Differential roles for glutamate receptor subtypes within commissural NTS in cardiac-sympathetic reflex

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Li, De-pei, David B. Averill, and Hui-Lin Pan. Differential roles for glutamate receptor subtypes within commissural NTS in cardiac-sympathetic reflex. Am J Physiol Regulat Integrative Comp Physiol 281: R935–R943, 2001.—Ischemic stimulation of cardiac receptors evokes excitatory sympathetic reflexes. Although the nucleus of the solitary tract (NTS) is an important site for integration of visceral afferents, its involvement in the cardiac-sympathetic reflex remains to be fully defined. This study examined the role of glutamate receptor subtypes in the commissural NTS in the sympathetic responses to stimulation of cardiac receptors. Renal sympathetic nerve activity (RSNA) was recorded in anesthetized rats. Cardiac receptors were stimulated by epicardial application of bradykinin (BK; 10 μg/ml). Application of BK significantly increased the mean arterial pressure by 2.9 mmHg and augmented RSNA by 10.220.32. However, microinjection of 10 pmol of 6-cyano-7-nitroquinoxaline-2,3-dione, a non-N-methyl-D-aspartate (NMDA) antagonist, into the commissural NTS eliminated the pressor and RSNA responses to BK application in 10 rats. Thus this study suggests that non-NMDA, but not NMDA and glutamate metabotropic, receptors in the commissural NTS play an important role in the sympathoexcitatory reflex response to activation of cardiac receptors during myocardial ischemia.

ACTIVATION OF CARDIAC RECEPTORS induces chest pain and initiates excitatory cardiovascular reflexes in patients with myocardial ischemia (27, 34). Cardiac primary afferents running in the sympathetic nerve, especially finely myelinated Aδ- and unmyelinated C-fiber afferents, are considered to be the essential pathways for transmission of cardiac nociception to the central nervous system during myocardial ischemia (7, 27, 32). Axonal tracing studies have shown that cardiac sympathetic afferents project to the dorsal horn of the upper thoracic spinal cord through the stellate ganglia and the sympathetic chain (7, 19). Stimulation of cardiac afferents excites neurons in the spinal ascending pathways, such as those located in the spinohalamic tract (5). The vasomotor neurons in the rostral ventrolateral medulla (RVLM) provide critical excitatory output to the preganglionic sympathetic neurons (26, 48).

In a recent study, we showed that stimulation of cardiac receptors with bradykinin (BK), an important ischemic metabolite (31), excites barosensitive neurons in the RVLM through cardiac sympathetic afferent pathways (21). However, it remains uncertain how the sensory input from cardiac sympathetic afferents is processed in the supraspinal sites.

The nucleus of the solitary tract (NTS) is an important synaptic station of the cardiopulmonary vagal afferents in the central nervous system and plays a key role in the modulation of the autonomic efferent activity to the cardiovascular system (1, 47, 50). The carotid and aortic baroreceptors (39, 49, 50), carotid chemoreceptors (18, 43), and cardiopulmonary vagal afferents (8, 47) make their first synapse in the NTS. Stimulation of NTS neurons typically modulates sympathetic outflow through excitation of neurons in the caudal ventrolateral medulla, which, in turn, inhibits vasomotor neurons in the RVLM (1, 17, 25). On the other hand, the ascending fibers from the dorsal horn of the cervical and thoracic spinal cord also terminate in the NTS (28). It has been suggested that this spinal-NTS pathway may play a role in certain excitatory somatomotor and viscerovisceral reflexes (20, 28). The direct sympathoexcitatory pathway from the NTS to the RVLM has been documented by electrophysiology and neuroanatomic techniques (2, 14, 18, 36, 42). In this regard, microinjection of L-glutamate into the NTS produces an increase in blood pressure, which is mediated by the RVLM (42). Also, stimulation of the NTS at the level of the obex (e.g., the commissural NTS) evokes monosynaptic excitatory postsynaptic potential on the RVLM neurons projecting to the spinal cord (14). Fur-
thermore, it has been shown that a portion of the NTS efferents directly projects to the RVLM (2, 36). The functional role of this NTS-RVLM pathway has been studied in sympathoexcitatory reflexes evoked by stimulation of carotid chemoreceptors (15, 43). For example, blockade of N-methyl-D-aspartate (NMDA) and non-NMDA receptors within the commissural NTS abolishes the chemoreflex response evoked by stimulation of the carotid chemoreceptors (43). Thus the NTS, especially its commissural subnucleus, may mediate the excitatory cardiac-sympathetic reflex (10, 43). Although activation of cardiac sympathetic afferents is known to excite RVLM vasomotor neurons and the sympathetic nervous system (21, 24, 30, 32, 41), the role of the commissural NTS and neurotransmitters utilized in this region in the cardiovascular reflex originating from the heart has not been studied. Therefore, in the present study, we tested a hypothesis that glutamate receptors in the commissural NTS mediate the sympathoexcitatory responses to stimulation of cardiac receptors.

