Sodium Appetite in Sheep Induced by Cerebral Ventricular Infusion of Angiotensin: Comparison with Sodium Deficiency

Abstract. *Intraventricular administration of supraphysiological amounts of renin, nerve growth factor preparation, or angiotensin II greatly increased the consumption of water and hypertonic sodium bicarbonate solution by sheep. These effects were antagonized by intraventricular administration of drugs that prevent the formation of angiotensin II or block its receptors. The fact that these angiotensin-blocking drugs did not change the sodium intake of sodium-deficient sheep challenges the idea that central angiotensin action is involved in sodium appetite due to a deficiency.*

Recent reports indicate that intracerebral (IC) administration of renin or angiotensin II (AII) causes intense thirst and also salt appetite in rats (*1*). Such effects, apparently caused by an isorenin contaminant of nerve growth factor (NGF) (*2*), have also been observed after IC administration of NGF preparation (2.5*S*) (*3*). Nerve growth factor is prepared from mouse submandibular gland, which is also a rich source of an isorenin (*4*). Although there has been extensive investigation over the last decade of the role of the renin-angiotensin system in water drinking, the effects on sodium intake have received less investigation. Thus it was of interest to determine whether IC administration of renin or AII would cause sodium appetite in sheep, and whether the evidence would point to a cerebral renin-angiotensin system that had a role in the physiological induction of sodium appetite in sodium deficiency.

We used sheep with permanent indwelling cannulas acting as guide tubes for access to the lateral cerebral ventricle (*5*), and infusions at 0.38 ml/hour or injections of 0.25 ml of test substance dissolved in an artificial cerebrospinal fluid (CSF) (*5*) vehicle were made into a lateral ventricle. We confirmed in the sheep, as in the rat, that intraventricular (IVT) administration of 0.025 Goldblatt units of partially pure sheep renin (1 U/mg) elicits rapid water drinking within 10 minutes and after a longer latency (6 to 12 hours) increases intake of a hypertonic sodium solution. A lower dosage of renin had no effect on sodium appetite or thirst (Table 1). The effects of IVT renin were blocked by central administration of the converting enzyme inhibitor teprotide (SQ 20881; Squibb) (Table 1). Both effects were also attenuated or blocked by IVT infusion of saralasin (Norwich Pharmacal; P113) at 10 or 30 μg/hour. Intraventricular AII (Hypertensin, Ciba; 1.9 μg/hour for 24 hours) more than doubled water intake but had no effect on sodium appetite despite the fact that within only 5 minutes the concentration of AII entering the third ventricle probably exceeded by a hundredfold the highest physiological concentration measured in sheep CSF (*6*). Infusion of 3.8 μg of AII per hour for 24 hours more than doubled daily intake of $NaHCO_3$ and water. This effect on sodium appetite was not reduced by IVT teprotide (100 μg every 6 hours for 24 hours) but was eliminated by IVT saralasin (30 μg/hour for 24 hours) (Table 1). The increased water intake with this dose of saralasin, but not with 10 μg/hour, may represent agonist activity on water intake.

Sheep were also injected intraventricularly with 50 μg of 2.5*S* NGF or 0.025 U of sheep renin; 75 minutes later CSF was withdrawn and assayed for AII (*7*). Concentrations of AII were elevated above the normal concentration in CSF of 15.1 ± 8.9 fmole/ml (N = 18) to 639 ± 119 fmole/ml with IVT NGF and to 816 ± 161 fmole/ml with IVT renin. On another day IVT injection of 50 μg of 2.5*S* NGF caused rapid water drinking within 5 to 20 minutes and large sodium appetite after 6 to 18 hours (Table 1). Both effects were abolished by IVT administration of teprotide each 6 hours (Table 1). The 2.5*S* NGF used in these experiments and those of Lewis *et al.* (*2*) was prepared by the method of Bochini and Angeletti (*8*) and is contaminated by mouse submandibular gland isorenin (*4*).

