Carotid sympathetic afferent stimulation impairs baroreflex control of renal sympathetic nerve activity in rats

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Gao, Lie, Zhen Zhu, Irving H. Zucker, and Wei Wang. Cardiac sympathetic afferent stimulation impairs baroreflex control of renal sympathetic nerve activity in rats. Am J Physiol Heart Circ Physiol 286: H1706–H1711, 2004;10.1152/ajpheart.01097.2003.—It is well known that cardiac sympathetic afferent reflexes contribute to increases in sympathetic outflow and that sympathetic activity can antagonize arterial baroreflex function. In this study, we tested the hypothesis that in normal rats, chemical and electrical stimulation of cardiac sympathetic afferents results in a decrease in the arterial baroreflex function by increasing sympathetic nerve activity. Under α-chloralose (40 mg/kg) and urethane (800 mg/kg ip) anesthesia, renal sympathetic nerve activity, mean arterial pressure, and heart rate were recorded. The arterial baroreceptor reflex was evaluated by infusion of nitroglycerin (25 μg iv) and phenylephrine (10 μg iv). Left ventricular epicardial application of capsaicin (0.4 μg in 2 μl) blunted arterial baroreflex function by 46% (maximum slope 3.5 ± 0.3 to 1.9 ± 0.2%/mmHg, P < 0.01). When the central end of the left cardiac sympathetic nerve was electrically stimulated (7 V, 1 ms, 20 Hz), the sensitivity of the arterial baroreflex was similarly decreased by 42% (maximum slope 3.2 ± 0.3 to 1.9 ± 0.4%/mmHg, P < 0.05). Pretreatment with intracerebroventricular injection of losartan (500 nmol in 1 μl of artificial cerebrospinal fluid) completely prevented the impairment of arterial baroreflex function induced by electrical stimulation of the central end of the left cardiac sympathetic nerve (maximum slope 3.6 ± 0.4 to 3.1 ± 0.5%/mmHg). These results suggest that the both chemical and electrical stimulation of the cardiac sympathetic afferents reduces arterial baroreflex sensitivity and the impairment of arterial baroreflex function induced by cardiac sympathetic afferent stimulation is mediated by central angiotensin type 1 receptors.

angiotensin type 1 receptor

The arterial baroreceptor reflex plays an important role in the adaptation and regulation of blood pressure in both physiological and pathophysiological situations (8). Despite the many advances made toward understanding arterial baroreflex function, very little is known concerning how the baroreflex is regulated by other cardiovascular reflexes. Our previous study showed that in the chronic heart failure (CHF) state, not only is the arterial baroreflex gain depressed (17, 18, 25, 26) but also the cardiac sympathetic afferent reflex gain is significantly enhanced (24, 28, 29). The cardiac sympathetic afferent reflex is a sympathoexcitatory reflex (12) and may contribute to the increase of sympathetic outflow in CHF (27). On the other hand, many studies (2, 6, 10, 23, 31) have solidly supported the idea that sympathetic activity can antagonize arterial baroreflex function in both humans and experimental animals. Chemical sympathectomy markedly potentiates the baroreceptor reflex in normal rats (5) and prevents the occurrence of the baroreceptor reflex impairment associated with chronic heart failure (15). We thus reasoned that because the cardiac sympathetic afferent reflex contributes to an increase in sympathetic outflow, this enhanced sympathetic activity may antagonize baroreflex function. If the cardiac sympathetic afferent reflex is augmented in CHF, this may be responsible for the suppressed arterial baroreceptor reflex associated with CHF. Thus it can be hypothesized that in normal rats, chemical and electrical stimulation of the cardiac sympathetic afferent reflex results in an increase in sympathetic nerve activity, followed by a decrease in the gain of arterial baroreflex. Therefore, the first goal of this study was to determine whether the chemical and electrical stimulation of cardiac sympathetic afferents impairs arterial baroreflex function in normal rats.