METHODS

Surgical Preparations and Procedures

Experiments were performed on male Sprague-Dawley rats (280–320 g; Harlan Industry, Indianapolis, IN). The experimental procedures and protocols were approved by the Institutional Animal Care and Use Committee and adhered to the Guide for the Care and Use of Laboratory Animals (US Public Health Service). Rats were anesthetized initially with halothane in an induction chamber. The femoral vein was cannulated, and the rats were ventilated artificially with 100% O2 by an appropriate level of anesthesia. The trachea was cannulated, and chloralose (20–25 mg/kg iv) were administered to maintain adequate depth of anesthesia was verified by the absence of responses to noxious pinch of the paw. Supplemental doses of α-chloralose (20–25 mg/kg iv) were administered to maintain an appropriate level of anesthesia. The trachea was cannulated, and the rats were ventilated artificially with 100% O2 using a rodent ventilator (model SAR-830/A, IITC/Life Science Instruments, Woodland Hills, CA). The left carotid artery was cannulated, and the arterial blood pressure was measured with a pressure transducer (model PT300, Grass Instruments, Quarry, MA). After an appropriate level of anesthesia was ensured, the rats were paralyzed with pancuronium bromide (1 mg/kg iv) during renal nerve recordings. The adequacy of the anesthesia level during neuromuscular blockade was judged by the stability of the arterial blood pressure and was tested after paralysis wore off and before the next dose of pancuronium was given. Lidocaine (2%) was infiltrated around the surgical wound to minimize the nociceptive afferent input after surgery (21). A limited left lateral thoracotomy was performed to expose the heart. Body temperature was maintained at 37–38°C with a heating lamp. At the end of the experiments, animals were killed by an intravenous injection of an overdose of pentobarbital sodium.

The head of the rat was fixed on a stereotaxic frame (David-Kopf Instruments, Tujunga, CA), and the dorsal surface of the medulla was exposed surgically through the atlantooccipital membrane. The microinjection pipette (tip diameter 20 μm) was placed into the commissural NTS through a hydraulic microdrive (Stoelting, Wood Dale, IL) under direct vision with the use of a surgical microscope (model M900, D. F. Vasconcellos, Sào Paulo, Brazil). Target stereotaxic coordinates for the commissural NTS were 0.5 mm caudal and 0.5 mm lateral to the caudal tip of the area postrema (calamus scriptorius) and 0.5 mm ventral from the dorsal surface of the medulla (15, 43). Drugs were ejected bilaterally from the pipette in a volume of 50 nl over a 2-s period by application of controlled pulses of pressurized air to the pipette using a microinjection system (Picospritzer II, General Valve, Fairfield, NJ), as described in detail previously (12).

