Metabotropic Glutamate Receptors in the Ventrolateral Medulla of Rats

Takuya Tsuchihashi and David B. Averill

We investigated the hypothesis that stimulation of metabotropic excitatory amino acid receptors in the ventrolateral medulla evokes cardiovascular responses. Thus, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD], a selective agonist of metabotropic excitatory amino acid receptors, was microinjected into the rostral or caudal ventrolateral medulla of halothane-anesthetized Sprague-Dawley rats. Microinjections of (1S,3R)-ACPD (100 pmol–1 nmol) into the rostral ventrolateral medulla produced dose-dependent increases in mean arterial pressure (+20±4 mm Hg by 100 pmol and +35±2 mm Hg by 1 nmol, p<0.01 versus artificial cerebrospinal fluid) and integrated splanchnic sympathetic nerve activity (+17±3% and +46±4%, respectively, p<0.01), whereas (1S,3R)-ACPD microinjected into the caudal ventrolateral medulla decreased mean arterial pressure (−28±2 mm Hg by 100 pmol and −48±6 mm Hg by 1 nmol, p<0.01 versus artificial cerebrospinal fluid) and splanchnic sympathetic nerve activity (−24±4% and −49±5%, p<0.01). The blockade of ionotropic excitatory amino acid receptors by the combined injection of 2-amino-7-phosphonoheptanoic acid (200 pmol) and 6,7-dinitroquinoxaline-2,3-dione (200 pmol), which effectively blocked the responses elicited by either N-methyl-D-aspartate (20 pmol) or a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (5 pmol), failed to affect the responses evoked by either (1S,3R)-ACPD (100 pmol) or L-glutamate (2 nmol) microinjected in the rostral and caudal ventrolateral medulla. These results suggest that metabotropic receptors are present and mediate cardiovascular responses evoked by l-glutamate injections into the rostral and caudal ventrolateral medulla. (Hypertension 1993;21:739–744)

KEY WORDS • vasomotor neurons • amino acids • blood pressure • sympathetic nervous system • receptors, glutamate

It is well appreciated that L-glutamate (Glu) is a primary excitatory neurotransmitter in the central nervous system. Glu or other excitatory amino acids (EAA) may act at receptors that are ligand-gated ion channels and are referred to as ionotropic EAA receptors.1 These receptors exhibit selective responsiveness to N-methyl-D-aspartic acid (NMDA), kainic acid, or a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA); hence, they have been classified as either NMDA, kainate, or AMPA receptors. However, recent evidence suggests that Glu also acts through a separate class of receptors that may alter phosphoinositide hydrolysis and intracellular calcium mobilization.2-3 This class of EAA receptors is termed metabotropic glutamate receptors (mGluR).4-5 The pharmacological and functional characteristics of mGluR have been disclosed by using a selective mGluR agonist, (±)-1-aminocyclopentane-

trans-1,3-dicarboxylic acid (trans-ACPD*).6-8 Recently, Pawloski-Dahm and Gordon9 showed that activation of mGluR by trans-ACPD injected in the nucleus tractus solitarii (NTS) evoked cardiovascular effects that were similar to those produced by Glu.

The rostral (RVLM) and caudal (CVLM) ventrolateral medulla oblongata represent counterbalancing pools of neurons that are integral components of the baroreceptor reflex.10 Although a number of studies have demonstrated that Glu as well as agonists selective for ionotropic EAA receptors in these two regions mediate cardiovascular responses,11-13 mGluR-mediated responses evoked by Glu in these regions have not been studied. Therefore, the purpose of the present study was to demonstrate that mGluRs exist in the RVLM and CVLM and that selective activation of mGluRs elicits sympathetically mediated changes in blood pressure.

Methods

Animal Preparation

Experiments were done in male Sprague-Dawley rats (270–350 g, Harlan Industries, Ind.). All experiments were carried out in accordance with the guiding principles for the care and use of animals as mandated by the American Physiological Society. Rats were anesthetized with halothane (0.9–1.1%) and breathed oxygen-en-
Microinjection Procedures

Microinjections were made from multibarrel micropipettes with tip diameters of 20–50 μm. The pipettes were made from calibrated microbore capillary glass tubing (Accu-Fill 90, Clay Adams, N.J.). Injections (50 nl) were made over a 30-second period with a handheld syringe, and the injected volume was measured by observing the movement of the fluid meniscus along a reticule in a microscope.

