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THE ACTIONS OF L-GLUTAMATE AT THE POSTSYNAPTIC MEMBRANE OF THE SQUID GIANT SYNAPSE

BY D. J. ADAMS[1] AND J. I. GILLESPIE[2]

The Laboratory of the Marine Biological Association, Plymouth PL1 2PB, UK, [1]Department of Pharmacology, University of Miami School of Medicine, PO Box 016189, Miami, FL 33101, USA and *[2]Department of Physiological Sciences, The Medical School, University of Newcastle upon Tyne, Newcastle upon Tyne NE2 4HH, UK*

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Summary

The actions of L-glutamate on the postsynaptic membrane of the squid giant synapse were investigated using two methods of application: ionophoresis and bath perfusion. Bath perfusion of 10 mmol l^{-1} sodium glutamate did not produce an appreciable depolarization of the postsynaptic membrane but reversibly blocked the neurally evoked postsynaptic potential (PSP). The postsynaptic membrane depolarized when L-glutamate was applied ionophoretically. The sensitivity to glutamate application was not uniform, but sharply localized to sites which may correspond to synaptic contacts made by branching colaterals from the postsynaptic axon. The relationship between membrane potential and amplitude of the glutamate-activated postsynaptic potential (PSP) examined under current-clamp conditions was linear over the voltage range studied (−110 to −60 mV) with an extrapolated reversal potential of −36 mV. The amplitude of the glutamate-activated PSP was reduced either by replacing Na^+ in the external solution with $Tris^+$ (Na^+-free) or by raising the extracellular K^+ concentration to 20 mmol l^{-1} and was abolished by removing both Na^+ and Ca^{2+} from the bath solution. The PSP amplitude was insensitive to changes in the extracellular Mg^{2+} concentration. The extrapolated reversal potential of the glutamate PSP was shifted to more positive potentials in both Na^+-free and raised-K^+ bathing solutions and was unchanged by anion substitution.

The depolarization induced by L-glutamate increased with increasing ionophoretic current and reached a maximum with large pulses. Double logarithmic plots of the coulomb dose–response relationship gave a limiting slope in the range 1·7–2·2, suggesting that two glutamate molecules are required for receptor activation. The time course of desensitization of the glutamate response was studied using a double-pulse method. The initial decrease in the ratio, PSP_2/PSP_1, is followed by a slower time-dependent recovery of the postsynaptic response with a time constant of 8·5 s. Prolonged perfusion of the squid giant synapse with concanavalin A failed to abolish desensitization of the glutamate-evoked PSP.

Key words: glutamate, squid giant synapse, calcium, dose–response, desensitization.

Introduction

Transmission of nerve impulses across the giant synapse of the squid stellate ganglion is mediated by a transmitter substance released from the presynaptic nerve terminal. Although the endogenous neurotransmitter is unknown, evidence supporting L-glutamate as a candidate includes the depolarization of the postsynaptic membrane in response to ionophoretically applied L-glutamate (Miledi, 1967, 1969) and blockade (desensitization) of the excitatory postsynaptic potential (EPSP) with the aortic perfusion or bath application of L-glutamate (Kelly & Gage, 1969; Kawai *et al.* 1983; Stanley, 1984). Evidence based on the action of glutamate receptor agonists and antagonists also supports L-glutamate as a transmitter substance of motoneurones innervating squid chromatophore muscles (Florey *et al.* 1985). However, the hypothesis that L-glutamate is the transmitter has been questioned by the observation that the depolarization evoked by glutamate has a different reversal potential from that of the EPSP (Miledi, 1969; Llinas *et al.* 1974). A similar discrepancy in measurement of the reversal potentials for the neurally evoked excitatory postsynaptic current and ionophoretically applied L-glutamate at some crustacean neuromuscular junctions has been attributed to inadequate spatial control of the postsynaptic membrane voltage (see Dekin, 1983).