The left kidney was exposed through a left flank incision via a retroperitoneal approach. A small branch of the renal nerve was isolated and carefully dissected free from the renal vessels with the aid of an operating microscope. The renal nerve filament was placed on a stainless steel electrode, and the nerve signal was amplified (20,000–30,000) and filtered (100–3,000 Hz) by an alternating-current amplifier (model P511, Grass Instruments). Renal sympathetic nerve activity (RSNA) was then monitored through an audioamplifier (model AM9, Grass Instruments) and displayed on an oscilloscope (model DSO 450, Gould, Essex, UK). The RSNA and blood pressure were simultaneously monitored and recorded on a thermal-sensitive recorder (model K2G, Astro-Med, West Warwick, RI). In addition, the RSNA was fed into a Pentium computer through an analog-to-digital interface card for subsequent off-line quantitative analysis. Discharge frequency was quantified using a software window discriminator (DataWave Technology, Longmont, CO). The quality of the RSNA was assessed by its pulse synchronous rhythmicity and by examination of the magnitude of decrease in RSNA in response to sinoaortic baroreceptor loading induced by intravenous injection of phenylephrine (1–4 μg). The renal nerve was cut distally to the recording electrode to ensure that the afferent nerve activity was not recorded. The accuracy of the threshold level of the window discriminator was verified by the baroreflex and again at the end of each experiment after the animal was killed. Respective noise levels were subtracted from the nerve recording data before percentage changes from baseline were calculated.

Experimental Protocols

Effect of epicardial application of BK on blood pressure and RSNA. The responses of blood pressure and RSNA to epicardial application of BK were studied in 12 rats. After a good-quality recording of RSNA was obtained, a stabilization period of ≥30 min was allowed. A 60-s control period of nerve activity and blood pressure was recorded. Then, BK (10 μg/ml; Sigma Chemical, St. Louis, MO), dissolved in normal saline, was applied to the anterior surface of the heart with a cotton applicator through an exposed window on the left chest (21, 41). After BK application, blood pressure and RSNA were monitored for 10 min. After each application, the heart and pericardial sac were flushed twice using cotton-tipped applicators soaked with saline to eliminate any residual BK. Responses of blood pressure and RSNA to application of BK were repeated twice, each separated by 15–20 min, to allow the discharge activity of the renal nerve and the blood pressure to return to the control levels. In the same animals, the pressor and RSNA responses to epicardial application of BK were also examined before and 15 min after microinjection of 50 nl of artificial cerebrospinal fluid (CSF; in mM: 124 NaCl, 3 KCl, 1.3 MgSO4, 2.4 CaCl2, 1.4 NaH2PO4, 10 glucose, and 26 NaHCO3, pH 7.4) into the commissural NTS. The artificial CSF was used for preparing different glutamate...
receptor antagonists (see below) and was delivered in the same manner as drug injection and served as vehicle control. Effects of blockade of glutamate receptors within NTS on pressor and RSNA responses to epicardial application of BK. The effects of microinjection of 6-cyano-7-nitroquinolinic acid-2,3-dione disodium (CNQX), an antagonist for non-NMDA receptors, (±)-2-amino-5-phosphonopentanoic acid (AP5), an antagonist for NMDA receptors, or α-methyl-4-carboxyphe- 

nylglicine (MCPG), an antagonist for metabotropic recep-
tors, into the commissural NTS on the pressor and RSNA responses to epicardial application of BK were studied in three separate groups of rats. These antagonists were purchased from RBI (Natick, MA) and dissolved in artificial CSF. The initial sympathetic response to epicardial application of BK was examined twice, separated by 15 min, to ensure that the blood pressure and renal nerve responses were reproducible. Subsequently, the selected antagonist was injected into the commissural NTS bilaterally through a glass pipette in a volume of 50 nl over a 2-s period. The pressor and RSNA responses to epicardial application of BK were measured again 15 min after microinjection. The effective concentrations of these glutamate receptor antagonists have been determined in previous studies (12, 43). Additionally, because the effect of MCPG lasts for only 2–3 min after microinjection into the medial NTS (12), separate experiments were performed to examine the effect of 0.1–1 nmol of MCPG 2 min after microinjection into the commissural NTS in five rats.

