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# INVOLVEMENT OF THE RENIN–ANGIOTENSIN SYSTEM IN CAPTOPRIL-INDUCED SODIUM APPETITE IN THE RAT

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## SUMMARY

1. The angiotensin converting enzyme inhibitor, captopril, given to rats in their drinking water (about 40 mg/day) for 6 days caused an increase in intake of hypertonic NaCl solution which began 1–2 days after the captopril was started and reached a plateau after 4–5 days.

2. Twice-daily subcutaneous injections of captopril (15 mg per injection) elicited a sodium appetite similar in pattern to that seen with oral administration.

3. The rats remained in sodium and fluid balance during oral captopril treatment and the haematocrit did not alter.

4. Captopril infused directly into the ventricles (12 $\mu$g/h), or captopril reaching the brain from the periphery across a leaky blood–brain barrier, suppressed the sodium appetite which normally follows oral captopril.

5. Continuous intravenous infusion of captopril at rates high enough to block angiotensin converting enzyme in the brain (25, 50 or 500 mg/day) did not cause sodium appetite. As soon as the rate was reduced to a low value (5 mg/day), NaCl intake increased.

6. In conclusion, moderate levels of circulating captopril which do not cross the blood–brain barrier in sufficient amounts to block cerebral angiotensin converting enzyme, result in an increase in circulating angiotensin I which stimulates sodium appetite when it is converted to angiotension II in the brain.

## INTRODUCTION

Within a week of adding the angiotensin converting enzyme inhibitor, captopril, to the diet rats increased their consumption of normally aversive NaCl solutions (0·25, 0·35 and 0·35 M-NaCl) when they were offered the choice between one of them and distilled water (Fregly, 1980). Since angiotensin injected into certain parts of the brain stimulates sodium appetite (Buggy & Fisher, 1974; Avrith & Fitzsimons, 1980; Bryant, Epstein, Fitzsimons & Fluharty, 1980), and since captopril-induced thirst depends mainly on activation of the renin–angiotensin system, it is possible that the stimulating effect of captopril on sodium appetite is also mediated by angiotensin.

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Lehr, Goldman & Casner (1973) found that water intake induced by various thirst stimuli was increased by another angiotensin converting enzyme inhibitor, teprotide. They suggested that drinking in response to thirst stimuli is enhanced by systemic teprotide because unconverted angiotensin I accumulates in the periphery and spills over into the brain where, in the absence of cerebral angiotensin converting enzyme blockade, it is converted to angiotensin II. This also appears to be the way in which captopril acts to increase water intake (Katovich, Barney, Fregly & McCaa, 1979; Barney, Katovich & Fregly, 1980; Elfont & Fitzsimons, 1983). Captopril increases renin secretion and angiotensin I formation by opening the negative feed-back loop at the point of conversion of angiotensin I to angiotensin II. The increased circulating angiotensin I stimulates water intake after its conversion to angiotensin II in the brain. In the present experiments we considered this as a possible explanation of the increased intake of hypertonic NaCl by animals treated with captopril. This is of interest because if it were true it would show that angiotensin formed in the circulation can stimulate sodium appetite, a hypothesis for which evidence has been lacking up to now.

We also examined two other possibilities. First, that captopril-induced sodium appetite is the result of excessive urinary sodium loss, since captopril is diuretic and natriuretic (McCaa, Hall & McCaa, 1978; Bengis, Coleman, Young & McCaa, 1978; Hall, Guyton, Smith & Coleman, 1979). Secondly, that the increased preference for sapid solution (saccharin and glucose as well as NaCl) observed by Fregly (1980) might have been a consequence of the taste of the drug in the food. The rats in Fregly's experiment ate significantly less of the diet containing captopril than they did a normal diet, so that they may have compensated for this by increasing their intake of another strongly tasting substance such as NaCl.

## METHODS

*Animals.* Male Sprague–Dawley and Wistar rats, weighing between 220 and 445 g at the beginning of the experiment, were housed individually in rooms with a 12 h light–dark cycle and constant temperature (Philadelphia) or a temperature which varied between 20 and 24 °C (Cambridge). The rats had access to tap water, hypertonic NaCl solution (3·0 %, Philadelphia; 2·7 %, Cambridge) and standard food pellets (Purina Rat Chow, Philadelphia; Dixon's Diet FFG(M), Cambridge) *ad libitum* except where otherwise specified. Solutions were presented in either glass burettes, graduated to 0·1 ml (Cambridge) or plastic graduated cylinders, graduated to 1 ml (Philadelphia), fitted with metal drinking spouts. Rats were tested in their home cages and each rat was used in one experiment only.

*Sodium appetite.* In the first experiment, following a control period of 1–3 days to establish base-line intakes of water and NaCl, sixteen rats were given captopril in their drinking water (1 mg/ml) for 4–6 days. Intakes were measured for 4 days more after stopping the captopril administration.

*Taste preference.* Four rats were given the choice of drinking either tap water or a 1 mg/ml solution of captopril in distilled water. The positions of the two fluids were alternated daily for 8 days.

*Fluid and electrolyte balance.* Fourteen rats in Philadelphia and eight rats in Cambridge were housed individually in metabolism cages for collection of urine and faeces. They were presented with tap water and either a 2·7 % (Cambridge) or 3·0 % (Philadelphia) NaCl solution in burettes or graduated cylinders, the spouts of which were recessed just outside the cage. Standard diets which contained 0·9 % NaCl (Purina) or 1·32 % NaCl (Dixon's) were available in powdered form from a hopper also recessed outside the cage. Food, water (with or without captopril) and the NaCl solution were available *ad libitum*. Body weight, and intake of water, NaCl and food were measured every 24 h. Urine and faeces were also collected. Base-line intakes and excretion were established over

4 days for each animal, after which the drinking water was replaced for 6 days with a 1 mg/ml solution of captopril in distilled water. Half the rats were deprived of their NaCl solution at the same time that the captopril was started. The other rats had uninterrupted access to NaCl. After the rats had received the captopril for 6 days, drinking water without captopril was returned. Measurements were continued for another 4 days.

Urinary sodium and potassium concentrations were measured by flame photometry. Faecal electrolytes were measured by flame photometry on simple aqueous extracts or on aqueous extracts made by treating the dried faeces with 100 volumes $H_2O_2$ at a low heat and dissolving the desiccated residue in distilled water.

