Effects of Acute AV3V Lesions on Renal and Hindlimb Vasodilation Induced by Volume Expansion

Débora S.A. Colombari, Sérgio L. Cravo

Abstract—The role of the anteroventral third ventricle (AV3V) region in the cardiovascular adjustments to volume expansion (VE) with 4% Ficoll (1% body weight, 1.4 mL/min) was studied in urethane-anesthetized rats. In sham-lesioned animals, VE produced a transitory (≤20 minutes) increase in mean arterial pressure, which peaked at 10 minutes (10 ± 3 mm Hg), and sustained increases of renal (123 ± 10% and 127 ± 6% of baseline, respectively, 10 and 40 minutes after VE) and hindlimb vascular (157 ± 19% and 153 ± 9% of baseline) conductance. After AV3V lesions, VE induced a sustained increase in mean arterial pressure. Although renal blood flow increased in response to VE, renal vascular conductance was unaffected, indicating that renal vasodilation was abolished. On the other hand, after AV3V lesions, the increases in hindlimb blood flow and vascular conductance were higher than those observed in sham-lesioned rats. Results obtained demonstrated that the AV3V region is essential for the renal vasodilation induced by VE. (Hypertension. 1999;34[part 2]:762-767.)

Key Words: blood flow velocity ■ brain ■ rats ■ blood pressure ■ water-electrolyte balance

The anteroventral third ventricle (AV3V) region is a critical area acting on fluid and electrolytic balance and maintaining cardiovascular homeostasis.1–2 Numerous studies have demonstrated that the AV3V is involved with behavioral and autonomic responses to changes in extracellular fluid (ECF) volume and composition. AV3V lesions markedly reduce thirst induced by central and peripheral stimuli such as hypertonic saline or angiotensin II infusion or by microinjection of a cholinergic agonist into the subfornical organ.3–6 Electrolytically produced lesions of this region also reduce the natriuretic responses to volume expansion (VE) or water deprivation.7–8 It has been shown that AV3V lesions reduce atrial natriuretic peptide (ANP) levels in several areas of the brain as well as the increase in plasmatic ANP that follows deprivation.2–8 That AV3V is also involved in cardiovascular regulation is suggested by the observation that in rats, the development of several forms of experimental hypertension is markedly reduced after AV3V lesion.1–2 AV3V electrical stimulation produces an integrated response comprising vasodilatation of the hindquarters and vasoconstriction of the renal and mesenteric beds, resulting in a net decrease in arterial blood pressure.11

Acute isotonic increases in blood volume produce several responses, resulting in marked diuresis and natriuresis until ECF basal conditions are restored. These responses include renal vasodilatation,12–16 decrease of renal sympathetic nerve activity,17–21 and release of ANP.9,10,22,23 Previously, we demonstrated that in urethane-anesthetized rats, acute VE produces hypertension and renal vasodilatation. Renal vasodilatation seems to involve both neural and humoral mechanisms, which are triggered by signals arising from baroreceptor afferents.24–25 In the present study, we investigated the role of the AV3V region in the cardiovascular adjustments induced by VE.

Methods

All experiments were performed on adult, male Wistar rats weighing 280 to 320 g. Rats were housed in a temperature-controlled room on a 12:12-hour light/dark cycle with food and tap water available ad libitum. The Medical Ethics Committee of the Universidade Federal de São Paulo approved all protocols in this study.

Animals were anesthetized with urethane (1.2 g/kg IV) after induction with halothane (2% in 100% O2). Catheters were inserted into both femoral veins and the right femoral artery for drug administration, VE, and blood pressure measurement, respectively. The arterial catheter was connected to a Statham P23Db pressure transducer attached to a Beckman recorder (model R 611 A) for pulsatile pressure and mean arterial pressure (MAP) recording. A tracheotomy was done to reduce airway resistance. The temperature was kept between 36°C and 37°C.

Blood flow was measured with a Doppler flowmeter (University of Iowa Bioengineering Facility, Iowa City) as described by Haywood et al.26 Through a midline laparotomy, the left renal artery and the inferior abdominal aorta were isolated, and miniaturized pulse-Doppler flow probes were implanted around each vessel to record renal blood flow (RBF) and hindquarter blood flow (HBF), respectively. Changes in blood flow velocity were recorded simultaneously on a Beckman recorder and a digital oscilloscope and were expressed as a percentage of baseline. Relative renal and hindquarter vascular conductances (RVC and HVC) were calculated as the ratio of Doppler pulsatile pressure and mean arterial pressure (MAP) recording. A tracheotomy was done to reduce airway resistance. The temperature was kept between 36°C and 37°C.

