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L-GLUTAMATE RECEPTORS ON THE CELL BODY MEMBRANE OF AN IDENTIFIED INSECT MOTOR NEURONE

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Summary

Current-clamp experiments on an identified neurone have demonstrated the presence of L-glutamate receptors in the insect central nervous system. The cell body of the fast coxal depressor motor neurone (D_f) in the metathoracic ganglion of the cockroach *Periplaneta americana* exhibits a hyperpolarizing response to L-glutamate, accompanied by an increase in membrane conductance. The response is dependent on both intracellular and extracellular chloride concentration, but is not affected by changes in potassium concentration. The hyperpolarization reverses at −82 mV (the equilibrium potential for chloride), is mimicked by the action of L-aspartate, blocked by the antagonists picrotoxin and γ-D-glutamylglycine (γ-DGG) at high concentrations ($1{\cdot}0\times10^{-4}$ mol l^{-1}), and is enhanced by L-amino phosphonobutyrate (L-APB). The response is insensitive to glutamate diethyl ester (GDEE), *cis*-2,3-piperazine dicarboxylic acid (*cis*-2,3-PDA) and D-amino phosphonobutyrate (D-APB). The L-glutamate-activated increase in chloride conductance does not cross-desensitize with the γ-aminobutyric acid (GABA) response on the same cell. It is less sensitive than the GABA response to block by picrotoxin. In addition, γ-DGG specifically blocks the L-glutamate receptor.

A depolarizing response is elicited by kainate and quisqualate; it is associated with an increase in conductance, and exhibits a much slower time course than the response to L-glutamate, indicating a different underlying mechanism. L-Cysteate produces a small depolarizing response of similar time course to that produced by L-glutamate. L-Homocysteate and *N*-methyl-D-aspartate (NMDA) are ineffective on the cell body membrane when applied at concentrations up to $1{\cdot}0\times10^{-3}$ mol l^{-1}. This first detailed description of the properties of L-glutamate receptors on an identified insect neurone reveals that they are not readily accommodated in the existing classification of receptor subtypes, based on vertebrate pharmacology.

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Key words: L-glutamate receptor, chloride channel, motor neurone, cockroach (*Periplaneta americana*).

Introduction

Considerable evidence now exists for a neurotransmitter function for L-glutamate in the central and peripheral nervous systems of both vertebrates and invertebrates. Subtypes of L-glutamate receptors in vertebrates were first proposed to consist of a 'glutamate-preferring' receptor, binding to the extended conformation of L-glutamate, and an 'aspartate-preferring' receptor binding to the folded conformation (Johnston *et al.* 1974). Watkins & Evans (1981) identified three subtypes of L-glutamate receptor in the vertebrate central nervous system (CNS), based on their agonist profiles and the selective actions of antagonists. *N*-Methyl-D-aspartate (NMDA)-type receptors activate channels permeable to monovalent cations and calcium (MacDermott *et al.* 1986), which are blocked by magnesium in a voltage-dependent manner (Mayer *et al.* 1984; Nowak *et al.* 1984). NMDA receptors are also blocked by a number of other selective antagonists such as D-APV (D-amino phosphonovalerate), PCP (phencyclidine) and MK-801 (Kemp *et al.* 1987; Mayer & Westbrook, 1987). The quisqualate-type receptor is preferentially activated by quisqualate and the conformationally restricted analogue 5-methyl-4-isoxazole propionic acid (AMPA), but no specific antagonists are known. Kainate-type receptors, activated preferentially by kainate, are thought to have different agonist selectivity from both NMDA and quisqualate receptors (Foster & Fagg, 1984). A number of broad-spectrum antagonists will reduce quisqualate and kainate responses in preference to NMDA responses. These include γ-DGG (γ-D-glutamylglycine), *cis*-2,3-PDA (*cis*-2,3-piperazine dicarboxylic acid), GDEE (glutamate diethyl ester), GAMS (γ-D-glutamyl aminomethyl sulphonic acid) and CNQX (6-cyano-7-nitroquinaline-2,3-dione) (Ganong *et al.* 1986; McLennan & Lodge, 1979; Mayer & Westbrook, 1987; Honoré *et al.* 1988). Both kainate and quisqualate responses are mediated by sodium and potassium (Mayer & Westbrook, 1987). In contrast, little is known of the properties and/or existence of subtypes of L-glutamate receptors in the insect central nervous system. Here we provide the first detailed description of an L-glutamate receptor on an identified insect neurone.

