Journal of Physiology (1991), **439**, *pp.* 15–35
With 12 figures
Printed in Great Britain

DIFFERENT TYPES OF GLUTAMATE RECEPTORS IN ISOLATED AND IDENTIFIED NEURONES OF THE MOLLUSC *PLANORBARIUS CORNEUS*

BY V. YU. BOLSHAKOV, SVETLANA A. GAPON AND L. G. MAGAZANIK*

From the I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Thorez pr. 44, 194223 Leningrad, USSR

(*Received 25 June 1990*)

SUMMARY

1. The membrane currents evoked by glutamate agonists on isolated and identified neurones of molluscan pedal ganglia were investigated using the voltage clamp technique.

2. The fast chloride current (E_r (reversal potential) = −41 mV) evoked in a Ped-9 neurone by application of glutamate, quisqualate and ibotenic acid could be blocked by furosemide (0·1 mM). The slow potassium current (E_r = −85 mV) evoked in Ped-8 and Ped-9 neurones by glutamate, quisqualate and kainate could be blocked by tetraethylammonium (50 μM).

3. *N*-Methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazole-proprionic acid (AMPA) failed to induce a response in neurones studies.

4. The spider venoms argiopine and argiopinine III (50–500 nM) selectively inhibited quisqualate-induced potassium current, but had no influence on glutamate-, ibotenate- or quisqualate-induced chloride and kainate-induced potassium currents. Glutamate-induced potassium current was partially inhibited by argiopine and argiopinine III.

5. The existence of several types of distinct glutamate receptors was confirmed in cross-desensitization experiments, and a lack of interaction was observed between quisqualate and kainate.

6. Potassium currents induced both by quisqualate and kainate strongly depended on temperature and could be blocked by pertussis toxin. Intracellular injection of the calcium chelator, EGTA, did not affect quisqualate and kainate responses.

7. In neurones loaded with non-hydrolysable GTP analogues, GTP-γ-S (guanosine-5′-*O*-(3-thio)triphosphate) or GppNHp (5′-guanylylimidodiphosphate), the potassium current was gradually induced in the absence of agonists. As this current progressed, the magnitude of the glutamate- or kainate-evoked current transients became smaller and finally negligible. The GTP-γ-S-induced current was not inhibited by argiopine.

8. These data indicate that in the molluscan neurones studied there are at least three pharmacologically distinct glutamate receptors: (1) a receptor of quisqualate–ibotenate type which directly controls chloride channel; (2) quisqualate

* To whom correspondence should be addressed.

MS 8591

and (3) kainate receptors which control in calcium-independent manner the common potassium channel by activation of GTP-binding protein.

INTRODUCTION

A variety of glutamate (Glu) responses differing in ionic mechanism (K^+, Cl^-, cationic) are typical for molluscan neurones. Separate depolarizing and hyperpolarizing responses to Glu and other agonists or biphasic responses have been observed (Kerkut & Walker, 1962; Gerschenfeld & Lasansky, 1964; Walker, Woodruff & Kerkut, 1971; Gerschenfeld, 1973; Ascher & Kehoe, 1975; Piggott, Kerkut & Walker, 1975; Walker, 1976; Kehoe, 1978; Kato, Oomura, Maruhashi & Shimizu, 1983; Jones, Rosser & Bulloch, 1987). The classification of Glu receptors in molluscan neurones and the mechanism of transduction of the agonist signal to transmembrane ionic current have not been fully elucidated. In this paper we have pursued the characterization of glutamate responses and the underlying receptors of identifiable neurones in pedal ganglia of the fresh-water mollusc *Planorbarius corneus* to clarify the correlation between the receptor type and ionic mechanism involved. Our findings suggest that at least three types of Glu receptors exist on these cells. Two separate receptors (of quisqualate- and kainate-type) control the potassium channel by G protein activation.

METHODS

The preparation and identification of neurones

The experiments were performed on the completely isolated identified neurones from the left and right pedal ganglia of the freshwater gastropod mollusc *Planorbarius corneus*. Several criteria of single neurone identification were used: localization of cells on ganglion surface, and position relative to nerves and statocytes (Gapon, 1983) (Fig. 1). We used the neurone nomenclature presented earlier. Single neurones were isolated as previously described (Kostenko, Geletyuk & Veprintsev, 1974). The circumoesophageal ganglionic ring was dissected and treated with 0·2% pronase type E (Serva) for 30–40 min. The sheath was removed mechanically, and single neurones were isolated together with a short part of axon (about 40–60 μm length).

Perfusion techniques and solutions

The isolated cell was transferred into the experimental chamber (about 1 ml) which was continuously perfused at 1 ml min^{-1} with a saline solution. The saline solution contained (in mM): NaCl, 50; KCl, 1·6; $CaCl_2$, 4; $MgCl_2$, 8; Tris base, 1; pH adjusted to 7·5 with H_2SO_4. This saline was in good accordance with the ionic composition of the haemolymph of *Planorbarius corneus* (Kostyuk, 1968). In some experiments the potassium concentration was increased twofold (3·2 mM) with a corresponding decrease of sodium content, or sodium content was doubled (addition of 25 mM-Na_2SO_4). Increased osmolarity of the latter solution (1·7 times) could be neglected since it is known that a prominent increase in osmolarity does not influence responses of these mollusc neurones (Katchman & Zeimal, 1982). The temperature was maintained at 20 ± 1 °C. Cooling of the bath solution to 10 °C could be achieved in 3–5 min. Agonists were applied either by conventional ionophoresis from a micropipette or by microsuperfusion from a drug-filled pipette with a tip of about 70–100 μm in diameter, or by perfusion of the bath with solution containing a drug. A microsuperfusion was favoured in most experiments since it allowed the neurone to be exposed (time constant $\approx$ 100 ms) to a known drug concentration (Ger, Katchman, Zeimal, 1979).

Electrical measurements

A routine voltage clamp technique was used. Double-barrelled micropipettes filled with either K_2SO_4 (0·5 M) or KCl (2·5 M) were used: one barrel for measuring membrane potential; the other for passing current required for maintaining the neurone at a given holding potential. The

resistance of each electrode was 6–20 MΩ, and the coupling resistance did not exceed 100 kΩ. The resultant data were plotted directly on an *X–Y* recorder or photographed from the screen of a storage oscilloscope.

Drugs

L-Glutamic and ibotenic acids were obtained from Serva. Quisqualic (QA) and kainic (KA) acids, *N*-methyl-D-aspartate (NMDA), glycine, pertussis toxin, tetralithium guanosine 5′-*O*-(3-thio)-triphosphate (GTP-γ-S), sodium salt of 5-guanylylimidodiphospate (GppNHp), ethyleneglycol-

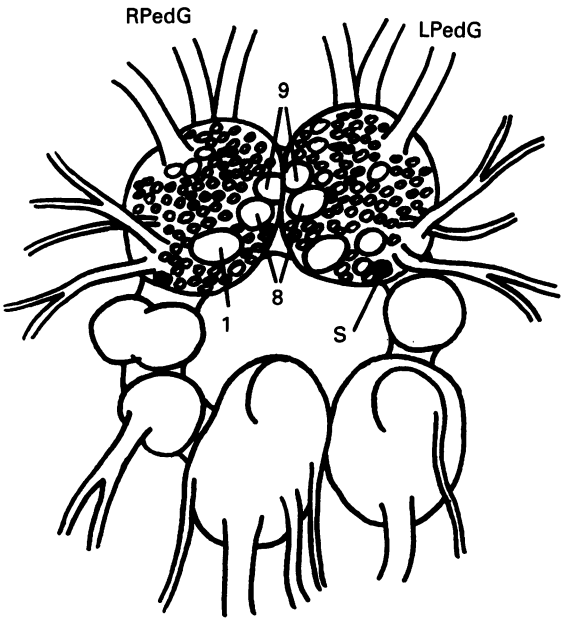

Fig. 1. Schematic drawing of circum-oesophageal ring of the mollusc *Planorbarius corneus*, showing the positions of identified neurones in pedal ganglia. RPedG and LPedG, right and left pedal ganglia; 1, 8 and 9, numbers of neurones studied; S, statocyte.

bis-(β-aminoethylether)*N,N′*-tetraacetic acid (EGTA), furosemide, tetraethylammonium bromide were obtained from Sigma. α-Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) was obtained from Tocris. Argiopine and argiopinine III were kindly supplied by Drs T. M. Volkova and E. V. Grishin, Shemyakin Institute of Bioorganic Chemistry, Moscow.

RESULTS

Identification of neurones responsive to L-glutamate

Twenty-five neurones have been identified on the ventral surface of the ganglionic ring of the fresh-water mollusc *Planorbarius corneus* (Gapon, 1983). Eighteen of those neurones responded to the application of L-glutamate (Glu). Cell-dependent depolarizations, hyperpolarizations and biphasic responses were observed. Only five of the neurones (RPed-1, RPed-8, RPed-9, LPed-8, LPed-9), all of them from the pedal ganglion (see Fig. 1) were responsive to relatively low concentrations of Glu (0·5–1 μM). All other neurones were 10–100 times less sensitive.

