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Physiology

Participation of the ascending serotonergic system in the stimulation of atrial natriuretic peptide release

(sodium excretion/water intake/volume expansion/dorsal raphé nucleus lesions/serotonin depletion)

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ABSTRACT **Results obtained in our laboratories have provided evidence for the participation of the hypothalamic atrial natriuretic peptide (ANP) neuronal system in the regulation of water and electrolyte homeostasis. The anterior ventral third ventricular (AV3V) region, a site of the perikarya of the ANP neurons, receives important afferent input from ascending serotoninergic axons. We hypothesized that the ascending serotoninergic tract might be involved in control of the liberation of ANP. Therefore, electrolytic lesions were produced in the mesencephalic dorsal raphé nucleus (DRN), the site of perikarya of serotonin (5-HT) neurons whose axons project to the AV3V region. Rats with sham lesions constituted the control group. In a second group of animals, the serotoninergic system was depleted of 5-HT by lateral ventricular administration of *p*-chlorophenylalanine (PCPA), an amino acid that causes depletion of 5-HT from the serotoninergic neurons. Control animals were injected with an equal amount of isotonic saline. The DRN lesions induced an increase of water intake and urine output beginning on the first day that lasted for 1 week after lesions were produced. There was a concomitant sodium retention that lasted for the same period of time. When water-loaded, DRN-lesioned and PCPA-injected animals showed diminished excretion of sodium, accompanied by a decrease in basal plasma ANP concentrations, and blockade of the increase in plasma ANP, which followed blood volume expansion by intraatrial injection of hypertonic saline. The results are interpreted to mean that ascending stimulatory serotoninergic input into the ANP neuronal system in the AV3V region produces a tonic stimulation of ANP release, which augments sodium excretion and inhibits water intake. Therefore, in the absence of this serotoninergic input following destruction of the serotoninergic neurons by DRN lesions or intraventricular injection of PCPA, an antinatriuretic effect is obtained that is associated with increased drinking, either because of sodium retention *per se* or removal of ANP-induced inhibition of release of the dipsogenic peptide, angiotensin II. The serotoninergic afferents also play an essential, stimulatory role in volume expansion-induced release of ANP and the ensuing natriuresis.**

Atrial natriuretic peptide (ANP), a hormone produced primarily by right atrial myocytes, plays an important role in hydromineral and cardiovascular homeostasis (1–3). In addition to the atrial myocytes, the peptide is produced in a brain ANP neuronal system. The cell bodies of the ANPergic neurons are located in the anterior, medial hypothalamus ranging dorsally from the paraventricular nuclei to the subfornical organ and to the anterior ventral third ventricular (AV3V) region ventrally. Axons from these neurons project to the median eminence and neural lobe of the pituitary gland (4–7). There the peptide is released into the hypophyseal portal vessels and gains access to the anterior pituitary sinusoids and thence to the systemic circulation. It is also released from axons of ANPergic neurons in the neural lobe and reaches the circulation through veins draining the neural lobe (8). Microinjection of ANP into the third ventricle (3V) can rapidly inhibit water and salt intake (9, 10). Stimulation of the AV3V, and presumably the ANPergic neurons located there, produces an increase in plasma ANP concentration and natriuresis, coupled with a decrease in the content of the peptide in the brain ANPergic system (11).

The brain ANPergic neurons appear essential to resting and volume expansion-induced ANP release, since lesions of the cell bodies or axons of these neurons lower basal plasma ANP levels and block volume expansion-induced ANP release (12). Furthermore, injection of antisera directed against the peptide into the 3V can lower basal ANP levels and interfere with volume expansion-induced release of ANP and natriuresis in rats (13) and sheep (14). Blockade of α-adrenergic or muscarinic receptors in the region of the AV3V also blocks the ANP response to volume expansion (15). The response is probably mediated by afferent input to the brain ANP neuronal system via baroreceptors since section of the carotid–aortic baroreceptors or renal baroreceptors blocks the ANP response to volume expansion (16). Thus, the participation of the hypothalamus and the ANP neuronal system in control of ANP release is established.