Permeability changes produced by the release of endogenous transmitter or L-glutamate at the synaptic region of crayfish muscle indicate that the postsynaptic channel is non-selective for cations (Onodera & Takeuchi, 1976; Dekin, 1983). The nerve-evoked EPSP at the squid giant synapse has been shown to be due to an increased sodium, potassium and, to a small extent, calcium permeability (Llinas *et al.* 1974; Manalis, 1974; Kusano *et al.* 1975). The present experiments were undertaken to examine the ionic dependence and dose–response characteristics of the glutamate-activated postsynaptic channels at the squid giant synapse.

Materials and methods

Experiments were made on the distal (giant) synapse of the isolated stellate ganglion of adult squid *Alloteuthis subulata* (Lamarck, 1798) (mantle lengths 5–10 cm) using methods described previously (Gillespie, 1979; Adams *et al.* 1985). The preparation was mounted in a Perspex bath (volume <1 ml) and the extracellular solution changed continuously at a rate of 1–5 ml min^{-1}. Artificial seawater (ASW) solutions contained (mmol l^{-1}): NaCl, 470; KCl, 10; $CaCl_2$, 11; $MgCl_2$, 25; $MgSO_4$, 30; Tris buffer 10; pH 7·8 and were oxygenated. Sodium-free solution (0 Na^+) was made by isosmolar substitution of TrisCl for NaCl. The nominal removal of $CaCl_2$ (0 Ca^{2+}) from sodium-free sea water and addition of KCl to ASW constituted 0 Ca^{2+} and 20 K^+ solutions, respectively. The extracellular Mg^{2+} concentration was reduced by omitting Mg^{2+} salts from the artificial seawater solution. Anion substitution (84% of the extracellular Cl^- concentration) was achieved by replacing NaCl with sodium methylsulphate. Crystalline salts of cadmium chloride were added to ASW to give the final concentration

stated. Concanavalin A was obtained from Sigma (grade IV). The experiments were carried out at 15–20°C.

Two microelectrodes were inserted into the postsynaptic axon, adjacent to the presynaptic nerve terminal and <200 μm apart: one filled with 3 mol l^{-1} KCl (resistances approx. 15 MΩ) for voltage recording and a second filled with 1·8 mol l^{-1} potassium citrate (resistances approx. 5 MΩ) for current injection. The current injected was measured *via* a current-to-voltage converter as the voltage drop across a 4·7 kΩ resistor. Postsynaptic potentials and ionophoretic currents were displayed on an oscilloscope and recorded on FM tape (Racal Store 4, 19 cm s^{-1}) for later analysis. Neurally evoked postsynaptic potentials were elicited using extracellular platinum electrodes to stimulate the preganglionic nerve. An extracellular pipette containing 1 mol l^{-1} sodium glutamate (pH 7·8; resistances >50 MΩ), located within 50 μm of the postsynaptic region of the axon, was used to apply L-glutamate ionophoretically. Bath application of exogenous glutamate was achieved by adding sodium glutamate (Sigma) to ASW to give a final concentration of 10 mmol l^{-1}.

Results

Postsynaptic sensitivity to L-*glutamate*

The effects of L-glutamate on the postsynaptic membrane of the squid giant synapse were investigated using two methods of application: ionophoresis and bath perfusion. Focal application of glutamate, *via* ionophoresis from a micropipette filled with 1 mol l^{-1} sodium glutamate depolarized the postsynaptic axon as shown in Fig. 1A. Ionophoresis of glutamate (>0·5 μC) typically produced a 10–15 mV depolarization from a resting membrane potential of −65 mV which was confined to the postsynaptic membrane adjacent to the presynaptic nerve terminal and not observed outside the synaptic region. The time-to-peak of the glutamate potential was usually between 0·5 and 1 s, but this was critically dependent on the location of the ionophoretic pipette. The glutamate-sensitive sites along the synaptic region were very circumscribed, and movement of less than 10 μm of the tip of the pipette reduced or abolished the response. The amplitude and time course of the membrane depolarization produced in response to ionophoretically applied glutamate were similar to the glutamate responses originally reported at this synapse by Miledi (1967, 1969). To exclude the possibility that the glutamate potential is a secondary effect due to the release of endogenous transmitter from the presynaptic nerve terminal, 1 mmol l^{-1} $CdCl_2$, which blocks evoked release of transmitter at the squid giant synapse (Llinas *et al.* 1981; Augustine & Eckert, 1984), was added to bath solution. The amplitude and time course of the glutamate-induced depolarization of the postsynaptic membrane was unchanged in the presence of Cd^{2+} (not shown).

