J. Physiol. (1980), **301**, *pp.* 365–382
With 6 text-figures
Printed in Great Britain

AROUSAL OF A SPECIFIC AND PERSISTENT SODIUM APPETITE IN THE RAT WITH CONTINUOUS INTRA-CEREBROVENTRICULAR INFUSION OF ANGIOTENSIN II

By R. W. BRYANT, A. N. EPSTEIN, J. T. FITZSIMONS and S. J. FLUHARTY

From the Institute of Neurological Sciences and Department of Biology, University of Pennsylvania, U.S.A. and the Physiological Laboratory, University of Cambridge, Downing Street, Cambridge CB2 3EG

(*Received 25 July 1979*)

SUMMARY

1. Prolonged exposure of the brain of the normal Na-replete rat to angiotensin II produced a marked and persistent Na appetite. In a first series of experiments, short-term, repeated systemic injections of isoprenaline or renin (both of which raise circulating angiotensin levels), and repeated intracranial injections of angiotensin II evoked increased ingestion of 2·7% NaCl. In the second series of experiments, continuous infusions of angiotensin II directly into the brain evoked extremely large intakes of 3% NaCl.

2. In addition to large intakes of hypertonic NaCl some rats drank daily volumes of water that exceeded their body weight.

3. Not only did the animals drink large volumes of 3% NaCl during continuous angiotensin II infusion, but after termination of the infusion they continued to ingest NaCl at a rate comparable to that of the adrenalectomized rat. In most of the animals the persistent NaCl intake diminished over several days, but other animals continued to drink NaCl for as long as their intake was measured (up to 7 months).

4. The response to continuous infusion of angiotensin II was dose-dependent. Both water and 3% NaCl intake increased over a dose range of 6 ng h^{-1} to 6000 ng h^{-1}. The persistence of the sodium appetite was also dose-dependent across the same range of doses.

5. Angiotensin-induced salt appetite is specific for Na. Animals did not drink 0·5 M-NH_4Cl and only occasionally drank minimal amounts of 0·5 M-KCl during continuous infusion.

6. The large water turnover was not responsible for the Na appetite. Rats given access to 3% NaCl only during infusion of angiotensin drank copiously. Animals that were not infused but were given saccharine-flavoured water in order to increase their water intakes did not drink 3% NaCl offered at the same time even though fluid intake was high. Rats that did not receive intracranial infusions but were infused intragastrically with volumes of water equal to or exceeding the amounts that were drunk during angiotensin infusion did not drink the 3% NaCl but did drink some water.

0022-3751/80/7880-0409 $07.50

7. Records of the drinking by rats infused with angiotensin show that firstly the onset of drinking after the start of angiotensin infusion varied from animal to animal, secondly, NaCl drinking was not temporally linked to water intake, although this was observed occasionally, and thirdly, most of the drinking occurred during the night although angiotensin was infused continuously throughout the nychthemeron.

8. Therefore, increases in angiotensin levels, probably with other factors such as increased levels of aldosterone or ACTH, result in Na appetite. The hormonal changes may alter the animals' preception of salt making it more acceptable. By means that are not yet understood the increased acceptability of salt persists after the termination of angiotensin infusion.

INTRODUCTION

The appetite for Na that is induced by disorders of Na metabolism is a classical phenomenon of neuropsychology. It was first described by Richter (1936) in the adrenalectomized rat and is induced, we know now, by a variety of challenges (Denton & Sabine, 1961; Falk, 1961; Fregly & Waters, 1966; Stricker, 1973; Wolf, 1964), some of which occur naturally (adrenal insufficiency, Na deprivation) others of which are inventions of the laboratory (I.P. dialysis, parotid fistulation, S.C. trauma with formalin, treatment with frusemide and other diuretics), all of which have in common a reduction of plasma Na. The ingestion of large amounts of Na even when offered at strong and normally avoided concentrations, by animals subjected to these challenges is an ideal example of self-regulatory behaviour.

But the appetite does not appear to be a simple expression of Na homoeostasis. It can be produced by pharmacological doses of the mineralocorticoids (aldosterone and desoxycorticosterone) which maintain animals in positive Na balance (Fregly & Waters, 1966; Wolf, 1964; Wolf & Handel, 1966), and it is not controlled by manipulations of plasma Na (Beilharz, Denton & Sabine, 1962; Falk, 1961; Nachman & Valentino, 1966). Decreases in the concentration of plasma Na do not appear to be the direct cause of the appetite.

The fact that angiotensin is an important intermediary in Na conservation by the kidney makes it a candidate for a role in the arousal of the appetite. Renin is released and angiotensin is subsequently produced in the plasma whenever Na is deficient, and it is primarily angiotensin, not hyponatraemia, that causes the secretion of aldosterone from the adrenal cortex which, in turn, promotes the reabsorption of Na by the renal tubule (Davis, 1971). This is one of the better established roles for angiotensin and it is tempting to assume that the hormone may also be the intermediary in the arousal of the Na appetite. This is suggested by the observation (Fitzsimons, 1964) that caval ligation, a known releaser of renin, evokes a delayed appetite for 1·8% NaCl, and was discussed by Fisher & Buggy (1975) who noted a tendency for increased intakes of isotonic saline solutions in rats receiving pulse injection of large doses of angiotensin into the cerebral ventricles. Intraventricular infusions of angiotensin have been shown to produce impressive intakes of 2·7% NaCl (Buggy & Fisher, 1974; Fisher & Buggy, 1975), but the intakes did not occur with high reliability when 0·9% saline solutions were used (Radio, Summy-Long, Daniels-Severs & Severs, 1972), and infusions lasting 8 h (Fisher & Buggy, 1975) were the longest reported. And, lastly, Chiaraviglio (1976) has restored salt drinking to the anephric rat with pulse injections of systemic renin or intracranial angiotensin.

Since sodium appetite accompanies Na deficiency it is associated with prolonged elevation of plasma angiotensin. If the hormone arouses sodium appetite as is suggested by the work of Chiaraviglio (1976), repeated intracranial injections of angiotensin should be effective in inducing an excess intake of hypertonic NaCl solutions, and it should be most effective when infused into the brain continuously for several days. The experiments described here were undertaken in pursuit of this idea. They show that angiotensin causes a specific and persistent appetite for Na.

METHODS

Animals. Male, Sprague–Dawley or Hotzman rats (280–487 g at the beginning of the experiment) were individually housed, some in temperature controlled rooms (Philadelphia experiments), with a light–dark cycle of 12 h on 12 h off. All animals were freely supplied with Purina rat pellets. Tap water and 2·7 % (Cambridge experiments) or 3·0 % NaCl (Philadelphia experiments) were usually available except when otherwise noted.

Cannulae assembly. The animals in Cambridge were equipped with intracranial cannulae as described previously (Epstein, Fitzsimons & Rolls, 1970). For the Philadelphia experiments cannula guide shafts (23-gauge), injection cannulae (30-gauge), and obturator dust-cap assemblies (Plastic Products, Roanoke, Virginia, U.S.A.) were prepared such that the tip of the cannula or the obturator when inserted into the guide was flush with its tip. A small hole was drilled in the centre of the screw-on dust cap just wide enough to admit the injection cannula. PE-50 tubing (approximately 1·2 m in length) was fitted to the injection cannula. A 25 cm length of extension spring was passed over the PE-50 tubing, one end of which was fixed to the modified dust cap–injection assembly with dental acrylic. The loose end of the PE-50 tubing was then passed through a modified plastic swivel (Epstein & Teitelbaum, 1962) and attached to a 50 μl Hamilton micro-syringe held in a Harvard syringe pump. The rats were free to move about in their cages with the spring protecting the PE tubing from damage. The rate of infusion in all experiments was 1 μl h^{-1}.

