Neural Control of Atrial Natriuretic Peptide Actions on Fluid Intake and Excretion (44044)

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Clinical studies involving patients with diabetes insipidus and the description of cases with brain lesions that had either hypo- or hypernatremia suggested an important role for the hypothalamus in the control of water and salt intake and excretion (1). In the early 1950s, Andersson and his associates showed that microinjection of hypertonic saline into the hypothalamus of goats could induce drinking (2).

Electrical stimulation in the same region that evoked drinking following injection of hypertonic saline caused reproducible drinking with very slight delay and with little aftereffect, such that the animals could drink a total of 30% to 40% of their body weight in water (3). Stimulation also could evoke milk ejection *via* release of oxytocin (OT) and antidiuresis *via* release of antidiuretic hormone (vasopressin [VP]). Stimulation in or around the paraventricular nucleus, a site of origin of OT and VPergic neurons, produced natriuresis (3). It is interesting to note that the area that induced natriuresis is also the site of cell bodies of atrial natriuretic peptide (ANP) neurons (4).

In dogs, lesions in the medial hypothalamus surrounding the third ventricle (3V), some of which extended into the anterior-ventral portion of the third ventricle (AV3V), produced complete adipsia, though the animals tended to recover. During the adipsic period, the animals would not drink water but would drink fluid food in the form of milk or broth. During the adipsic period, the animals were given water by stomach tube yet still developed pronounced hypernatremia (5). A similar syndrome occurs following AV3V lesions in rats (6).

Transmitters in the Hypothalamic Control of Fluid and Electrolyte Homeostasis

Much later, Andersson *et al.* (7) microinjected hypertonic saline into the 3V of goats and found that not only did it evoke reproducible drinking in the animals in contrast to the difficulty of repeatability following injections into the tissue, but it also evoked a marked natriuresis. This effect was then confirmed following 3V injection of hypertonic saline into rats (8), and the role of various brain transmitters in control of water, sodium chloride, and food intake, and sodium excretion was then studied. Intraventricular injection of carbachol induced a dramatic, rapid 15-fold increase in water intake, whereas none of the other adrenergic or cholinergic drugs was effective (9), which was in agreement with earlier findings of Grossman injecting drugs into hypothalamic tissue (10). Both carbachol and isoproterenol, a β adrenergic agonist, evoked large increases in salt intake. Again, other drugs failed to produce significant effects (9).

Hypertonic saline injected into the 3V produced a delayed increase in both water intake and food intake but did not alter salt intake (9). Therefore, it is clear that there is a cholinergic synapse in the pathways which mediate water intake (9, 10), whereas both cholinergic and adrenergic synapses are involved in mediation of salt intake (9). Little further research along these lines has been undertaken.

The role of various transmitters in the natriuretic response to the 3V injection of hypertonic saline was studied, and intraventricular injection of carbachol evoked a dramatic natriuretic response which mimicked the response to intraventricular hypertonic saline (8). Both natriuretic and kaliuretic responses and an increase in the sodium/potassium ratio were induced by intraventricular injection of norepinephrine or carbachol, whereas dopamine had no effect (13). The β receptor stimulator, isoproterenol, induced an

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This work was supported by National Institutes of Health Grants DK43900[SMMcC] and MH51853[SMMcC]. We would like to thank Judy Scott and Jason Holland for their excellent secretarial assistance.

0037-9727/96/2132-0117$10.50/0

antinatriuretic and antikaliuretic effect. To determine the nature of the receptors involved, adrenergic blockers were injected. The α adrenergic blocker, phentolamine, abolished the natriuretic response to intraventricular hypertonic saline and to norepinephrine and carbachol. In contrast, the β adrenergic blocker, propranolol, induced a natriuresis and kaliuresis when injected alone and had an additive effect when its injection was followed by that of norepinephrine or hypertonic saline. Propranolol potentiated the natriuretic response to carbachol. Cholinergic blockade with atropine diminished the response to norepinephrine and blocked the natriuretic response to hypertonic saline (13). It was suggested that sodium receptors in the ventricular wall (12) modify renal sodium excretion by a stimulatory pathway involving cholinergic and α adrenergic receptors and inhibit sodium excretion by a tonically active β receptor pathway (13). Much later, β receptors were described in the hypothalamus (14).

Covian, Antunes-Rodrigues, and their associates mapped the various pathways in the CNS controlling salt excretion and obtained similar results concerning the role of cholinergic and adrenergic responses. Cholinergic or adrenergic stimulation of the medial septal area, medial preoptic area, anterior lateral hypothalamus, and subfornical organ as well as the anterior portion of the AV3V induced dose-related natriuresis accompanied by a lesser kaliuresis. Thus, considerable evidence indicates that the medial preoptic area, anterior lateral hypothalamus, subfornical organ, AV3V, habenula, stria medullaris, supraoptic nucleus, and medial septal area are organized in a neural circuit involved in the regulation of water and sodium intake and excretion (15). Specific hypothalamic lesions in this circuit altered salt intake (16). The role played by the CNS in the control of renal sodium excretion was demonstrated by other investigators as well (12).

Natriuretic Hormones

During the 1960s much attention was paid to the possibility of the existence of a natriuretic hormone. The idea stemmed from the experiments of de Wardener and Clarkson (17) showing that natriuresis could occur following body fluid expansion even though factors such as increased glomerular filtration rate or changes in aldosterone secretion were eliminated. Davis and associates obtained evidence for a circulating natriuretic factor (18) in volume-expanded dogs by cross-circulation experiments.

