Cardiac sympathetic afferent stimulation by bradykinin in heart failure: role of NO and prostaglandins

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Cardiac sympathetic afferent stimulation by bradykinin in heart failure: role of NO and prostaglandins. Am. J. Physiol. 275 (Heart Circ. Physiol. 44): H783–H788, 1998.—I have shown that cardiac sympathetic afferent stimulation by epicardial application of bradykinin (BK) was significantly enhanced in pacing-induced heart failure (HF) dogs. This enhancement appeared to be mediated by prostaglandins. The present study was to determine whether nitric oxide is involved in this enhancement. Under α-chloralose (100 mg/kg iv) anesthesia, the renal sympathetic nerve activity (RSNA) response to BK was determined in 15 HF and 15 sham dogs in the sinoaortic-denervated and vagotomized state. The RSNA response to BK was significantly enhanced in HF. This enhanced RSNA response to BK was significantly reduced in the HF dogs after administration of the cyclooxygenase inhibitor indomethacin (5 mg/kg iv), but no significant change was found in the sham group. In contrast, RSNA responses to BK were significantly reduced in the sham dogs after administration of the nitric oxide synthase inhibitor N⁶-nitro-L-arginine methyl ester (L-NAME, 30 mg/kg iv), but no significant change was found in the HF group. These data suggest that the RSNA response to BK is mediated by nitric oxide to a large degree in the normal state but is primarily mediated by prostaglandins in the HF state.

METHODS

Surgical instrumentation. Thirty mongrel dogs of either sex and weighing between 20 and 30 kg were used in these experiments. All dogs were instrumented with the use of sterile techniques under pentobarbital anesthesia (30 mg/kg iv initially plus 1/10 of initial dose per hour). Through a right thoradomcy (4th interspace), catheters were implanted in the left atrium or left ventricle through a branch of a pulmonary vein. A pacing lead (Medtronic model 6917–357) was placed near the base of the right ventricle. Through a subcutaneous incision a catheter was also implanted in the aorta through the omocervical artery. Catheters were used for measurement of the respective vessel or chamber pressure. One week after recovery from surgery, the dogs were paced (right ventricular) at 210 beats/min using a Medtronic 8529 pacemaker after control hemodynamic measurements were made in conscious dogs. The pacing rate was increased to 250 beats/min in the second week and continued for the next 2–3 wk.

Hemodynamic measurements. Left ventricular pressure or left atrial pressure, arterial blood pressure, and heart rate (HR) were determined in conscious dogs in order to determine when to carry out the acute experiment. All pressures were measured using Hewlett-Packard pressure transducers. All catheters attached to external transducers were zeroed at the supraspinous process with the dog lying on its left side. All hemodynamic measurements were taken with the dog resting on a laboratory table with the pacemaker set to the inhibit mode.

Acute experiments. When dogs were paced for 3–4 wk and their left atrial pressure or left ventricular end-diastolic pressure (LVEDP) was significantly elevated (>15 mmHg), acute experiments were carried out. Each dog was anesthetized with α-chloralose (100 mg/kg iv) and intubated. A femoral artery was catheterized for systolic, diastolic, pulse, and mean arterial pressure (MAP) measurements. A femoral vein was canulated with administration of supplemental doses of anesthesia (1/2 of initial dose of α-chloralose per hour). Arterial blood gases were measured throughout the experiment and kept within normal limits (pH 7.35–7.45; Pco₂, 30–40 mmHg; Po₂, 85–95 mmHg).

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 bifurcation and the common carotid arteries were stripped of adventitial
tissue from ~1 cm below the bifurcation to 1 cm above. Finally, the same area was painted with a solution containing 10% phenol in ethanol. Each vagus nerve was then identified in the neck, tied, and sectioned. Through a left 5th intercostal space, the chest was opened. The heart was suspended from a pericardial cradle, exposing the anterior wall of the left ventricle. 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 and placed on a pair of platinum-iridium recording electrodes. The signal was amplified with a Grass DC preamplifier (model P18D, Grass Instrument) with low-frequency cutoff set at 30 or 100 Hz and high-frequency cutoff set at 1 or 3 kHz. The amplified discharge was monitored on a storage oscilloscope (model 121N, Tektronix) and connected to a neuronal spike analyzer (model N750, Mentor). A window discriminator was set just above the noise so that only the renal nerve discharge signal was discriminated. The discriminator pulses were fed into a rate meter (Frederick Haer) for quantification. The raw nerve activity, rate meter output, discriminator pulses, and arterial pressure were recorded on an electrostatic strip-chart recorder (model ES 1000B, Gould). Hemodynamics and nerve activity were also digitized and analyzed by a computer (MacLab System).

