± 0.9 ml/day to 26.3 ± 1.2 ml/day during captopril treatment (P < 0.001; n = 9) and urine output increased from 8.8 ± 0.5 ml/day to 16.1 ± 0.9 ml/day (P < 0.001, n = 9). The balance became significantly (0.01 > P > 0.001) less positive (before captopril, +11.9 ± 0.8 ml/day; during captopril, +10.2 ± 0.5 ml/day), i.e. output was closer to intake.

(b) Intakes and outputs of electrolytes. In both groups of animals there was a significant (0.05 > P > 0.02) reduction in food (and hence electrolyte) intake on the first day of captopril administration, and throughout the drug treatment food intakes remained slightly depressed (Fig. 2). The combination of reduced intake and some elevation in urinary sodium excretion (Fig. 2) resulted in a significant reduction in sodium balance in the Brattleboro rats (before captopril, +0.43 ± 0.06 mmol/day; during captopril, +0.08 ± 0.07 mmol/day; 0.01 > P > 0.001) and in the Long Evans rats (before captopril, +0.80 ± 0.07 mmol/day; during captopril, +0.55 ± 0.07 mmol/day; 0.01 > P > 0.001).

There were no significant changes in potassium balance in either the Brattleboro rats (before captopril, +0.16 ± 0.09 mmol/day; during captopril, +0.02 ± 0.07 mmol/day) or the Long Evans rats (before captopril, +0.69 ± 0.07 mmol/day; during captopril, +0.44 ± 0.09 mmol/day).

(c) Body weight. Before captopril, the mean body weight was 289 ± 17 g in the Brattleboro rats and 303 ± 5 g in the Long Evans rats. By the end of the period of captopril administration, neither group had significantly changed their weight (Brattleboro, 286 ± 16 g; Long Evans, 301 ± 6 g).

Experiment 2: effects of captopril on blood pressure and heart rate

The increases in fluid intake and urine output in response to captopril reported in experiment 1 (part B) were well reproduced in this experiment. In the Brattleboro rats, water intake increased from 233 ± 11 ml/day to 272 ± 15 ml/day (P < 0.001; n = 7) and urine output increased from 225 ± 11 ml/day to 263 ± 15 ml/day (P < 0.001). In the Long Evans rats, water intake increased from 15.9 ± 0.9 ml/day to 25.0 ± 1.8 ml/day (P < 0.001; n = 9), and urine output increased from 9.3 ± 0.4 ml/day to 16.9 ± 1.4 ml/day (P < 0.001). These changes in fluid intakes and outputs were accompanied by significant reductions in systolic blood pressures in both groups (Fig. 3). There was no difference between the systolic blood pressures of the two groups before captopril (Brattleboro, 130 ± 2 mmHg; Long Evans, 131 ± 2 mmHg). During the last 3 days of the captopril treatment, systolic blood pressure in the Brattleboro rats was 115 ± 1 mmHg (P < 0.001) and in the Long Evans it was 120 ± 2 mmHg (P < 0.001). Interestingly, the fall in blood pressure was not associated with any significant change in heart rate (Fig. 3).

![](https://example.com/fig3.png)
Experiment 3: influence of the route of administration on the effects of captopril on water intake and urine output

Neither subcutaneous nor intragastric administration of captopril significantly affected water intake or urine output in the two groups of rats.

Experiment 4: voluntary intake of captopril

When offered the choice of water or captopril to drink neither group of rats showed any preference for the captopril (Fig. 4). In the Brattleboro rats the average total fluid intake on the 3 days before captopril was given was 233 ± 12 ml/day, of which 128 ± 8 ml/day was taken from bottle A (the bottle in which the captopril solution was to be put). On the last 3 days of captopril being offered, the average total intake was 302 ± 1 ml/day ($P < 0.001$) of which 139 ± 6 ml/day was taken from bottle A (captopril intake 17 mg/kg). In the Long Evans rats, before captopril was offered the total fluid intake was 24 ± 1 ml/day, of which 13 ± 2 ml/day was taken from bottle A (in which the captopril was to be put). When captopril was given, the total intake was 25 ± 2 ml/day, of which 7 ± 3 ml/day was from bottle A (captopril intake 10 mg/kg).

Discussion

In the present study we consistently observed that when captopril was given to Brattleboro rats or to Long Evans rats in their drinking water, there was an increase in fluid intake and urine output. There was no effect on fluid turnover when the same dose of captopril was given as a bolus, either intragastrically or subcutaneously. One simple explanation for our findings would be that the increased fluid intake which occurred was due to a liking for the taste of the captopril solution. However, we demonstrated that this was not the case, since when the animals were given the choice to drink water or a captopril solution, they showed no preference for the captopril. Indeed, the Long Evans rats showed a tendency towards an aversion to the solution since their daily intake

![Graph](image.png)

**FIG. 4.** Voluntary intake of captopril by Long Evans rats ($n = 6$) and Brattleboro rats ($n = 6$). The total block of the histogram represents the daily fluid intake, which comprises intake from bottle A (hatched section) and intake from bottle B (open section). On the days included between the arrows, bottle A contained a captopril solution (Long Evans, 0.46 mg/ml; Brattleboro, 0.04 mg/ml). The position of the two bottles (left-hand side vs right-hand side of cage) was alternated each day, hence the variability in daily water intake from bottle A shown by the Long Evans rats illustrates a preference for drinking from the bottle on the right-hand side of the cage. The Brattleboro rats did not show this preference. Neither group showed a preference for the captopril solution.
of captopril solution was less than their daily intake of water when it was offered in the same bottle (Fig. 4).

