Vasoconstriction During Volume Expansion Is Independent of Central Control


We have previously demonstrated that, in the absence of the rapid acting neural and hormonal controllers of blood pressure, an acute blood volume expansion of only 5% in unanesthetized rats caused an increase in total peripheral resistance (TPR) of 22%. Either whole body autoregulation or the release of a putative ouabainlike factor from the central nervous system (CNS) could have explained these responses. The purpose of the present study was to investigate the possible contribution of a centrally released ouabainlike factor to the vasoconstriction response observed during volume expansion. Because the anteroventral third ventricle (AV3V) region is proposed to be important in the control of this putative factor, we compared the hemodynamic responses to blood volume expansion in rats with AV3V lesions (n=6), sham lesions (n=6), and total CNS ablation (n=6). The results of our studies showed that neither AV3V lesion nor CNS ablation reduced the increases of total peripheral resistance seen with blood volume expansion. We conclude that centrally released factors are not required for vasoconstriction in response to acute volume expansion and that regional autoregulatory mechanisms result in a net increase of systemic vascular resistance (i.e., whole body autoregulation). (Hypertension 1990;15:712-717)

W

We have recently reported that small (5%) increases of blood volume cause an increase of systemic vascular resistance in rats lacking reflex control of blood pressure.1 Two general hypotheses have been proposed for this response. The hypothesis of whole body autoregulation predicts that an expansion of blood volume would lead to an increase in cardiac output with a resulting elevation of systemic vascular resistance because each of the regional beds autoregulated blood flow.3 As the overperfusion of the tissues is diminished, the resultant hemodynamic profile would be an elevated arterial blood pressure with only small elevations of cardiac output. Evidence for these events has been reported in dogs,4,5 cats,6,7 and rats.1,8

An alternative hypothesis for increased systemic vascular resistance with volume expansion proposes that an increase in thoracic blood volume stimulates the release of a circulating Na⁺,K⁺-ATPase inhibitor, which causes natriuresis and vasoconstriction.9 Although the putative inhibitor has not been isolated or characterized, it is believed to be controlled by central mechanisms.10 The specific brain region proposed to influence the circulating levels of the factor is the anteroventral third ventricle (AV3V) area of the hypothalamus.11,12

The present study was designed to determine the extent to which the central nervous system (CNS) or factors released from the brain influence systemic vascular responses observed in conscious areflexic rats during blood volume expansion. We studied rats with AV3V lesions, which are presumably unable to regulate the circulating levels of the putative factor, and compared their responses with those in rats with sham lesions. We also studied the responses in a group of rats that had undergone total ablation of the CNS and thus lacked all central control of peripheral hemodynamics.

Methods

Anteroventral Third Ventricle Lesion

Male Sprague-Dawley rats weighing 325–400 g (King Animal Labs, Madison, Wisconsin) were housed individually for at least 1 week before experimentation and were maintained on normal Purina lab chow and tap water ad libitum. Animal room temperature was 23°C with a 12-hour light/dark cycle.

Rats were anesthetized with a mixture of ketamine (50 mg/kg i.m.) and acepromazine (5 mg/kg i.m.) and secured in a stereotaxic instrument. Lesions of the AV3V region were made using a single penetration of a Teflon-coated stainless-steel wire electrode 0.3 mm anterior to bregma and 0.9 mm lateral to the sinus. The electrode was lowered at an angle 7° to the vertical plane to a depth 7.6 mm below the top of the
were given penicillin G (20,000 units i.m.) and were placed in a Plexiglas restrainer inside a sound-proof ventilated chamber. Arterial pressure was measured in the arterial catheter with a pressure transducer (model P23 Dd, Statham Instr. Division, Gould Inc., Oxnard, California), and cardiac output was measured by an electromagnetic flowmeter (model S01D, Carolina Medical Electronics). Measurements of mean arterial pressure and cardiac output were recorded on a Grass polygraph recorder (model 7D, Grass Instr. Co., Quincy, Massachusetts). Total peripheral resistance (TPR) was calculated as the ratio of mean arterial pressure to cardiac output.

Pharmacological blockade of the major neurohumoral systems involved in arterial pressure regulation was performed as previously described. Chlorisondamine chloride (10 mg/kg) and methscopolamine bromide (0.5 mg/kg) were given to block ganglionic transmission of the autonomic nervous system. Captopril (1 mg/kg) was used to inhibit Ang II synthesis. A specific vascular vasopressin receptor antagonist, [d(CH2)3Tyr(Me)]arginine vasopressin (10 μg/kg), prevented the vasoconstrictor effects of circulating vasopressin. These drugs were first administered intravenously as a bolus injection and were then continuously infused throughout the experimental protocol. Mean arterial pressure in areflexic rats was maintained at control levels with a constant infusion of norepinephrine (0.5–1.0 μg/kg/min).