Since an afferent pathway to the AV3V region via serotoninergic (5-HTergic) neurons with cell bodies in the raphé nuclei has been demonstrated (17), we hypothesized that 5-HT (5-hydroxytryptamine) may play a role in the control of ANP neurons in the region of the AV3V. Consequently, we determined the effect of lesions in the dorsal raphé nuclei (DRNs) and depletion of 5-HT in these and other 5-HTergic neurons with *p*-chlorophenylalanine (PCPA) on resting and volume expansion-induced ANP release. The results indicate that the afferent 5-HTergic pathway to the AV3V plays an essential stimulatory role in controlling resting and volume expansion-induced release of ANP.

MATERIALS AND METHODS

Animals. Male Wistar rats (250–280 g) were maintained in our animal facility with constant lighting (lights on from 7:00

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Abbreviations: ANP, atrial natriuretic peptide; AV3V, anterior ventral third ventricular; DRN, dorsal raphé nucleus; 5-HT, serotonin (5-hydroxytryptamine); PCPA, *p*-chlorophenylalanine; AII, angiotensin II.

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to 19:00 h) and temperature (23 ± 2°C) with free access to laboratory chow and water.

Lesions of the DRN. Electrolytic lesions of the DRN were produced in rats anesthetized with tribromoethanol (2.5%, i.p.), which were fixed in a Kopf stereotaxic instrument. The lesions were produced by passing an anodal current of 2 mA for 10 sec. Confirmation of the location of lesions was made by histological examination of serial coronal sections through the brainstem (10 μm) stained with a Nissl stain.

PCPA Lesions. Depletion of 5-HT in 5-HTergic neurons is usually accomplished by systemic administration of PCPA (18, 19), an amino acid that competes with tryptophane, the substrate of tryptophane hydroxylase, the rate-limiting enzyme in the synthesis of indolamines (20). We used a modification of this method (21) and injected PCPA (3 mg/10 μl) directly into the right lateral ventricle via a cannula implanted in the lateral ventricle. This technique produced a 5-HT depletion in the brain of >50% 5 days after central injection (21). Hyperactive lateralized motor activity in the rats served as an indicator of 5-HT depletion as well. Control rats received an equal volume of isotonic NaCl.

Intraatrial Catheters. An indwelling intraatrial catheter was placed in the right atrium 24 hr before experiments while the rats were anesthetized with tribromoethanol (22).

Measurement of Water Intake and Urinary Excretion. Water intake, urine volume, sodium and potassium excretion, and urine osmolality were measured daily in rats with DRN lesions while they were maintained singly in metabolic cages.

Measurement of Urinary Excretion in Hydrated Rats. The animals were hydrated by two intragastric injections of water (5% of their body weight) with a 1-h interval between the injections. Urine was collected every 20 min while the animal was in a metabolic cage.

Blood Volume Expansion. This was performed 2 days after DRN lesions and 4 days after the intraventricular injection of PCPA. The animals were infused over a period of 1 min through the intraatrial catheter with 0.3 M NaCl (2 ml/100 g of body weight). Animals were decapitated at 0 time (just before infusion), 5 and 15 min postinfusion, and blood was collected from the trunk into polyethylene tubes containing proteolytic enzyme inhibitors (11). At termination of the experiments, the blood was centrifuged at low speed, and the plasma was separated and stored in aliquots of 1 ml at −70°C prior to radioimmunoassay.

Determination of Plasma ANP. ANP was determined by the method of Gutkowska (23).

Determination of Na^+, K^+, and Urine Osmolality. Na^+ and K^+ were determined by flame photometry and osmolality was determined by an osmometer.

