Cardiac sympathetic afferent stimulation augments the arterial chemoreceptor reflex in anesthetized rats

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Gao L, Pan Y-X, Wang W-Z, Li Y-L, Schultz HD, Zucker IH, Wang W. Cardiac sympathetic afferent stimulation augments the arterial chemoreceptor reflex in normal rats. Under anesthesia, renal sympathetic nerve activity (RSNA) and mean arterial pressure (MAP) were recorded. The chemoreceptor reflex was tested by unilateral intra-carotid artery bolus injection of potassium cyanide (KCN) and nicotine. We found that 1) left ventricular epicardial application of capsaicin increased the pressor responses and the RSNA responses to chemoreflex activation induced by both KCN and nicotine; 2) when the central end of the left cardiac sympathetic nerve was electrically stimulated, both the pressor and the RSNA responses to chemoreflex activation induced by KCN were increased; 3) pretreatment with intracerebroventricular injection of losartan (500 nmol) completely prevented the enhanced chemoreflex activation induced by KCN (34); and 4) bilateral microinjection of losartan (250 pmol) into the nucleus tractus solitarii (NTS) completely abolished the enhanced chemoreceptor reflex by epicardial application of capsaicin. These results suggest that both the chemical and electrical stimulation of the CSAR augments chemoreceptor reflex and that central ANG II, specially located in the NTS, plays a major role in these reflex interactions.

renal sympathetic nerve activity; mean arterial pressure; cardiac sympathetic afferent reflex; AT1 receptor

Several studies have shown that the chronic heart failure (CHF) is characterized by elevated sympathetic tone and depressed cardiac vagal tone, which have been shown to be associated with progression of the disease and a poor prognosis (5, 6, 8). Although the precise mechanisms responsible for this phenomenon are not completely established, the roles of enhanced peripheral chemoreflex function (29), augmented cardiac sympathetic afferent reflex (CSAR) (34), and depressed arterial baroreflex control (19) are of importance, among which the augmented CSAR might play an initial and essential role in the observed sympathetic overactivity in CHF because of the failing heart.

The chemoreflexes play an important role in regulation of sympathetic tone in both physiological and pathophysiological states. The peripheral chemoreceptors of the carotid bodies respond primarily to hypoxemia and arterial carbon dioxide tension (Paco2) and the central chemoreceptors of the brain stem respond to hypercapnia; the activation of both elicits the hyperventilation and sympathetic activation. Studies in animals (28, 29) and humans (25) have suggested the augmented peripheral chemoreflexes and the enhanced oxygen sensitivity in the carotid body in CHF, which might contribute to the sympathetic activation in this disease. However, the mechanism(s) underlying the enhanced arterial baroreceptor reflex in CHF remains unclear. An antagonistic interaction between the peripheral chemoreflex and the arterial baroreflex has been reported in animals and healthy subjects (18, 24, 27). The blunted baroreceptor response in CHF may result in a loss of this inhibitory interaction, leading to a further increase in excitatory activity of the peripheral chemoreceptors. However, there are no data regarding the association between the CSAR and the peripheral chemoreflex sensitivity in the CHF state.

Cardiac chambers have afferent connections to the brain stem and to the spinal cord. Cardiac vagal afferents mediate depressor responses and are activated by volume expansion, increased myocardial contractility, and the atrial natriuretic factor and other metabolic productions (1). Cardiac sympathetic afferents, on the contrary, are known to activate the cardiovascular system, leading to increases in blood pressure, HR, and myocardial contractile function (22), and they are activated by metabolic mediators, myocardial ischemia, and cardiac enlargement (2, 31, 32). In a previous study, our laboratory showed that, in the CHF state, the CSAR is enhanced (34), probably due to relative myocardial ischemia, ventricular dilatation, and increased metabolic products in this disease state. In a recent study, our laboratory found that cardiac sympathetic afferent stimulation inhibits the arterial baroreceptor reflex in normal rats (10) and that the enhanced CSAR in rats with CHF played an important role in the depression of arterial baroreceptor reflex function in this disease (9). In addition, evidence also indicated that the inputs from both carotid chemoreceptor and cardiac sympathetic afferents were terminated in the nucleus tractus solitarii (NTS) (17, 30), which is a well-known integrator and mediator from the peripheral to the central. We therefore hypothesize that cardiac sympathetic afferent stimulation enhances the arterial chemoreceptor reflex, and the NTS probably is the critical central site for the interaction of the two reflexes. The purpose of this study was to determine the effect of cardiac sympathetic afferent stimulation on arterial chemoreceptor reflex in normal...
rats and whether the NTS is involved in this interaction. In addition, based on our laboratory's previous work, we believe that central activation of the ANG II type 1 (AT₁) receptor mediates this effect (35). Thus this aspect of the CSAR-chemoreflex interaction was also examined.

