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Glutamate Receptors on the Somata of Dorsal Unpaired Median Neurons in Cockroach, *Periplaneta americana*, Thoracic Ganglia

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ABSTRACT—Effects of application of glutamate and glutamatergic ligands were studied to characterize the receptors for glutamate present on the soma membrane of the dorsal unpaired median (DUM) neurons in the thoracic ganglia of the cockroach, *Periplaneta americana*, using the intracellular recording technique. Application of L-glutamate did not block the GABA-response, and application of ß-guanidino-propionic acid, a competitive antagonist for GABA, failed to block the response to L-glutamate. These results indicate that most of L-glutamate action may not be mediated by a GABA-activated channel. To examine glutamate receptor types on the DUM neurons, glutamate receptor agonists were applied. The ionotropic glutamate receptor (iGluR) agonists evoked depolarizations with the following relative rank of order of potency: kainate>AMPA>quisqualate. Metabotropic glutamate receptor (mGluR) agonists also elicited membrane depolarizations or hyperpolarizations associated with an increase in membrane conductance. The mGluR agonists evoked depolarizations or hyperpolarizations with the following relative rank of order: L-CCG-I>1S, 3R-ACPD>L-AP4. Depolarization of the same DUM neuron was detected following exposure of kainate and L-CCG-I, suggesting the coexistence of distinct iGluR and mGluR types. A membrane permeable cAMP analog, CPT-cAMP, could not mimic the effect of mGluR agonists. The mGluR selective antagonists, MCCG and MCPG, failed to antagonize the response to mGluR agonists. The involvement of cAMP in the mGluR response was not confirmed in DUM neurons. Although the functional roles of these receptors are unknown, it might be possible then that these extrasynaptic receptors have a modulatory effect on the excitability of the DUM neurons.

Key words: glutamate receptor, dorsal unpaired median neuron, cockroach

INTRODUCTION

L-glutamate has been established as the major excitatory neurotransmitter not only in the vertebrate central nervous system, but also at the neuromuscular junction and in the central nervous system of most arthropods. In mammalian brains, glutamate receptors have been classified into two main categories termed ionotropic and metabotropic receptors. The ionotropic glutamate receptors (iGluRs) that are ligand-gated integral ion channels are further subdivided into three types, based on their sensitivity to the specific glutamate agonists, kainate, quisqualate/AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazoleprionic acid), and NMDA (N-methyl-D-aspartate) receptors. The quisqualate/ AMPA and kainate receptor channels mediate fast synaptic excitatory neurotransmission, whereas at NMDA receptor channels glutamate evokes currents with comparatively much slower kinetics (onset, offset and desensitization time-courses) (Seeburg, 1993). On the other hand, metabotropic glutamate receptors (mGluRs) are highly heterogeneous class of glutamate receptors that are coupled to multiple second messenger systems via GTP binding proteins (Schoepp and Conn, 1993). Based on their amino acid sequence identity, pharmacology, and transduction mechanism, mGluRs have been divided into three subgroups. Group I mGluRs, constituting of the mGluR1 and mGluR5 subtypes, increase inositol-1,4,5-triphosphate (IP_3) levels and are most effectively activated by quisqualate. Group II and III mGluRs, comprising the mGluR2 and 3, and mGluR4, 6, 7 and 8 subtypes, respectively, decrease adenosine 3', 5'-cyclic monophosphate (cAMP) formation and are selectively activated by (2S, 3S, 4S)- α-(carboxycyclopropyl)-glycin (L-CCG-I) and L-2-amino-4-phosphonobutyric acid (L-AP4), respectively (Pin and Bockaert, 1995; Pin and Duvoisin,1995). (1S, 3R)-1-amino- cyclopentane-1,3-dicarboxylic acid (1S, 3R-ACPD) is a broad-spectrum mGluR agonist acting on group I and II mGluRs.

The response to glutamate as the putative neurotransmitters of the soma membrane of the dorsal unpaired

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median (DUM) neuron in the cockroach thoracic ganglia was studied in a previous work (Washio, 1994). It was shown that glutamate and GABA receptors were present on the DUM somata. Both amino acids had an inhibitory effect on the DUM neuron whose responses were mediated by changes in the chloride conductance. Because the reversal potential was almost the same for L-glutamate and GABA (Washio, 1994), I wondered whether the glutamate action was mediated by a GABA-activated channel or not. To gain insight into this, first the interaction between glutamate- and GABA-activated channels has been studied. Secondly, to characterize the somal response to allow comparisons with the pharmacology of the mammalian central nervous system, the glutamate receptor type on the DUM neuronal somata was investigated, using glutamate receptor agonists. A brief account of some results has been reported in abstract form (Washio, 1997).

MATERIALS AND METHODS

Adult cockroaches, *Periplaneta americana* were used in all experiments. They were taken from stock colonies maintained at 26°C. The thoracic nervous system was isolated and fixed dorsal side up on a wax plate. The meta-, meso-, and prothoracic ganglia were desheathed by means of very fine forceps and a few drops of saturated neutral red saline were applied to the desheathed ganglia for about 10 min. The cell bodies of DUM neurons on the dorsal side of thoracic ganglia were stained pink (Tanaka and Washio, 1988). This aided penetration of the neuron somata with microelectrodes. The isolated preparation was pinned in about 2 ml perfusion chambers, and perfused by constant flow at a rate of 3.0 ml/min. Membrane potentials were measured with microelectrodes filled with 3M potassium chloride. The resistance ranged 30 to 50 MΩ. Currents were injected through the recording electrode under bridge recording mode with bridge balanced using a conventional amplifier (Nihonkoden CEZ-3100, Tokyo, Japan). Since current-voltage relations for DUM neurons are fairly linear from resting potentials to about 30mV more negative than rest (Washio and Tanaka, 1992), the membrane conductance was obtained directly from responses to constant current pulses (pulse duration, 80 ms). Drugs were applied topically to the preparation through the continuously flowing perfusion system. The following drugs were used: L-glutamic acid, γ-aminobutyric acid (GABA), β-guanidino-propionic acid (β-GP), kainic acid, and N-methyl-D-aspartate (NMDA) from Sigma Chemicals (St.Louis, MO); quisqualate, L(+)-2-amino-4-phosphonobutyric acid (L-AP4), (2S, 3S, 4S) -α- (carboxycyclopropyl) glycin (L-CCG-I), (+)-α-methyl-4-carboxylphenylglycine (MCPG) and (2S, 3S, 4S) -2-methyl-2- (carboxycyclopropyl) glycine (MCCG) from Tocris Cookson (Bristol, UK); α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), (1S, 3R)-1- amino- cyclopentane- 1,3-dicarboxylic-acid (1S, 3R-ACPD) and 6-cyano-7-nitroquinoxaline-2,3- dione (CNQX) from Research Biochemicals International (Natick,MA). 8- (4-chlorophenylthio)-adenosine-3': 5'-cyclic monophosphate (CPT-cAMP) from Boehringer (Germany). The antagonists, β-GP, picrotoxin, CNQX, MCPG and MCCG were bath-applied. The cockroach saline had the following composition (in mM); NaCl, 158; KCl, 10.8; CaCl, 5; MgCl, 2; HEPES buffer, 5. The pH of the solution was adjusted to 7.0 with NaOH. In the experiments of low chloride saline, all of the sodium chloride was substituted by the sodium isethionate. The concentration of chloride in the low chloride saline was as low as l4% of the control. Experiments were performed at room temperature (20–24°C). Numerical data in the text and in the table are expressed in the mean±sem. The value given in the change of membrane conductance is normalized mean±sem. The expression of the "typical" effect or response has been used only when the same result was obtained at least in five experiments.

