Cardiovascular Responses to Hypothalamic Arcuate Nucleus Stimulation in the Rat
Role of Sympathetic and Vagal Efferents

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Abstract—Experiments were carried out in urethane-anesthetized, artificially ventilated, adult male Wistar rats. Microinjections (50 nL) of N-methyl-D-aspartic acid (1, 5, and 10 mmol/L), but not artificial cerebrospinal fluid, into the hypothalamic arcuate nucleus (ARCN) elicited increases in mean arterial pressure (5.7±0.5, 13.2±1.4, and 17.3±1.1 mm Hg, respectively) and heart rate (24.3±4.3, 49.3±5.2, and 75.2±8.0 bpm, respectively). ARCN stimulation was accomplished by microinjections of a maximally effective concentration of N-methyl-D-aspartic acid (10 mmol/L). The tachycardic responses to the ARCN stimulation were significantly attenuated after bilateral vagotomy. Intrathecal injections of ionotropic glutamate receptor (iGLUR) antagonists completely blocked pressor responses to the ARCN stimulation, whereas the tachycardic responses were significantly attenuated but not abolished. Intrathecal injections of iGLUR antagonists at T9 to T10, combined with bilateral vagotomy, completely blocked the tachycardic responses to ARCN stimulation. ARCN stimulation with N-methyl-D-aspartic acid elicited increased activities of the greater splanchnic nerve (91.7±14.8%) and the renal nerve (109.3±13%). Intrathecal injections of iGLURs at T9 to T10 blocked the increase in the greater splanchnic nerve activity in response to ARCN stimulation. These results indicate the following: (1) the chemical stimulation of the ARCN elicits increases in mean arterial pressure, greater splanchnic nerve and renal nerve activity, and heart rate; (2) the increases in mean arterial pressure and sympathetic nerve activity are mediated via the activation of spinal cord iGLURs; and (3) the increases in heart rate are mediated via the activation of spinal cord iGLURs and decreases in vagal input to the heart. (Hypertension. 2009;54:1369-1375.)

Key Words: blood pressure ■ heart rate ■ intrathecal injection ■ microinjection ■ N-methyl-D-aspartic acid ■ sympathetic nerve activity

The hypothalamic arcuate nucleus (ARCN) is located bilaterally at the base of the third ventricle. Direct projections from the ARCN to the intermediolateral cell column of the spinal cord (IML), rostral ventrolateral medullary pressor area (RVLM), nucleus tractus solitarius, dorsal motor nucleus of the vagus, parabrachial nucleus, raphe nuclei, periaqueductal gray, and hypothalamic paraventricular nucleus (PVN) have been identified.1–3 These reports suggest that the ARCN may be involved in the central regulation of cardiovascular function. There are very few studies in which the ARCN has been stimulated chemically to evaluate its role in cardiovascular function. There are very few studies in which the ARCN has been stimulated chemically to evaluate its role in cardiovascular or sympathetic nerve regulation.4–6 Therefore, a systematic study was carried out to investigate the cardiovascular effects of ARCN stimulation.

Methods

General Procedures
Experiments were done in adult male Wistar rats (Charles River Laboratories, Wilmington, MA) weighing 300 to 360 g (n=91). All of the animals were housed under controlled conditions with a 12:12-hour light-dark cycle. Food and water were available to the animals ad libitum. The experiments were performed according to the National Institutes of Health Guide for the Care and Use of Laboratory Animals and with the approval of the institutional animal care and use committee of the university.

The general procedures have been described in detail elsewhere.7 Briefly, the rats were anesthetized with urethane (1.2 to 1.4 g/kg; injected IV in divided doses). The absence of a pressor response and/or withdrawal of the limb in response to pinching of a hind paw indicated that the rats were properly anesthetized. The rats were artificially ventilated, and end-tidal CO₂ was maintained at 3.5% to 4.5%. Rectal temperature was maintained at 37.0±0.5°C. Blood pressure and heart rate (HR) were recorded by standard techniques.

Vagotomy
Silk sutures were placed loosely around the vagus nerves bilaterally for subsequent identification and sectioning of the nerves.

Microinjections Into the ARCN
Details of microinjection procedure are mentioned elsewhere.7 The coordinates for the ARCN were as follows: 3.6 to 3.8 mm caudal to the bregma, 0.1 to 0.2 mm lateral to the midline, and 9.8 to 10.2 mm deep from the dura. The same coordinates for the ARCN were used.
for all of the experiments unless indicated otherwise. The volume and duration of all of the microinjections into the ARCN were 50 nL and 5 to 10 seconds, respectively.