There is good evidence that L-glutamate is an excitatory transmitter at many arthropod neuromuscular junctions (Shinozaki, 1988) and its action on these junctions has been extensively studied. Locally applied L-glutamate has been shown to activate receptors on crayfish muscle (Takeuchi & Takeuchi, 1964; Takeuchi & Onodera, 1973; Dudel, 1975; Shinozaki, 1980) and insect muscle (Jan & Jan, 1976; Lea & Usherwood, 1973; Usherwood, 1980). At the locust neuromuscular junction, a depolarizing response has been observed in response to L-glutamate and, at extrajunctional receptors, biphasic responses have been detected (Cull-Candy, 1976). The hyperpolarizing, 'H'-phase of this biphasic response is mediated by an enhanced chloride conductance, and a pure hyperpolarizing response can be elicited by applying ibotenate. Both hyperpolarizing responses are blocked by high concentrations of picrotoxin (Lea & Usherwood, 1973; Cull-Candy, 1976). Chloride-mediated, picrotoxin-sensitive responses to

L-glutamate have also been observed on certain crustacean gastric muscles (Lingle & Marder, 1981).

Crustacean and molluscan neurones exhibit three different types of response to L-glutamate (Marder & Paupardin-Tritsch, 1978; Roberts & Walker, 1982; Mat Jais *et al.* 1983; Walker *et al.* 1976; Yarowsky & Carpenter, 1976; Kehoe, 1978): a potassium-mediated, slow hyperpolarization; a fast, chloride-dependent hyperpolarization, sensitive to high concentrations of picrotoxin; and a fast, sodium-dependent depolarization.

Although the action of L-glutamate upon insect muscle has been well studied, relatively little is known of its effects in the insect central nervous system. L-Glutamate, L-aspartate, kainate and quisqualate elicit a variety of responses in unidentified cultured locust and cockroach neurones (Giles & Usherwood, 1985; Horseman *et al.* 1988), but no systematic examination of the actions of L-glutamate and L-glutamate receptor ligands has been performed in the insect nervous system. The identifiable fast coxal depressor (D_f) motor neurone in the metathoracic ganglion of the cockroach *Periplaneta americana* responds to application of L-glutamate, L-aspartate, kainate and quisqualate (Wafford & Sattelle, 1986). This cell offers a convenient preparation on which to carry out a detailed investigation of the pharmacology and channel properties of insect neuronal L-glutamate receptors.

Materials and methods

Male adult cockroaches *(Periplaneta americana)* were used in all experiments. They were reared at 24°C, with freely available food and water.

The cockroach nerve cord was isolated and the metathoracic ganglion desheathed using fine forceps. The preparation was mounted under saline in a 3 ml Perspex chamber and perfused with saline consisting of (in mmol l^{-1}): NaCl, 214; KCl, 3·1; $CaCl_2$, 9·0; sucrose, 50·0; Tes, 10·0, adjusted to pH 7·2 with 1·0 mol l^{-1} NaOH. Saline flow rate was approximately 0·5 ml min^{-1}, and all experiments were performed at 18–20°C. The fast coxal depressor motor neurone (D_f) was visually located and impaled with two 15–20 MΩ electrodes filled with 2·0 mol l^{-1} potassium acetate. Amino acids were ionophoresed as anions, using negative current, directly onto the surface of the cell from 5–10 MΩ micropipettes (filled with 1·0 mol l^{-1} solutions at pH 8·0). Leakage was prevented by applying a small outward retaining current (10–50 nA). Ionophoretic currents were measured using a virtual-earth circuit. Bath-applied drugs were dissolved in saline. Except where noted, cells were current-clamped at −60 mV and input resistance was monitored by passing 200 ms hyperpolarizing pulses of 1–2 nA at 2 s intervals. Because the I/V curve for motor neurone D_f is relatively linear between −40 mV and −120 mV (Pinnock *et al.* 1988), membrane conductances were calculated from input resistance measurements before and during drug application and conductance changes were used to plot dose–response relationships. Antagonists were bath-

applied, and agonists were tested at intervals up to 30 min after initial application. Responses were recorded on a pen recorder and oscilloscope. The quisqualate analogues, L-glutamic acid *N*-thiocarboxyanhydride (L-GANTA), D,L-hydantoin-propionic acid (DL-HPA) and methyl-D,L-2-thiohydantoin propionic acid were generously supplied by Dr D. Yamamoto from the Neurosciences Division of the Mitsubishi-Kasei Institute of Life-Sciences, Tokyo. L-Cysteate, L-homocysteate, *N*-methyl-D-aspartate (NMDA), D-amino phosphonobutyrate (D-APB), L-amino phosphonobutyrate (L-APB), γ-D-glutamylglycine (γ-DGG), *cis*-2,3-piperazine dicarboxylic acid (*cis*-2,3-PDA) and glutamate diethyl ester (GDEE) were obtained from Cambridge Research Biochemicals. All other compounds were purchased from Sigma Chemicals.

Results

When bath-applied or ionophoresed onto the surface of the cell body of motor neurone D_f, L-glutamate elicited a membrane hyperpolarization, together with an increase in membrane conductance. The response was faster than the hyperpolarization elicited by GABA on the same cell, and desensitized slightly following multiple applications. The response increased to a maximum on increasing the ionophoretic dose (Fig. 1), and Hill plots from such data yielded a coefficient of 2·5, indicating that more than one molecule of L-glutamate must be bound to activate the channel.