RESULTS

Separate receptors are activated by glutamate and GABA

In order to determine whether the L-glutamate and GABA share a common chloride channel, cross-desensitizing experiments between these amino acids were made. As shown in Fig. 1A, L-glutamate produced a depolarization followed by an application of GABA after subsided of the somal depolarization. GABA produced a large depolarization that was almost identical with the response obtained when GABA was applied alone (not shown), showing the absence of cross-desensitization between L-glutamate and GABA. In this experiment L-glutamate was not removed from bath. Fig. 1B shows the typical effect of β-guanidino-propionic acid (β-GP), a selective competitive antagonist of GABA (Feltz, 1971), on the L-glutamate- and GABA-evoked responses. Bath-application of 1 mM β-GP suppressed completely the response to GABA, but failed to suppress the response to the L-glutamate. These results provide clear evidence that most of the L-glutamate action may not be mediated by a GABA-activated channel.

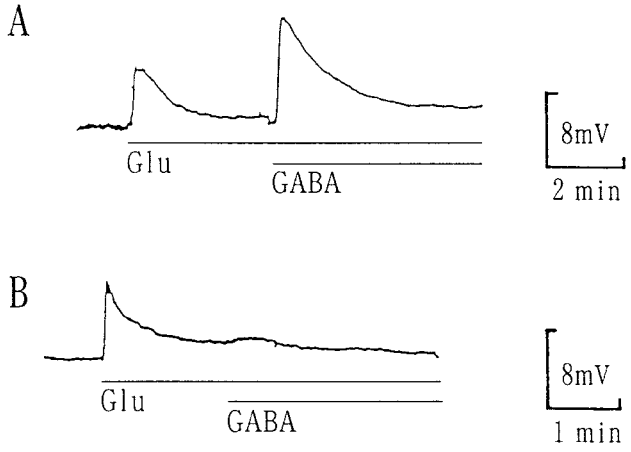

Fig. 1. (A) Absence of L-glutamate and GABA cross-desensitization in the DUM neuron. Response of a given DUM neuron to L-glutamate (0.1 mM) precedes a brief puff of GABA (0.1 mM). In this experiment L-glutamate was not removed from the bath. Resting potential, –45mV. (B) Suppression of GABA-, but not L-glutamate-induced responses by β-guanidino-propionic acid (β-GP). The response to GABA (0.1 mM) was suppressed completely, but the response to L-glutamate (0.1 mM) was not impaired by bath application of β-GP (1 mM), a selective competitive antagonist of GABA. Resting potential, -38mV. In this and the following figures duration of application of chemicals is denoted by bars under traces.

Ionotropic glutamate receptors (iGluRs)

To examine the glutamate receptor types on the DUM neurons, glutamate receptor agonists were bath-applied

