Tonic γ-Aminobutyric Acid–ergic Activity in the Hypothalamic Arcuate Nucleus Is Attenuated in the Spontaneously Hypertensive Rat

Tetsuya Kawabe, Kazumi Kawabe, Hreday N. Sapru

Abstract—We tested the hypothesis that tonic γ-aminobutyric acid–ergic activity in the hypothalamic arcuate nucleus (ARCN) modulates blood pressure control and attenuation of this inhibitory activity contributes to hypertension in the spontaneously hypertensive rats (SHR). Mean arterial pressure (MAP), heart rate (HR), and greater splanchnic nerve activity (GSNA) were recorded in urethane-anesthetized, artificially ventilated, adult male SHR and Wistar-Kyoto rats (WKY). Microinjections of gabazine into the ARCN elicited significantly smaller increases in MAP, HR, and GSNA in baroreceptor-intact SHR compared with baroreceptor-intact WKY. Attenuation of the responses to gabazine in SHR persisted, despite lowering of their baseline MAP to levels of WKY or barodenervation. Microinjections of N-methyl-D-aspartic acid (NMDA) into the ARCN elicited decreases in MAP and GSNA and increases in HR in baroreceptor-intact WKY. However, after microinjections of gabazine into the ARCN, microinjections of NMDA into the same nucleus elicited pressor responses in baroreceptor-intact WKY. In barodenervated WKY, increases in MAP and GSNA were elicited by ARCN stimulation by NMDA and the increases in HR were exaggerated. In baroreceptor-intact SHR, ARCN stimulation by NMDA elicited increases in MAP, GSNA, and HR which persisted, despite lowering of baseline MAP or barodenervation. Increases in MAP and GSNA elicited by ARCN stimulation by NMDA in barodenervated SHR were significantly greater than corresponding increases in barodenervated WKY. These results indicated that attenuated γ-aminobutyric acid–ergic activity in the ARCN and impaired baroreflex function may contribute to increases in blood pressure and sympathetic nerve activity after ARCN stimulation by NMDA and elevation of baseline blood pressure in SHR. (Hypertension. 2013;62:281-287.) ● Online Data Supplement

Key Words: baroreflex • blood pressure • heart rate • hypertension • sympathetic nervous system

We have reported that microinjections of N-methyl-D-aspartic acid (NMDA) into the hypothalamic arcuate nucleus (ARCN) in baroreceptor-intact (baro-intact) Wistar rats elicited decreases in mean arterial pressure (MAP) and sympathetic nerve activity (SNA) whereas the heart rate (HR) increased.1 The depressor responses in baro-intact Wistar rats were mediated via the γ-aminobutyric acid type A (GABA_A) and neuropeptide Y1 (NPY1) and opiate receptors in the hypothalamic paraventricular nucleus (PVN).1 However, in barodenervated Wistar rats, similar microinjections of NMDA into the ARCN elicited increases in MAP, SNA, and HR, indicating that interruption of baroreceptor afferents converts depressor responses elicited from the ARCN to pressor responses.2 Increases in MAP elicited from the ARCN in barodenervated Wistar rats were mediated via melanocortin 3/4 receptors and ionotropic glutamate receptors in the PVN.2 On the basis of this observation, it was hypothesized that microinjection of NMDA into the ARCN may elicit increases in MAP, SNA, and HR in the spontaneously hypertensive rat (SHR) because baroreceptor function has been reported to be attenuated in SHR.3 Furthermore, it was hypothesized that GABAergic activity in the ARCN may normally play a role in the modulation of blood pressure (BP) control and attenuation of this inhibitory activity may contribute to increase in baseline BP in SHR. Consistent with these hypotheses, microinjections of gabazine (a GABA_A receptor antagonist) into the ARCN of Wistar rats elicited increases in MAP, greater splanchnic nerve activity (GSNA), and HR revealing a tonic GABAergic activity in this nucleus.4 SHR is a commonly used model for studying various aspects of hypertension. There are no studies on the role of ARCN in cardiovascular regulation in SHR.

Methods

An expanded Methods section is available in the online-only Data Supplement.

General Procedures

Adult male, 14-week-old, Wistar-Kyoto rats (WKY) and age- and sex-matched SHR (Charles River Laboratories, Wilmington, MA) were used in this study.

The details of all procedures used in this study have been published in our previous reports.1,2 The rats were anesthetized by intravenous administration of pentobarbital (i.v.) in urethane-anesthetized, artificially ventilated, adult male SHR and Wistar-Kyoto rats (WKY). Microinjections of gabazine (a GABA_A receptor antagonist) into the ARCN of Wistar rats elicited increases in MAP, greater splanchnic nerve activity (GSNA), and HR revealing a tonic GABAergic activity in this nucleus.4 SHR is a commonly used model for studying various aspects of hypertension. There are no studies on the role of ARCN in cardiovascular regulation in SHR.