Some characteristics of Ped-8 and Ped-9 neurones

No difference was found in resting membrane potential between Ped-9 and Ped-8 neurones ($-42{\cdot}1 \pm 1{\cdot}2$ mV, $n = 32$ and $-41{\cdot}0 \pm 0{\cdot}8$ mV, $n = 42$ respectively). Both groups of cells usually fired spontaneously. Action potentials of about 80 mV

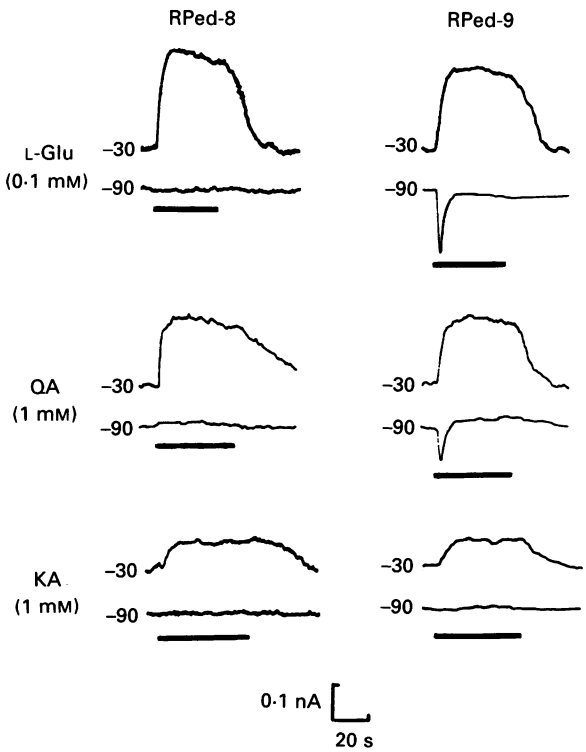

Fig. 2. Currents induced by application of glutamate (Glu), quisqualate (QA) and kainate (KA) on Ped-8 and Ped-9 neurones. The bar on each trace represents the period of agonist application by microsuperfusion from a drug-filled pipette. Clamped membrane potential levels (mV) and agonist concentrations are indicated on the left. Microelectrodes were filled with 2·5 M-KCl. Here and in subsequent figures, the upward and downward deflections in the traces are due to outward and inward current, respectively.

amplitude were followed by well-defined slow after-hyperpolarizations. In both cell types Glu application induced an inhibition of spontaneous impulse activity. Despite these similarities voltage-clamp analysis revealed that there were two distinct elements in the Glu response of Ped-9 neurone, but only one in Ped-8.

Glutamate-induced currents in Ped-8 and Ped-9 neurones

The application of 100 μM-Glu by microperfusion to both kinds of neurones clamped at a holding potential of -30 mV, which was close to or more positive than the resting level, induced a similar response: after a pronounced latency (about 1 s) a slowly developing outward current appeared. It reached a maximal amplitude in

10–20 s. Long-lasting application of Glu led to a slow and moderate decrease of current (10–25 % of initial level during 40–60 s) evidently due to desensitization (Fig. 2). The amplitude of Glu-induced current decreased with hyperpolarization and increased with depolarization of the neurone. Voltage–current relations in both kinds

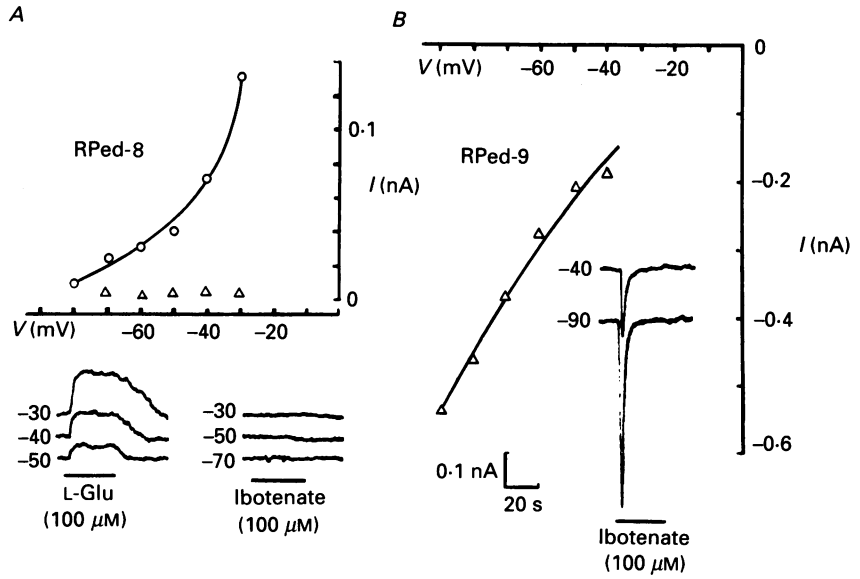

Fig. 3. Comparison of the ibotenate and glutamate effects on Ped-8 (*A*) and the ibotenate effect on Ped-9 (*B*) neurones. Agonist responses at different holding potential levels (shown on the left in mV) and *I–V* plots of these responses. ○, Glu; △, ibotenate. All records were obtained from the same cell in *A* and *B*. The interval between subsequent applications was not less than 3 min.

of neurones were practically identical (see below). When the neurones were clamped at −90 mV an obvious difference in Glu response in Ped-8 and Ped-9 neurones was recognized: in Ped-9 neurone a fast inward current with a very short latency could be recorded, which was never seen in Ped-8 neurones. This inward current decayed relatively rapidly in spite of continued Glu application. Depolarization of Ped-9 neurone induced a decrease of current amplitude. Neurones isolated from both right and left pedal ganglia gave the similar responses to Glu. Therefore no reference was made to the origin of the neurones, whether right or left ganglion, in our experiments.

Comparison of effects induced by Glu and some agonists

Quisqualic acid (QA) reproduced both kinds of response on Ped-9 neurone and only the monophasic responses on Ped-8 (Fig. 2). But QA was 6–10 times less potent than Glu for both responses, the threshold QA concentration being 1–5 μM.

On the contrary, application of kainic acid (KA) evoked only outward currents in all investigated neurones. The sensitivity of cells to KA was very low. The threshold concentration of KA was 50–100 μM. The outward current which was small, developed very slowly and there was no evidence of desensitization.

Application of ibotenate evoked only inward current in the Ped-9 neurone (Fig. 3). The Ped-8 neurone was completely insensitive to ibotenate action. The ibotenate-evoked current was quite similar to the inward current induced by Glu in the Ped-9 neurone: the current had a short latency, its rise and decay (evidently due to desensitization) were fast.

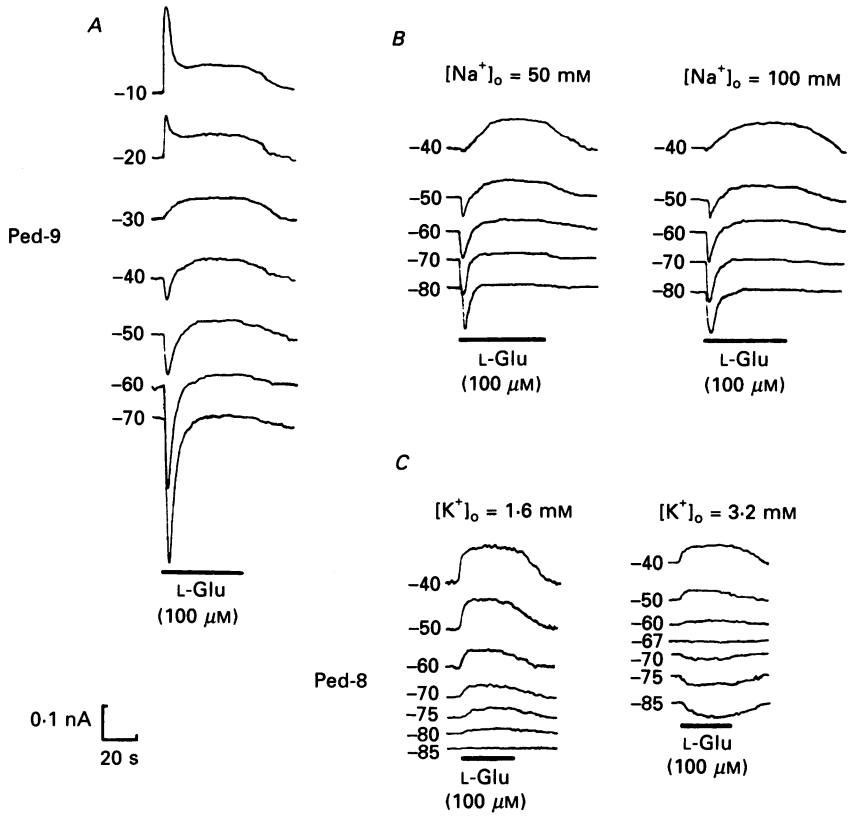

Fig. 4. Reversal potential (E_r) of Glu responses in Ped-8 and Ped-9 neurones. Glu-induced responses at different holding potential levels (shown on the left in mV). KCl (*A*) and K_2SO_4 (*B* and *C*) filled electrodes were used. *B*, before and after increasing of sodium concentration in the perfusing saline. *C*, before and after increasing of potassium concentration in the perfusing saline.

AMPA, the potent agonist selectively acting on quisqualate-preferring receptors in vertebrate neurone, was ineffective at concentration up to 1 mM being applied on both Ped-8 and Ped-9.

