Cardiovascular Actions of Vasopressin at the Ventrolateral Medulla

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Vasopressin acts at a number of sites in the central nervous system to alter arterial pressure. This study investigated the hypothesis that vasopressin acts at the rostral ventrolateral medulla to increase arterial pressure. The rostral pressor area of the medulla oblongata was exposed in urethane-anesthetized rats prepared for topical application of vasopressin. A 3-minute application of vasopressin (range 10^{-4} to 10^{-3} M) produced dose-dependent increases in arterial pressure that averaged between 2±1 and 65±11 mm Hg (p<0.01). Tachycardia was not a consistent response at any concentration of vasopressin. Intravenous administration of a V_1 vasopressin antagonist did not modify the pressor response produced by topical application of vasopressin (10^{-4} M). Application of the V_1 antagonist to the rostral pressor area, however, prevented the production of a pressor effect to subsequent topical application of vasopressin (10^{-4} M). These experiments suggest that vasopressin stimulates the activity of vasomotor neurons in the rostral ventrolateral medulla by a mechanism that involves a neuronal V_1 receptor. (Hypertension 1990;15(suppl I):I-102–I-106)

Currently, the role of neuropeptides in the central control of blood pressure is the subject of considerable attention. It has been shown that intracerebroventricular injections of angiotensin II (Ang II), vasopressin (VP), substance P, and enkephalin increase blood pressure. To better understand the pathways that can account for the effects produced by intracerebroventricular injection of peptides, we focused our attention on the ventrolateral medulla (VLM) because it plays an important role in the regulation of blood pressure. We showed that topical application of Ang II at a region overlying the rostral ventrolateral medulla (RVLM) increases blood pressure without producing changes in heart rate. The ventral medulla receives a projection of VP-containing fibers originating in the paraventricular nucleus of the hypothalamus. Additionally, high affinity binding sites for VP have been demonstrated recently in the vicinity of the VLM. Thus, we investigated the cardiovascular effect of VP applied topically to the rostral ventral medullary surface of the rat.

Methods

Experiments were performed in 22 male Sprague-Dawley rats (430–470 g) anesthetized with urethane (1.5–1.8 g/kg i.v.). Studies were conducted according to the guidelines set forth by the American Physiological Society. The trachea was cannulated and the rat permitted to breathe spontaneously. With the rat in a supine position, the head was fixed in a stereotaxic frame. The ventral aspect of the medulla was exposed as described by Feldberg and Guertzenstein. A femoral artery and vein were cannulated to monitor arterial pressure and to administer drugs and fluids, respectively. Peptides were diluted in saline (pH 7.4) and applied topically to both sides of the RVLM by means of Gelfoam pledgets (1 mm²). Pledgets were saturated in 1 µl solution of the peptide and positioned rostral to the hypoglossal nerve rootlets and lateral to the border of the pyramidal tract. In some experiments, the correct placement of the pledget was confirmed by the occurrence of a hypotensive response to topical application of sodium pentobarbital.

Arginine⁸-VP (Bachem, Inc., Torrance, Calif.) was applied topically to the RVLM in six different concentrations (range, 10^{-6}–10^{-3} M). Up to four doses were randomly tested in each rat (n=12). The peptide was left in place for 3 minutes. Immediately after removal of the peptide-soaked pledget, the area was dried and then thoroughly rinsed with saline. Repeated applications of the peptide were 30 minutes apart.

To exclude a vascular action of VP, the V_1 VP antagonist, d(CH₂)₅Tyr(Me) arginine vasopressin (AVP) (Bachem, Inc.), was given (20 µg/kg i.v.). Blockade of peripheral vascular V_1 VP receptors...
was confirmed by substantial reduction in the pressor response produced by bolus injection of VP (10^{-8} \text{ M i.v.}).

In four rats, the effects of local application of VP (10^{-4} \text{ M}) were determined before and after topical application of a V_1 antagonist (10^{-5} \text{ M}). In these rats, the V_1 antagonist was administered intravenously 30 minutes before application of either VP or the V_1 antagonist on the rostral pressor area.

One-way analysis of variance was used to evaluate differences among controls. The effect of drugs on blood pressure and heart rate was assessed by paired comparison of these variables before and after application of the drug of interest (i.e., paired t tests). All variables are reported as mean±SEM. A p value less than 0.05 was required for statistical significance.