Statistical Analysis. The data were analyzed statistically by two-way analysis of variance with repeated measures, and the significance of differences between means was determined by the Newman–Keuls test.

RESULTS

Effect of Lesions in the DRN on the Intake of Water and the Excretion of Urine and Electrolytes. The rats with DRN lesions showed a significant increase in water intake and urine volume that was already present on first measurement at 24 h and continued for 6 days postlesion ($P < 0.001$ to $P < 0.005$). The peak intake of water was on day 3 following lesions, whereas excretion of urine was maximal from day 1 to day 3 and then declined to equal that of controls on day 7 (Fig. 1). The urinary sodium excretion in the rats with lesions was significantly less than that in the sham-operated animals from day 1 to day 7 postlesions ($P < 0.001$ to $P < 0.05$). The urine osmolar excretion was even more reduced than the excretion of sodium (Fig. 2). The maximum decrease in the excretion of sodium and osmolality occurred at 24 h, and the excretion returned toward that of the controls and was not significantly different from that of the controls by 8 days postlesion.

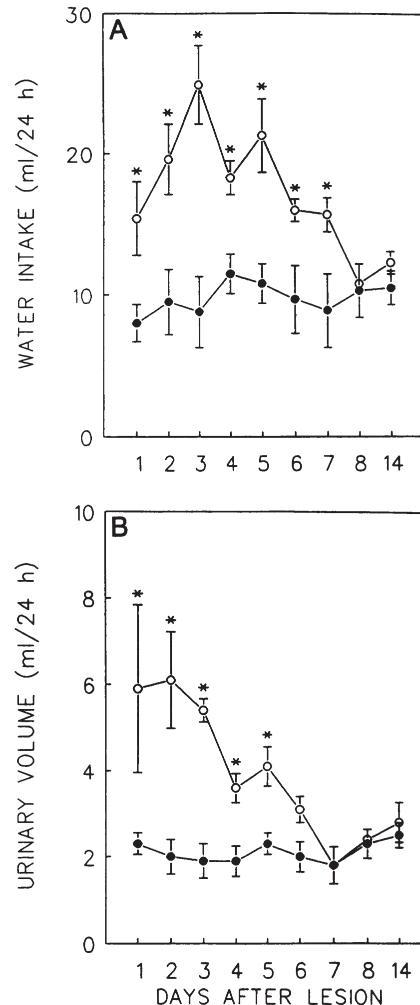

FIG. 1. Effect of electrolytic DRN lesions (lesioned rats; ○) or sham-DRN lesions (control rats; ●) on daily water intake (*A*) and urine volume (*B*). Vertical bars depict SEM. *, $P < 0.05$ (or better) compared to sham-lesioned rats.

Rats with DRN lesions were water-loaded as described, 2 days postlesion. There was a significant reduction in sodium excretion ($P < 0.01$ to $P < 0.05$) on comparison with the results in the sham-operated animals (Fig. 3). These effects were no longer significant after 4 or 14 days postlesion. In contrast, there was no significant reduction in kaliuresis and diuresis in the rats with lesions compared to the results in the sham-operated animals and the normal controls, respectively (data not shown).

Effect of PCPA Lesions on Urinary Excretion. Rats that received lateral ventricular injections of PCPA 5 days earlier were also hydrated, and they showed a similar significant reduction in natriuresis as that of rats with DRN lesions ($P < 0.01$ to $P < 0.05$) during the 60 min postwater-loading on comparison to the group of animals injected intraventricularly with saline (Fig. 4). The results differed from those in rats with lesions in that there was also a significant reduction in kaliuresis in PCPA-injected rats; however, diuresis was not affected (data not shown).