Experimental protocols. The same protocol that I used previously (31) was applied in this study. In brief, in sinoaortic-denervated (SAD) and vagotomized dogs (7 sham and 8 heart failure), after baseline RSNA were recorded, a 3-cm diameter piece of filter paper saturated with vehicle (isotonic saline) or bradykinin (5 µg in 0.5 ml and 50 µg in 0.5 ml) was applied to the epicardial surface of the anterior wall of the left ventricle. Each drug was applied for 30 s, and RSNA were averaged over the last 10 s. The filter paper was then removed, and the epicardium was rinsed three times with 20 ml of warm normal saline (38°C). Consistent with the previous study, these concentrations of BK evoked a significant RSNA response. These procedures were repeated 20 min after administration of the cyclooxygenase inhibitor indomethacin (5 mg/kg iv) and 20 min after the NO synthase inhibitor N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME, 30 mg/kg iv).

### Table 1. Hemodynamics of intact anesthetized sham and heart failure dogs

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<th>Sham</th>
<th>Heart Failure</th>
<th>P Value</th>
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<tr>
<td>LVSP, mmHg</td>
<td>139.6 ± 5.1</td>
<td>102.8 ± 3.0</td>
<td>&lt;0.01</td>
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<tr>
<td>LVEDP, mmHg</td>
<td>2.1 ± 0.5</td>
<td>22.5 ± 2.1</td>
<td>&lt;0.001</td>
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<tr>
<td>MAP, mmHg</td>
<td>118.8 ± 6.8</td>
<td>82.8 ± 3.2</td>
<td>&lt;0.05</td>
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<td>HR, beats/min</td>
<td>141.8 ± 5.7</td>
<td>153.7 ± 5.4</td>
<td>NS</td>
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Values are means ± SE; n = 15 dogs/group. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; MAP, mean arterial pressure; HR, heart rate. NS, not significant.

Fig. 1. Representative recording shows renal sympathetic nerve activity (RSNA) response to epicardial application of bradykinin (BK, 50 µg) in a sham (top) and a heart failure (HF, bottom) dog before indomethacin (control, left), after indomethacin (middle), and after indomethacin plus N\textsuperscript{G}-nitro-L-arginine methyl ester (L-NAME, right). Arrows show when BK was applied. ABP, arterial blood pressure.
In another eight sham and seven heart failure dogs, the order of administration of indomethacin and L-NAME was reversed.

Statistical analysis. A two-way repeated measure analysis of variance (ANOVA) associated with the Newman-Keuls test for post hoc analysis was used when multiple comparisons were made. ANOVA was used when sham versus heart failure before and after either indomethacin or L-NAME were compared. All statistical analyses were carried out using commercial computer software (Sigmastat, Jandel). The data are expressed as means ± SE; a P value of <0.05 was considered statistically significant.

RESULTS

Hemodynamics of anesthetized sham and heart failure animals. LVEDP, left ventricular systolic pressure, MAP, and HR were measured in anesthetized sham and heart failure groups. As seen in Table 1, the LVEDP was significantly elevated (22.5 ± 2.1 vs. 2.1 ± 0.5 mmHg, P < 0.001), and MAP was significantly decreased (82.8 ± 3.2 vs. 118.8 ± 6.8 mmHg, P < 0.05) in dogs with heart failure. It should be pointed out that these data were taken before SAD and vagotomy.

Effects of cycloxygenase inhibition followed by NO synthase inhibition on RSNA response to bradykinin. In seven sham and eight heart failure dogs with SAD and vagotomy, the effects of the cycloxygenase inhibitor indomethacin (5 mg/kg iv) and the NO synthase inhibitor L-NAME (30 mg/kg iv) on the RSNA response to bradykinin were examined. Figure 1 shows a representative recording from a sham and a heart failure dog. As shown in Fig. 1 and Fig. 2 as averaged data, RSNA responses to bradykinin were significantly enhanced in the heart failure dogs compared with those of the sham dogs (28.4 ± 6.8 vs. 12.0 ± 4.7%, P < 0.05, and 39.8 ±

<table>
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<th>Table 2. Effects of indomethacin or L-NAME on baseline MAP and RSNA in sinoaortic-denervated and vagotomized dogs</th>
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Values are means ± SE; n = number of dogs. RSNA, renal sympathetic nerve activity; HF, heart failure; L-NAME, N\textsuperscript{G}-nitro-L-arginine methyl ester.

Fig. 2. RSNA response to epicardial application of BK (A: 5 µg, B: 50 µg) in sham (left) and HF dogs (right) in control, 20 min after indomethacin (Indo), and 20 min after L-NAME. *P < 0.05, compared with the control; †P < 0.05 compared with sham.

Fig. 3. Bar graphs showing percent change in RSNA responses to epicardial application of BK (A: 5 µg, B: 50 µg) in sham (left) and HF dogs (right) in control (100%), 20 min after Indo, and 20 min after L-NAME. *P < 0.05 compared with the control.
Normal saline did not evoke any RSNA responses in either sham or heart failure animals.