**Results**

Topical application of VP to the RVL of the rat produced dose-dependent increases in mean arterial pressure (MAP). Figure 1 shows that the lower doses of VP (10^{-8} and 10^{-7} \text{ M}) did not significantly alter the baseline blood pressure. VP, in concentrations from 10^{-6} \text{ M} to 10^{-3} \text{ M}, however, produced significant increases (p<0.01) in arterial pressure. VP, throughout this dose range (10^{-8} to 10^{-3} \text{ M}), did not alter heart rate consistently (Figure 1). Because four different doses of VP were applied in random order in each rat, we examined whether variations in blood pressure affected the response to topical application of VP. Table 1 lists the baseline values of MAP and heart rate in rats receiving no previous treatment with a VP antagonist. Multiple comparison of the values for MAP did not show any differences in baseline blood pressure, whereas analysis of variance demonstrated that VP doses had a significant effect on the increase in MAP (F=22.0, p<0.0001). Also shown in Table 1 is the fact that significant differences existed among the baseline values of heart rate before application of the peptide. Analysis of variance, however, demonstrated that application of VP at various concentrations did not significantly alter heart rate. Topical application of VP produced a pressor response with a characteristic time course. Figure 2A illustrates that topical application of VP (10^{-4} \text{ M}) initiated an increase in blood pressure within 60 seconds after placement of the pledget on the ventral surface. By 4 minutes, MAP had increased by 50 mm Hg. This pressor response outlasted the removal of the pledget from the ventral surface. At higher VP concentrations (10^{-4} and 10^{-3} \text{ M}), the pressor response lasted for as much as 15 minutes after application of the peptide to the ventral surface.

To elucidate whether the pressor response produced by topical application of VP to the ventral medulla was the result of leakage of the peptide into the systemic circulation, six rats were pretreated with the V_1 VP antagonist. Adequacy of VP blockade was confirmed by elimination of the pressor response produced by intravenous bolus injection of 10^{-4} \text{ M VP}. Figure 2B illustrates the pressor response produced by topical application of the peptide (10^{-4} \text{ M}) in a rat that had been given the V_1 antagonist intravenously. The central pressor effect of VP in this rat pretreated with the VP antagonist was essentially the same as that obtained in a rat with no previous exposure to the VP antagonist (Figure 2A). In the group of rats that received the VP antagonist intravenously, there was a trend for MAP to decrease and heart rate to increase (see data in bottom row of Table 1). Comparison of the baseline values of MAP and heart rate for the two rats with no previous exposure to the VP antagonist in the group of rats that received the VP antagonist intravenously, there was a trend for MAP to decrease and heart rate to increase (see data in bottom row of Table 1). Comparison of the baseline values of MAP and heart rate for the two rats with no previous exposure to the VP antagonist.
groups of rats that subsequently received topical application of VP at 10^{-4} M concentration, however, did not reveal any difference in the baseline values of these variables whether the rats were untreated or pretreated with the V_1 VP antagonist intravenously. VP (10^{-4} M), applied topically to the ventral medulla of rats (n=6) given the V_1 antagonist intravenously, produced an average increase in blood pressure of 35±3 mm Hg (p<0.01). This pressor response was not different from the increase in blood pressure (35±4 mm Hg) obtained in a separate group of untreated rats (n=6). Topical application of VP during blockade of the peripheral V_1 VP receptors, however, was associated with a significant increase in heart rate (46±5 beats/min; p<0.01), which was not observed in untreated rats (32±13 beats/min; p>0.05).

To further establish the mode of action of VP at the ventral medulla, four of six rats that had received the V_1 antagonist intravenously were treated with the V_1 antagonist (10^{-5} M) applied topically to the ventral medulla for 3 minutes. VP was applied topically a second time, immediately after removal of the antagonist from the ventral surface. Before application of the VP antagonist to the ventral medulla, this group of rats had baseline values of 60±3 mm Hg for MAP and 440±25 beats/min for heart rate. The antagonist did not alter the baseline blood pressure (69±5 mm Hg, p>0.01), whereas, the V_1 antagonist was associated with a significant increase in heart rate to 475±20 beats/min (p<0.05). The topical application of the peptide during peripheral V_1 blockade increased MAP by 36±4 mm Hg (p<0.01). The second application of the peptide, however, did not significantly increase MAP (+5±2 mm Hg, p>0.10). Blockade of ventral medullary V_1 VP receptors had a similar effect on heart rate. Before central VP blockade, topical application of VP increased heart rate by 57±7 beats/min (p<0.01); whereas, after central VP blockade, topical application of the active peptide was without effect (+6±3 beats/min; p>0.10).

An additional group of rats received hexamethonium chloride (9 mg/kg i.v.) to assess the contribution of the sympathetic nervous system to the VP-induced pressor response. With the sympa-
thetatic nervous system intact, topical application of VP at 10^{-4} and 10^{-6} M (n=2, at each concentration) increased MAP by 23±7 and 27±1 mm Hg, respectively. After ganglionic blockade, a second application of VP was without effect (+7±3 and +1±7 mm Hg, respectively).

Discussion

The present study demonstrates that topical application of VP to the RVLM of the rat produces dose-dependent increases in arterial pressure but no changes in heart rate. The pressor response due to local application of VP to the ventral medullary surface is produced by a local agonist action of the peptide at neuronal elements of the ventral medulla rather than VP-mediated vasoconstriction secondary to leakage of the peptide into the circulation. The absence of a heart rate component suggests that VP acts at the ventral medulla to prevent a baroreceptor reflex–mediated influence on heart rate. The outflow pathway for this response seems to involve the sympathetic nervous system.