The absence of NMDA effects on investigated neurones

Application of 1 mM-*N*-methyl-D-aspartate (NMDA) evoked no responses in Ped-8 and Ped-9 neurones perfused in normal solution at any level of holding potential ($n = 7$). It is well known that in vertebrate neurones glycine potentiates and magnesium ions inhibit the response to NMDA (Nowak, Bregestovski, Ascher,

Herbet & Prochiantz, 1984; Johnson & Ascher, 1987). However, in our experiments ($n = 3$) the removal of Mg^{2+} ions from the solution and addition of 5 μM-glycine did not uncover a response to NMDA.

Ionic basis of the action of glutamate and glutamate agonists

To elucidate the ions responsible for the current flow through glutamate-activated channels the effect of altering the ionic gradient to specific ions on the reversal potential of the response was determined. In addition treatment with selective blockers of ion channels was used.

The fast phase of the glutamate response recorded in Ped-9 neurone was greatly diminished when the membrane was depolarized. The reversal potential (E_r) of fast current was $-28{\cdot}9 \pm 3{\cdot}9$ mV ($n = 14$) when recording electrodes were filled with KCl (Fig. 4*A*). With K_2SO_4-filled electrodes the reversal of fast currents occurred at a more negative potential, $E_r = -41{\cdot}2 \pm 2{\cdot}0$ mV ($n = 5$) (the difference was highly significant ($P < 0{\cdot}02$)) (Fig. 4*B*). The last value of E_r is close to the apparent mean value of chloride equilibrium potential (−42·7 mV) calculated on the basis of chloride intracellular concentration measurements in *Helix aspersa* neurones (Neild & Thomas, 1974). Evidently diffusion of Cl^- from the tip of the microelectrode can increase the intraneuronal Cl^- concentration and therefore shift the chloride potential, E_{Cl}, causing changes in the E_r of the Glu response.

Possible involvement of sodium ions was checked by increasing the sodium concentration 2-fold ($[Na^+]_o = 100$ mM). No amplitude change and shift of E_r of the Glu response were observed in the experiments on Ped-9 neurone using either chloride- or sulphate-filled microelectrodes (Fig. 4*B*). Experiments of this kind were done also on another neurone RPed-1 (Fig. 1), which responded to Glu in a biphasic manner. No shift of E_r of the initial fast inward component of Glu-induced current was found: $E_r = -22{\cdot}6 \pm 4{\cdot}0$ mV ($n = 8$) in normal solution and $E_r = -24{\cdot}3 \pm 4{\cdot}1$ ($n = 4$) in sodium-rich solution (chloride-filled microelectrodes).

Additional evidence that chloride mediates the fast inward current induced by Glu was obtained with furosemide treatment. The potency of furosemide to inhibit chloride-dependent responses evoked by GABA in frog spinal motoneurones (Nicoll, 1978) and by acetylcholine in isolated neurones of the mollusc *Planorbarius corneus* (Katchman & Zeimal, 1982) was shown in earlier work. In our experiments ($n = 4$) furosemide (0·1 mM) reduced or eliminated completely the fast inward current evoked by Glu application (Fig. 5*C* and *D*). This effect developed during several minutes of furosemide superfusion. The slow outward current was unaffected. The furosemide concentration used did not affect the membrane potential and steady-state conductance of the neurones. The fast chloride current induced by ibotenate and the fast phase of current induced by QA had $E_r = -28{\cdot}0 \pm 4{\cdot}6$ mV ($n = 5$) and $-33{\cdot}2 \pm 4{\cdot}2$ mV ($n = 6$) respectively. These values are close (the difference is statistically insignificant) to E_r of fast Glu-induced current ($-28{\cdot}9 \pm 3{\cdot}9$ mV, $n = 14$). Ibotenate- and quisqualate-induced chloride currents were also blocked by furosemide. Evidently all these inward currents are mediated by a selective increase in chloride conductance.

The slow Glu response evoked in both Ped-8 and Ped-9 neurones increased as the cell was depolarized. The *I–V* curve was also non-linear (Figs 3*A* and 5*D*), and the

zero level of glutamate-induced current was reached at $-84{\cdot}6 \pm 2{\cdot}6$ mV ($n = 22$). The reversion of slow currents was not obtained in either neurone in spite of hyperpolarization to -110 or -120 mV. The level of membrane potential when the response disappeared was taken for the E_r value. E_r was close to the potassium

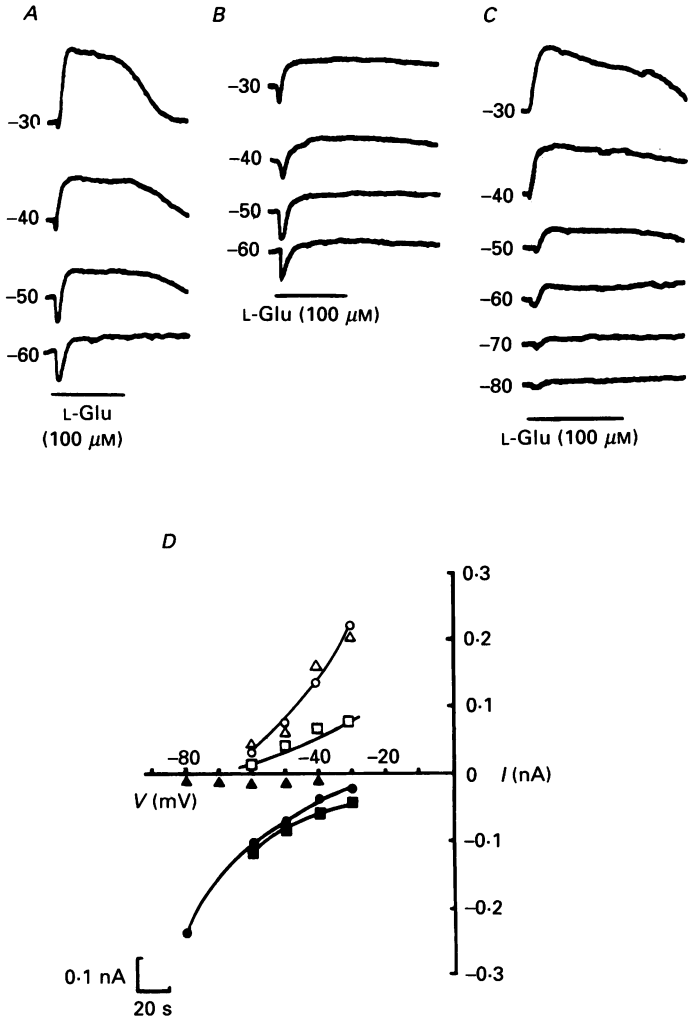

Fig. 5. Blocking effects of TEA and furosemide on the components of Glu biphasic response in Ped-9 neurone. Control (*A*), Glu responses at different holding potential levels. At -40 and -50 mV a biphasic pattern of Glu response is clearly seen. *B*, 10 min after addition of 50 μM-TEA to perfusion solution. *C*, 5 min after addition of 100 μM-furosemide into the bath. At least a 30 min interval was maintained after washing with standard saline between subsequent treatments with blocking drugs. *D*, *I–V* plots of these responses, open symbols correspond to outward currents (the late component of the biphasic response), filled symbols correspond to chloride currents (the initial component of the response). Circles, control; squares, in the presence of TEA; triangles, in the presence of furosemide.

equilibrium potential ($E_K = -87$ mV) calculated earlier from the results of direct intracellular measurements of potassium activity in neurones of *Planorbarius corneus* (Kostyuk, 1968). A twofold increase of $[K^+]_{out}$ (3·2 mM) shifted E_r in a more positive direction (by $17{\cdot}3 \pm 0{\cdot}8$ mV, $n = 3$, in accord with Nernst equation) and permitted reversal of the slow Glu currents by hyperpolarization (Fig. 4*C*). Application of tetraethylammonium (TEA) provided additional evidence that slow outward currents induced by Glu resulted from an increase in K^+ conductance. With a moderate concentration of TEA in the perfusion solution (50 μM), the slow outward Glu current recorded at a holding potential of −30 mV was reduced (Fig. 5*B*) to one-third (three cells).

The KA and QA-induced outward current had E_rs of $-86{\cdot}9 \pm 2{\cdot}0$ mV ($n = 5$) and $-85{\cdot}2 \pm 1{\cdot}8$ mV ($n = 10$), respectively. These values are quite close (the difference is statistically insignificant) to the E_r of the Glu-induced slow outward current ($-84{\cdot}6 \pm 2{\cdot}6$ mV, $n = 22$). Injection of EGTA into the neurones was performed ionophoretically from a micropipette filled with 0·2 M-EGTA. The efficiency of intracellular injection was proved by the disappearance of hyperpolarization following directly evoked action potentials. This procedure, which led to a large decrease of intracellular calcium concentration, did not influence either inward or outward agonist-induced currents ($n = 4$).

In summary, the fast chloride current induced by Glu, QA and ibotenate is due to the movement of chloride ions, while the slow outward current induced by Glu, QA and KA is due to a selective increase in potassium permeability.

