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The mapped pattern of kainate on blood pressure responses is similar to that of L-proline in the ventrolateral medulla of the rat

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Abstract

Kainate is an excitatory amino acid receptor agonist with a structure similar to the amino acid L-proline. Our previous studies demonstrated that microinjections of L-proline into the ventrolateral medulla (VLM) of the rat induce a mapped pattern of blood pressure responses distinct from L-glutamate, and the depressor response to L-proline in the caudal VLM (CVLM) is abolished by the kainate/AMPA receptor antagonist CNQX. The present study investigated whether kainate produces the L-proline-mapped pattern of responses in the VLM, compared with the pattern by AMPA. Kainate is known to activate AMPA receptors at higher concentrations. Therefore, responses to kainate were investigated at a low concentration. Microinjections of AMPA or NMDA showed the pattern of the L-glutamate-type; a pressor response in the rostral VLM and caudal pressor area (CPA) and a depressor response in the CVLM. Microinjections of kainate showed depressor responses in the CVLM but minor pressor responses in the rostral VLM, suggesting the same responses to L-proline. However, the response sites in the CPA did not enable us to clearly determine the L-proline-type. Further trials at sites defined by a pressor response to L-glutamate in the CPA, successive injections of L-proline and kainate produced no response, indicating that L-glutamate responding neurons in the CPA are not sensitive to L-proline and kainate. These results suggest that kainate stimulation in the VLM produces a mapped pattern of ABP responses similar to the mapped pattern with L-proline. Kainate receptors could therefore be involved in the depressor response to L-proline in the medulla.

Keywords: Kainate; L-Proline; Amino acid; Ventrolateral medulla; Arterial blood pressure; Rat

Kainate is a typical exogenous agonist for kainate receptors. Kainate receptors are defined as one of the ionotropic excitatory amino acid (EAA) receptors which include the NMDA (*N*-methyl-D-aspartate) and AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptors [2,5,8–11,15]. Interestingly, the molecular structure of kainic acid is similar to that of the non-essential amino acid L-proline. Previous studies have demonstrated that injection of L-proline into the rat brain induces various cardiovascular responses similar to those by induced by L-glutamate [20–23]. However, a study of mapped pattern of arterial blood pressure (ABP) responses to microinjections of L-proline into the ventrolateral medulla (VLM) in the rat showed a distinct result from that by L-glutamate [25]. The VLM contains three loci that regulate cardiovascular responses, including the rostral (R) VLM, the caudal (C) VLM and the caudal pressor area (CPA [6]). The RVLM sends pre-sympathetic efferent projections to the intermediolateral cell column. In addition, the cellular activity in the CVLM and CPA can influence the basal ABP through RVLM efferent projections [3,4,7,16,19]. Microinjections of L-glutamate in the RVLM and CPA increase ABP, whereas ABP is decreased following L-glutamate injections into the CVLM; however the mapped pattern for L-proline has depressor responses in the caudal areas of VLM with minor pressor responses in the RVLM [25]. Because L-glutamate stimulates all known EAA receptors [2,5,8–11,15], local injections of L-glutamate may result in a complicated response pattern in a region containing all subtypes of the ionotropic EAA receptors. Many physiological experiments have used ionotropic EAA receptor agonists to stimulate neurons. However, there are no systematic data on the mapped patterns of the responses in the VLM including the CPA with ionotropic EAA receptor agonists especially at low concentrations. L-Proline may influence the

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responses of a subgroup of EAA receptors, such as the structurally related kainate receptors. Indeed, if medullary kainate receptors are largely responsible for the ABP response to L-proline, selective microinjections of kainate into the VLM would be expected to result in the same pattern of ABP responses as those seen following L-proline administration. Kainate at high concentrations (0.1–4 nmol/microinjection), in the medulla, has commonly been used to destroy neurons that regulate cardiovascular output [12,13,28]. Kainate is also known to be a stimulant of AMPA receptors at higher concentrations [11]. To avoid damaging cells and to evaluate the physiological role of kainate at kainate receptors in cardiovascular regulation, lower picomolar concentrations of kainate were employed. Therefore, the mapped pattern of ABP responses to microinjections of kainate in the VLM of the anesthetized rat were initially investigated and compared to those following AMPA or NMDA receptor stimulation. The results from kainate injections indicated a marked response pattern in the RVLM and CVLM as L-proline produced, but unclear in the CPA. Therefore, the responses to L-proline or kainate following precise injections into a locus of the CPA were subsequently evaluated, after confirmation of the pressor response to AMPA or L-glutamate injection. Brief reports of this work have appeared previously in abstract form [24,27].

All protocols and surgical procedures used in this study were performed in accordance with the Guiding Principles for the Care and Use of Animals approved by the Council of the Physiological Society of Japan and the Guideline for the Committee of Animal Experimentation, Hiroshima University and the Committee of Research Facilities for Laboratory Animal Science, Natural Science Center for Basic Research and Development (N-BARD), Hiroshima University.

