Afferent pathways in cardiovascular adjustments induced by volume expansion in anesthetized rats

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Afferent pathways in cardiovascular adjustments induced by volume expansion in anesthetized rats. Am J Physiol Regulatory Integrative Comp Physiol 279: R884–R890, 2000.—The role of baroreceptors, cardiopulmonary receptors, and renal nerves in the cardiovascular adjustments to volume expansion (VE) with 4% Ficoll (Pharmacia; 1% body wt, 0.4 ml/min) were studied in urethan-anesthetized rats. In control animals, VE produced a transitory increase in mean arterial pressure (MAP), which peaked at 10 min (17 ± 4 mmHg) and increases in renal (128 ± 6 and 169 ± 19% of baseline at 10 and 40 min, respectively) and hindlimb vascular conductance (149 ± 6 and 150 ± 10%). These cardiovascular adjustments to VE were unaffected by bilateral vagotomy. After sinoaortic denervation, the increase in MAP induced by VE was greater than in control rats (30 ± 4 mmHg). However, renal vasodilation in response to VE was blocked, whereas hindlimb vasodilation was similar to that observed in control rats. After unilateral renal denervation (ipsilateral to flow recording), the initial renal vasodilation was blocked. However, 40 min after VE, a significant renal vasodilation (125 ± 4%) appeared. The hindlimb vasodilation and MAP responses were unaffected by renal denervation. These results demonstrate that the baroreceptor afferents are an essential component of cardiovascular adjustments to VE, especially in the control of renal vascular conductance. They also suggest that renal vasodilation induced by VE is mediated by neural and hormonal mechanisms.

METHODS

All experiments were performed on adult male Wistar rats weighing 280–320 g. Rats were housed in a temperature-controlled room on a 12:12-h light-dark cycle with food and tap water available ad libitum.

Animals were anesthetized with urethan (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 (R 611 A model) for pulsatile pressure and mean arterial pressure (MAP) recording. A tracheostomy was performed to reduce airway resistance. The temperature was thermostatically controlled. The animals were kept under controlled room conditions with 12-h cycles of light and dark, and temperature was maintained at 25°C.

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kept between 36 and 37°C. In experiments measuring right atrial pressure (RAP), the right jugular vein was dissected and a catheter was advanced to the right atria. The atrial catheter was connected to a Statham P23Db pressure transducer attached to a Beckman recorder.

Blood flow was measured with a Doppler flowmeter (University of Iowa Bioengineering Facility, Iowa City, IA) as described by Haywood et al. (13). Through a midline laparotomy, the left renal artery and the inferior abdominal aorta were isolated, and miniaturized pulse-Doppler probes were implanted around each vessel to record renal blood flow (RBF) and hindquarter blood flow (HBF), respectively. Changes in blood flow velocity measured in Hertz were recorded simultaneously with a Beckman recorder and a digital oscilloscope and were expressed as percentage of baseline. Relative renal and hindquarter vascular conductance (RVC and HVC) were calculated as the ratio of Doppler shift and MAP, and they were expressed as percentage of the baseline.

Sinoaortic baroreceptor denervation (SAD) was performed as described previously by Krieger (19) under 2%-halothane anesthesia. Rats were allowed to recover for 7-10 days before the experiment. Before VE, SAD was tested by an intravenous injection of phenylephrine (0.5-2.0 μg/kg). Only animals that presented a bradycardia of ≤10 beats/min in response to 25- to 40-mmHg increases in MAP were considered to have an effective denervation and be submitted to VE.

The surgical procedure to achieve bilateral vagotomy was similar to the SAD surgery, except that only the vagus nerves were sectioned. Bilateral vagotomy was performed immediately before the experiment.

The left kidney innervation was removed immediately before the placement of the probe around the renal artery as described previously (1). Briefly, the left kidney was exposed, and the renal nerves running along the renal artery were carefully removed under stereoscopic-microscopic observation; the renal artery was stripped of its adventitia, and the nerve bundles of the renal artery and hilum were sectioned.

All variables analyzed were stable for at least 20 min before VE. VE was obtained by infusion of 4% Ficoll (Pharmacia; 1% body wt, 0.4 ml/min). MAP, heart rate (HR), RBF, HBF, RVC, and HVC were recorded for 60 min after the onset of VE.

The results are presented as means ± SE. Groups were compared by analysis of variance followed by Fisher’s protected least-significant differences 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 Table 1. SAD rats had a lower MAP compared with control rats. Animals submitted to bilateral vagotomy exhibited a higher HR level compared with control animals. There were no differences among the groups in relation to body weight.

