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Carotid–aortic and renal baroreceptors mediate the atrial natriuretic peptide release induced by blood volume expansion

(plasma atriopeptin/vagus nerve/afferent neurons)

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ABSTRACT **Our previous studies have shown that stimulation of the anteroventral third ventricle (AV3V) region of the brain increases atrial natriuretic peptide (ANP) release, whereas lesions of the AV3V region or median eminence of the tuber cinereum block the release of ANP caused by blood volume expansion. These results suggest that participation of the central nervous system is critical to this response. The role of baroreceptors in the response was evaluated in the current research by studying the response of plasma ANP to blood volume expansion induced by intravenous injection of hypertonic saline solution (0.3 M NaCl, 2 ml/100 g of body weight, over 1 min) in conscious, freely moving male rats. Plasma samples were assayed for ANP by radioimmunoassay. In sham-operated rats, blood volume expansion induced a rapid increase in plasma ANP: 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 blood volume expansion in the deafferented rats were greatly reduced. Unilateral right vagotomy reduced resting levels of plasma ANP but not the response to blood volume expansion; 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 blood volume expansion 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 blood volume expansion. In contrast, baroreceptor impulses from the carotid–aortic sinus regions and the kidney are important pathways involved in the neuroendocrine control of ANP release. 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.**

Atrial natriuretic peptide (ANP), which is primarily localized to the atrial myocytes, plays an important role in control of body fluid homeostasis by decreasing salt and water intake and increasing salt and water excretion (1–7). When the blood volume is expanded—for example, by intravenous injection of saline solution—ANP is released into the circulation and induces natriuresis, in part by direct action on the kidneys (8, 9). Natriuresis is also promoted by direct suppression of the release of aldosterone from the adrenal glomerulosa by ANP (1). ANP also inhibits the release of renin from the juxtaglomerular apparatus of the kidneys (10, 11), which decreases the release of angiotensin II, further decreasing the release of aldosterone. Since angiotensin II is an important mediator of salt and water intake via its hypothalamic action (11), this leads to a decrease in the intake of both water and salt. ANP has a direct effect in the brain to inhibit water and salt intake and to antagonize the dipsogenic action of angiotensin II (4–7). Therefore, the release of ANP induced by increased blood volume may cause a further decrease in the intake of fluid and electrolytes in addition to increasing the output of water and sodium.

Expansion of the circulating blood volume has been thought to induce ANP release by stretching the atrial myocytes and directly releasing the peptide into the circulation from its storage granules (1); however, it has yet to be shown that the stretch induced by volume expansion is sufficient to release ANP directly (12).

A role for the central nervous system (CNS) in the control of renal sodium excretion was demonstrated long before the discovery of ANP (13–16). The central mechanism of sodium control is believed to reside in the anteroventral third ventricle (AV3V) region, since stimulation of this region enhances, and its destruction diminishes, excretion of sodium (17, 18). The AV3V region is the site of perikarya of ANP-containing neurons whose axons project to the external layer of the median eminence (19–21) and also to the neural lobe of the pituitary gland (21–23). These ANP neurons appear to play an important role in mediation of the effects of the AV3V region on natriuresis, since stimulation of the AV3V resulted in a rapid increase in plasma ANP, whereas its destruction resulted in a decrease in plasma ANP (24). Since the ANP content of the atria was not altered under these conditions but rapid changes in the CNS and pituitary content of the peptide occurred, we hypothesized (24) that release of ANP from the CNS by way of the median eminence and/or neural lobe of the pituitary gland might play a role in the stimulation-induced increase in plasma ANP.

The critical importance of the AV3V region and of its caudal projections to the neural lobe to the control of resting ANP levels and the response to blood volume expansion was supported in later studies in which lesions of the AV3V region lowered resting ANP levels and largely blocked the increase in plasma ANP that follows volume expansion (25–27). Similar results were obtained following more-caudal lesions in the ANP neuronal system in the median eminence or posterior pituitary (25, 26). Since a common denominator of the lesions was elimination of the brain ANP neuronal system, these results suggest that brain ANP plays an important role in the mediation of the release of ANP that

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Abbreviations: ANP, atrial natriuretic peptide; AV3V, anteroventral third ventricle; CNS, central nervous system.

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occurs after volume expansion. Since the content of ANP in this system is much less than in the atria, it is also possible that blockade of volume expansion-induced release of other brain peptides, such as endothelin (28), which can directly stimulate the release of ANP from the atria (29), may result in failure of the release of ANP from the atrial myocytes following volume expansion.