Effects of NO synthase inhibition followed by cyclooxygenase inhibition on RSNA response to bradykinin. In another eight sham and seven heart failure dogs with SAD and vagotomy, the effects of L-NAME and indomethacin on the RSNA response to bradykinin were determined. Table 2 shows baseline MAP and RSNA after L-NAME (30 mg/kg iv). Whereas there was a trend for MAP to increase after L-NAME, it did not reach statistical significance. Figure 4 shows that the RSNA response to bradykinin was significantly augmented in the heart failure group compared with the sham group (24.5 ± 2.7 vs. 13.3 ± 1.4% for BK 5 µg, P < 0.05, and 31.8 ± 1.7 vs. 15.2 ± 1.2% for BK 50 µg, P < 0.05, respectively). Again, when the control responses were set to 100%, in the sham group L-NAME significantly blunted the RSNA response to bradykinin (35.2 ± 5.9% for BK 5 µg and 40.3 ± 5.7% for BK 50 µg vs. 100% in control, Fig. 5). In contrast, L-NAME alone had no significant effect on the RSNA response to bradykinin in the heart failure group. In both sham and heart failure groups, L-NAME plus indomethacin prevented the RSNA response to bradykinin.

In six sham and six heart failure animals, epicardial application of vehicle (isotonic saline) was also tested.
DISCUSSION

Consistent with our previous studies (29, 31), 4 wk of ventricular pacing at 250 beats/min induced congestive heart failure with significantly elevated LVEDP and decreased MAP. A previous study from this laboratory has shown that the RSNA response to epicardial application of bradykinin is significantly enhanced in dogs with pacing-induced congestive heart failure, i.e., the cardiac sympathetic afferent reflex is augmented in the heart failure state (31). This enhancement was confirmed in the present study. It is well known that the effects of bradykinin are mediated, in part, by prostaglandins (3, 11, 25, 32). Nerdrum et al. (11) and our previous studies (30, 31) have shown that excitation of the cardiac sympathetic afferents induced by epicardial application of bradykinin can be partially prevented by the cyclooxygenase inhibitor indomethacin. In the present study, the RSNA response to epicardial application of bradykinin was also inhibited by indomethacin. In the heart failure group only 20% of the bradykinin response remained, whereas ~50% of the RSNA response remained in the sham group after indomethacin administration. This suggests that the enhanced RSNA response to epicardial application of bradykinin in heart failure is mainly mediated by an augmented prostaglandin synthesis.

In addition to prostaglandins, it has also been shown that some effects of bradykinin are mediated by NO (3, 27, 33, 34). NO synthase and NO-mediated responses are significantly reduced in the heart failure state (2, 7, 17, 24). Enhanced RSNA responses to epicardial application of bradykinin in the heart failure group were not significantly inhibited by the NO synthase inhibitor L-NAME. In contrast, the RSNA responses to bradykinin were significantly inhibited by L-NAME in the sham group. This suggests that the effect of epicardial bradykinin on the RSNA responses is predominantly mediated by NO in the sham group. In the present experiment, the RSNA responses to epicardial bradykinin were completely prevented by combined cyclooxygenase and NO synthase inhibition in both sham and heart failure groups. This is consistent with other studies (9, 25, 33) and indicates that the effects of bradykinin are mediated by both prostaglandins and NO. In addition to the augmented cardiac sympathetic afferent fiber response to bradykinin in heart failure, the vagal afferent C fiber response from the left ventricle is also augmented in pacing-induced heart failure (23). Moreover, indomethacin significantly attenuated the vagal afferent response to bradykinin in dogs with heart failure (23). The present study indicates that the enhanced RSNA response to BK in heart failure is mainly mediated by cyclooxygenase. It is not clear from the results of this study if cyclooxygenase-2 is upregulated in heart failure; however, Smith et al. (24) have shown a significant reduction in the expression of vascular endothelial NO synthase and cyclooxygenase-1 in dogs with pacing-induced heart failure. In the coronary vessels, chronic inhibition of NO synthase enhanced the production of prostaglandin through upregulation of cyclooxygenase in both in vivo (1) and in vitro (16) studies. It is also shown that inhibition of NO synthesis enhanced prostanoid production by upregulation of cyclooxygenase-2 (6).

In the present study, there was a tendency for baseline MAP to increase in both sham and heart failure groups after administration of indomethacin, with no change in baseline RSNA. This is consistent with other studies (28, 35). In the present study, however, there was also no significant increase in baseline MAP and RSNA following NO synthase inhibition, especially in the heart failure group. NO synthase inhibition showed significantly increased baseline MAP and sympathetic outflow (5, 13, 21). It is curious that increased baseline MAP and RSNA did not occur. One explanation may be that in the present study, the animals were anesthetized, and the baroreceptors were denervated and the vagi were cut bilaterally. Sympathetic outflow was likely to be much higher compared with the intact state, especially in the heart failure group. In addition, since NO synthesis is impaired (2, 7, 17, 24) in heart failure and the tonic release of NO is less, this might account for the lack of a change in RSNA following NO synthase inhibition. These data suggest that the tonic release of NO had little effect on baseline MAP and RSNA in both sham and heart failure groups in anesthetized baroreceptor-denervated and vagotomized dogs.

In summary, RSNA responses to epicardial application of bradykinin are significantly enhanced in the heart failure state. This enhanced RSNA response is predominantly mediated by prostaglandin synthesis, whereas the RSNA responses to bradykinin in the normal state are mainly mediated by NO oxide.

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