It has been shown that the renin-angiotensin (ANG) system is activated in human and experimental chronic heart failure (13, 33). Central ANG II plays an important role in both depressing the arterial baroreceptor reflex (33) and in enhancing the cardiac sympathetic afferent reflex associated with the CHF state (27). Blockade of the ANG II type 1 receptor in CHF animals not only normalized the enhanced cardiac sympathetic afferent reflex (11) and reduced sympathetic tone (4) but also restored the impaired arterial baroreflex function (16). Given the above-mentioned close relationship between the renin-ANG II system, arterial baroreflex, and cardiac sympathetic afferent reflex, the second goal of the present study was to test the hypothesis that the central ANG II mechanism is involved in the effect of electrical stimulation of cardiac sympathetic afferent stimulation on arterial baroreceptor reflex function in normal rats.

METHODS

Male Sprague-Dawley rats weighing between 350 and 420 g were used in these experiments. All experiments were approved by the Institutional Animal Care and Use Committee of the University of Nebraska Medical Center and were carried out under the guidelines of the American Physiological Society and the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Each rat was anesthetized with urethane (800 mg/kg ip) and α-chloralose (40 mg/kg ip). Supplemental doses of anesthesia were administered at 1/10 of the initial dose per hour. Body temperature was maintained with the use of a heating pad. A midline incision in the neck was made, and the trachea was cannulated to facilitate mechanical ventilation. Through the midline incision in the neck, the
right common carotid artery was exposed and cannulated with a catheter transducer (model SPR-524, Millar Instruments; Houston, TX) for measurement of mean artery pressure (MAP). Heart rate (HR) was derived from the arterial pressure pulse using a PowerLab model 16S (ADInstruments; Colorado Springs, CO). A femoral vein was cannulated with a polyethylene-20 catheter for administration of drugs.

Recording of renal sympathetic nerve activity. The left kidney, renal artery, and nerves were exposed through a left retroperitoneal flank incision. The renal sympathetic nerves were identified, dissected free of the surrounding connective tissue, and was placed on a pair of platinum-iridium recording electrodes. When an optimal signal-to-noise ratio was achieved, the electrode and the renal nerve were covered with a fast setting silicone (Kwik-Sil, World Precision Instruments; Sarasota, FL). The signal was amplified with a Grass direct current preamplifier (model P18D, Astro-Med; West Warwick, RI) with the low-frequency cutoff set at 30 to 100 Hz and high-frequency cutoff at 1 to 3 kHz. The amplified discharge was monitored on a storage oscilloscope (model 121 N, Tektronix; Beaverton, OR) and then imported to a computer system with other parameters. A voltage integrator (model 1801, Busco Electronics) was used for quantifying the raw renal sympathetic nerve activity (RSNA). The raw nerve activity, integrated nerve activity, arterial pressure, and HR were recorded using a Lab data-acquisition system (model 16S, AD-Instruments) and stored on disk until analyzed.

Epicardial application of capsaicin. The chest was opened through the fourth intercostal space. The pericardium was removed to expose the left ventricle. A piece of filter paper (3 × 3 mm) containing capsaicin (0.4 µg in 2 µl) was applied to the epicardial surface of the anterior surface of the left ventricle. Each drug was applied for ~2 min until the end of the baroreflex test, and then the filter paper was removed and the epicardium was rinsed three times with 10 ml of warm normal saline (38°C).

Electrical stimulation of cardiac sympathetic afferents. The chest was opened through the left second intercostal space. The left ventral ansa, which contains cardiac sympathetic afferent nerves, was identified, tied, and ligated. A pair of stainless steel stimulation electrodes was placed on the central end of this nerve. The stimulus (7 V, 1 ms, 3.8 mm ventral to the zero level. Losartan (500 nmol in 1 ml of warm normal saline (38°C)) was infused. At the end of the experiment, the cannula tip placement was confirmed by microinjection of fast green (1 µl).