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injections of urethane (1.2–1.4 g/kg). The end-tidal CO₂ was maintained at 3.5% to 4.5%. BP and HR were recorded by standard techniques. Microinjections of NMDA and gabazine into the ARCN induced increase in respiratory movements (our unpublished observations). To avoid cardiovascular effects secondary to respiratory changes after ARCN stimulation, the rats were paralyzed with intravenous administration of pancuronium bromide (initial bolus injection of 1.2 mg/kg followed by 0.6 mg/kg bolus injections every 40 minutes). Proper depth of anesthesia was confirmed by the absence of a BP increase and withdrawal of the limb in response to pinching of a hind paw before the administration of pancuronium. Confirmation of depth of anesthesia was repeated before every injection of pancuronium.

**Microinjections Into the ARCN**
Anesthetized rats were fixed in a prone position in a stereotaxic instrument with bite bar 3.3 mm below the interaural line. The coordinates for the ARCN were 1.9 to 4.1 mm caudal to the bregma, 0.2 to 0.4 mm lateral to the midline, and 9.6 to 9.9 mm deep from the dura. All microinjections into the ARCN were unilateral unless indicated otherwise. The volume and duration of all microinjections were 20 nL and 5 seconds, respectively. Control microinjections consisted of artificial cerebrospinal fluid (pH 7.4). At the end of the experiment, the microinjection sites in the ARCN were marked by microinjections (20 nL) of diluted green retrobeads IX (1:50) and the sites were identified using a standard atlas.

**Nerve Recording**
The central end of the greater splanchnic nerve was desheathed and its electric activity was recorded by standard techniques. At the end of the experiment, the nerve was sectioned centrally and the remaining activity was considered to be the noise level, which was subtracted from the recorded nerve activity.

**Barodenervation**
The carotid sinus, aortic depressor, and recurrent laryngeal nerves were sectioned bilaterally.

**Drugs and Chemicals Used**
CGP52432 (GABA<sub>B</sub> receptor antagonist),<sup>6</sup> gabazine (GABA<sub>A</sub> receptor antagonist),<sup>6</sup> green retrobeads IX, isoflurane, NMDA, pancuronium bromide, t-phenylephrine hydrochloride, sodium nitroprusside (SNP), and urethane. The vendors of these substances are mentioned in the online-only Data Supplement.

**Statistical Analyses**
The statistical analyses for MAP, HR, and GSNA are described in detail in the online-only Data Supplement. Changes in GSNA were expressed as percentages because of variability in the nerve discharge between rats.

**Results**
Baseline values for MAP in urethane-anesthetized WKY (n=70) and SHR (n=79) were 96.8±1.7 and 132.9±1.5 mm Hg, respectively. Baseline values for HR in WKY and SHR were 388.8±7.1 and 403.8±4.7 bpm, respectively.

**Concentration-Response of Gabazine in the ARCN**
Figure 1 shows the log concentration–effect curves for gabazine (0.1–10 mmol/L) in baro-intact WKY and SHR. The values for MAP and HR responses are shown in Table 1. Pressor responses elicited by microinjections of gabazine (1–10 mmol/L) into the ARCN of SHR were significantly smaller than the corresponding responses in WKY (Figure 1A).

Tachycardic responses were elicited by gabazine in baro-intact WKY and SHR. In SHR, tachycardic responses elicited by gabazine (2–10 mmol/L) were significantly smaller when compared with the corresponding responses in WKY (Figure 1B).

Increases in GSNA elicited by gabazine (2 mmol/L) in baro-intact WKY and SHR are shown in Table 1 and Figure 2C; increases in GSNA were significantly smaller in SHR compared with WKY.

The onset, peak, and duration of responses to gabazine (2 mmol/L) in baro-intact WKY and SHR were 10 to 20 seconds, 5 to 10 minutes, and 25 to 35 minutes, respectively. Microinjections of artificial cerebrospinal fluid alone into the ARCN did not elicit any MAP or HR responses in each strain of rats.

In both baro-intact WKY and SHR, bilateral microinjections of gabazine (2 mmol/L) into the ARCN elicited pressor and tachycardic responses, which were significantly (P<0.05) greater than the corresponding responses elicited by unilateral microinjections of the same concentration of gabazine (Table 1). Bilateral microinjections of gabazine elicited significantly (P<0.01) smaller increases in MAP and HR in baro-intact SHR when compared with the corresponding responses in baro-intact WKY (Table 1).