**Intrathecal Injections**
Details of this procedure are mentioned elsewhere. Combined solutions of the ionotropic glutamate receptor (iGLUR) antagonists (α-2-amino-7-phosphonooctane acid [D-AP7] and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzoquinoxaline-2,3-dione [NBQX] disodium salt) were injected intrathecally at T9 to T10 (volume: 20 μL; duration: 10 seconds). Intrathecal injections (20 μL) of artificial cerebrospinal fluid (aCSF) were used as controls. The location of the cannula tip was confirmed by postmortem examination under an operating microscope.

**Application of Drugs at T1 to T4**
Details of this procedure are mentioned elsewhere. The responses to ARCN stimulation were studied before and after the application of a tissue paper pledge soaked in combined iGLUR antagonist solution (20 μL) to the spinal cord surface at T1 to T4. The spinal cord surface was irrigated with aCSF after the application of drugs and a fresh aCSF-soaked paper pledge was applied.

**Nerve Recording**
The greater splanchnic and left renal nerves were exposed retroperitoneally in separate experiments, sectioned distally, and whole nerve activity was recorded from the central end of each nerve by standard techniques. At the end of the experiment, the nerves were sectioned centrally, and the remaining activity was considered to be the noise level.

**Histology**
Details of this procedure are mentioned elsewhere. The microinjection sites in the ARCN were marked by diluted India ink (50 nL) at the level from 3.60 to 3.80 mm caudal to the bregma (n = 10) and identified using a standard atlas.

**Drugs and Chemicals**
Details of the procedures used and their sources are mentioned elsewhere. The following drugs and chemicals were used: N-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA), D-AP7, NBQX, muscimol, L-glutamate monosodium (L-Glu), L-phenylephrine hydrochloride (L-Phe), and 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzoquinoxaline-2,3-dione (NBQX) disodium salt. Injections of NMDA were selected to be 1, 5, and 10 mmol/L into the ARCN (3.6 caudal to bregma, 0.2 lateral to the midline, and 9.0 deep from the dura); increases in MAP (19.6 ± 3.8 mm Hg) and HR (110.0 ± 17.0 bpm) were observed. There was no significant difference (P > 0.05) between the responses induced by NMDA or AMPA (10 mmol/L each).

**Reproducibility of NMDA Responses in the ARCN**
The increases in MAP in response to 3 consecutive microinjections of NMDA (10 mmol/L) in the ARCN were 15.2 ± 3.2, 15.8 ± 2.9, and 16.2 ± 3.1 mm Hg, respectively, and the increases in HR were 68.0 ± 14.7, 68.0 ± 17.0, and 65.0 ± 16.2 bpm, respectively (n = 5; P < 0.05), indicating that NMDA microinjections at 20-minute intervals did not exhibit tachyphylaxis. Therefore, the interval between the microinjections of NMDA was selected to be ≥ 20 minutes in all of the experiments.

**Site Specificity of NMDA-Induced Responses**
The NMDA-induced responses were tested at different sites within the ARCN (n = 18). The coordinates in the rostrocaudal, mediolateral, and dorsoventral directions were with reference to the bregma, midline, and dura, respectively. The coordinates of the ARCN sites (in millimeters) were as follows: (1) first site, 2.3 caudal, 0.2 lateral, and 9.8 deep; (2) second site, 3.6 caudal, 0.2 lateral, and 10.0 deep; (3) third site, 4.16 caudal, 0.2 lateral, and 10.0 deep; and (4) fourth site, 4.16 caudal, 0.5 lateral, and 10.2 deep. Microinjections of NMDA (10 mmol/L) into the first, second, third, and fourth ARCN sites elicited increases in MAP (13.8 ± 2.2, 16.1 ± 2.0, 15.2 ± 2.4, and 12.6 ± 1.8 mm Hg, respectively) and HR (56.4 ± 5.2, 69.1 ± 16.8, 42.8 ± 4.6, and 33.0 ± 4.3 bpm, respectively). There was no significant difference (P > 0.05) among these responses at 4 different ARCN sites.