Construction of arterial baroreflex curves and statistical analysis. RSNA was expressed as the percent change from baseline. Baroreflex curves were generated by measurement of RSNA responses to decreases and then increases in arterial pressure by intravenous infusions of nitroglycerin (25 µg iv) and phenylephrine (10 µg iv). MAP was altered at a rate of 1 mmHg/s during these infusions. The MAP data were acquired every 2 s from the threshold to the saturation points. A voltage derivative of the baroreceptor signal was filtered with the low-frequency cutoff set at 1 to 3 kHz. The amplification of discharge was monitored on a storage oscilloscope (model 121 N, Tektronix; Beaverton, OR) and then imported to a computer system with other parameters. A voltage integrator (model 1801, Busco Electronics) was used for quantifying the raw renal sympathetic nerve activity (RSNA). The raw nerve activity, integrated nerve activity, arterial pressure, and HR were recorded using a Lab data-acquisition system (model 16S, AD-Instruments) and stored on disk until analyzed.

RESULTS

Effects of epicardial application of capsaicin on arterial baroreflex function. Table 1 shows the effects of epicardial application of capsaicin on MAP, HR, RSNA, and several baroreflex curve parameters. Although there were no significant differences in HR, MAP and RSNA were significantly higher during application of capsaicin compared with baseline. Whereas capsaicin had no significant effects on the range of RSNA response, BP50, and minimum RSNA, it did reduce the average slope and Gainmax of the arterial baroreflex curve.

Figure 1 shows an original recording of arterial blood pressure changes induced by phenylephrine after the injection of nitroglycerin and attendant RSNA reflex responses before and during epicardial application of capsaicin in one rat. It is evident that the reflex RSNA response to phenylephrine is virtually absent during epicardial application of capsaicin. The group data shown in Fig. 2 indicates a significant attenuation of the Gainmax during stimulation of the cardiac sympathetic afferent nerve.

Effects of electrical cardiac sympathetic afferent stimulation on arterial baroreflex function. Table 2 shows the effects of electrical cardiac sympathetic afferent stimulation on MAP, HR, RSNA, and several of the baroreflex curve parameters. As shown in Table 2, there were no significant differences in HR levels; however, MAP and RSNA were significantly higher during electrical stimulation of cardiac sympathetic afferents compared with baseline. Electrical stimulation of cardiac sympathetic afferents had no significant effects on the range of RSNA response, BP50, or minimum RSNA, but it reduced the average slope and Gainmax of the arterial baroreflex curve.

Figure 3 shows an original recording of arterial blood pressure changes induced by phenylephrine injection after the injection of nitroglycerin and attendant RSNA reflex responses before and during electrical stimulation of cardiac sympathetic afferents in one rat. As seen for chemical stimulation, it is evident that the reflex RSNA response to phenylephrine is almost completely absent during electrical stimulation. This

Table 1: Effect of epicardial application of capsaicin on MAP, HR, RSNA, and baroreflex parameters

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Capsaicin</th>
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<tbody>
<tr>
<td>MAP, mmHg</td>
<td>90.3±5.5</td>
<td>105.4±4.9*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>325.2±12.1</td>
<td>327.9±15.8*</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>100</td>
<td>178.7±17.6*</td>
</tr>
<tr>
<td>Range of RSNA response, %</td>
<td>107.4±12.4</td>
<td>102.9±9.9*</td>
</tr>
<tr>
<td>Average slope, %/mmHg</td>
<td>0.13±0.03</td>
<td>0.07±0.01*</td>
</tr>
<tr>
<td>BP50, mmHg</td>
<td>90.1±10.7</td>
<td>89.3±10.5*</td>
</tr>
<tr>
<td>Minimum RSNA, %</td>
<td>25.9±3.7</td>
<td>26.3±5.4</td>
</tr>
<tr>
<td>Gainmax, %/mmHg</td>
<td>3.5±0.3</td>
<td>1.9±0.2*</td>
</tr>
</tbody>
</table>

*Values are means ± SE; n = 12 rats. MAP, mean arterial pressure; HR, heart rate; RSNA, renal sympathetic nerve activity; BP50, midpoint of RSNA blood pressure range; Gainmax, maximum gain. *P < 0.05, 1P < 0.01 compared with the control.
pattern was confirmed by the average group data shown in Fig. 4, in which the significantly blunted baroreflex slope is apparent during electrical stimulation.