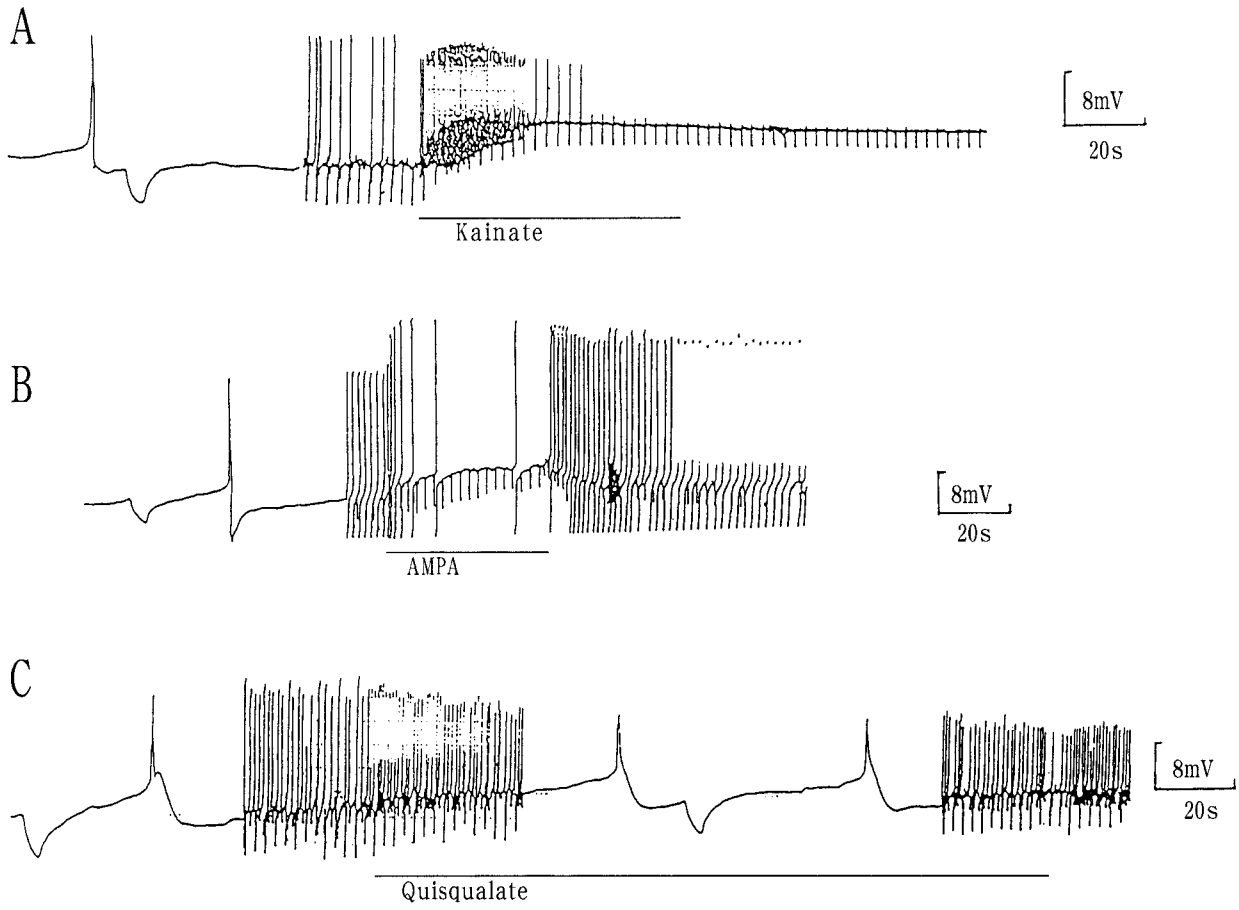

Fig. 2. Effects of ionotropic glutamate receptor (iGluR) agonists. (A) kainate (1 mM) application elicited burst firing, depolarization associated with an increase in membrane conductance and inhibited spontaneous spikes. Resting potential, –52 mV. (B) AMPA (1 mM) application also elicited depolarization and inhibited spontaneous spikes. Resting potential, –48 mV. (C) Effect of quisqualate (1 mM). Slight depolarization associated with an increase in membrane conductance and acceleration of spike frequency were observed but not inhibition of spikes. Resting potential, –45 mV. NR stands for normal Ringer's solution. The time scale is 60 times faster in the initial part than in the later part in A, B and C. Also, in some of the following figures the faster sweep of traces was used in the initial part of records.

onto the surface of the cell body of DUM neurons. Fig. 2 shows such experiments on iGluRs. Kainate (1 mM) and AMPA (1 mM) depolarized the membrane accompanied by an increase in membrane conductance, and eventually inhibited spontaneous spikes. The depolarization elicited by 1 mM kainate was large enough for inducing the burst firing. Quisqualate also caused a slight depolarization with accelerated spike frequency, but failed to inhibit spontaneous spikes in this cell. The effect of quisqualate was less effective than kainate and AMPA. The average values of the depolarization produced by iGluR agonists are summarized in Table 1A. From these results, the iGluR agonists evoked depolarizations with the following relative rank of order of potency: kainate > AMPA > quisqualate. On the other hand, NMDA was never observed to have a depolarizing effect on the neurons. Some examples are shown in Fig. 3A in which NMDA in a Mg-free saline accelerated the spike frequency. In Fig. 3A the duration of constant current pulses was not enough to reach a steady state in a NMDA-containing saline. The amplitude of the hyperpolarizing response might be larger than that shown in Fig. 3A to reach a steady state. Therefore, it appears most likely that a decrease in mem-

Table 1. Changes membrane potentials produced by iGluR and mGluR agonists.

	agonist	mM	mV[1)]	n[2)]
(A) iGluR agonists	Kainate	0.2	1.6±0.5	4
		1.0	7.8±0.2	8
	AMPA	1.0	2.5±0.3	6
	Quisqualate	1.0	1.7±0.1	15
(B) mGluR agonists	L-CCG-I	0.2	4.0±0.3[3)]	5
		1.0	7.5±0.45	4
	1S,3R-ACPD	1.0	3.3±0.2[4)]	8
	L-AP4	1.0	1.1±0.2	5

1) Averaged values of membrane potentials (mV) are mean±sem.
2) n Indicates the number of observations.
3) The hyperpolarization recorded in some neurons was excluded.
4) The hyperpolarization alone.

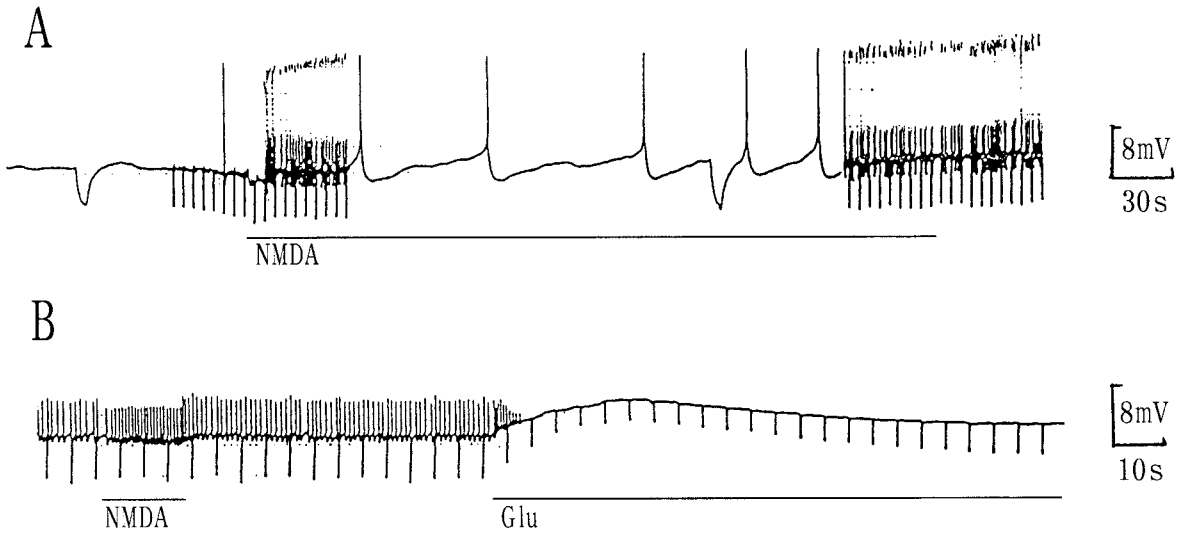

Fig. 3. Effect of iGluR agonist, NMDA, in a Mg-free saline. (A) NMDA (1 mM) application elicited acceleration of spike frequency in this neuron. Neither depolarization nor inhibition of spontaneous spikes was observed. Resting potential, –45 mV. (B) Effects of NMDA and L-glutamate (Glu). The response to NMDA (1 mM) was compared with the one to L-glutamate (0.1 mM). Resting potential, –48 mV.