Surgery. A single cannula guide shaft was implanted in each animal using a Kopf small-animal stereotaxic instrument (Model 1260) under Chloropent (Fort Dodge Laboratories, Iowa) or equithesin anaesthesia (0·3 ml 100 g^{-1} body weight I.P.). In Philadelphia the tip of the guide shaft was directed towards the most anterior ventral portion of the third ventricle. In Cambridge the cannulae opened either into the preoptic area or the lateral ventricle. Following surgery, all animals were allowed at least 5 days post-operative recovery before experiments were begun.

Preparation of solutions. Angiotensin II (Ile^5-angiotensin II, Schwarz–Mann and Beckman) (A II) was dissolved in sterile 0·9 % NaCl and divided into aliquots of 0·2–0·3 ml which were immediately frozen at − 23 °C.

All salt solutions (NaCl, KCl, NH_4Cl) and sodium saccharine solutions were made using reagent grade salts dissolved in distilled water. Carbachol (carbamylcholine chloride), isoprenaline (Isuprel, Winthrop), and renin (ICN Pharmaceuticals, Cleveland) were prepared fresh in isotonic saline before use.

Histology. At the conclusion of the Philadelphia experiments rats were perfused with 10 % formalin; the brain was removed and placed in formalin for at least 5 days before being blocked. Once embedded in celloidin 40 μm sections were taken through the cannula track, stained with cresyl violet, and then mounted.

Single intracranial and systemic injection experiments. Four groups of animals were studied. Group 1 (n = 10) received either A II (100 ng) or carbachol (300 ng) by intracranial injection. Group 2 (n = 5) received S.C. injection of isoprenaline (100 μg kg^{-1}), a known releaser of endogenous renin (Peskar, Meyer, Tauchmann & Hertting, 1970; Reid, Schreier & Early, 1972). Group 3 (n = 5) received exogenous renin (10 u, I.P.). And Group 4 (n = 5) received 2 M-NaCl or 2 M-sucrose I.P., in sufficient amounts to raise the animals' total osmotic pressure by about 10 %. The animals were removed from their individual home cages, held in the hand for all injections, and then returned to their cages where 2·7 % NaCl and water were available from graduated burettes. At all other times they had access to water only and food pellets. Water and NaCl intakes were measured to the nearest 0·1 ml at the end of the hour after the intracranial injections, and after 60 and 180 min following the systemic injections. Typically, 2 or 3 days of

rest followed each day of injection. The results yielded by animals with preoptic cannulae were indistinguishable from those from animals with cannulae opening directly into the lateral ventricle and they are treated as a single group. The data are given by injection–day in serial order. Omitted injection–days are redundant of the trends shown. For each injection day the mean (± S.E. of mean) water and NaCl intakes and the water to NaCl ratios are shown.

Schedule for intracranial infusion of A II. After the postoperative recovery period the rats were placed on the following schedule.

The animals were first given access to water and 3·0 % NaCl for 4 days but no intraventricular infusions were made (no-infusion condition). At approximately the same time each day (12.00 h) they were weighed and their food, water and 3·0 % NaCl intakes recorded. The positions of the water and NaCl were alternated daily. On the fifth day the continuous intracranial infusion of A II was started and allowed to continue for 4 days. On the ninth day, the infusion was terminated and the animals were returned to the no-infusion condition for 4 more days.

The volume of intraventricular infusion was 1 μl h^{-1}. The dose of A II was varied by infusing different concentrations of A II giving rates in the range 0 (0·9 % NaCl), 6, 60, 600, or 6000 ng h^{-1} A II. There were at least three rats in each group and no rat received more than one dose of A II, so that 'between animal' responsiveness was being compared. This was necessary because of the persistence of salt drinking after termination of A II infusion (Fig. 2).

Specificity of the phenomenon. Eight male, albino Holtzman rats were given a choice of water and 0·5 M-KCl or water and 0·5 M-NH_4Cl to drink before, during, and after intraventricular infusion of A II. The higher dose of 6 μg h^{-1} was used to maximize the possibility of unmasking nonspecific appetites that may be generated by this procedure.

Intragastric infusion. Three large (400–450 g) rats were equipped with nasopharyngeal–gastric tubes under Chloropent (3 ml/100 g, I.P.) anaesthesia. This is a length of PE-50 tubing that is passed through the nares into the stomach and is fixed to the skull by connexion to a piece of bent metal tubing (Epstein & Teitelbaum, 1962). After they had recovered spontaneous food and water intake, the animals were given continuous access to water and 3·0 % NaCl, and to Purina pellets. Three days later direct intragastric infusions of tap-water were begun and continued for 3–5 days by continuous peristaltic pump at rates from 5·3 to 26·9 ml h^{-1}. Water and 3·0 % NaCl intakes were measured daily. No intracranial infusions were made.

Single-solution experiment. Five rats in this experiment had access to both tap-water and 3·0 % NaCl for two days prior to intracranial A II infusion at 6 μg h^{-1}. During the infusion, three rats (CPR-17, 19, 20) had access to 3·0 % NaCl as the sole available fluid and two rats (CPR-22, 23) had access only to water. After 4 days of A II infusion the two rats which had had access only to water were given only 3·0 % NaCl to drink. Rats with access to NaCl only were given access to water without 3·0 % NaCl present for 2 h during the day (12.00–14.00 h). This was necessary since rats cannot survive solely on 3·0 % NaCl solution. Purina rat chow was always available to all animals.

Drinkometer analysis of pattern of onset. After the usual post-operative recovery period five rats were given access to tap water and 3·0 % NaCl for at least 2 days before intraventricular A II infusion at 6 μg h^{-1} began. At the beginning of the A II infusion every twelfth lick for both water and 3·0 % NaCl was recorded for at least 2 days using drinkometers (Grason–Stadler).

RESULTS

Repeated pulse injections

Animals were given short-term access to 2·7 % NaCl and water immediately after repeated intracranial injections of A II or carbachol or systemic injections of isoprenaline or hypertonic NaCl in the expectation that those injections which increased the amounts of angiotensin in the brain would result in an increase in NaCl intake.

As can be seen in Table 1, intakes of 2·7 % NaCl increased with repeated injections of A II. Note first that by the fifth injection of A II the intake of 2·7 % NaCl had increased seven-fold ($P < 0·01$, compared with first injection) reaching 3·72 ml with all ten animals drinking NaCl (range = 0·1–9·8 ml). The H_2O to NaCl ratio fell from

12·7:1 to 4·3:1 despite the accompanying progressive increase in water intake. The results of the 6th trial show that the increase in 2·7 % NaCl intake was not a mere consequence of increased water intake. Injection of carbachol elicited large water intakes in all animals, but only four animals drank 2·7 % NaCl and only in small amounts. Moreover, repeated injection of carbachol (injection days 10–15) did not increase intake of 2·7 % NaCl.

TABLE 1. Water and 2·7 % NaCl intakes during the hour immediately after repeated intracranial injections of 10 ng angiotensin II (A II) or 300 ng carbachol (CARB). Mean values for ten rats in ml ± S.E. of mean

Injection day	1	5	6	7	10	12	15
Treatment	A II	A II	CARB	A II	CARB	CARB	CARB
H_2O	7·51 ± 0·96	16·15** ± 1·19	9·64 ± 1·54	12·46 ± 1·83	10·09 ± 1·87	8·54 ± 1·15	7·10 ± 1·45
2·7 % NaCl	0·59 ± 0·16	3·72** ± 0·62	0·43 ± 0·26	4·04* ± 1·19	0·34 ± 0·15	1·04 ± 0·39	0·38 ± 0·13
H_2O:NaCl ratio	12·7:1	4·3:1	22·4:1	3·1:1	29·7:1	9:1	18·7:1

* $P < 0·05$, ** $P < 0·01$, paired t test, compared with injection day 1.

