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# The Specificity of the Dipsogenic Effect of Angiotensin II[1]

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ABRAHAM, S. F., D. A. DENTON AND R. S. WEISINGER. *The specificity of the dipsogenic effect of angiotensin II.* PHARMAC. BIOCHEM. BEHAV. 4(4) 363–368, 1976. – The specificity of choice in drinking by sheep was examined when a cafeteria of water and of 900 mmol/l solutions of NaCl and KCl was presented, during intracarotid infusion of angiotensin II (800–1200 ng/min) or 4M NaCl (1.6 ml/min), and following 48 hr of water deprivation or following Na depletion. Water was the fluid of predominant choice with angiotensin II, 4M NaCl infusion and water deprivation. The hypertonic NaCl was the clear choice of the Na deficient animals. With a cafeteria of 300 mmol/l solutions, there was no clear discrimination between NaCl and water with intracarotid angiotensin II infusion. A 2 choice study of taste preference for water or NaCl concentrations with free access indicated sheep elect to take more of higher NaCl concentrations than the rat, and that 300 mmol/l NaCl is not less preferred than water in sheep. The data indicated, overall, that the dipsogenic effect of supraphysiological cerebral blood concentrations of angiotensin II is biased to water drinking when the choice is between water and 900 mM NaCl and KCl solutions. It does not induce any specific salt appetite.

Angiotensin II  $Na^+$ depletion  Water deprivation  Sodium preference  Taste preference  Salt appetite

THE behaviour which follow various modes of administration of angiotensin II to rats [10] and sheep [2] appears to be specific for drinking. In most of the experimental work involving angiotensin II and thirst, water has been the only solution available. The small amount of data involving a 2 choice situation of water and sodium chloride (1.8% or 2.7%) is conflicting. Wirth and Fitzsimons [17] reported that intracranial administration of renin substrate and angiotensin II to sodium deprived adrenalectomized rats augmented the intake of water, and either diminished or did not effect the intake of 2.7% sodium chloride over a 1 hr period of measurement. Renin, and caval ligation have also been reported to produce drinking of water and not 1.8% saline over a 6 hr period [11]. Buggy and Fisher [8] also studied the drinking behaviour in rats induced by intracranial administration of high pharmacological doses of angiotensin II. In both satiated animals and animals with pre-existing fluid or sodium deficits, angiotensin II stimulated the intake of both water and sodium chloride (1.8% and 2.7%) over 1 or 8 hr.

The purpose of the study here was to establish in sheep the specificity of the drinking response to angiotensin II in a simple cafeteria choice situation. Does angiotensin II stimulate water appetite specifically – or does it stimulate water intake and intake of saline fluids indiscriminately? Earlier data from this laboratory [7] showed that intravenous angiotensin II infusion in high physiological doses caused a small but significant decrease of Na intake when Na deficient animals are presented with $NaHCO_3$ solution for 1 hr compared to control intake for the same deficient animals. That is, there was no stimulation of Na appetite.

## METHOD

The specificity of water appetite in angiotensin II induced thirst was examined by presentation of a cafeteria of sodium chloride, potassium chloride and water, in separate bins on the side of the animal's cage. A total of 78 experiments were performed on 6 sheep of merino and merino crossbreds (5 ewes and 1 wether, 35–45 kg body weight). These sheep had had previous experience with the presentation of a sodium solution following sodium depletion by acute cannulation of 1 parotid duct, and of water following water depletion.

Intracarotid artery infusions of angiotensin II in normal saline (800 or 1200 ng/min for 45 min) and 4M NaCl (1.6 ml/min for 15 min) were given to sodium and water replete sheep. This followed a control period of 30 min of intracarotid infusion of isotonic saline, as previously described [2]. The contralateral carotid artery was occluded throughout the infusion. This ensured that the ipsilateral carotid infusion was distributed over both sides of the brain [3,4]. The cafeteria with water, and potassium chloride and sodium chloride at a concentration of 900 mmol/l, was presented for the 30 min prior to and then, in the first series of experiments, during infusions of angiotensin II at 1200 ng/min (n = 6). A similar procedure was followed with intracarotid 4M NaCl infusion (n = 5). The volume of

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the solutions drunk was measured after the control infusion of normal saline for 30 min, and at the end of the experimental infusion. In control experiments under exactly the same conditions, solutions were also measured after 30 min and following 45 min except that no infusion took place (n = 8). In a second experimental series the 900 mmol/l cafeteria was also presented for 15 min after 48 hr of water deprivation (n = 9) and for 45 min following 24–48 hr of sodium depletion by parotid duct cannulation (n = 6). For comparison with this second series of experiments where no preperiod was feasible, the 900 mmol/l cafeteria was also first presented at the time of commencement of intracarotid artery infusion of angiotensin II at 1200 ng/min (n = 6) or 4M NaCl infusion at 1.6 ml/min (n = 6).