After neurohumoral reflex ablation, the rats were subjected to an acute blood volume expansion. Donor blood was infused through a venous catheter at a rate of 0.15 ml/min using an infusion pump (model 355, Sage Instr., Boston, Massachusetts). A total volume of 0.9 ml donor blood was infused in 6 minutes. To restore blood volume to control levels, 0.9 ml blood was then withdrawn through the arterial catheter approximately 3–5 minutes after the infusion of donor blood. These arterial blood samples were used to measure oxygen tension (PO2), carbon dioxide tension (PCO2), and pH by using a blood gas analyzer (model 168, Ciba-Corning-Medfield, Medfield, Massachusetts).

Donor blood was prepared as a mixture of washed blood cells and a plasmalike solution to eliminate any vasoactive agents. A detailed description of donor blood preparation has been previously published. Before use in an experimental protocol, donor blood electrolytes, gases, and pH were analyzed and verified to be within a normal physiological range.

Ablation of the Central Nervous System

Sprague-Dawley rats weighing 300–375 g were surgically instrumented for hemodynamic measurements as previously described. On the day of the experimental protocol, the rats were anesthetized with thiamylal sodium (10 mg/kg i.v.) and ventilated through a tracheostomy with a small-animal respirator (model 680, Harvard Apparatus, South Natick, Massachusetts). The spinal cord was exposed by a dorsal incision in the cervical region. A catheter was inserted into the vertebral column, and 90% ethanol was injected to destroy the spinal cord. A clamp was then placed around the neck to compress the cervical spinal cord and eliminate all blood supply to the brain. The head was removed with no trunk blood loss, and mean arterial pressure was restored to preablation levels with a constant infusion of norepinephrine (1.0–2.0 μg/kg/min). The rats with CNS ablation were then subjected to an acute blood volume expansion as previously described.

Statistical Analysis

The results of this study are expressed as mean±SEM. Differences between baseline hemodynamic variables before and after neurohumoral blockade or CNS ablation were assessed with a Student's t test for paired values. Differences in blood gases and blood pH between rats with sham lesions, AV3V lesions, and CNS ablation were determined by a one-way analysis of variance followed by a Duncan's multiple range test. Two-way analysis of variance with repeated measures on one variable was used to evaluate the changes in mean arterial pressure, cardiac output, and TPR during acute increases in blood volume in the rats with AV3V lesions, sham lesions, and CNS ablation. The Dunnett's multiple range test was used to determine significant differences in these hemodynamic changes as compared with control values. Linear regression analysis was
TABLE 1. Resting Hemodynamic and Blood Gases Before and After Neurohumoral Blockade or CNS Ablation

<table>
<thead>
<tr>
<th>Variables</th>
<th>Sham lesion (n=6)</th>
<th>AV3V lesion (n=6)</th>
<th>CNS ablation (n=6)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Intact</td>
<td>Areflexic</td>
<td>Intact</td>
</tr>
<tr>
<td>Mean arterial pressure (mm Hg)</td>
<td>117±2</td>
<td>113±5</td>
<td>118±2</td>
</tr>
<tr>
<td>Cardiac output (ml/min)</td>
<td>95±4</td>
<td>98±5</td>
<td>111±7</td>
</tr>
<tr>
<td>Total peripheral resistance (mm Hg • min/ml)</td>
<td>1.24±0.05</td>
<td>1.17±0.09</td>
<td>1.10±0.06</td>
</tr>
<tr>
<td>P02 (mm Hg)</td>
<td>...</td>
<td>90±6</td>
<td>...</td>
</tr>
<tr>
<td>PCO2 (mm Hg)</td>
<td>...</td>
<td>37±3</td>
<td>...</td>
</tr>
<tr>
<td>pH</td>
<td>7.45±0.02</td>
<td>7.45±0.01</td>
<td>7.37±0.02</td>
</tr>
</tbody>
</table>

Values are mean±SEM. Hemodynamic values and blood gases during areflexic conditions were measured in the presence of norepinephrine infusion. Blood oxygen tension (P02), carbon dioxide tension (PCO2), and pH are shown during areflexic conditions only. AV3V, anteroventral third ventricle; CNS, central nervous system.

*Difference between intact and areflexic (p<0.01).
†Different from values in rats with sham lesions and AV3V lesions (p<0.01).