**Microinjections of NMDA (10 mmol/L) into the dorsomedial hypothalamic nucleus (DMH; 3.6 caudal to bregma, 0.2 lateral to the midline, and 9.0 deep from the dura) directly dorsal to our microinjection site in the ARCN elicited increases in MAP (10.0 ± 3.5 mm Hg) and HR (78.0 ± 13.9 bpm; n = 5). After an interval of 20 minutes, muscimol (0.5 mmol/L; 50 nL) was microinjected into the same site. Within 3 minutes, NMDA was again microinjected at the same site; pressor and tachycardic responses to NMDA were abolished. Three minutes later, the micropipette was lowered to the same site; and microinjections (50 nL) of NMDA (10 mmol/L) were made into the ARCN (3.6 caudal to bregma, 0.2 lateral to the midline, and 10.0 deep from the dura); increases in MAP (19.6 ± 3.8 mm Hg) and HR (83.0 ± 6.6 bpm) were observed. The increases in MAP and other groups of rats. The onset and duration of cardiovascular responses to microinjections of NMDA (10 mmol/L) were 6.2 ± 0.8 seconds and 10.4 ± 1.0 minutes, respectively. The peak effect was observed at 46.6 ± 4.7 seconds. In this and other series of experiments, microinjections of aCSF (pH 7.4) alone into the ARCN did not elicit significant changes in MAP (0.8 ± 0.5 mm Hg) and HR (1.6 ± 1.0 bpm).

Microinjections (50 nL) of AMPA (10 mmol/L; n = 6) and L-Glu (100 mmol/L; n = 6) into the ARCN also elicited increases in MAP (16.3 ± 1.7 and 3.5 ± 1.1 mm Hg, respectively) and HR (74.1 ± 15.7 and 13.3 ± 2.1 bpm, respectively). There were no significant differences (P > 0.05) between the responses induced by NMDA or AMPA (10 mmol/L each). On the other hand, the responses to L-Glu (100 mmol/L) were significantly smaller (P < 0.01) when compared with those elicited by NMDA or AMPA (10 mmol/L each).

An expanded Methods section is available in the online Data Supplement at http://hyper.ahajournals.org.
HR induced by unilateral microinjections of NMDA into the ARCN after the inhibition of DMH were not significantly different from the increases in MAP and HR (16.1 ± 2.0 mm Hg and 69.1 ± 16.8 bpm, respectively) observed in other experiments in which DMH was not inhibited by microinjections of muscimol.

Effect of Vagotomy on the ARCN Stimulation
The initial increases in MAP and HR to microinjections of NMDA (10 mmol/L) into the ARCN were 23.4 ± 1.6 mm Hg and 61.0 ± 9.2 bpm, respectively (n = 5). Twenty minutes later, bilateral vagotomy was performed. After a 30-minute stabilization period, NMDA was again microinjected into the same ARCN site. Bilateral vagotomy did not significantly affect the increases in MAP (24.4 ± 1.4 mm Hg) induced by microinjection of NMDA into the ARCN. On the other hand, the tachycardic responses (24.0 ± 4.3 bpm) to the ARCN stimulation were significantly attenuated (P < 0.01), but not abolished, after bilateral vagotomy. The baseline MAP (83.0 ± 4.2 mm Hg) was not significantly (P > 0.05) altered by bilateral vagotomy. However, the baseline HR (462.0 ± 11.4 bpm) after bilateral vagotomy was significantly greater (P < 0.05) compared with the initial value (422.0 ± 23.3 bpm). Adding the change in HR to the baseline HR in the pre vagotomy (422.6 ± 483 bpm) and post vagotomy (462.24 ± 486 bpm) cases yielded nearly identical values for the peak HR responses, suggesting a “ceiling” effect.

Responses to ARCN Stimulation: Role of Spinal iGLURs
Initial increases in MAP and HR in response to microinjections of NMDA (10 mmol/L) into the ARCN were 22.6 ± 3.2 mm Hg and 82.0 ± 14.2 bpm, respectively (n = 5). Twenty minutes later, a combined solution of iGLUR antagonists (2 mmol/L of NBQX and 5 mmol/L of D-AP7; each 10 μL) was injected intrathecally at T9 to T10. After the intrathecal injections of the iGLUR antagonists, there was no pressor response to ARCN stimulation. The tachycardic responses (23.0 ± 4.0 bpm) to the ARCN stimulation were also significantly attenuated (71.8 ± 4.4% reduction; P < 0.01) but not abolished after intrathecal injection of NBQX and D-AP7 at T9 to T10. The baseline MAP and HR (61.2 ± 2.5 mm Hg and 340.0 ± 9.6 bpm, respectively) after intrathecal injection of NBQX and D-AP7 at T9 to T10 were significantly smaller (P < 0.01) compared with the corresponding initial values (81.4 ± 3.5 mm Hg and 380.0 ± 8.9 bpm, respectively). Two hours after intrathecal injection of NBQX and D-AP7 at T9 to T10, the increases in MAP (16.4 ± 2.2 mm Hg) and HR (52.0 ± 9.6 bpm) in response to NMDA-induced stimulation of the ARCN were not significantly different from the initial response.

