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Oxytocin mediates atrial natriuretic peptide release and natriuresis after volume expansion in the rat

(vasopressin/kaliuresis/diuresis/urine osmolality)

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ABSTRACT **Our previous studies have shown that stimulation of the anterior ventral third ventricular region increases atrial natriuretic peptide (ANP) release, whereas lesions of this structure, the median eminence, or removal of the neural lobe of the pituitary block ANP release induced by blood volume expansion (BVE). These results indicate that participation of the central nervous system is crucial in these responses, possibly through mediation by neurohypophysial hormones. In the present research we investigated the possible role of oxytocin, one of the two principal neurohypophysial hormones, in the mediation of ANP release. Oxytocin (1–10 nmol) injected i.p. caused significant, dose-dependent increases in urinary osmolality, natriuresis, and kaliuresis. A delayed antidiuretic effect was also observed. Plasma ANP concentrations increased nearly 4-fold ($P < 0.01$) 20 min after i.p. oxytocin (10 nmol), but there was no change in plasma ANP values in control rats. When oxytocin (1 or 10 nmol) was injected i.v., it also induced a dose-related increase in plasma ANP at 5 min ($P < 0.001$). BVE by intra-atrial injection of isotonic saline induced a rapid (5 min postinjection) increase in plasma oxytocin and ANP concentrations and a concomitant decrease in plasma arginine vasopressin concentration. Results were similar with hypertonic volume expansion, except that this induced a transient (5 min) increase in plasma arginine vasopressin. The findings are consistent with the hypothesis that baroreceptor activation of the central nervous system by BVE stimulates the release of oxytocin from the neurohypophysis. This oxytocin then circulates to the right atrium to induce release of ANP, which circulates to the kidney and induces natriuresis and diuresis, which restore body fluid volume to normal levels.**

Our previous studies have shown that the central nervous system controls atrial natriuretic peptide (ANP) release; osmotic, cholinergic, and noradrenergic stimulation of the anterior ventral third ventricular (AV3V) region induces ANP release (1). Conversely, lesions of the AV3V region decreased resting plasma ANP concentrations and largely blocked ANP release in response to blood volume expansion (BVE) (2). Neurons containing ANP, termed ANPergic neurons, have their perikarya in the AV3V region and axons that project to the median eminence and neural lobe of the pituitary gland (3–5). These appear critical to the volume expansion-induced release of ANP since antisera directed against ANP injected into the third ventricular region of rats (6) or sheep (7) can inhibit volume expansion-induced ANP release.

Lesions of the median eminence or neural lobe of the pituitary gland, which interrupt neuronal pathways projecting from the AV3V region to the neurohypophysis, blocked volume expansion-induced ANP release (2). Therefore, we hypothesized that release of one or more neuropeptides from the neurohypophysis caused the increase in ANP release after volume expansion. As indicated above, the axons of ANP neurons terminate in the neurohypophysis, and these could contribute to the ANP release from volume expansion. However, these neurons are unlikely to provide sufficient ANP to account for the volume expansion-induced release since their ANP content is 1000 times less than that of the right atrium (1). Consequently, we hypothesized that the ANP neurons might release another peptide from the neurohypophysis. Both vasopressin and oxytocin are stored in large amounts in the neural lobe of the pituitary and are the prime candidates to be released into the venous drainage of the neurohypophysis by volume expansion. These polypeptides could circulate to the atria to release ANP. Decreases in blood volume, such as occur with hemorrhage, stimulate vasopressin release via baroreceptor input to the brain stem (8). Therefore, one would predict that volume expansion would not elevate, and perhaps would suppress, vasopressin release. Therefore, we hypothesized that hypothalamic ANP neurons cause release of oxytocin, which triggers the release of ANP from the atria. Indeed, we report here that volume expansion causes a rapid increase in plasma oxytocin as well as ANP and that oxytocin can induce ANP release, which is followed by natriuresis. Furthermore, isotonic volume expansion decreased plasma vasopressin concentrations. The results support the hypothesis that volume expansion-induced ANP release and natriuresis are caused by release of oxytocin, which stimulates ANP release that in turn induces natriuresis.

