Role of Metabotropic Glutamate Receptors in Ventrolateral Medulla of Hypertensive Rats

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Abstract Evidence is accumulating for the role of metabotropic glutamate receptors in cardiovascular regulation. We sought to determine whether stimulation of metabotropic glutamate receptors in the rostral ventrolateral medulla would evoke enhanced cardiovascular responses in spontaneously hypertensive rats (SHR). Thus, we microinjected (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD], a selective agonist of metabotropic glutamate receptors, into the rostral ventrolateral medulla of urethane-anesthetized adult SHR and age-matched Wistar-Kyoto (WKY) rats. Microinjection of (1S,3R)-ACPD (1 nmol/50 nL) produced increases in mean arterial pressure and splanchnic sympathetic nerve activity in SHR (+41±6 mm Hg and +34±4%, respectively) that were significantly greater than those observed in WKY rats (+18±3 mm Hg and +22±3%, respectively). The pressor responses evoked by microinjection of L-glutamate (2 nmol), N-methyl-D-aspartate (20 pmol), or α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (5 pmol) were also significantly (P<.001) augmented in SHR (+55±3, +61±7, and +53±3 mm Hg, respectively, in SHR versus +31±1, +30±3, and +28±2 mm Hg in WKY rats). Results indicate that stimulation of metabotropic, as well as ionotropic, glutamate receptors in the rostral ventrolateral medulla evokes enhanced cardiovascular responses in SHR, which may contribute to hypertension in this model.

Key Words • receptors, glutamate • medulla oblongata • blood pressure • sympathetic nervous system • rats, inbred SHR

Glutamate is a major excitatory neurotransmitter in the central nervous system. According to results of biochemical, electrophysiologic, and molecular studies, receptors for glutamate are classified as ionotropic (ion channel linked) or metabotropic (G protein linked). Ionotropic receptors are further subdivided into the receptors for N-methyl-D-aspartate (NMDA) and non-NMDA receptors for kainate/α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA). Metabotropic receptors (mGluR) are coupled to phosphoinositide hydrolysis or cyclic AMP modulation. (1S,3R)-1-Aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD] is a selective agonist of these receptors. We have recently reported that microinjection of (1S,3R)-ACPD into the ventrolateral medulla oblongata produces cardiovascular responses in anesthetized Sprague-Dawley rats. Since the bulbospinal sympathoexcitatory neurons in the rostral ventrolateral medulla (RVLM) constitute an integral component of the baroreflex arc and therefore play an important role in cardiovascular regulation, alteration of responsiveness to the excitatory amino acids in the RVLM may be involved in the pathogenesis or maintenance of hypertension. Indeed, enhanced cardiovascular responses elicited in the RVLM of spontaneously hypertensive rats (SHR) by either electrical or chemical stimulation with L-glutamate (Glu) or ionotropic receptors agonists have been reported, but the role of mGluR in this region has not been investigated in this model. In the present study, we determined whether stimulation of mGluR in the RVLM would evoke an enhanced cardiovascular response in SHR.

Animal Preparation

All experiments were done in adult male SHR (13 to 18 weeks old, 339±9 g, n=19) and in age-matched Wistar-Kyoto (WKY) rats (358±5 g, n=23) obtained from Japan Charles River Co. This experiment was reviewed by the Committee on the Ethics of Animal Experimentation in the Faculty of Medicine, Kyushu University, and carried out following the guidelines for animal experimentation in the Faculty of Medicine, Kyushu University, and The Law (No. 105) and Notification (No. 6) of the government. Rats were anesthetized with urethane (1.5 g/kg IP). A femoral artery and vein were cannulated for measurement of arterial pressure and injection of drugs, respectively. Body temperature was maintained at 37.5±0.5°C by use of a heating pad.