The role of spinal iGLURs in the upper thoracic spinal cord in mediating the cardiovascular responses to microinjections of NMDA into ARCN was studied in another group of rats (n = 4). The spinal cord was exposed at the C8 to T5 level for direct application of drugs. The stimulation of the ARCN by microinjection of NMDA (10 mmol/L) elicited increases in MAP (25.2 ± 3.7 mm Hg) and HR (75.0 ± 8.4 bpm). After 20 minutes, a pledget of tissue paper soaked in combined solution of NBQX (2 mmol/L; 10 μL) and D-AP7 (5 mmol/L; 10 μL) was applied to the surface of the spinal cord at the T1 to T4 level. Ten minutes later, the pressor responses (24.7 ± 3.9 mm Hg) to the ARCN stimulation were not altered significantly (P > 0.05) after direct application of NBQX and D-AP7 at T1 to T4. On the other hand, the tachycardic responses (31.2 ± 3.1 bpm) to the ARCN stimulation were significantly attenuated (58.3 ± 1.6%; P < 0.01), but not abolished, after the application of NBQX and D-AP7 at T1 to T4. The reductions in tachycardic responses to NMDA-induced stimulation of the ARCN after intrathecal injections of iGLUR antagonists at T9 to T10 (71.8 ± 4.4%) and direct application of iGLUR antagonists at T1 to T4 (58.3 ± 1.6%) were not statistically different (P > 0.05). One hour after the application of NBQX and D-AP7 at T1 to T4, the NMDA-induced increases in HR elicited from the ARCN (65.0 ± 5.0 bpm) were not significantly different (P > 0.05) from the initial increases. The baseline HR (537.5 ± 24.1 bpm) after the application of NBQX and D-AP7 at T1 to T4 was significantly smaller (P < 0.01) compared with the corresponding initial value (381.2 ± 25.7 bpm, respectively). However, the baseline MAP (73.7 ± 1.9 mm Hg) after the application of NBQX and D-AP7 at T1 to T4 was not significantly different (P > 0.05) from the initial value (80.5 ± 3.6 mm Hg).

Responses to ARCN Stimulation: Role of the Vagus and Spinal iGLURs
Initial stimulation of the ARCN by microinjection of NMDA (10 mmol/L) elicited the expected increases in MAP (23.6 ± 2.1 mm Hg) and HR (79.0 ± 11.0 bpm; n = 5). After 20 minutes, bilateral vagotomy was performed; an increase in baseline HR (52.0 ± 7.3 bpm) was observed. After an interval of 30 minutes, NMDA was again microinjected into the same ARCN site. The pressor responses (24.4 ± 2.6 mm Hg) to the ARCN stimulation were not altered significantly (P > 0.05) after bilateral vagotomy, whereas the tachycardic responses (32.0 ± 7.3 bpm) were significantly (P < 0.01) attenuated. Twenty minutes after NMDA-induced stimulation of the ARCN, combined solution of NBQX (2 mmol/L) and D-AP7 (5 mmol/L) was injected intrathecally at T9 to T10. Ten minutes later, the pressor (1.6 ± 0.7 mm Hg) and tachycardic (5.6 ± 1.6 bpm) responses to the ARCN stimulation were nearly completely blocked after combined vagotomy and intrathecal injections of the iGLUR antagonists at T9 to T10. The baseline MAP after combined vagotomy and intrathecal injections of the iGLUR antagonists at T9 to T10 (61.4 ± 2.9 mm Hg) was significantly smaller (P < 0.01) compared with the initial value (104.8 ± 2.6 mm Hg). However, the baseline HR (391.0 ± 8.7 bpm) after combined vagotomy and intrathecal injections of the iGLUR antagonists at T9 to T10 was similar to the initial value (413.0 ± 9.6 bpm). Two hours after bilateral vagotomy and intrathecal injections of the iGLUR antagonists at T9 to T10, the pressor responses (20.6 ± 1.1 mm Hg) to the NMDA-induced ARCN stimulation were not significantly different from the pressor responses before the bilateral vagotomy and intrathecal injections of the iGLUR antagonists at T9 to T10, and the tachycardic responses (32.6 ± 6.0 bpm) recovered to the level comparable to the values at 30 minutes after vagotomy. A typical tracing depicting the effect of the combination of...
bilateral vagotomy and intrathecal injections of the iGLUR antagonists at T9 to T10 on the pressor and tachycardic responses to the NMDA-induced ARCN stimulation is shown in Figure 1.