MATERIALS AND METHODS

Animals. Adult male Wistar rats, weighing 250–330 g, were housed individually at room temperature (23–25°C), on a 12-hr light/12-hr dark cycle (lights on 0700 hr); unless otherwise stated, the animals had free access to rat chow and tap water. Experiments were conducted between 0800 and 1200 hr.

Urine Excretion Experiments. For the experiments measuring urinary excretion, to decrease stress influences, the rats were handled daily and trained for the gavage for 1 week prior to the experiments.

Experimental Schedule. After 14 hr of food deprivation, the animals were weighed, and water overloads (5% of body weight, 37°C) were administered by gavage, at 0 and 60 min, after which they were placed into individual metabolic cages without access to water and food. Six urine samples were then collected at 20-min intervals over a period of 120 min. Com-

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Abbreviations: ANP, atrial natriuretic peptide; AV3V, anterior ventral third ventricular; BVE, blood volume expansion; AVP, arginine vasopressin.

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plete voiding of urine was manually induced by gently pressing the suprapubic region of the animal at the end of each interval. After collection of the first urine sample, oxytocin (0.1, 1, or 10 nmol per rat in 0.5 ml of 0.15 M NaCl) or 0.15 M NaCl (0.5 ml per rat) was injected i.p.

ANP Experiments. *Effect of i.p. oxytocin on plasma ANP.* Another group of rats, kept under the same experimental conditions, were killed by decapitation just prior to (0 min) or 5, 20, and 40 min after i.p. injection of oxytocin (10 nmol in 0.5 ml of saline) or saline, 20 min after the second water overload. Trunk blood was collected in cooled test tubes containing proteolytic enzyme inhibitors (2 mg of EDTA, 20 μl of 1 mM phenylmethylsulfonyl fluoride, and 20 μl of 500 μM pepstatin A). The plasma was separated by centrifugation (4°C) and stored at −70°C until measurement of ANP by RIA (1).

Effect of i.v. oxytocin on ANP. The effect of i.v. oxytocin was determined in animals that were not water-loaded. Twenty-four hours before the experiments, a catheter was inserted into the right external jugular vein and advanced to the right atrium (9). Oxytocin (0.1, 1.0, or 10 nmol) or saline diluent was injected i.v., and the animals were killed by decapitation just prior to (0 min) or 5 or 15 min after injection. Trunk blood was collected for plasma ANP determination.

Effect of BVE Expansion. In rats with intra-atrial catheters, blood volume was expanded as described (2) by injection of 2 ml of hypertonic (0.3 M) or isotonic (0.15 M) saline per 100 g of body weight through the atrial catheter over 1 min. The animals were killed by decapitation, and the blood was collected as described above for measurement of plasma oxytocin, vasopressin, and ANP concentrations by RIA.

Urine Analysis. Urine volume was measured to the nearest 0.1 ml. Sodium and potassium concentrations were measured with a flame photometer (Micronal; model b262), and osmolality was measured by an osmometer (Fiske ostm), based on the freezing-point method. Na^+ (UNaV) and K^+ (UKV) excretion were expressed as the product of concentration (U) of the ion and urine flow per min (V).

Determination of ANP, Oxytocin, and Vasopressin. Plasma ANP was extracted by vycor glass and determined by specific RIA as described (10).

Oxytocin and vasopressin were measured using RIA kits (Peninsula Laboratories).

Statistical Analysis. Statistical significance was determined by analysis of variance for repeated measures followed by the Student–Newman–Keuls test for comparisons of individual means.