Effects of argiopine and argiopinine III

Argiopine (Arg), a neurotoxin isolated from the venom of the spider *Agiope lobata* (Grishin, Volkova, Arseniev, Reshetova, Onoprienko, Magazanik, Antonov & Fedorova, 1986) is a member of the family of spider neurotoxins of low molecular weight which effectively block non-NMDA-type Glu receptors in invertebrates (insect and crustacean muscles) and vertebrates (hippocampal and spinal cord neurones) (see review by Jackson & Usherwood, 1988). Recently another Arg derivative, argiopinine III (Arg III), was isolated from the venom of *Argiope lobata* (Grishin, Volkova & Arseniev, 1989). In the present experiments Arg III (50 nM–1 μM) strongly, but not completely, inhibited the slow potassium currents induced by Glu in Ped-8 and Ped-9 neurones. On the other hand, Arg III failed to affect the chloride Glu-induced current recorded in Ped-9 neurone (Fig. 6*A*). The inhibitory effect of Arg III developed slowly over a 30–40 min period and could be partially reversed during 60 min of washing. The inhibitory effect was potential dependent: at −70 mV the current was reduced to $35 \pm 5\%$ ($n = 5$) of initial level, but at −30 mV it fell to $64 \pm 3\%$, ($n = 11$). Evidently the depolarization of the neurone weakens the Arg III blocking action. Steady-state *I–V* curves recorded in the presence of Arg III did not differ from control. Consequently Arg III does not influence the neurone input resistance.

The inhibitory effects of Arg and Arg III were qualitatively quite similar: only the late potassium Glu-induced current was affected, the potential dependence of Arg action was the same (the shift of holding potential from −30 to −70 mV decreased the Arg blocking effect by 1·8 times), and the Arg effect was only partially reversible.

Arg had no influence (up to 500 nM) on the neurone input resistance. The only difference demonstrated was in the potency of these two neurotoxins. Apparent dissociation constants obtained from corresponding dose–response curves prove that Arg is 3 times less potent than Arg III: $K_d = 230 \pm 30$ nM ($n = 5$) and $K_d = 76 \pm 3$ nM ($n = 6$), respectively.

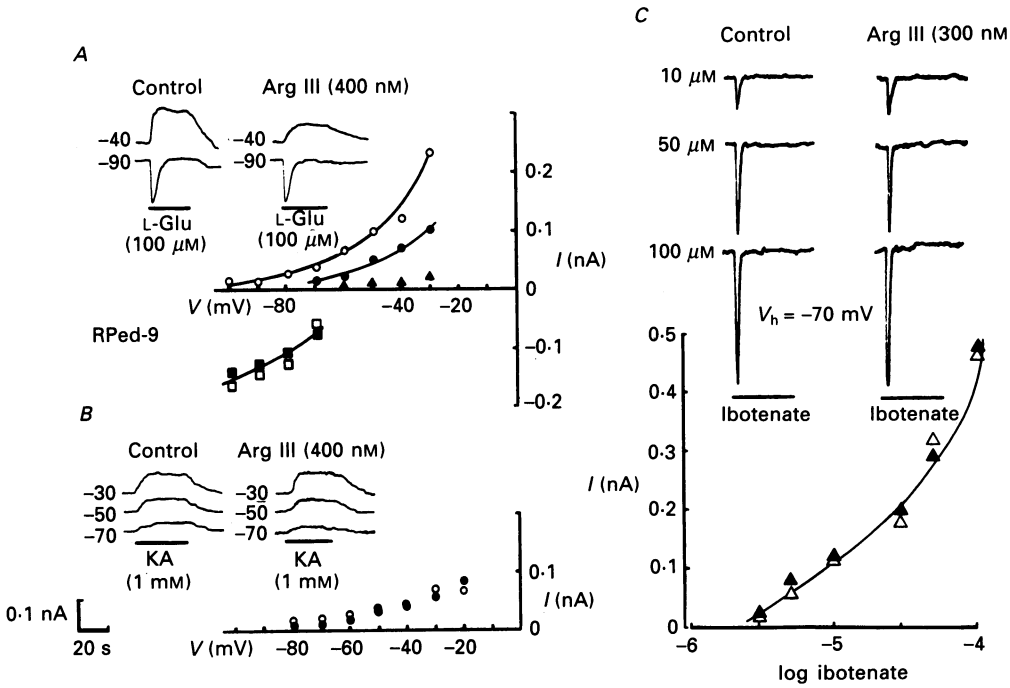

Fig. 6. Effect of argiopinine III on glutamate (*A*), kainate (*B*) and ibotenate (*C*) responses on a Ped-9 neurone. *A* and *B*, *I–V* plots for responses. Circles, amplitude of potassium currents; squares, amplitude of chloride currents. Open symbols, control responses; filled circles and squares, after 40 min treatment with 400 nM-Arg III; filled triangles, after an additional 30 min treatment with increased concentration of Arg III up to 1 μM. Insets: examples of currents recorded at different holding potential level. *C*, dose–response curves of ibotenate induced currents before (△) and after 40 min treatment with 300 nM-Arg III (▲). Insets: responses to different doses of ibotenate before and after Arg III. Holding potential, −70 mV.

The ability of Arg III to block the responses induced by superfusion of several agonists was compared. QA-induced potassium current was inhibited by Arg III just like the Glu-induced one: K_d values differed insignificantly ($K_d = 52 \pm 12$ nM, $n = 3$ in the case of QA responses). Arg III failed to block QA-induced chloride current as well. The most interesting difference was found in the experiments with KA application. In contrast to Glu- and QA-induced responses, Arg III (Fig. 6*B*) and Arg (up to 1 μM) had no influence on KA-induced potassium currents. Inward current induced in Ped-9 by ibotenate application was also resistant against Arg III blocking action (Fig. 6*C*).

Cross-desensitization experiments

The ability of agonists to induce desensitization was used for determining whether these agonists activate the same or different receptors. Possible interaction between agonist-induced responses was tested comparing the response to an agonist before

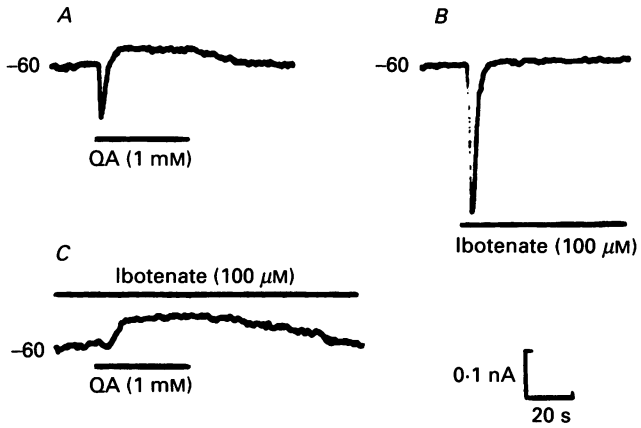

Fig. 7. Interaction between effects induced by quisqualate and ibotenate. QA (*A*) and ibotenate (*B*) single responses in Ped-9 neurone. A fast desensitization of chloride currents is seen. *C* is a continuation of *B*, QA was applied 3 min after beginning of ibotenate application: only potassium QA-induced current can be seen.

and during the continued presence of another agonist. In the experiments on Ped-9 the early component of the response to 1 mM-QA (Fig. 7*A*) and the response to 100 μM-ibotenate (Fig. 7*B*) completely decayed during the first second of application, evidently due to desensitization. Application of QA from the micropipette onto the neurone perfused with ibotenate-containing solution (100 μM) failed to evoke the typical biphasic response. Only the slow outward component of this response was observed (Fig. 7*C*). Evidently, pretreatment of the neurone with ibotenate induced the desensitization of the common receptor for ibotenate and QA which was involved in the activation of fast chloride current.

Application of 1 mM-KA on Ped-8 evoked a steady-state plateau-like outward current (Fig. 8*B* and *C*) in contrast to the QA (100 μM) response which slowly decayed and by the 6–8th min of a long-lasting application had completely disappeared (Fig. 8*A* and *C*). Repeating the KA application at this moment evoked a potassium current of the same amplitude as the control one (Fig. 8*C*). The absence of cross-desensitization, which was found in each of four neurones tested, spoke in favour of the existence of two different receptors for QA and KA. Evidently Glu interacted with both kinds of receptor since previous exposure to QA (1 mM) completely blocked the responses to QA but only partially to Glu (Fig. 8*A* and *B*). The remaining Glu response resembled the KA response: both of them were resistant to the QA desensitization and Arg blocking action (Fig. 8*B*, see also Fig. 6*B*).