Table 1. Effect on mean intake of 0.6*M* $NaHCO_3$ or water of IVT injection of renin or nerve growth factor preparation or IVT infusion of angiotensin II. These agents have been administered with or without IVT treatment with converting enzyme inhibitor injections (teprotide, 100 μg each 6 hours for 24 hours) or IVT infusions of saralasin (10 or 30 μg/hour for 24 hours). Baseline values represent the mean intake on the days before treatment (mean ± standard error of the mean). Data were analyzed by two-way analysis of variance and subsequent *t*-test. N.S., not significant.

Treatment	Sheep (No.)	Mean intake of 0.6*M* $NaHCO_3$ (millimoles per 24 hours)	Mean intake of water (milliliters per 24 hours)
Baseline	10	331 ± 122	1780 ± 127
0.0025 unit of renin	5	426 ± 193 N.S.	1610 ± 152 N.S.
Baseline	14	407 ± 65	1802 ± 99
0.025 unit of renin	7	979 ± 112*	3611 ± 564*
Baseline	8	551 ± 91	1681 ± 77
0.1 unit of renin	4	1432 ± 262*	6013 ± 469*
Baseline	10	395 ± 72	1715 ± 116
0.025 unit of renin + 100 μg of teprotide per 6 hours	5	210 ± 66†	1350 ± 127 N.S.
Baseline	10	258 ± 51	1770 ± 310
0.025 unit of renin + 10 μg of saralasin per hour	5	559 ± 153†	2130 ± 536 N.S.
Baseline	10	183 ± 63	2462 ± 252
50 μg of NGF	5	1266 ± 103*	6310 ± 1026*
Baseline	12	460 ± 88	1814 ± 176
50 μg of NGF + 100 μg of teprotide per 6 hours	6	287 ± 119 N.S.	1366 ± 83 N.S.
Baseline	4	135 ± 53	1450 ± 215
1.9 μg of AII per hour	4	180 ± 122 N.S.	4150 ± 618‡
Baseline	18	344 ± 67	1822 ± 179
3.8 μg of AII per hour	9	780 ± 242‡	4205 ± 570*
Baseline	10	210 ± 74	1812 ± 110
3.8 μg of AII per hour + 100 μg of teprotide per 6 hours	5	817 ± 264*	4455 ± 863*
Baseline	8	179 ± 60	1638 ± 137
3.8 μg of AII per hour + 10 μg of saralasin per hour	4	585 ± 220†	2388 ± 613 N.S.
Baseline	10	234 ± 68	1300 ± 59
3.8 μg of AII per hour + 30 μg of teprotide per hour	5	426 ± 137 N.S.	1980 ± 313‡

*P = .001. †P = .05. ‡P = .01.

0036-8075/81/1009-0195$01.00/0

The dipsogenic- and salt appetite–inducing actions of centrally administered crude NGF preparations in sheep, rats (*3*), and dogs (*9*) therefore appear to be due to generation of AII after the action of an isorenin contaminant of the NGF preparation.

There is early evidence against renal renin as a determinant of salt appetite in sodium deficiency (*10*). In sheep with IVT renin or AII there is a time delay of 6 to 18 hours before maximum effect on sodium appetite. In both instances, the concentrations in CSF required to induce salt appetite results in AII concentration therein at least a hundredfold greater than that measured in severe sodium deficiency (*11*). On the basis of a liberal estimate of the volume of the third ventricle of the rat, or volume of injection (1 μl) in the instance of cranial cannulas, the concentrations of AII contrived in the rat brain were probably