Control animals. VE was associated with immediate increases in MAP and HR levels in control rats. The MAP increase peaked at 10 min (17 ± 4 mmHg), remaining elevated until ~20 min after the onset of VE. A gradual return to basal levels was observed thereafter, and 40 min after VE basal MAP levels were reestablished. The HR increase peaked after 20 min (23 ± 11 beats/min), remaining elevated throughout the experiment. Immediately after VE, RBF and HBF increased and remained elevated throughout the experiment.

Table 1. Basal MAP, HR, body weight of control, VAGX, SAD, and RD rats

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>MAP, mmHg</th>
<th>HR, beats/min</th>
<th>Weight, g</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11</td>
<td>98 ± 6</td>
<td>358 ± 14</td>
<td>317 ± 4.6</td>
</tr>
<tr>
<td>VAGX</td>
<td>8</td>
<td>106 ± 5</td>
<td>451 ± 12*</td>
<td>310 ± 8.2</td>
</tr>
<tr>
<td>SAD</td>
<td>10</td>
<td>74 ± 2*</td>
<td>334 ± 10</td>
<td>302 ± 11.1</td>
</tr>
<tr>
<td>RD</td>
<td>8</td>
<td>95 ± 5</td>
<td>346 ± 13</td>
<td>292 ± 12</td>
</tr>
</tbody>
</table>

Values are means ± SE. MAP, mean arterial pressure; HR, heart rate; VAGX, vagotomized rats; SAD, sinoaortic-denervated rats; RD, renal-denervated rats. * Different from control (P < 0.05).

Blood pressure and HR. VE induced a greater increase in MAP in SAD rats. The peak of the response at 10 min (30 ± 4 mmHg) was significantly higher than in control animals. Moreover, although a progressive decline was observed in these animals, MAP did not return to basal levels during the experimental period (60 min). Tachycardic responses to VE were similar to those observed in control animals. After acute bilateral vagotomy, pressor response to VE was unaffected, and the increase in MAP 10 min after VE (14 ± 3 mmHg) was equivalent to that observed in control animals. Bilateral vagotomy abolished the tachycardic response to VE. Actually, in these animals, VE was associated with bradycardia, which peaked 10 min after the onset of VE (~18 ± 9 beats/min). Afterwards, a progressive recovery was observed, and 30 min after VE, HR was stabilized at basal levels. The MAP profile induced by VE after acute unilateral renal denervation was similar to that observed in control animals. After a transient (~20 min) increase that peaked at 10 min (24 ± 6 mmHg), MAP gradually returned to basal levels. Curiously, in this group, tachycardia induced by VE was augmented compared with control animals (Fig. 2).

RBF and RVC. In SAD animals, RBF was augmented 10 min after the onset of VE, remaining elevated throughout the experimental period. However, this increase was significantly lower than that observed in control animals at the response peak (10 min, 137 ± 10%) and throughout the whole experimental period. More important, calculated RVC levels after VE were not different from those observed during the control period, indicating that after SAD the renal vasodilation induced by VE was completely blocked. In vagotomized rats, RBF and RVC increases were also equivalent to those observed in control animals, indicating a similar level of renal vasodilation. After renal denervation, a sustained increase in RBF was observed, beginning 10 min after VE (127 ± 6%) and remaining elevated throughout the experimental period. Nonetheless, this increase in RBF was smaller compared with that verified in the control group. Cal-

experiment. Calculated vascular conductance of the renal and hindlimb vascular beds was significantly increased 10 min after VE, indicating vasodilation in both territories (128 ± 6 and 143 ± 6%, respectively). Sixty minutes after VE, RVC (175 ± 18%) and HVC (146 ± 9%) remained elevated in relation to basal levels (Fig. 1).
culated RVC levels indicated that, in the denervated kidney, the vasodilatory response was delayed, and a significant increase in RVC was observed only 40 min after VE (131 ± 4%). Although it remained elevated until the end of the experimental period, RVC levels were significantly lower than in control animals (Fig. 2).

HBF and HVC. SAD was ineffective in modifying hindlimb vasodilation in response to VE. The increases in HBF and HVC observed in these animals 10 min after VE (207 ± 25 and 146 ± 17%, respectively) were equivalent to those observed in control experiments. Also, similarly to what is observed in control animals, these values remained elevated throughout the experimental period. After bilateral vagotomy, the increases in HBF and HVC were similar to control animals. In renal-denervated animals, significant increases in HBF (224 ± 39%) and HVC (176 ± 30%) were observed.

Fig. 1. Effects of volume expansion (VE) (4% Ficoll, 1% body wt, 0.4 ml/min) on mean arterial pressure (MAP; A), heart rate (HR; B), renal blood flow (RBF; C) and vascular conductance (RVC; D), and hindlimb blood flow (HBF; E) and vascular conductance (HVC; F) in control rats (n = 11). *Different from baseline (0); P < 0.05. The horizontal bar indicates the duration of VE; bpm, beats/min.
10 min after VE, remaining elevated throughout the experimental period. Even though the HBF and HVC levels observed in renal-denervated animals were consistently higher than in control animals, these differences did not reach statistical significance (Fig. 2).