The Czech group (19) reported the purification of a hypothalamic natriuretic factor and claimed it was an OT analog. Orias and McCann confirmed that both VP and OT are natriuretic. Also α– and β–melanocyte-stimulating hormone (MSH) had natriuretic activity in conscious water-loaded male rats (20–22). It is now known that α-MSH is produced in neurons in the brain and is also released from the intermediate lobe of the pituitary, so it was possible that the natriuretic hormone is MSH; however, it is still not clear if MSH has a physiologic role in the induction of natriuresis.

Since there was considerable evidence suggesting that there was a natriuretic hormone in the hypothalamus and it could be related to OT, Morris *et al.* (23) evaluated the effect of median eminence lesions on the natriuretic responses to hypertonic saline and carbachol or norepinephrine injected into the 3V, since, if indeed there were a hypothalamic natriuretic hormone, it might be expected to gain exit to the general circulation *via* the neurohypophysis. These animals had lesions which destroyed most of the median eminence and thereby induced diabetes insipidus, because of interruption of the supraopticohypophyseal tract and consequent elimination of VP and OT secretion. These median eminence lesions blocked the natriuresis, kaliuresis, and antidiuresis that followed the injection of hypertonic saline or norepinephrine into the 3V. Sham lesions did not interfere with the responses (23). Hypophysectomy did not block the responses, which ruled out the participation of anterior pituitary hormones (23). Orias and McCann showed that the responses still occurred in rats with hereditary diabetes insipidus that lacked VP (24). Thus, although natriuretic, both VP and MSH were eliminated as essential components of the natriuretic responses. Therefore, we suggested that these lesions had interrupted the secretion of a natriuretic hormone involved in the induction of central natriuresis.

Atrial Natriuretic Peptide

We were amazed to become aware in 1983 of the discovery of atrial natriuretic factor (peptide; ANP) (25). It had been known that dilation of the atria could produce diuresis from the pioneering experiments of Gauer and Henry (26). At that time it was thought that distension of the atria activated impulses which traveled up the vagus to inhibit the release of antidiuretic hormone. The decreased release of this hormone was thought to be responsible for the diuresis. Immersion in baths had been known since the mid 19th century to evoke diuresis. Immersion probably increased venous return to the heart and dilated the atria.

Therefore, it was a great shock to find that this was not due to a reflex activation of the brain but instead was due to the release of ANP from the right atrium of the heart. Space does not permit the elaboration of the history of ANP, suffice it to say, the thinking then changed to the idea that natriuresis following volume expansion was due to secretion of this peptide from the atria which circulated to the kidneys and evoked natriuresis (27, 29).

The Brain ANPergic Neurons

The demonstration of ANP in extracts from various hypothalamic regions (28) and the evidence that ANP had opposite actions to those of angiotensin II (Ang II) in every site so far studied (29), led us to hypothesize that ANP might have opposite actions to those of Ang II in control of water intake and that it might be the long sought hypothalamic natriuretic hormone. Indeed, it is now known that ANPergic neurons are localized in the region extending from the paraventricular nucleus rostrally to the organum subfornicalis and ventrally to the organum vasculosum lamina terminalis, areas known to be implicated in thirst (30), and their axons also project caudally and ventrally to the median eminence and neural lobe (4). There, they terminate in proximity to either the long or short portal vessels, so that the peptide could be transported to the anterior pituitary and also into the general circulation.

The ANPergic Neurons in Water and Salt Intake

Injection of ANP into the 3V of water-deprived rats induced a dose-related inhibition of drinking with doses of 1.0 or 2.0 nmole of ANP injected into the ventricle. Inhibition could only be obtained following intravenous injection of the peptide at the higher 2.0-nmol dose, indicating a central action of the intraventricularly injected peptide (31). ANP also blocked Ang II-induced drinking. This inhibitory response was present at doses of intraventricularly injected Ang II ranging from 4.8 to 25 pmol; however, the 1.0 nmole dose of ANP given 5 min before Ang II infusion was unable to block responses to much higher doses of Ang II ranging from 96 to 956 pmol (31).

Since Ang II also increases salt intake, we speculated that ANP would have the opposite effect and inhibit saline intake when injected into the 3 of conscious salt-depleted rats. Animals were salt-depleted by 4 days of salt restriction followed by peritoneal dialysis with 5% glucose solution to produce hyponatremia. Intake of 1.5% sodium chloride solution was suppressed dramatically by a minimal effective dose of 0.2 nmole of ANP. There was no additional effect with a 10-fold higher dose. The suppression was maintained during the 24 hr after central injection of the peptide; however, in this case the inhibition was somewhat greater with the higher dose of ANP. In contrast, the relatively low intake of distilled water which was also offered to the animals was not affected by any dose of ANP (32). Consequently, it appears that this peptide can definitely suppress dehydration and Ang II-induced drinking and is even more potent to suppress salt intake.