Effect of DRN Lesions and Intraventricular PCPA Injections on Plasma ANP. ANP concentrations in animals 2 days after DRN lesions were significantly lower than those in sham-operated controls (Fig. 5). Following administration of hypertonic saline into the right atrium, plasma ANP

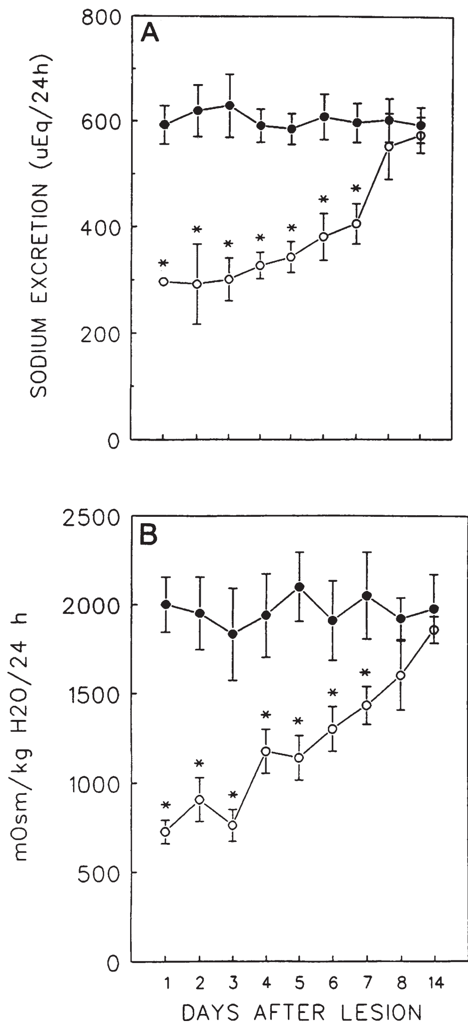

FIG. 2. Effect of electrolytic DRN lesions (lesioned rats; ○) or sham-DRN lesions (control; ●) on daily urine sodium excretion (*A*) and osmolality (*B*). *, $P < 0.05$ (or better) compared to sham-DRN lesioned rats.

concentrations increased dramatically in animals with sham lesions, and this increase was almost completely blocked by DRN lesions. By 15 min after expansion, values in sham-operated animals had returned nearly to control, whereas those in animals with lesions had risen slightly and were no longer significantly different from those of the sham-operated rats.

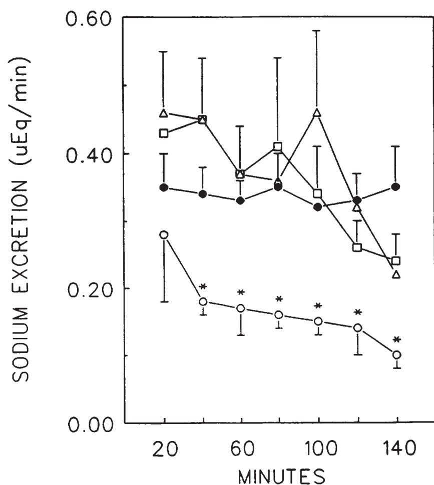

FIG. 3. Effect of electrolytic DRN lesions 2 (○), 4 (△), and 14 (□) days postlesion or the sham-DRN lesion (●) on sodium excretion in water-loaded rats. Vertical bars depict SEM. *, $P < 0.05$ (or better) compared to sham-lesioned rats.