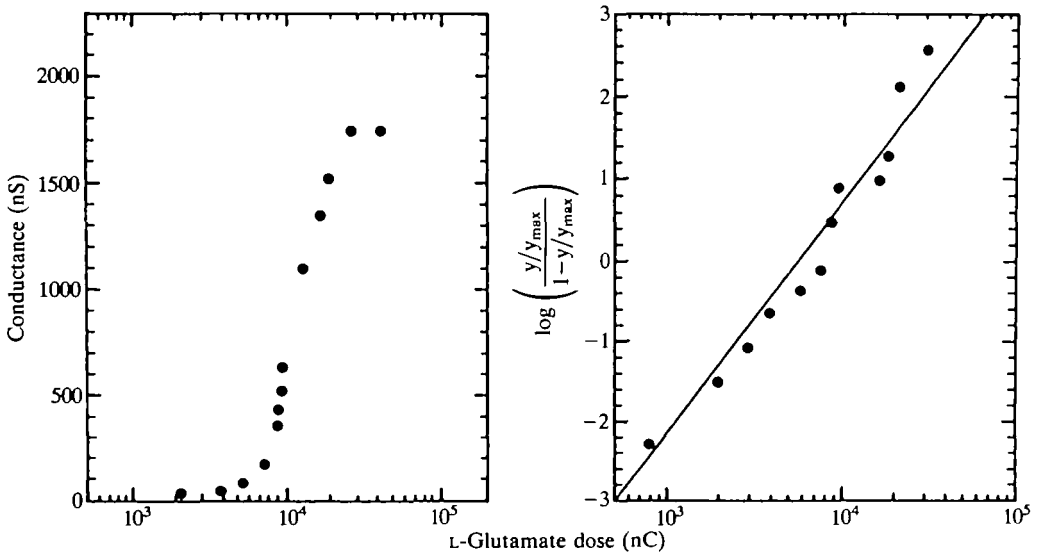

Fig. 1. (A) Dose–response curve, conductance (nS) *versus* dose (nC), for the action of L-glutamate on motor neurone D_f. L-Glutamate application is in 1 s pulses at low doses, increasing to 12 s at higher doses. Membrane potential is −60 mV. (B) Hill plot from the data shown in A; the Hill coefficient determined from the slope of the line is approximately 2·5. Data are from a single neurone, but are typical of four other experiments.

Ionic basis of the L-glutamate response

The reversal potential for the L-glutamate response was -82 ± 4 mV (mean ± S.E.M., $N = 8$), corresponding to the equilibrium potential for chloride (-80 ± 3 mV) in this particular cell (Pinnock *et al.* 1988). Substitution of caesium for potassium in the saline had no effect on the L-glutamate response (Fig. 2A,B). However, when chloride was completely replaced with isethionate, a positive shift in the L-glutamate reversal potential was observed 30 min after the substitution (Fig. 2C). Chloride was injected into the cell using 2·0 mol l^{-1} potassium chloride in the current-passing electrode. In this way the internal concentration was raised and the chloride equilibrium potential (E_{Cl}) was shifted to a more positive value. When L-glutamate was applied under these circumstances, a depolarizing response was elicited (Fig. 2D), again indicating a role for chloride ions in the actions of