We hypothesized that blood volume expansion detected by stretch of baroreceptors causes afferent input to the brain ANP system, thereby increasing release of the peptide from the median eminence and neural lobe. The present experiments were designed to evaluate this hypothesis by deafferenting the atrial baroreceptors by right and by bilateral vagotomy. The carotid–aortic baroreceptors were also deafferented to evaluate their role. Finally, baroreceptors described by Davis and coworkers (30, 31) in the kidney were deafferented to determine their possible role in the control of resting and volume expansion-induced ANP release.

MATERIALS AND METHODS

Male Wistar rats weighing 230–250 g were maintained on a stock diet with water ad libitum. They were communally housed before surgery. Thereafter, they were maintained in individual cages in a light-controlled (lights on 0700–1900) and temperature-controlled (23 ± 2°C) room before and during all the experiments.

Hemodynamic Studies. To measure arterial blood pressure a polyethylene catheter (PE-10 connected to PE-50, Clay Adams) was chronically inserted into the abdominal aorta via the femoral artery. To administer drugs a second catheter was inserted into the femoral vein. Both catheters were implanted under ether anesthesia. Arterial pressure was monitored by means of a Statham pressure transducer (P23D6) connected to a Hewlett-Packard four-channel recorder (model 7754A). Mean arterial pressure was obtained from the arterial pulse pressure through a Bioelectric Amplifier (model 8811A) provided with analog/digital interface to a Monydata-XT microcomputer, which processed arterial pressure signals.

Carotid–Aortic Sinus Deafferentation. This procedure was performed by the method of Krieger (32). Records of mean arterial pressure and arterial pressure lability of deafferented and sham-operated rats were initiated 48 hr after surgery. The efficacy of deafferentation was tested; rats that presented bradycardia of <20 beats/min in response to a 25- to 40-mmHg (1 mmHg = 133 Pa) increase in mean arterial pressure produced by injection of phenylephrine (0.5–2.0 μg/kg) were considered to have adequate deafferentation. Computer-aided sampling of individual points for mean arterial pressure was performed at a frequency of 30 Hz during 15 min (33–35). Arterial pressure lability was expressed as the standard deviation of the mean arterial pressure at these sampling points.

Right or Bilateral Vagotomy. The right vagus or both vagi were located, isolated, and ligated in the cervical region at the cricocartilage level, under ether anesthesia. Each nerve trunk was cut distal to the ligature. The sham-operated rats were submitted to the same steps of the surgical procedure leaving intact the vagus.

Renal Denervation. After dorsal abdominal incisions both kidneys were exposed and surgically denervated with the aid of a stereomicroscope while the rats were anesthetized with tribromoethanol (2.5% solution in 0.15 M NaCl, i.p., 1 ml/100 g of body weight). The denervation was accomplished by cutting all visible nerves along the renal artery and by stripping both renal artery and hilum of all nerve bundles. Immediately thereafter, the renal artery was surrounded with a cotton pledget that had absorbed a 10% (vol/vol) phenol solution in absolute ethanol (36–38). The sham-operated rats underwent all steps of the surgical procedure leaving untouched the renal artery.

The animals were used in the experiment 1 week after carotid–aortic and renal denervation and 24 hr after uni- or bilateral vagotomy. All rats received, at the end of the operation, a prophylactic injection of penicillin (60,000 units, s.c.).

Blood Volume Expansion. Twenty-four hours before the experiment, a catheter was inserted into the right external jugular vein and advanced to the right atrium as described (39). The blood volume expansion was performed by i.v. injection of 0.3 M NaCl in a volume of 2 ml/100 g of body weight, over 60 sec. The rats were decapitated just before (0 time) or 5 and 15 min after expansion. The trunk blood was collected into tubes cooled in crushed ice and containing the following proteolytic-enzyme inhibitors: 2 mg of EDTA, 20 μl of 1 mM phenylmethylsulfonyl fluoride (Sigma, P-7626), and 20 μl of 500 μM pepstatin A (Sigma, P-4265).

Plasma ANP Levels. The immunoreactive ANP was extracted from 1 ml of plasma by heat-activated Vycor glass (Corning no. 7930, mesh 140). The ANP plasma levels were measured by radioimmunoassay (2). The mean recovery of standard ANP was 75 ± 5% (mean ± SD). The intra- and interassay variances were 8% and 12%, respectively. The sensitivity of the assay was 1.5 pg per tube.