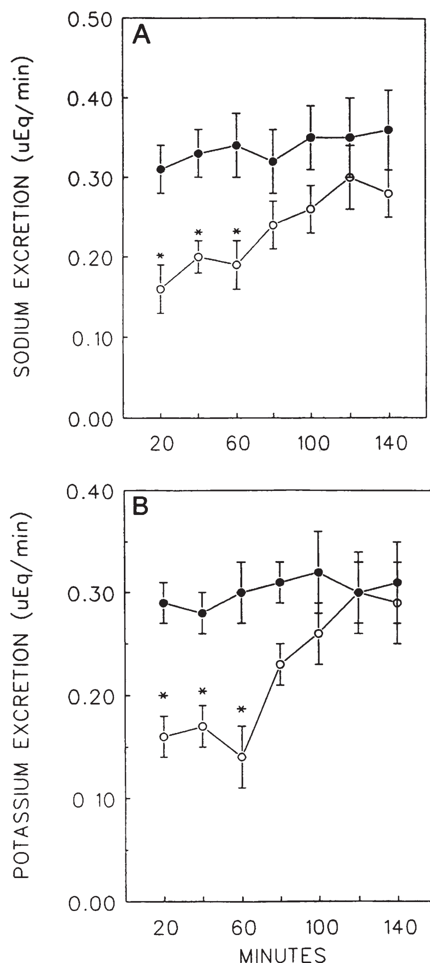

FIG. 4. Effect of previous lateral ventricular injection of PCPA (○) or saline (controls; ●) on sodium (*A*) and potassium (*B*) excretion in water-loaded rats. Vertical bars depict SEM. *, $P < 0.05$ (or better) compared to sham-DRN lesioned rats.

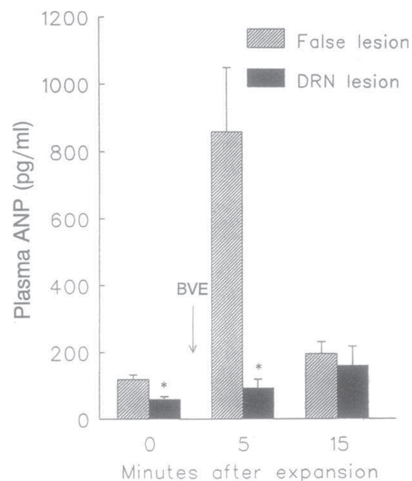

FIG. 5. Effect of electrolytic DRN lesions or sham-DRN lesions on basal plasma ANP concentrations and on the response of plasma ANP concentrations to blood volume expansion (BVE). Vertical bars depict SEM. *, $P < 0.05$ (or better) compared to sham-DRN lesioned rats.

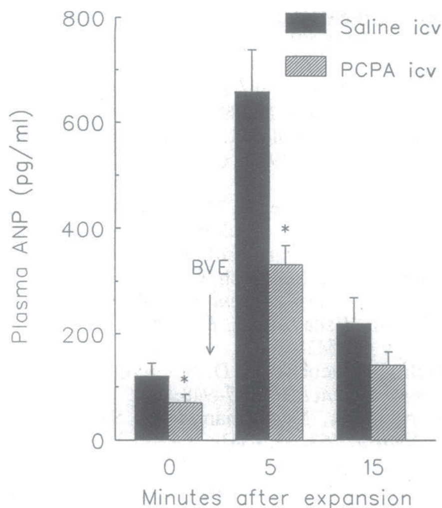

FIG. 6. Effect of previous lateral ventricular (icv) injection of PCPA or saline (control) on the resting plasma ANP concentrations and the response to blood volume expansion (BVE). Vertical bars depict SEM. *, $P < 0.05$ (or better) compared to saline-injected rats.

Five days after injection of PCPA into the lateral ventricle, plasma ANP concentrations were also significantly lower than those of control animals injected intraventricularly with saline (Fig. 6). Five minutes following volume expansion, plasma ANP concentrations had risen dramatically and this rise was highly significantly reduced in animals injected with PCPA; however, by 15 min the values in control animals had decreased dramatically and those in PCPA-injected animals had declined significantly and were no longer significantly different from those in saline-injected controls.

DISCUSSION

Lesions of the DRN or depletion of 5-HT from central nervous system neurons by PCPA inhibited the release of ANP into the circulation under resting and volume expansion-induced conditions. Therefore, the results support the existence of a tonically active 5-HTergic afferent pathway with cell bodies in the raphé nuclei and axons projecting to the AV3V region, there to stimulate the ANPergic neurons. Furthermore, other experiments with 3V and lateral ventricular injections of 5-HT antagonists or agonists implicate 5-HT as the effector, probably acting via 5-HT2 receptors (24–27).