TABLE 2. Water and 2·7 % NaCl intakes at 1 and 3 h after repeated S.C. injections of 100 μg kg^{-1} isoprenaline. Mean values for five rats in ml ± S.E. of mean

Injection day	1		4		8	
	1 h	3 h	1 h	3 h	1 h	3 h
H_2O	8·72 ± 0·38	8·78 ± 0·34	7·72 ± 0·98	9·0 ± 0·82	7·2 ± 0·82	9·6 ± 2·45
2·7 % NaCl	0·38 ± 0·27	0·84 ± 0·48	1·34 ± 0·54	2·92* ± 0·33	2·14 ± 0·85	3·8 ± 1·06*
H_2O:NaCl ratio	—	10·5:1	—	3·1:1	—	2·5:1

* $P < 0·50$, paired t test, compared with injection day 1.

TABLE 3. Water and 2·7 % NaCl intakes at 1 and 3 h after repeated I.P. injection of 10 Goldblatt u renin. Mean values for five rats in ml ± S.E. of mean

Injection day	1		4	
	1 h	3 h	1 h	3 h
H_2O	2·10 ± 0·08	3·98 ± 1·79	5·50 ± 1·05	8·00* ± 1·17
2·7 % NaCl	0·40 ± 0·29	1·02 ± 0·90	0·90 ± 0·49	2·62** ± 1·02
H_2O:NaCl ratio	—	2·7:1	—	3:1

* $P < 0·05$, ** $P < 0·01$, paired t test, compared with injection day 1.

Tables 2 and 3 show that procedures which raise plasma angiotensin, either by releasing endogenous renin pharmacologically with isoprenaline or by injection of exogenous renin, also caused a significant increase in intake of 2·7 % NaCl by the fourth trial compared with the first trial. The intake of 2·7 % NaCl during the first trial, however, was not significantly different from that of unstimulated rats. Table 4 shows that no such increase in intake of 2·7 % NaCl was produced by repeated injection of hypertonic NaCl or hypertonic sucrose, which cause cellular dehydration,

TABLE 4. Water and 2·7 % NaCl intakes at 1 and 3 h after repeated cellular dehydration produced by (*a*) 2 M-NaCl 5·83 ml kg^{-1}; (*b*) 2 M-sucrose, 11·66 ml kg^{-1}. Mean values for five rats in ml ± S.E. of mean. Repeated injections caused no significant change in intake of water or 2·7 % NaCl

(*a*)

Injection day	1		4	
	1 h	3 h	1 h	3 h
H_2O	9·25 ± 1·36	12·52 ± 0·64	11·18 ± 1·19	16·74 ± 1·86
2·7 % NaCl	0·14 ± 0·12	0·14 ± 0·12	0	0·6 ± 0·40
H_2O:NaCl ratio	—	89·4:1	—	27·9:1

(*b*)

Injection day	1		4	
	1 h	3 h	1 h	3 h
H_2O	4·58 ± 1·26	11·98 ± 1·94	3·82 ± 2·13	12·1 ± 2·49
2·7 % NaCl	0	0	0	0·52 ± 0·25
H_2O:NaCl ratio	—	—	—	23·3:1

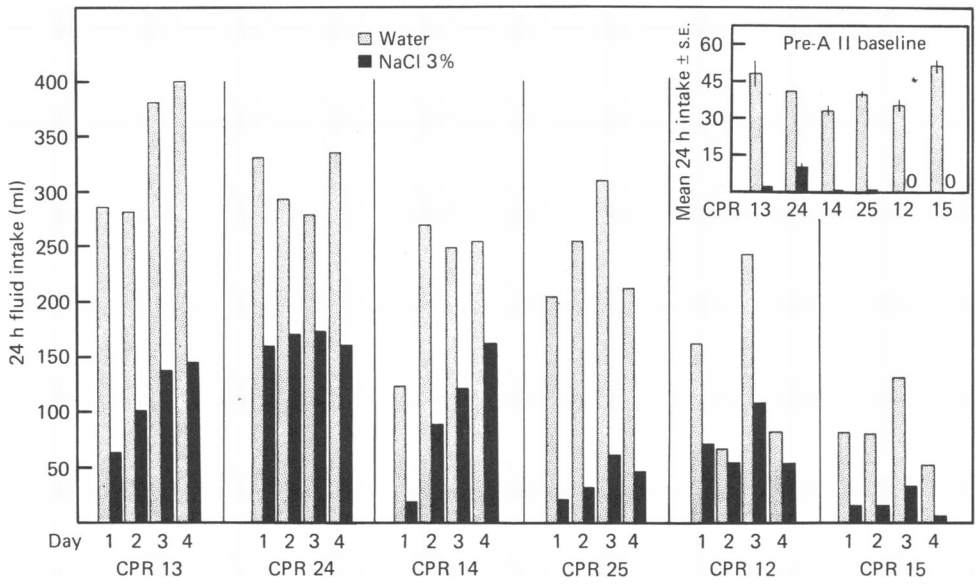

Fig. 1. Individual daily intakes for 4 days during A II infusion (6 $\mu g\ h^{-1}$) into the anterior ventral third ventricle. Inset (upper right) shows mean 24 h water (stippled bars) and 3 % NaCl (filled bars) intakes for the 4 days preceeding A II infusion in rats CPR 13, 24, 14, 25, 12, 15 (note the difference in inset and principle scales).

despite very similar increases in water intake. Even by the eighth injection of 2 M-NaCl only 2 out of the five animals drank NaCl, taking 1·1 and 1·9 ml each of 2·7 % NaCl although all five rats drank substantial amounts of water.

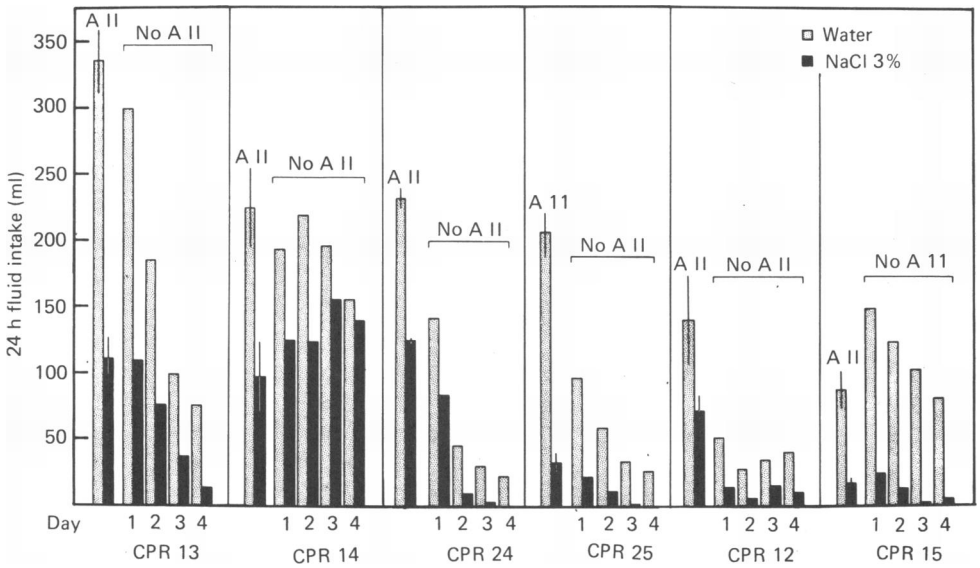

Fig. 2. Comparison of 24 h water and 3 % NaCl intakes during 4 days of A II infusion (first pair of bars show mean intakes ± S.E. of mean for each rat) and for the 4 days after termination of A II infusion (designated 'No A II'). Same animals as in Fig. 1.

