Volume expansion potentiates cardiac sympathetic afferent reflex in dogs

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Received 4 October 1999; accepted in final form 2 October 2000

Wang, Wei, Harold D. Schultz, and Rong Ma. Volume expansion potentiates cardiac sympathetic afferent reflex in dogs. Am J Physiol Heart Circ Physiol 280: H576–H581, 2001.—Our previous study (27) showed that the cardiac sympathetic afferent reflex (CSAR) was enhanced in dogs with congestive heart failure. The aim of this study was to test whether blood volume expansion, which is one characteristic of congestive heart failure, potentiates the CSAR in normal dogs. Ten dogs were studied with sino-aortic denervation and bilateral cervical vagotomy. Arterial pressure, left ventricular pressures, ventricular epicardial diameter, heart rate, renal sympathetic nerve activity were measured. Coronary blood flow was also measured and, depending on the experimental procedure, controlled. Blood volume expansion was carried out by infusion of isosmotic dextran into a femoral vein at 40 ml/kg at a rate of 50 ml/min. CSAR was elicited by application of bradykinin (5 and 50 μg) and capsaicin (10 and 100 μg) to the epicardial surface of the left ventricle. Volume expansion increased arterial pressure, left ventricular pressure, left ventricular diameter, and coronary blood flow. Volume expansion without controlled coronary blood flow only enhanced the RSNA response to the high dose (50 μg) of epicardial bradykinin (17.3 ± 1.9 vs. 10.6 ± 4.8%, P < 0.05). However, volume expansion significantly enhanced the RSNA responses to all doses of bradykinin and capsaicin when coronary blood flow was held at the prevolume expansion level. The RSNA responses to bradykinin (16.9 ± 4.1 vs. 5.0 ± 1.3% for 5 μg, P < 0.05, and 28.9 ± 3.7 vs. 10.6 ± 4.8% for 50 μg, P < 0.05) and capsaicin (29.8 ± 6.0 vs. 9.3 ± 3.1% for 10 μg, P < 0.05, and 34.2 ± 2.7 vs. 15.1 ± 2.7% for 100 μg, P < 0.05) were significantly augmented. These results indicate that acute blood volume expansion potentiated the CSAR. These data suggest that enhancement of the CSAR in congestive heart failure may be mediated by the concomitant cardiac dilation, which accompanies this disease state.

METHODS

Ten mongrel dogs of either sex, weighing between 20 and 30 kg, were used in these experiments. All procedures in the present study were approved by the University of Nebraska Institutional Animal Care and Use Committee and were performed under the Guidelines for the Care and Use of Experimental Animals of NIH and the American Physiological Society. All experiments were carried out under anesthesia with a-chloralose (100 mg/kg iv). Stable anesthesia was maintained by administration of supplemental a-chloralose (1/10 of the initial dose per hour) throughout the experiments. Arterial blood gases were measured and kept within normal limits (pH 7.35–7.45; Pco2 30–40 mmHg; Po2 85–95 mmHg) during the experiment. Measurement of hemodynamics. A femoral artery was catheterized for measurement of systolic, diastolic, and mean arterial blood pressure (SAP, DAP, and MAP, respectively) during ischemia excite these nerve endings (1, 22). It has been suggested that cardiac sympathetic afferent nerves contribute to reflex control of the circulation via spinal and supraspinal pathways in physiological and certain pathological conditions (12). Generally, this reflex, called the cardiac sympathetic afferent reflex (CSAR), is known as a sympatho-excitatory reflex (12).