The RVLM and CVLM were identified by injection of 2 nmol Glu based on the criteria of our previous study. The rostral pressor area (i.e., RVLM) was restricted to injection sites located 0.6–1.0 mm rostral to the most rostral rostral tip of the hypoglossal nerve: 1.7–1.9 mm lateral to the midline, and 0.5–0.8 mm below the ventral surface. The CVLM corresponded to injection sites located between the second and third rootlets of the hypoglossal nerve: 1.9–2.1 mm lateral to the midline and 0.7–0.9 mm below the ventral surface.

All drugs for microinjection were dissolved in artificial cerebrospinal fluid (aCSF) (in mM: NaCl 133.3, KCl 3.4, CaCl2 1.3, MgCl2 1.2, NaH2PO4 0.6, NaHCO3 32.0, and glucose 3.4, pH 7.4). Ten nanoliters of an emulsion of Alcian blue dye was injected from a separate barrel of tubing (Accu-Fill 90, Clay Adams, N.J.). Injections (50 nl) were made over a 30-second period with a handheld syringe, and the injected volume was measured by observing the movement of the fluid meniscus along a reticule in a microscope.

Histological Analysis

At the completion of experiments, rats were deeply anesthetized with pentobarbital sodium (50 mg i.v.). The animals were then 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. By using a 10-nl injection of Alcian blue dye, we could demonstrate discrete localization of injection sites in histological sections. 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.

Experimental Protocols

Dose response of (1S,3R)-ACPD in RVLM and CVLM.

Figure 1 shows that (1S,3R)-ACPD produced significant (p<0.001) dose-related increases in MAP and SNA for injections in the RVLM and significant (p<0.001) dose-related decreases in MAP and SNA for injections in the CVLM. HR was also increased by the injection of (1S,3R)-ACPD into the RVLM at a dose of 100 pmol (+7±2 beats per minute, p<0.05 versus controls).
aCSF) or 1 nmol (+20±4 beats per minute, p<0.01), and decreased by the injection into the CVLM (−13±4, p>0.05, and −39±16 beats per minute, p<0.05, respectively). Typical responses evoked by the injections of 1 nmol of (1S,3R)-ACPD into the RVLM or CVLM are shown in Figure 2. The peak MAP and SNA responses occurred within 1 minute after the initiation of injection. Thereafter, 8–10 minutes were required before MAP and SNA returned to baseline levels.

**Effect of L-AP3 or AP7+DNQX on (1S,3R)-ACPD responses**

The microinjection of L-AP3 (2 nmol) in the RVLM (n=6) produced transient and significant increases in MAP (+30±5 mm Hg, p<0.001 versus aCSF), HR (+14±3 beats per minute, p<0.001), and SNA (+58±13%, p<0.005). Likewise, L-AP3 (2 nmol) injected in the CVLM (n=6) transiently decreased MAP (−36±6 mm Hg, p<0.001) and SNA (−41±7%, p<0.001). However, L-AP3 failed to inhibit responses elicited by subsequent injection of 100 pmol of (1S,3R)-ACPD in either the RVLM or CVLM (Figure 3). In a limited number of experiments, we found that L-AP3 did not alter either the blood pressure or SNA responses to injection of Glu into either the RVLM or CVLM. In addition, the responses evoked by (1S,3R)-ACPD (100 pmol) were not affected by the blockade of ionotropic EAA receptors with AP7+DNQX (Figure 3).

**Effect of AP7+DNQX on Glu, NMDA, and AMPA Responses**

Microinjection of a solution containing the NMDA and non-NMDA antagonists, AP7 (200 pmol) and DNQX (200 pmol), respectively, into the RVLM (n=9) did not alter baseline MAP (−6±3 mm Hg, NS), HR (−4±4 beats per minute, NS), or SNA (−2±5%, NS). However, injection of the same solution containing AP7 and DNQX (200 pmol each) in the CVLM (n=10) elicited significant increases in MAP (+10±2 mm Hg, p<0.005 versus aCSF) and SNA (+23±4%, p<0.005) but no change in HR (+5±3 beats per minute, NS). As shown in Figure 4, the MAP and SNA responses evoked by NMDA (20 pmol) or AMPA (5 pmol) were significantly (p<0.05) attenuated by injection of the solution containing AP7 and DNQX in either the RVLM or the CVLM. HR responses were also attenuated. In contrast, the responses evoked by the microinjection of Glu were not affected by pretreatment with AP7+DNQX in either RVLM or CVLM.