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

RESULTS

Carotid–Aortic Deafferentation. To investigate the effects of deafferentation of carotid–aortic baroreceptors on the ANP release induced by blood volume expansion, three groups of rats that had undergone such deafferentation were studied in basal conditions and at 5 and 15 min after blood volume expansion. Each of these groups had its respective control group of sham-operated rats. Only those rats showing hemodynamic data compatible with effective carotid–aortic deafferentation (i.e., arterial pressure lability and absence of bradycardia in response to increase in mean arterial pressure) were submitted to blood volume expansion studies.

Carotid–aortic deafferented rats (n = 8) had significantly lower ($P < 0.001$) plasma ANP concentrations under basal conditions than the sham-operated rats (n = 7) (Fig. 1). Their plasma ANP levels 5 min after blood volume expansion were

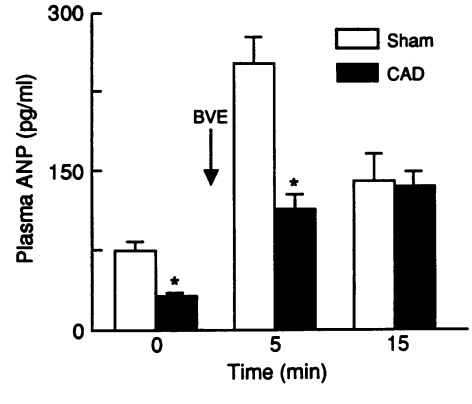

FIG. 1. Effect of carotid–aortic sinus denervation on basal plasma ANP concentrations and the response to blood volume expansion (BVE). There were seven sham-operated animals and eight carotid–aortic sinus deafferented (CAD) rats. In this and subsequent figures, the height of the column gives the mean and vertical lines indicate the standard error of the mean. ★, $P < 0.01$ vs. control at that point in time.

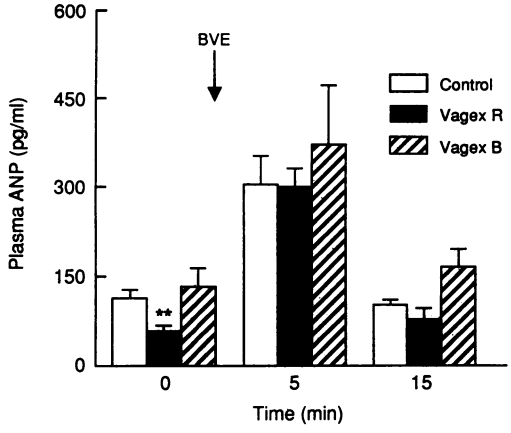

FIG. 2. Effect of right or bilateral vagotomy on basal plasma ANP concentrations and the response to blood volume expansion (BVE). There were 7 sham-operated rats (control), 10 right vagotomized (Vagex R), and 6 bilaterally vagotomized (Vagex B) animals. **, $P < 0.005$.

also significantly lower ($P < 0.001$) than those of the sham-operated controls. There was no statistical difference between the still slightly elevated values in both experimental groups 15 min after expansion.

Right or Bilateral Vagotomy. Unilaterally vagotomized rats ($n = 10$) had plasma ANP values that were significantly lower ($P < 0.005$) in basal conditions than values observed in sham-operated rats ($n = 7$) (Fig. 2). No significant differences were observed in basal plasma ANP values when rats with bilateral vagotomy ($n = 6$) were compared with the sham-operated controls. The increases in plasma ANP concentrations in response to blood volume expansion were similar in the unilaterally or bilaterally vagotomized rats when compared with those of the sham-operated rats ($n = 7$) at 5 and 15 min.

Renal Deafferentation. Although renal deafferented ($n = 8$) and sham-operated ($n = 8$) rats had similar basal plasma ANP values, 5 min after blood volume expansion the renal deafferented rats showed a smaller increase ($P < 0.001$) in ANP plasma concentrations than their controls (Fig. 3). No significant differences were observed in plasma ANP concentrations from both renal deafferented and sham-operated groups 15 min after expansion.

DISCUSSION

Previous results with stimulation (21) and lesions (22) in the ANP neuronal system of the CNS suggest that the CNS is essential to the control of ANP release, since the lesions not only lowered basal ANP levels but largely blocked the ANP release induced by volume expansion. The present results further implicate the CNS in the control of ANP release because deafferentation of carotid–aortic baroreceptors and renal deafferentation largely blocked the increase in plasma ANP in response to volume expansion. Deafferentation of carotid–aortic baroreceptors even lowered resting plasma ANP levels, in agreement with the independent results of Morris and Alexander (40).