Our previous study (27) has shown that the renal sympathetic nerve activity (RSNA) responses to epicardial application of bradykinin and capsaicin are significantly enhanced in sino-aortic denervated (SAD) and vagotomized dogs with pacing-induced heart failure (27). Further experiments in our laboratory (26) demonstrated that sensitization of the sympathetic afferents was one mechanism leading to this enhancement. However, the mechanisms responsible for the sensitization of afferent endings and enhancement of the CSAR are not well understood. Because congestive heart failure (CHF) is associated with blood volume expansion (16), it is possible that distension of cardiac chambers induced by expanded blood volume in this disease state provides more intense stimulation of sympathetic afferent endings and, in turn, elicits over-activity of the CSAR. Therefore, the aim of the present study was to test the hypothesis that the CSAR is enhanced by plasma volume expansion and left ventricular distension in normal dogs.
and heart rate (HR). A left thoracotomy was performed through the fourth intercostal space, and the heart was exposed and suspended in a pericardial cradle. A catheter was positioned within the left ventricular cavity via the apex of the left ventricle for the measurement of left ventricular systolic pressure (LVSP) and left ventricular end-diastolic pressure (LVEDP) using transducers. The proximal portion of the left circumflex coronary artery was carefully dissected free from surrounding tissues. An ultrasonic flow probe (2.5SB172, Transonic Systems, Ithaca, NY) was placed around the left circumflex coronary artery and connected to an ultrasonic flow meter (model T106, Transonic Systems) for measurement of coronary blood flow (CBF). A 1-0 silk snare was placed around the left main coronary artery to control for changes in CBF. All hemodynamic data were digitized at a sampling rate of 400/s and monitored continuously by a computer system (MacLab System, AD Instruments, Milford, MA).

Measurement of left ventricular diameter. A pair of piezo-electric crystals (2 mm-XTAL-34C, Sonometrics, Ontario, Canada) were symmetrically sutured to the anterior and posterior epicardial surface of the left ventricle. Ventricular diameters were measured by a sonomicrometer (Triton Technology, San Diego, CA), the output of which was fed into the MacLab system.

Baroreceptor denervation. Through a midline incision in the neck, the carotid sinus area was exposed bilaterally. Each carotid sinus nerve was identified, ligated, and cut. All other visible nerve fibers in the area of the carotid sinus were cut. The carotid sinus bifurcation and the common carotid arteries were stripped of adventitial tissues from ~1 cm below the bifurcation to 1 cm above. Each vagus was then identified in the neck, tied, and sectioned. The effectiveness of baroreceptor denervation was determined by recording the change in HR to bolus injection of nitroglycerin (25 μg/kg). This dose evoked decreases in blood pressure of between 25 and 40 mmHg. Baroreceptor denervation was assumed to be complete if the HR did not change more than 5 beats/min to this intervention.

Volume expansion. Acute volume expansion was induced by intravenous injection of isotonic and isosmotic dextran solution (40 ml/kg) via a femoral vein. The rate of volume loading was 50 ml/min.

RSNA recording. A left flank incision was made, and a retroperitoneal dissection was used to expose the renal artery and nerves. The renal sympathetic nerves were identified, and a branch was carefully dissected free of the surrounding connective tissue. The nerve was immersed in a warm mineral oil bath (37°C) and placed on a pair of platinum-iridium recording electrodes. The signal was amplified with a Grass direct current preamplifier (model P18D, Grass Instruments, Quincy, MA) with the low-frequency cutoff set at 2 or 3 kHz. The amplified discharge was monitored on a storage oscilloscope (model 121N, Tektronix, Beaverton, OR) and further fed into the MacLab system. RSNA was quantified by setting the level recorded before volume expansion by manipulation of the silk snare prepositioned around the left main coronary artery. Each procedure was separated by at least 20 min to recover from the previous procedure.

Statistical analysis. Because it is not possible to accurately assess RSNA in absolute terms among animals, the RSNA is expressed and calculated as the percent change from control. All data are expressed as means ± SE. A one-way analysis of variance for repeated measurements associated with the Newman-Keuls test for post hoc analysis was used to compare the control response with the responses after different interventions in the present study. All statistical analysis was carried out using commercial computer software (Sigma-Stat, Jandel). A P value of <0.05 was considered statistically significant.

RESULTS

Changes in hemodynamics after acute volume expansion with and without control of CBF. SAP, DAP, MAP, HR, LVSP, LVEDP, and CBF measured before volume expansion were taken as control values. After isosmotic dextran (40 ml/kg body wt) was acutely added into the circulatory system, all variables except HR were significantly elevated (Table 1). LVEDP and CBF were increased by approximately four- and twofold, respectively, from their corresponding control values. The increase in hemodynamics was sustained when CBF was reduced to the control level by partial occlusion of the left main coronary artery. These data are summarized in Table 1.