If the potassium outward currents evoked by activation of these two receptors resulted from entirely separate mechanisms one would expect that the maximal currents generated by the two agonists would summate. This is not the case. No

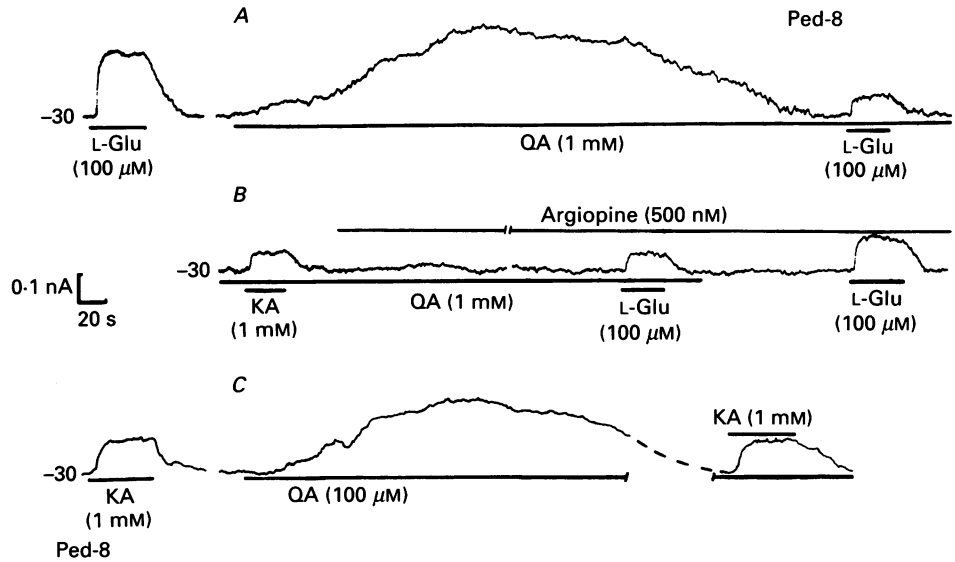

Fig. 8. Interaction between effects induced by quisqualate, glutamate and kainate. *A* and *B*, after the control response to Glu the continuous bath application of QA (marked by line below the record in *A* and *B*) was done; test-responses to short applications of agonists (Glu, and KA) and application of Arg (marked by line above the record) were superimposed during continuous application of QA. Gap in the recording (25 min) is shown by break of line in *B*. *C*, experiment from another neurone. After the control KA response the continuous application of QA induced a prominent decrease of potassium current due to desensitization. Test application of KA at this moment induced the same response as in control. The dashed line shows the break of recording (3 min).

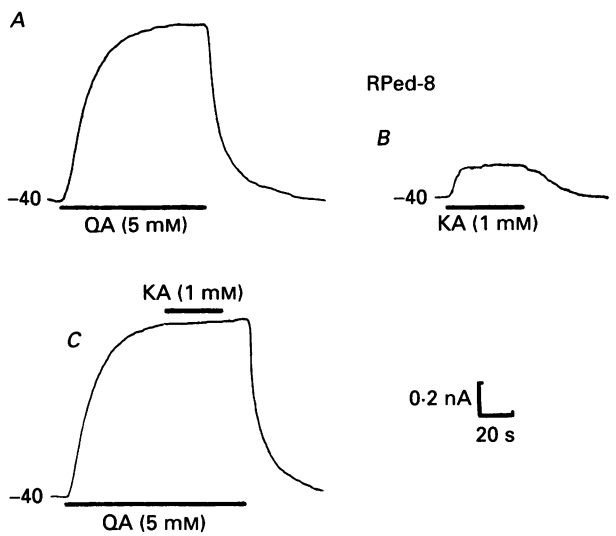

Fig. 9. Non-additive interaction of kainate and maximal quisqualate responses. *A*, the ceiling response to high dose of QA. *B*, test response to KA. *C*, superimposed application of KA on the height of QA response failed to induce the additional potassium current. Holding potential −40 mV.

increment of current can be observed when the maximal (saturating) concentration of QA (5 mM) was used, and at the moment of maximal response ($0{\cdot}79 \pm 0{\cdot}15$ nA, $n = 4$) a testing dose of KA (1 mM) was applied (Fig. 9).

Temperature dependence of glutamate and agonist responses

The responses of neurones to application of Glu, QA and KA were tested at different temperatures (from 11 to 21 °C). The amplitude of chloride currents induced

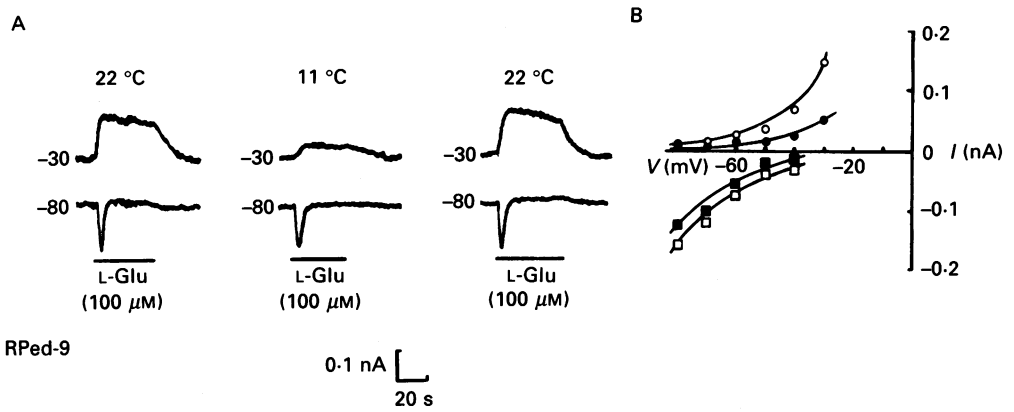

Fig. 10. Temperature dependence of glutamate response in Ped-9 neurone. *A*, Glu-induced currents recorded at different temperatures (values are shown). Holding potential (in mV) is shown on the left. *B*, *I–V* plots of Glu responses recorded at two different temperatures. Circles, potassium currents; squares, chloride currents. Open symbols, 22 °C ; filled ones, 11 °C.

by Glu or QA was only slightly sensitive to temperature change: temperature coefficient $Q_{10} = 1{\cdot}3 \pm 0{\cdot}1$, ($n = 3$) and $1{\cdot}2 \pm 0{\cdot}1$, ($n = 3$), respectively. The amplitude of potassium currents induced by Glu, QA and KA revealed a prominent temperature dependence: $Q_{10} = 3{\cdot}1 \pm 0{\cdot}4$, ($n = 4$); $3{\cdot}2 \pm 0{\cdot}3$, ($n = 3$) and $3{\cdot}2 \pm 0{\cdot}4$, ($n = 3$), respectively (Fig. 10). The rate of current onset and decay became slower at lower temperatures. The data observed suggest that some intracellular metabolic mechanisms might take part in the generation of potassium currents induced by Glu and agonists. In particular, the involvement of G proteins and some secondary messenger systems in the transduction process could be proposed.

Effects of pertussis toxin on glutamate and agonist responses

The effect of pertussis (PTX), which inactivates some G proteins by ADP-ribosylation of their α-subunits, was examined. The ganglionic ring was placed overnight (14–15 h) at room temperature in the bathing solution containing 1–2 μg ml^{-1} of PTX. Treated isolated neurones were then clamped and tested for their response to Glu, KA and QA. Ped-9 exhibited normal fast chloride currents but failed to show the slow potassium one (Fig. 11). The average amplitude of Glu-induced slow currents in PTX-treated cells ($n = 11$) was 18·5% of the control value estimated in similar experiments (100 μM-Glu, holding potential −40 mV) on untreated cells. The same results were obtained when QA (100 μM) and KA (1 mM)

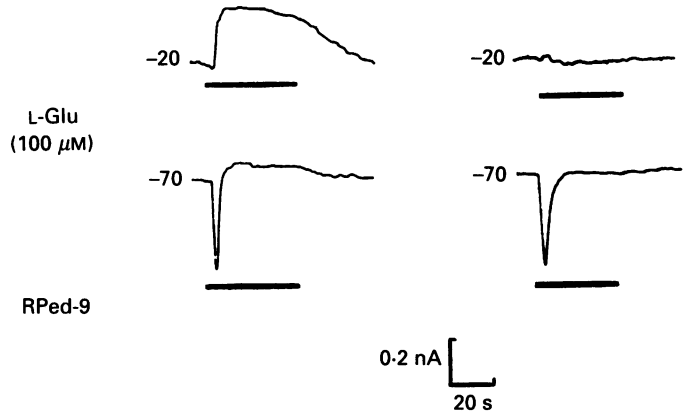

Fig. 11. Effect of pertussis toxin (PTX) on glutamate responses in Ped-9 neurone. On the left, control Glu responses at two potentials. On the right, the same experiment on the same neurone pretreated with 2 μg ml^{-1} of PTX during 15 h. The control experiment was done on the preparation held in standard saline under the same conditions.

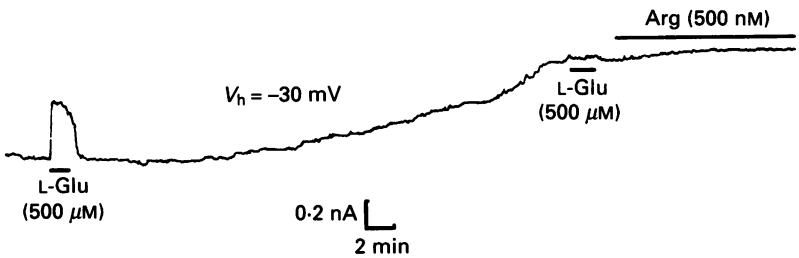

Fig. 12. Effect of intracellular injection of GTP-γ-S into Ped-8 neurone. Ionophoretic injection of GTP-γ-S was done from the recording microelectrode. Glu was applied at the beginning of injection and at the height of GTP-γ-S-induced potassium current. Application of argiopine at this moment also had no influence on the amplitude of the current. Holding potential, −30 mV. Recording microelectrode contained 10 mM-GTP-γ-S.

were applied: 21·4% (n = 6) and 23·0% (n = 6), respectively. PTX-treated cells did not differ from control ones in their resting membrane potential, input resistance and ability for spontaneous firing.