RESULTS

Effects of i.p. Injection of Oxytocin on Urinary Sodium, Potassium, Osmolarity, and Volume. In water-loaded rats undergoing diuresis, oxytocin induced a significant ($P < 0.01$), dose-related increase in Na^+ and K^+ excretion, as well as in urine osmolarity (Fig. 1 *A–C*). The lowest (0.1 nmol) dose was ineffective (data not shown). A dose–response relationship was evident by 20 min with sodium and by 40 min with

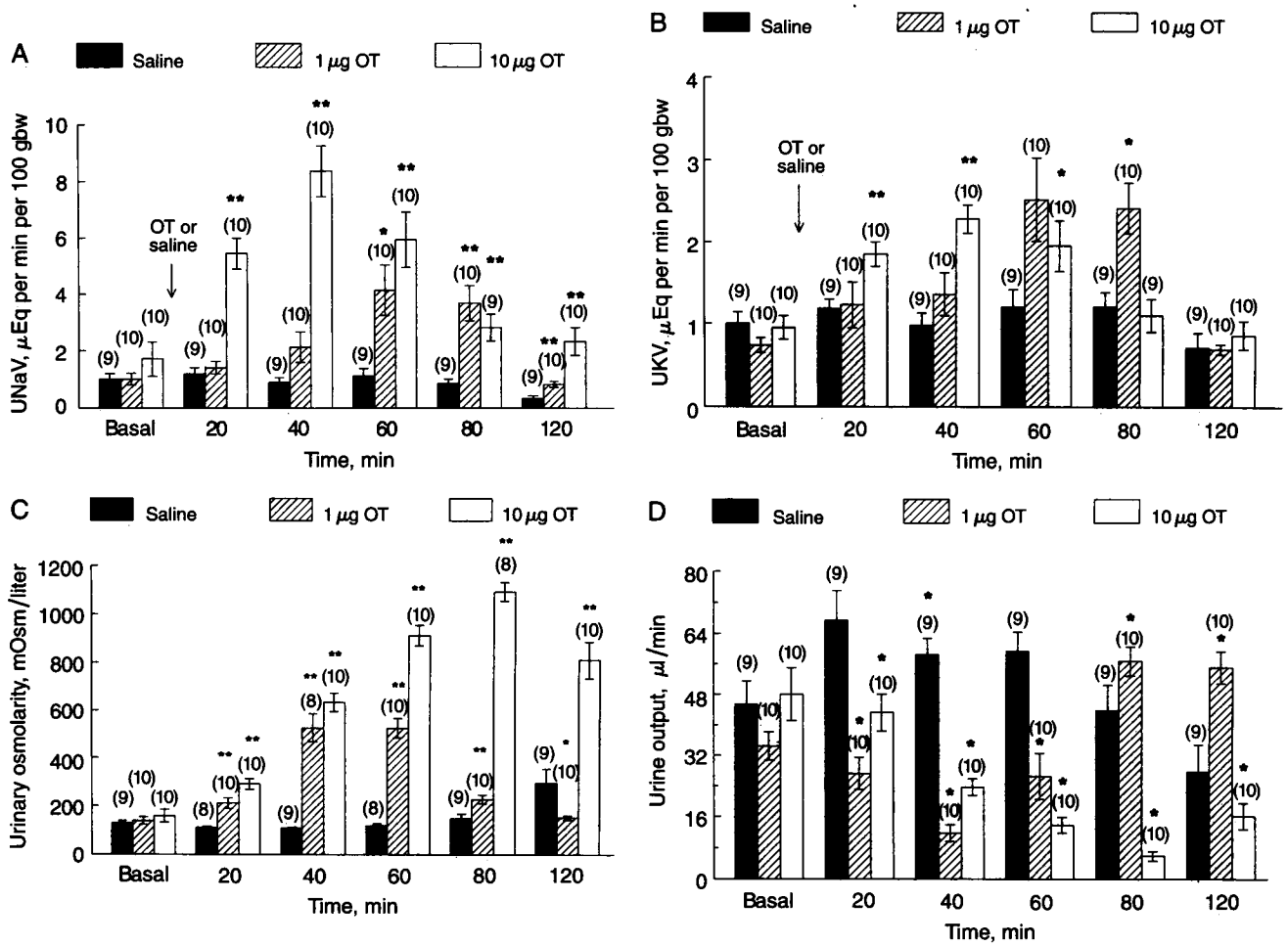

FIG. 1. Na^+ excretion (*A*), K^+ excretion (*B*), urine osmolarity (*C*), and urine flow (*D*) in water-loaded rats after i.p. injection of 0.15 M NaCl (Saline) (control; $n = 9$), 1.0 μg (1.0 nmol) of oxytocin (OT) ($n = 10$), or 10.0 μg of oxytocin ($n = 10$). ★, $P < 0.05$; ★★, $P < 0.01$ compared to 0.15 M NaCl injection. UNaV and UKV, Na^+ and K^+ excretion, respectively; bw, body weight; μEq, microequivalents.