*Haematocrit.* Blood samples were collected under ether anaesthesia by cardiac puncture from ten rats with access to water and 2·7 % NaCl, twelve rats with access to water containing captopril (1 mg/ml) and 2·7 % NaCl, and nine rats with access to water containing captopril only. A single haematocrit measurement was made on each rat, on days 1–5 of a particular treatment, after which the rat was destroyed.

*Intracerebroventricular infusion.* The procedure for intracerebroventricular (I.C.V.) infusion has been described elsewhere (Bryant *et al.* 1980). An intracranial guide cannula (Plastic Products Guide Cannula C313G) aimed at the anterior third ventricle and passing through the left lateral ventricle was implanted under equithesin anaesthesia in twenty-eight rats. Four rats underwent a sham cannulation operation in which a non-functional cannula was attached to the outside of the skull but no hole was drilled through the skull. All the rats were allowed at least 3 days to recover from surgery before the start of the experiment and a minimum of 6 days before the start of the infusion.

Infusions were made through an injector (Plastic Products Internal Cannula C313I) which had its tip cut flush with the end of the implanted guide cannula. The injector was connected by a 60 cm length of polyethylene tubing (PP20, Portex) enclosed in a stainless-steel spring through a water-tight swivel joint (Nicolaïdis, Rowland, Meile, Marfaing-Jallat & Pesez, 1974) to a 100 $\mu$l Hamilton microsyringe driven by a Sage infusion pump. The infusion system was first completely filled with 0·9 % NaCl. Then a 5 $\mu$l air bubble was aspirated into the injector before drawing up the solution to be infused in order to separate the latter from the 0·9 % NaCl in the syringe. The infusion system was flushed through with 0·9 % NaCl and the old infusion solution was replaced with a fresh solution once a day. After the infusion was started it was continued for 9 days without interruption except for 30–60 min each day for cleaning and reloading.

All I.C.V. infusions were at a rate of 4 $\mu$l/h. The infusion schedule was as follows: days 1–3, no infusion; days 4–6, I.C.V. infusion of 0·9 % NaCl (vehicle); days 7–12, I.C.V. infusion of vehicle or captopril (3 $\mu$g/$\mu$l, i.e. 12 $\mu$g/h).

The rats were divided into five groups, A, B, C, D and E. Group A consisted of sham-cannulated rats. The rats in groups B, C, D and E were cannulated. During the first 3 days of the experiment (no infusion) rats were screened for spontaneous NaCl intake. Four rats consumed 5 ml or more 2·7 % NaCl on two consecutive days and were excluded from the experiment. Three other animals were excluded because they became ill during the course of the infusion.

Penetration of the cannula into the cerebral ventricle was confirmed at the conclusion of the experiment by injecting 2 $\mu$l of concentrated Trypan or Evans Blue through the cannula and then perfusing the brain through the circulation with 10 % formalin immediately after injection of the dye. Good ventricular spread of the dye was observed in all rats.

For the first 3 days of the I.C.V. infusion (days 4–6) groups B, C and D received vehicle only. Group E rats had injectors inserted into their guide cannulae and they were connected to empty infusion systems that were not switched on. Group A rats were connected to non-functioning infusion systems. Starting on day 4 of the infusion the drinking water of groups A, C, D and E was replaced with 1 mg/ml solution of captopril in distilled water.

*Intravenous infusion.* Twenty-seven animals had intravenous catheters implanted into the jugular vein under ether or equithesin anaesthesia. The catheter was connected by a length of polyethylene tubing sheathed by a metal spring to an infusion pump. All intravenous infusions were at approximately 6·6 $\mu$l/min. The infusion ran continuously except for daily interruptions lasting no longer than 30 min to reload the systems with fresh solution. Reloading did not require disconnecting the infusion system from the animal. 3–6 days before the start of the infusion, water and 2·7 % NaCl intakes were measured and any rats with spontaneous sodium appetite (as defined in the previous section) were left out of the experiment. Animals which fell ill or whose catheters became non-functional during the infusion were also excluded. Six rats in all were left out.

All rats received intravenous infusion of 0·9% NaCl for the first 3–5 days of the experiment. Following the control infusion captopril was infused for 6 days at various concentrations in order to achieve rates of administration of approximately 5 mg/day in four rats, 25 mg/day in seven rats, 50 mg/day in seven rats and 500 mg/day in three rats. The infusion was then terminated or the rate changed as follows. Of the seven rats infused at 25 mg/day, the infusion was terminated in one rat, in two rats it was lowered to 5 mg/day for 3 days, in three rats the 25 mg/day infusion was continued for another 3 days, and in one rat it was continued for another 5 days and then lowered to 5 mg/day for 2 days. Of the seven rats infused at 50 mg/day, the infusion was terminated in one rat, in four rats it was lowered to 5 mg/day for 2–5 days, and in two rats the 50 mg/day infusion was continued for another 3 days. In two of the 500 mg/day rats the infusion rate was lowered to 5 mg/day for 4 days, and in one rat the infusion was terminated. In two of the 5 mg/day rats the rate was raised to 10 mg/day for 2 days and then to 20 mg/day for 2 days more, in one rat the rate was raised to 25 mg/day for 3 days, and in one rat the rate was lowered to 1 mg/day.

*Subcutaneous captopril.* Eight rats with continuous access to water and 2·7% NaCl were given twice-daily subcutaneous injections of 0·9% NaCl at 8·30 and 19·30 h for 3 days followed by twice-daily subcutaneous injections of captopril (1·5 ml per injection of 10 mg/ml solution of captopril dissolved in 0·9% NaCl) according to the same schedule.

*Statistics.* Student's paired *t* test was used to compare observations made on the day before captopril administration with those made on each subsequent day except where stated otherwise in the text.

## RESULTS

### *Captopril-induced sodium appetite*

Rats given captopril in their drinking water (1 mg/ml, i.e. about 40 mg/day per rat) increased their consumption of an ordinarily aversive NaCl solution (2·7 or 3·0%; Fig. 1). Cambridge and Philadelphia rats generally behaved similarly and their results have therefore been combined. The increase in NaCl intake reached statistical significance by day 2 of captopril administration. Two animals showed a substantial increase in NaCl intake after only 1 day. The initial increase in NaCl consumption occurred without an increase in water consumption. The small rise in water intake towards the end of captopril administration was probably secondary to the large NaCl intakes at this time (Fig. 1). The NaCl intake of these animals remained significantly elevated above pre-captopril levels for the 4 days after stopping the drug during which intake was measured.