In a third series of experiments a cafeteria with water, and with sodium chloride and potassium chloride at a concentration of 300 mmol/l was presented for 30 min prior to and during intracarotid artery infusion of angiotensin II at 800 ng/min (n = 6) and 1200 ng/min (n = 6), 4M NaCl at 1.6 ml/min (n = 6) and for 15 min following 48 hr of water deprivation (n = 11).

In order to eliminate positional preferences the bins containing the solutions were placed on the animal's cage such that in any 3 consecutive experiments each solution was presented in a different position. Drinking behaviour was observed from an adjacent room via closed circuit television.

All data are presented as the mean (standard error of the mean). Where appropriate Student's $t$ test for paired or unpaired data was used for comparison of the volumes of solutions drunk under different experimental conditions.

*Taste Preference Curve for Sodium Chloride Solutions [16]*

The preference for various concentrations of sodium chloride, relative to water, was tested in 2 sheep. They had not previously experienced drinking sodium chloride solutions. Each animal had continuous access to 4 l of water and 4 l of a sodium chloride solution, in separate identical bins on the side of their cage. The position of the bins was reversed every 24 hr. Each concentration of the sodium chloride solution was available for 48 hr. The concentration of the sodium chloride solution presented was increased progressively from 2.5 to 1000 mmol/l in increments of 2.5, 5, 10, 20, 25, 50 and 450 mmol/l up to 20, 50, 100, 200, 400, 550 and 1000 mmol/l respectively.

For 4 days prior to these experiments the animals were tested for bin preference. Water was available in both bins. These bins were alternated every 24 hr. No evidence of a bin position preference was observed.

## RESULTS

*Drinking Following Presentation of Randomly Placed Bins of Water, Sodium Chloride (900 mmol/l) and Potassium Chloride (900 mmol/l) for 30 min Before, and During Intracarotid Infusion of Angiotensin (1200 ng/min), and 4M NaCl (1.6 ml/min)*

In all experiments the animals sampled each solution at least once immediately upon presentation. The mean volume (ml) of water, sodium chloride (900 mmol/l) and potassium chloride (900 mmol/l) drunk in the 30 min prior to and during intracarotid artery infusion of angiotensin II (1200 ng/min) for 45 min or during 4M NaCl (1.6 ml/min) for 15 min, or during 45 min observations without infusion are shown in Table 1. In the initial 30 min of cafeteria presentation, sodium chloride was the only solution drunk in any significant amount by animals (Table 1). This drinking was completed in the first 10 min of the 30 min access, and there was no further drinking of the solutions in the following 45 min as indicated by the no infusion group (Table 1). Intracarotid artery infusion of 4M NaCl (1.6 ml/min) and angiotensin II (1200 ng/min) resulted in water drinking (Table 1). The volumes of water drunk in response to angiotensin II and 4M NaCl infusion were significantly greater than that drunk in the same time period by the no infusion group ($p<0.001$ and $p<0.001$ respectively). A small amount of potassium chloride was drunk on some occasions. This was not peculiar to any 1 animal.

In Table 2 the mean volumes (ml) of water and the solutions (900 mmol/l) drunk in response to 48 hr of water deprivation, or after 24–48 hr of sodium depletion by parotid cannulation are shown. For comparison with this data, where no control period of exposure to the solutions was feasible, experiments were made where 900 mmol/l solutions and water were presented simultaneous with the commencement of intracarotid infusion of angiotensin II