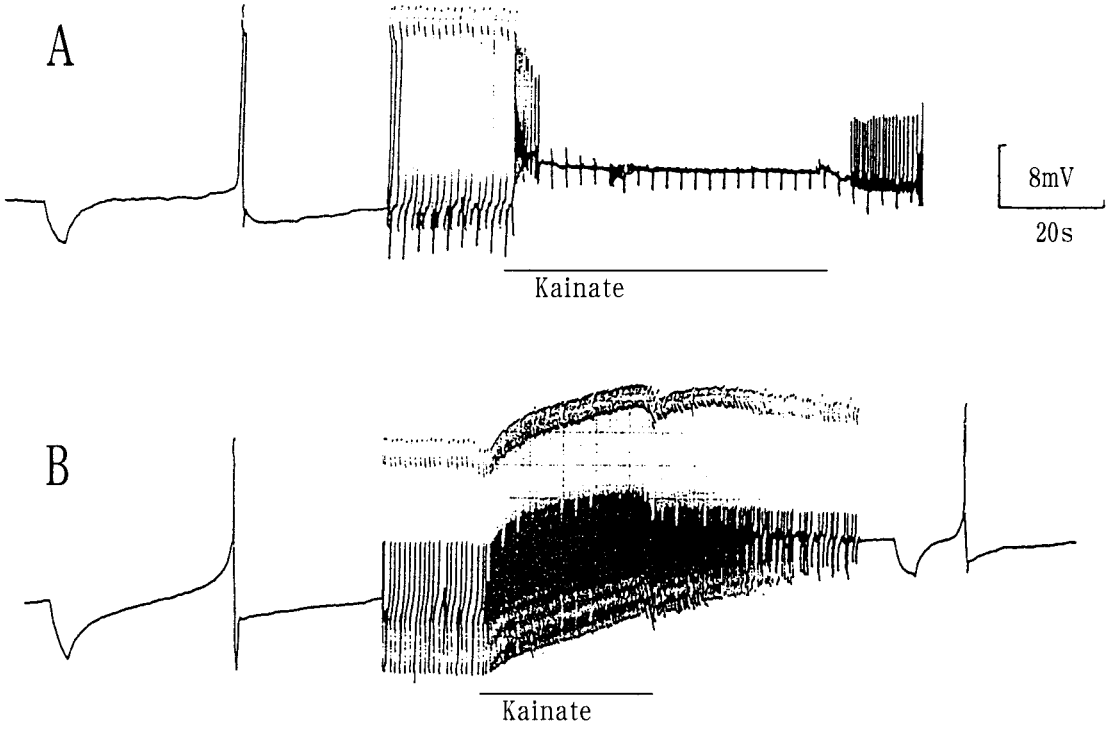

Fig. 4. Effects of reduction in the extracellular chloride concentration and of picrotoxin on the response to kainate (1 mM). (A) All of sodium chloride in a saline was substituted by sodium isethionate. The reduction in chloride failed to cause any changes in the response to kainate. Resting potential, –52 mV. (B) Picrotoxin (0.5 mM), a blocker of GABA-mediated chloride channels, also failed to cause any changes in the response to kainate. Resting potential, –48 mV. Calibration for A also for B.

brane conductance takes place in a saline containing 1 mM NMDA. The experiments of NMDA were made in a Mg-free saline, because voltage-dependent Mg^{2+} block of the NMDA receptor channel has been demonstrated (Nowak *et al.*, 1984). In Fig. 3B the response to NMDA was compared with the response to L-glutamate that elicited a noticeable depolarization associated with an increase in membrane conductance and inhibited spontaneous spikes.

As reported before (Washio, 1994), glutamate has an inhibitory effect on the excitability of the DUM neuron whose response is mediated by changes in the chloride conductance. To examine an involvement of chloride ions on the

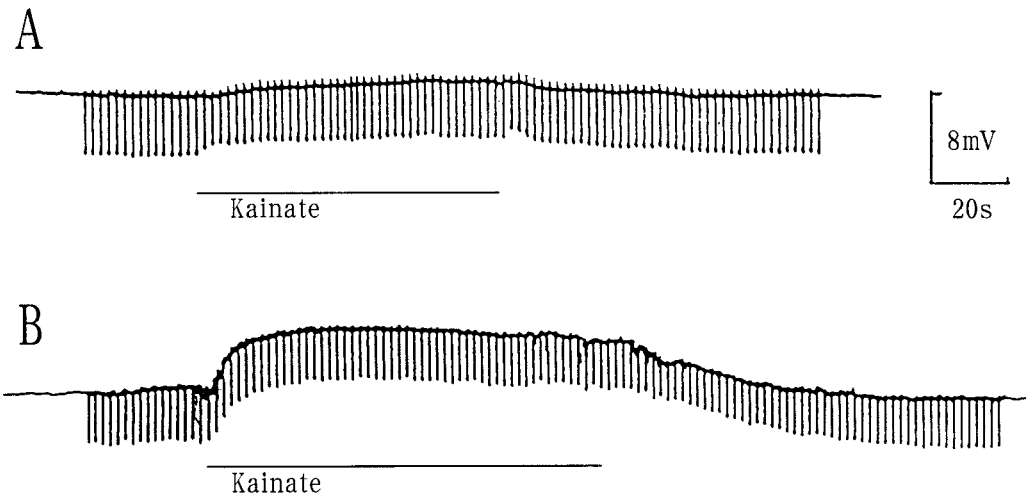

Fig. 5. Effect of CNQX, a selective antagonist for vertebrate AMPA-kainate receptor, on the response to kainate (1 mM) in the same cell. (A) In the presence of CNQX (50 μM), kainate application did not elicit any depolarization. (B) In a normal Ringer's solution kainate elicited a membrane depolarization. In this experiment, the preparation was pretreated with a CNQX-containing saline for about 10min. After recording the response to kainate (as shown in A), the extracellular medium was replaced with a normal saline for 30min before taking the record in B. Resting potential, –42 mV. Calibration for A also for B.