**Gabazine Responses in the ARCN of SHR: Effect of Lowering Baseline MAP**
Pressor responses elicited by microinjections of gabazine (2 mmol/L) into the ARCN of baro-intact SHR did not change by lowering baseline MAP to the levels observed in baro-intact WKY (intravenous infusion of SNP was used to lower MAP in SHR; Table 1; Figure 2A). However, the pressor responses in baro-intact SHR, in which baseline MAP was lowered using SNP infusion, were significantly smaller than those...
in baro-intact WKY, despite the fact that baseline MAP was similar in the 2 groups of rats; baseline MAP was 97.1±3.4 in WKY and 94.8±3.1 mm Hg in baro-intact SHR with lowered baseline MAP.

Tachycardic responses elicited by gabazine (2 mmol/L) in baro-intact SHR did not change by lowering baseline MAP (Table 1; Figure 2B). However, the tachycardic responses in baro-intact SHR with lowered baseline MAP were significantly smaller than those in baro-intact WKY. Baseline HR in baro-intact SHR with lowered baseline MAP using SNP infusion was 414.0±27.1 bpm.

Similar results were obtained using microinjections of higher concentration of gabazine (4 mmol/L) into the ARCN of baro-intact SHR in which MAP was lowered by SNP infusion (Table 1; Figure 2D and 2E). In these experiments, baseline MAP in baro-intact SHR after SNP infusion (96.8±2.2 mm Hg) was not significantly different from baseline MAP in baro-intact WKY (94.6±3.0 mm Hg). Baseline HR in baro-intact SHR with lowered baseline MAP using SNP infusion was 419.4±20.7 bpm. The MAP or HR responses elicited by 4 mmol/L of gabazine in baro-intact SHR with lowered baseline MAP were not different from those elicited by 2 mmol/L of gabazine in baro-intact SHR with lowered baseline MAP.

**Microinjections of Gabazine into the ARCN: Effects of Barodenervation**

Bilateral barodenervation elicited increases in baseline MAP in WKY (50.8±3.1 mm Hg) and SHR (35.8±2.4 mm Hg), which returned to the levels before barodenervation within 60 minutes.

Pressor responses elicited by microinjections of gabazine (2 mmol/L) into the ARCN of barodenervated WKY and SHR are shown in Table 1 and Figure 2A. The pressor responses in barodenervated SHR were significantly smaller than those in barodenervated WKY.

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**Table 1. Responses to Microinjections of Gabazine into the ARCN**

<table>
<thead>
<tr>
<th>Conc.</th>
<th>n</th>
<th>WKY</th>
<th>SHR</th>
<th>WKY</th>
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<td>68.3±13.7</td>
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**Baro-intact rats: unilateral microinjections using SNP infusion**

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**Baro-intact rats: bilateral microinjections**

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<th>SHR</th>
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<td>6</td>
<td>49.3±3.8</td>
<td>28.5±1.3</td>
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</tbody>
</table>

**Barodenervated rats: unilateral microinjections**

<table>
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<th>SHR</th>
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<td>6</td>
<td>49.3±3.8</td>
<td>28.5±1.3</td>
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</table>

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The results of statistical analyses of these data are shown in Figures 1 and 2. Data are expressed as mean±SEM. ARCN indicates arcuate nucleus; Baro-intact, baroreceptor-intact; Conc, concentration (mmol/L); GSNA, greater splanchnic nerve activity; HR, heart rate; MAP, mean arterial pressure; SHR, spontaneously hypertensive rats; SNP, sodium nitroprusside (150–300 µg/kg per hour, IV); and WKY, Wistar-Kyoto rats.
than those in barodenervated WKY and significantly greater than those in baro-intact SHR. The pressor responses in barodenervated WKY were significantly greater than those in baro-intact WKY.

Bilateral barodenervation elicited increases in baseline HR; 62.3±5.9 bpm in WKY and 42.7±9.8 bpm in SHR. Tachycardic responses elicited by gabazine (2 mmol/L) in barodenervated SHR and WKY are shown in Table 1 and Figure 2B. The tachycardic responses in barodenervated SHR were significantly smaller than those in barodenervated WKY. The tachycardic responses in barodenervated WKY were significantly greater than those in baro-intact WKY. The tachycardic responses in barodenervated SHR were not different from those in baro-intact SHR.

Increases in GSNA elicited by gabazine (2 mmol/L) in barodenervated WKY and SHR are shown in Table 1 and Figure 2C. The increases in GSNA in barodenervated SHR were significantly smaller than those in barodenervated WKY. The increases in GSNA in barodenervated WKY were significantly greater than those in baro-intact WKY. The increases in GSNA in barodenervated SHR were not different from those in baro-intact SHR.