These 5-HTergic neurons control the basal level of plasma ANP and sodium excretion under resting conditions and may also exercise a tonic suppressive effect on the angiotensin II (AII) neuronal control of water intake, probably by stimulating the ANPergic neurons. Therefore, when lesions are made, the inhibition of AII release is removed and central release of AII stimulates drinking. Reduction of ANP release resulting in decreased natriuresis and the increased water intake leads to a decrease in plasma osmolality and inhibition of vasopressin release resulting in a marked increase in urine volume of low osmolality.

Although the ANP neuronal system inhibits dehydration and hemorrhage-induced vasopressin release (28), we hypothesize that it has little, if any, tonic inhibitory action on basal vasopressin secretion. Therefore, decreased ANP release following the lesions or PCPA injections would not increase vasopressin release, which would have an antidiuretic effect and minimize the ensuing hypotonic diuresis.

When the rats with DRN lesions were water-loaded at 2 days but not 4 or 14 days postlesion, they showed a suppression of sodium, but not potassium excretion, which indicates that the antinatriuretic effect of the lesions could be demonstrated under water-loaded conditions that must have completely suppressed vasopressin secretion. Similarly, in animals previously injected intraventricularly with PCPA and water-loaded 5 days later, sodium excretion was markedly inhibited, but in this case, in contrast to the results in rats with lesions, the excretion of potassium was also significantly suppressed, possibly because of a greater reduction in 5-HT input to the AV3V region.

The measurement of resting concentrations of plasma ANP indicated that the release of ANP was inhibited by the DRN lesions or the previous injection of PCPA to deplete 5-HT in 5-HTergic neurons. The decrease in plasma ANP could account for the antinatriuretic effects observed. Both types of lesions dramatically inhibited the increase in plasma ANP concentrations induced within 5 min after volume expansion.

Therefore, these lesions produced a profound defect in the release of ANP under resting and volume expansion-induced stimulation. In view of the fact that stimulation of the medial raphé nuclei, also a source of cell bodies of 5-HTergic neurons that project to the AV3V region, with kainic acid caused natriuresis and an increase in plasma ANP (29), we believe that the results establish a crucial role for the ascending 5-HTergic tract in the stimulatory control of ANP release. The animals recovered from the effects of DRN lesions or PCPA injections, presumably because of incomplete destruction of 5-HTergic neurons or incomplete 5-HT depletion, respectively.

Our previous work has indicated the essentiality of the AV3V region and its ANPergic neurons in the control of ANP release in response to volume expansion and indicated that α-adrenergic and muscarinic receptors are critical in mediating these responses (11–13, 15, 16). We now add 5-HT2 receptors as essential for the mediation of the response. We have schematically diagramed the possible pathways of the 5-HTergic stimulatory input into the ANP neuronal system (Fig. 7). We postulate that there is a tonic stimulatory input of the 5-HTergic pathway through the ANP neurons, which results in a tonic inhibition of AII neurons, thereby limiting water intake. Volume expansion presumably augments this 5-HTergic stimulation of the ANP neurons. Either baroreceptor stimulation by volume expansion directly stimulates the raphé nuclei or, alternatively, the stimulation of afferent adrenergic and cholinergic neurons directly stimulates the ANP neurons, but this stimulation is only effective in the presence of tonic 5-HTergic drive. The loss of any of these three drives to the ANP neurons causes a blockade of the resting release of ANP and the response to volume expansion.