Action of GTP-γ-S

Intracellular ionophoretic injection of GTP-γ-S from the tip of a micropipette (10 mM-GTP-γ-S was added to 2·5 M-KCl solution filling the pipette) caused a potassium current (Fig. 12). The rise of the current was extremely slow, generally taking 60–80 min to reach the maximal amplitude (0·82 ± 0·16 nA, at holding potential −30 mV, n = 4). As the intracellular GTP-γ-S-induced activation of the current progressed, the magnitude of the Glu-induced current transient became smaller and finally negligible. Long-lasting (45–50 min) treatment of neurones with 0·5 μM-Arg did not inhibit this current. Application of QA and KA on the neurones loaded with GppNHp (n = 2) also failed to evoke an additional potassium current.

DISCUSSION

The major findings of the present study are: (1) Glu induces an increase of potassium and chloride conductance in neurones of *Planorbarius corneus* similar to those reported in other molluscan neurones (Kerkut, Horn & Walker, 1969; Szczepaniak & Cottrell, 1973; Oomura, Ooyama & Sawada, 1974; Kehoe, 1978; Jones *et al.* 1987). (2) A number of pharmacologically distinct Glu receptors involved in these responses may be revealed by agonist and antagonist actions and by cross-desensitization. (3) Potassium current induced by Glu or agonists in molluscan neurones is mediated by pertussis toxin-sensitive GTP-binding protein. (4) This potassium channel is controlled by two distinct Glu-receptors of QA- and KA-type.

Ionic basis of Glu response

Several pieces of evidence were obtained that proved the chloride and potassium nature of Glu-induced currents. The slow outward current elicited (at resting potential level) by Glu is due to an increase of potassium permeability: (1) its E_r is very close to E_K estimated earlier (Kostyuk, 1968); (2) the effect of $[K^+]_o$ change on E_r of the Glu response is in accordance with the Nernst equation predictions. Inhibition of the slow Glu-induced outward current in TEA-treated neurones could be considered as a direct effect of potassium channel blocker TEA on channels activated by Glu. The blocking effect of an extracellular application of 200 μM-TEA on acetylcholine-induced potassium current was observed in the experiments on *Aplysia* neurones (Kehoe, 1972) and 50 μM-TEA on the muscarinic potassium response in the experiments on *Planorbarius corneus* neurones (Ger & Zeimal, 1977). But even 1 mM-TEA failed to block the potassium currents evoked in neurones by intracellular injection of GppNHp (our preliminary observations). Similar results have been obtained in the experiments on *Aplysia* neurones (Brezina, 1988). Thus the precise mechanism of TEA blocking effect on the potassium current evoked by activation of the non-cholinergic receptors is not clear yet. The Glu-activated potassium channels appear to differ from the widely distributed Ca^{2+}-sensitive potassium channels because in the present experiments the injection of the Ca^{2+} chelator, EGTA, into the neurone inhibits the slow after-hyperpolarization but fails to affect the Glu response. The analogous results were obtained in *Aplysia* neurones when the potassium conductance was activated by arecoline application (Kehoe, 1985).

The fast initial component of Glu-induced current is due to an increase of chloride permeability: (1) its E_r becomes more negative when sulphate-filled microelectrodes are used instead of chloride-filled ones; (2) injection of Cl^- ions from a microelectrode induces a predicted shift of E_r of the Glu response; (3) furosemide inhibits selectively the fast Glu-induced chloride current. Unfortunately, there is no information about chloride intracellular activity, $(a_{Cl})_i$, in *Planorbarius corneus* neurones. The possible value of E_{Cl} in these neurones could be calculated if the chloride intracellular activity $(a_{Cl})_i$ is taken to be the same as that in *Helix aspersa* neurones (8·3 mM according to the data by Neild & Thomas, 1974). Then with 75·6 mM-chloride in the bathing solution and the activity coefficient of 0·77, E_{Cl} would be -49 mV. E_r of the fast initial component of Glu-induced current was $-41{\cdot}2 \pm 2{\cdot}0$ mV, $n = 5$. The difference

observed may reflect a higher $(a_{Cl})_i$ of isolated neurones in our experiments. An existence of small Na^+ component of this current can be also discussed. An excitatory Na^+-dependent Glu response has been described in molluscan neurones (Lowagie & Gerschenfeld, 1973; Kehoe, 1978). Some attempts to reveal the involvement of Na^+ ions in Glu-evoked inward current were made in the present experiments on two types of neurones, Ped-1 and Ped-9. However, a 2-fold increase of $[Na^+]_o$ had no influence on the biphasic Glu response; in particular, no shift of E_r was observed. The loop diuretic furosemide is well-established as an inhibitor of the Na^+–K^+–$2Cl^-$ co-transport system (Geck & Heinz, 1986). It has also been reported that furosemide inhibits neurotransmitter-activated chloride currents: GABA responses in frog spinal motoneurones (Nicoll, 1978) and acetylcholine responses in molluscan neurones (Katchman & Zeimal, 1982). In Nicoll's and in the present experiments little or no change in agonist reversal potential during the blocking effect of furosemide was observed. In these cases it appears that furosemide has a direct effect on receptor-controlled chloride channels rather than on the Cl^- intracellular concentration.

Thus in the neurones studied here only Cl^- ions take part in the generation of the initial fast depolarizing component of the biphasic Glu response as was the case in the biphasic responses studied by other authors (Szczepaniak & Cottrell, 1973; McCreery & Carpenter, 1984; Jones *et al.* 1987). The possibility that the chloride inward current may underlie the excitatory postsynaptic potential is quite low since the E_r of this response is close to the resting potential of the neurones. More likely, both potassium and chloride Glu-evoked currents are involved in the inhibitory reactions of the neurones studied.

Pharmacological characteristics of glutamate receptors

The receptors studied appear to belong to the non-NMDA group of excitatory amino acid receptors, since all our attempts to reveal NMDA receptors in molluscan neurones were to no avail. Some correlation between the ionic mechanism and the type of Glu receptor involved was found. In *Aplysia* neurones (Kehoe, 1978) quisqualate (QA) was found to activate the K^+ conductivity, but not the Cl^- one. Here we found that both types of responses are reproduced by QA. But ibotenic acid and kainate (KA) possess the ability to evoke only chloride and potassium currents, respectively. The selective effect of ibotenate on chloride permeability was observed earlier in *Aplysia* neurones (Kehoe, 1978) and in locust muscle (Dudel, Franke, Hatt & Usherwood, 1989). The identity of the receptor which can be activated either by Glu or by QA and ibotenic acid was proved in cross-desensitization experiments. This type of Glu receptor controls the chloride conductivity. Although the potassium conductance can be activated both by QA and KA as well as Glu, the desensitization experiments spoke in favour of the existence of two distinct types of Glu receptors. Specifically, long-lasting desensitizing applications of QA failed to prevent the KA-induced response. The opposite order of agonist application, when KA was applied before QA, did not reveal cross-desensitization. This is not surprising, since the ability of KA to activate receptors without desensitization has been shown with hippocampal neurones (Kiskin, Krishtal & Tsyndrenko, 1986). It should be noted that the partial block of Glu responses by QA-induced desensitization is probably

due to the ability of Glu also to activate the separate KA receptors. Support for this hypothesis may be found in our experiments with selective blockade of QA receptors.

The potent and selectively-acting antagonist, argiopine, which was isolated from the venom of *Argiope lobata* spider (Grishin *et al.* 1986) was found to distinguish between the Glu receptors. Arg and various related toxins block Glu-activated channels of the quisqualate type in lobster, crayfish, locust, and blowfly muscles (Abe, Kawai & Miwa, 1983; Bateman, Boden, Dell, Duce, Quicke & Usherwood, 1985; Magazanik, Antonov, Fedorova, Volkova & Grishin, 1986; Miwa, Kawai, Saito, Pan-Hou & Yoshika, 1987; Antonov, Dudel, Franke & Hatt, 1989) and in vertebrate neurones (Saito, Kawai, Miwa, Pan-Hou & Yoshika, 1985; Antonov, Grishin, Magazanik, Shupliakov, Vesselkin & Volkova, 1987; Magazanik, Antonov, Fedorova, Volkova & Grishin, 1987; Magazanik & Antonov, 1988; Kiskin, Krishtal, Tsyndrenko, Volkova & Grishin, 1989; Antonov, Kalinina, Kurchavyi, Magazanik, Shupliakov & Vesselkin, 1990). In the present experiments on molluscan neurones Arg and another related toxin, argiopinine III (Grishin *et al.* 1989), selectively inhibited Glu- and QA-activated slow potassium current. Both toxins failed to affect the fast chloride current evoked by Glu-, QA- and ibotenate and the slow potassium current evoked by KA. Thus the selective effect of these antagonists indicated the existence in molluscan neurones of two distinct Glu receptors (QA- and KA-type) controlling the potassium conductivity, in agreement with our cross-desensitization experiments. The inability of Arg to block in molluscan neurones the Glu-activated chloride channels of the QA-type is in good accordance with the results obtained in the experiments on *Aplysia* neurones (Ikemoto & Akaike, 1988) and crayfish muscles (J. Dudel, personal communication). The latter chloride channels are also gated by ibotenate, but not by QA (Dudel *et al.* 1989).