### *Possible effects of the aversive taste of captopril*

When rats were allowed to choose between water and water containing captopril (1 mg/ml) they clearly preferred unadulterated water. On the first day of such a choice four rats drank $30{\cdot}5 \pm 10{\cdot}3$ ml of water and $5{\cdot}5 \pm 4{\cdot}5$ ml water containing captopril. By day 7 these values had become $40{\cdot}7 \pm 3{\cdot}1$ ml and $0{\cdot}7 \pm 0{\cdot}5$ ml respectively. Since in the experiment of Fig. 1, captopril was administered in the rats' drinking water it was necessary to establish whether the taste of captopril was causing the rats to switch to NaCl. Two groups of rats were tested. One group of rats had access to NaCl throughout the period during which captopril was in the drinking water. In the other group of rats, NaCl was only made available after stopping the captopril administration.

The eleven rats with continuous access to NaCl showed an increase in NaCl consumption similar to that found in the first experiment except that the increase reached significance after only 1 day of captopril treatment (Fig. 2). The eleven rats

that were deprived of NaCl during captopril treatment consumed large amounts of the NaCl solution when it was restored in the first 24 h after stopping captopril. In this series, the seven Philadelphia rats drank 5·9 ± 1·1 ml 3 % NaCl in the first 15 min immediately following the return of the NaCl solution and a cumulative total of 28·7 ± 6·5 ml in the succeeding 24 h with one animal taking as much as 67 ml. The

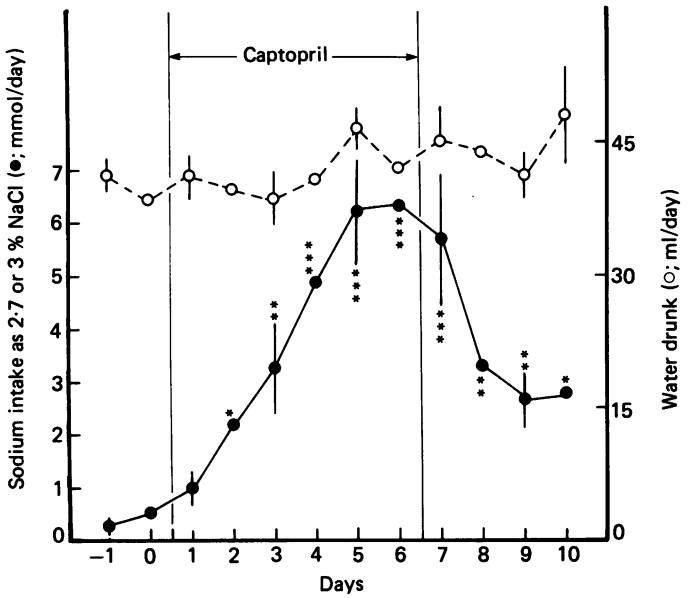


Fig. 1. Mean 24 h intake (± S.E. of mean) of sodium (mmol) taken as 2·7 or 3·0 % NaCl (filled symbols, left-hand ordinate), and water (ml; open symbols, right-hand ordinate), of rats with access to both fluids and receiving captopril in the drinking water (1 mg/ml) on days 1–6 inclusive. Sodium intake excludes sodium in the food. $n = 16$, up to and including day 4, $n = 11$ thereafter. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$, compared with day 0 by paired $t$ test.

four Cambridge rats drank less, ingesting a mean of 6·2 ± 3·9 ml 2·7 % NaCl in 24 h. In the series which had continuous access to NaCl during captopril, the seven Philadelphia rats and the four Cambridge rats showed similar NaCl intakes.

After stopping captopril the rats which had not been allowed access to NaCl during captopril treatment chose to drink large amounts of NaCl even though they were now also given access to water which no longer contained captopril. The increased NaCl intake produced by adulteration of the rat's drinking water with captopril, cannot therefore have been the result of the animal trying to reduce its intake of one aversive solution (water containing captopril) by supplementing it with intake of another aversive solution (hypertonic NaCl).

The rats of both series tended to drink more water despite the fact that it was adulterated with captopril. The increase in water intake reached statistical significance on days 4 and 5 of captopril treatment in the group of rats that were not allowed access to NaCl ( = total fluid intake, days 1–6, for this group, Fig. 2), but did not reach significance in the other group of animals (see Fig. 1).