Anesthetized rats were placed in a supine position with the head fixed in a stereotaxic frame (David Kopf Instruments). The trachea and esophagus were transected in the lower neck and reflected rostrally. The distal trachea was cannulated to facilitate ventilation. After retraction of the bilateral longus capitis muscles, the inferior occipital bone was removed to provide a 5x6-mm window to the surface of the ventral medulla oblongata. After incision and retraction of the dura, the ventral surface of the medulla was kept moist with either artificial cerebrospinal fluid (aCSF, pH 7.4) or endogenous CSF. After paralysis had been induced with d-tubocurarine (0.8 mg/kg IV), the tracheal cannula was connected to a ventilator (model 681D, Harvard Apparatus), and the rats were artificially ventilated at a rate of 60 strokes per minute and a tidal

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volume of 3.0 mL to maintain arterial blood gases and pH within physiological limits (pH 7.35 to 7.45; $P_02$, 100 to 140 mm Hg; $P_{CO2}$, 40 to 45 mm Hg). In a limited number of experiments, the abdominal plexus was exposed through a transverse incision of the lateral abdominal wall, and the inferior nerve bundle accompanying a superior mesenteric artery was placed over bipolar silver electrodes. Nerves and electrode tips were immersed in mineral oil to preserve the integrity of the nerve fibers. Splanchnic sympathetic nerve activity (SNA) was amplified and filtered (bandwidth 100 to 3000 Hz). At the end of each experiment, the noise level associated with SNA recording was determined after the injection of hexamethonium bromide (30 mg/kg IV). Since the absolute value of SNA could vary among animals and during the course of an experiment, the effects of substances microinjected into the RVLM on SNA were evaluated by using the percent change in SNA where the level of SNA immediately preceding the microinjection was used as a normalizing factor.

**Microinjection Procedures**

Microinjections used multibarrel micropipettes with tip diameters of 20 to 50 μm. The pipettes were made from calibrated microbore capillary glass tubing (Accu-Fill 90, Clay Adams). Injections (50 nL) were made unilaterally over a 30-second period with a hand-held syringe. The injection volume was measured by observing the movement of the fluid meniscus along a reticule in a microscope.

The RVLM was identified by the injection of Glu (2 nmol) based on the following criteria: (1) latency to the onset of the change in blood pressure produced by Glu was no more than 5 seconds, (2) a response plateau occurred within 20 seconds after microinjection of Glu, and (3) change in blood pressure was at least 25 mm Hg. The RVLM was restricted to injection sites located 0.6 to 1.0 mm rostral to the most rostral rootlet of the hypoglossal nerve, 1.7 to 1.9 mm lateral to the midline, and 0.5 to 0.8 mm below the ventral surface.

All drugs were dissolved in aCSF (mmol/L: NaCl 133.3, KCl 3.4, CaCl$_2$ 1.3, MgCl$_2$ 1.2, NaH$_2$PO$_4$ 0.6, NaHCO$_3$ 32.0, and glucose 3.4, pH 7.4).

**Histological Analysis**

In a limited number of experiments, 10 nL of Alcian blue dye was injected from a separate barrel of the pipette to mark the injection site. At the completion of the experiments, the rats were deeply anesthetized with pentobarbital sodium (50 mg/kg IV) and perfused transcardially with 150 mL of 0.9% NaCl followed by 150 mL of 10% phosphate-buffered formaldehyde solution. The brain stem was sectioned in the coronal plane (50 μm) and stained with neutral red. Microinjection sites were identified by the deposition of Alcian blue dye and referred to standard anatomic structures of the caudal brain stem according to the atlas of Paxinos and Watson.¹²

**Experimental Protocols**

**Dose Response of (1S,3R)-ACPD in the RVLM**

(1S,3R)-ACPD at a dose of either 0.1 or 1 nmol was microinjected into the RVLM of SHR and WKY rats. At each injection site, only one dose of the drug was examined. aCSF was also injected as a vehicle control.

**Effect of NMDA and AMPA in the RVLM**

For comparison of the cardiovascular responses of SHR and WKY rats to ionotropic receptor agonists, NMDA (20 pmol) or AMPA (5 pmol) was microinjected.

**Statistical Analysis**

Data are expressed as mean±SEM. One-way ANOVA was used to compare results in the two strains. One-way ANOVA followed by multiple comparisons by Duncan's multiple range test was also used in the analysis of the dose response to (1S,3R)-ACPD in each strain. Differences in dose response between the strains were analyzed by two-way ANOVA. Probability values less than .05 were considered statistically significant.