Changes in left ventricular diameter after acute volume expansion with and without control of CBF. Figure 1 shows the mean data for LVESD and LVEDD before and after volume expansion. The left ventricle was enlarged by loading a large volume (40 ml/kg at 50 ml/min) of isosmotic dextran into the circulation, which was indicated by the significant increments of LVESD and LVEDD. As shown in Fig. 1, the increase in the left ventricular diameter was not affected by control of CBF.
Effects of acute volume expansion with and without control of CBF on CSAR. The CSAR was evoked by topical administration of bradykinin (5 and 50 μg in 0.5 ml, respectively) or capsaicin (10 and 100 μg in 0.5 ml, respectively) to the epicardial surface of the left ventricle in all 10 SAD and vagotomized dogs. The changes in arterial pressure and RSNA were taken as indicators of the degree of activation of this reflex. Figure 2 is a representative recording showing the changes in the hemodynamic and RSNA responses to 100 μg capsaicin after volume expansion with and without control of CBF. In this representative case, acute volume expansion not only resulted in increases in baseline hemodynamics but also caused greater increase in arterial blood pressure and RSNA in response to activation of the cardiac sympathetic afferent endings by capsaicin (Fig. 2, middle). These responses were further elevated when CBF was held at the level before volume expansion (Fig. 2, right). Comparisons of the MAP, RSNA, and HR responses to two doses of bradykinin and capsaicin before and after volume expansion with and without control of CBF are illustrated in Figs. 3 and 4. It is shown that both bradykinin (Fig. 3) and capsaicin (Fig. 4) elicited greater increases in the MAP and RSNA after volume expansion. Volume expansion without controlled CBF (Table 1) only enhanced the RSNA response to the high dose (50 μg) of bradykinin (17.3 ± 1.9 vs. 10.6 ± 4.8%, \( P < 0.05 \); Fig. 3). However, volume expansion significantly enhanced the RSNA responses to epicardial application of all doses of bradykinin and capsaicin when CBF was held at the prevolume expansion level (Table 1). The RSNA responses to bradykinin (16.9 ± 4.1 vs. 5.0 ± 1.3% for 5 μg, \( P < 0.05 \), and 28.9 ± 3.7 vs. 10.6 ± 4.8% for 50 μg, \( P < 0.05 \)) and capsaicin (29.8 ± 6.0 vs. 9.3 ± 3.1% for 10 μg, \( P < 0.05 \), and 34.2 ± 2.7 vs. 15.1 ± 2.7% for 100 μg, \( P < 0.05 \)) were significantly augmented (Figs. 3 and 4). Epicardial bradykinin and capsaicin did not alter CBF at any dose in control, volume expansion without controlled CBF, and volume expansion with controlled CBF groups.

The primary finding in the current study was that acute volume expansion with controlled coronary flow enhanced the responses of the RSNA to application of bradykinin and capsaicin to the epicardial surface of the left ventricle in SAD and vagotomized dogs.

It is known that the CSAR participates in reflex control of the circulation via spinal and supraspinal pathways in physiological and under some pathological conditions (12). The sympathetic afferent endings innervating the heart are mechano- and chemosensitive. Either distension of cardiac chambers (22) or algesic agents (1) can activate the receptors of the CSAR and evoke sympatho-excitatory effects (3, 12). Consistent with the data from other studies (2, 9), volume expansion in the current study increased left ventricular pressures and dimensions as indicated by elevations in the LVSP, LVEDP, and the left ventricular diameters. In addition, CBF was also increased after volume expansion. Under this condition, the RSNA responses to bradykinin and capsaicin were augmented, suggesting that the chemosensitive afferent endings of the CSAR were sensitized. This augmentation was significantly potentiated when the increased CBF associated with volume expansion was maintained at the prevolume expansion level. These results suggest that increases in left ventricular pressure and distension not only activated the CSAR, as reported by other investigators (3, 12), but also sensitized this reflex to chemical stimulation as well.