To evaluate the physiologic significance of various peptides in the control of water intake, we injected into the 3V highly purified antibodies against peptides thought to be involved and injected control animals with normal rabbit serum in the same volume. Since the evidence was already quite strong that Ang II might be involved in the drinking which follows hemorrhage and depletion of extracellular fluid volume, we decided to evaluate its possible role in dehydration-induced drinking. Antiserum directed against Ang II was microinjected into the 3V of rats that had been deprived of water overnight. We had previously found that frequently there is a delay following intraventricular injection of antiserum against peptides before they are effective. These delays may represent time for the antiserum to be absorbed from the ventricle and to diffuse to the site of action of the peptide. In this case, if water was offered immediately after injection of the antiserum, drinking was not altered. If water was offered 1 hr after injection, drinking was largely blocked. At 3 hr after injection of the antiserum, drinking was completely abolished. These results indicate that Ang II is required to induce the drinking that follows dehydration (33).

Previous attempts had been made to block dehydration-induced drinking with saralasin, an antagonist of Ang II; however, except for one experiment in the rat in which lateral ventricular infusion of saralasin commencing 30 min prior to giving the animals access to water partially suppressed drinking (34), these experiments with saralasin have been negative (35). These results contrast strikingly with the dramatic effectiveness of Ang II antiserum to block dehydration-induced drinking. We believe that the discrepancy is probably related to the short duration of action of saralasin, plus the failure to distribute it to the activate sites following infusions into the lateral ventricle which would not distribute the antagonist uniformly bilaterally and therefore might not completely inactive the Ang II receptors. We conclude that Ang II, either reaching the brain *via* the circulation and uptake *via* the circumventricular organs, or more likely released from neurons containing Ang II within the hypothalamus, which have been found in close association with ANP neurons, plays an essential role in dehydration-induced drinking. By contrast, Ang II appears to play no role in the normal prandial drinking that occurs concomitantly with feeding when the lights are turned off, because the antiserum injected 3 hr before lights off had no effect on prandial drinking (33).

Brain ANPergic Neuronal System and Release of ANP

The next question was the role of the brain ANPergic neuronal system in CNS-induced natriuresis brought on by intraventricular injection of hypertonic saline or activation of brain cholinergic and adrenergic circuits by carbachol or norepinephrine. We had earlier thought that these effects were brought about by

the release of a hypothalamic natriuretic hormone. Alternatively, they could be brought about by neural activation of the release of ANP from the neurohypophysis or the atria.

Therefore, we evaluated the possible role of brain ANP in evoking the changes in renal sodium excretion that followed stimulations or lesions of the AV3V, a region further implicated in control of sodium excretion by earlier experiments (6). Injection of carbachol into the AV3V produced the expected natriuresis on the basis of our earlier experiments, which was accompanied by a dramatic rise in the plasma ANP concentration and a rise in ANP content in the medial basal hypothalamus, the neurohypophysis and particularly the anterior hypophysis but without alterations in the content of ANP in the lungs or the right or left atrium (36).

The dramatic increase in plasma ANP after carbachol stimulation of the AV3V was accompanied by marked elevations in content of the peptide in basal hypothalamus and neuro- and adenohypophysis, suggesting that the natriuresis resulting from this stimulation is brought about at least in part by release of ANP from the brain.

Conversely, there was a dramatic decline in plasma ANP at both 24 and 120 hr after AV3V lesions had been placed (37). This was accompanied by a slight decline in the content of the peptide in the lungs. There was no change in its content in the right atrium at 24 hr after lesions, but there was a significant increase at 120 hr. These small changes contrasted sharply with the dramatic decline in content of the peptide in the medial basal hypothalamus, median eminence, neurohypophysis, choroid plexus, anterior hypophysis, and olfactory bulb. These declines persisted or became greater at 120 hr, except in the olfactory bulb in which the decline was no longer significant.

Therefore, lesions which destroyed the perikarya of ANP-ergic neurons caused a decline in ANP content in presumed projection areas of these neurons to the olfactory bulb, where they are probably involved in control of salt and water intake (37). ANP content of the choroid plexus also declined probably because of loss of input from the AV3V neurons. There is evidence that the ANP neuronal projection to the choroid plexus may be involved in cerebrospinal fluid formation. Destruction of the AV3V also caused loss of ANP from caudal axonal projections to the neurohyphophysis, whereas only a delayed increase in right atrial ANP content occurred, probably related to decreased release of the peptide in the presence of continued synthesis which led to increased tissue content of the peptide (37).

The dramatic decline in plasma ANP after AV3V lesions was accompanied by a very dramatic decline in content of ANP in regions containing the caudal axonal ANP neuronal connections to the median eminence and neural lobe of the pituitary gland, which was probably caused by release of these stores of the peptide that could not be replenished by axoplasmic flow of the peptide from the destroyed perikarya.

In view of the much larger quantities of the peptide stored in the atria, it is probable that changes in atrial release contribute to the alterations in plasma ANP observed after stimulation or ablation of the AV3V region; however, these results suggest that the dramatic changes in plasma ANP that followed these manipulations may be due to altered release of the peptide from brain structures as well as the atria and lungs (36, 37). These stimulation and lesion experiments support a crucial role of the CNS in controlling ANP release.

Role of Hypothalamic ANPergic Neurons in Volume Expansion-Induced Release of ANP

Expansion of the blood volume causes a release of ANP that is believed to be important in induction of the subsequent natriuresis and diuresis, which in turn acts to reduce the increase in blood volume. Since stimulation of the AV3V induced a rapid elevation of plasma ANP, whereas lesions of the AV3V were followed by a marked decline in plasma concentration of the peptide, we hypothesized that release of ANP from the brain ANP neuronal system might be important to the control of plasma ANP. As already described, the perikarya of the ANP-containing neurons are densely distributed in the AV3V and their axons project to the median eminence and neural lobe (30).