Similar results for MAP and HR responses were obtained using microinjections of higher concentration of gabazine (4 mmol/L) into the ARCN in barodenervated rats (Table 1; Figure 2D and 2E). The MAP or HR responses elicited by 4 mmol/L of gabazine in barodenervated WKY or SHR were not different from those elicited by 2 mmol/L of gabazine in barodenervated WKY or SHR.

Typical tracings of responses in HR, MAP, and GSNA elicited by microinjections of gabazine (2 mmol/L) into the ARCN of baro-intact and barodenervated WKY and SHR are shown in Figure 3.

Microinjections of a GABA<sub>B</sub> receptor antagonist (CGP52432) into the ARCN elicited increases in MAP and GSNA, but no change in HR; these responses were not significantly different between WKY and SHR (Table S1 in the online-only Data Supplement).

**Concentration-Response of NMDA in the ARCN**

In baro-intact WKY, depressor responses were elicited by microinjections of NMDA into the ARCN. However, pressor responses were elicited by similar microinjections in baro-intact SHR (Table 2). In each strain of rats, the amplitudes of MAP responses elicited by 10 mmol/L of NMDA were significantly greater than those elicited by 2.5 and 5 mmol/L of NMDA.

Microinjections of NMDA elicited tachycardia in baro-intact WKY and baro-intact SHR (Table 2). Tachycardic responses elicited by NMDA (10 mmol/L) in baro-intact SHR were significantly greater than those elicited by NMDA (10 mmol/L) in baro-intact WKY. In each strain of rats, tachycardic responses elicited by 10 mmol/L of NMDA were significantly greater than the responses to 2.5 mmol/L of NMDA. Therefore, 10 mmol/L of NMDA was selected for further studies.

Microinjections of NMDA (10 mmol/L) in baro-intact WKY and SHR elicited decreases and increases in GSNA, respectively (Table 2).

The onset, peak, and duration of responses to NMDA (10 mmol/L) in baro-intact WKY and SHR were 5 to 15 seconds, 1 to 4 minutes, and 15 to 20 minutes, respectively.

**Stimulation of the ARCN by NMDA in WKY: Effect of Gabazine**

In baro-intact WKY (n=5), microinjections of NMDA (10 mmol/L) elicited decreases in MAP (−14.8±0.9 mmHg). After 20 minutes, gabazine (2 mmol/L) microinjected into the same ARCN sites elicited increases in MAP (31.4±2.6 mmHg). At the peak responses of gabazine, microinjections of NMDA (10 mmol/L) into the same ARCN sites elicited increases in MAP (14.6±2.1 mmHg).

**Stimulation of the ARCN by NMDA in SHR: Effect of Lowering Baseline MAP**

In baro-intact SHR, baseline MAP was lowered to 100.7±3.8 mmHg, which was comparable with baseline MAP of baro-intact WKY (103.1±4.2 mmHg), by SNP infusion. The lowering of baseline MAP of baro-intact SHR did not alter MAP or HR responses to microinjections of NMDA (10 mmol/L) into the ARCN (Table 2). The tachycardic responses elicited by NMDA (10 mmol/L) in baro-intact SHR were significantly greater than those in baro-intact WKY (Table 2). Baseline HR in baro-intact SHR with lowered baseline MAP using SNP infusion was 416.8±19.9 bpm.

**Stimulation of the ARCN by NMDA: Effect of Barodenervation**

Pressor responses elicited by microinjections of NMDA (10 mmol/L) into the ARCN of different groups of barodenervated rats (SHR) elicited smaller increases in arterial pressure (MAP), and greater increases in heart rate (HR), mean arterial pressure (MAP), and greater splanchnic nerve activity (GSNA).
rats are shown in Table 2. The pressor responses in barodenervated SHR were significantly greater than those in barodenervated WKY and those in baro-intact SHR. The pressor responses in barodenervated WKY were significantly different from depressor responses elicited by NMDA (10 mmol/L) in baro-intact WKY.

Microinjections of NMDA into the ARCN of barodenervated WKY and SHR elicited tachycardic responses (Table 2). The tachycardic responses in barodenervated SHR were not different from those in barodenervated WKY or those in baro-intact SHR. Tachycardic responses in barodenervated WKY were significantly greater than those in baro-intact WKY.

Increases in GSNA were elicited by microinjections of NMDA in barodenervated WKY instead of decreases in GSNA. Increases in GSNA in barodenervated WKY and SHR were significantly greater than those in barodenervated WKY and those in baro-intact SHR (Table 2).

Typical tracings of responses in HR, MAP, and GSNA elicited by microinjections of NMDA (10 mmol/L) into the ARCN of baro-intact and barodenervated WKY and SHR are shown in Figure 4.

**Histology**

Typical microinjection sites are shown in Figure S1.