TABLE 1

THE MEAN (SEM) VOLUMES (ml) OF WATER, SODIUM CHLORIDE (900 mmol/l), POTASSIUM CHLORIDE (900 mmol/l) DRUNK FOR THE CONTROL 30 MIN PRIOR TO (PRE), AND DURING (EXP) INTRACAROTID ARTERY INFUSION OF ANGIOTENSIN II (1200 ng/min FOR 45 MIN), 4M NaCl (1.6 ml MIN FOR 15 MIN) AND IN CONTROL EXPERIMENTS IN WHICH THERE WAS NO INFUSION (45 MIN). n = NUMBER OF EXPERIMENTS

| Volume drunk of Solution at 900 mmol/l | No Infusion | | Angiotensin II 1200 ng/min | | 4M NaCl | |
|---|---|---|---|---|---|---|
| | Pre | Exp. | Pre | Exp. | Pre | Exp. |
| Water | 0 (0) | 0 (0) | 0 (0) | 1142*(152) | 0 (0) | 1180*(60) |
| Sodium Chloride | 181 (51) | 0 (0) | 287 (99) | 0 (0) | 365 (153) | 0 (0) |
| Potassium Chloride | 6 (6) | 0 (0) | 0 (0) | 10 (10) | 0 (0) | 10 (10) |
| n | 8 | 8 | 9 | 9 | 5 | 5 |

For each solution, the volumes drunk in the pre and Exp. periods were tested against each other. Only significant differences are indicated.

*$p<0.001$.

TABLE 2

THE MEAN (SEM) VOLUME (ml) OF WATER, SODIUM CHLORIDE (900 mmol/l) POTASSIUM CHLORIDE (900 mmol/l) DRUNK FOLLOWING 48 HR OF WATER DEPLETION (15 MIN ACCESS), SODIUM DEPLETION (45 MIN ACCESS), AND DURING INTRACAROTID ARTERY INFUSION OF ANGIOTENSIN II (1200 ng/MIN FOR 45 MIN ACCESS) AND 4M NaCl (1.6 ml/MIN FOR 15 MIN OF ACCESS). n = NUMBER OF EXPERIMENTS

| Volume drunk of Solution at 900 mmol/l | Angiotensin II 1200 ng/min | 4M NaCl 1.6 ml/min | Water Deprivation | Sodium Depletion |
|---|---|---|---|---|
| Water | 983 (162) | 1000 (132) | 1344 (267) | 92*(73) |
| Sodium Chloride | 83 (45) | 42 (27) | 211 (79) | 467†(51) |
| Potassium Chloride | 0 (0) | 0 (0) | 3 (3) | 0 (0) |
| n | 6 | 6 | 9 | 6 |

For each solution, all volumes drunk were tested against each other. Only significant differences are indicated.
*$p<0.001$.
†$p<0.05$.

TABLE 3

INDIVIDUAL AND MEAN (SEM) SALIVA VOLUME (ml), SALIVARY Na LOSS (mmol), AND SODIUM CHLORIDE (900 mmol/l) DRUNK DURING 45 MIN OF ACCESS FOLLOWING 24–48 HR SODIUM DEPLETION

| Animal | Saliva volume (ml) | Amount Na drunk (mmol) | Amount Na (900 mmol/1 NaCl) drunk in 45 min (mmol) |
|---|---|---|---|
| Olla | 3300 | 547 | 540 |
| Gundi | 3900 | 588 | 585 |
| Priscilla | 2400 | 364 | 360 |
| | 2000 | 312 | 360 |
| Kanutus | 1800 | 293 | 315 |
| | 2750 | 445 | 360 |
| Mean | 2691 | 424 | 420 |
| SEM | 326 | 50 | 45 |

There was a significant correlation between salivary Na loss (mmol) and sodium chloride intake (mmol) during 45 min of access. ($r = 0.93$, $p<0.01$; $y = 0.85 x + 57.58$).

(1200 ng/min) or 4M NaCl (1.6 ml/min). Water was the solution of choice following water deprivation and infusions of angiotensin II and 4M NaCl. The volumes of water drunk in response to angiotensin II and 4M NaCl with (Table 1), or without (Table 2), a prior 30 min access period to the cafeteria were not significantly different. The water intake after sodium depletion by parotid cannulation for 24–48 hr (293 to 588 mmol $Na^+$) was significantly lower than the water drunk after water depletion and during infusion of angiotensin II or 4M NaCl ($p<0.001$).