Arg elicits either a closed or open channel block. In the blowfly preparation both types of block were manifested in the same concentration range and differed only in the direction of their potential dependence: hyperpolarization enhanced the blocking effect of Arg on the open channel but reduced that on closed ones (Magazanik *et al.* 1986; Magazanik & Antonov, 1988). In crayfish preparations, the block induced by 1–100 nM-Arg only reduced the number of Glu-activated channels and did not influence the channel kinetics (Antonov *et al.* 1989). In the present experiments the blocking effect of Arg and Arg III on potassium current induced by Glu and QA was more pronounced at -70 mV than at -30 mV, but it cannot serve as a good basis for believing that the antagonists preferentially interacted with open potassium channels. On the contrary the inability of Arg to inhibit the KA effect speaks against this possibility. The simultaneous application of maximal (saturating) concentrations of QA and KA did not reveal summation of their effects. There are some reasons to believe that QA and KA may activate the same population of potassium channels but through different receptors. Only one of these receptors, namely the QA-type, is liable to the Arg and Arg III inhibitory effect.

Although the blocking effect of Arg and Arg III was concentration dependent, the Glu response could not be blocked completely even in the long-lasting presence of 1 μM-Arg III. Thus it appears that the resting part of Glu response is due to activation of KA receptors, which are resistant to the Arg blocking effect. It would be reasonable to extend this explanation to the fact that QA-induced desensitization

effectively prevented the QA response but only diminished the Glu response (Fig. 8). The blocking effect of Arg was not additive in this case because the QA desensitization revealed the existence of separate KA receptors. Thus Glu can activate both QA and KA receptors.

The mechanism of transduction of the Glu response

The prominent temperature dependence and slow kinetics of the QA- and KA-induced potassium currents, unlike the QA- and ibotenate-induced chloride ones, suggested that a different transduction mechanism was associated with different receptor actions. Many neurotransmitter receptors are connected with GTP-binding proteins (G-proteins) as one of the intermediate links between the receptor and effector, namely ion channel (see for review: Strange, 1988; Smart, 1989). The involvement of G-proteins in signal transduction processes can be detected by the help of specific molecular probes: (1) bacterial exotoxins, which induce the ADP-ribosylation of some G-proteins and modify the transmitter response (Gilman, 1987); (2) non-hydrolysable GTP analogues, which activate G-proteins and thereby imitate the transmitter action (Sasaki & Sato, 1987). Pretreatment of neurones with pertussis toxin (PTX) selectively inhibited the slow potassium currents evoked by both QA and KA application but failed to affect the fast chloride current. These responses to QA or KA were mimicked by intracellular injection of GTP-γ-S or GppNHp. Glu, QA or KA application at the height of GTP-γ-S-induced potassium current could not evoke an additional response. The average maximal amplitudes of currents induced by QA application and GTP-γ-S injections were practically equal. These results and the above-mentioned data suggest that two distinct Glu receptors (of QA- and KA-type) can share a common potassium channel by means of G-protein activation. Similarly the coupling of a set of different agonist-specific receptors to a single K^+ channel has been previously demonstrated (Ascher & Chesnoy-Marchais, 1982; Andrade, Malenka & Nicoll, 1986; Sasaki & Sato, 1987; Brezina, 1988). The Glu receptor of the quisqualate–ibotenate type directly operates the chloride channel as evidenced by the fast kinetics, the low Q_{10} coefficient of activation and the insensitivity to PTX action.

The absence of an Arg inhibitory effect towards GTP-γ-S-evoked potassium current (Fig. 12) indicates that Arg fails to block the open potassium channels. This result is in a good accordance with the lack of Arg effect on the KA response. The activation of pharmacologically unidentified Glu receptors induced a potassium conductivity in lobster nerve terminals that was blocked by PTX but insensitive to the blocking effect of Joro spider toxin (JSTX, Miwa, Kawai & Ui, 1987). It seems that high concentrations of Arg and related antagonists (e.g. argiotoxin$_{(636)}$, JSTX) block the open state of cation low-selective channels similar to those that are found in arthropod muscles (Bateman *et al.* 1985; Magazanik *et al.* 1986; Kerry, Ramsey, Sansom & Usherwood, 1988; Antonov *et al.* 1989), in hippocampal neurones (Kiskin *et al.* 1989), and those expressed in *Xenopus* oocytes after injection of rat brain mRNA (Sugiyama, Ito & Hirono, 1987).

The distinguishing features of Glu receptors found in *Planorbarius corneus* neurones are summarized in Table 1. Thus at least three distinct Glu receptors could be revealed which resembled the mixed quisqualate–ibotenate (I), the proper quisqualate (II) and the kainate (III) types. The receptor of the II type is 6–10 times

less sensitive to QA than Glu, but even so we prefer to classify it as a quisqualate receptor since it can not be activated by other agonists and is selectively blocked by Arg. Receptors of I and II type differ from QA-receptors of vertebrate neurones being insensitive to QA-type agonist, AMPA (Krogsgaard-Larsen, Honore, Hansen, Curtis & Lodge, 1980). Further tests would be necessary using suitable activating

TABLE 1. Types of glutamate receptors in identified neurones of the mollusc *Planorbarius corneus*

Types	I	II	III
Agonists	Glu, QA, Ibotenate	Glu, QA	Glu, KA
Cross-desensitization	QA〈+〉Ibotenate Glu〈+〉QA	Glu〈+〉QA	Glu〈+〉KA* QA〈−〉KA
Effect of Arg and Arg III	no**	inhibition (Arg K_D = 230 nM; Arg III K_D = 76 nM)	no**
Effects of putative channel blockers:			
Furosemide (0·1 mM)	+	−	−
TEA (0·05 mM)	−	+	+
Response at resting potential***	D	H	H
E_r (mV)	−41	−85	−87
Ionic nature	Cl^-	K^+	K^+
Q_{10} of activation	1·3	3·2	3·2
Effect of PTX	No	Inhibition	Inhibition

* Glu prevents the KA response but not *vice versa*. ** No effect at 1 μM. *** D, depolarization; H, hyperpolarization.

and blocking agents to elucidate the possible role of any secondary messengers in transduction of QA- and KA-receptor activation leading to an increase of potassium conductivity.

REFERENCES

ABE, T., KAWAI, N. & MIWA, A. (1983). Effects of a spider toxin on the glutaminergic synapse of lobster muscle. *Journal of Physiology* **339**, 243–252.

ANDRADE, R., MALENKA, R. C. & NICOLL, R. A. (1986). A G protein couples serotonin and GABAb receptors to the same channels in hippocampus. *Science* **234**, 1261–1265.

ANTONOV, S. M., DUDEL, J., FRANKE, CH. & HATT, H. (1989). Argiopine blocks glutamate-activated single-channel currents on crayfish muscle by two mechanisms. *Journal of Physiology* **419**, 569–587.

ANTONOV, S. M., GRISHIN, E. V., MAGAZANIK, L. G., SHUPLIAKOV, O. V., VESSELKIN, N. P. & VOLKOVA, T. M. (1987). Argiopine blocks the glutamate responses and sensorimotor transmission in motoneurones of isolated frog spinal cord. *Neuroscience Letters* **83**, 179–184.

ANTONOV, S. M., KALININA, N. I., KURCHAVYJ, G. G., MAGAZANIK, L. G., SHUPLIAKOV, O. V. & VESSELKIN, N. P. (1990). Identification of two types of excitatory monosynaptic inputs in frog spinal motoneurones. *Neuroscience Letters* **109**, 82–87.

ASCHER, P. & CHESNOY-MARCHAIS, D. (1982). Interactions between three slow potassium responses controlled by three distinct receptors in *Aplysia* neurones. *Journal of Physiology* **324**, 67–92.

ASCHER, P. & KEHOE, J. S. (1975). Amine and amino acid receptors in gastropod neurons. In *Handbook of Psychopharmacology*, vol. 4, ed. IVERSON, A., IVERSON, L. & SNYDER, A., pp. 265–310. Plenum Press, New York.

BATEMAN, A., BODEN, P., DELL, J. R., DUCE, I. R., QUICKE, D. L. J. & USHERWOOD, P. N. R. (1985). Postsynaptic block of glutamatergic synapse by low molecular weight fractions of spider venom. *Brain Research* **339**, 237–244.

BREZINA, V. (1988). Guanosine 5-triphosphate analogue activates potassium current modulated by neurotransmitters in *Aplysia* neurones. *Journal of Physiology* **407**, 15–40.

DUDEL, J., FRANKE, C., HATT, H. & USHERWOOD, P. N. R. (1989). Chloride channels gated by extrajunctional glutamate receptors (H-receptors) on locust leg muscle. *Brain Research* **481**, 215–220.

GAPON, S. A. (1983). The effect of putative transmitters on identified neurones of the gastropod mollusc *Planorbarius corneus*. *Journal of Evolutionary Biochemistry and Physiology* **19**, 38–45 (in Russian).

GECK, P. & HEINZ, E. (1986). The Na–K–2Cl cotransport system. *Journal of Membrane Biology* **91**, 97–105.

GER, B. A., KATCHMAN, A. N. & ZEIMAL, E. V. (1979). Biphasic acetylcholine responses of molluscan neurones: their dependence on acetylcholine concentration. *Brain Research* **167**, 426–430.

GER, B. A. & ZEIMAL, E. V. (1977). Pharmacological study of two kinds of cholinoreceptors on the membrane of identified completely isolated neurones of *Planorbarius corneus*. *Brain Research* **121**, 131–149.

GERSCHENFELD, H. M. (1973). Chemical transmission in invertebrate central nervous systems and neuromuscular junctions. *Physiological Reviews* **53**, 1–119.