**Discussion**

Major findings in this study are as follows: (1) GABA<sub>α</sub> receptor blockade in the ARCN elicited increases in MAP, GSNA, and HR in WKY, as well as SHR but the increases in SHR were smaller, (2) microinjections of NMDA into the ARCN of baro-intact SHR elicited decreases in MAP, GSNA, and HR, whereas similar microinjections into the ARCN of baro-intact WKY elicited decreases in MAP and GSNA and increases in HR, (3) in baro-intact WKY, microinjections of NMDA into the ARCN elicited pressor responses after microinjections of gabazine into the same nucleus, and (4) in barodenervated SHR, microinjections of NMDA into the ARCN elicited increases in MAP and GSNA, which were exaggerated when compared with increases in MAP and GSNA elicited by NMDA in barodenervated WKY.

Tonic GABAergic activity was revealed in the ARCN of both WKY and SHR by microinjections of gabazine and CGP52432 into the ARCN. There was no difference in MAP and GSNA responses elicited by CGP52432 between WKY and SHR. Microinjections of gabazine into the ARCN elicited smaller increases in MAP, SNA, and HR in SHR than those in WKY, indicating that tonic GABAergic activity mediated via GABA<sub>α</sub> receptors in the ARCN was attenuated in the SHR. The mechanism by which attenuation of tonic GABAergic activity in the ARCN can contribute to increase in BP in SHR can be explained as follows. It is known that NPY/GABA and pro-opiomelanocortin/glutamate (POMC/Glu) neurons are present in the ARCN. Activation of POMC/Glu neurons in the ARCN elicits increases in MAP, SNA, and HR via activation of PVN neurons. POMC/Glu neurons in the ARCN may be tonically inhibited by NPY/GABA neurons located in the same nucleus. Attenuation of tonic GABAergic activity of NPY/GABA neurons in the ARCN may cause increase in activity of POMC/Glu neurons in the same nucleus. This conclusion is supported by our observation that pressor responses were elicited by microinjections of NMDA into the ARCN of baro-intact WKY after microinjections of gabazine into the same nucleus.

**Attenuation of the cardiovascular responses to microinjections of gabazine in the ARCN** was not a result of elevated baseline MAP in SHR compared with WKY because tonic GABAergic activity in the ARCN remained attenuated in SHR when the baseline MAP was lowered by infusions of SNP to the levels comparable with WKY.

Microinjections of NMDA into the ARCN elicited increases in MAP, GSNA, and HR in baro-intact SHR, whereas similar
Microinjections in baro-intact WKY elicited decreases in MAP and GSNA and increases in HR. These observations can be explained as follows. Activation of NPY/GABA and POMC/Glu neurons in the ARC elicits decreases and increases in MAP and SNA via the PVN, respectively. In WKY, microinjections of NMDA stimulate NPY/GABA and POMC/Glu neurons in the ARCN. Stimulation of NPY/GABA neuronal projections to the POMC/Glu neurons in the ARCN results in suppression of the activity of latter group of neurons allowing the activity of NPY/GABA neurons in the ARCN to predominate. Predominance of NPY/GABA neuronal activity elicits decreases in MAP and GSNA in WKY. However, in SHR, attenuation of GABAergic activity of NPY/GABA neurons in the ARCN results in the predominance of excitatory projections from the POMC/Glu neurons in the ARCN to the PVN.

As mentioned above, microinjections of NMDA into the ARCN of baro-intact WKY elicited increases in HR, whereas MAP and GSNA were decreased. This observation can be explained as follows. The mechanism of tachycardic responses elicited by the microinjections of NMDA may involve activation of sympathetic input and decreases in vagal input to the heart. However, vagal input to the heart is known to be more dominant in regulating HR. Therefore, the microinjections of NMDA in WKY may elicit increase in HR via decrease in the activity of vagal input to the heart while decrease in SNA mediated the decrease in MAP.

Baroreflex function has been reported to be attenuated in SHR. It may be argued that the differences of responses to microinjections of NMDA into the ARC between WKY and SHR are attributable to differences in baroreceptor function between the 2 strains of rats. However, this explanation is not likely to be true because increases in MAP and GSNA elicited by microinjections of NMDA in barodenervated SHR were greater than the increases in these variables in barodenervated WKY.