**RAP.** The effect of VE on RAP was evaluated in a separate group of animals. VE induced a transitory increase in RAP that peaked 10 min after the onset of VE (2.2 ± 0.8 mmHg). After that, RAP immediately declined, and 20 min after, VE basal levels were re-established. A further decline was usually observed, and RAP levels remained just below basal levels throughout the experimental period (Fig. 3).

**DISCUSSION**

Several studies have examined the afferent pathways involved in the cardiovascular adjustments induced by acute VE. However, the use of different species, expanders (saline, blood, or macromolecules),
duration, and magnitude of VE impairs comparison of the results obtained. In the present study, urethane-anesthetized rats were submitted to an acute volume load of 1% body wt with the use of an isotonic 4% solution of a macromolecule, in a single infusion period of 6- to 8-min duration, with a low infusion rate. We demonstrated that, under these conditions, acute VE induced a transient (<30 min) hypertension and sustained (>60 min) increases in HR, RVC, and HVC. These results are comparable to those obtained by Lovick et al. (22) with the use of the same protocol for VE, although in that study the transient nature of the induced hypertension was not described, probably due to the shorter period of observation after VE (20 min). Guo and Richardson (12) also observed hindlimb vasodilation in response to VE in anesthetized rats.

After SAD, renal vasodilation was abolished, whereas the increase in HVC was unaffected. Bilateral vagotomy was ineffective in modifying the induced renal or hindlimb vasodilation, whereas tachycardic responses were abolished. Renal denervation reduced the magnitude and increased the latency of renal vasodilation without affecting pressure, HR, or hindlimb vasodilation. These results suggest that arterial baroreceptors represent the main afferent pathway involved in the renal vasodilation induced by the VE paradigm used in this study.

A different conclusion was reached in previous studies characterizing the effects of acute VE on RSNA and the afferent pathways involved. The decrease in RSNA induced by VE is abolished or remarkably reduced after bilateral vagotomy in dogs or rats, although this response is not affected by SAD (14, 25, 31, 35).

Several reasons may contribute to the apparent discrepancies between those studies and the results reported here. As mentioned before, in the present study, VE was obtained after a single dose at a low infusion rate over a period of 6-8 min. Data obtained in experiments recording RAP demonstrated that the VE protocol we used induced just a discrete rise in RAP. At its peak, RAP was just above the threshold for activation of right atrial receptors (2–5 mmHg) (37). It is conceivable that this VE represents a much lesser stimulus for cardiopulmonary receptors than that obtained in other studies using continuous infusion and/or higher infusion rates (14, 31, 35).

Comparison of the results obtained recording RSNA or RBF is also especially difficult because, in the kidney, the relationship between the activity of sympathetic efferent fibers and vascular resistance is highly complex, involving several distinct components. Within the renal nerve, different groups of sympathetic efferents regulate RBF, renin secretion, and tubular sodium reabsorption (18). Several lines of evidence indicate that these functions are controlled by specific, functionally diverse sympathetic fibers. Renal sympathetic efferents involved in urinary flow have a greater diameter and lower threshold than sympathetic efferents regulating RBF (9). Furthermore, significant reductions of RSNA can be obtained by stimulation of cardiopulmonary afferents without changes in renal hemodynamics (25, 28). Therefore, it is conceivable that different components of RSNA may be associated with specific sets of afferents. Maybe sympathetic fibers associated with control of sodium reabsorption are preferentially associated with cardiopulmonary afferents, whereas sympathetic fibers controlling RVC are primarily associated with arterial baroreceptor afferents.

Volume load induced a significant increase in MAP in all experimental groups. In control animals, the induced hypertension peaked 10 min after the onset of volume infusion, then declining gradually to basal levels after 40 min. Numerous previous studies demonstrated that the activation of arterial baroreceptors produced decreases in sympathetic nerve activity and vasodilation in renal, mesenteric, and the hindlimb beds (3, 6, 10). It is therefore possible that the renal and hindlimb vasodilation produced by VE is secondary to baroreceptor stimulation. However, it is noteworthy that increases in RVC and HVC were maintained even after MAP returned to basal levels. These observations suggest that baroreceptors are essential to induce a reflex vasodilation in the renal and hindlimb vascular beds and are also responsible for the onset of a long-lasting response that, once started, does not depend on blood pressure levels.

Several lines of evidence indicate that arterial baroreceptors may have a more significant role in regulation of blood volume than initially suspected (reviewed in Ref. 37). These lines of evidence indicated that arterial baroreceptors are as sensitive as cardiopulmonary receptors to detect small changes in blood volume, and that unloading of arterial baroreceptors may be the main signal triggering hormonal responses to blood-volume reduction. Results obtained in the present study are compatible with these observations,
because they demonstrated that, under the conditions of the VE paradigm used, arterial baroreceptors represent the main afferent pathway triggering cardiovascular responses to an increase in blood volume.