Animals were fixed in a stereotaxic apparatus (David Kopf Instruments) with the incisor bar 3.4 mm below the interaural line. A monopolar, stainless steel wire electrode, bared at the tip, was...
positioned 0.1 mm rostral to the bregma, in the midline, to a depth of 7.0 to 7.2 mm from the sinus. The anodal lesion was made with a 2-mA direct current for 30 seconds. In sham-lesioned rats, the electrode was positioned in the same place, except that the depth was 5.5 mm from the sinus and no electrical current was passed. Ten minutes after AV3V (n=8) or sham lesions (n=8), animals were subjected to VE (see below).

At the end of the experiments, animals were perfused through the heart with saline, followed with 10% formaldehyde. Brains were removed, sectioned coronally at 40-μm thickness, and stained with 1% neutral red. The position of lesions was determined, photographed, and plotted on drawings of sections from the atlas of Paxinos and Watson. A typical AV3V lesion is shown in Figure 1.

Figure 1. Photomicrographs of coronal sections of the forebrain of a representative AV3V lesion (A, arrows) and an equivalent section of a sham-lesioned rat (B). ac indicates anterior commissure; LPO, lateral preoptic area; and oc, optic chiasm.
Basal Values (Mean±SE) of Mean Arterial Pressure (MAP), Heart Rate (HR), and Body Weight of Sham and AV3V-Lesioned Rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mm Hg</th>
<th>HR, bpm</th>
<th>Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham lesion</td>
<td>8</td>
<td>96±7</td>
<td>371±6</td>
<td>305±12.9</td>
</tr>
<tr>
<td>AV3V lesion</td>
<td>8</td>
<td>95±3</td>
<td>353±11</td>
<td>295±7.5</td>
</tr>
</tbody>
</table>

All variables analyzed were stable for at least 20 minutes before lesions. VE was obtained by infusion of 4% Ficoll in isotonic saline (Pharmacia), 1% body weight, 0.4 mL/min. MAP, heart rate (HR), RBF, HBF, and vascular conductance were recorded for 60 minutes after onset of VE.

The results are represented as mean±SE. Groups were compared by ANOVA followed by Fisher’s least significant difference test when the global F ratio was significant. All values reported as significant are at the P<0.05 level.

Results

Body weight and baseline MAP and HR are shown in the Table. There were no differences among the groups in relation to these variables.

Sham-Lesioned Animals

Basal levels of all variables were unaffected by placement of the electrode at the AV3V vicinity. Ten minutes later VE was initiated. Cardiovascular responses to VE in sham-lesioned animals were identical to those described in the introduction. Immediately after VE an increase in MAP was observed, which peaked at 10 minutes (10±3 mm Hg). After that, a gradual return to basal levels was observed, and 40 minutes after VE, MAP basal levels were reestablished. The RBF and HBF also increased immediately after VE and remained elevated throughout the experimental period. Calculated values for RVC and HVC vascular beds were significantly increased 10 minutes after VE (123±10% and 157±19%, respectively), remaining elevated 60 minutes after VE (maximum period observed). These results indicated that in AV3V sham-lesioned rats, VE was associated with a sustained vasodilation of both renal and hindlimb vascular beds (Figure 2).

AV3V-Lesioned Animals

Arterial blood pressure, RBF, HBF, RVC, and HVC were unaffected by AV3V lesions. However, HR levels of AV3V-lesioned rats were significantly elevated when compared with those observed in the prelesion period or in sham-lesioned animals. In AV3V-lesioned rats 10 minutes after VE, an increase in MAP (8±2 mm Hg) was observed. This increase was similar to that observed in sham-lesioned animals. However, in these animals, MAP remained significantly elevated throughout the experimental period. Similarly, in AV3V-lesioned animals, HR increased progressively, peaking 30 minutes after VE onset and remaining elevated all through the experimental period. Ten minutes after the onset of VE, RBF increased significantly (111±5%), remaining at this level throughout the experiment. Although it seemed that the increases in RBF observed in AV3V-lesioned rats were lower than those observed in sham-lesioned animals, this difference was not at statistical levels. Calculated RVC demonstrated that the renal vasodilation induced by VE was completely blocked in AV3V-lesioned rats. VE produced an increase in HBF starting at 10 minutes (132±25%) and was maintained at high levels until the end of the recording. When HVC was calculated, we observed an enormous vasodilation in this bed, which remained elevated for the entire observed period. The increase in HBF and HVC was significantly higher than in sham animals for at least 30 minutes after VE (Figure 2).

To further ensure that the blockade of renal vasodilation induced by VE in AV3V-lesioned rats was not due to a nonspecific decrease in RVC, in a separate group of animals (n=5) all variables were observed for 70 minutes after AV3V lesions. Results obtained in this group of animals demonstrated that AV3V lesions were ineffective for modifying MAP, RBF, HBF, RVC, and HVC (Figure 3). However, AV3V lesions induced a progressive increase in HR, and 30 minutes after the lesion, it was significantly elevated when compared with prelesion values, remaining elevated throughout the experimental period. Actually, this increase in HR closely resembled the HR response observed after VE in AV3V-lesioned animals.