METHODS

Male Sprague-Dawley rats weighing between 310 and 400 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 intraperitoneally administered at one-tenth of the initial dose per hour. Body temperature was maintained using a heating pad. A midline incision in the neck was made, and the trachea was cannulated. Animals were artificially ventilated using a mechanical respirator (model 683, Harvard Apparatus; tidal volume: 2.5 ml; frequency: 60 breaths/min) throughout the experiment by inhalation of an air-O₂ mixture. Through a midline incision in the neck, the right common carotid artery was exposed and the peripheral end catheterized for measurement of mean arterial pressure (MAP) and heart rate (HR). The central end of the right common carotid artery was also with a polyethylene catheter for chemoreceptor reflex stimulation.

Recording of renal sympathetic nerve activity. The left kidney, renal artery, and renal nerves were exposed through a left retroperitoneal flank incision. The renal sympathetic nerves were identified, dissected free of the surrounding connective tissue, and 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 (Wacker Sil-Gel). The signal was amplified with a Grass direct-current preamplifier (model P18D, Astro-Med, West Warwick, RI) with low-frequency cutoff set at 30–100 Hz and high-frequency cutoff at 1–3 kHz. The amplified discharge was monitored on a storage oscilloscope (model 121 N, Astro-Med, West Warwick, RI) with low-frequency cutoff set at 30–100 Hz and high-frequency cutoff at 1–3 kHz. The amplified signal was imported to a computer with other parameters. A voltage integrator (model 1801, Buxco 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 on a PowerLab data-acquisition system (model 16S, AD Instruments, Mountain View, CA) and stored on disk until analyzed.

Epicardial application of capsaicin. The chest was opened through the fourth intercostals space. The pericardium was removed to expose the left ventricle. Filter paper (3 × 3 mm) containing capsaicin (0.04 and 0.4 µg in 2 µl) was applied to the epicardial surface of the anterior surface of the left ventricle. Capsaicin was applied for ~2 min until the end of chemoreflex test, and then the filter paper was removed and the epicardium was rinsed three times with 10 ml of warm (38°C) normal saline. The MAP, HR, and RSNA responses of CSAR were determined by averaging the values from the 5-s period during the application of capsaicin.

Electrical stimulation of cardiac sympathetic afferents. The chest was opened through the left second intercostal space. The left stellate ganglion was identified. The branch innervating the heart was tied and cut as distal as possible. A pair of stainless steel stimulation electrodes was placed on the central end of this nerve. The stimulus (7 V, 1 ms, 20 Hz) was delivered with a square-wave stimulator (Grass S88, Astro-Med) and a stimulus isolation unit.

Microinjection of losartan into right cerebral ventricle (intracerebroventricular) and NTS. The rats were placed in a stereotaxic instrument (Stoelting, Chicago, IL), and the skull was exposed through an incision on the midline of the scalp. For intracerebroventricular (icv) injection, a cannula (outer diameter 0.5 mm and inner diameter 0.1 mm) connected to a microsyringe (model 7001, Hamilton, Reno, NV) was implanted into the right cerebral ventricle. The coordinates were determined from the Paxinos and Watson rat atlas (23), which is 0.8 mm posterior, 1.4 mm lateral to the bregma, and 3.8 mm ventral to the zero level. For NTS injection, the dorsal surface of medulla was exposed by removing the atlanto-occipital membrane and a portion of the occipital bone. Coordinates of the NTS ranged from 0.3 to 0.5 mm rostral to the calamus scriptorius, 0.5 mm lateral to midline, and below 0.5 mm from the dorsal surface of the medulla. A total of 100 nl volume of NTS microinjection for each side was made over 15-s period. The time interval between bilateral microinjections was within 60-s period.

At the end of experiments, the cannula tip placement and NTS injection site were confirmed by microinjection of fast green dye (1 µl for icv injection, 100 nl for NTS microinjection), and then the rats were euthanized with an overdose of anesthetic (pentobarbital sodium 100 mg/kg iv). The brains were removed from the skulls, placed in 10% formalin, and sectioned to view the microinjection sites.

Chemoreflex test. In the present study, the chemoreflex was activated by unilateral intracarotid artery bolus injections of potassium cyanide (KCN) (5, 10, and 20 µg in 100 µl) and nicotine (0.1, 1, and 10 µg in 100 µl). Dose-response curves for the changes in MAP and RSNA were obtained. These experiments were performed on the same animals before and after denervation of the carotid bifurcation according to the method described by Franchini and Krieger (7). The apex of the MAP and RSNA was used as the chemoreflex response values.