GERSCHENFELD, H. M. & LASANSKY, A. (1964). Action of glutamic acid and other naturally occurring amino acids on snail central neurons. *International Journal of Neuropharmacology* **3**, 301–314.

GILMAN, A. G. (1987). G-proteins: transducer of receptor-generated signals. *Annual Reviews of Biochemistry* **56**, 615–649.

GRISHIN, E. V., VOLKOVA, T. M. & ARSENIEV, A. S. (1989). Antagonists of glutamate receptors from the venoms of *Argiope lobata* spider. *Bioorganicheskaja Khimia* **14**, 883–832 (in Russian).

GRISHIN, E. V., VOKOLVA, T. M., ARSENIEV, A. S., RESHETOVA, O. S., ONOPRIENKO, V. V., MAGAZANIK, L. G., ANTONOV, S. M. & FEDOROVA, I. M. (1986). Structure–functional characterizations of an ion channel blocker from the venom of spider *Argiope lobata*. *Bioorganicheskaja Khimia* **12**, 1121–1124 (in Russian).

IKEMOTO, Y. & AKAIKE, N. (1988). The glutamate-induced chloride current in *Aplysia* neurones lacks pharmacological properties seen for excitatory responses to glutamate. *European Journal of Pharmacology* **150**, 313–318.

JACKSON, H. & USHERWOOD, P. N. R. (1988). Spider toxins as tools for dissecting elements of excitatory amino acid transmission. *Trends in Neurosciences* **11**, 278–283.

JOHNSON, J. W. & ASCHER, P. (1987). Glycine potentiates the NMDA response in cultured mouse brain neurones. *Nature* **325**, 529–531.

JONES, P. G., ROSSER, S. J. & BULLOCH, A. G. M. (1987). Glutamate suppression of feeding and the underlying output of effector neurons in *Helisoma*. *Brain Research* **437**, 56–68.

KATCHMAN, A. N. & ZEIMAL, E. V. (1982). Ionic mechanisms of the rapid (nicotinic) phase of acetylcholine response in identified *Planorbarius corneus* neurones. *Brain Research* **241**, 95–103.

KATO, M., OOMURA, Y., MARUHASHI, J. & SHIMIZU, N. (1983). Chemical characteristics of the L-glutamate receptor on the *Onchidium* neuron. *Journal of Neuroscience* **3**, 549–556.

KEHOE, J. S. (1972). Three acetylcholine receptors in *Aplysia* neurones. *Journal of Physiology* **225**, 115–146.

KEHOE, J. S. (1978). Transformation by concanavalin A of the response of molluscan neurones to L-glutamate. *Nature* **274**, 866–869.

KEHOE, J. S. (1985). Synaptic block of calcium-activated potassium conductance in *Aplysia* neurones. *Journal of Physiology* **369**, 439–474.

KERKUT, G. A., HORN, N. & WALKER, R. J. (1969). Long lasting synaptic inhibition and its transmitter in the snail *Helix aspersa*. *Comparative Biochemistry and Physiology* **30**, 1061–1074.

KERKUT, G. A. & WALKER, R. J. (1962). The chemical sensitivity of *Helix* nerve cells. *Comparative Biochemistry and Physiology* **7**, 277–288.

KERRY, C. J., RAMSEY, R. L., SANSOM, M. S. P. & USHERWOOD, P. N. R. (1988). Single channel studies of non-competitive antagonism of a quisqualate-sensitive glutamate receptor by argiotoxin$_{(636)}$ – a fraction isolated from orb-web spider venom. *Brain Research* **459**, 312–327.

KISKIN, N. I., KRISHTAL, O.A. & TSYNDRENKO, A. V. (1986). Excitatory amino acid receptors in hippocampal neurons: kainate fails to desensitize them. *Neuroscience Letters* **63**, 225–230.

KISKIN, N. I., KRISHTAL, O. A., TSYNDRENKO, A. V., VOLKOVA, T. M. & GRISHIN, E. V. (1989). Argiopine, argiopinines and pseudoargiopinines as glutamate receptor blockers in hippocampal neurons. *Neurofiziologia* **21**, 748–756 (in Russian).

KOSTENKO, M. A., GELETYUK, V. I. & VEPRINTSEV, B. N. (1974). Completely isolated neurones in the mollusc *Lymnaea stagnalis*. A new objective for nerve cell biology investigation. *Comparative Biochemistry and Physiology* **49A**, 89–100.

KOSTYUK, P. G. (1968). Ionic background of activity in giant neurons of molluscs. In *Symposium on Neurobiology of Invertebrates*, ed. SALANKI, J., pp. 145–167. Plenum Press, New York.

KROGSGAARD-LARSEN, P., HONORE, T., HANSEN, J. J., CURTIS, D. R. & LODGE, D. (1980). New class of glutamate agonist structurally related to ibotenic acid. *Nature* **284**, 64–66.

LOWAGIE, C. & GERSHENFELD, H. M. (1973). Mécanisme ionique des reponses au glutamate des neurones centrauz d'*Helix aspersa*. *Journal de Physiologie* **67**, 350–351.

MCCREERY, M. J. & CARPENTER, D. O. (1984). Modulation of neuronal responses to *l*-glutamate in *Aplysia*. *Cellular and Molecular Neurobiology* **4**, 91–95.

MAGAZANIK, L. G. & ANTONOV, S. M. (1988). Activation and blockade of glutamate-sensitive ion channels. *Symposia Biologica Hungarica* **36**, 105–116.

MAGAZANIK, L. G., ANTONOV, S. M., FEDOROVA, I. M., VOLKOVA, T. M. & GRISHIN, E. V. (1986). Argiopin – a naturally occurring blocker of glutamate-sensitive synaptic channels. *Biologischeskie Membrany* **3**, 1204–1219 (in Russian).

MAGAZANIK, L. G., ANTONOV, S. M., FEDOROVA, I. M., VOLKOVA, T. M. & GRISHIN, E. V. (1987). Argiopine – a naturally occurring blocker of glutamate-sensitive synaptic channels. In *Receptors and Ion Channels*, ed. HUCHO, F. & OVCHINNIKOV, YU. A., pp. 305–312. de Gruyter, Berlin.

MIWA, A., KAWAI, N., SAITO, M., PAN-HOU, H. S. & YOSHIKA, M. (1987). Effects of spider toxin (JSTX) on excitatory postsynaptic current at neuromuscular synapse of spiny lobster. *Journal of Neurophysiology* **58**, 319–326.

MIWA, A., KAWAI, N. & UI, M. (1987). Pertussis toxin blocks presynaptic glutamate receptors – a novel 'glutamate$_B$' receptor in the lobster neuromuscular synapse. *Brain Research* **416**, 162–165.

NEILD, T. O. & THOMAS, R. C. (1974). Intracellular chloride activity and the effects of acetylcholine in snail neurones. *Journal of Physiology* **242**, 453–470.

NICOLL, R. A. (1978). The blockade of GABA mediated responses in the frog spinal cord by ammonium ions and furosemide. *Journal of Physiology* **283**, 121–132.

NOWAK, L., BREGESTOVSKI, P., ASCHER, P., HERBET, A. & PROCHIANTZ, A. (1984). Magnesium gates glutamate-activated channels in mouse central neurones. *Nature* **307**, 462–465.

OOMURA, Y., OOYAMA, H. & SAWADA, M. (1974). Analysis of hyperpolarizations induced by glutamate and acetylcholine on *Onchidium* neurones. *Journal of Physiology* **243**, 321–341.

PIGGOTT, S. M., KERKUT, G. A. & WALKER, R. J. (1975). Structure-activity studies of glutamate receptor sites of three identifiable neurones in the sub-oesophageal ganglia of *Helix aspersa*. *Comparative Biochemistry and Physiology* **51C**, 91–100.

SAITO, M., KAWAI, N., MIWA, A., PAN-HOU, H. & YOSHIKA, M. (1985). Spider toxin (JSTX) blocks glutamate synapse in hippocampal pyramidal neurons. *Brain Research* **346**, 397–399.

SASAKI, K. & SATO, M. (1987). A single GTP-binding protein regulates K channels coupled with dopamine, histamine and acetylcholine receptors. *Nature* **259**, 260–262.

SMART, T. G. (1989). Excitatory amino acids: the involvement of second messengers in the signal transduction process. *Cellular and Molecular Neurobiology* **9**, 193–206.

STRANGE, P. G. (1988). The structure and mechanism of neurotransmitter receptors. Implications for the structure and function of the central nervous system. *Biochemical Journal* **249**, 309–318.

SUGIYAMA, H., ITO, J. & HIRONO, C. (1987). A new type of glutamate receptor linked to inositol phospholipid metabolism. *Nature* **325**, 531–533.

SZCZEPANIAK, A. C. & COTTRELL, G. A. (1973). Biphasic action of glutamic acid and synaptic inhibition in an identified serotonin-containing neurone. *Nature New Biology* **241**, 62–64.

WALKER, R. J. (1976). The action of kainic acid and quisqualic acid on the glutamate receptors of three identifiable neurones from the brain of the snail, *Helix aspersa*. *Comparative Biochemistry and Physiology* **55C**, 61–67.

WALKER, R. J., WOODRUFF, G. N. & KERKUT, G. A. (1971). The effect of ibotenic acid and muscimol on single neurons of the snail, *Helix aspersa*. *Comparative and General Pharmacology* **2**, 168–174.