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GLUTAMATE-INDUCED CURRENTS IN ACUTELY DISSOCIATED GUINEA PIG LOCUS COERULEUS NEURONS

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INDEXING WORDS

locus coeruleus; acutely dissociated neuron; glutamate; NMDA receptor; non-NMDA receptor; patch-clamp technique

SYNOPSIS

To investigate the properties of L-glutamate (Glu) receptors in single locus coeruleus (LC) neurons at mature stage, we recorded the Glu-induced currents in the LC neurons acutely dissociated from adult guinea pigs, using the whole-cell patch-clamp technique. The concentration-current relationships show that Glu-induced currents in LC neurons might be predominantly mediated by non-NMDA receptors rather than NMDA receptors. The current-voltage relationship of NMDA or non-NMDA receptor-mediated currents indicated that both subtypes of the Glu-receptors operate non-selective cation channels, and that the NMDA receptor-operated channels but not the non-NMDA receptor-operated channels are permeable to Ca^{2+}. These results suggest that in LC nerons, the normal excitatory neurotransmission may be mediated through non-NMDA receptors, and that NMDA receptors may be involved in intracellular signal transduction by their Ca^{2+} permeability.

INTRODUCTION

The locus coeruleus (LC) is the largest nucleus of noradrenaline-containing neurons in the brain,[6] innervating almost the entire central nervous system.[10] The LC is involved in various physiological functions, including modulation of sleep and waking,[10] respiration,[16] micturition,[21] blood pressure[20] and responses to noxious stimuli.[11] L-glutamate (Glu) and aspartate, major excitatory neurotransmitters in the mammalian central nervous system, exert their effect through glutamate receptors, NMDA and non-NMDA receptors.[24] As well in the LC neurons, *in vivo*

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studies reported that microinfusion of Glu into the LC regions induced various physiological effects,[16,17,20] while intracoerulear microinjection or systemic application of the NMDA and/or non-NMDA receptor antagonists reduced an activity of the LC neurons.[2,7,11] These studies indicate that various physiological effects regulated by LC neurons were mediated by glutamatergic inputs. On the other hand, it has been demonstrated that the both subtype of receptors exist in the rat LC neurons by recent investigations using a selective antibody or expression patterns of messenger RNAs.[22,23] However, the relative contribution of each receptor subunit to the excitatory effects by Glu varies among the central neurons, which would result in the diversity of the functional properties.[23] As well in case of the LC neurons, some studies suggested that responses of the LC neurons to Glu may be predominantly mediated by non-NMDA receptors.[3,5,8,9] In the present experiment, therefore, in order to investigate the properties of Glu receptors in single LC neurons, we recorded the currents activated by extracellularly applied Glu from LC neurons acutely dissociated from adult guinea pigs, using the whole-cell patch-clamp technique.

MATERIALS AND METHODS

LC neurons were acutely dissociated from adult male Hartley guinea pigs using a procedure reported elsewhere.[15] Male guinea pigs weighing 200-250g were decapitated under urethane anesthesia. The brainstem was rapidly removed and coronally cut into slices (500 μm thick) with a razor blade on the level of the inferior colliculus. The slices with the LC were placed in solution, containing (in mM) : 120 NaCl, 5 KCl, 1 $CaCl_2$, 1 $MgCl_2$, 25 D-glucose and 20 piperazine-N,N'-bis-(2-ethanesulfonic acid) (Pipes) (pH 7.0 with NaOH), gently bubbled with 100 % O_2 for 30 min at room temperature (20-24℃). Then, the slices were incubated in the solution supplemented with 7 mg/ml -trypsin (type XI, Sigma, U.S.A.) for 120 min at 32 ℃. Bilateral LC regions of the slice were punched out using a pipette tip with 1.0 mm-internal diameter. The LC regions were dissociated by gently triturating with serially smaller fire-polished Pasteur pipettes in Dulbecco's modified Eagle's Medium (Gibco, U.S.A.) containing 25 mM N-2-hydroxy- ethylpiperazine-N'-2-ethanesulfonic acid (Hepes) and 25 mM D-glucose. The dissociated cells were then allowed to settle on glass coverslips coated with concanavalin A. Whole-cell patch-clamp recordings were obtained at room temperature (20-24℃). The ion compositions of extracellular solutions and intracellular pipette solutions are shown in Table 1. Glu and other drugs were applied through a glass pipette with an internal tip diameter of about 3 μm placed about 50 μm distant from the neuron by positive gas pressure (0.14 kg/cm^2) for 2 s. The liquid junction potentials between the external and the internal solutions were negligible, and the actual membrane potentials were not corrected by them. The Ag-AgCl electrode connecting with a capillary filled with 150 mM-NaCl agar was used as a reference. Whole-cell membrane currents were recorded by the standard patch-clamp method.[12] The whole-cell membrane currents and potentials were recorded with a patch-clamp amplifier, CEZ-2300 (Nihon Kohden, Japan). Current traces were low-pass filtered