To test the hypothesis that these neurons are involved in volume expansion–induced ANP release, we destroyed the AV3V, the site of the perikarya, in male rats by electrolytic lesions. Other lesions were made in the median eminence and posterior pituitary, sites of termination of the axons of these neurons, and also hypophysectomy was performed in other animals (38).

In conscious freely moving animals, volume expansion and stimulation of postulated sodium receptors (12) in the hypothalamus were induced by intravenous injection of hypertonic NaCl solution (0.5 or 0.3 *M* NaCl; 2 ml/100 g body wt). Volume expansion alone was induced with the same volume of an isotonic solution (NaCl or glucose). In the sham-operated rats, volume expansion with hypertonic or isotonic solutions caused equivalent rapid increases in plasma ANP that peaked at 5 min and returned nearly to control values by 15 min. Lesions caused a decrease in the initial levels of plasma ANP on comparison with values from the sham-operated rats, and each type of lesion induced a highly significant suppression of the

response to volume expansion on testing 1–5 days after lesions were made.

Because a common denominator of the lesions was elimination of the brain ANP neuronal system, these results suggest that the brain ANP plays an important role in the mediation of the release of ANP that occurs after volume expansion. Since the content of ANP in this system is 1000-fold less than that in the atria, it is likely that release of brain ANP associated with this stimulus cannot account for the 4-fold increase in plasma ANP within 5 min of volume expansion. Therefore, a large increase in release from the atrium must occur. This could be mediated by efferent neural input to the atrium but this cannot account for the ability of neural lobectomy to block volume-expansion-induced ANP release since the lesions would not directly injure the CNS. Therefore, we hypothesized that volume expansion–induced release of other neurohypophyseal hormones, such as VP, OT, or endothelin, may induce release of ANP from atrial myocytes (37).

In other experiments, we determined the essentiality of the brain ANP neuronal system to the BVE-induced ANP release induced by hypertonic saline by injecting antiserum directed against ANP into the AV3V prior to inducing volume expansion. The antiserum had no effect on resting levels of ANP; however, it partially blocked the increase in ANP and the natriuresis which followed BVE (38). Other experiments in sheep had given similar results (39). Therefore, it appears that the essentiality of the nervous system in these responses of ANP to volume expansion is conferred by the ANP neuronal system.

Previously, we had shown that cholinergic and adrenergic synapses within the hypothalamus mediated the natriuresis induced by 3V injection of hypertonic saline (12). Therefore, we evaluated their role in the ANP release evoked by volume expansion. The receptor-blocking agents were injected into the 3V 30 min prior to BVE as previously described. These blockers had no effect on resting levels of the hormone just prior to BVE; however, a highly significant blockade of the response was induced by the prior injection of the muscarinic cholinergic receptor blockers, atropine sulphate (5 nmole in 2 ml 0.9% NaCl) or methyl atropine at a similar dose. Microinjection of the α receptor blocker, phentolamine (5 nmole in 2 ml saline) also markedly suppressed the ANP response (40).

To determine whether this was a central or possibly a systemic effect of the blockers, methyl atropine (0.01 μmole/100 g body wt), which does not cross the blood brain barrier, was injected ip 30 min before volume expansion. It also had no effect on basal levels of plasma ANP, but, in striking contrast to the blockade of the response to volume expansion induced by intraventricular injection of methyl atropine, the response to volume expansion was markedly enhanced by ip injection of methyl atropine. The results therefore indicate that hypothalamic muscarinic and α adrenergic synapses are essential to release of ANP in response to volume expansion (40).

Thus, the results to this point indicated the crucial participation of the CNS and the brain ANP neurons in the response of ANP and natriuresis to volume expansion. We considered the possibility that the baroreceptors, when they were stretched by volume expansion, would activate the brain ANP neurons, which would then produce the release of ANP and the ensuing natriuresis. Therefore, we determined the role of the baroreceptors in affecting the increase in plasma ANP from volume expansion induced by iv injection of hypertonic saline solution (0.3 *M* NaCl, 2 ml/100 g body wt, over 1 min) into conscious, freely moving male rats (40). In sham-operated rats, blood volume expansion (BVE) induced a rapid increase in plasma ANP as before. The concentration peaked at 5 min and remained elevated at 15 min after saline injection. One week after deafferentation of the carotid-aortic baroreceptors, basal plasma ANP concentrations were highly significantly decreased on comparison with values of sham-operated rats; plasma ANP levels 5 min after BVE in the deafferented rats were greatly reduced. Unilateral right vagotomy reduced resting levels of plasma ANP but not the response to BVE; resting concentrations of plasma ANP and responses to expansion were normal in bilaterally vagotomized rats. In rats that had undergone renal deafferentation, resting levels of ANP were normal but the response to BVE was significantly suppressed.

The evidence indicates that afferent impulses *via* the right vagus nerve may be important under basal conditions, but they are not required for the ANP release induced by BVE. In contrast, baroreceptor impulses from the carotid-aortic sinus regions and the kidney are important pathways involved in neuroendocrine control of ANP release. Others have also found that the carotid-aortic baroreceptors are important in mediating the response (42). The evidence from these experiments and our previous stimulation and lesion studies indicates that the ANP release in response to volume expansion is mediated by afferent baroreceptor input to the AV3V region, which mediates the increased ANP release *via* activation of the hypothalamic ANP neuronal system (38).