potassium. The natriuresis was maximal at 40 min. The kaliuretic response was smaller in magnitude than that of sodium. There was also a dose-related decrease in urine volume, which was maximal at 40 min with the 1.0 nmol (1.0 μg) dose and at 80 min for the 10.0 nmol dose (Fig. 1*D*). Control injections of isotonic saline did not induce any significant changes in the values for urine volume, Na^+ and K^+ excretion, or urine osmolarity.

Effect of i.p. Oxytocin on Plasma ANP. Plasma ANP concentrations increased significantly ($P < 0.01$) and maximally at 20 min after i.p. injection of oxytocin (10 nmol), which induced the greatest natriuretic response (Fig. 2). In saline-injected rats (controls), there were no significant changes in plasma ANP.

Effect of i.v. Oxytocin on Plasma ANP. In rats that were not water-loaded, there was a dose-related increase ($P < 0.001$) in plasma ANP concentrations at 5 min after i.v. injection. The minimally effective dose was 1 nmol (Fig. 3). The plasma ANP levels remained elevated for 15 min only with the highest dose (10 nmol) of oxytocin. No changes in plasma ANP levels were observed in control rats.

The Effect of BVE on Plasma Oxytocin Concentration. BVE is a well-known stimulus for ANP release (2, 8), and we wished to determine the effect of BVE on release of oxytocin, the putative stimulator of ANP release. BVE with either isotonic or hypertonic saline induced a rapid increase in plasma oxytocin concentration that was highly significant at 5 min (Fig. 4). Although the release of oxytocin was greater with the hypertonic saline solution, it was not significantly greater than that caused by 0.15 M NaCl. By 15 min, plasma oxytocin levels returned to basal in the case of isotonic saline but were still significantly elevated ($P < 0.05$) after hypertonic saline-induced volume expansion. The results are expressed in terms of picograms per milliliter of plasma, which is equivalent to the picomolar concentration. Previous experiments have shown that these isotonic or hypertonic volume expansions are associated with nearly equivalent increases in plasma ANP (2) with a similar time course as that found for plasma oxytocin concentrations in this experiment.

Effect of BVE on Plasma AVP. Isotonic BVE caused a significant decrease in plasma arginine vasopressin (AVP) concentrations at 5 min (Fig. 5), and the values remained low at 15 min. However, hypertonic expansion induced a significant increase in plasma AVP ($P < 0.01$) at 5 min. Values were no longer significantly elevated by 15 min.

DISCUSSION

The present results have demonstrated that the i.p. administration of oxytocin can cause a concomitant increase in plasma ANP concentrations and urinary Na^+ and K^+ excretion. The effects on urinary sodium excretion were dose-related and accompanied by a dose-related, lesser stimulation of K^+ excretion. The effective doses also caused a dose-related increase in urinary osmolality and decrease in urine output. The minimal effective dose of i.v. oxytocin needed to increase plasma ANP concentrations was the same as the dose that induced natriuresis after i.p. injection. The increase in plasma ANP after i.p. injection of oxytocin was correlated with the increase in urinary concentrations of Na^+ and K^+ at 20 min after injection. Therefore, these results are consistent with the hypothesis that oxytocin circulates to the atria and acts on receptors there to cause the release of ANP from atrial myocytes, which then mediates the urinary effects of oxytocin.

Noteworthy was the fact that even the lowest dose of i.p. oxytocin that induced natriuresis also increased urinary osmolality and decreased urine volume, suggesting an action of vasopressin. This could be caused by the vasopressin-like action of high doses of oxytocin at all sites, which is related to their similar structure and possible utilization of the V2 receptor responsible for the renal actions of vasopressin (11). Indeed, the V2 receptor could be responsible for the release of ANP and concomitant urinary changes, since we have recently shown that vasopressin and a V2 receptor agonist increased plasma ANP in doses that produce antidiuresis (unpublished data).