Bilateral barodenervation converted decreases in MAP and GSNA elicited by microinjections of NMDA into the ARCN of WKY to increases in MAP and GSNA. The mechanism of this observation can be explained as follows. Barodenervation abolishes excitatory inputs from baroreceptor afferents to the nucleus tractus solitarius neurons involved in baroreflex. Consequently, the activity of excitatory glutamatergic nucleus tractus solitarius neurons projecting to the caudal ventrolateral medullary depressor area decreases, the activity of inhibitory GABAergic inputs from the caudal ventrolateral medullary depressor area to the rostral ventrolateral medullary pressor area (RVLM) decreases, and sympahtetic RVLM neurons are disinhibited. Microinjections of NMDA into the ARCN stimulate both POMC/Glu and NPY/GABA neurons projecting to the PVN. RVLM is the major pathway via which cardiovascular responses are elicited from the PVN. When GABAergic inhibitory input from the caudal ventrolateral medullary depressor area to the RVLM is withdrawn by barodenervation, pressor responses elicited from the PVN predominate. Removal of inhibitory restraint on the RVLM neurons may also explain exaggeration of MAP, GSNA, and HR responses elicited by microinjections of gabazine into the ARCN of barodenervated WKY and exaggeration of pressor responses elicited by microinjections of NMDA and gabazine into the ARCN of barodenervated SHR.

Microinjections of NMDA and gabazine into the ARCN elicited exaggerated increases in MAP in barodenervated SHR. However, increases in MAP elicited by microinjections of NMDA and gabazine into the ARCN of baro-intact SHR were not altered by lowering of baseline BP using SNP infusions. This difference in responses may be explained by the degree of interruption of baroreceptor input by these 2 procedures; barodenervation completely abolishes the baroreceptor afferent input, whereas some baroreceptor afferent activity persists when baseline MAP is lowered in SHR. This explanation is supported by our observation that barodenervation elicited an increase in baseline HR (42.7±9.8 bpm), although the increase in baseline HR was relatively small (17.4±7.4 bpm) after lowering of MAP using SNP infusion in SHR.

Baroreflex is one of the established regulatory mechanisms of BP control and includes a GABAergic mechanism that tonically inhibits the sympathetic RVLM neurons. In this and other studies, we have identified tonic GABAergic activity in the ARC as another mechanism modulating BP control. Tonic GABAergic activity in the ARC may suppress activities of excitatory ARC neurons (probably POMC/Glu neurons). Both regulatory mechanisms are normal in normotensive rats. ARC stimulation by NMDA elicited decreases in BP and SNA in WKY. In SHR, baroreflex function is attenuated, which results in attenuation of GABAergic input to the presympathetic RVLM neurons. In this study, we report attenuation of tonic GABAergic activity in the ARC of SHR, which may explain increases in BP and SNA elicited by the ARC stimulation and increase in baseline BP in this strain of SHR.
rats. Collectively, our results indicated that GABAergic activity in the ARCN normally plays a role in the modulation of BP control and attenuation of this inhibitory activity contributes to hypertension in SHR.

ARCN plays an important role in food intake, energy expenditure, and cardiovascular regulation.\textsuperscript{1–7} Abnormal function of NPY neurons in the ARCN may result in obesity. NPY neurons in the ARCN are known to contain GABA.\textsuperscript{3} In this study, we observed attenuation of tonic GABAergic activity in the ARCN of SHR, which may contribute to hypertension in these rats. Both obesity and hypertension are risk factors included in the metabolic syndrome. Thus, attenuation of GABAergic activity in the ARCN may also contribute to diet-induced obesity hypertension and metabolic syndrome.

Perspectives

Previous reports from this and other laboratories have addressed the emerging role of ARCN in cardiovascular regulation in normotensive rats.\textsuperscript{1,2,7–9} This study extends the role of ARCN to regulation of cardiovascular function in SHR. The data presented provide a new platform for further studies on the role of ARCN in cardiovascular regulation in normal and disease states, such as hypertension, obesity, and metabolic syndrome.

Sources of Funding

This study was supported by National Institutes of Health grants HL024347 and HL076248 awarded to H.N. Sapru.

Disclosures

None.

References


Novelty and Significance

What Is New?

- γ-Aminobutyric acid type A receptor blockade in the arcuate nucleus (ARCN) elicited increases in mean arterial pressure (MAP), greater splanchnic nerve activity, and heart rate (HR) in Wistar-Kyoto rats, as well as spontaneously hypertensive rats, but the increases in spontaneously hypertensive rats were smaller.
- ARCN stimulation by N-methyl-D-aspartic acid (NMDA) elicited increases in MAP and sympathetic nerve activity in baro-intact spontaneously hypertensive rats, whereas this stimulation elicited decreases in MAP and sympathetic nerve activity in baro-intact Wistar-Kyoto rats.
- In barodenervated Wistar-Kyoto rats, increases in MAP and sympathetic nerve activity were elicited by ARCN stimulation by NMDA, which were smaller than increases in MAP and sympathetic nerve activity by NMDA in barodenervated spontaneously hypertensive rats.

What Is Relevant?

