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Atrial Natriuretic Factor Inhibits Vasopressin Secretion in Conscious Sheep (42544)

JONGEUN LEE, RICHARD L. MALVIN, JOHN R. CLAYBAUGH, AND BING S. HUANG

Department of Physiology, The University of Michigan, Ann Arbor, Michigan 48109; and Department of Clinical Investigation, Tripler Army Medical Center, Honolulu, Hawaii 96859

Abstract. To test the hypothesis that atrial natriuretic factor (ANF) has a centrally mediated action on body fluid homeostasis, the effects of intracerebroventricularly (ICV) infused ANF on plasma vasopressin (AVP) concentration and urinary water and electrolyte excretion were investigated in euhydrated and water-deprived conscious sheep. ICV ANF decreased plasma AVP concentration and increased urinary free water excretion in euhydrated sheep, with excretion of Na and K unaltered. However, ICV ANF did not affect urinary volume, free water clearance, or excretion of Na and K in dehydrated animals, although plasma AVP concentration was significantly decreased. The relationship between urine volume and plasma AVP concentration was fitted by a power curve: urine volume = $0.79 \cdot [\text{AVP}]^{-0.71}$; urine volume changes very little as a function of AVP concentration at the higher ranges. Intravenous infusion of the same amount of ANF was without effect on plasma AVP concentration or urinary excretion in both euhydrated and dehydrated animals. Mean arterial pressure was unchanged throughout all experiments. These results are consistent with the hypothesis that central ANF inhibits AVP secretion.

Atrial natriuretic factor (ANF)-like immunoreactivity in brain regions (1, 2) suggests a central role for this factor. Indeed, evidence for an inhibitory effect of ANF on vasopressin (AVP) secretion was recently shown in the rat (3–5). In contrast, an ANF-induced release of AVP from the isolated posterior lobe of the rat hypophysis was also reported (6).

Although these reports are in conflict, they are in agreement with a possible role for ANF in the regulation of AVP secretion. It is difficult to evaluate the physiological effectiveness of ANF in terms of body fluid homeostasis, since they were done without measuring renal excretion.

The present study was done to evaluate whether ANF has a central action in the regulation of body fluid homeostasis. Changes in urinary excretion and the concentration of AVP in plasma in response to intracerebroventricular (ICV) infusion of ANF were observed in conscious sheep.

Materials and Methods. *Animal preparations.* Nine female sheep (*Ovis aries,* 50–65 kg) were used. They were fed commercial sheep pellets and hay. A chronic indwelling cannula was placed in the right lateral cerebral ventricle as described previously (7). A skin loop containing segments of common carotid artery and jugular vein was constructed in the neck as described by McDonald *et al.* (8).

Experimental protocols. Normally hydrated animals, which had been allowed free access to water, were used for one set of experiments. Artificial cerebrospinal fluid (ACSF) was infused ICV at the rate of 40 μl/min throughout the protocol. During the experimental periods, ANF (atriopeptin III, Peninsula; 100 pmole/min) dissolved in the ACSF vehicle was infused either ICV ($N = 8$) or IV ($N = 4$).

In the dehydration group, the sheep were deprived of water for 20 to 24 hr. The experimental protocol for this group was the same as that for the euhydrated group. They had ANF-infused ICV ($N = 7$) or IV ($N = 4$).

No animal was used more than once in the same set of experiments. A minimum of 3 weeks separated any two experiments in the same animal.

On the day of experiment, the animal was loosely restrained in a sling, and a Foley catheter was inserted into the bladder to collect the urine samples. Cannulas were placed in the common carotid artery and jugular vein

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to allow continuous monitoring of mean arterial pressure and intravenous infusion, respectively.

ICV infusion of ACSF was started and an equilibration period of no less than 1 hr was allowed until the first basal clearance period began. Each period lasted 20 min. Four experimental periods were bracketed by two basal and three recovery periods.

At the end of each period, urine was collected by flushing the bladder with 20 ml of distilled water followed by 30 ml of air injected through the urinary catheter.

Blood samples (8 ml) were taken at the midpoint of each period from the common carotid artery and were immediately replaced by an equal volume of saline. Due to technical problems, mean arterial pressure could not be monitored and the blood samples were taken from the jugular vein in 3 of the 23 experiments: one from the euhydrated ICV group, and the other two from the dehydrated ICV group. Samples were centrifuged at 4°C, and the plasma was kept at −20°C until analyzed. The first two experimental and the first recovery samples were not analyzed for AVP.