The mechanisms for the enhancement of the CSAR induced by acute volume expansion are unclear but may be related to an imbalance between myocardial O₂ delivery and demand. It has been demonstrated that myocardial ischemia can activate the sensory endings that activate the CSAR in humans and various species.

**Table 1. Hemodynamic changes after acute volume expansion with and without control of CBF**

<table>
<thead>
<tr>
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<th>Control</th>
<th>VE−Ctrl CBF</th>
<th>VE+Ctrl CBF</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>98.3 ± 5.8</td>
<td>124.6 ± 9.8*</td>
<td>121.5 ± 6.5*</td>
</tr>
<tr>
<td>LVSP, mmHg</td>
<td>120.0 ± 4.8</td>
<td>157.2 ± 11.3*</td>
<td>155.6 ± 9.9*</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>4.3 ± 0.6</td>
<td>18.3 ± 2.6*</td>
<td>18.0 ± 1.8*</td>
</tr>
<tr>
<td>LCBF, ml/min</td>
<td>39.6 ± 3.9</td>
<td>85.9 ± 8.2*</td>
<td>40.2 ± 6.5</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>174.5 ± 8.2</td>
<td>170.9 ± 8.1</td>
<td>171.3 ± 10.6</td>
</tr>
</tbody>
</table>

Values are means ± SE. CBF, coronary blood flow. VE−control (Ctrl) CBF, volume expansion (VE) without controlled coronary flow; VE+Ctrl CBF, volume expansion with controlled coronary flow; MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; LCBF, left circumflex coronary blood flow; HR, heart rate. \( * \) \( P < 0.05 \), significant difference compared with corresponding controls.
In these studies, myocardial ischemia induced by coronary artery disease or experimental occlusion of the coronary artery evoked obvious sympatho-excitation, such as tachycardia, hypertension, and an increase in RSNA. These responses were abolished by removal or blockade of cardiac sympathetic afferent fibers, suggesting that these sympatho-excitatory effects were mediated by the CSAR. Furthermore, the degree of activation of the CSAR was related to the severity of myocardium ischemia (14). Minisi and colleagues (14) measured the responses of arterial pressure and efferent sympathetic nerve activity during occlusion of the left anterior descending (LAD alone) coronary artery and during LAD artery occlusion with a circumflex stenosis (LAD + CIRC) in anesthetized dogs with sinoaortic and vagal deafferentation (14). They observed significantly greater CSAR-induced increases in renal and cardiac nerve activity during LAD + CIRC than during LAD alone. By measuring myocardial blood flow, they confirmed that LAD + CIRC elicited more severe ischemia than did LAD alone. Interestingly, myocardial ischemia not only activated the CSAR but also increased the sensitivity to other stimuli. It has been demonstrated that high-threshold stimulation of the superficial peroneal nerve (nociceptive stimulation), which was used to evoke the CSAR in the presence of a severe coronary stenosis, elicited greater sympatho-excitatory effects than high-threshold stimulation in the intact coronary artery (21). In the present study, left ventricular pressure was elevated, and cardiac chambers were enlarged, after volume expansion. This cardiac distension likely increased wall tension. Because HR was relatively constant, an increase in wall tension would increase cardiac O_2 consumption (5, 10). Although we did not measure myocardial O_2 consumption, it is conceivable that volume expansion resulted in some degree of relative ischemia given the significant elevation in LVEDP. When CBF was not allowed to rise after volume loading, this ischemia would be more severe. We speculate that this volume expansion-induced ischemia was severe enough to excite or sensitize cardiac sympathetic sensory endings and consequently enhance the CSAR to substances such as bradykinin and capsaicin.

It has been shown that myocardial ischemia-induced activation of the CSAR is primarily attributed to a variety of substances released from ischemic cardiac tissues (12). These include potassium, hydrogen ion, hydrogen peroxide, bradykinin, adenosine, and prostaglandins (7, 8, 23–25, 27). These metabolic products and chemicals not only activate the CSAR but may interact to potentiate their actions. One example was recently provided by Gneccchi et al. (7), which showed that adenosine potentiated the excitation of cardiac sympathetic afferent fibers induced by coronary occlusion. The same situation may exist in the present study. Volume expansion-induced ischemia might result in the release of several substances from the ischemic myocardium. With the control of CBF, more ischemia by-products would be produced and accumulated locally. This environment may excite and/or sensitize the afferent endings of the CSAR and result in the enhancement of this reflex, which was observed in this study.

