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Evidence for Hormonal Participation in the Natriuretic and Kaliuretic Responses to Intraventricular Hypertonic Saline and Norepinephrine[1] (39336)

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The microinjection of hypertonic sodium chloride solution into the third ventricle elicits natriuresis, kaliuresis, and a decline in urine volume in several species (1-4). Natriuresis also follows the intraventricular injection of carbachol (5) or norepinephrine (4), suggesting that adrenergic and cholinergic synapses may participate in the pathway concerned with the elicitation of central natriuresis and kaliuresis. The mechanism by which these agents induce increased excretion of monovalent cations has not been resolved. To determine if pituitary or hypothalamic hormones might play a role in mediating these responses, lesions were placed in the median eminence of the tuber cinereum of rats and the effects of the lesions on the response to third-ventricular injection of hypertonic saline and norepinephrine were studied.

Materials and methods. Male Holtzman rats (250-350 g) were housed communally under conditions of controlled temperature and lighting, and were given free access to tap water and laboratory chow. Using ether anesthesia, bilateral electrolytic lesions were placed in the median eminence region using cathodal current (3 mA) passed for 17 sec as previously described (6). Sham lesions were placed by inserting the electrodes bilaterally to a depth of 5 mm beneath the calvarium without passage of current. After at least 5 days the animals were anesthetized with ether and subjected to the second series of procedures (cannulation of the third ventricule, jugular vein, and urinary bladder) as previously described (4). They were used for the experiments 1-2 days later. Daily water intake was measured in sham-operated rats and those with median eminence lesions.

For the experimental procedure, the animals were placed in individual metabolic cages and an infusion into the external jugular vein of 5% dextrose (0.19 ml/min) was begun. Urine samples were collected at 15-min intervals and when the rate of urine flow had reached a plateau, 2 μl of 2 *M* NaCl or 5 μg of norepinephrine bitartrate in 2 μl of 0.9% NaCl were injected into the third ventricle. This was followed by the collection of an additional six urine samples. Urine volume and Na^+ and K^+ were measured in each sample. The site and extent of the lesion was determined by microscopic examination of frozen frontal hypothalamic sections cut at 15-μm and stained with thionin.

Significance of differences between means of two groups was determined by Student's *t* test.

Results. Effect of median eminence lesions on the response to hypertonic saline. An increase in the sodium concentration of the cerebrospinal fluid (CSF) elicits a natriuretic effect in the conscious animal (4). This effect was still observed when hypertonic saline (2 μl of 2 *M* NaCl) was injected into the third ventricle in the animals with sham lesions (Fig. 1); however, destruction of the median eminence region prevented this natriuresis. Likewise, there was no increase in potassium excretion following the intraventricular injection of hypertonic saline into the rats with electrolytic lesions. The increase in CSF sodium concentration was effective in producing an antidiuresis in both groups although the degree of antidiuresis was significantly greater in the sham-operated animals.

Effect of median eminence lesions on the response to norepinephrine. Third ventricular injection of the neurotransmitter, norepinephrine, produced a natriuresis, kaliuresis, and antidiuresis in normal (4) or

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sham-operated animals (Fig. 2). Median eminence lesions were effective in preventing the increases in sodium and potassium excretion and in reducing the antidiuretic response elicited by this catecholamine.

Localization of the lesions. Thionin-stained sections of the brains of rats with lesions were examined microscopically to determine the extent and the site of the damage. The rostral limit of the lesions was the optic chiasm and they extended caudally so as to destroy 1/2 to 2/3 of the median eminence. Damage to the wall of the ventricle was variable; however, in some animals, it appeared to be largely intact. There was considerable damage to the anterior part of the arcuate nucleus and variable damage to the ventromedial nucleus.

Daily water intake was increased after lesions in all animals. On the seventh post-

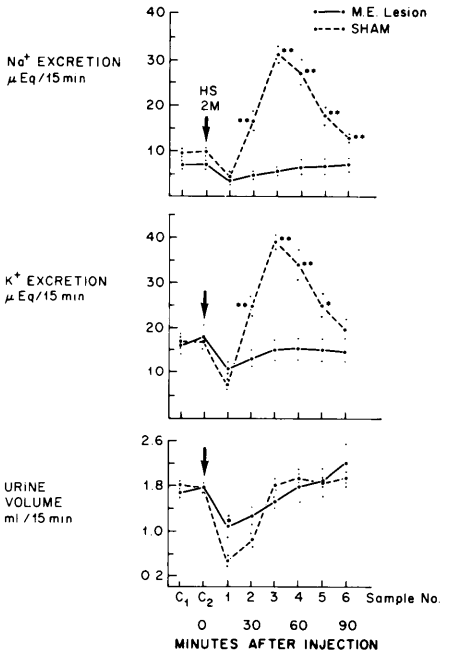

FIG. 1. Effect of median eminence lesions on the natriuresis, kaliuresis, and urine output following injection of hypertonic saline (HS) (2 *M* NaCl, 2 μl) into the third ventricle. Values for sham-operated rats are indicated by · – – ·, whereas those from rats with lesions are indicated by · — ·. Standard errors are indicated by the small dots above and below each mean. * = $p < 0.05$ vs the sham-operated animals; ** = $p < 0.01$ vs sham-operated controls. $N = 8$ for the group with lesions, and 7 for the sham-operated controls.