Analytical techniques. Plasma concentrations of AVP were determined by radioimmunoassay as described previously (9). Briefly, 4 ml of heparinized plasma, to which 0.4 ml of 1 *N* HCl had been added prior to freezing, was extracted on octadecylsilane cartridges (Sep-Pak C18; Waters Associates, Milford, MA). The recovery of added AVP to sheep plasma was 88.3 ± 2.3% (mean ± SE, $N = 7$). There were no corrections made for recoveries. The within- and between-assay coefficients of variability were 5.2 and 10.8%, respectively. All samples from one sheep were assayed in a single assay. With this volume of plasma extracted, the limit of sensitivity of the assay was 0.16 μU/ml of plasma. The AVP standard used was purchased from Sigma (St. Louis, MO).

Na and K concentrations were measured by flame photometry, and osmolalities by freezing point depression.

Statistics. The two basal measurements were averaged to obtain a single basal value in each experiment. Results for grouped values were expressed as means ± SE. Statistical significance was determined using a paired *t* test to compare the experimental and recovery values to their own basal values, and a profile analysis was used to compare differences between the groups over time. Computed probabilities were adjusted by the Bonferroni correction factor where applicable. A probability of less than 0.05 was considered significant.

Results. *Effects of ANF on urinary excretion and blood pressure.* The alterations in urine volume and free water clearance following the infusion of ANF in euhydrated sheep are presented in Fig. 1. A diuretic response parallelled by an increase in free water clearance was observed in the ICV-infused group. In contrast, IV infusion of the same dose of ANF had no effect on urine volume or free water clearance.

Figure 2 presents the data for the dehydrated group. Neither IV nor ICV infusion of ANF had any effect on urinary water excretion.

Neither IV nor ICV infusion of ANF in both euhydrated and dehydrated sheep altered urinary excretion of Na and K, or mean arterial pressure (Table I).

Effects of ANF on plasma AVP concentration. Figure 3 depicts the plasma concentrations of AVP before, during, and after ANF infusion. AVP levels measured at the fourth

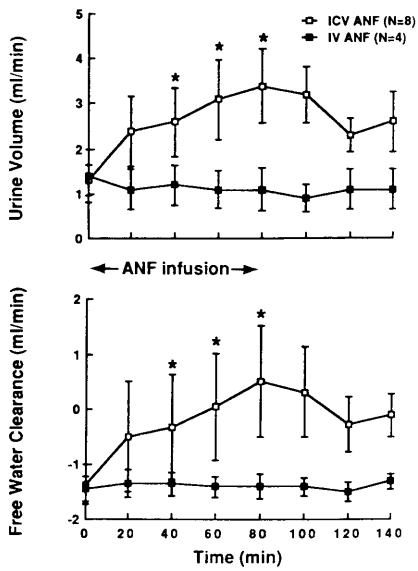

FIG. 1. Effect of atrial natriuretic factor on urine volume and free water clearance in euhydrated sheep. ANF (100 pmole/min) was infused for 80 min either ICV or IV. *N* = number of animals. *$P < 0.05$, profile analysis between the groups.

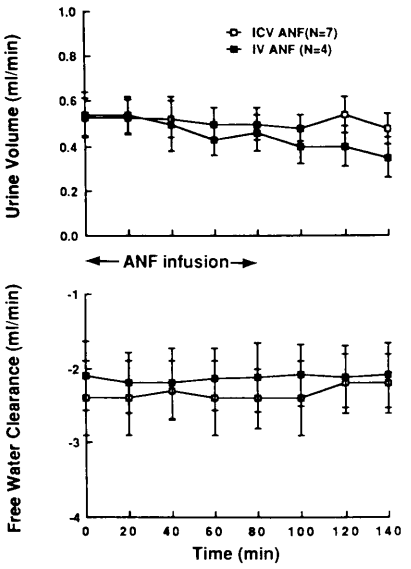

FIG. 2. Effect of ANF on urine volume and free water clearance in dehydrated sheep. Legend as in Fig. 1.

period during ICV infusion of ANF showed a significant reduction in both euhydrated (top panel) and dehydrated sheep (bottom panel). After termination of the infusion of ANF, the AVP values returned toward the basal values.

Figure 4 presents the data obtained relating urine volume to the simultaneous concentration of AVP in plasma. The relationship is clearly not linear and can be fitted reasonably well to a power function, where urine volume $= 0.79 \cdot [\text{AVP}]^{-0.71}$ ($r = 0.69$, $P < 0.001$).

Discussion. Our results show that centrally infused ANF decreases the concentration of AVP in plasma of both euhydrated and water-deprived conscious sheep. An associated increase in urinary free water excretion was shown in euhydrated animals, with urinary excretion of Na and K unaltered.