response to the iGluR agonists, the effect of low chloride ions and picrotoxin, a blocker of GABA-mediated chloride channels, was investigated. The response to kainate was not altered by the low chloride saline in an experiment in which all of sodium chloride was substituted by sodium isethionate (Fig. 4A). Picrotoxin at concentrations of 0.5 mM did not affect the response to kainate (Fig. 4B), either. CNQX, a selective antagonist for vertebrate AMPA-kainate receptor (Honoré *et al*., 1988), was tested to find whether it selectively counteracts the effect of kainate on the DUM neurons. The depolarization produced by kainate was blocked by CNQX at concentrations of 50 μM as shown in Fig. 5. In this experiment the preparation was pretreated with a CNQX- containing saline followed by the replacement with a standard saline. The neuron was desensitized after application of kainate. Therefore, the preparation was washed with a standard saline for more than 30 min before a second application of kainate.

Metabotropic glutamate receptors (mGluRs)

A typical response to a selective potent agonist for the group II mGluRs, L-CCG-I, is shown in Fig. 6. In this DUM neuron as shown in Fig. 6, L-CCG-I at concentrations of 0.2 mM elicited a membrane depolarization (2.2mV) associated a conspicuous increase (about 6.5 times as compared with the initial value) in membrane conductance (see MATERIALS AND METHODS). Eventually, the agonist inhibited spontaneous spikes. Although no systematic studies in the changes in membrane conductance has been made, the amount of the increase in membrane conductance produced by the application of L-CCG-I was quite different from neuron to neuron (compare the results obtained in Fig. 6, 7 and 8). The normalized amount of the increase was 3.29±0.89 (n=5). The average values of the depolarization produced by L-CCG-I are shown in Table 1B. Interestingly, in the low chloride saline the response to L-CCG-I was appreciably reduced as shown in Fig. 7B. Also the response to L-CCG-I was blocked partially by 0.1 mM picrotoxin (Fig. 7C), a blocker of GABA-mediated chloride channels. These results suggest a possible involvement of changes in chloride conductance with the response to the mGluR agonist. In some DUM neurons, L-CCG-I elicited a small hyperpolarization

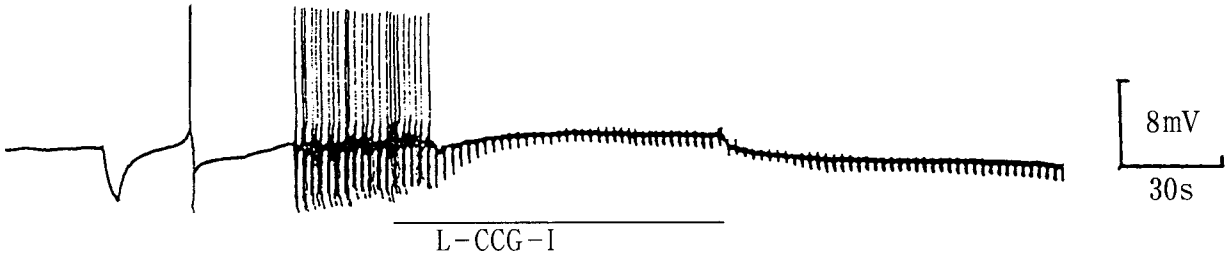

Fig. 6. A typical response to a selective agonist of the group II mGluR, L-CCG-I (0.2 mM). This mGluR agonist elicited a slight depolarization (2.2mV), an increase (about 6.5 times as compared with the initial value) in membrane conductance and inhibited spontaneous spikes. Resting potential, –45 mV.

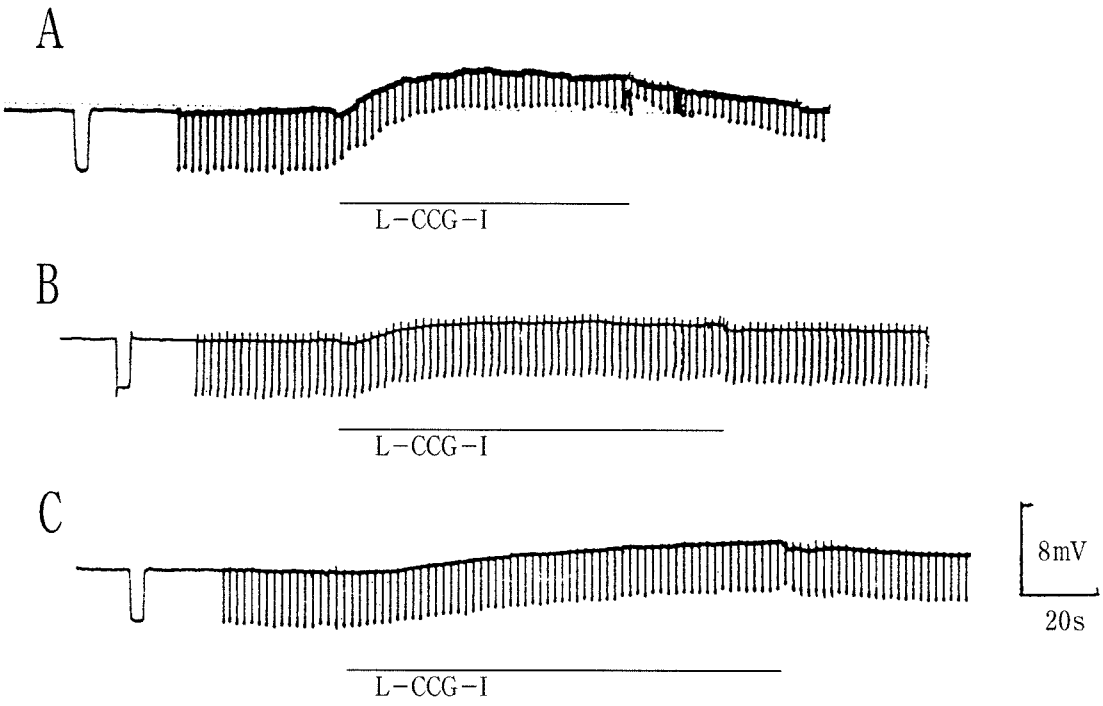

Fig. 7. Effects of reduction in the extracellular chloride concentration and of picrotoxin on the response to L-CCG-I (0.2 mM). (A) control. (B) All of sodium chloride in a saline was substituted by sodium isethionate. Reduction in chloride caused impairment of the response to L-CCG-I. Records A and B were obtained from the same cell. Membrane potential, –45 mV. (C) In the presence of picrotoxin (0.1 mM), a blocker of GABA-mediated chloride channel, also the response to L-CCG-I was impaired. Membrane potential, –40 mV. Calibration for C also for A and B.