The solution of choice in response to sodium depletion was sodium chloride (Table 2). The amount drunk (Table 3) was linearly related to the degree of Na deficit ($p<0.01$). However, in 2 experiments 100 and 450 ml of water was drunk. This occurred in the latter part of the 45 min access period after completion of sodium chloride drinking. The sodium chloride intakes following water deprivation and during infusion of angiotensin II or 4M NaCl (Table 2) were not significantly different from the sodium chloride intakes of the no infusion group in the first 30 min of access to the cafeteria (Table 1). The volume of sodium chloride drunk following sodium depletion (Table 2) was significantly greater than that drunk by the no infusion group in the first 30 min of access ($p<0.005$) (Table 1) and following water depletion ($p<0.05$) (Table 2) angiotensin II (1200 ng/min ($p<0.05$) (Table 2) and 4M NaCl (1.6 ml/min) ($p<0.05$) (Table 2).

*Drinking Following Presentation of Randomly Placed Bins of Water, Sodium Chloride (300 mmol/l) and Potassium Chloride (300 mmol/l) for 30 min Before, During Intracarotid Infusion of Angiotensin II, and 4M NaCl, and Following Water Deprivation for 48 hr*

With the exception of 1 animal, all solutions were sampled at least once by the 5 animals immediately upon access.

The mean volumes (ml) of water, sodium chloride (300 mmol/l) and potassium chloride (300 mmol/l) drunk in the 30 min prior to and during intracarotid artery infusion of angiotensin II (800 or 1200 ng/min for 45 min) or 4M NaCl (1.6 ml/min for 15 min) are shown in Table 4. The mean volume (ml) of these solutions drunk when they were presented after 48 hr of water deprivation are also shown in Table 4. In the 30 min control periods, sodium chloride was the only fluid drunk in any amount. There were no significant differences between the experimental groups as regards the volume of sodium chloride drunk during control periods.

Approximately equal volumes of water and sodium chloride were drunk during infusion of angiotensin II (800 or 1200 ng/min) or following water deprivation. The total water and sodium chloride intakes in response to angiotensin II (1200 ng/min) were not significantly different from the volumes of water drunk with the same stimulus during presentation of the 900 mmol/l cafeteria. However, water was the solution of choice during intracarotid infusion of 4M NaCl. The volume of water drunk in response to 4M NaCl infusion was not significantly different from the value obtained during presentation of the 900 mmol/l cafeteria. The total fluid intakes during angiotensin II at 800 ng/min and 1200 ng/min were not significantly different. Following water deprivation, large amounts of

TABLE 4

THE MEAN (SEM) VOLUME (ml) OF WATER, SODIUM CHLORIDE (300 mmol/l) AND POTASSIUM CHLORIDE (300 mmol/1) DRUNK 30 MIN PRIOR TO AND DURING INTRACAROTID ARTERY INFUSION OF ANGIOTENSIN II (1200 ng/MIN FOR 45 MIN AND 800 ng/MIN FOR 45 MIN), 4M NaCl (1.6 ml/MIN FOR 15 MIN) AND FOR 15 MIN AFTER 48 HR OF WATER DEPRIVATION. n = NUMBER OF EXPERIMENTS

| Volume of Solution 300 mmol/1 (ml) | Angiotensin II 800 ng/min | | Angiotensin II 1200 ng/min | | 4M NaCl 1.6 ml/min | | Water Deprivation |
|---|---|---|---|---|---|---|---|
| | Pre | Exp. | Pre | Exp. | Pre | Exp. | Exp. |
| Water | 0 (0) | 325 (108) | 0 (0) | 579 (171) | 8 (8) | 1050 (59) | 1114 (292) |
| Sodium Chloride | 50 (32) | 296 (67) | 100 (68) | 433 (262) | 342 (155) | 25 (11) | 909 (368) |
| Potassium Chloride | 0 (0) | 83 (28) | 0 (0) | 92 (63) | 8 (8) | 17 (17) | 218 (189) |
| n | 6 | 6 | 6 | 6 | 6 | 6 | 11 |

300 mmol/l sodium chloride were ingested on 4 of the 11 occasions. In 3 experiments following water deprivation on one animal, it showed a definite bin position preference. This also accounts for the higher mean potassium chloride intake in this experimental group (Table 4). Potassium chloride was drunk on some occasions other than the one involving bin position reference. This was not peculiar to any 1 animal.