**Histological localization of microinjection sites.** Accurate pipette location and spread of injection within the commis-
sural NTS were verified histologically. At the end of the experiment, 50 nl of 2% Chicago blue dye were ejected from the same microinjection pipette at the same site. After euthanasia, the brain stem was removed rapidly and fixed in 10% buffered formalin. Frozen 40-μm coronal sections were made on a freezing microtome, mounted on the slides, and stained with cresyl violet (Sigma Chemical). The dye spot and spread area were identified and plotted on standardized sections according to the atlas of Paxinos and Watson (33). Data were excluded if the microinjection site and spread were not localized in the commissural NTS.

**Data Analysis**

Values are means ± SE. The RSNA was averaged during control and the entire response period (usually 2.5 min) after BK application. RSNA data are expressed as percentage changes from control values because of the variability in baseline RSNA in each animal. Comparisons between the control and experimental interventions were made by the Student’s paired t-test or repeated-measures analysis of vari-
ance. Differences were considered to be statistically signifi-
cant when \( P < 0.05 \).

**RESULTS**

A total of 43 rats was used to study the effects of microinjection of glutamate antagonists into the com-
misural NTS on the sympathetic response to epicard-
dial application of BK. The resting mean arterial pres-
sure in all rats studied was 79.2 ± 2.5 mmHg, and the baseline heart rate was 342 ± 12 beats/min. Figure 1 shows the distribution of microinjection sites into the commissural NTS. The size of the dye spread area was 0.1–0.2 mm around the microinjection site. Four rats were excluded from the study because of misposition of the microinjection pipette. We observed that microin-
jection of CNQX into the site outside the commissural NTS in these four rats did not change the baseline blood pressure, RSNA, and responses to epicardial application of BK.

**Responses of RSNA and Blood Pressure to Epicardial Application of BK**

Epicardial application of BK (10 μg/ml) significantly increased the blood pressure and RSNA in 12 rats. The RSNA evoked by BK increased by 38.5 ± 2.5% (\( P < 0.05 \)) after a latency of ~10 s. Topical application of BK (10 μg/ml) also significantly increased the mean arterial blood pressure from 78.2 ± 2.2 to 97.5 ± 2.9 mmHg (\( P < 0.05 \)). Maximal pressor and RSNA responses were usually reached within 40 s and lasted for 2.5–3.0 min. Epicardial application of saline had no effect on the blood pressure and RSNA (data not shown). Microin-
jection of artificial CSF (50 nl) into the commissural NTS did not alter the pressor and RSNA responses to epicardial application of BK (Fig. 2).

**Effect of CNQX Microinjection Into the Commisural NTS on the Cardiac-Sympathetic Reflex**

The effect of CNQX microinjection into the commis-
sural NTS on the cardiac-sympathetic reflex was studied in 10 rats. Figure 3 is a representative trace showing the effect of microinjection of CNQX, the non-NMDA-receptor antagonist, on the pressor and RSNA responses to epicardial application of BK. Before CNQX microinjection, topical application of BK (10 μg/ml) significantly increased RSNA and blood pres-
sure (Figs. 3 and 4). Microinjection of CNQX (10 pmol) into the commissural NTS increased the baseline discharge activity of RSNA (18.4 ± 2.4%) 3–4 min after microinjection. Pressor and RSNA responses to epicard-
dial application of BK were abolished 15 min after CNQX injection (Figs. 3 and 4). At 30–45 min after CNQX injection, the responses of RSNA (from 102 ± 3.2 to 135 ± 2.7%, \( P < 0.05 \)) and blood pressure (from 79.2 ± 1.9 to 98.3 ± 2.9 mmHg, \( P < 0.05 \)) to BK application were restored to the preinjection levels (Figs. 3 and 4).