The effect of the combination of bilateral vagotomy and direct application of iGLUR antagonists at T1 to T4 on the cardiovascular responses to microinjections of NMDA into ARCN was also studied in another group of rats (n=4). The stimulation of the ARCN by microinjection of NMDA (10 mmol/L) elicited increases in MAP (24.0±1.8 mm Hg) and HR (86.2±20.5 bpm). Thirty minutes after bilateral vagotomy, a pledget of tissue paper soaked in a combined solution of NBQX (2 mmol/L; 10 µL) and D-AP7 (5 mmol/L; 10 µL) was applied to the surface of the spinal cord at the T1 to T4 level. Ten minutes later, the pressor responses (23.0±1.8 mm Hg) to the ARCN stimulation were not altered significantly (P>0.05). On the other hand, the tachycardic responses (5.5±1.6 bpm) to the ARCN stimula-

Figure 1. Combined bilateral vagotomy and intrathecal injections of iGLUR antagonists at T9 to T10 block responses to ARCN stimulation. Top trace, HR (bpm); middle trace, pulsatile arterial pressure (PAP; millimeters of mercury); bottom trace, MAP (millimeters of mercury). A, Microinjections (50 nL) of NMDA (10 mmol/L) into the ARCN elicited increases in HR, PAP, and MAP. B, Twenty minutes later, bilateral vagotomy increased the baseline HR. Within 30 minutes, an increase in HR elicited by ARCN stimulation was attenuated by vagotomy. C, Twenty minutes later, NBQX (2 mmol/L) and D-AP7 (5 mmol/L) were injected intrathecally (20 µL) at T9 to T10; the baseline HR, PAP, and MAP decreased and reached a nadir within 10 minutes. At this time, the responses to microinjections of NMDA were abolished. D, The increases in HR and MAP elicited by NMDA-induced stimulation of the ARCN showed partial and complete recovery, respectively, within 120 minutes.

Effect of the ARCN Stimulation on Sympathetic Nerve Activity

Typical tracings of a recording of efferent GSNA are shown in Figure 2. An IV bolus injection of PE (10 µg/kg) increased MAP, which, in turn, elicited reflex bradycardia and inhibition of efferent GSNA discharge (Figure 2A). Ten minutes later, when the effects of PE subsided, microinjection of NMDA (10 mmol/L) into the ARCN elicited an increase in the GSNA (Figure 2B). After 20 minutes, NBQX (2 mmol/L) and D-AP7 (5 mmol/L) were injected intrathecally at T9 to T10. The baseline GSNA decreased gradually and reached nadir within 10 minutes. At this time, microinjection of NMDA into the same ARCN site failed to elicit the increase in the GSNA (Figure 2C).

Figure 2. Blockade of GSNA elicited by ARCN stimulation. Top trace, HR (bpm); second trace, PAP (millimeters of mercury); third trace, MAP (millimeters of mercury); fourth trace, integrated GSNA (∫GSNA; µV/50 ms); and bottom trace, whole GSNA (microvolts). A, Reflex inhibition of GSNA elicited by pressor response induced by PE (10 µg/kg, IV) indicated that the GSNA was barosensitive. B, Ten minutes later, microinjection of NMDA (10 mmol/L) into the ARCN increased HR, PAP, MAP, integrated GSNA, and whole GSNA. C, Twenty minutes later, intrathecal injection (20 µL) of NBQX (2 mmol/L) and D-AP7 (5 mmol/L) at T9 to T10 decreased the baseline HR, PAP, MAP, integrated GSNA, and whole GSNA, which reached a nadir within 10 minutes. At this time, microinjection of NMDA failed to elicit an increase in PAP, MAP, integrated GSNA, and whole GSNA responses, whereas the increase in HR was attenuated.
Group data (n=5) for changes in GSNA were as follows. An IV bolus injection of PE (10 μg/kg) elicited a significant (P<0.01) decrease in the GSNA (88.3±3.9%). Microinjection of NMDA (10 mmol/L) into the ARCN elicited a significant increase (P<0.05) in the GSNA (91.7±14.8%). After intrathecal injections of iGLUR antagonists, NMDA-induced stimulation of the ARCN did not elicit a significant increase (4.7±2.0%).