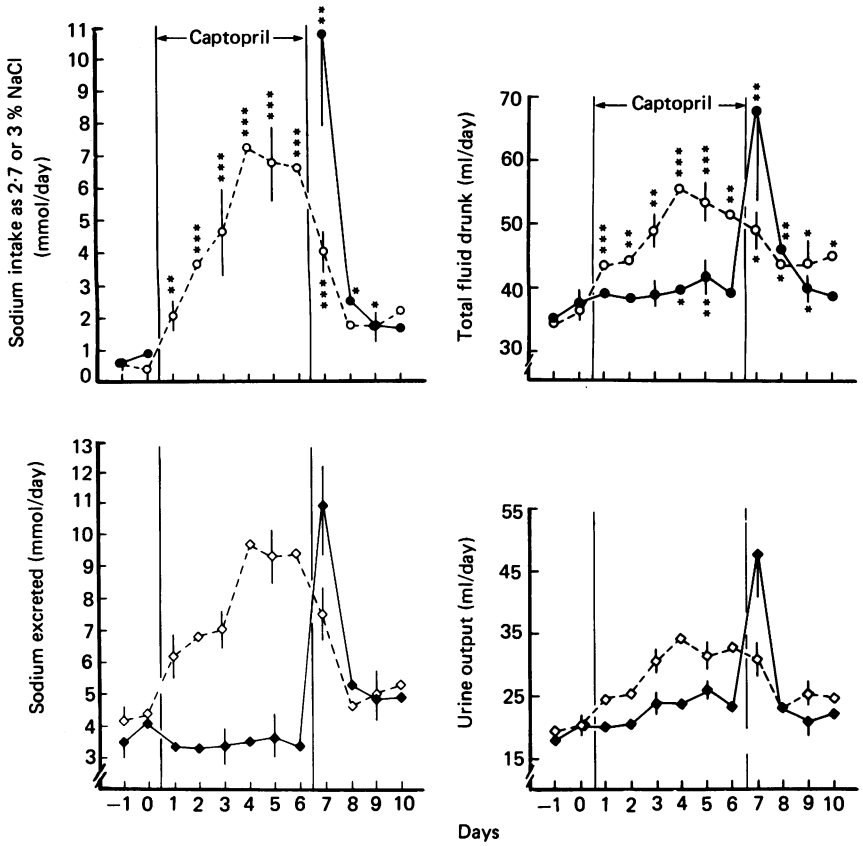


Fig. 2. Above: 24 h intakes of sodium (mmol) taken as 2·7 or 3% NaCl (left), and total fluid (NaCl+water) (ml; right), of eleven rats with continuous access to NaCl (open symbols), and eleven rats with restricted access (filled symbols). Sodium intake excludes sodium in the food. Drinking water, containing captopril (1 mg/ml) on days 1–6 inclusive, was available throughout the experiment. Below: 24 h urinary sodium (mmol; left) and urinary volume (ml; right) in the same two groups of rats as above (symbols as before). Mean values ±S.E. of mean. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$, compared with day 0 by paired $t$ test.

*Fluid and sodium balance*

Sodium excretion and urine flow increased in parallel with the increase in NaCl and water consumption in rats with continuous access to NaCl (Fig. 2). Rats deprived of NaCl also showed some increase in urine flow, but sodium excretion tended to decrease. The change in sodium balance on each day of captopril administration compared with the balance on the day before the start of captopril was calculated for each rat as follows: (sodium intake minus sodium output) on the day in question minus (sodium intake minus sodium output) on day 0. Change in water 'balance' (excluding insensible water loss) was similarly calculated. In neither group of rats did the changes in non-cumulative 24 h sodium or water balance reach statistical significance during captopril administration (Fig. 3). After stopping captopril, the rats which had had continuous access to NaCl during captopril went briefly into

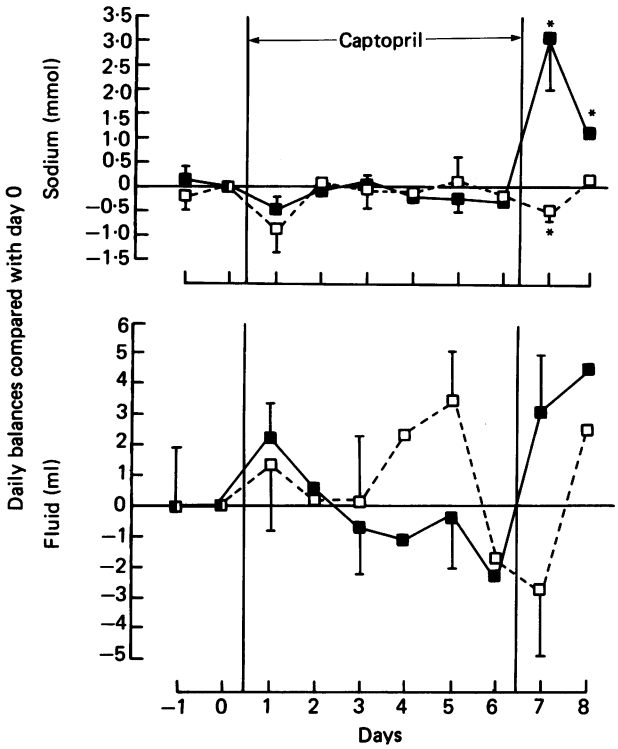


Fig. 3. Non-cumulative changes in 24 h sodium balance (upper) and 24 h fluid 'balance' (lower) compared with day 0 of the same two groups of rats as in Fig. 2. Continuous access to NaCl (open symbols); restricted access (filled symbols). Mean values with one S.E. of mean. Only after captopril was stopped did any of the changes reach statistical significance. *$P < 0{\cdot}05$ compared with day 0 by paired $t$ test.

negative sodium balance as they reduced their intake of NaCl, whereas the rats that had been deprived of NaCl during captopril went into positive sodium balance since they now had access to and drank large amounts of NaCl.

Captopril did not alter the daily food consumption of either group compared with day 0 (paired $t$ tests) or by comparison of the pooled intakes before and during captopril treatment. Pooled intakes of eleven rats deprived of NaCl were $25{\cdot}6 \pm 0{\cdot}5$ g/day for the 4 days before captopril and $25{\cdot}6 \pm 0{\cdot}4$ g/day for the 6 days during captopril; pooled intakes of eleven rats with access to NaCl were $27{\cdot}6 \pm 0{\cdot}5$ g/day for the 4 days before captopril and $28{\cdot}3 \pm 0{\cdot}4$ g/day for the 6 days during captopril. Animals in both groups continued to gain weight throughout the experiment, but the gain in weight on day 6 was less than the gain on day 1. The eleven rats deprived of NaCl gained $5{\cdot}1 \pm 1{\cdot}1$ g on day 1 and $0{\cdot}8 \pm 1{\cdot}3$ g on day 6, a significant difference ($P < 0{\cdot}05$); the eleven rats with access to NaCl gained $7{\cdot}2 \pm 1{\cdot}5$ g on day 1 and $2{\cdot}1 \pm 0{\cdot}9$ g on day 6, also a significant difference ($P < 0{\cdot}05$).

The ratio of urinary sodium concentration to potassium concentration in seven rats which were not allowed access to hypertonic NaCl solution and whose only electrolyte intake was in the food and therefore almost constant, did not change significantly over a 6 day period of captopril administration compared with the pre-captopril value

on day 0 by paired $t$ test. This suggests that aldosterone secretion was not increased by captopril. It is even less likely that there was any increase in aldosterone secretion in the rats that were allowed to drink the increasing amounts of hypertonic NaCl resulting from treatment with captopril.

Since hypovolaemia is a reliable cause of sodium appetite and since the fluid and electrolyte balance experiment only gives a measure of over-all changes in hydration the haematocrit was used to estimate changes in blood volume. A two-way analysis of variance, where treatment (2·7 % NaCl and water; 2·7 % NaCl and water containing captopril; water containing captopril) and the duration of treatment (days elapsed before sampling) were the independent variables and the haematocrit was the dependent variable, showed that the haematocrit did not vary significantly with treatment ($F(2,16) = 0·09$) or with the duration of treatment ($F(4,16) = 0·16$) and that the interaction of treatment and duration of treatment did not significantly affect the haematocrit ($F(8,16) = 0·54$). The mean haematocrit was $40·3 \pm 0·6$ % for the ten control rats presented with water and 2·7 % NaCl, $40·6 \pm 0·9$ % for the twelve rats presented with water containing captopril and 2·7 % NaCl, and $39·9 \pm 1·1$ % for the nine rats presented with water containing captopril only, the results being pooled across days in each case. Therefore, it is unlikely that hypovolaemia is the mechanism through which captopril produces sodium appetite.