Perfusion of the isolated stellate ganglia with artificial sea water containing 10 mmol l^{-1} sodium glutamate did not produce appreciable depolarization (<2 mV) of the postsynaptic membrane. However, after approximately 15 min of

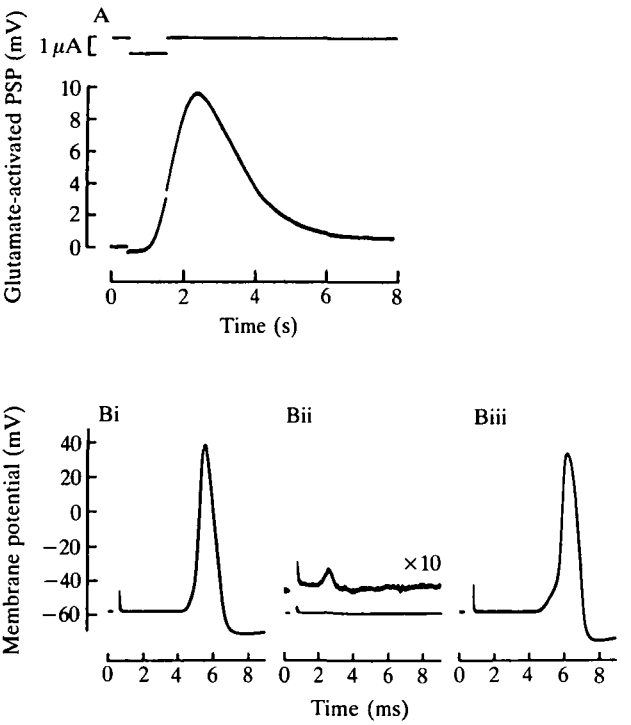

Fig. 1. Postsynaptic actions of L-glutamate. (A) Depolarization of the postsynaptic membrane in response to ionophoretically applied L-glutamate (1 μA, 1 s pulse). Temperature 20°C. (B) Neurally evoked postsynaptic response obtained in the absence (i, iii) and presence (ii) of bath applied sodium glutamate (10 mmol l^{-1}). (i) Control response before glutamate application. (ii) Evoked postsynaptic response following exposure to ASW containing 10 mmol l^{-1} sodium glutamate. High-gain (×10) record shows the presence of the extracellular presynaptic action potential. (iii) Recovery of the evoked postsynaptic action potential 40 min after washout of L-glutamate.

perfusion, stimulation of the presynaptic nerve failed to evoke a postsynaptic response (Fig. 1B). The blockade of the evoked postsynaptic potential is seen in records (e.g. Fig. 1B) of the postsynaptic response in the presence and absence of the external solution containing 10 mmol l^{-1} L-glutamate. Recovery of the evoked postsynaptic action potential followed approximately 30 min washout of glutamate sea water. The depression of synaptic transmission by exogenous glutamate is consistent with previous studies which attributed the effect of glutamate to receptor desensitization (Kelly & Gage, 1969; Stanley, 1984).