The effect of ARCN stimulation on RSNA was also studied (n=5). Typical tracings of a recording of efferent RSNA are shown in Figure 3. An IV bolus injection of PE (10 μg/kg) elicited reflex inhibition of efferent RSNA (Figure 3A). Ten minutes later, when the effects of PE subsided, microinjection of NMDA (10 mmol/L) into the ARCN elicited an increase in the RSNA (Figure 3B). Group data (n=5) for this experiment were as follows: intravenous injection of PE elicited a significant (P<0.01) decrease (89.7±1.7%) and microinjection of NMDA into the ARCN elicited a significant increase (P<0.05) in the RSNA (109.3±13.0%). The changes in GSNA and RSNA refer to comparison with basal nerve activity.

Histology
A typical ARCN site marked with India ink (50 nL) is shown in Figure 4A. Figure 4B and 4C represent composite diagrams of marked ARCN sites. The sites were located 3.60 to 3.80 mm caudal to the bregma.

Discussion
The major findings of the present study were as follows: (1) microinjections of NMDA and AMPA into the ARCN elicited increases in MAP and HR; (2) microinjections of L-Glu into the ARCN elicited similar, but significantly smaller, responses; (3) microinjections of NMDA into the ARCN elicited increases in GSNA and RSNA; (4) the increases in HR elicited by microinjections of NMDA into the ARCN were mediated via both inhibition of vagal outflow and activation of sympathetic outflow to the heart; and (5) the sympathetic activation contributing to the pressor and tachycardic responses was mediated via spinal cord iGLURs.

Unilateral microinjections of NMDA into the ARCN elicited an increase in MAP and efferent GSNA. ARCN stimulation also increased RSNA, which is involved in the long-term regulation of blood pressure.11 The increase in HR in response to the NMDA-induced stimulation was also partly mediated via sympathetic activation, because direct application of iGLUR antagonists at T1 to T4 attenuated this response.
Microinjections of NMDA at different rostral-caudal and mediolateral levels of the ARCN elicited similar pressor and tachycardic responses. The diameter of the diffusion sphere of a 50-nL microinjection has been reported to be \( \approx 500 \mu m \). Therefore, it is likely that the microinjections were restricted to the ARCN. This was confirmed by microinjections of India ink into the ARCN. The possibility that NMDA injected into the ARCN may have spread to the DMH was excluded, because the pressor and tachycardic responses to ARCN stimulation remained unaltered after the injection of DMH by microinjections of muscimol. The concentrations of NMDA that elicited pressor and tachycardic responses when microinjected into the ARCN (ie, 10 mmol/L; 50 nL), did not elicit a response when injected IV, indicating that leakage of the drug, if any, from the microinjection site in the ARCN to the peripheral circulation was not responsible for the observed responses. Thus, the cardiovascular responses elicited from the ARCN were site specific.

Direct projections from ARCN to PVN, raphe nuclei, IML, and RVLM\(^4\),\(^{13,14}\) may mediate the increases in MAP and GSNA induced by chemical stimulation of the ARCN, because these responses were abolished after intrathecal injections of iGLUR antagonists at T9 to T10, which are likely to block iGLURs in the IML of thoracolumbar spinal cord. The concentrations of the iGLUR antagonists (D-AP7 and NBQX) used in this study were specific for blocking iGLURs on the basis of our previous studies.\(^8\)\(^-\)\(^{10}\) The role of projections of the ARCN to other brain areas (eg, nucleus tractus solitarius, dorsal motor nucleus of the vagus, parabrachial nucleus, and periaqueductal gray) in mediating these responses remains to be investigated.\(^3\)\(^,\)\(^4\)

Because bilateral vagotomy attenuated the increase in HR induced by the chemical stimulation of the ARCN, decrease in vagal input to the heart may have partly contributed to the tachycardia. The tachycardic responses to the microinjection of NMDA into the ARCN were also attenuated by direct application of iGLUR antagonists at T1 to T4. When the bilateral vagotomy was combined with direct application of iGLUR antagonists at T1 to T4, the tachycardic responses to the microinjection of NMDA into the ARCN were abolished. These results indicate that the tachycardic responses are mediated by both the inhibition of vagal outflow to the heart and the activation of spinal cord iGLURs.