at 1 kHz. In the experiments for the current-concentration relationships, the peak amplitude of whole-cell currents was normalized by the cell capacitance as current density (in AF^{-1}). All statistical results are given as mean ± standard error mean (S.E.M.).

TABLE I. Ion composition of solutions used (mM).

External solution	NaCl	KCl	$CaCl_2$	pH
1	145	5	2.4	7.4 with NaOH
2	141.1	5	5	7.4 with NaOH
Internal solution	**KCl**		**CsCl**	**pH**
A	150		——	7.2 with KOH
B	——		150	7.2 with KOH

All external solutions contained 10 mM-Hepes, 10 mM-glucose, 3 μM-tetrodotoxin. All internal solutions contained 10 mM-Hepes and 5 mM-EGTA.Osmolalities for all solutions used ranged from 310 to 320 (mOsm/kg.H_2O).

RESULTS

The LC regions were easily recognized in the slice preparations and correctly punched out using a pipette tip with 1.0 mm-inner diameter. Therefore, dissociated cells from the regions, which had approximately 20 μm-diameter with well defined multiple processes (three or more), were able to be identified as LC neurons (Fig. 1) and distinguished from neurons in the mesencephalic nucleus of the trigeminal nerve, situated along the lateral border of the LC, because of these morphological characteristics ,as reported in the histological studies of the guinea pig LC neurons.[13,15] The cell capacitance of a single LC neuron was 33.1 ± 1.0 pF (n=121) and the resting membrane potential was -53.3 ± 1.8 mV (n=16), when the external and the internal solutions were Solution 1 and Solution A (150 mM-KCl), respectively.

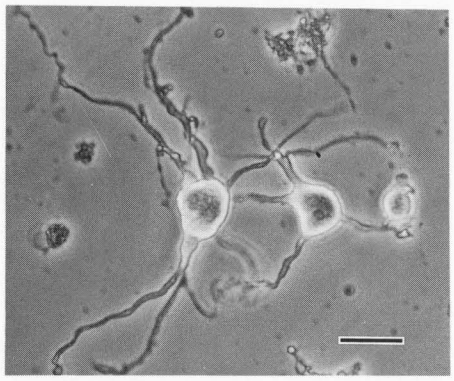

Figure 1. Photomicrograph of two locus coeruleus neurons acutely dissociated from adult guinea pigs. LC neurons could be identified by the morphological characteristics; the cell bodies were 15-20μm in diameter with well defined multiple processes. Scale bar, 20 μm.

Fig. 1.

To study the contribution of each Glu receptor subtype to the membrane conductance activated by Glu in LC neurons, specific antagonists were added to the external solution. Control Glu-induced currents, mediated by NMDA receptors and non-NMDA receptors were recorded with 5 μM-glycine in Solution 1. Moreover, to isolate NMDA receptor-mediated currents, a non-NMDA receptor antagonist, 6,7-dinitroquinoxaline-2,3 (1H,4H)-dione (DNQX 10 μM) was added. For isolation of non-NMDA receptor-mediated currents, a NMDA receptor antagonist, D-2-amino- 5-phosphonovaleric acid (APV 100 μM) together with 1 mM-$MgCl_2$ wad added, while glycine was omitted. Compared to the control, 10 μM- Glu-induced currents were more distinctly diminished in the presence of 10 μM- DNQX, than those in the presence of APV (Fig. 2A). The current density-concentration relationships of Glu-induced currents at a holding potential of -60 mV with 100 μM- APV, 10 μM-DNQX or without either antagonist in an external solution are shown in Fig. 2B. Judging from the half-maximal concentration (EC_{50}) of Glu in each condition, when a concentration of the applied Glu was less than about 100 μM, currents were much more carried through non-NMDA receptor-channels (EC_{50} = 8.8 μM) than NMDA receptor-channels (EC_{50} = 109.1 μM) at a holding potential of -60 mV. Figure 3A shows an example of Glu-induced currents in a single LC neuron at different voltages (-60 to +40 mV). The extracellular solution was Solution 1 and the intracellular pipette solution was Solution B (150 mM-CsCl). The peak current-voltage relationship for the control Glu-induced currents exhibited linearity with a reversal potential (E_{rev}) of +2.7 ± 0.7 mV (n=5).