**Results**

Baseline mean arterial pressure (MAP) and heart rate (HR) were significantly higher in SHR (115±2 mm Hg and 410±6 beats per minute, respectively) than in WKY rats (85±2 mm Hg and 357±8 beats per minute, respectively, $P<.001$). Fig 1 summarizes the cardiovascular responses evoked by Glu (2 nmol). Glu increased MAP, HR, and SNA in both SHR and WKY rats. However, the increases in MAP and SNA were significantly greater in SHR than in WKY rats. Fig 2 presents typical tracings showing the cardiovascular responses produced by microinjection of (1S,3R)-ACPD (1 nmol) into the RVLM. (1S,3R)-ACPD increased MAP, HR, and SNA in both strains, but the pressor and sympathoexcitatory re-

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**Fig 1.** Bar graphs show effects of microinjection of L-glutamate (2 nmol) into the rostral ventrolateral medulla of spontaneously hypertensive rats (SHR) (closed bars) and Wistar-Kyoto (WKY) rats (open bars). Increases in mean arterial pressure (MAP, top) and splanchnic sympathetic nerve activity (SNA, bottom) were significantly greater in SHR than WKY rats. *$P<.05$, †$P<.001$ vs WKY rats. Number of rats is shown in parentheses. HR indicates heart rate.
Fig 2. Polygraph tracings illustrate cardiovascular responses to (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD, 1 nmol] microinjected into the rostral ventrolateral medulla of a spontaneously hypertensive rat (SHR) (A) and a Wistar-Kyoto (WKY) rat (B). Increases in arterial pressure and splanchnic sympathetic nerve activity (SNA) were greater in the SHR than the WKY rat.

Responses were clearly greater in an SHR (Fig 2A) than in a WKY rat (Fig 2B). Fig 3 shows the comparison of dose responses to (1S,3R)-ACPD. (1S,3R)-ACPD elicited significant (P<.001) dose-related increases in MAP and HR in both strains. However, the increase in MAP evoked by either 0.1 or 1 nmol of (1S,3R)-ACPD was significantly (P<.005) greater in SHR than in WKY rats. A two-way ANOVA also revealed that the dose response to (1S,3R)-ACPD was significantly (P<.001) different between SHR and WKY rats. Similarly, the increase in SNA elicited by 1 nmol of (1S,3R)-ACPD was significantly greater in SHR (34±4%, n=6) than in WKY rats (22±3%, n=5, P<.05). In contrast, increases in HR were not different between the two strains. For comparison of the cardiovascular responses evoked by ionotropic receptor agonists, either NMDA (20 pmol) or AMPA (5 pmol) was microinjected (Fig 4). As with the responses induced by (1S,3R)-ACPD, the increase in MAP elicited by either NMDA or AMPA was significantly (P<.001) greater in SHR than in WKY rats. In addition, the increase in SNA evoked by NMDA tended to be greater in SHR (77±17%, n=4) than in WKY rats (31±4%, n=3, P=.07), and the increase in SNA elicited by AMPA was significantly greater in SHR than in WKY rats (104±16%, n=3 versus 41±6%, n=5; P<.01). Histological examination demonstrated that the injection sites were located in the area that encompassed the dorsolateral aspect of the lateral paragigantocellular nucleus and the region dorsolateral to this nucleus. This area lies at the caudal end of the facial nucleus.

Discussion

The main finding of the present study was that stimulation of mGluR in the RVLM evoked enhanced pressor and sympathoexcitatory responses in SHR. The presence of glutamate receptors other than ionotropic receptors in the medulla oblongata has been hypothesized based on evidence that kynurenic acid, a broad-spectrum ionotropic receptor antagonist, failed to inhibit the cardiovascular responses produced by injection of Glu into the nucleus tractus solitarius. After the discovery of mGluR and the selective agonist trans-ACPD, Pawloski-Dahm and Gordon reported that stimulation of mGluR in the nucleus tractus solitarius of anesthetized rats produces a depressor response that is not blocked by kynurenic acid. In our previous study, we added evidence that mGluR also participates in cardiovascular regulation in the ventrolateral medulla of anesthetized Sprague-Dawley rats. The mGluR activity seems to be ubiquitous in the central nervous system; e.g., seizures and brain injury can be elicited by selective activation of mGluR, and these effects cannot be blocked by ionotropic receptor antagonists.