NMDA concentrations used to stimulate the ARCN were generally higher than those needed for stimulating medullary cardiovascular areas. The reason for the necessity to use higher concentrations of NMDA, AMPA, and L-Glu to stimulate the ARCN may be that the ARCN neurons are tonically inhibited by \( \gamma \)-aminobutyric acid and NO.\(^{15,16}\) Another possibility is that microinjections of excitatory amino acid receptor agonists into the ARCN activated inhibitory projections emerging from the ARCN to different brain regions, which resulted in attenuation of the effects of excitatory projections from the ARCN to RVLM and IML. In this context, it may be noted that ARCN sends excitatory projections to the ventrolateral periaqueductal gray, which, in turn, sends inhibitory projections to the RVLM.\(^{14}\) ARCN is also known to project to the nucleus tractus solitarius,\(^2\)\(^,\)\(^3\) which, in turn, inhibits the RVLM via activation of the caudal ventrolateral medullary depressor area.\(^17\) However, after the microinjection of NMDA in the ARCN, the projections to the RVLM and IML predominated, and the net effects were pressor and tachycardic responses.

Pressor responses to chemical stimulation of the ARCN have been reported to be mediated via activation of vasopressinergic neurons in the PVN.\(^6\) In our study, the pressor responses to chemical stimulation of the ARCN were abolished by intrathecal injections of iGLUR antagonists at T9 to T10, suggesting that vasopressin release did not contribute to the pressor responses elicited from the ARCN. However, the contribution of the PVN to blood pressure and sympathetic nerve responses elicited by chemical stimulation of the ARCN cannot be excluded, because ARCN projects to the PVN,\(^3\) and chemical stimulation of the PVN elicits pressor and tachycardic responses via the iGLURs in the spinal cord and inhibition of vagal input to the heart.\(^8\)

The ARCN includes different populations of neurons containing diverse neuroactive substances, including pro-opiomelanocortin, cocaine- and amphetamine-regulated transcript, neuropeptide Y/agouti-related protein, \( \gamma \)-aminobutyric acid, and glutamate.\(^{15}\) Although the functions of all of the phenotypes of ARCN neurons have not been established, it is known that pro-opiomelanocortin and cocaine- and amphetamine-regulated transcript neurons are involved in catabolic activity (decrease in food intake and increase in energy expenditure, including increase in sympathetic nerve activity), whereas the neuropeptide Y/agouti-related protein neurons are involved in anabolic activities (increase in food intake and reduction in energy expenditure, including decrease in sympathetic nerve activity). Microinjections of NMDA are expected to excite most of the ARCN neurons regardless of the phenotype. However, it may be speculated that NMDA-induced activation of neurons involved in catabolic activity (pro-opiomelanocortin/cocaine- and amphetamine-regulated transcript neurons) may have predominated in view of our observation that NMDA-induced stimulation of ARCN increased GSNA, as well as RSNA.

Perspectives
The ARCN is located in close proximity to the median eminence, which has a high capillary density (1079/mm\(^2\)), high capillary blood flow (2433 \( \mu L/g \) per minute), and lacks a blood-brain barrier.\(^{18}\) This anatomic arrangement of the ARCN may favor the accessibility of the ARCN neurons to physiologically active substances circulating in the blood.\(^5\) To test this hypothesis, experiments need to be designed to study the alterations in blood pressure and sympathetic nerve responses to physiologically active circulating substances after the inhibition of ARCN neurons. The results of the present article provide the first step for designing future studies on the role of ARCN in mediating cardiovascular responses to circulating physiologically active substances.

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Cardiovascular Responses to Hypothalamic Arcuate Nucleus Stimulation in the Rat: Role of Sympathetic and Vagal Efferents

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Short title: Arcuate nucleus: cardiovascular function

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Methods

Microinjections into the ARCN
The rats were placed in a prone position in a stereotaxic instrument with bite bar 11 mm below the interaural line. A hole (8-10 mm in diameter) was drilled in the midline at the junction of the two parietal bones caudal to the bregma. The microinjections were made through this hole, on either side of the midline, using multi-barreled glass-micropipettes (tip size 20–40 μm). The coordinates for the ARCN were: 3.6-3.8 mm caudal to the bregma, 0.1-0.2 mm lateral to the midline, and 9.8-10.2 mm deep from the dura; the same coordinates for the ARCN were used for all experiments unless indicated otherwise. The volume and duration of all microinjections into the ARCN were 50 nl and 5-10 s, respectively. Microinjections of artificial cerebrospinal fluid (aCSF, pH 7.4) into the ARCN were used as controls.