Relationship between membrane potential and amplitude of glutamate-activated postsynaptic potentials

The evidence against L-glutamate being the endogenous neurotransmitter released from presynaptic nerve terminal is the observation that the reversal (zero-current) potential for the evoked synaptic potential (+28 mV) is different from

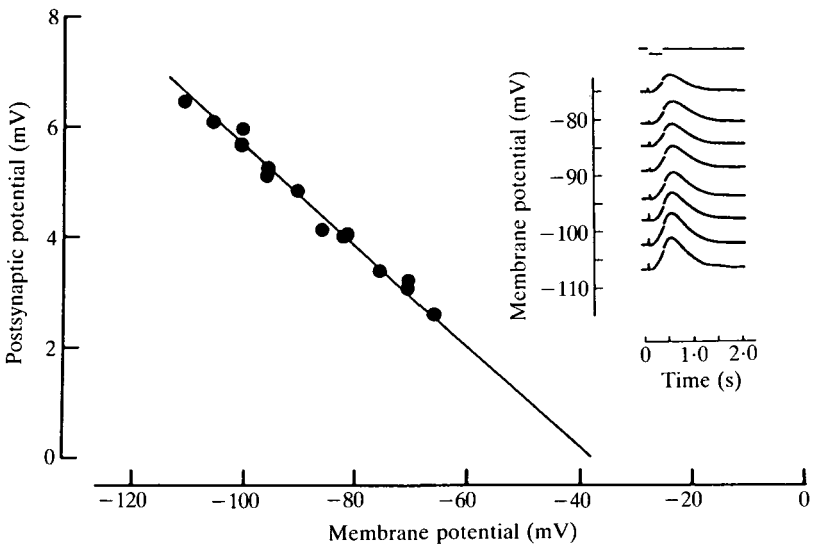

Fig. 2. Relationship between membrane potential of the post-axon and amplitude of the glutamate-induced postsynaptic potential at the same synapse. The line is a least-squares fit to the data points. Inset, responses to ionophoretic application of L-glutamate. Ionophoretic current pulse 1 μA, 250 ms.

that of the glutamate-induced PSP (−22 mV) (Miledi, 1969). The relationship between the postsynaptic membrane potential and the amplitude of the glutamate potential was re-investigated under current-clamp conditions. The membrane potential of the postsynaptic axon was altered by passing a constant current through an independent electrode located distal (<200 μm) to the voltage-recording electrode. Excitatory potentials evoked by ionophoretic pulses of L-glutamate applied to the postsynaptic axon are shown in Fig. 2 (inset) for a limited range of membrane potentials (−75 to −110 mV). The relationship between the membrane potential of the postsynaptic axon and the amplitude of the glutamate-induced potential (filled symbols) is shown in Fig. 2. Although quaternary ammonium ions were not injected into the postsynaptic axon to reduce the delayed K^+ conductance and permit direct measurement of the reversal potential (Miledi, 1969), the extrapolated reversal potential obtained from the least-squares fit to the data points for the glutamate-evoked postsynaptic potentials was $-36 \pm 1{\cdot}6$ mV (S.E.M., $N = 4$).

Serious considerations in the measurement of the voltage-dependence of PSP amplitude and reversal potential of ionophoretically applied agonists are the morphology and cable properties of the postsynaptic region. The postsynaptic axon can be considered as an infinite cable the response of which, V, to a prolonged step current change, I_o, under steady-state conditions may be calculated using the equation (Jack *et al.* 1975):

$$V = (r_a I_o \lambda/2) \exp(-x/\lambda) , \quad (1)$$

where r_a (the intracellular resistance per unit length of cable) $= R_i/\pi a^2$, and x is the distance from the site of current injection. The input resistance $R_i = V/I_o$ and a is the cable radius. The membrane space constant $\lambda = r_m/r_a$, where r_m is the membrane resistance per unit length of cable.

During the postsynaptic conductance change, we calculate that the space constant, λ, is reduced approximately fivefold (to $<0{\cdot}7$ mm) (Martin, 1955) which would attenuate the spatial voltage control of the postsynaptic region. The measurement of the voltage-dependence of PSP amplitude is valid only if the agonist is focally applied or released at the site of voltage recording as performed in our experiments. However, the relationship between the neurally evoked PSP amplitude and membrane potential and the determination of the reversal potential would be prone to error because the synaptic contacts, and hence presumably transmitter release sites, are distributed along the entire length of the synapse (0·5–1·2 mm; Young, 1973; Martin & Miledi, 1986).