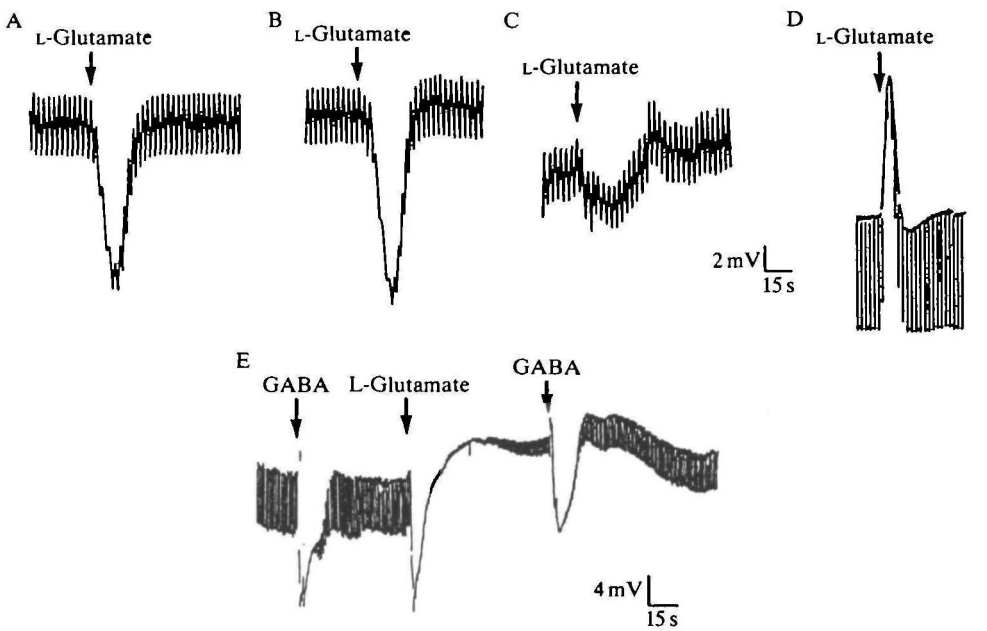

Fig. 2. Ionic basis of the L-glutamate response on motor neurone D_f. Ionophoretic application of L-glutamate (5000 nA for 2 s) under different conditions: (A) in normal saline, 2·0 mol l^{-1} potassium acetate electrodes; (B) in potassium-free saline (substituted with CsCl), 2·0 mol l^{-1} potassium acetate electrodes; (C) in chloride-free saline (substituted with isethionate), 2·0 mol l^{-1} potassium acetate electrodes; (D) in normal saline, employing a 3·0 mol l^{-1} potassium chloride current-injection electrode. Recordings A–C are from a single neurone, and D is from a separate cell. Data are typical of three similar experiments. Membrane potential is −60 mV. (E) Absence of GABA and L-glutamate cross-desensitization on motor neurone D_f. A dose of ionophoretically applied GABA (1000 nA for 2 s) precedes an excess dose of L-glutamate, directly applied into the bath (final concentration, $1{\cdot}0 \times 10^{-2}$ mol l^{-1}), and is followed almost immediately by a repeated application of the initial dose of GABA. Data are from a single neurone, but are typical of three such experiments. Membrane potential is −60 mV.

L-glutamate on this cell. Cross-desensitizing experiments were attempted. In these, GABA was applied ionophoretically, producing a hyperpolarization. This was followed by a large dose of bath-applied L-glutamate, then a repeat, identical ionophoretic dose of GABA (Fig. 2E). The second GABA response was not reduced by the intervening high dose of L-glutamate.

Actions of L-glutamate agonists

A variety of L-glutamate agonists were either bath-applied or ionophoresed onto the cell body membrane of motor neurone D_f. Kainate and quisqualate produced depolarizations with a long time course, when bath-applied at $1{\cdot}0\times10^{-5}$ mol l^{-1} (Fig. 3A,B), kainate being the most potent.

L-Aspartate elicited a smaller hyperpolarization than the equivalent dose of L-glutamate (Fig. 4), though reversal potentials for L-glutamate- and L-aspartate-induced responses were similar. L-Cysteate produced a small depolarization, accompanied by an increase in conductance (Fig. 4). In the same cell L-glutamate elicited a hyperpolarization, suggesting either a different ionic mechanism to that of the L-glutamate and L-aspartate responses or a mixed receptor response. L-Homocysteate had no effect when bath-applied at concentrations up to $1{\cdot}0\times10^{-3}$ mol l^{-1}. NMDA was also tested by bath-application onto motor neurone D_f, with no effect observed at concentrations up to $1{\cdot}0\times10^{-3}$ mol l^{-1}. A series of analogues of quisqualate known to have agonist activity at the insect neuromuscular junction (Fukami, 1986; Miyamoto *et al.* 1985) were also tested for agonist potency on this receptor. L-Glutamic acid-*N*-thiocarboxyanhydride (L-GANTA), D,L-hydantoin propionic acid (DL-HPA) and methyl-D,L-2-thiohydantoin propionic acid (MO-105) (Fig. 3) were each bath-applied at $1{\cdot}0\times10^{-3}$ mol l^{-1} but all were without effect on motor neurone D_f.

Actions of putative L-glutamate antagonists

A number of different antagonists were tested on the L-glutamate response of motor neurone D_f. Picrotoxin produced a dose-dependent, non-competitive block (Fig. 5A), but very little reduction of the response was detected at concentrations below $1{\cdot}0\times10^{-5}$ mol l^{-1}, demonstrating a much lower sensitivity to picrotoxin than the GABA response, where complete block was detected at $1{\cdot}0\times10^{-6}$ mol l^{-1}. The effects of the phosphono analogues of L-glutamate, L- and D-amino phosphonobutyrate (L- and D-APB), were examined. At concentrations up to $1{\cdot}0\times10^{-4}$ mol l^{-1} D-APB was ineffective, whereas L-APB produced an enhancement of the L-glutamate response at $1{\cdot}0\times10^{-5}$ mol l^{-1} (Fig. 6), shifting the dose–response curve to the left. The dipeptide antagonist γ-D-glutamylglycine (γ-DGG) inhibited the L-glutamate response non-competitively at $1{\cdot}0\times10^{-4}$ mol l^{-1} (Fig. 5B), but was inactive on GABA responses. The other antagonists tested, glutamate diethyl ester (GDEE) and *cis*-2,3-piperazine dicarboxylic acid (*cis*-2,3-PDA), had no effect on the L-glutamate response. As NMDA was without effect, the selective NMDA receptor antagonists L-AP5 and L-AP7 were not tested.