Nonselective activation of the glutamate receptors by microinjection of Glu was augmented in SHR in the present study. This observation is in accord with our
previous findings.\textsuperscript{18} Miura et al\textsuperscript{11} also reported that the threshold dose of Glu or NMDA to evoke a pressor response in the RVLM was 10 to 12 times lower for SHR than for WKY rats. In contrast, Muratani et al\textsuperscript{19,20} and Smith and Barron\textsuperscript{21,22} observed comparable pressor responses to Glu in SHR and WKY rats, although they did not evaluate SNA. The noticeable difference between the current study and the studies by Muratani et al\textsuperscript{19,20} is whether the rats were paralyzed and artificially ventilated. Since microinjection of Glu into the RVLM evokes not only cardiovascular but also respiratory (hypoaxemic or apneic) responses, the blood pressure responses observed in their study could be modified by the secondary change to the altered respiration in spontaneously breathing rats. On the other hand, Smith and Barron\textsuperscript{21,22} used paralyzed and artificially ventilated rats and found similar pressor responses to Glu in SHR and WKY rats. Although slight differences including the age of the rats and the dose or volume of Glu injection exist between their study and ours, we are unable to explain completely the disparate results. Obviously, further studies evaluating the influences of spontaneous breathing, rat age, and anesthetized type on pressor and sympathoexcitatory responses to various Glu doses are necessary. In the present study, however, the pressor and sympathetic responses to selective activation of either metabotropic or ionotropic glutamate receptors were also augmented in SHR. Therefore, it seems reasonable that nonselective activation of the glutamate receptors by microinjection of Glu produces enhanced responses in SHR. One may argue that the higher baseline blood pressure observed in SHR can affect the greater blood pressure increase induced in SHR by either nonselective or selective glutamate receptor agonists. However, the differences between SHR and WKY rats in the pressor response produced by each agonist were still significant when we analyzed the results for relative increase from baseline (percent change) instead of for absolute change. Furthermore, sympathetic activation elicited by Glu or selective agonists was also augmented in SHR. Thus, we speculate that the greater blood pressure increase elicited by stimulation of mGluR as well as ionotropic receptors in SHR may be attributable to an augmented sensitivity to the excitatory amino acids in the RVLM. Since microinjections were made unilaterally, it may be possible that the blunted baroreflex-mediated sympathoinhibitory response through the contralateral RVLM could contribute to the accentuated response to glutamate receptor agonists injected into the RVLM of SHR. We are also unable to exclude the possibility that the altered responsiveness of sympathetic
preganglionic neurons of SHR could contribute at least in part to the augmented blood pressure and sympathetic responses. Nevertheless, considering the reported evidence that the firing pattern of RVLM neurons differed in SHR and WKY rats, the altered electrophysiological properties and the enhanced responsiveness of the RVLM to various stimuli may contribute to the pathogenesis or maintenance of hypertension in SHR.

The differential role of metabotropic and ionotropic glutamate receptors in the RVLM is a matter of great interest. In our previous study, blockade of ionotropic receptors by microinjection of antagonists, which effectively abolished the cardiovascular responses evoked by exogenously injected ionotropic receptor agonists, failed to affect the prevailing blood pressure. This observation raises the possibility that mGluR makes a major contribution to the maintenance of blood pressure in the RVLM. To explore the role of endogenous mGluR in cardiovascular regulation, however, use of a selective mGluR antagonist is required. We found that L-2-amino-3-phosphonopropionate (L-AP3), which had been described as a specific mGluR antagonist, failed to inhibit the cardiovascular responses elicited by (1S,3R)-ACPD in the RVLM. The failure of L-AP3 to inhibit the cardiovascular responses evoked by trans-ACPD in the nucleus tractus solitarius was also reported. More recently, phenylglycine derivatives have been reported to be specific mGluR antagonists in vitro, but they too were unable to block the (1S,3R)-ACPD responses in our preliminary experiments (data not shown). Since several forms of mGluR exist in the brain, the particular subtype of mGluR in the RVLM may not be sensitive to L-AP3 or phenylglycine derivatives.

In conclusion, stimulation of mGluR in the RVLM produced enhanced pressor and sympathoexcitatory responses in SHR, suggesting that mGluR may participate in cardiovascular regulation in this animal model. The development of effective mGluR antagonists in the RVLM may aid our ability to discern the physiological and pathophysiological role of this class of glutamate receptors in cardiovascular regulation.

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