*Intracerebroventricular infusions*

In order to determine whether intracerebral conversion of angiotensin I to angiotensin II is necessary for captopril-induced sodium appetite, captopril (12 μg/h) or vehicle (0·9 % NaCl) was given by I.C.V. infusion to rats also taking captopril (1 mg/ml) in their drinking water, as described under Methods.

I.C.V. infusion of captopril by itself (group B) had no effect on drinking behaviour, but it prevented the sodium appetite generated by oral captopril (group C), compared with oral captopril given alone to sham-cannulated rats connected to non-functioning infusion systems (group A) (Fig. 4). But this suppression of captopril-induced sodium appetite was not unique to I.C.V. infusion of captopril. I.C.V. infusion of vehicle (group D) or the presence of a cannula system in the rat's brain but with no I.C.V. infusion (group E) was sufficient to suppress the sodium appetite induced by oral captopril. Since third ventricular cannulation at the same stereotaxic coordinates does not interfere with the sodium appetite produced by a number of other procedures (e.g. I.C.V. angiotensin II infusion, adrenalectomy, subcutaneous deoxycorticosterone acetate (DOCA) and because brain cannulation is known to cause a break-down of the blood–brain barrier (Davson, 1967; Wood, 1980), it is possible that captopril entered the brain through a locally deficient blood–brain barrier in sufficient quantities to block conversion of angiotensin I to angiotensin II in the brain as well as peripherally. This would account for oral captopril's inability to elicit sodium appetite in cannulated rats. That sodium appetite is suppressed if captopril gains access to the brain is compatible with the idea that conversion of angiotensin I to angiotensin II by the brain's own angiotensin converting enzyme is important in captopril-induced sodium appetite.

*Intravenous infusions*

In high doses peripherally administered captopril crosses the blood–brain barrier and inhibits thirst (Evered, Robinson & Richardson, 1980); low doses on the other hand stimulate thirst (Katovich *et al.* 1979; Barney *et al.* 1980; Elfont & Fitzsimons, 1983). The I.C.V. infusion experiments just described suggest that sodium appetite

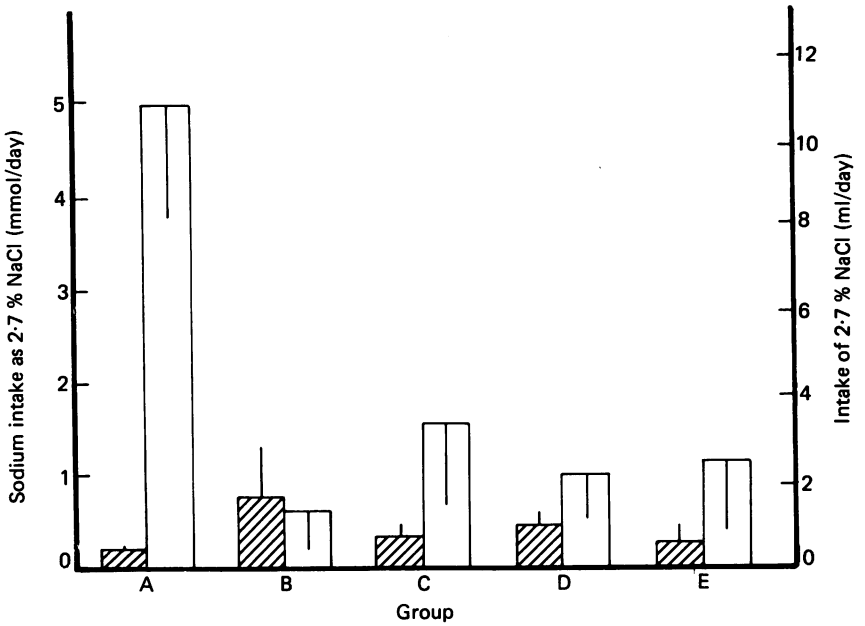


Fig. 4. Mean 24 h intake (with one S.E. of mean) of sodium (mmol; left-hand ordinate), taken as 2·7 % NaCl (ml; right-hand ordinate), of rats on day 0, before captopril (hatched columns), and on day 6 of oral captopril (open columns). Sodium intake excludes sodium in the food. Water was also available to drink. Group A, sham cannulation and oral captopril ($n = 4$). Group B, I.C.V. captopril (but no oral captopril) ($n = 4$). Group C, I.C.V. and oral captopril ($n = 5$). Group D, I.C.V. 0·9 % NaCl and oral captopril ($n = 7$). Group E, cannulated (but no I.C.V. infusion) and oral captopril ($n = 5$). Oral captopril produced a significant ($P < 0{\cdot}01$) increase in intake in group A (open compared with hatched columns). None of the differences in intake in the other groups was significant.

is also antagonized by captopril crossing into the brain. Therefore by varying the systemic dose and hence the amount of captopril reaching the brain it should be possible to enhance or suppress the sodium appetite induced by the blood-borne drug in a similar way to the effects of variation in dose on thirst. Intravenous administration was chosen because captopril could be given at high and controlled rates which would have been impossible with oral administration. Furthermore, giving captopril by a parenteral route would help to establish if there were anything peculiar about the oral route in eliciting sodium appetite.