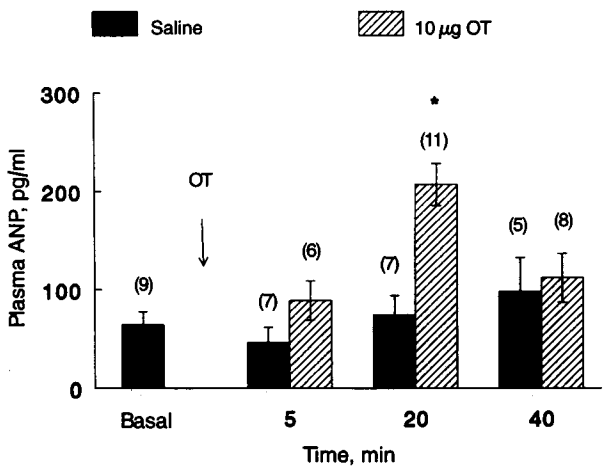

FIG. 2. Effects of i.p. oxytocin (OT) injection (10 μg) or 0.15 M NaCl (Saline) on the plasma ANP concentrations in water-loaded rats. Values are from blood samples obtained just prior to (Basal) or 5, 20, and 40 min after saline or oxytocin injection. ★, $P < 0.01$ compared to 0.15 M saline (n = 6–10).

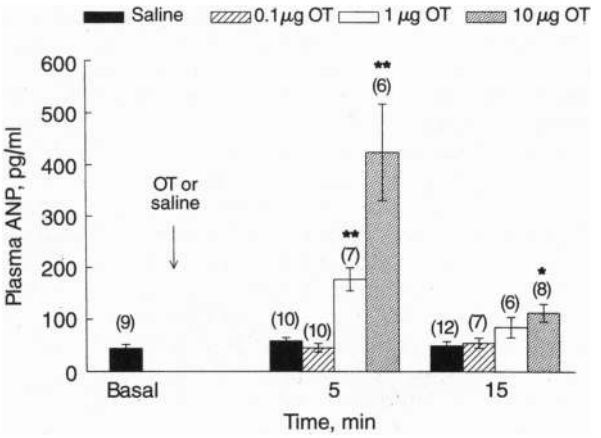

FIG. 3. Effects of i.v. oxytocin (OT) injection (0.1, 1.0, or 10.0 μg) or 0.15 M NaCl (Saline) on plasma ANP concentrations in hydrated rats. Values are from blood samples obtained just prior to (Basal) or 5 and 15 min after injection. ★, $P < 0.01$; ★★, $P < 0.001$ compared to saline (n = 6–12).

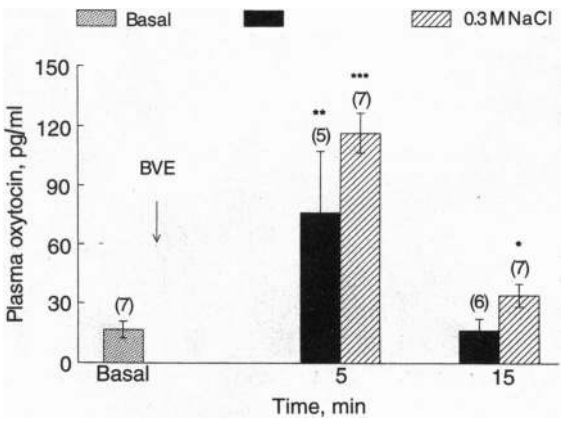

FIG. 4. Effect of isotonic or hypertonic blood volume expansion on plasma oxytocin concentrations. The numbers of observations are given above the bars. ★, $P < 0.05$; ★★, $P < 0.01$; ★★★, $P < 0.001$ versus basal.

