Role of Metabotropic Glutamate Receptors in Ventrolateral Medulla of Hypertensive Rats

Takuya Tsuchihashi, Isao Abe, Masatoshi Fujishima

Abstract Evidence is accumulating for the role of metabotropic, as well as ionotropic, 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%, P<.005 and P<.05, 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±5 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. (Hypertension. 1994;24:648-652.)

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, electrophysiological, 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.

Methods

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...
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, CaCl₂ 1.3, MgCl₂ 0.6, NaH₂PO₄ 0.6, NaHCO₃ 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 10% phosphate-buffered formaldehyde solution. The brainstem 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-
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 elicited 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 dorsal 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
FIG 3. Bar graphs show effects of microinjection of (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD] or artificial cerebrospinal fluid (aCSF, vehicle control) into the rostral ventrolateral medulla of spontaneously hypertensive rats (SHR) (closed bars) and Wistar-Kyoto (WKY) rats (open bars). (1S,3R)-ACPD elicited significant (P<.001) dose-dependent increases in mean arterial pressure (MAP, top) and heart rate (HR, bottom) in both strains. However, the increase in MAP evoked by either 0.1 or 1 nmol of (1S,3R)-ACPD was significantly greater in SHR than in WKY rats. tP<.005 vs WKY rats. Number of rats is shown in parentheses.

Previous findings 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 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 is whether the rats were paralyzed and artificially ventilated. Since microinjection of Glu into the RVLM evokes not only cardiovascular but also respiratory (hypopneic 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 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 Smith and Barron is whether the rats were paralyzed and artificially ventilated. Since microinjection of Glu into the RVLM evokes not only cardiovascular but also respiratory (hypopneic 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 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 Smith and Barron is whether the rats were paralyzed and artificially ventilated.

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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 RVLN neurons differed in SHR and WKY rats, the altered electrophysiological properties and the enhanced responsiveness of the RVLN 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 RVLN 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.\(^6\) This observation raises the possibility that mGlur makes a major contribution to the maintenance of blood pressure in the RVLN. 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,\(^24\) failed to inhibit the cardiovascular responses elicited by (1S,3R)-ACPD in the RVLN.\(^5\) The failure of L-AP3 to inhibit the cardiovascular responses evoked by trans-ACPD in the nucleus tractus solitarius was also reported.\(^15\) More recently, phenylglycine derivatives have been reported to be specific mGlur antagonists in vitro,\(^25,26\) 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,\(^27\) the particular subtype of mGlur in the RVLN may not be sensitive to L-AP3 or phenylglycine derivatives.

In conclusion, stimulation of mGlur in the RVLN 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 RVLN may aid our ability to discern the physiological and pathophysiological role of this class of glutamate receptors in cardiovascular regulation.

References

Role of metabotropic glutamate receptors in ventrolateral medulla of hypertensive rats.

T Tsuchihashi, I Abe and M Fujishima

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