When fluid intakes and fluid outputs both change, it is often difficult to determine which is cause and which effect. There are several changes that might occur during treatment with captopril which could secondarily promote drinking. One such change is a reduction in ANG II production and/or a potentiation of the kallikrein-kinin system [9–11], causing a natriuresis accompanied by a diuresis; drinking would then occur in response to volume depletion. In the present study, a natriuresis occurred only when captopril was given in the non-steady state (experiment 1, part A), whereas an increase in fluid turnover occurred in both parts of that experiment. Furthermore, the maximal increment in sodium excretion occurred later than the maximal change in water intake. For these reasons it seems unlikely that the polydipsia which occurred in response to captopril was due to renal salt loss. It is unclear why a natriuresis occurred in response to captopril only when the animals were not in a steady state, but it may have been due to changes in fluid and electrolyte balance with individual housing [16].

Another indirect mechanism through which captopril could promote drinking is by inhibiting vasopressin secretion [12], thereby causing a diuresis; again the drinking would be in response to volume depletion [17, 18]. This, however, cannot be the explanation for the present results since captopril increased fluid turnover in Brattleboro rats, which have no vasopressin.

A third possibility is that captopril lowers blood pressure by reducing peripheral vascular resistance and that drinking occurs in response to the hypotension. In the present study, captopril lowered blood pressure in both strains of rat: thus the increased drinking may have been a response to the fall in blood pressure. If this was so, it might be argued that the animals should have retained the ingested fluid and blood pressures should have returned to normal. However, the fluid that was being drunk was responsible for the fall in blood pressure. Furthermore, if captopril was also acting to inhibit vasopressin secretion in the Long Evans rats [12] then both they and the Brattleboro rats (with the congenital inability to synthesize vasopressin) may have been incapable of effectively retaining the ingested fluid.

A reduction in peripheral vascular resistance in response to captopril could be due to a reduction in circulating ANG II levels, to interference with the sympathetic nervous system [19] or to inhibition of kininase, thereby potentiating the kallikrein-kinin system and prostaglandin synthesis [20]. If the effect was due to a reduction in circulating ANG II levels it would be expected to be more profound under conditions where the renin-ANG II system was activated. We observed a similar reduction in blood pressure in the Long Evans rats and in the Brattleboro rats in response to captopril administration, but it is only the latter strain in which the renin-ANG II system is reported to be chronically stimulated [1–3]. This suggests either that plasma ANG II levels were elevated in the two strains under the conditions of our experiment or that the response which we observed was unrelated to the prevailing levels of ANG II.

Apart from these indirect modes of action, it has been suggested that there is a primary change in fluid intake in response to captopril administration [7, 21]. The evidence used to support the notion that the dipsogenic response is primary is that a diuresis does not occur when the animals are not allowed to increase their fluid intake [21] and that drinking occurs in response to captopril in rats with ureteric ligation [7]. However, blood pressures were not measured in either of those studies and it is possible therefore that the drinking was in response to hypotension.

It remains to explain why the same dose of captopril given as a bolus either intragastrically or subcutaneously did not affect fluid turnover in the present study. It has been suggested [7, 22] that a dipsogenic response to captopril occurs when only peripheral converting enzyme activity is inhibited; this causes a build-up of ANG I which enters the central nervous system, where it is converted to ANG II, which then stimulates drinking. Under conditions where central converting enzyme activity is also inhibited, this cannot occur [22]. Bolus administration of captopril by gavage (10 mg/kg) or subcutaneously (5 mg/kg) has been shown to inhibit drinking responses to central administration of renin or ANG I when given up to 2 h before the test, but the response was back to normal within 24 h [23]. Thus it is possible that, in the present work, bolus administration of captopril caused a transient inhibition of central and peripheral converting enzyme activity and hence did not stimulate drinking, whereas with administration of the same total dose of captopril in the drinking water throughout the day, the plasma levels were only sufficient to inhibit peripheral converting enzyme activity. Clearly more evidence is required to support this proposal.

There is no ready explanation for the difference between our results and those of Henderson et al. [4, 5]. We suggest that the dipsogenic response which we observed was due to the hypotension caused by captopril. It is likely that with hypo-
tension induced by peripheral converting enzyme inhibition, the increase in peripheral ANG I and its subsequent conversion into ANG II in the central nervous system (see above) elicits a greater drinking response than would be observed for the same degree of hypotension caused by some other disturbance.

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