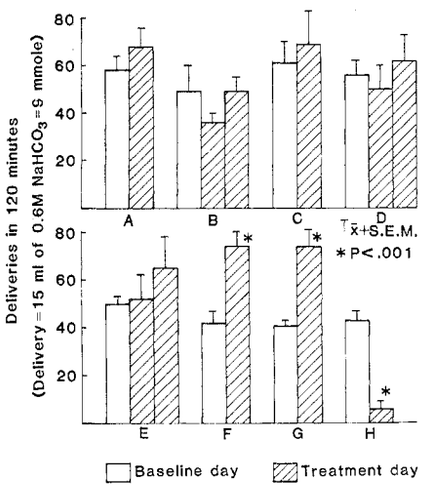

Fig. 1. The effect of various treatments on the bar-pressing behavior of sodium-deficient sheep. Animals were made deficient by loss of saliva for 22 hours and were then allowed to bar press for 0.6*M* $NaHCO_3$ (15 ml per delivery = 9 mmole). The baseline shown by the open bar is the number of deliveries on the preceding day. Treatments (shown by the hatched bar) were (A) injection of 100 μg of teprotide into a lateral ventricle every 6 hours for 24 hours (N = 4); (B) injection of captopril (20 μg each 6 hours for 48 hours) into a lateral ventricle (N = 6); (C) infusion of saralasin (10 μg/hour for 24 hours) into a lateral ventricle (N = 4); (D) infusion of saralasin (30 μg/hour for 48 hours) into a lateral ventricle (N = 5); (E) infusion of angiotensin II (3.8 μg/hour for 48 hours) into a lateral ventricle (N = 5); (F) sodium deprivation for 48 hours (N = 7); (G) infusion of 0.7*M* mannitol-CSF at 1 ml/hour for 3 hours into a lateral ventricle beginning 1 hour prior to bar pressing (N = 6); and (H) administration of a sodium load of 600 mmole of $NaHCO_3$ into the rumen 3 hours prior to bar pressing. Mean and standard error of the mean are shown. Statistical analyses were made by two-way analysis of variance (repeated measures design) or t-test, or both.

massive (*12*) in the experiments of Fitzsimons and Wirth (*10*) and of Avrith and Fitzsimons (*1*).

The possibility exists that AII measured in CSF is locally generated in the brain; persistence of AII in CSF after nephrectomy supports this (*13*). Accordingly, the very high concentration in CSF required for appetite effect may reflect that required to cause appropriate concentration at a distant site, such as is generated by local production under some stimulus associated with sodium deficiency.

An alternative explanation is that a precursor of AII is directly synthesized in neurons and that AII acts as the neurotransmitter or a modulator of peptidergic neurons (*14*), which subserve salt appetite. The massive IVT doses required may reflect penetration at sufficient concentration to a distant synaptic site in the brain substance, whereas the rapid action of AII on thirst may be because of the juxtaventricular situation of the reactive elements.

Our next step was to probe the relevance of angiotensin-induced sodium appetite to physiological salt appetite caused by sodium deficiency. The blocking, by saralasin infusion, of thirst and salt appetite effects caused by IVT renin in sheep and rats (*2*) shows that the competitive antagonist can effectively pass to sites where AII acts. Sheep with parotid fistulas were made sodium-deficient by a 22-hour salivary loss (deficits, 350 to 550 mmole of sodium). The sheep were then allowed to bar press for $NaHCO_3$ for 2 hours (each delivery to drinking cup was 15 ml of 600 m*M* $NaHCO_3$, which is equivalent to 9 mmole of sodium). The effect of IVT administration of drugs that block the renin-angiotensin system was investigated in these sheep. The IVT infusion of saralasin at 10 or 30 μg/hour for 24 hours had no effect on sodium or water intake during sodium deficiency (Fig. 1). In addition to giving saralasin at the dosage that over a period of 24 or 48 hours blocked the effect of IVT AII, teprotide was given IVT for 24 hours with the same procedure that blocked completely the effect of IVT renin or 2.5*S* NGF. Similarly, IVT teprotide or captopril (SQ 14225; Squibb) had no effect on sodium or water intake in sodium deficiency (Fig. 1).