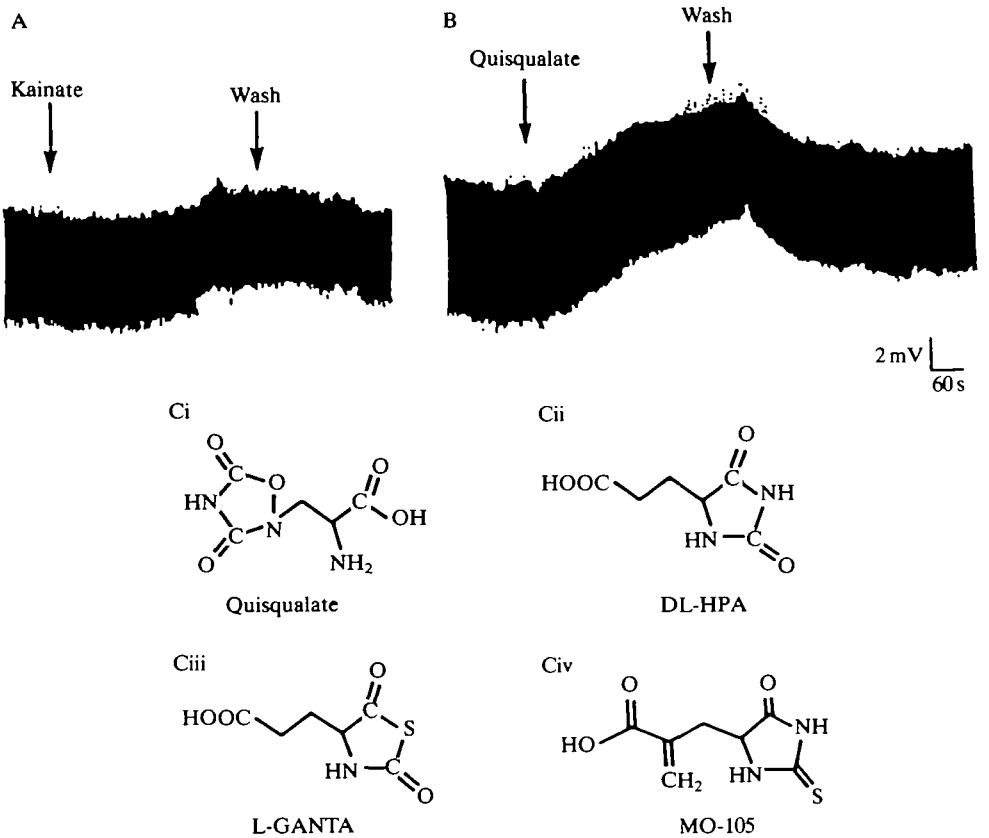

Fig. 3. Effects of kainate and quisqualate on membrane potential and input resistance of motor neurone D_f. (A) Kainate is bath-applied ($1{\cdot}0\times10^{-5}$ mol l^{-1}). (B) Quisqualate is bath-applied ($1{\cdot}0\times10^{-4}$ mol l^{-1}). Constant-current hyperpolarizing pulses (2 nA, 400 ms) are applied through the current-injecting electrode to measure membrane resistance. Data are typical of three such experiments. Membrane potential is −60 mV. (C) Structures of the analogues of quisqualate applied to the cell body of motor neurone D_f: (i) quisqualate; (ii) DL-HPA (D,L-hyantoin propionic acid); (iii) L-GANTA (L-glutamic acid *N*-thiocarboxyanhydride); (iv) MO-105 (methyl-D,L-2-thiohydantoin propionic acid).

Discussion

The hyperpolarizing response of the cockroach fast coxal depressor motor neurone (D_f) to L-glutamate demonstrates the presence of an inhibitory L-glutamate receptor in insects. This is the first observation of this type of response in the insect nervous system *in vivo*, although hyperpolarizations have been seen in unidentified cultured insect neurones (Giles & Usherwood, 1985; Horseman *et al.* 1988), and at extrajunctional sites on locust muscle (Cull-Candy, 1976). Inhibitory responses to L-glutamate are also found in the molluscan and crustacean central nervous system and have been the subject of a number of studies (Mat Jais *et al.* 1983; Piggott *et al.* 1975; Marder & Eisen, 1984).