**Effect of Injection of AP5 Into the Commisural NTS on the Cardiac-Sympathetic Reflex**

Neither the pressor nor the RSNA response to epi-
cardial application of BK was affected significantly by microinjection of 100 pmol of AP5 into the commis-
sural NTS. Figure 5 summarizes the responses of blood pres-
sure and RSNA to epicardial application of BK in eight animals before and 15 min after AP5 (100 pmol) microinjection into the commissural NTS. Furthermore, microinjection of a higher concentration of AP5 (1 nmol) into the commissural NTS also failed to alter significantly the responses of RSNA and blood pressure to epicardial application of BK in the same animals (\( n = 8 \)). Before injection of 1 nmol of AP5, BK application increased blood pressure from 79.1 ± 2.3 to 102.7 ± 2.5 mmHg and excited RSNA by 37.9 ± 2.5%. After AP5 injection, blood pressure increased from
79.5 ± 2.2 to 101.6 ± 2.0 mmHg, and RSNA was augmented by 38.8 ± 2.3% in response to epicardial BK application.

Effect of Microinjection of MCPG Into the Commissural NTS on the Cardiac-Sympathetic Reflex

The pressor and RSNA responses to stimulation of cardiac receptors with BK were not altered 2 min after microinjection of 100 pmol of MCPG into the commissural NTS (Fig. 6; n = 5). Microinjection of 1 nmol of MCPG into the commissural NTS also failed to attenuate significantly the responses of RSNA and blood pressure to epicardial application of BK in the same animals (n = 5). Before microinjection of 1 nmol of MCPG, BK application increased blood pressure from 77.8 ± 2.5 to 104.5 ± 2.1 mmHg and excited RSNA by 35.8 ± 2.5%. After MCPG injection, blood pressure increased from 78.3 ± 1.9 to 103.9 ± 2.2 mmHg, and RSNA was augmented by 36.9 ± 2.1% in response to epicardial BK application. In eight additional animals, BK-elicited pressor and RSNA responses were assessed 15 min after MCPG (100 pmol) was injected into
the commissural NTS. The pressor and RSNA responses to epicardial application of BK were not attenuated 15 min after MCPG injection into the commissural NTS (n = 8).

DISCUSSION

The brain stem nuclei are important for the fine tuning of cardiovascular control, including cardiac-sympathetic reflexes (2, 18, 21, 36, 46, 49). The present study focused on the functional role of the commissural NTS in the sympathoexcitatory response initiated by stimulation of cardiac receptors. We found that microinjection of a non-NMDA-receptor antagonist, CNQX, into the commissural NTS eliminated the excitatory responses of RSNA and blood pressure to activation of cardiac receptors. However, these cardiac-sympathetic responses were little affected by microinjection of AP5, an NMDA-receptor antagonist, or MCPG, a glutamate metabotropic receptor antagonist, into the commissural NTS. Therefore, this study provides new information that non-NMDA glutamate receptors in the commissural NTS play an important role in the excitatory cardiac-sympathetic reflex.

Fig. 2. Repeatability of the responses of renal sympathetic nerve activity (RSNA) and mean arterial blood pressure (MAP) to epicardial application of bradykinin (BK) before and after microinjection of artificial cerebrospinal fluid (CSF) into the ComNTS. Values are means ± SE (n = 12). *P < 0.05 compared with respective controls.

Fig. 3. Original traces showing responses of blood pressure (BP) and RSNA to epicardial application of BK before (A) and 15 and 30 min after (B and C, respectively) microinjection of 10 pmol of 6-cyano-7-nitroquinoxaline-2,3-dione disodium (CNQX) into the ComNTS in 1 rat.
pressor responses to intrapericardial BK in rats. Also, we recently showed that the vagal afferent pathway is not important for the response of RVLM barosensitive neurons to epicardial application of BK (21). It is difficult to reconcile the differences in the above-mentioned studies in the role of cardiac vagal afferents in the cardiac-sympathetic reflex. Future studies are needed to delineate the sites and mechanisms of possible interaction between cardiac vagal and sympathetic afferents in various brain stem nuclei. Because epicardial application of BK induced profound and consistent sympathetic and pressor responses in our preparations, we did not propose to study the interaction between cardiac vagal and sympathetic afferents. It is unlikely that attenuation of RSNA by glutamate antagonist microinjection into the commissural NTS is due to an enhancement of the cardiac vagal input. The NTS has long been known for its role in integration of sensory input from baroreceptors and visceral afferents traveling in the vagal nerve (39, 47, 49). The sympathoinhibitory pathways of the baroreflex and the Bezold-Jarisch reflex involve an excitatory projection from the NTS to the caudal ventrolateral medulla and an inhibitory projection from the caudal ventrolateral medulla to the RVLM (1, 17, 25). However, activation of the peripheral chemoreceptors also excites the sympathetic system, possibly through a direct or indirect pathway from the NTS to the RVLM (14, 16, 42). Using neuroanatomy and electrophysiology techniques, a different approach is needed to study the interaction between cardiac vagal and sympathetic afferents.