Following saline infusion to expand blood volume, there is a stretch of the baroreceptors in the aortic and carotid sinus and of renal baroreceptors that send afferent input to the nucleus tractus solitarius. Neurons in the nucleus tractus solitarius project to the locus ceruleus. From there noradrenergic axons project to the hypothalamus to activate cholinergic interneurons, which then activate the ANP neurons via muscarinic cholinergic receptors. These ANP neurons inhibit the intrahypothalamic AII neurons, thereby suppressing water intake (Fig. 7). Activation of the ANP neurons also causes release of ANP from the anterior and neural lobe of the pituitary gland. ANP neurons may also activate the oxytocinergic neurons in the supraoptic nucleus, which project to the neural lobe. Oxytocin would circulate to the atria and may directly activate release of ANP from the atrial myocytes since we have shown that i.p. injection of oxytocin, which is a natriuretic peptide, not only increases sodium excretion but also concomitantly elevates plasma ANP (unpublished observations). Since the quantity of ANP in the hypothalamus is 1000-fold less than that in the atria, we postulate that the ANP release from the neurohypophysis would be quite small relative to that released from the right

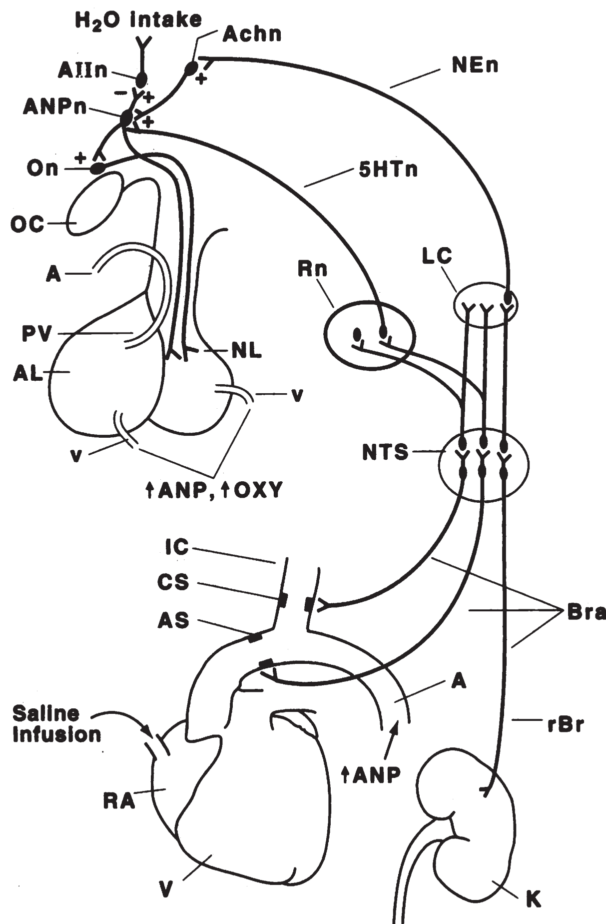

FIG. 7. Schematic diagram of the ANP neuronal control of ANP release. For explanation, see *Discussion*. AIIn, AII neuron; Achn, acetylcholinergic neuron; NEn, norepinephrinergic neuron; ANPn, ANPergic neuron; On, oxytocinergic neuron; 5HTn, 5-HTergic neuron; OC, optic chiasm; A, artery; PV, portal vessel; AL, anterior lobe of the pituitary gland; NL, neural lobe of the pituitary gland; v, vein; Rn, raphé nuclei; LC, locus ceruleus; NTS, nucleus tractus solitarius; IC, internal carotid artery; CS, carotid sinus; AS, aortic sinus; A, aorta; RA, right atrium; V, ventricles; Bra, baroreceptor afferents; rBr, renal baroreceptor receptors; K, kidney.

atrium. Oxytocin is present in the neural lobe in large quantity, which could reach the atria in high concentration and release ANP from atrial myocytes.

The present data indicate that activation of the nucleus tractus solitarius must cause neurons there to activate the raphé nuclei, thereby stimulating the 5-HTergic neurons that project to the region of the AV3V to activate ANP release. Alternatively, tonic activity in this pathway has a permissive effect, permitting the activation induced by the ascending norepinephrine neurons.

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