Effects of intracerebroventricular losartan on baroreflex function after electrical cardiac sympathetic afferent stimulation. Table 3 shows the effects of intracerebroventricular losartan (500 nmol in 1 μl artificial cerebrospinal fluid in 1 min) on the response to electrical cardiac sympathetic afferent stimulation and baroreflex function. No significant differences in HR, MAP, RSNA, or any of the baroreflex curve parameters were observed after intracerebroventricular losartan compared with baseline. However, intracerebroventricular losartan prevented electrical stimulation of cardiac sympathetic afferents from enhancing MAP and RSNA and from lowering the average slope and Gainmax of the arterial baroreflex function (Fig. 5). Figure 6 shows the composite baroreflex curves generated before and after intracerebroventricular losartan. These curves are nearly superimposable and there were no significant differences. The average Gainmax was 3.3 ± 0.2%/mmHg before losartan and 3.6 ± 0.4%/mmHg after losartan. Figure 7 shows the composite baroreflex curves generated before and during cardiac sympathetic afferent electrical stimulation after intracerebroventricular losartan. These curves are also nearly superimposable. The average maximum gain was 3.6 ± 0.4%/mmHg before electrical stimulation and 3.1 ± 0.5%/mmHg after electrical stimulation.

DISCUSSION

The major new finding of our study is that, in anesthetized rats, both left ventricular epicardial application of capsaicin and electrical stimulation of left cardiac sympathetic afferents impair the baroreflex control of RSNA mainly via reducing the average slope and Gainmax of the arterial baroreflex curve. This is the first time, to our knowledge, that it has been demonstrated...
strated that the chemically and electrically evoked cardiac sympathetic afferent reflex inhibits arterial baroreflex function. In addition, we found that pretreatment with intracerebroventricular losartan inhibits the response of electrical stimulation of cardiac sympathetic afferents from suppressing baroreflex control of RSNA. This indicates that the inhibition of baroreflex function by stimulation of the cardiac sympathetic afferent reflex is mediated by central ANG type 1 receptors.

Sympathetic nerves innervating the heart contain sensory fibers, which enter the spinal cord via upper thoracic dorsal roots (19). Various substances that may be augmented in the myocardium 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). In the present study, during application of capsaicin to the left ventricle or electrical stimulation of the left cardiac sympathetic afferent nerves, we observed sympathoexcitation and the subsequent elevation in MAP. This was essentially similar to previous studies using rats and dogs (29, 32). It is notable that the elevated MAP occurs subsequent to the increase in RSNA without an increase in HR, suggesting that the change of MAP induced by evoked cardiac sympathetic afferent reflex in this study was primarily due to a change in peripheral resistance.

It is now well accepted that the cardiac sympathetic afferent reflex is augmented (24, 28, 29) and the arterial baroreflex is depressed (17, 18, 25, 26) in the CHF state. However, it is not clear what, if any, relationship exists between these two cardiovascular reflexes. Because the cardiac sympathetic afferent reflex is a sympathoexcitatory reflex (12) and contributes to the elevation in sympathetic tone (27), which antagonizes arterial baroreflex function (2, 6, 10, 23, 31), it is possible that the

**Table 3. Effect of electrical cardiac sympathetic afferent stimulation on resting MAP, HR, RSNA, and baroreflex parameters after icv losartan in normal rats**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Losartan</th>
<th>Electrical Stimulation + Losartan</th>
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<tr>
<td>MAP, mmHg</td>
<td>93.7±10.2</td>
<td>90.2±7.6</td>
<td>92.7±8.3*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>331.6±13.6</td>
<td>326.3±17.5</td>
<td>329.4±15.3</td>
</tr>
<tr>
<td>RSNA, %</td>
<td>100</td>
<td>96.6±7.5</td>
<td>99.4±6.2†</td>
</tr>
<tr>
<td>Range of RSNA response, %</td>
<td>106.6±13.4</td>
<td>110.3±9.9</td>
<td>106.7±8.5</td>
</tr>
<tr>
<td>Average slope, %/mmHg</td>
<td>0.13±0.05</td>
<td>0.13±0.04</td>
<td>0.12±0.02†</td>
</tr>
<tr>
<td>BP&lt;sub&gt;sys&lt;/sub&gt;, mmHg</td>
<td>88.6±9.7</td>
<td>94.2±11.3</td>
<td>91.3±10.1</td>
</tr>
<tr>
<td>Minimum RSNA, %</td>
<td>27.2±4.3</td>
<td>23.3±4.2</td>
<td>26.7±4.5</td>
</tr>
<tr>
<td>Gain&lt;sub&gt;max&lt;/sub&gt;, %/mmHg</td>
<td>3.3±0.2</td>
<td>3.6±0.4</td>
<td>3.1±0.5†</td>
</tr>
</tbody>
</table>