**Discussion**

The aim of the present study was to demonstrate that stimulation of mGluR in the ventrolateral medulla evokes cardiovascular responses. To this end we used (1S,3R)-ACPD, which has been shown to act as an agonist selective for the mGluR. Activation of mGluR by (1S,3R)-ACPD has been shown to stimulate phosphoinositide hydrolysis and neuronal depolarization in slices of brain tissue. When this drug was injected in the RVLM of rats, it produced dose-dependent increases in blood pressure and SNA typical of activation of RVLM neurons projecting to sympathetic preganglionic neurons. On the other hand, (1S,3R)-ACPD injected in the CVLM produced dose-dependent decreases in blood pressure and SNA that are characteristic of stimulation of sympathoinhibitory neurons of this region. These observations bear a striking resemblance to the findings of Pawloski-Dahm and Gordon, who showed that trans-ACPD injected in the NTS evoked depressor responses. Although our data represent the first observations in rats that mGluR in the ventrolateral medulla may participate in cardiovascular regulation, our findings are in agreement with the preliminary report of McManigle et al. that trans-ACPD injected in the CVLM of cats decreased blood pressure and HR. However, in contrast to our findings, these investigators did not demonstrate a potential role for mGluR in cardiovascular regulation when trans-ACPD was injected into the RVLM.

The results of our experiments and those of other investigators support the view that mGluR as well as ionotropic EAA receptors participate in the cardiovascular and sympathetic responses evoked by Glu microinjected in the ventrolateral medulla. However, knowledge that (1S,3R)-ACPD acts at only mGluR to evoke cardiovascular responses requires the demonstration that this agent does not also act at ligand-gated ion channels. Therefore, we examined the cardiovascular
responses to (1S,3R)-ACPD after both NMDA and non-NMDA ionotropic EAA receptors had been blocked by AP7 and DNQX, respectively. The observation that blockade of ionotropic EAA receptors in either the RVLM or CVLM did not alter the cardiovascular and sympathetic nerve responses to (1S,3R)-ACPD argues that this drug selectively activates mGluR in these brain regions. In addition, we observed that blockade of ionotropic EAA receptors in the CVLM and RVLM did not alter the cardiovascular responses that could be evoked by Glu injection at these sites. Pawloski-Dahm and Gordon\(^9\) have also observed that blockade of ionotropic EAA receptors by the broad-acting antagonist kynurenic acid did not alter the cardiovascular response to Glu injection in the NTS. Thus, the well-known cardiovascular responses that can be evoked by Glu in the RVLM or CVLM may be mediated through activation of mGluR.

L-AP3 has been reported as a specific mGluR antagonist that inhibits phosphoinositide hydrolysis\(^{20}\) and the mobilization of intracellular calcium.\(^{16}\) However, the failure of L-AP3 to inhibit trans-ACPD responses have been delineated both in vitro\(^{21,22}\) and in vivo.\(^9\) In the present study, we provide additional evidence that L-AP3 does not affect the neuronal mechanisms responsible for the cardiovascular responses evoked by (1S,3R)-ACPD or Glu in the VLM. Our dose of L-AP3 (2 nmol) was nearly three times the dose of L-AP3 (740 pmol) that Pawloski-Dahm and Gordon\(^9\) showed did not block the cardiovascular responses evoked by Glu or trans-ACPD microinjected in the NTS. Glum and Miller\(^{23}\) observed that (1S,3R)-ACPD depolarized NTS neurons and that this effect did not require changes in intracellular calcium concentration. Although L-AP3 failed to block the effects of (1S,3R)-ACPD, it produced agonistic actions in both the RVLM and CVLM. This observation is in accordance with previous observations that suggest that L-AP3 possesses agonistic properties\(^{24,25}\) or induces nonspecific membrane effects.\(^{23}\) Taken together, these

**FIGURE 2.** Polygraph tracings illustrate the cardiovascular responses to (1S,3R)-l-aminocyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD]. Panel A: (1S,3R)-ACPD (1 nmol) microinjected into the rostral ventrolateral medulla increased mean arterial pressure, heart rate, and splanchnic sympathetic nerve activity. Panel B: (1S,3R)-ACPD (1 nmol) microinjected into the caudal ventrolateral medulla decreased mean arterial pressure, heart rate, and sympathetic nerve activity. Solid horizontal bars represent 1 minute.
observations suggest that multiple isoforms of mGluR may exist in the brain.