In addition to these findings, sodium-depleted animals responded predictably to physiological manipulation of sodium status. If 1.0 liter of 600 m*M* $NaHCO_3$ was given into the rumen by tube 3 to 4 hours before bar pressing, mean intake was reduced to a very low level (Fig. 1). Giving 1.0 liter of water in the same manner on the three preceding days had no effect. Allowing the animals to become sodium-depleted for 46 hours instead of 22 hours almost doubled the number of bar-press deliveries (Fig. 1). Furthermore, IVT infusion of isotonic (*15*) or 0.7*M* mannitol in artificial CSF begun 60 minutes beforehand (which lowered sodium concentration in CSF by 10 to 20 mmole/liter) acted to double sodium intake (Fig. 1), whereas an increase of sodium concentration in CSF by 10 to 20 mmole/liter, produced by IVT infusion (at 1 ml/hour) of 500 m*M* NaCl in artificial CSF, more than halved sodium intake (*15*). However, IVT AII (3.8 μg/hour for 48 hours) in sodium-depleted animals had no influence on salt appetite in 22 hours and caused an increase that was not statistically significant in the 22- to 46-hour period (Fig. 1). There was a significant effect on intake of water which was continuously available. The IVT AII infusion given to the same fistulated animals, when maintained sodium-replete by immediate pumping of saliva back into the rumen, similarly caused a large increase of water intake and increase of sodium intake, the increase of sodium intake being significant during the access period after 48 hours of infusion.

Although physiological change of sodi-

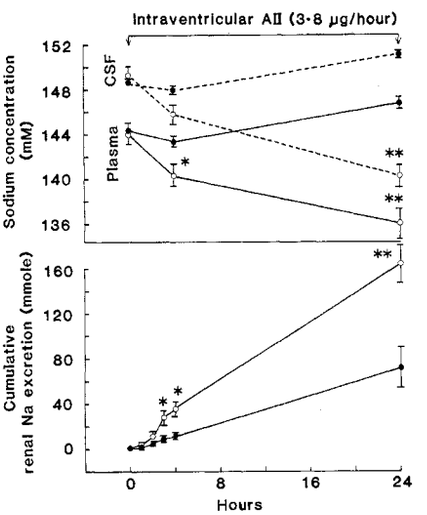

Fig. 2. Effect of infusion of angiotensin II (○) (3.8 μg/hour for 24 hours) (N = 7) or control artificial CSF (●) (N = 5), at 0.38 ml/hour, into a lateral ventricle on sodium concentration of plasma and CSF and on the renal sodium excretion of water-restricted sheep. Animals were allowed access to food and 0.5 liter of water 4 hours after the start of the IVT infusions. Normal daily water intake of these sheep was 2 liters. Mean and standard error of the mean are shown. Comparison of the effects of IVT AII with control IVT artificial CSF by Student's t-test is denoted by $^*P < .05$ and $^{**}P < .01$.

um status produced a marked change of appetite, as expected, the IVT infusions of blocking agents of the renin-angiotensin system at doses that eliminate effects of IVT renin and NGF had no significant quantitative effect on salt appetite in response to sodium deficiency. In addition, AII added to CSF of sodium-deficient sheep did not cause further increase in sodium intake, despite the fact that more severe sodium depletion did. By contrast, lowering the concentration of sodium in CSF with IVT mannitol consistently and rapidly doubles sodium intake (*15*).

While not being completely conclusive, these data suggest that the sodium appetite caused by IVT renin or AII may not be a physiological regulatory action. It could result from direct pharmacological actions of AII or be secondary to effects AII may have on systemic sodium balance and ionic concentrations.

Thus, the data with IVT renin and AII may reflect the effect of AII in vitro in altering a variety of ionic fluxes between cells and tissue fluid (*16*), including sodium fluxes between extracellular and intracellular fluid, as described for jejunum and skin (*17*). In supraphysiological concentration, the induction of such sodium movements in the neuronal systems subserving sodium appetite may mimic events occurring physiologically with sodium deficiency. Such ionic movements within the physiological range may also be contrived by IVT infusion of mannitol (*15*), a proven stimulus for sodium appetite in sheep.