Ionic dependence of the glutamate-induced postsynaptic potential

To determine which of the extracellular cations contributed to the glutamate-activated excitatory postsynaptic potential, the ionic composition of the external solution was altered. The postsynaptic response to repetitive ionophoresis of L-glutamate at frequencies of 0·02–0·1 Hz was monitored during changes in the ionic composition of the external solution. A continuous record of the glutamate-induced PSP amplitude during exposure to and recovery from sodium-free, sodium + calcium-free, and high-potassium external solutions is shown in Fig. 3A. Substitution of extracellular sodium ions with $Tris^+$ reduced the PSP amplitude by approximately half, whereas replacement of sodium with $Tris^+$ and removal of extracellular Ca^{2+} completely abolished the postsynaptic response to glutamate. The decrease in amplitude of glutamate-activated PSPs in the absence of sodium and calcium was completely reversible and not due to any significant change in the membrane potential of the postsynaptic fibre. Removal of calcium (nominally Ca^{2+}-free), while leaving the extracellular Na^+ concentration unchanged or lowering the external Mg^{2+} concentration to 2·5 mmol l^{-1}, produced a negligible change in the PSP amplitude (not shown). Raising the extracellular potassium ion concentration from 10 to 20 mmol l^{-1} also reduced the glutamate-activated PSP amplitude by approximately half. The decrease in PSP amplitude was greater than would be obtained for a 12–15 mV depolarization of the membrane potential produced by doubling the extracellular K^+ concentration. The rise in extracellular K^+ concentration might result in a tonic release of transmitter which could lead to the desensitization of postsynaptic glutamate receptors.

The voltage-dependence of the glutamate-activated PSP in the presence and absence of sodium and potassium is shown in Fig. 3B. The relationship between membrane potential and peak amplitude of the PSP was linear over the limited voltage range (−110 to −60 mV) studied in the various external solutions. Equimolar replacement of external sodium with Tris resulted in a fivefold decrease