Chronic infusion of A II, basic phenomenon

Continuous intraventricular infusion of A II into six rats evoked marked increases in intake of water and 3·0 % NaCl. The inset in the upper right of Fig. 1 shows that the pre-A II base line (no-infusion condition) intake of 3·0 % NaCl was virtually zero in all animals except CPR-24, and water intakes were in the usual range (30–50 ml 24 h^{-1}). The remainder of Fig. 1 shows that the water and salt intakes increased enormously for each rat on all 4 days of A II infusion. Rat CPR-13's 3·0 % NaCl intake increased from approximately 1 ml/24 h to 62 ml for the first 24 h of A II infusion and reached 145 ml after the fourth day of A II infusion. Even the poorest responder, CPR-15, increased its 3·0 % NaCl intake from 0 ml/24 h to a maximum of 30 ml/24 h. CPR-24 drank a maximum of 172 ml/24 h of salt and 334 ml/24 h of water on the third infusion day.

It should be emphasized that all rats, not only in this experiment, but in all experiments described below, showed excessive 3·0 % NaCl intakes as well as water intake in response to continuous infusion of A II. There have been no exceptions to this in 41 cases.

Fig. 2 shows that the excessive 3·0 % NaCl intake induced by intraventricular A II persisted after termination of the infusions. Representative results for six rats for only the first 4 days after termination of A II infusion are shown. We observed CPR-12, 13, 14 and one other animal for 7 months after termination of the A II

infusion, during which time they continued to consume from 10 to 125 ml 3% NaCl per day. In all the experiments reported in this paper no animal has stopped drinking 3·0% NaCl immediately after termination of A II infusions. Of the six rats shown in Figs. 1 and 2, 4 slowly reduced their 3·0% NaCl intake to 0·4 ml by the third post-infusion day, and one rat continued to consume substantial volumes of 3·0% NaCl during the usual post-infusion observation period of 4 days. Furthermore, Fig. 4 (right panel, Post-A II Infusion) shows that the persistence of 3·0% NaCl intake also occurred when A II was infused at lower rates (60 and 600 ng h^{-1}). The three rats infused at 600 ng h^{-1} were observed for 6 days after termination of the infusion during which time their 3·0% NaCl intakes were never lower than six times in excess of the pre-infusion base line levels. In addition, six other rats which were infused at 6 μg h^{-1} were observed for periods ranging from 21 to 65 days during which time their intakes of 3·0% NaCl were never less than from 5 to 66 times their pre-infusion base line intakes. The intake of 3% NaCl of these six animals for the pre-infusion base line period was $0·9 \pm 0·1$ ml (mean $\pm$ S.E. of mean), and for the post-infusion period it was $27·3 \pm 2·6$ ml. Therefore, although intakes of 3% NaCl do not remain at the enormously high levels that are reached during continuous infusion, intakes of previously infused rats do continue at elevated levels long after the treatment has been terminated.

Fig. 3 shows representative coronal sections from the brain of CPR-20 (lower half of Fig. 3) and CPR-22 (upper half of Fig. 3). The tips of the guide cannulae open into the anterior third ventricle and nearby parenchyma. They were positioned to assure rapid intraventricular distribution of infused solutions. These sections are representative of cannula placement in all of the Philadelphia experiments. No gross neural pathology was observed in any animals in this series. Representative locations of the preoptic and lateral ventricle cannulae of the Cambridge experiments have been described previously (Epstein *et al.* 1970).

Dose–response relationship

After establishing that chronic infusion of A II at high rates evokes massive intake of 3·0% NaCl and water a dose–response study was undertaken on a further seventeen rats. The left panel of Fig. 4 shows that there is no ingestion of 3·0% NaCl during infusion of 0·9% saline, but at 6 ng h^{-1} of A II a small (mean = 7·8 ml) but statistically insignificant amount of 3·0% NaCl was consumed. At rates of 60, 600, and 6000 ng h^{-1}, marked ingestion of 3·0% NaCl occurred (means: 58·1, 39·0, 100·9 ml, respectively). The 6 ng h^{-1} rate appears to be very close to threshold; three out of five rats on at least 2 days during A II infusion did not drink any 3·0% NaCl although excessive water intake was observed. It is interesting to note that there was no significant difference between the intakes evoked by the 60 and 600 ng h^{-1} rates. The 6000 ng h^{-1} rate was significantly more potent than all other doses ($P < 0·01$). The right panel of Fig. 4 presents results showing that the persistence of the 3·0% NaCl intake after termination of A II infusion was also dose dependent.

Fig. 5 shows that the dose–response curve for water intake is similar to the salt intake curve (Fig. 4). The lowest rate of infusion (6 ng h^{-1}) evoked a proportionately larger increase in water intake than in 3·0% NaCl intake although the increase was not statistically significant. There were no significant differences between the intakes of

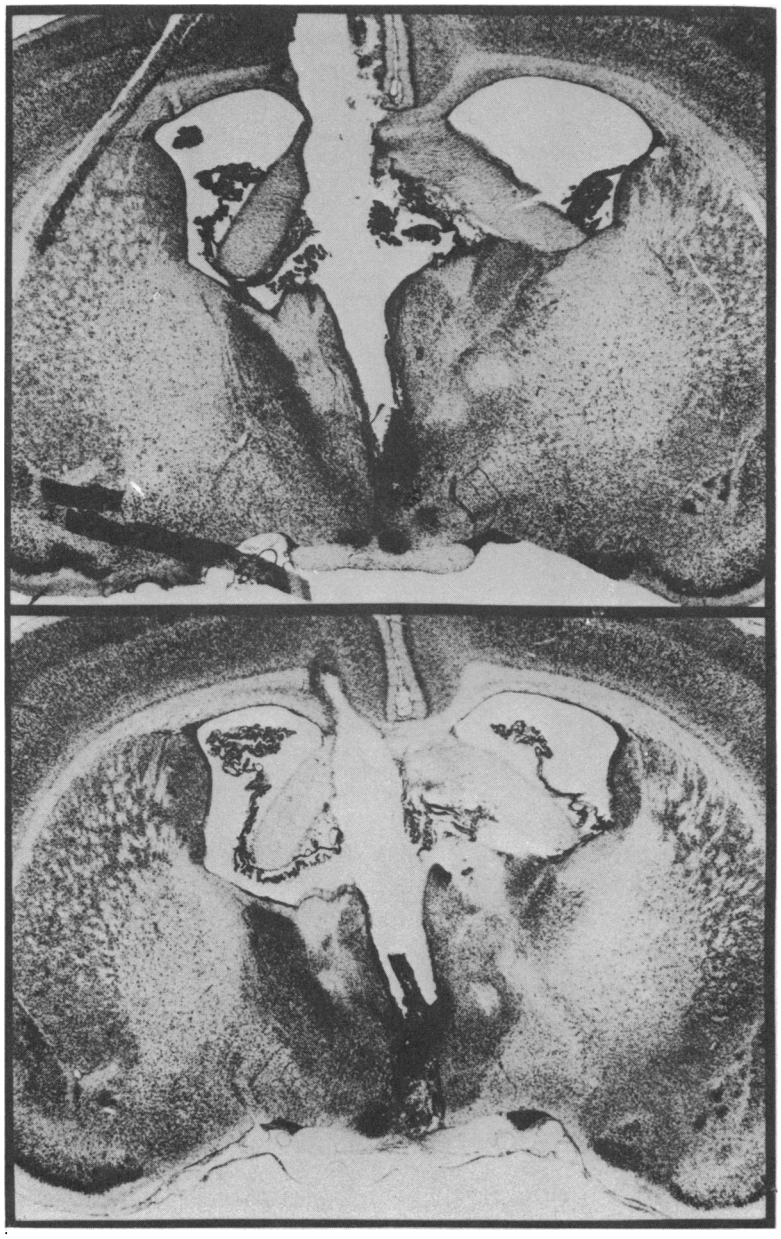

Fig. 3. Cresyl violet stained coronal sections showing cannula locations representative of all placements of the Philadelphia series (upper half CPR-22, lower half CPR-20). Cannula tips open into the anterior third ventricle at the level of the foramina of Monro.