*Taste Preference Behaviour Towards Sodium Chloride Solutions*

The mean sodium chloride preferences (volume of sodium chloride solution drunk in 48 hr divided by the total volume of sodium chloride and water drunk in 48 hr) of 2 sheep when sodium chloride solutions of concentrations varying from 2.5 to 1000 mmol/1 were presented are shown in Fig. 1. There was a preference for sodium chloride over water (>60%) at concentrations of sodium chloride of approximately 50–160 mmol/1 and correspondingly a preference for water (>60%) over sodium chloride solutions of concentrations greater than 400 mmol/1. Animals drank approximately equal volumes of water and sodium chloride between 160 and 400 mmol/1.

## DISCUSSION

Water was drunk as the solution of choice in response to intracarotid artery infusions of angiotensin II (1200 ng/min) when sodium chloride and potassium chloride at 900 mmol/1 concentration and water were offered to sheep. As recorded in the experiments of Abraham *et al.* 1975, virtually all the water intake occurred in the first 15 min of the infusions [2]. This rate of angiotensin II infusion produced supraphysiological concentrations of angiotensin II in cerebral blood [2]. These animals differentiated between solutions at this concentration as shown by the predominate selection of water in response to intracarotid artery infusion of 4M NaCl (1.6 ml/min) and water deprivation, and conversely the selection of sodium chloride in

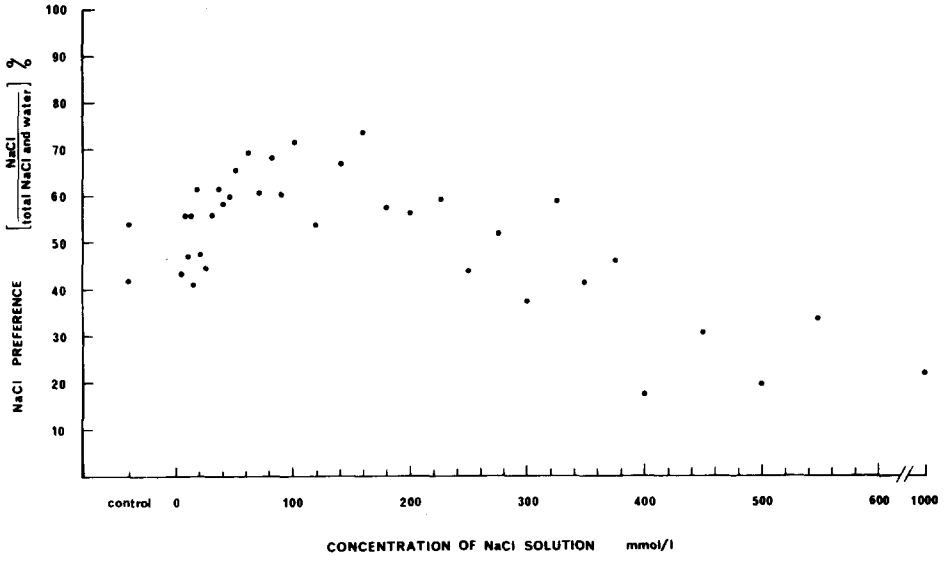


FIG. 1. Two choice NaCl preference for 2 normal sheep offered various concentrations of NaCl solution (presented in ascending order) and tap water. Each concentration of NaCl was offered for 2 consecutive days and each mean value plotted is the total NaCl intake/total fluid intake expressed in percent for the 2 days. During the control period, water was available from the 2 bins.

response to sodium depletion. This would indicate that angiotensin II does not stimulate a specific appetite for sodium in sheep and its dipsogenic effect is biased to intake of water under these conditions of choice.