We have measured the sodium concentration of CSF and plasma in sodium-replete sheep that were treated with IVT AII (3.8 μg/hour) and had access to water and 0.6*M* $NaHCO_3$. The CSF and plasma sodium concentration fell 11 ± 1 and 13.5 ± 2.6 m*M* (N = 4), respectively, over 24 hours. This occurred also in control experiments in which AII-treated animals were subjected to water restriction (0.6 liter in the 24 hours) and were not allowed access to 0.6*M* $NaHCO_3$. Therefore, the fall of the sodium concentration is not due solely to increased water intake (Fig. 2). Because IVT AII causes a rapid onset of natriuresis in which the sodium concentration of the urine is usually greater than that of plasma, this phenomenon will contribute to lowering the sodium concentration of CSF and plasma caused by IVT AII (Fig. 2). The increased water drinking and vasopressin release attributable to AII will augment this effect. In the light of our recent data in sheep showing the powerful sodium appetite–inducing effect of lowering the sodium concentration of CSF (*15*), this may be an important consideration in sodium appetite induced by IVT renin or AII. Caution is urged before assigning any primary role for cerebral AII in the physiological induction of sodium appetite in sodium deficiency. Evidence over and above results derived from introduction of pharmacological amounts of renin and angiotensin into the CSF or brain substance will be required to give a credible basis to such a hypothesis.

J. P. COGHLAN, P. J. CONSIDINE
D. A. DENTON, D. T. W. FEI
L. G. LEKSELL,* M. J. MCKINLEY
A. F. MULLER,† EVA TARJAN‡
R. S. WEISINGER
Howard Florey Institute of Experimental Physiology and Medicine, University of Melbourne, Parkville, Victoria, 3052, Australia
R. A. BRADSHAW
Department of Biological Chemistry, Washington University School of Medicine, St. Louis, Missouri 63110