water in response to the 6000 ng h^{-1} rate and to the 60 and 600 ng h^{-1} rates of A II although the highest rate produced the largest intakes of water (334, 380 and 400 ml/24 h). There were, however, significant differences between 60, 600 and 6000 ng rates and 0·9 % saline control ($P < 0{\cdot}01$).

It should be noted that rats infused with A II usually ate and gained weight

normally throughout the experiment. They showed no signs of upset during or after the infusions.

Specificity of phenomenon

An important question is whether the 'salt' appetite evoked by intraventricular infusion of A II is specific for the sodium ion. This experiment sought to answer that question by substituting other salts for NaCl. Table 5 (lower half) shows that very

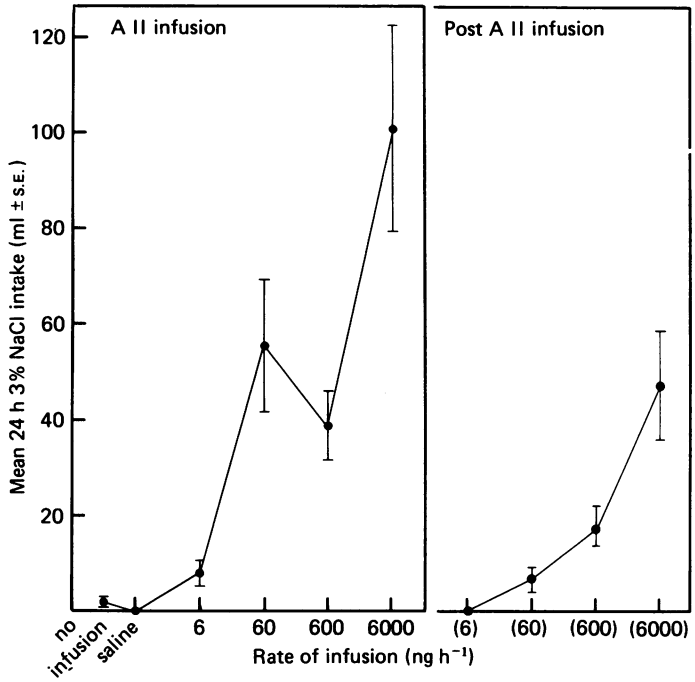

Fig. 4. Dose–response curves showing mean 24 h 3% NaCl intake during no infusion, 0·9% saline infusion, and the last 2 days of a 4-day infusion of A II at 6, 60, 600 or 6000 ng h^{-1} (left panel, A II infusion). The right panel (post A II infusion) shows mean 3·0% NaCl intake for the 4 days following termination of A II infusion in same animals arranged according to previous dose of A II (doses in parentheses).

little ingestion of 0·5 M-KCl was observed during A II infusion although water intakes were substantially elevated. The NH_4Cl data are presented in the upper half of Table 5 showing that no rat consumed any 0·5 M NH_4Cl during A II infusion, but again water intakes increased markedly. These results show that the observed salt appetite is primarily a Na appetite. Some KCl was consumed, but as reported previously (Falk, 1965; Richter, 1956) KCl ingestion is sometimes observed in Na-depleted rats, which is not surprising considering the similarity of taste between NaCl and KCl (Nachman, 1962).

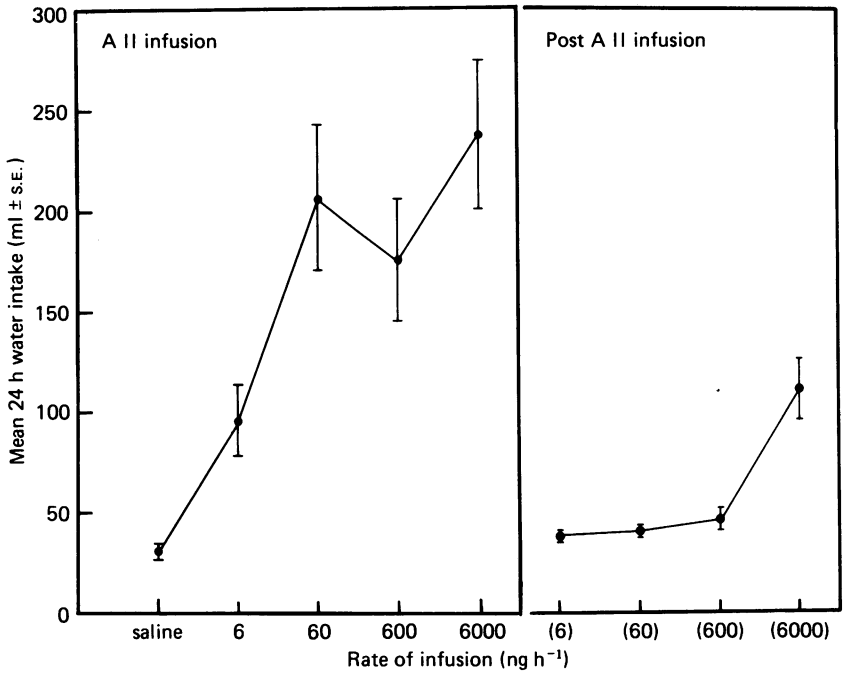

Fig. 5. Dose–response curves showing mean 24 h water intake during the last 2 days of 0·9% saline infusion and A II infusion at 6, 60, 600, 6000 ng h^{-1} (left panel, A II infusion). The right panel (post A II infusion) shows mean water intake for the 4 days following termination of A II infusion in the same animals arranged according to previous dose of A II (doses in parentheses).

TABLE 5. Fluid intakes of rats given either NH_4Cl (0·5 M) and water or KCl (0·5 M) and water to drink during chronic infusion of angiotensin II (6 μg h^{-1}). All values are in ml

	CPR-26		CPR-27		CPR-28	
Day	NH_4Cl	Water	NH_4Cl	Water	NH_4Cl	Water
1	0	72	0	198	0	66
2	0	175	0	146	0	—
3	0	224	0	260	0	172
4	0	194	0	274	0	164
Mean ± S.E. of mean	0	166·2 ± 28·5	0	219·5 ± 25·5	0	134 ± 27·8
	BA-4		BA-5		BA-6	
Day	KCl	Water	KCl	Water	KCl	Water
1	0	74	0	41	0	70
2	5	117	0	210	5	104
3	9	114	0	108	13	174
4	0	166	0	100	2	74
Mean ± S.E. of mean	3·5 ± 1·8	117·7 ± 16·3	0	114·7 ± 30·3	5 ± 2·4	105·5 ± 20·8

Independence of NaCl intake from water intake

A possible explanation of Na appetite evoked by infusion of A II is that the NaCl intake is secondary to the massive water intake produced by intracranial A II. In the following experiment that question was explored using three different approaches. First, rats were offered water sweetened with saccharine in order to increase their fluid intake. Secondly, rats were subjected to passive over-hydration using a naso-pharyngeal–gastric tube. Neither of these groups of rats received intraventricular infusions. And, thirdly, rats were infused with A II but were given only 3·0 % NaCl to drink.