Values are means ± SE. icv, intracerebroventricular. *P < 0.05 compared with values of electrical stimulation of cardiac sympathetic afferent without icv losartan.
augmented cardiac sympathetic afferent reflex impairs arterial baroreflex function in CHF via its sympathoexcitatory effects. The results of this study imply a central interaction between the cardiac sympathetic afferent reflex and the arterial baroreflex. This interaction may be of profound importance in the CHF state as well as in other abnormalities in which this reflex is evoked such as during coronary ischemia.

The exact mechanism(s) by which arterial baroreflex function is impaired by stimulation of the cardiac sympathetic afferent reflex are not clear. However, it is well known that ANG II modulates sympathetic function at many loci in the central nervous systems (14, 30). ANG II has been shown to augment sympathetic outflow in various portions of the hypothalamus and medulla (21, 33). It has also been shown that central administration of losartan reduced RSNA and restored baroreflex sensitivity in rats with chronic myocardial infarc-

Fig. 5. Pretreatment with intracerebroventricular losartan abolishes the effects of electrical cardiac sympathetic afferent stimulation on MAP (A), RSNA (B), and arterial baroreflex function (C and D). *P < 0.05, **P < 0.01 compared with before losartan, n = 12.

Fig. 6. Composite arterial baroreflex curves generated before and after intracerebroventricular losartan in normal rats (n = 12). Inset, gain curves of these mean baroreflex curves.

Fig. 7. Composite arterial baroreflex curves generated before and during electrical stimulation of cardiac sympathetic afferent with pretreatment of intracerebroventricular losartan (n = 12). Inset, gain curves of these mean baroreflex curves.
that the effect of intracerebroventricular administration of losartan in the present study was due to a peripheral action of losartan because our previous study indicated that intravenous administration of the same dose of losartan significantly decreased baseline MAP, which did not occur in the present study. Furthermore, administration of the same dose of losartan intravenously has no effect on the cardiac sympathetic afferent reflex sensitivity (32).

Our previous finding (32) showed that intracerebroventricular administration of losartan normalized the enhanced cardiac sympathetic afferent reflex evoked by epicardial application of both bradykinin and capsaicin in rats with CHF; but had no significant effects on the cardiac sympathetic afferent reflex in sham rats. However, in the present study we found that intracerebroventricular administration of losartan prevented the electrical stimulation of cardiac sympathetic afferents from increasing RSNA and elevating MAP in normal rats. The reason for these differences may be related to the method used to evoke the cardiac sympathetic afferent reflex or the preparation of the animals. In our previous experiment, we used the epicardial application of bradykinin and capsaicin to evoke the cardiac sympathetic afferent reflex, which may be involved in both sympathetic and parasympathetic components (3). In addition, the animals we used in that experiment were sino-aortic denervated, which might have an influence on the magnitude of the response to stimulation of cardiac sympathetic afferents.

In summary, the present results show that chemical and electrical stimulation of the cardiac sympathetic afferent reflex reduces arterial baroreflex control of RSNA in anesthetized normal rats and that this effect is mediated by a central ANG II mechanism. The results observed in this study provide new insight and a possible explanation for the blunted arterial baroreflex function in CHF, a condition in which the cardiac sympathetic afferent reflex is markedly enhanced.

GRANTS
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REFERENCES