After SAD, basal MAP levels were significantly reduced. To minimize surgical procedures, SAD was performed 7-10 days before the VE experiment. During this period, sinoaortic-denervated animals did not show a reduction in water or food ingestion, and body weight in this group was equivalent to that in control animals. It is possible that SAD reduced the ability to counterbalance the depressor effects of anesthesia. In SAD animals, hypertension induced by VE is increased, and the return of MAP to basal levels is much slower, and even after 60 min, a significant hypertension is still observed in this group. These observations are consistent with the role of the arterial baroreceptors in buffering fast changes in arterial blood pressure.

Pressor responses to VE in renal-denervated rats were equivalent to those obtained in control animals. However, increases in RBF observed after VE were smaller, and the initial renal vasodilation was abolished. Only 40 min after the onset of VE, a significant increase in RVC was observed, although it was lower than the one observed in control rats. These results suggest that the initial stages of renal vasodilation and part of the late response are due to a reduction in sympathetic outflow to the kidney. Because renal denervation was performed unilaterally, renal afferents from the contralateral kidney were intact, and signaling from renal chemo- and mechanoreceptors during VE was maintained. Therefore, it is reasonable to assume that the reduction in renal vasodilation observed in the denervated kidney was due to the removal of an efferent mechanism rather than an impairment of renal afferents. It also suggests that the late renal vasodilation observed in this group may be due to mechanisms other than a reduction in RSNA.

Numerous previous studies indicated that besides RSNA, several vasoactive agents, which plasmatic levels are modified by VE, also modulate RBF. Accordingly, VE is associated with reductions of catecholamines (30), vasopressin (4, 30), and renin activity (4), and it is also associated with increases of kinin (11, 24) plasmatic levels. Another possible mechanism involved in the late vasodilation would be the release of ANP. It is well known that different forms of VE promote an increase of plasma ANP levels (1, 20, 27, 32). Once released, besides the observed natriuresis and diuresis (5, 7, 32), ANP can induce a significant renal vasodilation (5, 26, 38). We observed that the VE model used in this study is capable to induce ANP release in unanesthetized or anesthetized rats (unpublished observations). Furthermore, Antunes-Rodrigues et al. (1) showed that the ANP release after acute VE is severely reduced after SAD, but it is not affected by bilateral vagotomy. Together, these observations present the ANP release as an appealing possibility for the hormonal mechanisms responsible for the renal vasodilation induced by VE.

Taken together, these results indicate that the renal vasodilation induced by VE seems to depend on two mechanisms. Both are activated by baroreceptor afferents: the initial fast increase in RVC that probably involves a reduction in RSNA and a late, long-lasting vasodilation that is not dependent on the integrity of renal innervation and may be induced by the release of vasoactive peptides (e.g., ANP).

Different kinds of mechanisms seem to participate in the vasodilation of the hindquarter induced by VE. Comparing the increase observed in HBF and HVC, we observed that these increases were all long lasting and not affected by SAD, bilateral vagotomy, or renal denervation.

In contrast to what was observed in the renal bed, in SAD animals, the VE was able to induce hindquarter vasodilation comparable to that of control animals. Although previous studies have shown that the arterial baroreceptor has stronger control of the hindquarter bed (10, 23), our data suggest that several mechanisms of vascular regulation are present.

In a study similar to ours, Guo and Richardson (12) observed that the increase in hindlimb conductance after volume-expanding rats with blood was not affected by baroreceptor denervation or bilateral vagotomy alone. Also, atrial and ventricular receptor afferent fibers present in the sympathetic cardiac nerves (2) may signal VE and modulate HVC. Previous results indicated that VE reduced lumbar sympathetic nerve activity in rabbits (36), suggesting that the increase in the HVC observed here may be due to a reduction of sympathetic vasoconstrictor tone.

The results obtained in this study demonstrate that baroreceptor afferents are essential components of cardiovascular adjustments to VE, especially in the control of RVC. They also suggested that renal vasodilation induced by VE is mediated by neural and hormonal mechanisms.

**Perspectives**

A growing amount of evidence suggests that, besides their function in blood pressure signaling, baroreceptor afferents may also be involved in the regulation of blood volume. Our results indicate that a volume load producing a transient increase in arterial blood pressure stimulates baroreceptor afferents, triggering neural and hormonal mechanisms and resulting in a long-lasting increase in RVC. Previous studies and preliminary evidence from our laboratory indicate that arterial baroreceptors may mediate a release of ANP. The study of central pathways and structures involved in this response could lead to a better understanding of the relationship between blood volume and arterial pressure regulation.

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