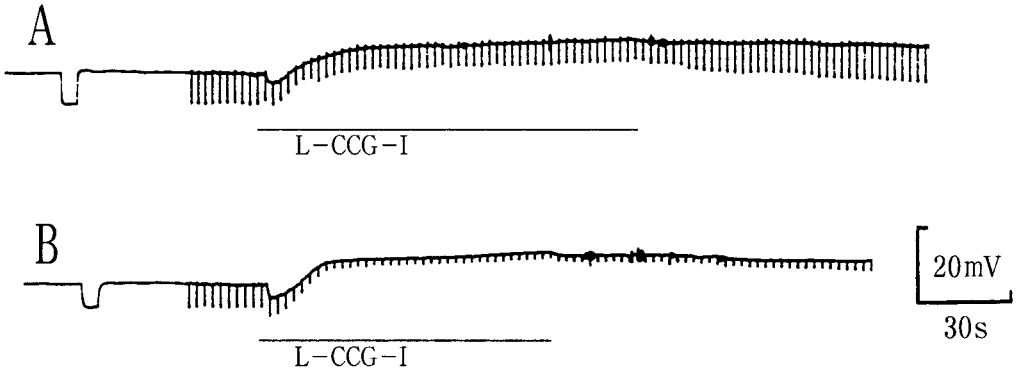

Fig. 8. Effects of a selective mGluR antagonist MCCG on the response to L-CCG-I (0.2 mM). (A) In the presence of MCCG (1.0mM), L-CCG-I elicited a hyperpolarization followed by a long-lasting depolarization. In the experiment, the preparation was pretreated with a MCCG- containing saline for about 20 min. (B) After recording the response to L-CCG-I, the extracellular medium was replaced with a normal Ringer's solution for 30 min. before taking the record in B. Records A and B were obtained from the same cell. Resting potential, –52 mV. Calibration for B also for A.

followed by a long-lasting depolarization as shown in Fig. 8. The depolarization and hyperpolarization produced by L-CCG-I was not affected by the presence of MCCG, the group II- specific antagonist (Roberts, 1995), at concentrations of 1 mM as shown in Fig. 8A. Application of 1S, 3R-ACPD, an agonist acting on group I and II mGluRs, at concentrations of 1 mM resulted in a membrane hyperpolarization in approximately 67% of DUM neurons examined (18 of 27 neurons) as shown in Fig. 9B and C. The membrane hyperpolarization was associated with a slight increase in membrane conductance. In other 9 neurons the agonist had no clear effect on the membrane potentials, but inhibited spontaneous spikes about a few minutes after application of the agonist (Fig. 9A). The hyperpolarization was not affected by the mGluR selective antagonist MCPG (Watkins and Collingride,1994) at concentrations of 0.5 mM in DUM neurons (Fig. 9C). The potassium channel blocker, tetraethylammonium chloride (TEA) at concentrations of 0.5 mM was found not to affect the hyperpolarization, either (not shown). L-AP4, a specific group III mGluR agonist had almost the

