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Role of Natriuretic Factor in Central Nervous System (CNS)-Induced Natriuresis (42044)

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Abstract. The presence of a natriuretic factor in the plasma of rats in which a 350 m*M* Na (high Na) artificial cerebrospinal fluid (CSF) was infused into the lateral ventricle was tested. Blood was obtained from control rats and rats which received an infusion of high Na CSF intraventricular (IVT) for 15 min. The plasma was incubated for 30 min at room temperature, acidified, placed in a boiling-water bath, and then centrifuged. The plasma supernate was assayed for natriuretic activity in pentobarbital anesthetized bioassay rats. Sodium excretion increased 6.5 ± 1.1 μeq/kg · min in rats which received an infusion of a control saline solution, 13.3 ± 3.2 μeq/kg · min in rats which received infusion of control plasma supernates, and 32.1 ± 8.3 μeq/kg · min in those rats which received plasma supernates from rats infused with high Na CSF IVT. Blood pressure was unchanged in all groups. The increment in sodium excretion elicited by plasma supernate from the high Na IVT group was significantly greater than that elicited by either control saline solution or control plasma extracts. Therefore, it is concluded that a heat-stable and nonpressor natriuretic factor is present in the plasma of rats infused IVT with high Na CSF.

Considerable evidence has indicated that a humoral factor or "natriuretic hormone" may be involved in the physiological control of sodium excretion (1). This evidence includes the demonstration of natriuretic activity in both plasma and urine extracts using a rat bioassay preparation. Other evidence includes the demonstration that plasma or urine extracts inhibit Na transport and Na/K-ATPase activity *in vitro,* which is consistent with the hypothesis that "natriuretic hormone" inhibits the tubular reabsorption of sodium. One group has demonstrated a factor in the plasma of volume-expanded animals which causes a natriuresis in assay rats (2). Further purification of the plasma from volume-expanded animals revealed a fraction containing a heat-stable factor which inhibits Na transport (3) and hog brain ATPase *in vitro* (4). Another group has presented evidence for a heat-stable circulating inhibitor of vascular Na/K-ATPase in volume-expanded animals (5).

Other observations have indicated that the central nervous system (CNS) may play an important role in the control of sodium excretion. One of these observations is that intraventricular infusion of a hypertonic artificial cerebrospinal fluid (CSF) results in a natriuresis (6–8). In rats the natriuresis appears to be mediated by a hormone other than antidiuretic hormone, aldosterone, or angiotensin II (8). We hypothesized that the humoral factor which mediates this CNS-induced natriuresis is "natriuretic hormone." The following experiments test the hypothesis that a heat-stable humoral factor is involved in the natriuresis which follows intraventricular (IVT) infusion of hypertonic CSF in rats. Extracts were prepared from plasma of control rats and rats infused with high Na CSF IVT, and the natriuretic activities of the extracts were tested in bioassay rats.

Materials and Methods. Male Sprague–Dawley rats (260–420 g) were used, both as bioassay animals and plasma donors. Rats were fed Purina Rat Chow (1% NaCl) and tap water *ad libitum.* In some of the rats, a chronic guide cannula was implanted above the right lateral ventricle at least 1 week prior to the experiment, as previously described (8). On the day of the experiment, all rats were anesthetized with sodium pentobarbital (50 mg/kg ip).

Donor rats. Two groups of plasma donor rats were used: (a) nontreated rats, and (b) rats in which an artificial high Na (350 m*M* Na) CSF was infused into the lateral ventricle (IVT). The ionic composition of the CSF was (in m*M*) 350 Na, 333 Cl, 3.1 K, 1.2 Ca, 1 Mg, 24.5 HCO_3, and 5.0 glucose. The CSF was infused for 15 min at a rate of 3.3 μl/min. The rats were then bled by aortic puncture (8–10 ml), into a heparinized syringe.