The animals also showed some hedonic liking for the sodium chloride solution (900 mmol/l) which was evident from the preference study and from experiments in which the cafeteria was presented prior to infusion to animals which were sodium and water replete (see control preperiod of Table 1). This small intake of NaCl in the control preperiod did not influence or determine the water intake in response to intracarotid angiotensin II since (1) the intake of water (Table 1) was no different from that recorded in Table 2 when no preperiod of access of the solutions preceded intracarotid angiotensin II. (2) There was no water drinking over the 45 min in the no infusion series in Table 1. Similarly, with 4M NaCl infusion, the water intake was virtually the same whether the control preperiod with NaCl drinking was permitted (Table 1) or was not included (Table 2). A small intake of sodium chloride was present also when the cafeteria was presented at commencement of infusion of angiotensin II, 4M NaCl, and following 48 hr of water deprivation (Table 2). It would not be possible to delineate from our data whether this was in the initial context of sampling by a highly motivated animal in the course of settling upon the water intake primarily manifest, or whether it reflected some measure of hedonic selection despite the primary drive. With Na depletion, the selection of sodium was primary with little interest in water except towards the end of the observation period which most likely reflected osmotic effects of the very hypertonic saline ingested at the beginning of the period. Overall the results in this regard are consistent with the data already published on sheep with water or Na depletion or concurrent depletion of both sodium and water [5] or rats with Na depletion or concurrent water and Na depletion [18]. These results are also consistent with the findings from the sodium chloride and water relative preference study when solutions of water and sodium chloride at concentrations of 400 to 1000 mmol/l were continuously available. These animals drank small amounts of NaCl over this concentration range though, the preference was for water.

When the 300 mmol/l cafeteria was presented, water was the solution of choice in response to the infusion of 4M NaCl. However, the animals did not show a preference between water and NaCl (300 mmol/l) in response to water deprivation and to intra-arterial infusions of angiotensin II at 800 and 1200 ng/min – the animals drank 1 solution or the other or both. The data is suggestive that angiotensin II does not cause any specific thirst for water as does a sudden large osmotic stimulus when the choice involves fluids of this concentration. Attribution of any specificity to water at these concentrations with the osmotic stimulus must also be with reserve since the intracarotid 4M NaCl infusion would raise $[Na^+]$ of lingual artery blood 15–30 mmol/l which may, of itself, alter taste and acceptability. The clear specificity of appetite for 320–420 mmol/l $NaHCO_3$ solutions over water during Na depletion in sheep [5] and for 1M NaCl solution in Na depleted rats [18] has been described previously. Water deprived sheep have been found to drink a $NaHCO_3$ solution of concentration 317 mmol/l when offered a $NaHCO_3$ solution and water, but not when the concentration of the $NaHCO_3$ was 420 mmol/l [4]. Andersson has recorded that the goat will not accept a solution of 2.5% NaCl (427 mmol/l) when water depleted [1].

The drinking of NaCl (300 mmol/l) and/or water in response to 48 hr water deprivation is not physiologically appropriate in the light of increased plasma sodium concentration [5]. This increase in plasma sodium concentration is of the same order as that seen with intra-arterial infusion of 4M NaCl at 1.6 ml/min. Water deprivation does involve another effect – hypovolaemia.

The data here on relative preference for saline or water in sheep together with some earlier observations [9] is not nearly as comprehensive as that emerging from the studies on rats [15,16] but is broadly confirmatory. A preference for saline over water begins in the range of 40–60 mmol/l and reverses in the range of 200–300 mmol/l. Our limited observations would suggest some movement of the taste preference curve to the right relative to the data on the rat. This may throw light on the lack of specificity of intake with the 300 mmol/l cafeteria in the face of water deprivation as well as angiotensin II infusion since with this free choice situation without stimuli, 300 mmol/l NaCl and $H_2O$ were about equally preferred. With the 900 mmol/l concentration the specificity was relatively clear. The movement of the taste preference curve to the right in the sheep, apart from reflecting the particular importance of sodium in the metabolic-digestive organization of this species [6], may also parallel a greater kidney concentrating capacity [13,14]. A number of sheep in our laboratory have been maintained in good health for many months with access only to a 270 mmol/l solution of sodium chloride (unpublished observations), and the same may be true for a 300 mmol/l solution as the upper level of this functional capacity was not explored.

Small amounts of 300 mmol/l KCl solution were drunk on some occasions including the 1 instance of bin position preference. This was not peculiar to any 1 animal. On only one occasion was KCl (300 mmol/l) drunk during the preperiod.

Overall the data of this study indicates that the dipsogenic effect of angiotensin II upon intracarotid infusion is orientated to water and not to sodium intake. This conclusion requires for its demonstration the condition that the choice is between water and saline solution as concentrated as 900 mmol/l. However, under these conditions the Na deficient animal does choose specifically Na solution, and the water depleted animals chooses water, and in the instance of concurrent depletion of water and Na in the rat, the animal will take both [5,18]. However, the specificity of effect is not as strong as with the sudden increase of osmotic pressure of cerebral blood since there is no differential choice between 300 mmol/l NaCl and water – as is also true in the free choice taste preference study. The data here is consistent with earlier published material from this laboratory indicative that intravenous infusion of angiotensin II at high physiological levels does not augment sodium appetite of Na deficient sheep [7], and data of Fitzsimons and Stricker in the rat which is also against a role of the renin-angiotensin system in Na appetite [12].