TABLE 6. Fluid intakes during access to either a single bottle of water or 3 % NaCl during or after A II infusion. All values are in ml

		Rats given only 3 % NaCl for 22 h/day				
	Day	CPR-17	CPR-19	CPR-20	CPR-22	CPR-23
A II infusion 6 μg h^{-1}	1	5	1	15	22	5
	2	7	9	24	42	11
	3	15	25	25	33	27
	4	29	29	50	41	45
Mean ± S.E. of mean		14 ± 4·7	18·5 ± 7·3	28·5 ± 6·5	35·5 ± 4	22 ± 7·7
Post infusion	5	35	8	18	45	48
	6	12	12	2	2	21
	7	6	22	7	0	20
	8	16	25	7	—	—
Mean ± S.E. of mean		17·2 ± 5·4	16·7 ± 3·4	8·5 ± 2·9	15·6 ± 11·9	29·6 ± 7·4

Saccharine experiment. Five rats were given access to water and 3·0 % NaCl for 2 days and then switched to 0·2 % Na saccharine (Merck, U.SP.) solution and 3·0 % NaCl for 2 days. No intraventricular infusions were administered. Animals voluntarily drinking large volumes of sweetened water did not drink 3·0 % NaCl as a consequence of their large fluid intakes. Saccharine intakes as high as 104, 121 and 124 ml 24 h^{-1} did not evoke any 3 % NaCl ingestion. The maximum salt intake observed was 1 ml in one rat.

Nasopharyngeal gastric tube experiment. There was very little spontaneous 3·0 % NaCl intake in these three rats. During the intragastric infusion of 121–620 ml water for 3–5 days intake of 3·0 % NaCl remained zero, except for rat CPR-32 which drank 1 ml 3·0 % NaCl on day 4. The animals remained in excellent health throughout. They continued to eat and drink variable amounts of water (5–27 ml/24 h).

Single-bottle experiment. In all five rats offered 3·0 % NaCl only, intake of 3 % NaCl solution increased over the 4 day infusion period (Table 6). In addition, all continued to drink the 3·0 % NaCl after termination of infusion, but the persistence was more variable among them than among the animals in the previous experiments which had had both 3·0 % NaCl and water available. At the extremes, CPR-22 reduced its intake of 3 % NaCl to zero in 3 days after the infusion ended, whereas CPR-23 continued to drink at least 20 ml per day as long as its intake was measured. The 'salt only' animals hydrated themselves at near normal levels during the 2 h

maintenance period when they had access to water. Intakes of water ranged from 15 to 42 ml, with a mean ± S.E. of 25 ± 1 ml for the five rats over 4 days.

Therefore large voluntary intakes of saccharine solution and gastric infusions of large volumes of water comparable to the intakes observed during intraventricular A II infusion, did not result in intake of 3·0 % NaCl. It seems clear, therefore, that the salt appetite produced by A II infusion is not secondary to a large turnover of water. The results obtained from the single bottle experiment show that water intake is not necessary for the development of the A II-induced Na appetite. Moreover, the 3·0 % NaCl intakes persist in the absence of large water intakes when the infusions were terminated.

Drinkometer analysis of pattern of onset

In this experiment the drinkometer was used to determine the pattern of the onset and the temporal relationship between the water and 3·0 % NaCl intake during intraventricular infusion of A II (6 μg h^{-1}).

Representative drinkometer records (Fig. 6) illustrate three main points. First, the onset of 3·0 % NaCl drinking after the start of A II infusion varied from animal to animal. Some rats, such as CPR-44, began drinking NaCl immediately, whereas others did not even sample 3·0 % NaCl until over 6 h into the first day and did not start drinking copiously until almost 14 h after the start of A II infusion. Fig. 6 shows that on the second (CPR-48) or third (CPR-44, CPR-25) day of A II infusion 3·0 % NaCl intake began immediately or within the first 45 min (CPR-25) of the renewal of the solutions and re-insertion of the injector, and this was typical of the larger group of all animals infused. Once started, NaCl intake was slow and sporadic in some animals and rapid in others. In addition, there seemed to be no consistent relationship between a rat's predisposition to drink hypertonic salt solutions spontaneously and the onset of salt drinking after the start of A II infusion.

It should also be noted that although the animals were infused continuously until 12.00 h, at which time they were weighed and the infusion system refilled they drank very little water or 3·0 % NaCl during the several hours before noon. As soon as the new infusion began, however, all rats began drinking avidly. This may have been a result of degradation of the A II which was at room temperature throughout the infusion period.

Secondly, intake of 3·0 % NaCl was not temporally linked to water ingestion, although this did occur occasionally. All three rats depicted in Fig. 6 show that there is a great deal of NaCl drinking without concomitant water ingestion (for example, CPR-48, day 2). Rats did not appear to be mixing an 'isotonic cocktail'. An animal may alternate between 3·0 % NaCl and water (CPR-44, day 3, 21.00 h) but there were long bouts of 3·0 % NaCl drinking without water and vice versa (CPR-25, day 1, 3; CPR-48, day 2).

The third main point is that most drinking, of both NaCl and water, occurs during the dark even though the animals were continuously infused throughout both light and dark periods. Therefore the rat continues to show a nocturnal pattern of drinking despite the continuous infusion of A II.

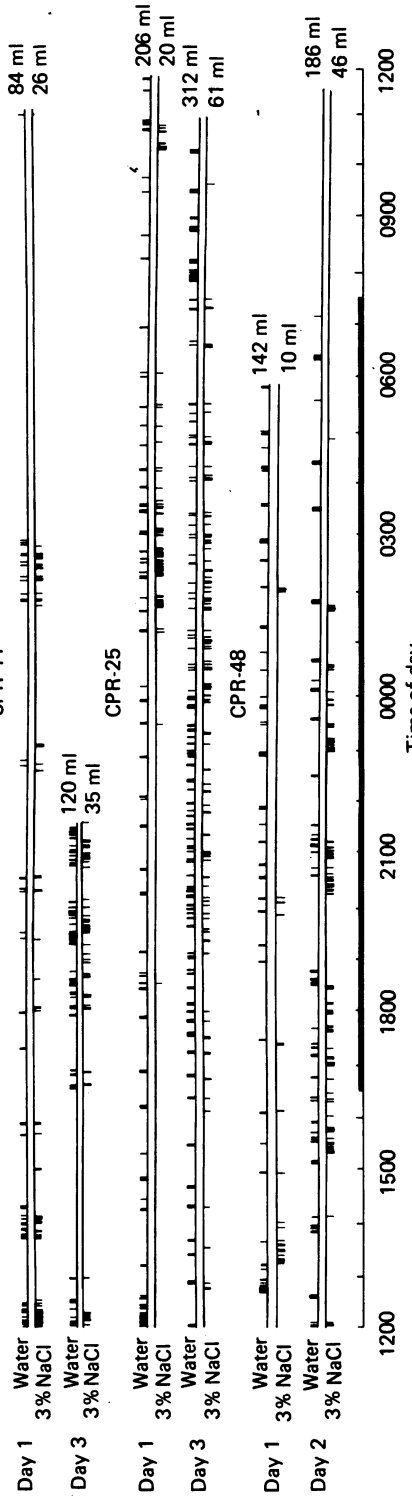

Fig. 6. Representative drinkometer records from CPR-44, 25, 48. Time of day is indicated at the bottom with the dark line representing the dark phase of the day. Each pen excursion represents 12 licks. The total amount of water (upper record of each pair) and 3% NaCl (lower record of each pair) drunk in 24 h is presented at the right of each day's records. The truncated records of CPR-44 day 3, CPR-25 day 3, and CPR-48 day 1 are a result of recorder failure. Water and 3·0% NaCl intakes are nevertheless for the entire 24 h period. A II was infused at 6 μg h^{-1}, beginning at 12.00 h in all records.