These findings provide direct evidence of a central inhibitory effect of ANF on the basal rate of secretion of AVP, since the same amount of ANF was without effect when infused IV. In addition, the diuretic response to ICV ANF was not associated with any alterations in mean arterial pressure. Taken together with the presence of ANF-containing neurons in the brain (1, 2), our results are consistent with the hypothesis that an endogenous ANF system in the brain modulates AVP secretion.

TABLE I. EFFECTS OF INTRACEREBROVENTRICULAR AND INTRAVENOUS INFUSION OF ANF ON URINARY EXCRETION OF Na AND K, AND MEAN ARTERIAL PRESSURE[a]

			Experimental (min)		Recovery (min)	
	Group	Basal	40–60	60–80	20–40	40–60
			Euhydrated groups			
UNaV (μeq/min)	ICV ($N = 8$)[b]	44 ± 34	39 ± 21	35 ± 19	34 ± 16	30 ± 14
	IV ($N = 4$)	16 ± 12	13 ± 9	15 ± 12	25 ± 17	26 ± 15
UKV (μeq/min)	ICV ($N = 8$)	131 ± 24	117 ± 24	114 ± 24	112 ± 21	121 ± 21
	IV ($N = 4$)	178 ± 46	141 ± 32	111 ± 23	90 ± 15	73 ± 17
MAP (mm Hg)	ICV ($N = 7$)	96 ± 3	95 ± 3	95 ± 3	95 ± 3	95 ± 3
	IV ($N = 4$)	98 ± 2	98 ± 2	98 ± 2	98 ± 2	97 ± 2
			Dehydrated groups			
UNaV (μeq/min)	ICV ($N = 7$)	23 ± 20	19 ± 15	9 ± 5	15 ± 10	19 ± 10
	IV ($N = 4$)	13 ± 2	12 ± 2	14 ± 3	13 ± 3	13 ± 3
UKV (μeq/min)	ICV ($N = 7$)	199 ± 47	150 ± 43	163 ± 55	138 ± 53	127 ± 42
	IV ($N = 4$)	181 ± 28	167 ± 29	172 ± 27	175 ± 36	183 ± 30
MAP (mm Hg)[c]	ICV ($N = 5$)	97 ± 2	96 ± 2	97 ± 2	96 ± 3	95 ± 3
	IV ($N = 4$)	96 ± 2	96 ± 2	94 ± 2	95 ± 2	95 ± 2

[a] Values are means ± SE.
[b] N = number of animals.
[c] MAP, mean arterial pressure.

Indeed, the inhibitory effect of ANF on AVP secretion has recently been reported in rats both *in vivo* (3, 4, 10) and *in vitro* (5). In a teleological sense, it is reasonable for ANF to have an overall antagonistic effect on mechanisms leading to expansion of extracellular fluid volume, including a reduction in AVP concentration. One would not expect a stimulatory effect of ANF on AVP secretion as was shown in the isolated posterior lobe of the rat hypophysis (6).

Our study not only confirms the inhibitory effect of ANF on basal or stimulated AVP secretion in conscious sheep, but also adds evidence of a centrally mediated renal effect. Previous studies which reported decreases in AVP secretion were done without measuring renal excretion. Thus, it was not possible to evaluate the physiological effectiveness of ANF in terms of the body fluid homeostasis. In the present study, the decrease in the concentration of AVP in plasma is in accordance with the diuretic response in euhydrated animals, suggesting that the hormonal change is of functional significance.

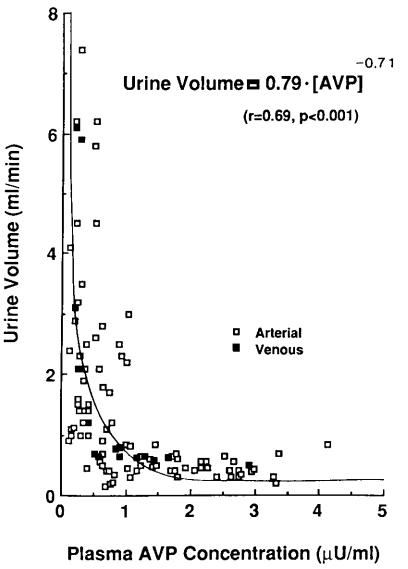

FIG. 4. Relation between urine volume and plasma AVP concentration. Each point represents urine volume during a 20-min collection period and the plasma AVP concentration at the midpoint of that period. Filled squares represent the plasma samples taken from the jugular vein, and empty squares represent the samples taken from the common carotid artery.