Role of the Locus Ceruleus and Raphé Nuclei in Transmission of Afferent Input to the AV3V Region

Since baroreceptor afferents terminate in the nucleus tractus solitarius (NTS), we hypothesized that

baroreceptor impulses to the NTS might be relayed to the locus ceruleus, which would then transmit the information by axons of noradrenergic neurons located there to the AV3V region. Indeed, lesions of the locus ceruleus lowered resting ANP levels and blocked the response of ANP volume expansion (Franci J *et al.*, 1992, unpublished). We speculate that the axons of these noradrenergic neurons projecting to the AV3V region activate cholinergic interneurons there, which in turn stimulate the hypothalamic ANPergic neurons (40). These neurons would activate efferent neurohumoral or neural pathways, which induce the release of ANP from the brain and in much greater quantities from the atria.

An afferent pathway to the AV3V region *via* serotonergic (5-HTergic) neurons with cell bodies in the raphé nuclei has been demonstrated (43). Therefore, we hypothesized that 5-HT may play a role in the control of ANP neurons in the region of the AV3V. Indeed, earlier studies had shown that injection of 5-HT agonists into the third or lateral ventricles could increase plasma ANP, and that the responses were prevented by 5-HT_2 receptor blockers (44). To determine the effect of loss of 5-HT input into the AV3V region, bilateral lesions were placed in the dorsal raphé nuclei (DRN), a major source of 5-HT neurons that project to the AV3V region, and in other animals depletion of 5-HT from 5-HTergic neurons was accomplished by systemic administration of parachlorophenylalanine (PCPA), an amino acid that competes with tryptophane, the substrate of tryptophane hydroxylase, the rate-limiting enzyme in the synthesis of indolamines (45).

Rather surprisingly at first glance, the DRN lesions produced a diabetes insipidus-like state in which there was a highly significant increase in water intake and urine volume beginning on the first day following lesions, reaching a peak of water intake at 3 days, followed by a gradual decline in water intake and urine volume to control levels a week after the lesions had been placed. During the diuresis, the osmolality of the urine was dramatically reduced as was the sodium excretion. When the animals were water-loaded and sodium excretion measured on Day 2 after lesions, the excretion of sodium was drastically lowered. However, this had recovered by 4 and 14 days post lesions.

We believe that these changes were due to a drastic suppression of ANP release since the basal levels of ANP were highly significantly lowered and concluded that the serotonergic system has a tonic stimulatory drive on the release of ANP. When this ANP drive is removed by the lesions, there may be a removal of tonic inhibition by ANP of all Ang II-secreting neurons within the AV3V region resulting in increased Ang II release which then brings about an increase in water intake. At the same time, the reduction in ANP output causes a reduction in renal sodium excretion. Consequently, water intake is increased leading to reduction in VP release and a hypotonic urine with drastically reduced sodium concentration. The animals recovered, probably because the DRN is not the only source of serotonergic input, which is also delivered through the median raphé nuclei. Depletion of the 5-HT from these serotonergic cells also produced a similar picture (45).

When the rats with PCPA lesions were water-loaded 5 days after the lesions, they showed a similar reduction in natriuresis as that of the rats with DRN lesions. The results differed from those in rats with DRN lesions only in that there was also a significant reduction in kaliuresis in the PCPA-injected rats. These effects were probably also due to reduced serotonergic input to the AV3V region and thereby reduced stimulation of the ANP neurons with resultant reduction in plasma ANP. As in the case of the DRN lesions, not only were the initial levels of plasma ANP significantly lowered, but also the response of plasma ANP to BVE was significantly reduced, although the reduction was not complete as was the case for the DRN lesion group of animals.

Therefore, we concluded that there was a tonic stimulatory input from the 5-HT neurons to the hypothalamic ANP neurons, which when removed resulted in disinhibition of Ang II release causing increased water intake and decreased ANP release into the circulation resulting in sodium retention. The raphé nuclei may be stimulated by afferent input from the baroreceptors *via* the NTS and this then may be in part responsible for the stimulation of ANP release which occurs following volume expansion. Alternatively, a tonic stimulatory drive *via* these neurons may be all that is required to have the volume expansion induced release of ANP, and the major stimulation may be *via* the locus ceruleus with increased noradrenergic drive to the AV3V region. Further work will be necessary to distinguish between these two possibilities.

We have illustrated (Fig. 1) the putative pathway of activation of ANP release and natriuresis *via* volume expansion. It involves distension of baroreceptors in the right atria, carotid and aortic sinuses, and the kidney, which alters their afferent input to the brain stem in the nucleus tractus solitarius. Impulses from there activate the locus ceruleus since lesions of the locus ceruleus, a major source of noradrenergic axons to the hypothalamus lower resting ANP. The axons of these noradrenergic neurons projecting to the AV3V region activate cholinergic interneurons there, which in turn stimulate the hypothalamic ANPergic neurons. These neurons would activate efferent neurohumoral or neural pathways which induce the release of ANP from the brain and the atria.