Male Wistar rats, weighing 325–370 g, were anesthetized with urethane (1.1–1.3 g/kg, I.P., Sigma), and the fur around the ventral neck and inguinal region was shaved with electric clippers. After placing the rat in a supine position on a stereotaxic frame (Narishige), insertions of carotid arterial and femoral venous tubing (PE50) and a tracheal catheter were performed as described previously [25]. The tracheal catheter was coated with atropine sulfate ointment (Santen) to prevent secretion. A microscope was used to visualize the brain stem region. The ventral surface of the medulla oblongata was exposed by opening a window in the basioccipital bone using a dental drill. The dura and arachnoid were lifted carefully to avoid tearing the surface vessels. The rats were ventilated by a rodent respirator (Shinano) to maintain normocapnia ($PaCO_2$: 35–45 mmHg) as described in a previous report [25], and were then immobilized by an I.V. infusion of pancuronium bromide (Sankyo) at a flow rate of 0.4 mg/kg/h. An adequate depth of anesthesia was assessed based on ABP stability and/or the absence of a withdrawal response to a firm toe pinch during a stoppage of the infusion of the neuromuscular blockade. Supplemental doses of urethane (75 mg/kg/injection, I.V.) were given as needed. The rectal temperature of the rats was maintained at $37.0 \pm 0.5\,^{\circ}C$ by a cooling bag or a heating mat. The direct and mean ABP was recorded on a pen-writing oscillograph (RJG4024, Nihon Kohden).

A glass micropipette (20–30 μm o.d. at both tips, borosilicate glass capillaries, 1 mm o.d. and 0.58 mm i.d., Clark Electromedical instruments) was made using a micropipette processor (PE-2, Narishige). One end of the micropipette was tightly connected to polyethylene tubing with a chemical bond and the needle of a Hamilton microsyringe (filled with distilled water) attached to a micromanipulator was inserted into the other end of the tubing. The graduation of the micromanipulator had been calibrated previously using the microsyringe. After the capillary system was filled with the solution to be injected, the micropipette was mounted onto a pipette holder. Artificial cerebrospinal fluid (ACSF, see [23]) was used as the vehicle for all drug solutions: L-proline and L-glutamate were obtained from nacalai tesque (Japan), and NMDA and AMPA were obtained from Sigma (St. Louis, MO). Kainic acid (nacalai tesque, Japan) was first dissolved in a small volume of distilled water and then diluted with ACSF. Injections of several doses of kainic acid and drug solutions were repeatedly delivered through the same micropipette. The capillary system was carefully cleaned with distilled water between each drug concentration or solution. A marker (zero) point was identified at the caudolateral edge of the beginning of the basilar artery as described previously [25]. When the location of the basilar artery differed markedly from the expected location, the rostral end of the second rootlet of cranial nerve XII was used as an additional reference point, located on average 1.11 mm rostral from the basilar artery zero point [25].

The concentration of kainic acid that effectively evoked changes in ABP was determined in the CVLM and compared with the depressor response to L-glutamate (0.34 nmol, 34 nl) in three rats. The concentrations of AMPA (0.4 pmol/34 nl) and NMDA (2 pmol/34 nl) were determined in a previous study [26]. The ABP responses to microinjections of kainic acid (59 loci in 10 rats), AMPA (52 in 5 rats) and NMDA (42 in 7 rats) in the RVLM, CVLM, and CPA were subsequently mapped. Micropipettes were lowered 0.7–1.0 mm in the CVLM and CPA, and 0.5–0.7 mm in the RVLM to the ventral surface of the medulla [25]. The microinjections were repeated when the ABP was stable and returned to the pre-injection level. In the case of no change in the ABP, the intervals between injections were at least 3 min. The patterns of the responses to AMPA and NMDA were clearly determined to be L-glutamate-type based on the response geometry, but the responses to kainate were unclear geometrically in the CPA but not in the RVLM and CVLM. In the second experiment, the loci in the functionally defined CPA that produce the pressor response to L-glutamate and/or AMPA were identified and successive injections of L-proline and kainic acid were performed to determine the effects of these compounds on ABP in six rats. The functionally identified CPA showed consistently no response to L-proline and kainic acid in all rats, indicating that the geometrically deduced CPA, as L-proline induced depressor acting area, in the previous study [25] could be involved in the CVLM depressor region or include neurons not sensitive to L-glutamate/AMPA. To locate sites after the injections, methylene blue (0.1%, 68 nl) was injected into the functionally defined CPA in two rats. Ten minutes later the rat was euthanized with an overdose of sodium pentobarbital (100 mg/kg, I.V., Dainippon). The brain stem was removed and