**Fig. 4.** Responses of RSNA and BP to epicardial application of BK before and 15 min after CNQX microinjection into the ComNTS. Values are means ± SE (n = 10). *P < 0.05 compared with the respective baseline controls. #P < 0.05 compared with BK-evoked response obtained before CNQX.

**Fig. 5.** Responses of RSNA and BP to epicardial application of BK before and 15 min after (±)-2-amino-5-phosphonopentanoic acid (AP5) microinjection into the ComNTS. Values are means ± SE (n = 8). *P < 0.05 compared with respective controls. NS, not significantly different from initial response.
rect pathway from the NTS to the RVLM neurons projecting to the thoracic spinal cord has been substantiated (2, 18, 36). Also, electrical stimulation of neurons in the NTS at the obex level excites RVLM descending neurons to the thoracic spinal cord (14). Several anatomic and physiological studies have indicated that the commissural NTS plays a unique role in the excitatory cardiovascular reflex (10, 15, 42, 43). For example, microinjection of L-glutamate into the commissural NTS produces a pressor response (9, 22). Electrolytic lesion of the commissural NTS eliminates the pressor response induced by microinjection of L-glutamate into the medial NTS (10). In addition, the excitatory chemoreflex is blocked by glutamate receptor antagonists injected into the commissural NTS (43, 50). Thus it is likely that the commissural NTS is also involved in processing ascending cardiac afferents from the spinal dorsal horns. However, the involvement of the commissural NTS in the sympathoexcitatory response induced by activation of cardiac receptors has not been studied previously.

The cardiac sympathetic afferents are known to project to the deep dorsal horn of the upper thoracic spinal cord (19). The supraspinal sites involved in integration of sensory input from cardiac sympathetic afferents are poorly understood. Although many spinal dorsal horn neurons project to the thalamus and hypothalamus (3, 6, 13), a direct spinal dorsal horn-NTS projection pathway also has been demonstrated (28). Furthermore, electrical stimulation of thestellate ganglia is capable of activating some neurons in the NTS (40). L-Glutamate is a major excitatory neurotransmitter in the central nervous system and is released in the NTS during stimulation of the peripheral chemoreceptors (29). In the present study, we determined the functional significance of this spinal-NTS pathway and the role of glutamate receptors in the excitatory cardiac-sympathetic reflex. We found that microinjection ofCNQX into the commissural NTS abolished the sympathetic responses to stimulation of cardiac receptors. These data indicate that non-NMDA receptors in the commissural NTS are important for the integration of cardiac sympathetic afferents.