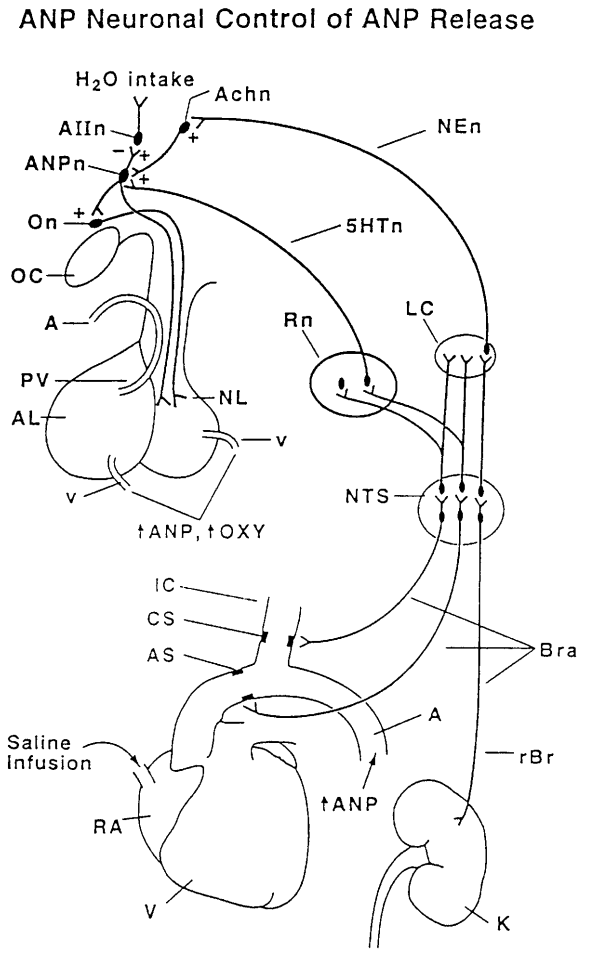

Figure 1. Schematic diagram of the ANP neuronal control of ANP release. For explanation see the text. AIIn, angiotensin II neuron; Achn, acetylcholinergic neuron; NEn, norepinephrinergic neuron; ANPn, ANPergic neuron; On, oxytocinergic neuron; 5HTn, 5HTergic neuron; OC, optic chiasm; A, artery; PV, portal vessel; AL, anterior lobe of the pituitary gland; NL, neural lobe of the pituitary gland; v, vein; Rn, raphè 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 baroreceptors; K, kidney. (From Reis *et al.* [45], with permission.)

Efferent Pathways by Which Volume Expansion Stimulates ANP Release

Some of the ANP neurons terminate in the median eminence and neural lobe of the hypophysis. It is probable that their activation leads to release of the peptide into the vasculature draining the median eminence or the neural lobe. Since the quantity of ANP is more than 1000-fold less in these structures than in the atria (36), we believe that ANP released from the brain plays a minor role in the response. Rather, we would suggest that these ANPergic neurons activate descending pathways which then activate efferent pathways to the heart with consequent release of ANP from the cardiac myocytes. Combined release from both sources then accounts for the increase in plasma ANP concentrations which mediate the ensuing natriuresis. We do not believe that the efferent pathway to the heart is principally neural. It cannot be cholinergic since bilateral section of vagi does not block the response to volume expansion. It is unlikely that it is a sympathetic efferent pathway, since volume expansion by elevating blood pressure should, if anything, diminish sympathetic outflow. There is a possibility that there could be an unknown efferent pathway reaching the atria, perhaps peptidergic in nature, or even nitricoxidergic.

Instead, we believe that release of brain peptides induced by ANP neurons is probably the major pathway. These peptides circulate to the atria and there act directly on atrial myocytes to stimulate the release of ANP. Because there was a large amount of endothelin discovered in the neural lobe, we evaluated the possibility that this could be the activator of the release of ANP by the atrial myocytes. However, our data indicate that this is unlikely (46). α-MSH is also natriuretic and we evaluated its effect on ANP release, but so far the results have not been impressive. The major peptides of the neurohypophysis are VP and OT. Both are natriuretic in the rat, but OT is by far the most potent of these natriuretic peptides and in our earlier experiments, we considered the possibility that OT was indeed the natriuretic peptide (22).

Therefore, we reevaluated the role of OT in the natriuresis and ANP release induced by volume expansion (48) OT (1–10 nmole) injected ip in water-loaded conscious rats caused significant dose-dependent increases in urinary osmolality, natriuresis, and kaliuresis, results exactly similar to those obtained by volume expansion in the conscious water-loaded rat under the same conditions used in all our prior experiments. Plasma ANP concentrations increased nearly 4-fold 20 min after this dose of OT, but there was no change in plasma ANP values in control animals. OT (1 or 10 nmole) injected iv induced a dose-related increase in plasma ANP peaking at 5 min. Therefore, we have demonstrated that OT can indeed induce natriuresis and kaliuresis, and furthermore that it induces a concomitant release of ANP in the concentrations and with the same time course as that previously found with BVE. Furthermore, OT (10^{-6} or 10^{-7} *M*) evoked a release of ANP from incubated rat atria which was blocked by an OT antagonist (Ferring, Malmo, Sweden). The antagonist (10^{-6} and 10^{-9} *M*) reduced basal ANP release. The data suggest that OT may be a physiologically significant releaser of ANP (Favaretto AL, Ballejo G, Antunes Rodrigues J, McCann SM, in preparation, 1996).