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The blood was processed by methods similar to the initial purification steps described by Gruber and Buckalew (3). It was centrifuged at 4°C and 1600*g* for 10 min, and the plasma was separated and left at room temperature for 30 min in order to potentiate any natriuretic activity (3). An equal volume of distilled water was then added to the plasma and the pH adjusted to 5.5 with 10% acetic acid. The plasma was placed in a boiling-water bath for 5 min, in order to precipitate large proteins, and centrifuged (1600*g*) at 4°C for 60–150 min. The supernate was then brought to room temperature and infused into a bioassay rat.

Bioassay rats. Catheters were implanted in the bladder, jugular vein, and in some cases, carotid artery. Following surgery, and during the entire protocol, anesthesia was maintained by infusing sodium pentobarbital (88 μg/min) in isotonic saline at a rate of 7 μl/min. In rats with carotid arterial catheters, blood pressure was monitored throughout the experiment by connecting the catheters to a Statham pressure transducer. Urine was collected during a 1-hr control period, at 15-min intervals. Plasma supernate (6–7 ml) was then infused into the jugular vein at a rate of 3.3 ml/min. Urine collections were continued for 1 hr. Three groups of bioassay rats were used. Rats in the first group received a plasma supernate from a control rat, and rats in the second group received a plasma supernate from a rat which had been infused with high Na CSF IVT. A third group received a similar infusion of a control saline solution which had been diluted 1:1 with distilled water. The composition of the diluted control saline solution was 75 m*M* Na, 67 m*M* Cl, 1.5 m*M* K, 0.6 m*M* Ca, 0.5 m*M* Mg, 22.3 m*M* HCO_3, and 0.25 m*M* H_2PO_4.

Na and K analysis. Na and K concentrations of urine, plasma supernates, and the control saline solution were determined by flame photometry.

Statistical analysis. Comparisons between group means were performed using analysis of variance with pairwise comparison of group means. A value of $P < 0.05$ was regarded as significant.

Results. The effects of infusion of either a control saline solution, control plasma supernate, or plasma supernate from a rat infused with high Na CSF IVT on Na, K, and water excretion rates are shown in Figs. 1, 2, and 3 respectively. Sodium excretion increased following the infusion in all groups, and was greatest from 60 to 75 min, the period in which the supernates were infused. The increment in sodium excretion over the average control value which occurred in rats receiving plasma supernates from the high Na IVT group was significantly greater than in rats receiving either the control saline solution ($P = 0.004$) or supernates of control plasma ($P = 0.016$). However, the increments in sodium excretion of rats receiving control saline solution and those receiving supernates of control plasma were not significantly different from each other ($P = 0.42$).

Potassium excretion and urine flow also increased in all groups following infusion of either plasma supernate or control saline solution, and was greatest during the 15-min period in which the supernates were infused. Potassium excretion was not increased differently between the three groups of bioassay rats ($P = .068$). In contrast, the increase in urine flow elicited by the plasma supernates from rats which were infused IVT with high Na CSF was significantly different from the increase elicited by either control saline solution ($P = 0.016$), or control plasma super-

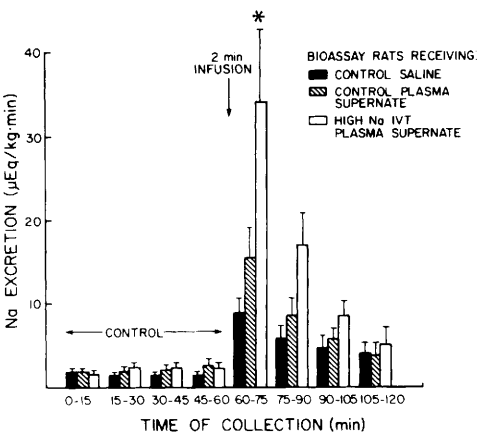

FIG. 1. Sodium excretion of bioassay rats (Mean ± SEM). Either a control saline solution ($n = 7$), control plasma supernate ($n = 13$), or plasma supernate from a rat infused with high Na CSF IVT ($n = 12$) was rapidly infused at 60 min. *$P < 0.02$ significantly different from control plasma supernate.