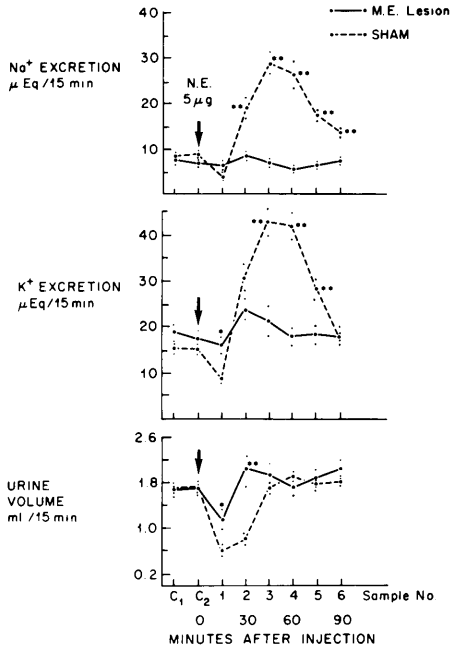

FIG. 2. Effects of median eminence lesions on the natriuresis, kaliuresis, and urine output after the injection of norepinephrine (NE) (5 μg, 2 μl) into the third ventricle. $N = 12$ for rats with lesions, and 8 for sham-operated controls.

operative day, water intake in sham-operated animals was 32.6 ± 4.3 (8)[2] ml/day, whereas in rats with median eminence lesions, it was 139.1 ± 4.0 (8) ml/day. Thus, the rats with lesions had diabetes insipidus as a result of at least partial interruption of the supraopticohypophyseal tract.

Discussion. The results of this study clearly show that damage to the median eminence region was effective in abolishing the natriuresis and kaliuresis produced by the third ventricular injection of either hypertonic saline or norepinephrine and in significantly reducing the antidiuresis which ensued. Even though a decrease in urine volume still followed these injections in animals with lesions, there was no increase in either the excretion or concentration (not shown) of sodium or potassium ions.

Since these lesions are known to produce interference with the release of a number of pituitary hormones, it is logical to suggest that the failure to release one or more of these hormones may be responsible for the

[2] Mean ± SE (number of rats).

absence of natriuresis and kaliuresis following intraventricular injection of hypertonic saline or norepinephrine. Previous studies have shown that lesions of this type interfere with the release of ACTH (7), growth hormone (8), TSH (9), FSH and LH (6), whereas they enhance the release of prolactin (6) and perhaps that of MSH (10). Intraventricular hypertonic saline has not been shown to be capable of altering release of any adenohypophyseal hormone; however, intraventricular norepinephrine has been reported to stimulate gonadotropin and possibly prolactin release (see 11 for review of the literature). Neither of these hormones has been shown to be natriuretic in the rat.

In addition to producing these alterations in adenohypophyseal hormone secretion, these lesions by their interruption of the supraopticohypophyseal tract also interfere with the release of both oxytocin (12) and vasopressin (13).

The evidence for interruption of the supraopticohypophyseal tract was the presence of diabetes insipidus in all of the rats with lesions. We have previously shown that similar lesions block suckling-induced release of oxytocin (12). Even though the animals had diabetes insipidus, the urinary output following infusion of 5% glucose solution was similar in animals with lesions and in the sham-operated controls. Presumably, this was because of the induction of a water diuresis in response to the infusions. Thus, although the absence of these responses following the lesions suggests hormonal participation, there is a wide range of possibilities as to which hormone or hormones may be involved in mediating the natriuretic responses in the intact animal. Antidiuretic hormone (ADH), oxytocin, and melanocyte stimulating hormone (MSH) are all natriuretic under the conditions of a water diuresis similar to those employed in the present experiment (14, 15).

ADH does not appear to be essential for the production of central natriuresis since it still occurred in animals with hereditary diabetes insipidus and the complete absence of ADH (16). Increases in the blood levels of ADH have been observed following intraventricular injection of norepinephrine in the rat (17) and the direct application of hypertonic saline or norepinephrine to the supraoptic nucleus of the cat (18). In view of the retention of the natriuretic response in the rat with hereditary diabetes insipidus and the fact that large doses of pitressin did not influence the urinary response to intraventricular hypertonic saline (17), it appears that ADH does not play an essential role in central natiuresis.

It is quite possible that an increased release of oxytocin, MSH and/or the proposed natriuretic hormone (19) may be involved in the mediation of central natriuretic effects; however, the natriuretic response to intraventricular carbachol persisted in hypophysectomized rats which should be completely deficient in MSH and largely deficient in oxytocin (5). Thus, the results are most consistent with mediation of the natriuretic response by a natriuretic hormone (19).

The possibility that the lesions may interrupt a neural pathway distinct from their effects on pituitary hormonal secretion cannot be ruled out. Central natriuresis has also been explained on the basis of alterations in blood pressure, glomerular filtration rate, or renal neural input; however, we have previously reported a lack of correlation between blood pressure changes and the natriuretic effects of hypertonic saline and norepinephrine (20).

Summary. To examine if hypothalamic or pituitary hormones are involved in the induction of the natriuresis which follows the injection of hypertonic saline or norepinephrine into the third ventricle, lesions were placed in the median eminence and the responses to intraventricular norepinephrine or hypertonic saline were evaluated. Sham lesions in which the electrode was lowered into the brain but stopped short of the hypothalamic region did not interfere with the natriuresis, kaliuresis, and antidiuresis induced by the third ventricular injection of either hypertonic sodium chloride or norepinephrine. Lesions in the median eminence which induced diabetes insipidus as evidenced by an increase in water consumption to approximately four times normal completely abolished the natriuresis and kaliuresis in response to intraventricular hypertonic saline or norepinephrine and di-

minished the antidiuresis. The observations suggest the possibility that a natriuretic hormone(s) is involved in the induction of central natriuresis.

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