Statistical analysis. All values are expressed as means ± SE. Data were analyzed with a paired t-test when comparing effects of capsaicin, electrical stimulation, and losartan in each group. A one-way analysis of variance followed with the Newman-Keuls test for post hoc analysis was used when multiple comparisons were made. A value of P < 0.05 was considered statistically significant.

RESULTS

Activation of CSAR by chemical and electrical stimulation of cardiac sympathetic afferents. Table 1 shows the baseline values of MAP, HR, and RSNA responses of CSAR were determined by averaging the values from the 5-s period during the application of capsaicin.
MAP, HR, and RSNA, and the effects of chemical and electrical stimulation of cardiac sympathetic afferents on them in anesthetized normal rats. There are no significant differences in the baseline variables between the four groups. However, the chemical stimulation of cardiac sympathetic afferents induced a dose-dependent increase in all the three variables examined.

Effects of activation of carotid chemoreceptors on MAP and RSNA. Right intracarotid artery bolus injection of KCN produced a dose-dependent pressor response and an increase in RSNA, and the dose of 10.0 µg KCN is the initial threshold to induce the chemoreflex (Fig. 1). The cardiovascular and RSNA responses to KCN are exclusively due to the activation of carotid chemoreceptors, because denervation of the carotid sinus area completely abolished these responses.

Effects of epicardial application of capsaicin on cardiovascular and RSNA changes induced by activation of carotid chemoreceptors with KCN and nicotine. Two doses of capsaicin (0.04 and 0.4 µg) were applied to the epicardial surface to evoke the CSAR, and three doses of KCN (5, 10, and 20 µg) or nicotine (0.1, 1, and 10 µg) were administrated to activate carotid chemoreceptors in this experiment. The MAP and RSNA responses to KCN injection were measured after epicardial application of saline (control) or capsaicin. Figure 2 presents original representative recordings of changes in MAP and RSNA in response to right carotid artery bolus injection of KCN (10 µg in 100 µl) after left ventricular epicardial application of saline (left) or capsaicin (0.4 µg; right). The activation of carotid chemoreceptors with KCN produces a typical pressor response and an increase in RSNA. These responses were enhanced following epicardial administration of capsaicin compared with saline treatment. Figure 2 also shows the increased baseline MAP and RSNA evoked by left ventricular epicardial application of capsaicin (0.4 µg in 2 µl; right) compared with saline (left), indicating that the CSAR was activated by capsaicin. Figures 3 and 4 show average MAP and RSNA responses to KCN and nicotine injection after epicardial application of capsaicin. Capsaicin induced a dose-dependent...
increase in MAP and RSNA responses to both KCN and nicotine injection. Figure 5 shows the effects of bilateral microinjection of losartan (250 pmol) into the NTS on the enhanced arterial chemoreflex by CASR, which indicates a complete abolishment of the interaction between these two reflexes.

Effects of electrical stimulation of cardiac sympathetic afferents on cardiovascular and RSNA changes induced by activation of carotid chemoreceptors with KCN. Similar to capsaicin application, electrical stimulation of the central end of the left cardiac sympathetic afferents evoked significantly enhanced responses of MAP and RSNA to intracarotid KCN injection compared with the control (saline) group. On the other hand, icv pretreatment with losartan (500 nmol) normalized the effect of electrical afferent stimulation on the KCN response (Fig. 6). However, no significant differences in MAP and RSNA were observed after icv losartan pretreatment compared with baseline.

DISCUSSION

Chemoreflex, baroreflex, and CSAR are the three major cardiovascular reflexes to regulate the cardiovascular activity via their potent influences on autonomic outflow. However, the interaction among these reflexes is still unknown. Our laboratory’s recent study has demonstrated that the CSAR evoked by chemical and electrical stimulation of the cardiac sympathetic afferents blunts arterial baroreflex sensitivity in normal rats (10) and that this negative effect of CSAR activation also plays a critical role in the impaired arterial baroreflex function in CHF (9). Therefore, the purpose of the present study was to assess the effect of activation of the CSAR with chemical and electrical stimulation on arterial chemoreflex responses in normal rats. We found that, in anesthetized rats, both left ventricular epicardial application of capsaicin and electrical stimulation of the central end of the left cardiac sympathetic afferents enhanced the pressor responses and increased RSNA induced by right carotid artery bolus injection of KCN and nicotine. This confirmed that stimulation of the CSAR augmented the hemodynamic and sympathetic responses to arterial chemoreflex stimulation.