Hill plots of the dose–response data yielded a coefficient greater than one for the L-glutamate-induced conductance change on motor neurone D_f, suggesting that a minimum of two L-glutamate molecules must bind in order to open the L-glutamate-activated ion channel. The L-glutamate response was abolished in low-chloride saline, reversed when chloride was injected into the cell and was sensitive to picrotoxin, indicating coupling of the receptor to a chloride channel. Chloride-dependent inhibitory responses to L-glutamate and distinct chloride-linked L-aspartate-specific hyperpolarizations have been recorded in *Aplysia* (Yarowsky & Carpenter, 1976). The hyperpolarizations in locust muscle were observed as part of a biphasic response to L-glutamate (Lea & Usherwood, 1973; Cull-Candy, 1976), and pure hyperpolarizing L-glutamate responses have been seen in crustacean gastric muscle (Lingle & Marder, 1981), where they were mediated by chloride ions and blocked by picrotoxin. There is no evidence for a potassium contribution to the motor neurone D_f response to L-glutamate, but potassium-mediated hyperpolarizations and sodium-mediated depolarizations are often observed in molluscan and crustacean central neurones (Oomura *et al.* 1974;

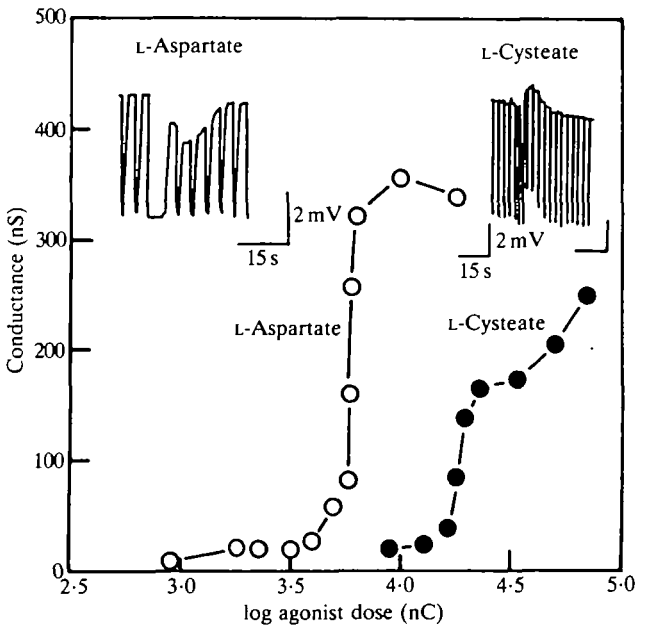

Fig. 4. Dose–response curves for ionophoretically applied L-aspartate (○) and L-cysteate (●). Application is by 1 s pulses at low doses, increasing to 12 s at higher doses. Data are from two separate neurones and are representative of four similar experiments (as shown in the inset, responses to L-cysteate are depolarizing, whereas L-aspartate responses are hyperpolarizing). Membrane potential is held at −60 mV for L-aspartate application and −70 mV for L-cysteate application. Inset shows typical current-clamp recordings of a response to L-aspartate (dose of 6300 nC) and a response to L-cysteate (dose of 25 000 nC).