In addition to a peripheral site, other sites in the CSAR pathway may also constitute the components for the augmentation of the CSAR induced by volume expansion. As described above, volume loading could activate the CSAR and elevate sympathetic outflow. Augmented sympathetic outflow to the kidneys may stimulate renin release from juxtaglomerular cells,
which, in turn, increases ANG II concentration in the circulation. In addition to its general sympatho-excitation properties (17), ANG II is also involved in the central integration of the CSAR, as reported in a previous study (11). From this laboratory, in addition to ANG II, other factors may play role in the modulation of the sensitivity of the CSAR after acute volume expansion as well. For instance, a recent study (13) showed that acute volume loading elevated the concentration of plasma brain natriuretic peptide. Therefore, the changes in the levels of various hormones in the central nervous system after volume expansion might affect the central sensitivity of the CSAR and constitute one mechanism for the results observed in the present study.

We noted that the MAP and HR responses to bradykinin and capsaicin were not affected by volume expansion either with or without control of CBF. This could be explained by the experimental interventions used in this study. All dogs used in the current study were SAD and vagotomized. Therefore, the baroreflexes in these dogs have been eliminated. Removal of these sympatho-inhibitory reflexes resulted in a much higher sympathetic tone, which was indicated by an increase in baseline MAP and HR after the intervention. Under these conditions, vascular smooth muscle and the sinoatrial node may be unable to respond to further elevation of sympathetic nerve activity (16). Thus, although activation of the CSAR by bradykinin and capsaicin after volume loading elevated sympathetic outflow (RSNA), blood vessel responses to the augmented sympathetic output might have been weakened and, consequently, MAP did not have significant change. In addition, bilateral vagotomy may also contribute to the lack of a significant change in HR responses to bradykinin and capsaicin.

The data presented here have some correlation to the CHF state. Water retention and elevated LVEDP are characteristics of CHF (11, 16, 27). It has been shown that CBF is decreased (18) and O2 consumption is increased in at least one form of experimental

**Fig. 3.** The mean arterial pressure (MAP; top), RSNA (middle), and heart rate (HR; bottom) responses to topical administration of bradykinin before (control) and after volume expansion without controlled CBF and after controlled CBF. BK, bradykinin; BPM, beats/min. *P < 0.05, significant difference compared with corresponding control values; #P < 0.05, significant difference compared with volume expansion without controlled coronary flow.

**Fig. 4.** MAP (top), RSNA (middle), and HR (bottom) responses to topical administration of capsaicin before (control) and after volume expansion without and with controlled CBF and after controlled CBF. Cap, capsaicin. *P < 0.05, significant difference compared with corresponding control values; #P < 0.05, significant difference compared with volume expansion without controlled coronary flow.
heart failure (20). In the anesthetized SAD and vagotomized dogs with CHF, the CSAR was enhanced, and this enhancement was attributed to sensitization of afferent endings and central components of this reflex (11, 26, 27). This abnormality of the CSAR was, to some extent, mirrored by acute volume expansion in the current study, suggesting that an increase in blood volume (which occurs in CHF) may be one mechanism to cause augmentation of the CSAR in this condition. It has to be mentioned that it might be some difference between acute volume loading in normal state and chronic volume expansion in heart failure. A recent study by Feigenbaum et al. (6) has shown that the intravascular volume in a patient with CHF was significantly lower than that in normal subjects. This study indicates that this lower volume in a patient with heart failure may be related by deconditioned state or excessive diuresis or both.

In summary, acute volume expansion raised left ventricular pressure and diameter to levels seen in CHF. With the control of increased CBF associated with volume loading, the RSNA responses to epicardial application of bradykinin and capsaicin to the left ventricle were potentiated. These data suggest that volume expansion sensitizes the CSAR. The results observed in this study provide new insight to explain the enhanced CSAR in CHF, a condition in which blood volume loading in patients with ischemic heart disease. Acta Cardiol 52: 261–272, 1997.

References