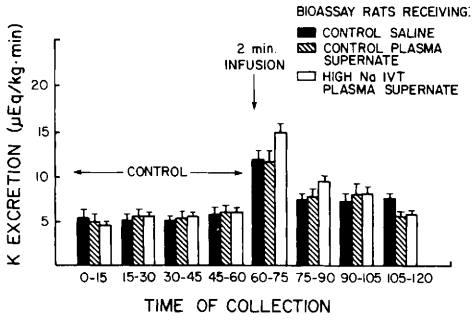

FIG. 2. Potassium excretion of bioassay rats (Mean ± SEM). Either a control saline solution (n = 7), control plasma supernate (n = 13), or plasma supernate from a rat infused with high Na CSF IVT (n = 12) was rapidly infused at 60 min.

nates (P = 0.035). The increase in urine flow was not significantly different between those rats receiving control saline solution and those receiving control plasma supernates (P = 0.59). Average Na and K concentrations of the infusates were 72.3 meq/liter Na, 1.89 meq/liter K and were not significantly different between the three groups.

Table I shows the effect of infusion of plasma supernates on blood pressure. Blood pressure remained unchanged in all three groups, indicating that the natriuresis was not due to an increase in renal perfusion pressure.

Discussion. Previous studies indicated that the natriuresis elicited by IVT infusion of hypertonic CSF in rats is mediated by an

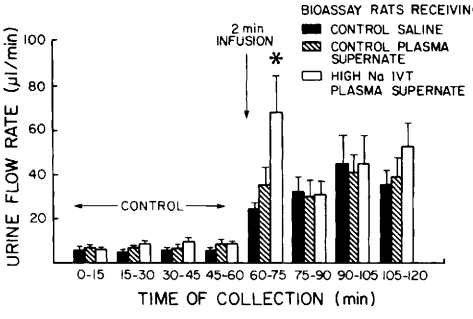

FIG. 3. Urine flow of bioassay rats (Mean ± SEM). Either a control saline solution (n = 7), control plasma supernate (n = 13), or plasma supernate from a rat infused with high Na CSF IVT (n = 12) was rapidly infused at 60 min. *P < 0.04 significantly different from control plasma supernate.

TABLE I. EFFECT OF INFUSION OF PLASMA SUPERNATES ON BLOOD PRESSURE OF BIOASSAY RATS

Urine collection period	Mean arterial pressure (mm Hg)		
	Saline	Control	High Na
1	128 ± 4	141 ± 2	138 ± 4
2	128 ± 4	141 ± 3	140 ± 3
3	128 ± 3	141 ± 3	141 ± 2
4	128 ± 5	142 ± 4	140 ± 4
5	127 ± 5	138 ± 7	142 ± 6
6	123 ± 5	141 ± 6	144 ± 3
7	121 ± 5	140 ± 7	144 ± 2
8	127 ± 6	138 ± 6	129 ± 9

Note. Values are means ± SE. Urine collection periods lasted 15 min. A control saline solution or plasma supernate was infused during period 5. Saline-control saline solution (n = 7); control = control plasma supernate (n = 6); high Na = plasma supernate from rats infused with high Na CSF IVT (n = 7).

unknown humoral factor(s) (8). In those studies it was shown that the natriuresis was not mediated by the renal nerves, antidiuretic hormone, aldosterone, angiotensin II, or increases in renal perfusion pressure. The present studies provide direct evidence for the presence of a humoral natriuretic factor in the plasma of rats which have been infused IVT with 350 mM Na artificial CSF. The factor was not detectable in the plasma of control rats. The increase in sodium excretion following infusion of supernates of control plasma was not significantly different from the increase which followed the infusion of a control saline solution of similar ionic composition. In contrast, infusion of a plasma supernate from rats which had been infused with high Na CSF IVT caused a significantly greater increase in sodium excretion than either the plasma supernates obtained from nontreated rats or the control saline solution. Plasma supernates were obtained by diluting, acidifying, boiling, and centrifugation. Such results provide direct evidence that a heat-stable factor is present in the plasma of rats infused IVT with high Na CSF. In addition, these studies indicate that this humoral factor does not increase sodium excretion via an elevation of renal perfusion pressure, since mean arterial pressure was not affected by infusion of the plasma extract.