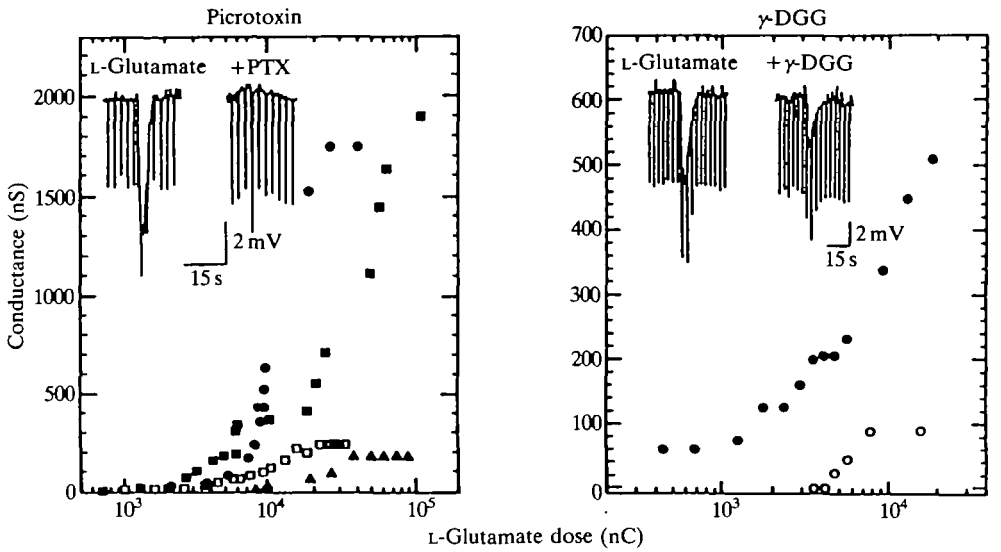

Fig. 5. (A) Picrotoxin actions on the L-glutamate response of motor neurone D_f. Dose–response curves show the effects of ionophoretically applied L-glutamate after 30 min application of bath-applied picrotoxin at various concentrations: (●) control dose–response curve for L-glutamate. Dose–response following: (■) $1{\cdot}0\times10^{-5}$ mol l^{-1} picrotoxin; (□) $1{\cdot}0\times10^{-4}$ mol l^{-1} picrotoxin and (▲) $1{\cdot}0\times10^{-3}$ mol l^{-1} picrotoxin. Data are from a single neurone but are typical of five similar experiments. Membrane potential is −60 mV. Inset shows a typical current-clamp recording of an L-glutamate hyperpolarization (dose 2000 nC) in the absence and presence of $1{\cdot}0\times10^{-3}$ mol l^{-1} picrotoxin (PTX). (B) Effects of γ-D-glutamylglycine (γ-DGG) on the L-glutamate response of motor neurone D_f. Dose–response curves for ionophoretically applied L-glutamate: (●) in normal saline; (○) after 30 min application of $1{\cdot}0\times10^{-4}$ mol l^{-1} γ-DGG. Data are from a single neurone but are typical of three such experiments. Membrane potential is −60 mV. Inset shows a typical current-clamp recording of an L-glutamate hyperpolarization (dose 5000 nC) in the absence and presence of $1{\cdot}0\times10^{-4}$ mol l^{-1} γ-DGG.

Yarowsky & Carpenter, 1976; Marder & Paupardin-Tritsch, 1978; Roberts & Walker, 1982).

The response of motor neurone D_f to L-glutamate can be desensitized by repeated applications in close succession. However, the desensitization is small and recovery is rapid. For this insect cell it has been shown that L-glutamate does not cross-desensitize with GABA, indicating that the two amino acids are acting on separate receptor populations. This is supported by a differential sensitivity to picrotoxin, and the selective antagonism of L-glutamate by γ-DGG. In *Aplysia* neurones, cross-desensitization has been observed with L-glutamate and GABA, leading to the suggestion that the two receptors are linked to the same ion channel (King & Carpenter, 1987).

Kainate and quisqualate, when applied to motor neurone D_f, elicited long, slow, depolarizing responses. This suggested the activation of different ion

channels from those controlled by L-glutamate. In invertebrates, kainate has only been seen to elicit a depolarization, whereas quisqualate shows agonist activity at both depolarizing and hyperpolarizing receptors (Walker, 1976; James *et al.* 1980). Kainate elicits depolarizations in cultured locust neurones (Giles & Usherwood, 1985) and locust nerve cord (Evans & Kirkpatrick, 1983).

In the cockroach motor neurone, quisqualate may be activating the same 'kainate-type' receptor, as a similarly slow time course is observed. In crustacean muscle, quisqualate is 500–1000 times more potent than L-glutamate (Shinozaki & Shibuya, 1974) and it is also a potent agonist at the locust neuromuscular junction. Studies using analogues of quisqualate show the receptor to be highly specific, with only small alterations in structure significantly reducing the activity (Boden *et al.* 1986). A number of structural analogues, active at the quisqualate receptor in the muscle of the mealworm larva *Tenebrio molitor*, were tested on motor neurone D_f. None of these compounds was more active than quisqualate on the muscle preparation (Miyamoto *et al.* 1985) and none of them elicited any response when bath-applied at $1{\cdot}0\times10^{-3}$ mol l^{-1}.

The sulphonic analogue of L-aspartate, L-cysteate, elicited a fast depolarizing

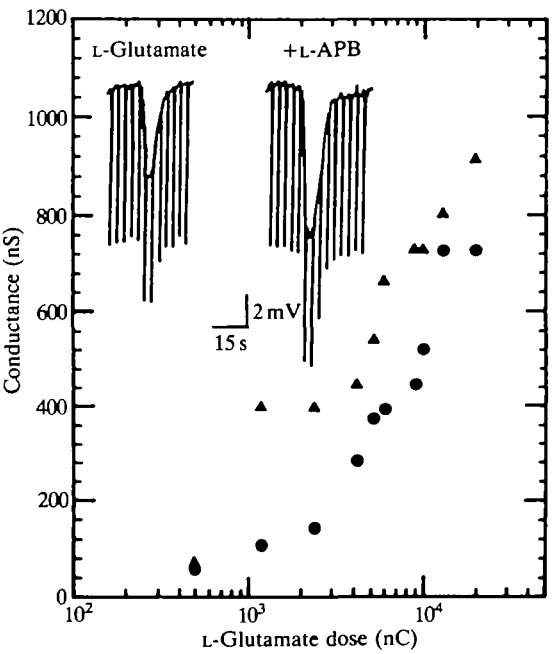

Fig. 6. Effects of L-amino phosphonobutyrate (L-APB) on the L-glutamate response of motor neurone D_f. Dose–response curves for ionophoretically applied L-glutamate: (●) in normal saline; (▲) after 30 min application of $1{\cdot}0\times10^{-4}$ mol l^{-1} L-APB. Data are from a single neurone but are typical of four similar experiments. Membrane potential is −60 mV. Inset shows typical current-clamp recordings of an L-glutamate hyperpolarization (dose 2500 nC) in the absence and presence of $1{\cdot}0\times10^{-4}$ mol l^{-1} L-APB.