Continuous intravenous infusion of captopril at constant rates of 5, 25, 50 or 500 mg/day for 6–11 days failed to evoke a consistent sodium appetite (Fig. 5), though there were transient increases on two occasions (3·3 ml on day 6 of an infusion of 25 mg/day, and 8·5 ml on day 5 of an infusion of 500 mg/day). In each of these

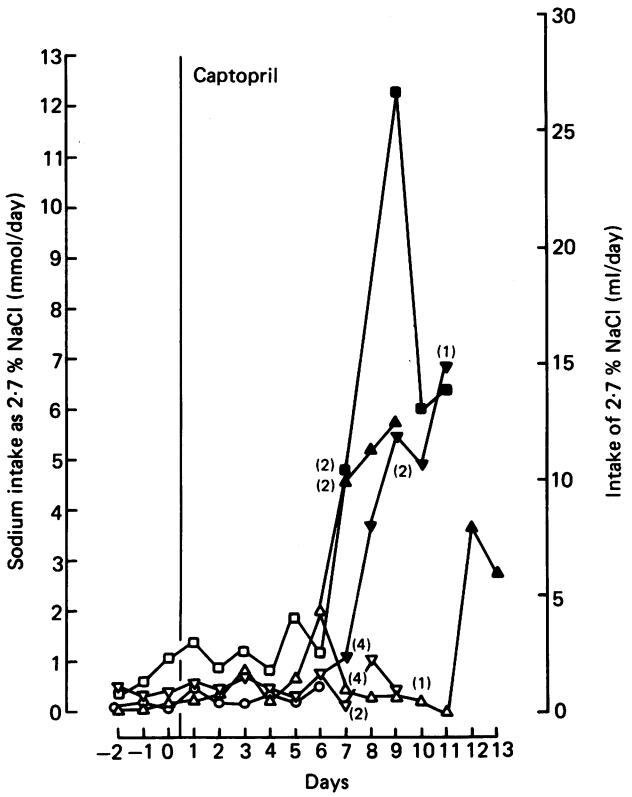


Fig. 5. Mean 24 h intakes of sodium (mmol; left-hand ordinate), taken as 2·7 % NaCl (ml; right-hand ordinate), of rats infused intravenously with captopril at 5 mg/day (○, $n = 4$), 25 mg/day (△, $n = 7$), 50 mg/day (▽, $n = 7$) and 500 mg/day (□, $n = 3$) for at least 6 days. After this, rats which had received captopril at the three higher rates had the rate of infusion lowered to 5 mg/day (filled symbols). Sodium intake excludes sodium in the food. All rats had drinking water available as well as 2·7 % NaCl. After day 6, as animals had their infusions terminated or changed to the lower rate, the numbers of animals remaining in the original groups or in the new lower-rate subgroups are indicated in parentheses next to the group or subgroup on the day in question.

cases the infusion system had leaked on the previous night. But when the rate of intravenous infusion of captopril was reduced from high rates (25–500 mg/day) to a low rate (5 mg/day), increases in 2·7 % NaCl intake occurred reliably (Fig. 5). Abrupt termination of the captopril infusion (four rats at 25 mg/day, two rats at 50 mg/day), as opposed to reducing the rate, did not elicit sodium appetite (not shown in Fig. 5). Increasing the rate of captopril infusion from 5 mg/day to 10–25 mg/day or lowering the rate from 5 mg/day to 1 mg/day (also not shown in Fig. 5) also failed to increase NaCl consumption.

*Subcutaneous injections*

Because rats drink mainly at night, giving captopril in the drinking water results in the circulating levels of the drug waxing and waning over the 24 h. Since a decrease in the rate of infusion was necessary to elicit sodium appetite with intravenous

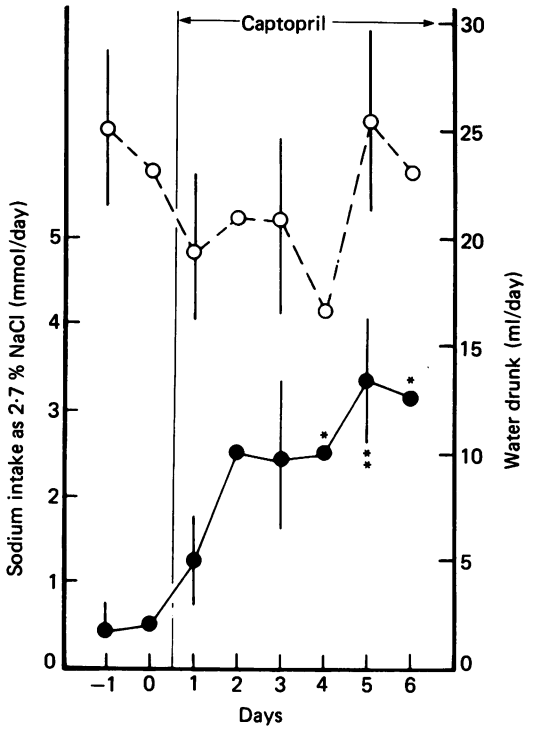


Fig. 6. Mean 24 h intakes (±S.E. of mean) of sodium (mmol), taken as 2·7 % NaCl (filled symbols, left-hand ordinate), and water (ml, open symbols, right-hand ordinate) of eight rats injected twice daily with subcutaneous captopril (15 mg per injection). Sodium intake excludes sodium in the food. $*P < 0{\cdot}05$, $**P < 0{\cdot}01$ compared with day 0.

infusion of captopril it seemed possible that fluctuation in the amounts of captopril in the blood could account for the effectiveness of oral administration in eliciting sodium appetite. This was tested by giving twice-daily subcutaneous injections of captopril in order to produce periodically fluctuating captopril levels. As shown in Fig. 6, these injections produced a pattern of increasing intake of NaCl which is similar to the pattern which developed during oral administration.

## DISCUSSION

The present finding that captopril given orally caused the rat to increase its sodium appetite over a period of several days supports the finding of Fregly (1980), but not that of Dean & Ingham (1978) who found that the drug reduced NaCl intake in DOCA-treated rats offered 1 % NaCl only to drink. Twice-daily subcutaneous injection of captopril or reducing the rate of a continuous intravenous infusion, as will be discussed later, also resulted in increased sodium appetite. Our demonstration that captopril at a concentration of 1 mg/ml in the drinking water has an aversive taste may explain why the rats in Fregly's experiment, which were given captopril in their food (0·7–1·05 mg/g), ate significantly less than the controls and therefore differed in weight from them at the end of the experiment, whereas our rats ate

normally. The rats' decreased consumption of the food containing captopril could have accounted for their increased ingestion of sweet solutions when these were made available in the experiment of Fregly. Our experiments also support Fregly's findings that chronic administration of captopril increased NaCl consumption without also increasing water consumption when both fluids were available, but did increase water consumption when it was the only fluid present.