Can we define the relative roles of ionotropic EAA receptors versus mGluR in the VLM with respect to cardiovascular regulation? In our experiments blockade of ionotropic EAA receptors by unilateral injection of AP7 and DNQX in the RVLM did not alter baseline blood pressure or SNA. Although this finding might diminish a role of ionotropic EAA receptors in the tonic activity of pressor neurons of the RVLM, ionotropic EAA receptors would appear important in mediating certain types of afferent input to rostral pressor neurons. Injection of kynurenic acid into the RVLM reduced the pressor and sympathoexcitatory effects evoked by hypothalamic stimulation,24 whereas selective blockade of NMDA receptors by AP7 microinjection attenuated the hypertension induced by bilateral common carotid artery occlusion.25 Ionotropic EAA receptors in the CVLM mediate glutamatergic input impinging on CVLM neurons that inhibit rostral pressor neurons.11 When the mixture of AP7 and DNQX was microinjected in the CVLM, we observed significant increases in baseline blood pressure and SNA. This finding is compatible with the observation of Jung et al.,12 who showed that kynurenic acid injection in the CVLM increased blood pressure. This glutamatergic input to CVLM neurons appears to represent a component of the baroreceptor reflex arc since injection of kynurenic acid26 or NMDA antagonists11 were effective in abolishing the depressor response to aortic depressor nerve stimulation. The availability of drugs that are effective antagonists at ionotropic EAA receptors have aided greatly in our ability to discern the role of this class of EAA receptors in cardiovascular regulation. However, as described above, we were not able to antagonist the actions of (1S,3R)-ACPD with L-AP3. Thus, our knowledge about the functional role of mGluR in the VLM remains incomplete. Further studies using more selective antagonists may provide insight into the physiological significance of this novel class of EAA receptor.

In conclusion, the results of this study provide evidence for the existence of mGluR on cardiovascular neurons of the VLM. Stimulation of mGluR by (1S,3R)-ACPD elicited cardiovascular and sympathetic nerve responses that had the same pharmacological profile as

**Figure 3.** Bar graphs show the effects of prior injection of L-2-amino-3-phosphono-propionate (L-AP3) (2 nmol), 2-amino-7-phosphonoheptanoic acid (AP7) (200 pmol) plus 6,7-dinitroquinazoline-2,3-dione (DNQX) (200 pmol), or vehicle (−) on the changes in mean arterial pressure (MAP) and sympathetic nerve activity (SNA) evoked by subsequent injection of 100 pmol of (1S,3R)-1-amino-cyclopentane-1,3-dicarboxylic acid [(1S,3R)-ACPD] in the rostral ventrolateral medulla oblongata (RVLM) (left panel) and the caudal ventrolateral medulla oblongata (CVLM) (right panel). Neither L-AP3 nor AP7+DNQX attenuated the blood pressure and sympathetic nerve responses to (1S,3R)-ACPD. Number of rats is shown in parentheses.

**Figure 4.** Bar graphs show the effects of 2-amino-7-phosphonoheptanoic acid plus 6,7-dinitroquinazoline-2,3-dione (AP7+DNQX) (200 pmol each) on l-glutamate (L-Glu) (2 nmol), N-methyl-D-aspartic acid (NMDA) (20 pmol), and a-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) (5 pmol) responses in the rostral ventrolateral medulla oblongata (RVLM) (left panel) and the caudal ventrolateral medulla oblongata (CVLM) (right panel). The increases or decreases in mean arterial pressure (MAP) and sympathetic nerve activity (SNA) evoked by NMDA or AMPA were significantly attenuated by AP7+DNQX, whereas the responses elicited by L-Glu were not affected. *p<0.05 and **p<0.01 vs. before AP7+DNQX. Number of rats is shown in parentheses.
that observed for Glu injection in the VLM. When antagonists that selectively block the cardiovascular responses of (1S,3R)-ACPD become available, we may explore the possibility that mGluR participate in cardiovascular regulation.

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References

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