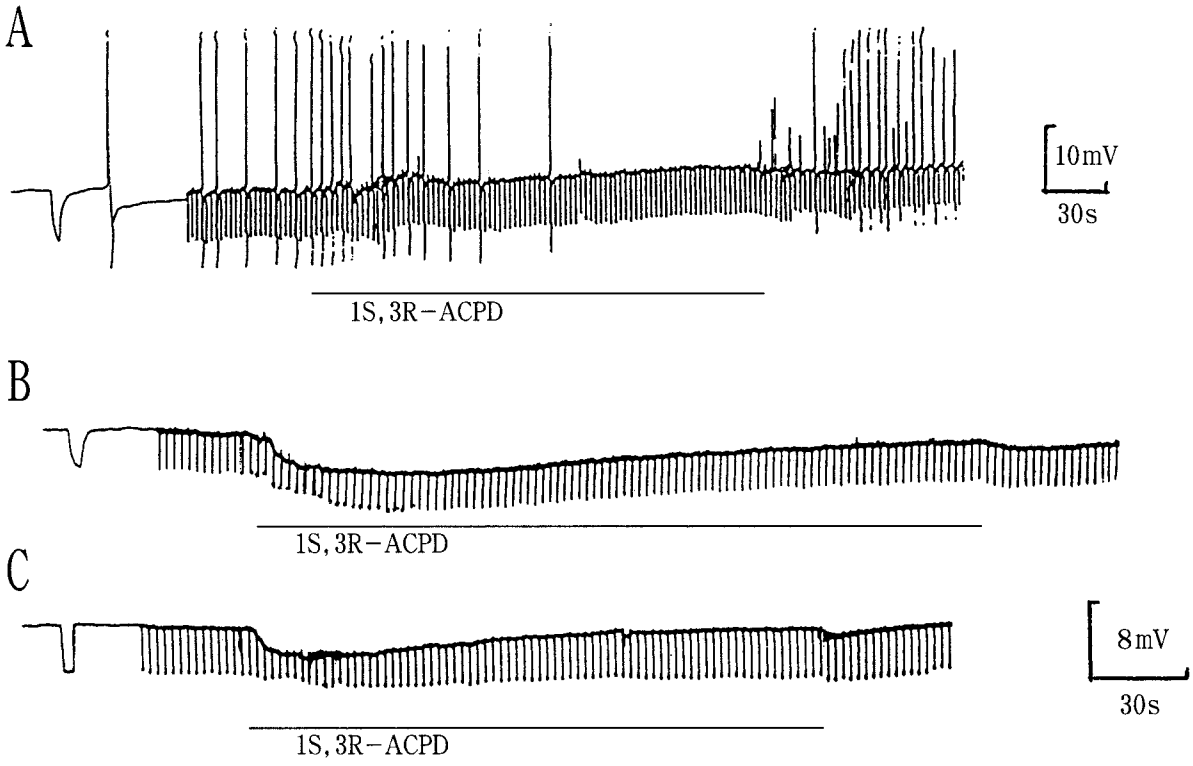

Fig. 9. Effects of group I and II mGluR agonist, 1S, 3R-ACPD (1 mM). (A) The agonist application inhibited spontaneous spikes, but had no clear effect on the membrane potential and membrane conductance in this neuron. Membrane potential, –48 mV. (B) The agonist application elicited a hyperpolarization and a slight increase in membrane conductance in the beginning of the agonist application . Membrane potential, –42 mV. (C). The response to the agonist was not affected by the presence of MCPG (0.5 mM), the mGluR-selective antagonist. Membrane potential, –45 mV. Calibration for C also for B.

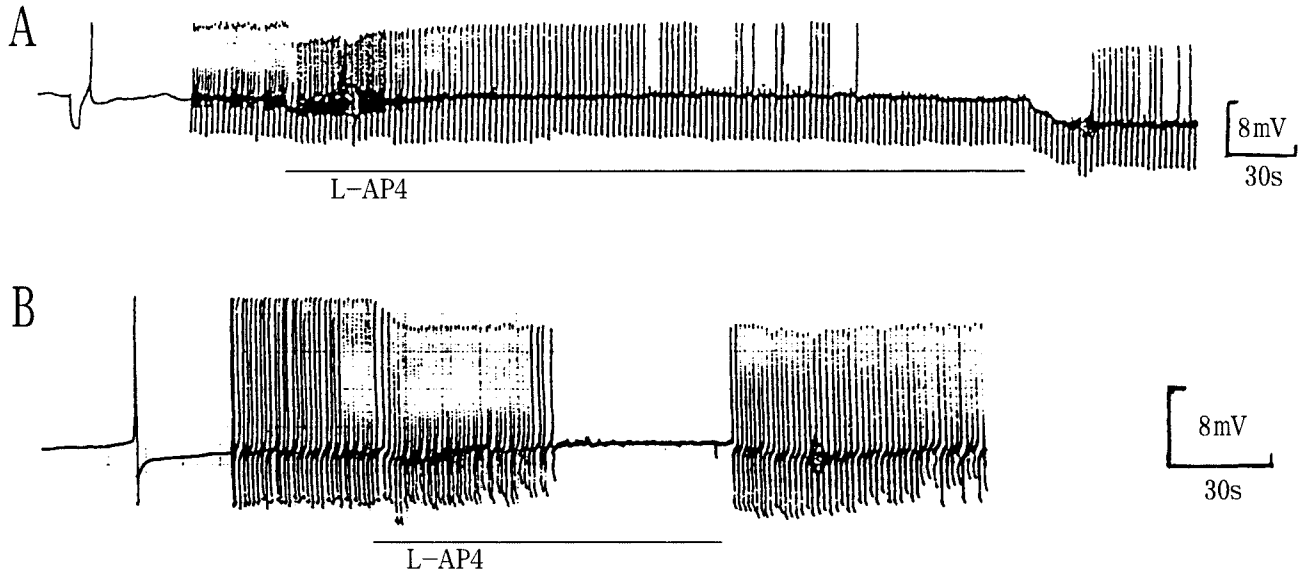

Fig. 10. Effects of a specific group III mGluR agonist, L-AP4 (1 mM). (A) and (B) L-AP4 application elicited acceleration of spike frequency and finally inhibited spontaneous spikes. In B, hyperpolarizing current pulses were not applied. Resting potential, –40 mV in A; –48 mV in B.

same effect as other mGluR agonists had. Two examples are shown in Fig. 10, in which L-AP4 at concentrations of 1mM elicited a small depolarization and inhibited spontaneous spikes in both neurons illustrated. These effects were readily reversed on wash out of the agonist. The average values of the depolarization or hyperpolarization produced by mGluR agonists are summarized in Table 1B. From the results the mGluR agonists evoked depolarization or hyper-

Fig. 11. Depolarizations evoked by exposure to iGluR agonist, kainate (1mM) followed by exposure to mGluR agonist, L-CCG-I (0.1 mM) obtained from the same DUM neuron. Resting potential, –46 mV.

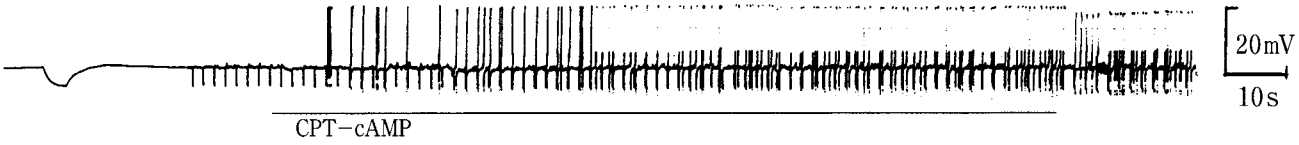

Fig. 12. Effects of a cAMP analog, CPT-cAMP, at concentrations of 1.5 mM on the DUM neuron. Some spikes in the beginning of the record were retouched. Resting potential, –52 mV.

polarization with following relative rank of order of potency: L-CCG-I>1S, 3R-ACPD>L-AP4. Depolarization of the same DUM neuron was detected following exposure to certain iGluR and mGluR agonists. Fig. 11 shows such an experiment in which application of 1 mM kainate was followed by application of 0.2 mM L-CCG-I to the cell body membrane of the same DUM neuron. The result strongly suggests the coexistence of distinct iGluR and mGluR types on the same neuron.

In order to obtain evidence that second messengers are involved in the response to the mGluR agonists, the effect of a membrane permeable analog of cAMP, CPT-cAMP, was tested. Fig. 12 shows a typical response of CPT-cAMP at concentrations of 1.5 mM. The analog of cAMP was never observed to induce a depolarization and changes in membrane conductance in the response, instead induced spontaneous spikes. Thus, the pharmacological properties of CPT-cAMP were not similar to those of mGluR agonists.