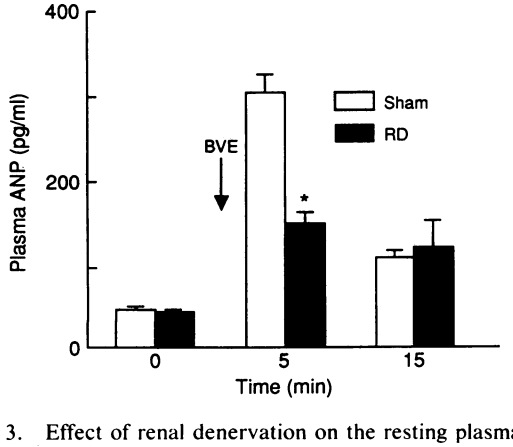

FIG. 3. Effect of renal denervation on the resting plasma ANP concentrations and the response to blood volume expansion (BVE). There were eight sham and eight renal deafferented (RD) rats.

At face value this would suggest that tonic impulses in baroreceptor afferents stimulate the release of ANP. Increased activity of these receptors brought about by volume expansion presumably causes increased afferent impulses in peripheral nerves and, in turn, within the brain to stimulate the release of ANP that follows. This ANP may be released directly from the brain ANP system via the median eminence and neural lobe, and/or activation of the brain ANP system may cause release of other hypothalamic peptides, such as endothelin (28), into the circulation that act directly on the atria to induce ANP release (29).

Alternatively, efferent pathways may descend from the hypothalamus and activate ANP release via the parasympathetic or sympathetic innervation to the heart. Since bilateral vagotomy did not alter resting ANP levels or volume expansion-induced increases in plasma ANP, we can exclude the obligatory participation of vagal afferent and efferent pathways in the mediation of the ANP release from volume expansion. Bilateral vagotomy failed to alter not only the response to volume expansion but also the basal levels of plasma ANP. Surprisingly, right vagotomy lowered basal values but did not modify the response to volume expansion. A lowering of the basal plasma ANP concentrations in the rats with right vagotomy is consistent with a stimulatory role of right atrial stretch receptors whose afferents reach the hypothalamus via the right vagus, which predominantly innervates the right atrium; however, it is clear that vagal afferents and efferents are not critical to the response to volume expansion, because of the normal response following bilateral vagotomy.

Renal denervation, although it did not alter basal levels of plasma ANP, was highly effective in blocking the response to volume expansion. The reduction was almost the same as that in animals in which the carotid-aortic baroreceptors had been denervated. Thus, it appears that distention of these renal baroreceptors plays a role via afferent input to the brain in the induction of ANP release following volume expansion.

None of the denervations completely blocked the response to volume expansion at 5 min, and none of them altered the response at 15 min. Thus, there was clearly a residual response that was not eliminated by carotid–aortic or renal denervation. Since we injected hypertonic saline, it is possible that this residual response may have been caused by stimulation of sodium receptors in the AV3V region (41), which could evoke ANP release independently of baroreceptor influences. Because our previous work (25, 26) showed no significant difference in the response to volume expansion with hypertonic versus isotonic solutions, albeit the response to the latter was less, we are inclined to believe that this is not the total cause of the failure of complete blockade by the deafferentations. A more likely explanation for the failure of the deafferentations to block completely the response is that the response is due to combined input from a variety of baroreceptors, the most important of which are likely those in the sino–aortic region and the kidney. However, there may be a minor component also from baroreceptors in the atria that was disclosed only by right vagotomy. This surgery would eliminate most of the input from baroreceptors located in the right atrium. To test this hypothesis requires combined denervation surgery.

Another possibility is that the residual response is due to direct activation of the release of ANP from the atria by stretch of the atrial myocytes (12). Our findings with deafferentation of baroreceptors strongly support the previous results with stimulation and lesions of the brain ANP system and indicate a crucial importance of the CNS in mediating the control of ANP release. The CNS may control circulating ANP either by directly releasing the peptide from the brain or via stimulation by brain ANP of the release of other brain peptides into the circulation via release from the median eminence and neural lobe. These would circulate to the heart and act directly on the atria to stimulate ANP release. A candidate for such a role is endothelin, which is found in neuronal terminals of the neurohypophysis and has been reported to stimulate release of ANP from the atrium (28). Finally, the baroreceptor stimulation could act in part via sympathetic efferents to the atria, but not via parasympathetic efferents, since bilateral vagotomy did not interfere with the ANP response to volume expansion.

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