Captopril-induced sodium appetite resembles the sodium appetite produced by intravenous infusion of angiotensin II (Findlay & Epstein, 1980) by causing increased intake of NaCl without first increasing water intake. Captopril-induced sodium appetite also resembles angiotensin-induced appetite (intravenous and I.C.V.) by the fact that NaCl ingestion occurred mainly at night, and by the NaCl intake sometimes remaining elevated for days after cessation of the drug (Bryant *et al.* 1980; Findlay & Epstein, 1980; Avrith & Fitzsimons, 1983).

Captopril has numerous effects, many of which reflect the widespread actions of the renin–angiotensin system in the body, though there are non-angiotensin effects of captopril as well. First, the unpleasant taste of captopril cannot account for appearance of the sodium appetite because rats that had been deprived of access to NaCl solution while receiving captopril in their drinking water consumed large quantities of NaCl after withdrawal of the drug and the return of unadulterated water and NaCl solution. Also, they drank the water containing captopril in normal or greater than normal amounts when it was the only fluid available. The fact that parenteral administration of captopril also elicited sodium appetite shows that the unpleasant taste of the drinking water was unimportant in evoking sodium appetite.

Secondly, the diuresis and natriuresis produced by circulating captopril (Bengis *et al.* 1978; McCaa *et al.* 1978; Hall *et al.* 1979) do not appear to be the primary causes of captopril-induced sodium appetite. The balance experiment shows that the rats deprived of hypertonic NaCl during administration of captopril as well as those with continuous access to NaCl showed no significant changes in fluid and sodium balance. It is possible that these changes would have become statistically significant had the captopril administration been continued for longer than 6 days. However, any tendency towards negative fluid balance that may occur after prolonged treatment with captopril seems an insufficient explanation to account for the appearance of a sodium appetite after only 1 day of captopril administration. Captopril treatment did not cause excessive loss of potassium in the urine which would have indicated a decrease in intracellular volume, nor was there any rise in haematocrit which would have indicated a decrease in extracellular fluid volume. The urinary sodium concentration fell and the amount of sodium excreted remained unchanged or fell slightly in the rats deprived of NaCl during captopril treatment. Although the changes in non-cumulative 24 h sodium balance in rats deprived of NaCl or with continuous access to NaCl never reached statistical significance, the possibility that sodium loss is involved in captopril-induced sodium appetite deserves further consideration.

If the NaCl ingestion of the rats with continuous access to NaCl were in fact secondary to sodium loss this might not be detected in a 24 h balance experiment because an overshoot in NaCl intake would continually offset transient sodium deficits, and in this way obscure any possible direct role for sodium loss in the

appetite. Since there could be no excessive NaCl intake in the rats deprived of NaCl, we would expect them to show sodium losses initially roughly similar in amount to the sodium intakes seen in the rats with continuous access to NaCl, or, at least to show sodium deficits increasing over the course of captopril treatment in similar proportion to the increase in NaCl intake of the rats with continuous access. No such pattern of sodium loss was found. Further evidence against the hypothesis that the captopril-induced increase in NaCl ingestion is simply an attempt by the rat to correct a sodium deficit is the fact that on any of the 6 days of captopril administration the NaCl intake of the rats with continuous access did not correlate significantly with the sodium balance on that day or on the preceding day. Secondly, the NaCl intake of the NaCl-deprived rats on the day that NaCl was returned to them did not correlate significantly either with the daily sodium balance on each of the 6 days of captopril treatment or with the 6 day cumulative sodium balance.

To put the degree of captopril-induced sodium loss into perspective; at the time the increase in sodium appetite had reached significance in the rats with continuous access to NaCl, the net sodium loss of the NaCl-deprived rats was below the minimum amount of sodium which has to be removed by peritoneal dialysis to arouse sodium appetite (Tang & Falk, 1979), and it was no greater than the imbalance incurred by rats deprived of food for 24 h (Jalowiec & Stricker, 1970), or deprived of food for 24 h following I.C.V. carbachol (Avrith & Fitzsimons, 1983), procedures that do not evoke sodium appetite.

Although the captopril-induced sodium loss was small and unlikely to have caused arousal of a sodium appetite by itself, the possibility cannot be discounted that sodium imbalance, especially on day 1, could have played some role, perhaps a permissive one, in the development of the appetite. In this respect also, captopril-induced sodium appetite resembles angiotensin-induced sodium appetite. It has been shown that centrally administered angiotensin II causes natriuresis in the goat (Andersson, Dallman & Olsson, 1969) and the rat (Severs, Daniels-Severs, Summy-Long & Radio, 1971), and that though central infusion of angiotensin II can initiate sodium appetite in rats in positive sodium balance (Avrith & Fitzsimons, 1980; Bryant *et al.* 1980), it also causes natriuresis. After the initial appetite, in some circumstances it appears that the continuing intake of hypertonic NaCl may depend on a developing negative sodium balance (Fluharty & Manaker, 1983). Even when this occurs, however, it is not clear whether angiotensin II, released endogenously owing to the sodium deficit, is contributing to the appetite.

Thirdly, the intravenous captopril experiments show that the sodium appetite could not have been the consequence of a direct action by captopril on the kidney or circulation. In these experiments high rates of infusion of captopril, which should have had natriuretic and hypotensive effects at least as great as those produced by the orally administered drug, failed to elicit a sodium appetite. Furthermore, the presence of a cannula in the brain prevented the sodium appetite usually seen with oral captopril even though the direct effects of the drug on the kidney and circulation should not have been affected by this.

It is unlikely that aldosterone, another known stimulus to increased sodium appetite in the rat (Wolf & Handal, 1966; Fregly & Waters, 1966), was responsible for the increase in sodium appetite caused by captopril. All the published evidence

indicates that under a variety of conditions captopril either has no effect on or inhibits aldosterone secretion (e.g. McCaa, McCaa, Bengis & Guyton, 1979; MacGregor, Markandu, Roulston, Jones & Morton, 1981). In the present experiments there were no changes in urinary sodium to potassium concentration ratios in the NaCl-deprived rats suggesting that circulating aldosterone did not increase during captopril treatment.

Angiotensin converting enzyme is also the bradykinin inactivating enzyme (Erdös, 1977). However, none of the actions of bradykinin on the central nervous system (Clark, 1979) or the periphery can account for the findings described here. Bradykinin causes diuresis and natriuresis (Ward & Margolius, 1979), yet diuresis and natriuresis did not seem to be the primary cause of captopril-induced sodium appetite. If elevated circulating bradykinin were responsible for the increased sodium appetite, then the continuous intravenous infusion of captopril should have been a powerful stimulus to sodium appetite, yet this was not the case.