The pressor effect of VP applied topically to the RVLM most likely involves neurons of this region with known projections to sympathetic preganglionic neurons. In the cat, this site corresponds to the glycine sensitive area. In a limited number of experiments, pentobarbital was applied to the ventral surface to define functionally that the pledgets had been placed at a site consistent with the rostral pressor area. Moreover, in all experiments, the anatomic coordinates for the placement of the pledgets corresponded to the region at which Keeler et al obtained maximal depressor or pressor responses to topical application of γ-aminobutyric acid (GABA) and bicuculline, respectively. Although we did not assess the penetration of topically applied VP into the neural substrate of the ventral medulla, Keeler et al have shown that [3H]GABA does not penetrate much farther than 750 μm below the ventral surface. A similar finding was obtained by us in cats given iodine-125-labeled Ang II. Thus, we suspect that VP acted at chemosensitive neural elements of the superficial ventral medulla as well as at more deeply located neural elements that have a facilitatory action on sympathetic nerve activity. Although VP in the pledgets might have diffused to other pressor sites of the medulla oblongata, we suspect that these sites would not explain the observed responses because this would have to occur at very low concentrations of the peptide.

The contention that VP had a primary central action is supported by the finding that peripheral administration of a V1 antagonist before application of VP prevented the pressor response. This observation suggests that VP acts at a V1 receptor. A brain V1 receptor has been implicated in the production of neurogenic cardiovascular actions of VP at the nucleus tractus solitarius (NTS) and spinal cord.

A central site of action is also reinforced by the demonstration that topical application of the V1 antagonist before application of VP prevented the pressor response. This observation suggests that VP acts at a V1 receptor. A brain V1 receptor has been implicated in the production of neurogenic cardiovascular actions of VP at the nucleus tractus solitarius (NTS).

Data suggest that neuronal elements of the VLM might be sensitive to localized ischemia. Evidence, however, would weigh against the idea that topical application of VP elicits a sympathetically mediated response secondary to local ischemia of the ventral medulla. First, systemic administration of the V1 antagonist did not alter the pressor response to topically applied VP. Second, Lassof and Altura showed that local application of VP to pial arterioles of anesthetized rats does not change microvascular diameter or blood flow. Additionally, Katsunos et al have shown that VP in a dose range of 10^{-10}–10^{-6} M produced a concentration-dependent relaxation of canine basilar artery rings that had been contracted by prostaglandin F2α. The possibility of a local vascular effect of VP in the ventral medulla is not ruled out because Long et al have shown that intrathecal VP (10–100 pmol) caused significant reductions in lumbosacral spinal cord blood flow. Further, this effect of VP on spinal cord blood flow was blocked by the V1 antagonist.

Preliminary experiments were performed to define the autonomic pathway involved in the pressor response of VP. After ganglionic blockade, topical application of VP had no influence on blood pressure or heart rate. Thus, it would seem that sympathetic activation is an important component of the pressor response produced by topical application of VP to the ventral medulla. In this regard, our results bear similarity to the involvement of the sympathetic nervous system in the pressor response produced by intrathecal injection of VP in anesthetized rats. Because the topically applied VP would be at highest concentrations at the ventral surface of the medulla in our experiments, it is probable that VP acted at sympathoexcitatory neurons of the VLM to increase arterial pressure. One of the interesting observations of this study was the absence of a reflex decrease in heart rate when VP was applied to the ventral medulla. This might be the outcome of a complex set of pathways having competing effects toward sympathoexcitatory neurons of the VLM. It is possible that the VP applied topically to the ventral medulla excited sympathoexcitatory neurons of the VLM, and this excitatory influence counteracted an inhibitory drive from baroreceptor reflex pathways originating in the NTS. Another consideration is that some of the VP applied to the ventral medullary surface gained access to the dorsomedial medulla where it would have to act at very low concentrations to attenuate baroreceptor reflex–mediated bradycardia. This mode of action would be consistent with the dem-
ontrast of Michelini and Bonagamba that microinjection of VP into the medial NTS attenuated baroreceptor reflex bradycardia associated with pressor infusions of phenylephrine. Our data also suggest that VP receptors at sites lacking a blood-brain barrier might contribute to the responses produced by topical application of the peptide in the presence of the V1 VP antagonist. We observed that tachycardia accompanied the pressor response of VP applied topically after blockade of peripheral V1 VP receptors. This phenomenon might reflect blockade of V1 VP receptors at blood-brain barrier-deficient sites implicated in the potentiation of baroreceptor reflex sensitivity. Alternatively, blockade of peripheral V1 VP receptors might have uncovered an effect involving V1 VP receptors at an undetermined central site.

Although our study did not assess the potential physiological role for VP at the VLM, the data are pertinent to the observation of Porter and Brody who showed that lidocaine or kainic acid injection of the V1 antagonist. These observations implicate a physiological role for VP at the VLM, the data are significant.

In this regard, the ventral medulla as well as the NTS, locus coeruleus, and spinal cord are sites where VP acts to modulate cardiovascular function. Although the direct effect of VP on "cardiovascular" neurons was beyond the scope of the present study, one might suggest the peptide has excitatory effects similar to those found for lateral horn cells of the spinal cord. In a number of studies, the excitatory effects of VP have been successfully blocked by the central administration of the V1 antagonist. These observations implicate a V1 receptor as mediating these effects.

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