Other investigators have demonstrated na-

triuretic factor by measuring the sodium excretion of a bioassay rat following injection of either urine, whole plasma, or a plasma extract obtained from a donor animal (2, 9–13). Using the rat bioassay, natriuretic factor has been demonstrated in the plasma and urine of uremic patients (9, 10), normal men undergoing water immersion (11), and salt-loaded man and animals (12, 13). The present study is the first demonstration of a natriuretic factor in animals infused with hypertonic solutions IVT. This natriuretic factor was released as a result of stimulation of the central nervous system and not volume expansion, as the total amount of CSF infused was minimal (50 μl).

Previous studies failed to detect natriuretic factor in the urine of anesthetized dogs undergoing ventriculocisternal perfusion with high Na CSF (7). The urine was extracted by the methods of Bourgoignie *et al.* (9) and tested for inhibition of short-circuit current in the toad bladder. These techniques were not only different from those used in the present study but may also test for a different natriuretic factor. However, the apparent discrepancy between the two studies could also be due to species differences. In support of this hypothesis, evidence has been presented that antidiuretic hormone mediates at least a portion of the natriuresis in dogs (14), but not in rats (8).

In previous studies using the rat bioassay model, the assay animal was sensitized by methods such as partial nephrectomy (2, 9–11), salt loading (9–11), water loading (2, 12, 13), or using rats with mild diabetes insipidus (13). The bioassay rats used in this study had both kidneys intact and were not volume expanded prior to the injection. However, it should be noted that a considerable amount of half isotonic plasma extract was infused (6–7 ml, or approximately 2% of body weight). Thus, the natriuretic effect of the humoral factor was coupled with the natriuretic effect of volume expansion. It is likely that both stimuli contributed to the increase in sodium excretion.

Our plasma extracts were prepared using methods similar to those previously used by other investigators. Gruber *et al.* have demonstrated that plasma extracts partially purified by high-pressure liquid chromatography inhibit Na transport in toad bladder (3) and hog brain Na/K-ATPase *in vitro* (4). These properties of the plasma fraction are thought to be due to the same humoral factor which is natriuretic in the bioassay rat (2). In the present study we used methods similar to the initial processing steps used by Gruber *et al.* Another group has presented evidence for a circulating inhibitor of vascular Na/K-ATPase in both volume-expanded (5) and hypertensive animals (15, 16) using plasma extracts prepared by similar methods. The partially purified plasma fraction isolated by Gruber *et al.* (4) also increases the sensitivity of the vasculature to pressor agents *in vivo* (17), providing a link between natriuretic factor and hypertension.

It is not known whether the humoral factor which is found in plasma following infusion of hypertonic CSF IVT and that released during volume expansion are one and the same. However, it should be noted that the central nervous system appears to play a role in the natriuresis following volume expansion. Bealer *et al.* (18) have found that the natriuresis induced by volume expansion is attenuated in rats with anteroventral third ventricular (AV3V) lesions, while the appearance of a factor in plasma which inhibits Na transport in toad bladder is also blocked. In addition, rats with AV3V lesions do not respond with a natriuresis during infusion of hypertonic CSF IVT (19).

Other putative natriuretic factors have been identified in tissues such as bovine hypothalamus (20) and atrial tissue (21). The factor present in bovine hypothalamus inhibits renal Na/K-ATPase activity and Na transport across toad bladder (20), whereas that found in atrial tissue does not (22, 23). It is not known whether the factor released by infusion of high Na CSF IVT inhibits Na/K-ATPase, but clearly this is of interest. It is interesting to note that the factor present in atrial tissue increases GFR (24, 25), an effect which is similar to infusion of hypertonic CSF IVT (8, 19).

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