The hypothesis which best fits the present results is that captopril induces sodium appetite as a result of its effects on the renin–angiotensin system. Blocking peripheral conversion of angiotensin I to angiotensin II releases the kidney from the inhibitory feed-back of angiotensin II on renin secretion (Davis & Freeman, 1976) and this results in increased circulating concentrations of renin and angiotensin I. Angiotensin I gains access to the brain into which captopril has not penetrated in sufficient amounts to block cerebral converting enzyme activity completely and it is there converted to angiotensin II by this cerebral converting enzyme activity. The centrally formed angiotensin II acts directly on the brain to stimulate sodium appetite. Intracranial administration of angiotensin II has been shown to be a reliable way of inducing sodium appetite (Buggy & Fisher, 1974; Avrith & Fitzsimons, 1980; Bryant *et al.* 1980). When captopril is injected directly into the brain, or when it gains access to the brain either because circulating levels are high or because of deficiencies in the blood–brain barrier, central as well as peripheral conversion of angiotensin I to angiotensin II is blocked and consequently no sodium appetite is induced. This view of the action of captopril on sodium appetite is consistent with analogous observations of its effects on thirst and cerebral converting enzyme activity. First, low to moderate doses of captopril cause increased water intake or potentiate intake of water in response to various angiotensin-dependent dipsogenic stimuli whereas high doses are antidipsogenic (Katovich *et al.* 1979; Barney *et al.* 1980; Elfont & Fitzsimons, 1983). Secondly, high doses of captopril injected peripherally inhibit drinking in response to centrally injected angiotensin I but low doses do not (Evered *et al.* 1980). Thirdly, oral captopril has been shown to reduce converting enzyme activity in the brain of spontaneously hypertensive rats (Cohen & Kurz, 1982).

The penetration of captopril into particular regions of the brain across the blood–brain barrier could account for the failure of high rates of continuous intravenous infusion to elicit sodium appetite. One interpretation of these results is that there is a narrow range of circulating blood captopril levels that if reached and maintained for a certain length of time would produce sodium appetite, but if exceeded would not (Fig. 7). According to this hypothesis infusions of 5 mg/day or less give levels of captopril that are too low to cause enough renin secretion for circulating angiotensin I to reach the critical level to penetrate the brain, whereas

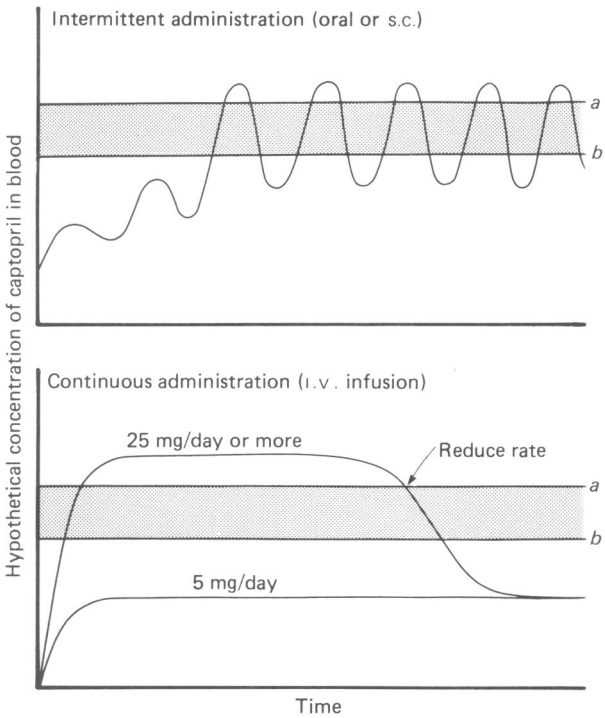


Fig. 7. The hypothetical patterns of blood concentrations of captopril which result from repeated subcutaneous injection or spontaneous oral intake (above), or from intravenous infusion (below) at a low rate, or at a high rate (25 mg/day or more) which is then reduced to a low rate. The zone above line *a* represents concentrations of captopril high enough to enter the brain and completely block both central and peripheral angiotensin converting enzyme. The zone below line *a* represents concentrations at which angiotensin converting enzyme blockade is effective only in the periphery. The zone below line *b* represents incomplete blockade of peripheral converting enzyme so that the amount of angiotensin I formed in the plasma is insufficient to enter the brain in appreciable amounts. The zone between lines *a* and *b* is therefore the critical range of captopril concentrations within which circulating angiotensin I is high enough to enter the brain and there be converted to angiotensin II and stimulate sodium appetite.

the higher rates of infusion of captopril block cerebral as well as peripheral converting enzyme activity so that the angiotensin I that does reach the brain is not converted to angiotensin II. Changing the intravenous infusion of captopril from a high to a low rate would have allowed the circulating levels of the drug to decrease gradually so that with the recovery of cerebral converting enzyme activity, the circulating angiotension I levels maintained by the continuing peripheral converting enzyme blockade would still have been high enough to enter the brain and produce increased sodium appetite after conversion to angiotensin II. Similarly, when captopril is given orally or by subcutaneous injection the blood levels of captopril would have oscillated daily and would have passed repeatedly into and out of the critical range over which peripheral converting enzyme blockade is complete but cerebral is not (Fig. 7).

The unexpected finding that I.C.V. cannulation suppressed the sodium intake produced by oral captopril is consistent with our hypothesis that central conversion

of angiotensin I to angiotensin II is responsible for sodium appetite. If captopril were able to gain access to the brain's endogenous converting enzyme by crossing a blood–brain barrier made locally deficient by the trauma and inflammation associated with the implantation and manipulation of an indwelling cannula, then little or no angiotensin II could be formed in the brain despite increased levels of circulating angiotensin I and no sodium appetite would be produced. Partial inhibition of thirst induced by low systemic doses of captopril has also been produced by preoptic cannulae in the rat (Elfont & Fitzsimons, 1983).

In conclusion, levels of circulating captopril which block peripheral conversion of angiotensin I to angiotensin II, but which do not cross the blood–brain barrier in sufficient amounts to block cerebral angiotensin converting enzyme activity, cause increases in circulating angiotensin I which when converted to angiotensin II in the brain stimulate sodium appetite. When cerebral as well as peripheral conversion is prevented by high doses of captopril sodium appetite is not aroused by captopril.

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