- Tonic γ-aminobutyric acid–ergic activity in the ARCN and baroreflex activity modulate blood pressure control and cardiovascular responses to ARCN stimulation by NMDA.

Summary

Attenuation of tonic γ-aminobutyric acid–ergic activity in the ARCN, which may increase activity of excitatory ARCN neurons and downstream presynaptic neurons, may contribute to elevated blood pressure in hypertension.
Tonic γ-Aminobutyric Acid–ergic Activity in the Hypothalamic Arcuate Nucleus Is Attenuated in the Spontaneously Hypertensive Rat
Tetsuya Kawabe, Kazumi Kawabe and Hreday N. Sapru

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Tonic GABAergic Activity in the Hypothalamic Arcuate Nucleus Is Attenuated in the Spontaneously Hypertensive Rat

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Short title: Arcuate nucleus: role in hypertension

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Methods

Ethics Statement
Experiments were done according to the NIH guide for “The Care and Use of Laboratory Animals, 7th Edition, 1996” and the protocols for the experiments were approved by the Institutional Animal Care and Use Committee of this institution.

General Procedures and Anesthesia
The animals were housed in the animal care facility of this institution under controlled conditions with a 12:12-hour light-dark cycle. Food and water were allowed to the animals ad libitum. The rats were anesthetized with inhalation of isoflurane (2–3% in 100% oxygen). A tracheostomy was performed and the rats were artificially ventilated using a rodent ventilator (model 683; Harvard Apparatus, Holliston, MA, USA). One of the femoral arteries was cannulated for monitoring BP. Mean arterial pressure (MAP) and HR were derived electronically from BP waves. One of the femoral veins was cannulated and urethane (1.2–1.4 g/kg) was injected intravenously in 8-9 aliquots at 2-min intervals (total volume of the anesthetic solution was 0.4–0.45 ml injected over a period of about 16–18 min). Isoflurane inhalation was terminated as soon as urethane administration was completed. Rectal temperature was maintained at 37 ± 0.5°C using a temperature controller (model TCAT-2AC, Physitemp Instruments, Clifton, NJ, USA). All of the tracings were stored on a computer hard drive using a data acquisition system obtained from Cambridge Electronic Design Ltd (CED; Cambridge, UK). At the end of the experiment, the rats were deeply anesthetized with a high dose of urethane (2 g/kg, i.v.), a pneumothorax was produced by an incision in one of the intercostal muscles and cessation of heart beat indicated that euthanasia was complete.

Microinjections into the hypothalamic arcuate nucleus (ARCN)
The rats were placed in a prone position in a stereotaxic instrument (David Kopf Instruments, Tajunga, CA, USA) with bite bar 3.3 mm below the interaural line. The bregma was visually identified and a small hole was drilled in the parietal bone. Multi-barreled glass-micropipettes (tip size 20–40 μm) were used for microinjections. For microinjection into the ARCN, the micropipettes were inserted into the brain perpendicularly. The coordinates for microinjections into the ARCN were: 1.9-4.1 mm caudal to the bregma, 0.2–0.4 mm lateral to the midline, and 9.6–9.9 mm deep from the dura.

Histology
At the end of the experiment, diluted green retrobeads IX (1: 50) were microinjected into the ARCN as a marker to confirm microinjection sites. The animals were perfused and fixed with 2% paraformaldehyde and serial sections of the hypothalamus were cut (40 μm) in a vibratome and mounted on subbed slides, covered with Citifluor mountant medium (Ted Pella Inc., Redding, CA, USA) and coverslipped. The microinjection sites were identified under a microscope (model AX70, Olympus Provis, Middlebush, NJ, USA) and the sections were photographed (Neurolucida software, version 7.5, MicroBrightField Inc., Williston, VT, USA) and compared with a standard atlas.1
Greater splanchnic nerve recording
The greater splanchnic nerve (GSN) was identified under an operating microscope (OPMI-1, Carl Zeiss, Thornwood, NY, USA) using a retroperitoneal approach. The GSN was sectioned at its junction with the celiac ganglion, a small segment was desheathed and its activity was recorded using a bipolar silver wire hook electrode. The activity of whole nerve (GSNA) was amplified (×10,000–20,000), filtered (100–5000 Hz), digitized and stored on a computer hard drive. The digitized signals were full-wave rectified and integrated over consecutive 1 sec intervals using Spike 2 program (CED, UK). At the end of the experiment, the nerve was sectioned centrally and the remaining activity was considered to be the noise level which was subtracted from the GSNA amplitude.

Barodenervation
The carotid sinus, aortic depressor and recurrent laryngeal nerves were identified under an operating microscope and sectioned bilaterally. Lack of bradycardia and inhibition of GSNA in response to intravenous bolus injections of phenylephrine (10 µg/kg) indicated that the barodenervation was complete.