Our laboratory’s previous studies indicated that central ANG II plays an important role in the regulation of CSAR function (33). For example, chronic icv infusion of ANG II potentiates the CSAR via central AT1 receptors in normal animals (15), and icv injection of losartan significantly attenuates the enhanced central gain of the CSAR in dogs with CHF (16). In the present study, we also found that pretreatment with icv losartan...
prevented the enhancement of the peripheral chemoreflex MAP and RSNA responses by electrical stimulation of cardiac sympathetic afferents, indicating that the augmentation of the evoked CSAR on peripheral chemoreflex function is mediated by central AT1 receptors.

The exact central locations for the interactions of these two reflexes are uncertain. However, it seems reasonable to suggest that one potential site is the NTS. The medial and lateral commissural subnucleus of the NTS has been shown to be the primary site of termination of cardiovascular afferent fibers, receiving inputs from carotid chemoreceptors, arterial baroreceptors, and cardiopulmonary receptors (11, 17, 21). In addition, extracellular single-unit recording also indicates that stimulation of cardiac sympathetic afferents excites neurons in the NTS (30). In this study, we found that microinjection of losartan into the NTS completely abolished the epicardial application of capsaicin-induced augmentation of arterial chemoreflex MAP and RSNA responses to nicotine, which is consistent with our another finding in the present experiment obtained following icv administration of losartan. These results suggest the ANG II pathway in the NTS plays a critical role in the interaction between these two reflexes. Indeed, several pieces of evidence have demonstrated the important role of ANG II mechanisms in the NTS on regulation of cardiovascular reflexes. For example, bilateral microinjection of ANG II into the NTS significantly and dose-dependently suppressed the baroreceptor reflex responses, and on the other hand, blocking the endogenous activity of the ANG II by microinjection into the bilateral NTS of losartan elicited a significant enhancement of the baroreceptor reflex responses (14). These findings were supported by other reports in anesthetized rats (3), conscious rats (20), and 10- to 20-day-old rats (12). In the most recent study, Chen et al. (4) nicely demonstrated an involvement of ANG II pathway within NTS in the cardiovascular regulation by the in vivo small-interference RNA-mediated AT1 receptor gene silence technique. More interestingly, in a rabbit model of acute myocardial ischemia (MI), Rosario et al. (26) found that activation of carotid chemoreflex elicited a greater increase of blood pressure and bradycardia after MI and that this was partially reversed by losartan microinjection into the NTS after MI. The same enhancement of cardiovascular carotid chemoreflex was also observed after administration of capsaicin on the ventricular surface but not after procainamide, which provides a powerful support to our present finding.

It is now well accepted that both the CSAR (34) and the arterial chemoreflex is augmented (29) in CHF. Therefore, it will be interesting and important to test whether the augmented
CSAR contributes to the enhancement in arterial chemoreflex in this disease state. This hypothesis is important to investigate in future studies.

As indicated by Korner (13), the central interactive processes induced by cardiovascular regulatory reflex inputs include summation and integration, both of which might be involved in the present study. For instance, the increase in MAP evoked by 0.4 μg of epicardial capsaicin was ~12 mmHg and the intracarotid injection of 10 μg of KCN evoked an increase in MAP of around 14 mmHg, the algebraic sum of which is 26 mmHg. The combination of these two reflexes produced a 29-mmHg increase of MAP, a value very close to the algebraic sum of the two reflexes. On the other hand, the RSNA responses appear to be more complex. A dose of 0.4 μg of capsaicin evoked an increase in RSNA of 17%. Intracarotid 10 μg KCN increased RSNA by 58%. However, the combined effect of these two reflexes evoked an increase in RSNA of 163%, a value clearly higher than the simple sum of the two responses. These data may suggest a central integrative effect of the input from these two cardiovascular reflexes on sympathetic outflow.

In summary, the present results show that chemically and electrically stimulating the cardiac sympathetic afferent reflex enhances the chemoreflex-induced pressor and RSNA responses in anesthetized normal rats, and this effect is mediated by a central (spatially located in the NTS) ANG II mechanism. The present data provide new insights into the interaction between CSAR and chemoreflex. However, because of the acute observation for the CSAR and chemoreflex in the present experiment, it is not known whether the same relationship exists in such a chronic pathological condition as CHF. Moreover, we cannot rule out the involvement of other mechanisms than AT1 receptors of NTS in this interaction between CSAR and chemoreflex by this experiment. Because of the decrease in vagal activity in heart failure state, it is also worthy to determine whether the vagal activity plays a role in the enhanced arterial chemoreflex in heart failure state.

GRANTS

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