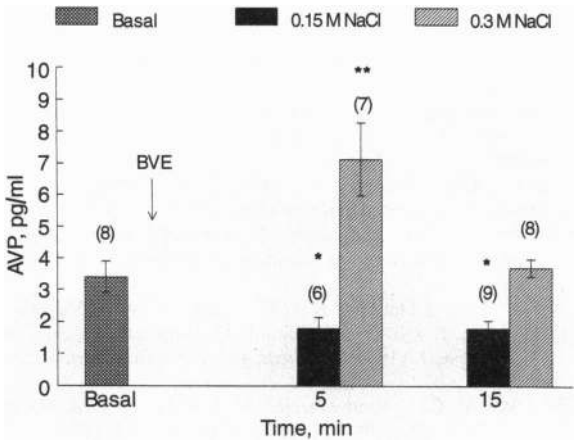

FIG. 5. Effect of isotonic or hypertonic blood volume expansion on plasma AVP concentrations. The numbers of observations are given above the bars. ★, $P < 0.05$; ★★, $P < 0.01$ versus basal.

If oxytocin mediates BVE-induced natriuresis via release of ANP from the atria, BVE should induce an increase in plasma levels of oxytocin; indeed, our experiments bore this out, and the time course and relative elevation of plasma oxytocin were similar to those obtained with plasma ANP concentrations (2, 12–14). These concentrations of oxytocin have been shown in earlier work to induce natriuresis (15).

The magnitude of the release of oxytocin after BVE was even greater than that which followed suckling in lactating rats, the classical stimulus for oxytocin release. The oxytocin release by suckling was also associated with an increase in plasma ANP (unpublished data).

Our hypothesis advanced at the beginning of this research is that BVE induces stretch of carotid-aortic and renal baroreceptors, which increases afferent baroreceptor input to the nucleus tractus solitarius in the brain stem (Fig. 6). These neurons send projections to the locus coeruleus, which activate the noradrenergic neurons located there. These in turn send axons to the AV3V region and stimulate cholinergic interneurons, which activate the ANPergic neurons (6, 12). These ANP neurons stimulate release of oxytocin from the neurohypophysis. Input to the AV3V from the serotoninergic neurons in the raphè nuclei is also essential for basal and volume expansion-induced ANP release (14). The released oxytocin circulates to the atria and triggers ANP release, which then induces the natriuresis. Further studies with receptor blockers of V1, V2, oxytocin, and ANP receptors and/or antisera directed against oxytocin and ANP will be needed to substantiate this hypothesis. However, since oxytocin causes ANP release and since ANP has natriuretic activity, it would appear that this mechanism accounts, at least in part, for the natriuresis observed after BVE. Others have shown receptors for oxytocin (15–17), ANP (18, 19), and vasopressin (15–17) in the kidney, so part of the natriuretic response may be due to the direct action of oxytocin on the kidneys.

As demonstrated here, isotonic volume expansion inhibits vasopressin release, but hypertonic expansion also induces the release of vasopressin, which has natriuretic activity. Indeed, we have shown that administration of vasopressin in even lower doses than those required for oxytocin release induces ANP release (unpublished data). Since a V2 receptor agonist also stimulated ANP release (unpublished data), the natriuretic effects of both vasopressin and oxytocin may be mediated via ANP through activation of putative V2 receptors and not oxytocin receptors in the atria. Furthermore, hypertonic volume expansion-induced release of vasopressin may participate in the ANP release and natriuresis that we have obtained under those conditions.