Drugs and Chemicals
All of the solutions for the microinjections were freshly prepared in artificial cerebrospinal fluid (aCSF, pH 7.4). The composition of aCSF was as follows: NaCl (128 mM), KCl (3 mM), CaCl_2 (1.2 mM), MgCl_2 (0.8 mM), dextrose (3.4 mM) and HEPES (5 mM). Where applicable, the concentration of drugs refers to their salts. The vendors for different drugs and chemicals were as follows: CGP52432 (R&D Systems, Minneapolis, MN, USA), isoflurane (Piramal Critical Care, Bethlehem, PA, USA), green retrobeads IX (Lumafluor Inc., Durham, NC, USA). All other drugs and chemicals were obtained from Sigma Chemicals (St. Louis, MO, USA).

Statistical Analyses
The means and standard error of the means (S.E.M.) were calculated for maximum changes in MAP and HR in response to microinjections of different drugs into the ARCN. One-way analysis of variance (ANOVA) followed by Tukey-Kramer's multiple test was used for determination of concentration-response of gabazine and NMDA responses in the ARCN. Student’s unpaired t-test was used for comparison of the following responses: increases in MAP and HR induced by the microinjections of gabazine, CGP52432 and NMDA into the ARCN in different groups of both Wistar-Kyoto rats (WKY) and spontaneously hypertensive rats (SHR). For analyses of the GSNA, the integrated signals obtained just before the microinjections of gabazine, CGP52432 and NMDA into the ARCN were averaged over a period of 60 sec. When the responses to these treatments were maximal, the integrated signals were averaged over a period of 60–90 sec. The percentage changes in GSNA elicited by these treatments were calculated and compared between different groups of rats by using Student's unpaired t-test. In all cases, the differences were considered significant at P < 0.05.
Results

**GABA<sub>B</sub> Receptor Blockade in the ARCN**
The concentration of CGP52432 (20 mmol/L) used in these experiments, was selected from published literature. In all groups of WKY and SHR, CGP52432 microinjected in the ARCN elicited increases in MAP and GSNA, but no change in HR (Table S1). There was no significant difference in MAP and GSNA responses elicited by CGP52432 between WKY and SHR. Lower concentration (e.g., 10 mmol/L) of CGP52432 did not elicit any significant change in MAP. Concentrations of CGP52432 higher than 20 mmol/L could not be used because of the lack of solubility of this antagonist in aCSF at these concentrations.

The onset, peak, and duration of responses to microinjections of CGP52432 into the ARCN were 10-25 seconds, 3-7 minutes and 20-35 minutes, respectively. Cardiovascular responses elicited by microinjections of CGP52432 (20 mmol/L) into the ARCN were smaller than those elicited by gabazine (2 mmol/L) (Tables 1 and S1).

**References**
Figure S1. Histological Identification of Microinjection Sites in the ARCN.

A: A typical microinjection site in the ARCN marked with green retrobeads IX (20 nL) in SHR. B-D: composite diagrams of ARCN sections at selected levels 2.40 mm (rostral region), 3.24 mm (middle region) and 3.84 mm (caudal region) showing microinjection sites in WKY (open squares) and SHR (dark triangles); the coordinates mentioned below each drawing are caudal to the bregma. In both WKY and SHR, microinjections were made unilaterally (left or right side) as well as bilaterally. In the diagrams, for simplification, microinjection sites in WKY are shown at the left side and those in SHR are shown at right side. Bar in panel A = 500 μm. DMN: the hypothalamic dorsomedial nucleus; PH: posterior hypothalamic nucleus; PMV: ventral premammillary nucleus; VMN: the hypothalamic ventromedial nucleus; 3V: the third ventricle.
Table S1. Responses to Microinjections of CGP52432 into the ARCN.

<table>
<thead>
<tr>
<th>Concentration (mmol/L)</th>
<th>n</th>
<th>Increase in MAP (mmHg)</th>
<th>Increase in GSNA (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>WKY</td>
<td>SHR</td>
<td>WKY</td>
</tr>
<tr>
<td>Baro-intact rats: unilateral microinjections</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>6</td>
<td>10.1 ± 1.3</td>
<td>8.6 ± 0.9</td>
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<tr>
<td></td>
<td>20</td>
<td>5</td>
<td>11.0 ± 1.5</td>
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<td>Barodenervated rats: unilateral microinjections</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>5</td>
<td>11.2 ± 1.4</td>
<td>10.0 ± 1.2</td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>5</td>
<td>12.2 ± 1.9</td>
</tr>
</tbody>
</table>

The values shown for WKY and SHR were not statistically different. n: number of rats.