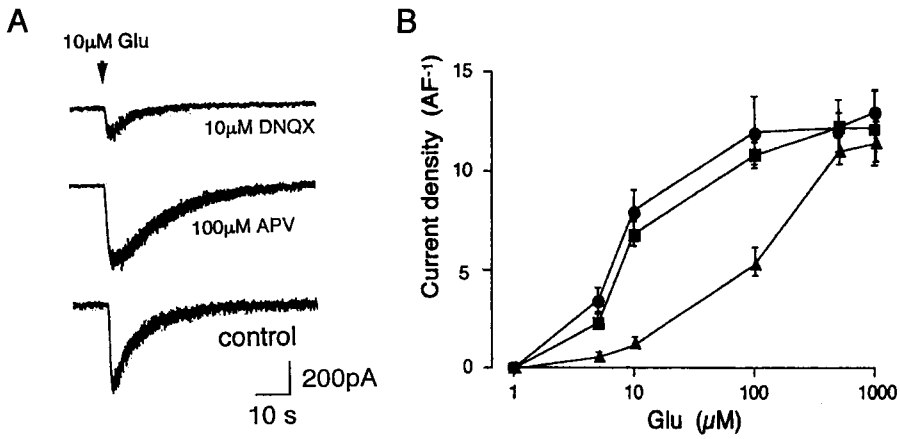

Fig. 2.

Figure 2. Effects of DNQX or APV on Glu-induced currents; the external and internal solution were Solution 1 and B, respectively. DNQX or APV was added to Solution 1. A : examples of 10 μM-Glu-induced currents with 10 μM-DNQX (top), 100 μM-APV (middle) or without either antagonist (bottom). Vh : -60 mV. B : current density-concentration relationship of Glu-induced currents in the presence of 10μM-DNQX (▲), 100μM-APV (■) or in the absence of both antagonists (●). Vh : -60 mV. Each point represents mean ± S.E.M. of 5-10 cells.

The Glu-induced currents with 100 μM-APV or 10 μM-DNQX in the external solution reversed at +3.8 ± 0.7 mV (n=6) or +2.5 ± 1.2 mV (n=5), respectively (Fig. 3B). Each E_{rev} was near 0 mV, suggesting that the Glu-induced currents were carried through non-selective cation channels.[19] To study the permeability of each Glu receptor-channel subtype to calcium, the extracellular calcium concentration was increased. In the presence of high concentration of calcium (>5 mM), the cells became rapidly round and finally detached from the glass coverslip. Therefore, the extracellular calcium concentration was increased up to 5 mM by replacing NaCl with CaCl2 on an isotonic basis (Solution 2). The Glu-induced currents with Solution 2 in the bath reversed at +0.6 ± 1.1 mV (n=6) or +8.8 ± 1.9 mV (n=6), in the presence of 100 μM- APV or 10 μM-DNQX, respectively (Fig. 4). As the extracellular calcium concentration increased, Erev shifted in the positive direction in the presence of DNQX but not in the presence of APV ($p < 0.05$; Student's paired t-test), indicating that in the guinea pig LC neurons, NMDA receptor-operated channels are permeable to Ca^{2+} and non-NMDA receptor-operated channels are not.

DISCUSSION

Recent *in vivo* studies reported that microinfusion of Glu into the LC regions induced various physiological effects, such as an augmentation of respiration,[16] decrease in blood pessure and heart rate[20] and facilitation of bladder contraction,[17] while intracoerulear microinjection or systemic application of the NMDA and/or non-NMDA receptor antagonists reduced an activity of the LC neurons.[2,7,11] These studies indicate that various physiological effects regulated by LC neurons were mediated by glutamatergic inputs. On the other hand, a recent study using a selective antibody showed that the NMDAR1 subunit was present in most nuclei including the LC in the rat brain.[22] Expression patterns of messenger RNAs revealed that non-NMDA receptor subunits (GluR1-4) also exist in the rat LC neurons.[23] However, the relative contribution of each receptor subunit to the excitatory effects by Glu varies among the central neurons, which would result in the diversity of the functional properties.[23] As well in case of the LC neurons, some studies suggested that responses of the LC neurons to Glu may be predominantly mediated by non-NMDA receptors.[3,5,8,9] To elucidate these mechanism, it is necessary that functional properties of the Glu receptor subtypes in single LC neurons should be investigated. However, any study of Glu receptor subtypes in dissociated single LC neurons has not been reported up to this time. Therefore, we studied the Glu-induced currents in the LC neurons acutely dissociated from adult guinea pigs. Another merit of using acutely dissociated neurons was that we were able to use the neurons of mature stage. In customary available culture methods, we can use only neurons of viviparous or neonatal stage, however, the receptors and ion channels of these neurons should be different from those of mature stage in the appearances and properties.[1]