DISCUSSION

The present experiments have shown that, in addition to iGluRs, mGluRs are also present on the DUM neurons in the cockroach thoracic ganglia. The iGluR agonists, kainate, AMPA, and quisqualate, depolarized the membrane accompanied by an increase in membrane conductance. The isolation and functional characterization of cDNA encoding a *Drosophila* kainate-selective glutamate receptor that is specifically expressed in the central nervous system have been reported (Ultsch *et al*., 1992). They showed that a *Drosophila* GluR subunit exhibited significant homology to mammalian subunits. NMDA had no appreciable changes in membrane potentials at concentrations of 1 mM, but accelerated the spike frequency in DUM neurons in this experiment. No invertebrates have been shown to exhibit the entire spectrum of agonist and antagonist affinities and ion channel properties described for the vertebrate NMDA receptors, while invertebrates are not entirely insensitive to NMDA (Glantz and Pfeiffer-Linn, 1992). Later, a cDNA which encodes a putative invertebrate NMDA receptor protein was isolated from *Drosophila melanogaster* (Ultsch *et al*., 1993). They suggested the existence of activity-dependent synaptic plasticity in the insect brain. The mGluR agonists, L-CCG-I, 1S,3R-ACPD and L-AP4, also depolarized or hyperpolarized the membrane. Application of 1S, 3R-ACPD at concentrations of 1 mM resulted in membrane hyperpolarization in DUM neurons. Hyperpolarizing responses produced by activation of postsynaptic mGluRs with 1S, 3R-ACPD also have been shown in neurons of the rat basolateral amygdala (Rainnie *et al*., 1994; Holmes *et al*., 1996). They suggested from their studies that the hyperpolarization is G-proteine mediated and results from activation of a TEA-sensitive, calcium-dependent potassium conductance. However, the hyperpolarizing response was insensitive to TEA in DUM neurons. Recently, Parmentier *et al*., (1996) isolated a cDNA encoding mGluR (DmGluRA) from *Drosophila meranogaster*, homologous to the mammalian mGluR family and especially to group II mGluRs. They showed that group II agonists 1S, 3R-ACPD and L-CCG-I were active, and among group III specific agonist, L-AP4 gave a small response, using inositol phosphate (IP) assay to establish the pharmacology of DmGluRA. Their order of potency of the different agonists of DmGluRA was glutamate>L-CCG-I >quisqualate>1S,3R-ACPD>L-AP4, in accordance with the order in DUM neurons obtained in the present experiments. MCCG-I, a specific antagonist of mGluR group II (Roberts, 1995), has been found to be a potent inhibitor of IP production in their work on the pharmacology of DmGluRA (Parmentier *et al*. 1996). On the other hand, in DUM neurons the mGluR selective antagonist MCPG (Watkins and Collingride, 1994) was ineffective to the hyperpolarizing response to 1S, 3R-ACPD at concentra-

tions of 0.5 mM. Also MCCG, the group II-specific antagonist, at concentrations of 1 mM, failed to antagonize the effect of L-CCG-I. Recently, Zhang *et al*. (1999) reported that activation of mGluRs enhances synaptic transmission at the *Drosophila* neuromuscular junction. In their work a transient increase in frequency of miniature synaptic currents was obtained using mGluR agonists, S-4C3HPG, DCCG-IV or 1S, 3S-ACPD, but not iGluR agonists, NMDA, AMPA or kainate, suggesting a potentiation of synaptic transmission via a process involving cAMP.

As mentioned above glutamate has an inhibitory effect on the DUM neuron whose response is mediated by changes in the chloride conductance (Washio, 1994). GABA receptor antagonist picrotoxin blocked completely the response to L-glutamate as well as the response to GABA in the DUM neurons (Washio, 1994). In the present study, picrotoxin has been found to block partially the response to mGluR group II agonist, L-CCG-I, but not iGluR agonist, kainate. The result suggests that a possible involvement of changes in chloride conductance in the response to this mGluR agonist. Recently 1S, 3R-ACPD-induced EPSP-spike (E-S) potentiation was shown to be blocked in the presence of the $GABA_A$ receptor antagonist, picrotoxin (Breakwell *et al*.,1996). From their data it was suggested that the E-S potentiation may be mediated by a reduction in GABAergic transmission from their data. Also, inhibitory glutamate receptor (IGluR) channels have been cloned from *Caenorhabditis elegans* and *Drosophila melanogaster* (Cully *et al*., 1994,1996). In the crustacean stomatogastric ganglion (STG), IGluRs have been shown to mediate recurrent synaptic inhibition central to the rhythmogenic capabilities of its embedded neural circuits (Cleland and Selverston, 1998). They showed failure of direct efforts to cross-activate or cross-desensitize the IGluR expressed in cultured spiny lobster STG neurons with high concentration of GABA, indicating that L-glutamate and GABA activate distinct receptors in this neurons. The result is in accord with the one obtained in DUM neurons (Fig. 1). However, L-glutamate and GABA cross-activate and/or cross-desensitize the same receptor channels in several species (Cleland, 1996). Interestingly, millimolar concentrations of quisqualate, kainate, AMPA and NMDA each failed to evoke any response in this cultured neurons (Cleland and Selverston, 1995).

In the present work, activation of iGluR and mGluR agonists have been studied using DUM neurons, and the action of these agonists on the same DUM neuron have also been examined (Fig. 11). The result strongly suggests the coexistence of distinct iGluR and mGluR types on the same DUM neuron with a “mixed” pharmacological property in the analogy of a “mixed” nicotinic-muscarinic pharmacology of housefly brain (Eldefrawi and O’Brien,1970). Coexistence of distinct nicotinic and muscarinic receptor subtypes on the same neuron has been reported in insect nervous tissues (Lapied *et al*., 1990; Bai *et al*., 1992; Benson, 1992). On the other hand, attempts to obtain the evidence that second messengers are involved in the response to the mGluR agonists have not been successful in the DUM neurons. Further studies are required to find out whether or not mGluR are coupled to second messenger systems in the DUM neurons. All taken together, the extrasynaptic glutamate receptors on DUM neuronal somata are pharmacologically distinct from the corresponding mammalian glutamate receptors. In conclusion, although the functional roles of these receptors are unknown, it might be possible then that these extrasynaptic receptors have a modulatory effect on the excitability of the DUM neurons.

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