Intrathecal injection
The atlanto-occipital membrane was incised, the tip of a polyethylene tubing (PE-10), connected to a Hamilton microsyringe and filled with a drug or aCSF, was inserted under the dura mater and advanced caudally 6 cm to T9-T10 level. Combined solutions of the iGLUR antagonists (D-AP7 and NBQX) were injected intrathecally (volume 20 µl and duration 10 s). Intrathecal injections (20 µl) of aCSF were used as controls.

Application of drugs at T1-T4
The rats were placed in a prone position in a stereotaxic instrument. The dorsal surface of the spinal cord from C8 to T5 level was exposed by laminectomy and the dura was sectioned. The responses to ARCN stimulation were studied before and after the application of a tissue paper pledget soaked in combined iGLUR antagonist solution (20 µl) to the spinal cord surface at T1-T4. The spinal cord surface was irrigated with aCSF after the application of drugs and a fresh aCSF-soaked paper pledget was applied.

Nerve recording
The greater splanchnic and left renal nerves were exposed retroperitoneally in separate experiments, sectioned distally and whole nerve activity was recorded from the central end of each nerve by standard techniques. At the end of the experiment, the nerves were sectioned centrally and the remaining activity was considered to be the noise level.

Histology
The microinjection sites in the ARCN were marked by diluted India ink (50 nl) at the level 3.60-3.80 mm caudal to the bregma (n = 10). The animals were perfused and fixed with 4% paraformaldehyde, serial sections of the hypothalamus were cut (30 µm) and stained with cresyl violet. The microinjection sites were identified using a standard atlas.
Drugs and chemicals
The following drugs and chemicals were used: N-methyl-D-aspartic acid (NMDA), (±)-α-amino-3-hydroxy-5-methyl-isoxazole-4-propionic acid hydrobromide (AMPA; non-NMDA receptor agonist), D(-)-2-amino-7-phosphono-heptanoic acid (D-AP7; NMDA receptor antagonist), 2,3-dioxo-6-nitro-1,2,3,4-tetrahydrobenzo-[f]quinoxaline-7-sulfonamide disodium (NBQX disodium salt; non-NMDA receptor antagonist), muscimol (GABA_A receptor agonist), L-glutamate monosodium (L-Glu), L-phenylephrine hydrochloride (PE), isoflurane, and urethane. All of the solutions for the microinjections and intrathecal injections were freshly prepared in aCSF (298 ± 2 mOsmol/kg; pH 7.4). Where applicable, the concentration of drugs refers to their salts. The sources of different drugs and chemicals were as follows: AMPA and D-AP7 (Tocris Cookson Inc., Ellisville, MO, USA) and isoflurane (Baxter Pharmaceutical Products, Deerfield, IL, USA). All other drugs and chemicals were obtained from Sigma Chemicals (St. Louis, MO, USA).

Statistical analyses
Mean and standard error of mean (SEM) were calculated for maximum changes in mean arterial pressure (MAP) and HR. Comparisons of maximum changes in MAP and HR in different groups of rats were made by one-way analysis of variance (ANOVA) followed by Tukey-Kramer’s multiple comparison. In experiments testing for tachyphylaxis and effect of spinal cord iGLUR block or combined vagotomy and spinal cord iGLUR block, comparisons of the maximum changes in MAP and HR elicited by microinjection of NMDA into the ARCN were made by repeated measures ANOVA followed by Tukey-Kramer’s multiple comparison test. Student’s paired t-test was used in all other statistical analyses. For the analysis of nerve activity, control value represented the average amplitude of integrated greater splanchnic nerve activity (GSNA) and renal nerve activity (RSNA) during 35 sec period before the i.v. administration of PE or the microinjections of drugs into the ARCN. Maximum change in GSNA and RSNA amplitude, induced by i.v. administration of PE or microinjections of drugs into the ARCN, was expressed as percent change from the control value of GSNA and RSNA amplitude. The mean values of the integrated nerve signals were compared using Student’s paired t-test. In all cases, the differences were considered significant at P < 0.05.

References