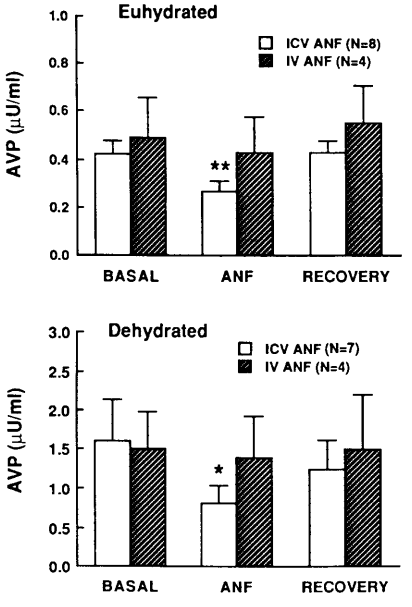

FIG. 3. Effect of ANF on plasma concentration of AVP in euhydrated (top panel) and dehydrated sheep (bottom panel). Basal, the last ANF-infused, and the last recovery period values are shown. $*P < 0.05$, $**P < 0.01$; paired t test compared to the basal values.

However, although the plasma AVP levels decreased significantly in the dehydration group, there was no appreciable diuretic effect. This is most likely a reflection of the nonlinear relationship between urine volume and plasma AVP concentration as shown in Fig. 4: urine volume changes very little as a function of AVP concentration at higher ranges. A similar curve was previously reported in anesthetized dogs (11).

In summary, our study demonstrates a central inhibitory effect of ANF on the basal secretory rate of AVP. This was shown by both a decreased plasma AVP concentration and an associated diuretic response following ANF infusion centrally, but not intravenously. The centrally mediated renal action of ANF may be functionally obscured by a preexisting fluid state of the individual, such as dehydration, in spite of the fall in the plasma concentration of AVP.

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1. Jakobowitz DM, Skofitsch G, Keiser HR, Eskay RL, Zamir N. Evidence for the existence of atrial natriuretic factor-containing neurons in the rat brain. Neuroendocrinology **40**:92–94, 1985.
2. Saper CB, Standaert DG, Currie MG, Schwartz D, Geller DM, Needleman P. Atriopeptin-immunoreactive neurons in the brain: Presence in the cardiovascular regulatory areas. Science **227**:1047–1049, 1985.
3. Samson WK. Atrial natriuretic factor inhibits dehydration and hemorrhage induced vasopressin release. Neuroendocrinology **40**:277–279, 1985.
4. Iitake K, Share L, Crofton JT, Brooks DP, Ouchi Y, Blaine EH. Central atrial natriuretic factor reduces vasopressin secretion in the rat. Endocrinology **119**: 438–440, 1986.
5. Obana K, Naruse M, Inagami T, Brown AB, Naruse K, Kurimoto F, Sakurai H, Demura H, Shizume K. Atrial natriuretic factor inhibits vasopressin secretion from rat posterior pituitary. Biochem Biophys Res Commun **132**:1088–1094, 1985.
6. Januszewicz P, Gutkowska J, Lean AD, Thibault G, Garcia R, Genest J, Cantin M. Synthetic atrial natriuretic factor induces release (possibly receptor-mediated) of vasopressin from rat posterior pituitary. Proc Soc Exp Biol Med **178**:321–325, 1985.
7. Brooks VL, Malvin RL. An intracerebral, physiological role for angiotensin: Effects of central blockade. Fed Proc **38**:2272–2275, 1979.
8. McDonald IR, Goding JR, Wright RD. Transplantation of the adrenal gland of the sheep to provide access to its blood supply. Aust J Exp Biol **36**:83–96, 1958.
9. Shiraki K, Konda N, Sagawa S, Claybaugh JR, Hong SK. Cardiorenal–endocrine responses to head-out immersion at night. J Appl Physiol **60**:176–183, 1986.
10. Yamada T, Nakao K, Morii N, Itoh H, Shiono S, Sakamoto M, Saito Y, Ohno H, Kanai A, Katsuura G, Eigyo M, Matsushita A, Imura H. Central effect of atrial natriuretic polypeptide on angiotensin II-stimulated vasopressin secretion in conscious rats. Eur J Pharmacol **125**:453–456, 1986.
11. Reid IA, Ahn JN, Trinh T, Shackelford R, Weintraub M, Keil LC. Mechanism of suppression of vasopressin and adrenocorticotropic hormone secretion by clonidine in anesthetized dogs. J Pharmacol Exp Ther **229**: 1–8, 1984.

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