## REFERENCES


1. Andersson, B., P. A. Jewell and S. Larsson. An appraisal of the effects of diencephalic stimulation of conscious animals in terms of normal behaviour. In: *Neurological Basis of Behaviour,* edited by Wolstenholme. London: Ciba, 1958, p. 76.
2. Abraham, S. F., R. M. Baker, E. H. Blaine, D. A. Denton and M. J. McKinley. Water drinking induced by angiotensin – a physiological or pharmacological effect? *J. comp. physiol. Psychol.* **88:** 503–518, 1975.
3. Baldwin, B. A. and F. R. Bell. The anatomy of the cerebral circulation of the sheep and ox. The distribution of the blood supplied by the carotid and vertebral arteries to cranial regions. *J. Anat.* **97:** 203–215, 1963.
4. Beilharz, S., E. Bott, D. A. Denton and J. R. Sabine. The effect of intracarotid infusions of 4M NaCl on the sodium drinking of sheep with a parotid fistula. *J. Physiol.* **178:** 80–91, 1965.
5. Beilharz, S., D. A. Denton and J. R. Sabine. The effect of concurrent deficiency of water and sodium on the sodium appetite of sheep. *J. Physiol.* **163:** 378–390, 1962.
6. Blair-West, J. R., E. Bott, G. W. Boyd, J. P. Coghlan, D. A. Denton, J. R. Goding, S. Weller, M. Wintour and R. D. Wright. General biological aspects of salivary secretion in ruminants. In: *Physiology of Digestion in the Ruminant,* edited by R. W. Dougherty. Washington: Butterworths, 1965, pp. 198–219.
7. Bott, E., D. A. Denton and S. Weller. The effect of angiotensin II infusion, renal hypertension and nephrectomy on salt appetite of sodium-deficient sheep. *Aus. J. exp. Biol. med. Sci.* **45:** 595–612, 1967.
8. Buggy, J. and A. E. Fisher. Evidence for a dual central role for angiotensin in water and sodium intake. *Nature* **250:** 733–735, 1974.
9. Denton, D. A. and J. R. Sabine. The selective appetite for $Na^+$ shown by $Na^+$-deficient sheep. *J. Physiol.* **157:** 97–116, 1961.
10. Epstein, A. N., J. T. Fitzsimmons and B. J. Rolls. Drinking induced by injection of angiotensin into the brain of the rat. *J. Physiol.* **210:** 457–474, 1970.
11. Fitzsimons, J. T. The role of a renal thirst factor in drinking induced by extracellular stimuli. *J. Physiol.* **201:** 349–368, 1969.
12. Fitzsimons, J. T. and E. M. Stricker. Sodium appetite and the renin-angiotensin system. *Nature* **231:** 58–60, 1971.
13. Gottschalk, C. W. and M. Mylle. Micropuncture study of the mammalian urinary concentrating mechanisms: evidence for the countercurrent hypothesis. *Am. J. Physiol.* **196:** 927–936, 1959.
14. Kinne, R., W. V. Macfarlane and O. E. Budtz-Olsen. Hormones and electrolyte excretion in sheep. *Nature* **192:** 1084–1085, 1961.
15. Pfaffmann, C. The sense of taste. In: *Handbook of Physiology. Neurophysiology,* Vol. 2, edited by J. Field. Washington: American Physiological Society, 1959.
16. Richter, C. P. Salt appetite of mammals; its dependence on instinct and metabolism. In: *L'Instinct dans le Comportement des Animaux et de l'Homme.* Paris: Masson et cie, 1956, pp. 557–632.
17. Wirth, J. B. and J. T. Fitzsimons. The renin-angiotensin system and Na appetite. Proceedings of the International Symposium on Physiology of Food and Fluid Intake. Jerusalem, 1974, p. 138.
18. Wolf, G. and E. M. Stricker. Sodium appetite elicited by hypovolemia in the adrenalectomized rats. Re-evaluation of the "reservoir" hypothesis. *J. comp. physiol. Psychol.* **63:** 252–257, 1967.