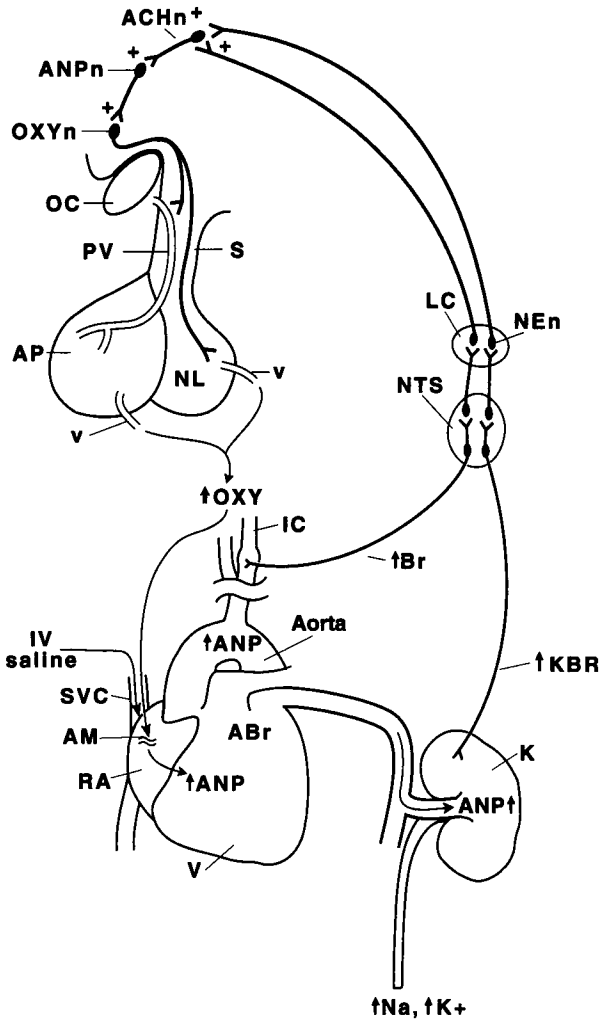

FIG. 6. Schematic diagram of the mechanism of natriuresis following BVE by injection of isotonic saline into the right atrium. OXYn, oxytocinergic neuron; ACHn, acetylcholinergic neuron; NEn, norepinephrinergic neuron; ANPn, ANPergic neuron; OC, optic chiasm; PV, portal vessel; AP, anterior lobe of the pituitary gland; NL, neural lobe of the pituitary gland; v, vein; LC, locus ceruleus; NTS, nucleus tractus solitarius; IC, internal carotid artery; RA, right atrium; V, ventricles; Br, baroreceptor afferents; KBR, renal baroreceptor afferents; K, kidney; S, pituitary stalk; AM, atrial myocyte; SVC, superior vena cava.

Our previous experiments showed that pituitary neural lobectomy, which largely abolishes release of oxytocin and vasopressin after volume expansion, nearly completely blocked the volume expansion-induced release of ANP (2). This led us to believe that neurohypophyseal hormones are the major mediators of central nervous system-induced release of ANP. The evidence is clear that direct release of ANP can occur by atrial stretch (8), but this does not contribute appreciably to the response to BVE under our conditions, since it can be almost completely blocked by denervation of baroreceptors and lesions of the AV3V or its caudal projections of ANP, oxytocinergic, and vasopressinergic neurons to the neurohypophysis (2, 12–14).

In view of the fact that oxytocin and possibly ANP cause the excretion of not only Na^+ but also K^+, it would appear that the physiologic function of oxytocin and ANP to reduce extracellular fluid volume is accompanied by a parallel, but lesser, reduction of intracellular fluid volume by induction of kaliuresis since K^+ is the principal intracellular cation. Thus, oxytocin and ANP induce a concomitant reduction of extra-

cellular and intracellular fluid volume in states of increased body fluid volume.

Another possibility that should be carefully evaluated is that oxytocin may not only cause release of ANP, which mediates natriuresis, but that oxytocin also acts independently to induce natriuresis and that there may be synergistic effects at the kidney level between the natriuretic actions of ANP, oxytocin, and even vasopressin. This will require studies with administration of various combinations of doses of the three peptides and their antagonists. α-Melanocyte-stimulating hormone (α-MSH) can also induce natriuresis, and the mechanism appears to depend on ANP release since it was released in natriuretic concentrations when α-MSH was injected systemically (D. Picanto-Diniz, and J.A.-R., unpublished results). Could there also be interactions at the kidney level between the α-MSH receptors and those for the three other peptides just discussed?

It is critical to perform experiments with ANP and oxytocin antagonists to distinguish between possible parallel natriuretic actions of ANP and oxytocin directly on the kidney to induce natriuresis and an action of oxytocin to release ANP, which then induces natriuresis, without the participation of oxytocin. In addition to inducing natriuresis, oxytocin and ANP also inhibit sodium intake (20, 21). The function of oxytocin in males is largely unknown. Its principal function in both sexes may be to control sodium intake and output and thereby body fluid homeostasis.

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