Results

Hemodynamic Responses to Blood Volume Expansion

The resting hemodynamics before and after neurohumoral blockade or CNS ablation are shown in Table 1. In the conscious areflexic rats with sham lesions and AV3V lesions, a constant norepinephrine infusion resulted in baseline hemodynamic values similar to the intact state. The rats with CNS ablation, however, had significant differences in cardiac output and TPR before and after CNS ablation despite the norepinephrine infusion. Also shown in Table 1 are the blood P02 and PCO2 and pH values measured during the areflexic state. These values were well within the normal physiological range; however, rats with CNS ablation had pH values slightly lower (p<0.01) than rats with sham lesions and AV3V lesions.

Figure 1 represents the average hemodynamic values observed before and during a 6-minute infusion of donor blood in rats with AV3V lesions and rats with sham lesions. Volume expansion caused similarly small but significant increases in cardiac output in rats with AV3V lesions (+11±1 ml/min) and sham lesions (9±2 ml/min). Mean arterial pressure increased 26±4 mm Hg in rats with AV3V lesions and 25±2 mm Hg in rats with sham lesions. The calculated increases in TPR were 0.13±0.04 and 0.15±0.03 mm Hg • min/ml in rats with AV3V lesions and rats with sham lesions, respectively.

The hemodynamic responses to blood volume expansion in rats with CNS ablation (n=6) are shown in Figure 2. Despite a constant norepinephrine infusion and a normal baseline mean arterial pressure, baseline cardiac output remained low in these rats, which resulted in a high baseline TPR. Nonetheless, blood volume expansion in rats with CNS ablation also resulted in only a small but significant increase in cardiac output (7±1 ml/min), whereas mean arterial pressure and TPR substantially increased by 23±3 mm Hg and 0.21±0.09 mm Hg • min/ml, respectively.

Pressure-Flow Relations

The autoregulatory strength of the systemic circulation is graphically represented in Figure 3 by the normalized pressure-flow relation. With no systemic autoregulation, this pressure-flow relation is charac-
Autoregulation is characterized by a regression line with a slope equal to 1. Complete autoregulation is characterized by a pressure-flow relation with a slope equal to 0. The data plotted in Figure 3 represent the fractional change in pressure (ΔP/P) and the fractional change in flow (ΔF/F) during each minute of blood infusion. The ratios ΔP/P and ΔF/F were determined before volume expansion (time=0 minutes) and at 1-minute intervals during the 6-minute protocol. The regression lines for the systemic pressure-flow relations were determined by seven points in each rat using linear regression analysis. The calculated slopes of these relations were 0.31±0.03, 0.43±0.02, and 0.46±0.03 in rats with AV3V lesions, sham lesions, and CNS ablation, respectively. These data indicate that all three groups demonstrated significant autoregulatory ability and that autoregulation in rats with AV3V lesions was actually significantly greater than in rats with sham lesions and rats with CNS ablation.

Anteroventral Third Ventricle Lesion Verification

Brain tissue histological analysis revealed the following three types of lesions: sham, partial, and complete AV3V lesions. All rats considered to have complete AV3V lesions sustained damage to ependymal and periventricular tissue on the anterior wall of the third ventricle ventral to the anterior commissure. Traces of the ventral median preoptic nucleus and organum vasculosum of the lamina terminalis were apparent in a few rats, although the majority had complete destruction of these two nuclei. Bilateral damage extended posteriorly to the anterior hypothalamus. Only the medial borders of the medial preoptic and anterior hypothalamic nuclei were damaged. Partial AV3V lesions were defined as lesions with incomplete destruction of the subnuclei located within the AV3V area. These lesions tended to be centered off midline and thus had asymmetrical bilateral damage. Sham lesions were associated with an intact AV3V area.

Rats with AV3V lesions manifested a profound weight loss, and were adipsic immediately after lesion surgery, which reversed with time. Rats with histologically verified AV3V lesions lost 41±5 g body wt as compared with 33±4 and 6±2 g body wt for rats with partial AV3V lesions and rats with sham lesions, respectively. Rats with sham lesions drank 24±3 ml water the day after lesion surgery, whereas rats with partial and complete AV3V lesions drank 24±3 ml water the day after lesion surgery, whereas rats with partial and complete AV3V lesions drank 32±2 and 1±0.7 ml, respectively. Rats with AV3V lesions were also refractory in their drinking responses to Ang II. The average 60-minute water intakes in two tests after subcutaneous injections of Ang II for rats with sham, partial, and complete AV3V lesions were 2.5±0.6, 1.7±0.9 and 0.3±0.2 ml, respectively. In our analysis of the effects of AV3V lesions on the auto-
regulatory response, we included in the AV3V-lesion condition only rats that satisfied both the histological and functional criteria.

**Discussion**

We have reported previously that in the absence of neurohumoral control systems, increases of blood volume as small as 5% in unanesthetized rats resulted in TPR elevations of 22%, and conversely, reductions of blood volume of 5% resulted in rapid TPR reductions of 16%. The mechanism we favored for the changes of TPR was that of whole body autoregulation, where the summed effects of regional blood flow autoregulation would dominate the control of systemic vascular resistance.