To determine if indeed BVE induces OT as well as ANP release, intraatrial injections of isotonic saline

were given (2 ml/100 g body wt), which induced a rapid (5 min postinjection) increase in plasma OT and ANP concentrations and a concomitant decrease in plasma arginine VP concentration (48). When hypertonic volume expansion was produced by injection of 0.3 *M* NaCl, which should also stimulate putative osmo- or sodium receptors and might be expected to cause a secretion of VP, there was a greater increase in plasma ANP and also OT, but not significantly different from the increases in the isotonic volume-expanded animals. However, in contrast to isotonic volume expansion, there was a transient (5 min) increase in plasma arginine VP.

We have already shown that suckling, which causes the release of OT, increases plasma ANP in rats, and that an OT antagonist injected 15 min prior to suckling, completely blocked the increase in ANP from suckling (unpublished data). Experiments are currently underway to determine if OT antagonists can similarly block the ANP release from volume expansion.

Consequently, we have developed the hypothesis that baroreceptor activation of the CNS by BVE stimulates the release of OT from the neurohypophysis (Fig. 2). This OT circulates to the right atrium to induce release of ANP. ANP circulates to the kidney and induces natriuresis and diuresis which restores body fluid volume to normal levels. It is not clear whether or not OT has a separate, independent natriuretic action which is additive or synergistic with the action of ANP. Studies with antisera directed against the peptides and receptor blockers will be necessary to reveal this.

It has been noteworthy throughout all of these studies that smaller changes in potassium excretion parallel those in sodium excretion, although they are not so pronounced. We found this also to be the case with injection of OT. Similarly, ANP produces a greater natriuresis but a significant kaliuresis (48). Consequently, we concluded that OT and ANP can account for the kaliuresis which also follows volume expansion, and for the decrease in potassium excretion which also occurs along with the decrease in sodium excretion when there is a reduction in ANP release. This view may need modification with the recent discovery of a kaliuretic peptide, which produces kaliuresis with little or no natriuresis, and is the 20–amino acid peptide amino terminal extension of ANP (49).

Furthermore, it has now been shown in humans that water-immersion to the neck, as would be expected from results obtained even in the middle ages, produces a diuresis, natriuresis, and kaliuresis. Vesely *et al.* (50) have shown that in this situation there is an elevation of plasma concentrations of both kaliuretic peptide and ANP. However, the time course of the

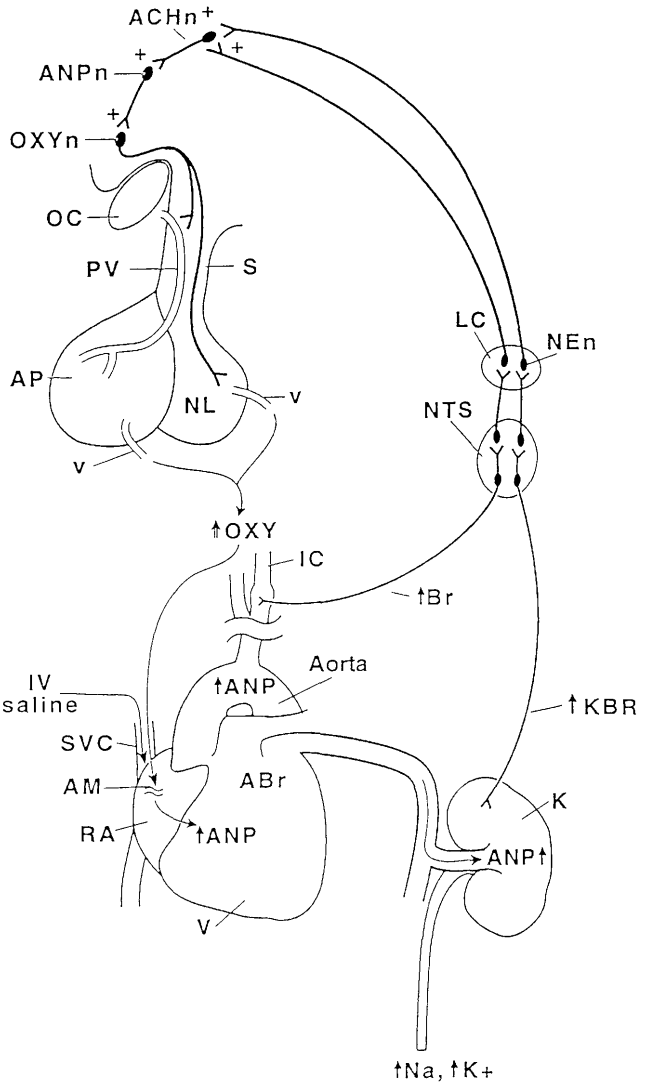

Figure 2. Schematic diagram of the mechanism of natriuresis following BVE by injection of isotonic saline into the right atrium. OXYn, oxytocinergic neuron; Achn, acetylcholinergic neuron; NEn, norepinephrinergic neuron; ANPn, ANPergic neuron; OC, optic chiasm; A, artery; PV, portal vessel; AP, anterior 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; A, aorta; RA, right atrium; V, ventricles; Br, baroreceptor afferents; KBR, renal baroreceptor afferents; K, kidney. (From Haanwinckel *et al.* [48], with permission.)

ensuing kaliuresis is more easily explained on the basis of increased concentrations of kaliuretic peptide than ANP. Therefore, it is possible that stimuli which either increase or decrease plasma ANP also produce a concomitant stimulation or inhibition of the release of kaliuretic peptide. Further experiments are needed to determine whether both peptides are involved in the changes or only ANP.