References and Notes

1. D. B. Avrith and J. T. Fitzsimons, *J. Physiol. (London)* **282**, 40 (1978); *ibid.* **301**, 349 (1980).
2. D. B. Avrith, M. E. Lewis, J. T. Fitzsimons, *Nature (London)* **285**, 248 (1980).
3. M. S. Perkins, D. L. Margules, I. L. Ward, *Brain Res.* **161**, 351 (1979); M. E. Lewis, D. B. Avrith, J. T. Fitzsimons, *Nature (London)* **279**, 440 (1979).
4. C. Cozzari, P. U. Angeletti, J. Lazar, H. Orth, F. Gross, *Biochem. Pharmacol.* **22**, 1321 (1973).
5. D. R. Mouw, S. F. Abraham, J. S. McKenzie, *Lab. Anim. Sci.* **24**, 505 (1974).
6. The volume of CSF in a lateral ventricle of a sheep based on measurements we have made on ventricular casts of sheep is 2 to 3 ml, CSF production rate is approximately 12 ml/hour [S. R. Heisy, D. Held, J. R. Pappenheimer, *Am. J. Physiol.* **203**, 775 (1962)] and there is almost no angiotensinase activity in CSF [I. A. Reid, in *Regulation of Blood Pressure by the Central Nervous System*, G. Onesti, M. Fernandez, K. E. Kim, Eds. (Grune & Stratton, New York, 1976), p. 161]. Infusion of AII into a lateral ventricle at 1.9 μg/hour would give an AII concentration of at least 12,000 pg/ml after 5 minutes of infusion. The highest AII concentration measured in sheep CSF during severe sodium deficiency is 120 pg/ml (*11*).
7. D. T. W. Fei, J. P. Coghlan, R. T. Fernley, B. A. Scoggins, *Clin. Exp. Pharmacol. Physiol.* **6**, 129 (1979).
8. V. Bochini and P. U. Angeletti, *Proc. Natl. Acad. Sci. U.S.A.* **64**, 787 (1969).
9. D. J. Ramsay, T. N. Thrasher, L. C. Keil, in *Proceedings of the International Union of Physiological Sciences* (Hungarian Physiological Society, Budapest, 1980), vol. 14, p. 655.
10. E. Bott, D. A. Denton, J. R. Sabine, *Aust. J. Exp. Biol. Med. Sci.* **45**, 595 (1967); J. T. Fitzsimons and E. M. Stricker, *Nature (London)* **231**, 58 (1971); J. T. Fitzsimons and J. B. Wirth, *J. Physiol.* **274**, 63 (1978).
11. S. F. Abraham, R. M. Baker, E. H. Blaine, D. A. Denton, M. J. McKinley, *J. Comp. Physiol. Psychol.* **88**, 503 (1975); S. F. Abraham *et al.*, in *Abstracts* (Sixth International Congress on Endocrinology, Melbourne, 1980), p. 725.
12. The lowest rate of infusion of AII into the third ventricle that increased sodium intake was 16 fmole/min (16 pg/min). The volume of the third ventricle of the rat is 1 to 2 μl based on a stereotaxic atlas of rat brain [J. F. R. König and R. A. Klippel, *The Rat Brain* (Williams & Wilkins, Baltimore, 1963)]. After 2 minutes of infusion the concentration of AII may be about 16,000 pg/ml. Concentrations of AII up to 359 pg/ml have been measured in rat CSF [J. B. Simpson, W. A. Saad, A. N. Epstein, in *Regulation of Blood Pressure by the Central Nervous System*, G. Onesti *et al.*, Eds. (Grune & Stratton, New York, 1976), pp. 191–202].
13. D. Ganten, J. S. Hutchinson, P. Schelling, U. Ganten, *Clin. Exp. Pharmacol. Physiol.* **2**, 103 (1976).
14. K. Fuxe, D. Ganten, T. Hokfelt, P. Bohme, *Neurosci. Lett.* **2**, 229 (1976).
15. R. S. Weisinger, P. Considine, D. A. Denton, M. J. McKinley, D. R. Mouw, *Nature (London)* **280**, 490 (1979).
16. P. Vecsei, E. Hackenthal, D. Ganten, *Klin. Wochenschr.* **56** (Suppl. 1) (1978).
17. A. D. Crocker and K. A. Munday, *J. Physiol. (London)* **206**, 323 (1970); R. D. McAfee and W. Locke, *Endocrinology* **81**, 1301 (1967).
18. This work was supported by a grant from the National Health and Medical Research Council of Australia, and by the Robert J., Jr., and Helen C. Kleberg Foundation.
* Permanent address: Department of Physiology, Royal Karolinska Institute, Stockholm.
† Permanent address: Department of Medicine, Hôpital Cantonal, Geneva.
‡ Permanent address: Department of Physiology, Semmelweis Medical University, Budapest.

4 February 1981

Vitamin D–Dependent Calcium Binding Protein: Immunocytochemical Localization in Chick Kidney

Abstract. *A vitamin D–dependent calcium binding protein in the chick kidney that was detected by immunocytochemical techniques was localized exclusively in the distal convoluted tubule, the initial collecting tubule, and the early part of the collecting tubule. The intercalated (mitochondria-rich) cells in these tubular segments were negative for the calcium binding protein. Subcellularly, the protein was found in the cytosol and the nucleus of the tubular cells. The results suggest a role for vitamin D–dependent calcium binding protein in intracellular calcium metabolism rather than a direct involvement in membrane-mediated calcium reabsorption in the avian kidney.*

The maintenance of the calcium and phosphorus homeostasis is essential for the normal functioning of cells and tissues (*1*) and principally involves the integrated actions of the intestine, bone, and kidney. This is accomplished by an endocrine system in which the peptide hormones calcitonin and parathyroid hormone (PTH) interact with vitamin D and its two chief biologically active metabolites 1,25-dihydroxyvitamin D_3 [1,25$(OH)_2D_3$] and 24,25-dihydroxyvitamin D_3 [24,25$(OH)_2D_3$] (*2, 3*). Both of these dihydroxylated metabolites are produced in the proximal tubule of the avian (*4*) and mammalian kidney (*5*). 1,25$(OH)_2D_3$

0036-8075/81/1009-0197$01.00/0