response in motor neurone D_f. This, together with the related compound L-homocysteate, shows agonist-like activity on vertebrate and invertebrate excitatory amino acid receptors. However, L-homocysteate had no effect on motor neurone D_f when bath-applied at concentrations up to $1{\cdot}0\times10^{-3}$ mol l^{-1}. L-Cysteate may be relatively more active on preparations from invertebrates than those from vertebrates, being similar in potency to L-aspartate on snail neurones (Szczepaniak & Cottrell, 1973; Piggott *et al.* 1975). Binding studies also confirm this relatively high affinity for L-cysteate in insect nervous tissue (Sherby *et al.* 1987). The time course of the L-cysteate response on motor neurone D_f differed considerably from that of kainate and quisqualate, suggesting a different mechanism of action.

NMDA had no effect on motor neurone D_f when bath-applied at concentrations of $1{\cdot}0\times10^{-3}$ mol l^{-1}. This is consistent with all other studies on invertebrates in which the effects of NMDA have been examined. Evidence available so far indicates that NMDA receptors are only present in vertebrates.

Of the antagonists tested on the L-glutamate response of motor neurone D_f, picrotoxin effected a non-competitive type of inhibition at $1{\cdot}0\times10^{-4}$ mol l^{-1} and complete block at $1{\cdot}0\times10^{-3}$ mol l^{-1}. This is a higher concentration of picrotoxin than that required to block GABA responses from the same neurone, where effects could be seen at $1{\cdot}0\times10^{-6}$ mol l^{-1} (Sattelle *et al.* 1988). Other studies of the actions of picrotoxin on inhibitory L-glutamate receptors also show a low sensitivity to this antagonist (Cull-Candy, 1976; Mat Jais *et al.* 1983; Marder & Paupardin-Tritsch, 1978; Lingle & Marder, 1981).

On motor neurone D_f, γ-DGG produced non-competitive inhibition at $1{\cdot}0\times10^{-4}$ mol l^{-1}. No effect was observed on the GABA response. This dipeptide antagonist blocks all three vertebrate receptor subtypes with low potency, being slightly more effective at NMDA and kainate receptors than at quisqualate-sensitive sites (Crunelli *et al.* 1985; Davies & Watkins, 1979). In leech Retzius neurones γ-DGG reduces responses to L-glutamate, ibotenate, kainate and quisqualate, blocking both excitatory and inhibitory phases at $5{\cdot}0\times10^{-4}$ mol l^{-1}. In this annelid preparation, γ-DGG is more effective on responses to L-glutamate and ibotenate than it is on quisqualate responses (Mat Jais *et al.* 1984*a*,*b*).

D-APB was ineffective at $1{\cdot}0\times10^{-4}$ mol l^{-1}, but the L-isomer enhanced the L-glutamate response at $1{\cdot}0\times10^{-5}$ mol l^{-1}, having no effect on the GABA response. DL-APB acts as an L-glutamate antagonist on the locust neuromuscular junction and inhibits binding of L-glutamate to hydrophobic proteolipids extracted from locust muscle (Cull-Candy *et al.* 1976). The enhancement by L-APB of L-glutamate responses in motor neurone D_f may be due to an allosteric interaction with an associated site, just as benzodiazepines can potentiate the GABA response (Sattelle *et al.* 1988). It may also be due to a reduced uptake of L-glutamate (Evans, 1975; Faeder & Salpeter, 1970), thereby increasing the concentration of L-glutamate in the vicinity of the receptors. The non-specific antagonists GDEE and *cis*-2,3-PDA did not affect the inhibitory L-glutamate response on motor neurone D_f at concentrations up to $1{\cdot}0\times10^{-4}$ mol l^{-1}.

These results provide evidence for an L-glutamate receptor on motor neurone D_f, linked to a chloride channel. The pharmacological properties of this site are broadly similar to those of the chloride-channel-linked receptors identified on molluscan neurones, crustacean neurones and locust muscle, but with a number of important differences, notably the effects of γ-DGG and L-APB. There is also evidence for a kainate–quisqualate-type receptor on motor neurone D_f. It seems likely that this depolarizing receptor does not normally respond to L-glutamate, and further work is required to analyse this response and characterize its pharmacology. Thus there is growing evidence that invertebrate receptors cannot readily be assimilated into the existing classification of L-glutamate receptors, based on vertebrate studies, and detailed characterization of these insect receptors may provide targets for new, safer, more selective insecticides.

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