Hormonal Effects of the Brain ANP System

In addition to altering water and salt intake and excretion, ANP has important actions on the release of anterior and posterior pituitary hormones. The peptide

can inhibit the release of corticotrophin (ACTH) (37, 50), and prolactin (37, 47), anterior pituitary hormones that are released during stress. To determine the physiologic significance of ANP in the control of basal and stress-induced release of anterior pituitary hormones, the same highly specific antiserum against the peptide (AB-ANP) which we had employed earlier (35) was microinjected into the 3V of conscious, freely moving male rats to immunoneutralize hypothalamic ANP (47). In the initial experiment, the antiserum or control normal rabbit serum (NRS) was injected into the 3V to determine the effect of the antiserum on basal release of pituitary hormones. The antiserum had no effect on the concentrations of plasma ACTH, prolactin, or thyroid-stimulating hormone (TSH) for 3 hr after the injection; however, plasma growth hormone concentration, although unchanged for 2 hr, was markedly elevated at 3 hr. These results indicate that although ANP appears to have no effect on the basal release of the other hormones, it has a physiologically significant inhibitory effect on growth hormone release. The delay of the effect is probably related to the time required for the antiserum to diffuse to the site of action of the peptide, presumably at some distance from the ventricle.

Since this effect was demonstrable only after 3 hr, in the stress experiment, the antiserum or NRS was microinjected into the 3V 3 hr prior to application of ether stress. The rapid elevation of plasma ACTH in NRS-injected rats was markedly augmented by AB-ANP. Ether also induced a rapid increase in plasma prolactin in the NRS-injected animals, as expected. Contrary to the ACTH response, the maximal increase in plasma prolactin after ether was attenuated in animals preinjected with AB-ANP.

In the NRS-injected animals, there was a significant decline in plasma growth hormone after the application of ether that was significantly accentuated by AB-ANP, but this was probably the result of the higher initial levels of plasma growth in the ANP-AB group followed by its disappearance with a half-time similar to that of the NRS-injected group. The decline in plasma TSH after ether stress was unaltered in the animals injected with AB-ANP.

The results of these immunoneutralization studies suggest that endogenous ANP does not play a role in TSH release. On the other hand, the endogenous peptide appears to have a physiologically significant inhibitory role in suppressing ACTH release during stress, mediated at least partly by suppression of VP release (28). Endogenous ANP has a pathophysiologic role in augmenting the prolactin release in stress either by inhibiting release of prolactin-inhibiting factors or, alternatively, by enhancing release of prolactin-releasing factors, such as OT. Endogenous ANP appears to inhibit resting, without altering stress-induced inhibition of growth hormone–releasing hormone release or by stimulating resting somatostatin release or by both actions (47).

The results of our experiments appear to indicate that the increased release of ANP during stress dampens a number of responses to the stressful stimulus. For example, the inhibition by ANP of stress-induced VP release would increase renal water loss and the diminished ACTH release induced by the peptide would lead to reduction in aldosterone secretion thereby diminishing the sodium retention which characterizes stress. The ANP released in stress probably acts intrahypothalamically to suppress VP secretion and since VP augments ACTH secretion during stress, this diminution in VP release would lead to the decreased ACTH secretion. The increased prolactin release in stress induced by the increased endogenous ANP release probably would not augment sodium excretion since, at least under certain circumstances, prolactin appears to have a direct antinatriuretic effect on the kidney (52).

In contrast to these results with GH, ACTH, and prolactin, there was not only no effect of the antiserum on resting TSH values but also no alteration in the suppression of TSH release induced by ether stress. In other studies, we have discovered (53) the physiological significance of the suppressive action of ANP on basal luteinizing hormone but not follicle-stimulating hormone release.

In summary, these immunoneutralization experiments support the concept that in states of volume expansion the increased ANP released would act centrally to inhibit release of VP and ACTH. The inhibition of VP release would augment renal water loss and the reduction of aldosterone release consequent to decreased ACTH release would augment water and Na^+ loss, respectively, amplifying the natriuresis induced by ANP and hastening the return of the blood volume to normal.

Conclusions

In conclusion, it is now clear that Ang II and ANP in the brain play important and opposite roles in the control of water and salt intake, Ang II promoting the intake of both and ANP inhibiting intake of both, perhaps by inhibiting the secretion within the brain of Ang II. In fact, there is probably a reciprocal relationship between the activity of these two neuronal systems. Again, on the output side, the two are related since ANP release is stimulated in situations of volume expansion to increase renal sodium and potassium loss, and at the same time, ANP not only blocks the release of renin from the kidney, thereby decreasing Ang II stimulation of aldosterone release but also directly inhibits aldosterone secretion. Decreased aldosterone secretion results in increased natriuresis. ANP neu-

rons in the hypothalamus inhibit the release of corticotrophin-releasing factor and VP, thereby ACTH release which also reduces aldosterone secretion.

Therefore, both of these peptides play a key role in the maintenance of body fluid homeostasis in the body. ANP acts slowly *via* natriuresis to reduce effective circulating blood volume. The rapid release of ANP following BVE would first produce vasodilatation by activating particular guanylate cyclase, leading to the production of cyclic GMP, which would relax vascular smooth muscle. These combined actions would result in a rapid reduction in the effective circulating blood volume. The longer-term effects would be mediated not only by diuresis and natriuresis but also by decreased intake of salt and water.

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