Discussion

The results obtained demonstrate that in urethane-anesthetized rats, VE induced a transient hypertension and a sustained vasodilation in both renal and hindlimb vascular beds. HR responses were variable but most frequently consisted of a moderate tachycardia. Acute AV3V lesions abolished renal but not hindlimb vasodilation. Actually, after AV3V lesions, pressor responses and hindlimb vasodilation induced by VE were greater than those observed in sham-lesioned animals. AV3V lesions induced a significant tachycardia.

These results are comparable to those obtained by previous studies describing increases in arterial blood pressure and renal and hindlimb vasodilation in response to VE.15,16,19,21,24,25,28 These adjustments are part of the animal’s integrated response to a sudden increase in the ECF volume. Increases in ECF activate several mechanisms, resulting in natriuresis and diuresis until basal ECF conditions are restored. The kidney is the main effector organ in this response. Accordingly, the renal sodium excretion rate represents the integration of activity of several humoral and neural mechanisms. Renal vasodilation is a significant factor contributing to the increase in water and sodium excretion after VE.29

Previously, we demonstrated that in anesthetized rats, the increase in RVC induced by the same VE protocol appeared to involve both neural and humoral mechanisms.24,25 As reported in the present study, RVC increased immediately after VE and remained elevated for at least 1 hour. After unilateral renal denervation, the initial phase of renal vasodilation was abolished. However, starting 30 minutes after VE onset, a significant increase in RVC was still observed, indicating that this response was not mediated by renal sympathetic efferents.25

Results obtained by Lovick et al15 demonstrated that renal vasodilation induced by VE was abolished by chemical lesions of the paraventricular nucleus of the hypothalamus, indicating that this response is mediated by the central nervous system. We further extend these results, indicating that AV3V may be part of this neural pathway.
Distinct observations indicate that the blockade of renal vasodilation after AV3V lesions may not be attributed to a nonspecific and diffuse increase in vasomotor tone. First, hindlimb vasodilation induced by VE was not reduced after AV3V lesions. In fact, a greater increase in HVC was observed after VE in AV3V-lesioned animals. Also, results obtained from the group of animals with AV3V lesions that were not subjected to VE indicate that lesions alone did not induce any significant changes in blood pressure, RBF, HBF, RVC, and HVC throughout the experimental period (60 minutes). Similarly, Bealer et al. demonstrated that a significant increase in MAP was not observed until 2 hours after AV3V lesions. According to these authors, the increased blood pressure contributed to the natriuresis observed after these lesions. Contrariwise, AV3V lesions induced a marked increase in HR levels. Actually, comparison of the HR profile of these animals with those obtained during VE in AV3V-lesioned rats suggests that most of the observed tachycardia may be attributed to the lesion itself.

Previous studies have indicated that the AV3V is involved in several components of cardiovascular control and the integrated response to ECF expansion. AV3V lesions increase catecholamine release, modulate RVC, and may mediate renal vasodilation in response to VE. AV3V is also involved in the neural control of cardiovascular function. The findings presented in this study suggest a specific role for AV3V in the regulation of renal vasodilation and blood pressure response to VE.
control of ANP and circulating vasopressin levels. Accordingly, after AV3V lesions, both ANP basal levels and the ANP release induced by VE are reduced.9–10 Similarly, previous studies have suggested that AV3V modulates ANP release induced by central angiotensin II or hypertonic saline.5

Results of the present study demonstrated that very shortly after AV3V lesions, pressor responses to VE were augmented in magnitude and duration. The integrative role of AV3V in cardiovascular control and in the response to acute ECF expansion may explain this observation. Because AV3V lesions impair the efficiency of several compensatory mechanisms to VE, blood pressure returns to basal levels after longer periods. The maintenance of increased blood pressure levels after VE may be due to a combination of several effects of AV3V lesions, like increased levels of circulating catecholamines, the increased circulating volume, and decreased levels of circulating ANP.

Several sets of afferents including baro-, cardiopulmonary, and intrathoracic receptors mediate the increase in HVC in response to VE.32 Interestingly, control of HVC seems to be more related to blood pressure than to blood volume control. Accordingly, hindlimb vasodilation in response to VE is maintained after bilateral vagotomy.24,25,28 The continuance of the hindlimb vasodilation induced by VE after AV3V lesions and the increased hindlimb vasodilation in face of increased levels of arterial blood pressure observed in these
animals are consistent with this hypothesis. Also, the increased hindlimb vasodilation induced by VE after AV3V lesions may be a compensatory mechanism for the lack of renal vasodilation in these animals. Finally, it is also conceivable that AV3V may exert an inhibitory control over hindlimb vasodilation during VE. Additional studies are necessary to elucidate these mechanisms.

In conclusion, the results obtained in the present study demonstrated that the AV3V region is essential for the renal vasodilation induced by VE and that it contributes to our understanding of the role of this region in the control of ECF and the responses to acute VE.

Acknowledgments

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References


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