In the present study, microinjection of an NMDA-receptor antagonist, AP5, or a metabotropic glutamate receptor antagonist, MCPG, into the commissural NTS did not significantly alter the cardiac-sympathetic reflex. These results suggest that NMDA and metabotropic glutamate receptor subtypes are less important than non-NMDA receptors in the commissural NTS in mediating this cardiogenic sympathetic reflex. Because microinjection of a higher concentration of AP5 or MCPG did not significantly alter the cardiac-sympathetic reflex, it is unlikely that the lack of actions of AP5 and MCPG in the commissural NTS was due to inadequate blockade of these two types of glutamate receptors. Our findings are consistent with several studies on the role of glutamate receptors in the NTS in other types of cardiovascular reflexes (15, 47, 49). The pressor response induced by intravenous injection of potassium cyanide is not affected by microinjection of AP5 into the NTS, indicating that the sympathoexcitatory effect is not mediated by NMDA receptors (15). Non-NMDA receptors have been shown to play a major role in the synaptic transmission of baroreceptor afferents in the NTS (49). Although metabotropic receptors can be activated by exogenous L-glutamate injected into the medial NTS, blockade of the metabotropic glutamate receptors with MCPG alone does not attenuate the cardiovascular responses to glutamate injection (12). We observed that neither the pressor nor the RSNA response to stimulation of cardiac receptors was affected by microinjection of MCPG. In fact, we found that microinjection of a metabotropic glutamate receptor agonist, trans-15,3R-aminocyclopentane-1,3-dicarboxylic acid (12), into the commissural NTS in rats also had no effect on the baseline blood pressure and RSNA (data not shown). It is important to recognize that MCPG does not block all metabotropic glutamate recep-

**Fig. 6.** Responses of RSNA and BP to epicardial application of BK before and 2 min after microinjection of 100 pmol of α-methyl-4-carboxyphenylglycine (MCPG) into the ComNTS. Values are means ± SE (n = 5). *P < 0.05 compared with respective controls. NS, not significantly different from initial response.
tors have been identified, and they can be categorized into three groups on the basis of their amino acid sequence identity (11). Although groups I and II of metabotropic glutamate receptors can be antagonized by MCPG, no antagonist blocks all types of metabotropic glutamate receptors. However, there is no compelling evidence to implicate a third type of metabotropic glutamate receptor in this reflex response, because CNQX abolished completely the pressor and sympathetic activation elicited by epicardial BK. Furthermore, when we did examine other possibilities (NMDA and the groups I and II of metabotropic glutamate receptors), we did not uncover a contribution to this reflex response. Microinjection of CNQX into the commissural NTS increased the baseline RSNA. Although the injection site and the dye spread area are within the commissural NTS, this effect still may be due to a spread of the drug solution to other regions of the NTS involved in baroreflex control. Furthermore, the medial and commissural NTS are involved in processing sensory input from peripheral baroreceptors (39), chemoreceptors (43, 50), and cardiopulmonary vagal afferents (47). It is likely that the commissural NTS is not a homogenous structure, and non-NMDA receptors in this region are also important for the baroreflex input (47, 49). Thus, alternatively, an increase in the sympathetic basal tone after CNQX injection could be the result of blockade of non-NMDA receptors and attenuation of sensory input from the baroreceptors and vagal afferents in the commissural NTS. In summary, we found in the present study that the commissural NTS is involved in integration of the cardiac-sympathetic reflex. Activation of non-NMDA, but not NMDA and glutamate metabotropic, receptors in the commissural NTS is important for the excitatory sympathetic reflex originating from the heart. Thus these data provide new information for our understanding of the regulatory mechanisms in the commissural NTS in the cardiovascular reflex occurring during myocardial ischemia.

**Perspectives**

Stimulation of cardiac sympathetic afferents during myocardial ischemia is known to elicit an increased sympathetic outflow. Our understanding of the supraspinal mechanisms in the integration of cardiac afferent input and generation of sympathetic output is incomplete. The NTS has a heterogeneous population of neurons and contains neurons that are involved in multiple afferent pathways. The present study demonstrates an important role of the commissural NTS in the sympathoexcitatory response to activation of cardiac receptors. However, the involvement of other subnuclei in the NTS in the cardiac-sympathetic reflex remains unclear. Stimulation of cardiac receptors with BK can excite the RVLN barosensitive neurons (21). Thus it is likely that cardiac afferent input is relayed by the commissural NTS neurons, which may provide excitatory synaptic inputs to the RVLN. In this regard, the response patterns of neurons located in the commissural NTS and other subnuclei in the NTS to stimulation of cardiac sympathetic afferents require further studies. Also, the direct functional link between the commissural NTS and RVLN in the cardiac-sympathetic reflex response needs to be established in future studies.

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