DISCUSSION

These experiments and those of Avrith & Fitzsimons (1978, 1979) show clearly that prolonged exposure of the brain to A II produces an appetite for Na. In the work reported here the appetite was produced first by single intracranial injections of A II repeated on successive days. After several days of such treatment hypertonic NaCl solution (2·7 %) which had previously been rejected was drunk in significant volumes, and it continued to be ingested in moderate amounts for as long as the injections were maintained. In addition, repeated systemic injections of renin or of isoprenaline eventually produced the same moderate and consistent appetite for salt. When, in the subsequent experiments reported here, the hormone was infused continuously into the anterior third ventricle the appetite occurred in all animals and was never less than that seen after adrenalectomy despite the fact that all animals were probably in positive Na balance, as shown in the accompanying paper (Avrith & Fitzsimons, 1979), and in some rats was expressed in massive intakes of 3·0 % NaCl. At rates of 60 ng h^{-1} to 6 μg h^{-1}, 46 % of the animals drank at least 100 ml/day and 76 % drank at least 59 ml/day. Furthermore, at these rates all animals drank at least 20 ml/day after the first day of A II infusion and for as long as the infusion continued. The brain mechanisms for the appetite are clearly responsive to angiotensin and can be driven by it to extremes of excess.

The appetite induced by intracranial A II has the essential characteristics of the natural phenomenon. First, it is specific for sodium. The rats did not drink NH_4Cl and only an occasional animal drank increased amounts of KCl, as in the case after adrenalectomy (Richter, 1956) and intraperitoneal dialysis (Falk, 1965), but in even the most extreme of these the intake was less by an order of magnitude than the amount of NaCl ingested during infusion with comparable doses of A II. Secondly, as has been reported for the natural phenomenon (Epstein & Stellar, 1955; Stricker, 1973), the intake of hypertonic NaCl solution was persistent. Once initiated by A II infusion the appetite continued after the termination of the treatment. A majority of the animals continued to drink 3·0 % NaCl in the post-infusion period usually in progressively diminishing amounts. Several animals drank it in large amounts for as long as their intake was followed. Although considerably less than the massive intakes induced by the infusion, the intakes in the period of persistence were nevertheless the equivalent of those seen after pathological disturbances of sodium balance. Richter's (1936) animals, for example, drank 15–25 ml 3·0 % NaCl solution/day after adrenalectomy.

Since it is a potent dipsogen, especially when given intracranially, A II in the doses used here generated very large daily water intakes, sometimes exceeding the animals' body weights. The large water intake was not, however, the cause of the Na appetite. Animals that were drinking large amounts of sweetened water ignored 3·0 % NaCl offered simultaneously, as did another group of rats that were passively infused with volumes of water that equalled or exceeded the amounts that were drunk during the A II infusion. Nor were the animals drinking the NaCl in order to ingest an isotonic mixture of water and hypertonic NaCl. First, because the infusions induced excess hypertonic NaCl intake when it was the only fluid offered, and secondly because records of the animals' drinking behaviour revealed that the ingestion of the water and of the hypertonic NaCl were not concurrent.

It is impossible with the present evidence to specify the effective dose of A II for induction of the appetite. We have infused the hormone at an unknown distance from unknown receptors and have utilized as medium the CSF, which is probably an unphysiological route of access to the brain for A II. However, the rates are not larger than those used to elicit thirst by intraventricular injection of the hormone. At the lowest effective rate (6 ng h^{-1}) the animals were receiving only 100 pg min^{-1} which is not an excessive dose for production of the dipsogenic response. Nor is it possible to specify where the receptors for the induction of Na appetite by A II are, nor comment on their relationship to the forebrain structures that have been implicated in the mediation of the appetite by Covian & Antunes-Rodrigues (1963) and Wolf (1967, 1968, 1971). Infusions were made through cannulae that opened into the anterior cerebral ventricles and the hormone was therefore dispersed widely in the brain, undoubtedly gaining access to the circumventricular organs and to the extracellular fluid and parenchyma of the brain wherever the cannulae penetrated the ependyma.

We can conclude, however, that a role for A II in the arousal of the Na appetite is likely from the evidence presented here and in the accompanying paper by Avrith & Fitzsimons (1979). Such a role for the hormone is most reasonable when it is recalled that, with the exception only of pharmacological doses of the mineralocorticoids, all known causes of the appetite are associated with decreases in plasma Na and with prolonged elevation of plasma A II, and it is an appealing simplification to think of A II as mediating both the conservation of Na by the kidney and the urge for salt ingestion by the brain. The hormone, of course, cannot be the sole cause of the appetite because in some species, for example sheep, the appetite survives nephrectomy (Bott, Denton & Weller, 1967); the appetite is produced by pharmacological doses of DOCA and aldosterone, both of which severely depress plasma renin, as well as by treatment with ACTH and the hormones of pregnancy (Denton, McKinley, Nelson & Weisinger, 1977), and because the appetite persists long after the withdrawal of A II treatment. The appetite is clearly a complex phenomenon that may require interaction of A II and aldosterone for its arousal and it may be sustained in the absence of endocrinological determinants by a conditioning process during which the taste of strong NaCl is associated with the benefits of sodium ingestion.

There is some controversy over the possible role of plasma A II in generating an appetite for Na. Chiaraviglio (1976) found that when Na appetite induced by Na deficiency was reduced by nephrectomy, it could be restored by I.P. renin or by intracranial A II. Na appetite was also diminished by intracranial saralasin, a competitive inhibitor of A II. Chiaraviglio's animals were offered a highly preferred concentration of NaCl (1 %) and they were made acutely Na deficient by intraperitoneal dialysis. On the other hand, a single I.P. injection of renin did not restore Na appetite in the formalin-treated rat whose Na appetite had been reduced by nephrectomy (Fitzsimons & Stricker, 1971). Nor did activation by various means of the renal renin–angiotensin system affect Ma appetite in the adrenalectomized rat, although such animals showed an increase in intake of 2·7 % NaCl in response to intracranial angiotensin stimulation (Fitzsimons & Wirth, 1978). Adrenalectomized animals are amply experienced at drinking hypertonic NaCl in order to repair Na deficiencies and they might therefore be less responsive to occasional manipulation of the renin–angiotensin system by

pharmacological or other means. However, there is probably a more important difference between the work described in these two papers and previous experiments which may account for the apparently contradictory outcomes. Whereas previous experiments have involved short-lived stimulation of the renin–angiotensin system by single injections of renin, isoprenaline, etc., together with relatively short periods of observation (up to 8 h), in the present experiments prolonged stimulation of the renin–angiotensin system by repeated injections and infusions over many days have been carried out. It seems that the duration and frequency of exposure of the mechanisms involved in Na appetite to A II are critical factors in the elicitation of Na appetite by this type of stimulation.