In the central neurons, Glu activates membrane conductance through NMDA and

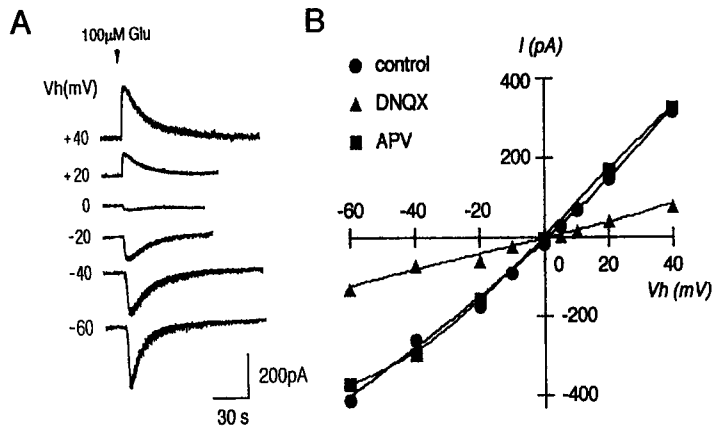

Fig. 3.

Figure 3. The peak current-voltage relationship of the Glu-induced currents. A : a typical example of the control currents activated by 100 μM-Glu in a LC neuron at different voltages (-60 ~ +40 mV). The external and internal solutions were the standard external solution (Solution 1) and Solution B, respectively. B : I-V relation of the peak currents activated by 100 μM-Glu with 10 μM-DNQX (▲), 100μM-APV (■) or without either antagonist (●, control) in the standard external solution (Solution 1), which recorded from an each different LC cell. Measured Erev was 2.5, 0.6 or 1.7 mV, respectively.

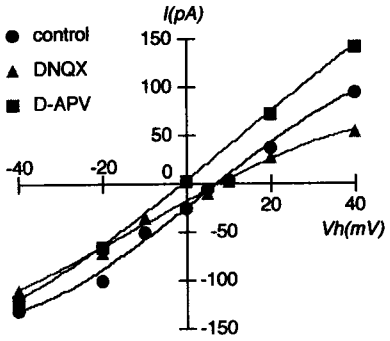

Fig. 4.

Figure 4. I-V relationships of the peak currents activated by 100 μM-Glu in 5 mM-Ca^{2+} external solutions (Solution 2) with 10 μM-DNQX (▲), 100 μM -APV (■) or without either antagonist (●, control), which were obtained from an each different LC cell. The internal solution was Solution B. Measured Erev was 9.0, -0.7 or 7.5 mV, respectively.

non-NMDA receptors, which directly couple to non-selective cation channels.[19] In the present study, the currents activated by application of Glu in LC neurons acutely dissociated from adult guinea pigs were carried through both NMDA and non-NMDA receptor channels. At a holding potential of -60 mV, which is near the resting potential of the LC neurons studied (-53.3 mV), the concentration-current relationship with DNQX (10 μM) in the bath shifted distinctly to the right more than that with APV (100 μM) from the control without both antagonists, suggesting that

Glu-induced currents in LC neurons might be predominantly mediated by non-NMDA receptors in the normal excitatory neurotransmission. On the other hand, application of Glu with a specific non-NMDA receptor antagonist, DNQX in the bath activated the currents at concentrations greater than 10 μM. And as the extracellular calcium concentration increased, E_{rev} shifted to the positive direction significantly with DNQX but not with APV in the bath ($p < 0.05$), indicating that NMDA receptor-operated channels are permeable to Ca^{2+} and non-NMDA receptor-operated channels are not in the LC neurons. Therefore, the NMDA receptors may be involved in intracellular signal transduction by their Ca^{2+} permeability. Meanwhile under certain conditions such as brain anoxia or ischemia, in which extracellular Glu concentration could increase to greater than 300 μM,[4,14] the NMDA receptor-mediated currents could be excessively activated, subsequently leading to Ca^{2+} influx through NMDA receptor-channel, which would cause neuronal damage of LC neurons.

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