An alternative hypothesis that we did not eliminate, was that of an ouabainlike factor thought to be released from the brain in response to volume expansion, which positively inhibits Na⁺,K⁺-ATPase pump activity and results in vasoconstriction and natriuresis. A decrease in Na⁺,K⁺-ATPase pump activity as indicated by a decrease in ouabain-sensitive rubidium uptake has been shown in arteries from rats that were volume expanded with acute saline infusion. Additionally, decreased pump activity has been demonstrated in vascular tissue from one-kidney, one clip Goldblatt hypertensive rats, reduced renal mass hypertensive rats, and deoxycorticosterone acetate (DOCA)-hypertensive rats. Conflicting results, however, have been reported in one-kidney, one clip Goldblatt hypertensive rats and Dahl salt-sensitive hypertensive rats.

Although the putative Na⁺,K⁺-ATPase pump inhibitor has not been isolated and characterized, the regulation of circulating levels of the factor is proposed to occur in the hypothalamus from which it can be released. An ouabainlike substance has been partially purified from bovine hypothalamus, and the AV3V area of the hypothalamus is thought to control the release of the factor. AV3V lesions prevent the inhibition of Na⁺,K⁺-ATPase pump activity in isolated vessels from volume-expanded rats. Additionally, Bealer et al confirmed the importance of the AV3V region by demonstrating a reduced natriuretic response to volume loading in rats with AV3V lesions. Studies that were performed to determine the relations between the AV3V region, Na⁺,K⁺-ATPase pump activity, and DOCA hypertension indicated that DOCA hypertension was associated with a decreased pump activity and that this was prevented by pretreatment with an AV3V lesion.

The whole body autoregulation hypothesis and the ouabainlike factor hypothesis have both been proposed to explain the increase in vascular resistance during volume expansion or volume-dependent hypertension. The goal of the present study was to determine the possible contribution of a centrally mediated factor and reevaluate the vasoconstrictor responses to blood volume expansion in rats. The complex reflex responses to volume expansions were eliminated in the present studies so the effects of any released factors could be assessed directly and without the problems associated with anesthetics. Because the AV3V region is thought to be important in the release of the putative factor, we compared the hemodynamic responses in rats with AV3V lesions and rats with sham lesions. We observed that conscious areflexic rats with AV3V lesions showed an increased TPR during blood volume expansion that was not diminished when compared with rats with sham lesions.

To completely eliminate the contribution from any centrally mediated circulating factor, we evaluated the vasoconstrictor response to blood volume expansion in rats with CNS ablation. Although these rats had a baseline cardiac output and TPR that were not typical of the conscious rats, we observed significant vasoconstriction in response to blood volume expansion. The pressure-flow relations in rats with CNS ablation were similar to those in conscious rats with sham lesions.

The results of this study provide evidence and indicate that a centrally released factor is not necessary for the increase in TPR during volume expansion. The results support the conclusion that the vasoconstriction is a result of local mechanisms. Granger and Guyton demonstrated whole-body autoregulation in dogs with CNS ablation. In their study, however, blood volume was not increased to values higher than control values; instead, blood volume was increased from volume-depleted states to control values.

We observed that the whole body autoregulation response in this model was similar to that predicted from a theoretical analysis of the contribution of regional autoregulation responses to total resistance and flow. We have also observed that changes in TPR in response to changes in blood volume occur within 1–2 minutes after blood volume begins to change. Additionally, TPR returns to control values within 1–2 minutes after blood volume is restored to normal (unpublished observations from our laboratory). The time course of these responses is similar to that observed in autoregulation responses in isolated vessels. In summary, our results indicate that no centrally mediated ouabainlike factor or any other central factor is responsible for the vasoconstriction we observed during acute blood volume expansion. The results support the hypothesis of whole body autoregulation.
Acknowledgments
The donations of chlorisondamine by CIBA Pharmaceutical Company and captopril by the Squibb Institute for Medical Research are gratefully acknowledged. We also thank Rosalie Zamiatowski and Shari Stoner for their technical assistance, and Helen Uhan for her secretarial assistance.

References

Key Words • blood pressure • central nervous system • vasoconstriction • cardiac output • blood volume • homeostasis
Vasoconstriction during volume expansion is independent of central control.
C Hinojosa-Laborde, R L Thunhorst and A W Cowley, Jr

Hypertension. 1990;15:712-717
doi: 10.1161/01.HYP.15.6.712

Hypertension is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1990 American Heart Association, Inc. All rights reserved.
Print ISSN: 0194-911X. Online ISSN: 1524-4563

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://hyper.ahajournals.org/content/15/6_Pt_2/712