It is clearly too early for conclusions about the role of A II in Na appetite, but we can suggest that in animals which have had no prior experience with salt drinking during sodium deficiency, prolonged elevations of A II may alter the mechanisms for salt perception, enhancing the hedonic value of strong solutions and making them more acceptable. This could occur through peripheral mechanisms as Richter (1936) suggested when he first speculated about the cause of the appetite. But because the infusions discussed here were made directly into the brain, and because the alteration in the perception of salt which we believe to be the essential mechanism of the appetite was produced by low doses of the hormone, the hedonic enhancement is likely to have occurred in the brain itself.

The first experiments were done by Fitzsimons and Epstein in the Physiological Laboratory at Cambridge. Subsequent experiments were done by Bryant, Epstein and Fluharty in the Department of Biology at the University of Pennsylvania where support was provided by NINCDS 03469. They were the subject of a preliminary report (Bryant, Fluharty & Epstein, 1978).

REFERENCES

AVRITH, D. & FITZSIMONS, J. T. (1978). Intracranial angiotensin II stimulates sodium appetite. *J. Physiol.* **282**, 40–41*P*.

AVRITH, D. & FITZSIMONS, J. T. (1979). Induction of delayed sodium appetite in the rat by intracranial administration of components of the renin–angiotensin system. *J. Physiol.* (In the Press).

BEILHARZ, S., DENTON, D. A. & SABINE, J. R. (1962). The effect of concurrent deficiency of water and sodium on the sodium appetite of sheep. *J. Physiol.* **163**, 378–390.

BOTT, E., DENTON, D. A. & WELLER, S. (1967). The effect of angiotensin II infusion, renal hypertension and nephrectomy on salt appetite of sodium deficient sheep. *Aust. J. exp. Biol. med. Sci.* **45**, 595–612.

BRYANT, R. W., FLUHARTY, S. J. & EPSTEIN, A. N. (1978). Excessive drinking of sodium solutions by rats receiving continuous intracranial infusion of angiotensin. *Fedn Proc.* **37**, 323.

BUGGY, J. & FISHER, A. E. (1974). Evidence for a dual role for angiotensin in water and sodium intake. *Nature, Lond.* **250**, 733–735.

CHIARAVIGLIO, E. (1976). Effect of renin–angiotensin system on sodium intake. *J. Physiol.* **255**, 57–66.

COVIAN, M. R. & ANTUNES-RODRIGUES, J. (1963). Specific alterations in sodium chloride intake after hypothalamic lesions in the rat. *Am. J. Physiol.* **205**, 922–926.

DAVIS, J. O. (1971). The renin–angiotensin system in the control of aldosterone secretion. In *Kidney Hormones*, ed. FISHER, J. W., pp. 173–205. New York: Academic.

DENTON, D. A., MCKINLEY, M. J., NELSON, J. F. & WEISINGER, R. S. (1977). Pregnancy, lactation, and hormone-induced mineral appetite. In *Food Intake and Chemical Senses*, ed. KATSUKI, Y., SATO, M., TAKAGI, S. F. & OOMURA, Y., pp. 247–261. Tokyo: Japan Scientific Press.

DENTON, D. A. & SABINE, J. R. (1961). The selective appetite for Na^+ shown by Na^+ deficient sheep. *J. Physiol.* **157**, 97–116.

EPSTEIN, A. N., FITZSIMONS, J. T. & ROLLS, B. J. (née SIMONS) (1970). Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol.* **210**, 457–474.

EPSTEIN, A. N. & STELLAR, E. (1955). The control of salt preference in the adrenalectomized rat. *J. comp. physiol. Psychol.* **48**, 167–172.

EPSTEIN, A. N. & TEITELBAUM, P. (1962). A watertight swivel joint permitting chronic injection into moving animals. *J. appl. Physiol.* **17**, 171–172.

FALK, J. L. (1961). The behavioral regulation of water-electrolyte balance. In *Nebraska Symposium on Motivation*, ed. JONES, M. R., pp. 1–33. Lincoln: University of Nebraska Press.

FALK, J. L. (1965). Limitations to the specificity of NaCl appetite in sodium-depleted rats. *J. comp. physiol. Psychol.* **60**, 393–396.

FISHER, A. E. & BUGGY, J. (1975). Central mediation of water and sodium intake: a dual role for angiotensin. In *Control Mechanisms of Drinking*, ed. FITZSIMONS, J. T., PETERS, G. & PETERS-HAEFELI, L., pp. 139–147. Berlin: Springer-Verlag.

FITZSIMONS, J. T. (1964). Drinking caused by constriction of the inferior vena cava in the rat. *Nature, Lond.* **204**, 479–480.

FITZSIMONS, J. T. & STRICKER, E. M. (1971). Sodium appetite and the renin–angiotensin system. *Nature, Lond.* **231**, 58–60.

FITZSIMONS, J. T. & WIRTH, J. B. (1978). The renin–angiotensin system and sodium appetite. *J. Physiol.* **274**, 63–80.

FREGLY, M. J. & WATERS, I. W. (1966). Effect of mineralocorticoids on spontaneous sodium chloride appetite of adrenalectomized rats. *Physiol. & Behav.* **1**, 65–74.

NACHMAN, M. (1962). Taste preferences for sodium salts by adrenalectomized rats. *J. comp. physiol. Psychol.* **55**, 1124–1129.

NACHMAN, M. & VALENTINO, D. A. (1966). Roles of taste and postingestional factors in the satiation of sodium appetite in rats. *J. comp. physiol. Psychol.* **62**, 280–283.

PESKAR, B., MEYER, D. K., TAUCHMANN, U. & HERTTING, G. (1970). Influence of isoproterenol, hydralazine and phentolamine on the renin activity of plasma and renal cortex of rats. *Eur. J. Pharmacol.* **9**, 394–396.

RADIO, G. J., SUMMY-LONG, J., DANIELS-SEVERS, A. & SEVERS, W. B. (1972). Hydration changes produced by central infusion of angiotensin II. *Am. J. Physiol.* **223**, 1221–1226.

REID, I. A., SCHRIER, R. W. & EARLEY, L. E. (1972). Effect of beta-adenergic stimulation on renin release. *Adv. exp. Med. Biol.* **17**, 49–64.

RICHTER, C. P. (1936). Increased salt appetite in adrenalectomized rats. *Am. J. Physiol.* **115**, 155–161.

RICHTER, C. P. (1956). Salt appetite in mammals – its dependence on instinct and metabolism. In *L'instinct*, ed. AUTOURI, M., pp. 577–629. Paris: Masson et Cie.

STRICKER, E. M. (1973). Thirst, sodium appetite and complementary physiological contributions to the regulation of intravascular fluid volume. In *The Neuropsychology of Thirst*, ed. EPSTEIN, A. N., KISSILEFF, H. R. & STELLAR, E., pp. 73–98. Washington, D.C.: Winston.

WOLF, G. (1964). Sodium appetite elicited by aldosterone. *Psychon. Sci.* **1**, 211–212.

WOLF, G. (1967). Hypothalamic regulation of sodium intake: relations to preoptic and tegmental function. *Am. J. Physiol.* **213**, 1433–1438.

WOLF, G. (1968). Thalamic and tegmental mechanisms for sodium intake: anatomical and functional relations to lateral hypothalamus. *Physiol. & Behav.* **3**, 997–1002.

WOLF, G. (1971). Neural mechanisms for sodium appetite: hypothalamus positive–hypothalamofugal pathways negative. *Physiol. & Behav.* **6**, 381–389.

WOLF, G. & HANDEL, P. J. (1966). Aldosterone-induced sodium appetite: dose–response and specificity. *Endocrinology* **78**, 1120–1124.