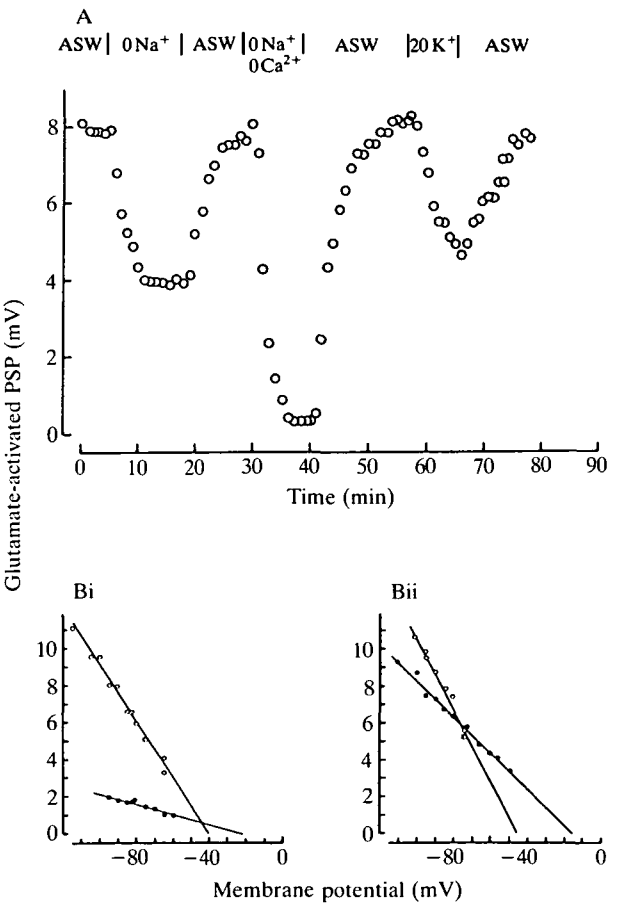

Fig. 3. Ionic dependence of the glutamate-activated response. (A) Continuous record of the postsynaptic potential amplitude in response to ionophoretic application of L-glutamate (1 μA, 1 s) during ion substitutions. Post-axon membrane potential: −64 mV. $0Na^+$, sodium replaced isosmotically by $Tris^+$; $0Ca^{2+}$, nominal removal of calcium ions from ASW solutions; $20K^+$, doubling of the ASW potassium ion concentration (membrane potential −47 mV). (B) Relationship between membrane potential and amplitude of the glutamate-activated postsynaptic potential (i) before (470 mmol l^{-1} Na^+) (○) and after (●) substitution of sodium with $Tris^+$ (0 mmol l^{-1} Na^+); (ii) in the absence (0 mmol l^{-1} K^+) (○) and presence (●) of a high external potassium concentration (20 mmol l^{-1} K^+).

in the slope of the relationship between glutamate-activated PSP amplitude and membrane potential from −0·16 to −0·03, respectively. The extrapolated reversal potential was correspondingly shifted by >30 mV in the positive direction, which could be explained by an increased calcium permeability of the open glutamate-activated channel upon the removal of external sodium ions. To determine the contribution of potassium ions to the glutamate-activated response, the PSP amplitude was measured in K^+-free and 20 mmol l^{-1} K^+ sea water. The slope of

the relationship between membrane potential and glutamate-activated PSP amplitude obtained in 20 mmol l^{-1} K^+ was approximately half of that obtained in the absence of external K^+ (0 mmol l^{-1} K^+), and the extrapolated reversal potential was −17 mV (Fig. 3Bii). The positive shift of the reversal potential upon raising the extracellular K^+ concentration would be consistent with a shift in the K^+ equilibrium potential and K^+ being permeant. The difference in the slope conductance obtained for the I–V relationships in 20 mmol l^{-1} K^+ and K^+-free solutions could not be attributed to a significant change in the resting membrane conductance as monitored by constant-current pulses. Lowering the extracellular Cl^- concentration from 562 to 92 mmol l^{-1} by substituting methylsulphate for chloride produced a small decrease in the slope conductance, but did not shift the apparent reversal potential.

Concentration-dependence of glutamate-activated responses

The coulomb dose–response relationship and kinetics of desensitization of glutamate receptors at the squid giant synapse were studied using a two-pulse method. To determine the dose–response relationship, L-glutamate was ionophoretically applied to the postsynaptic axon with a 4 s interval between the first (prime) and second (test) pulses (1 s duration) and repeated every 100 s. The ionophoretic current of the prime pulse was increased with successive trials and the PSP amplitude in response to the prime and test pulse measured (Fig. 4A). Increasing the ionophoretic current of the prime pulse produced an increase in PSP amplitude and a concomitant decrease in the amplitude of the PSP in response to the constant current of the test pulse. The concentration–response relationship was determined in normal ASW and at a resting membrane potential of −67 mV. The depolarization induced by L-glutamate varied as a graded function of the ionophoretic current, although the response reached a maximum with large pulses (Fig. 4B). For the action of glutamate on postsynaptic receptors at the squid giant synapse, the value of the limiting slope of the double logarithmic plot shown in Fig. 4B was 2·2 (range 1·7–2·2, $N = 4$) for the response to the prime pulse. This relationship illustrates the dependence of the response on glutamate concentration and the low glutamate-sensitivity of the postsynaptic membrane of the squid axon. The concentration of applied glutamate at the postsynaptic membrane at the mean peak of the glutamate-induced PSP was calculated from the diffusion equation (Crank, 1975) assuming diffusion from a micropipette located <70 μm from the receptor plane. The peak concentration (C) at a point distance r from the point source is given by:

$$C(t) = q/4\pi Dr \operatorname{erfc}\{r/2(Dt)^{\frac{1}{2}}\} \quad (t < t') , \tag{2}$$

where q is the rate of glutamate application for the period t′, D is the diffusion coefficient, and t is the time-to-peak of the response. The time-to-peak t is related to the distance r by the equation $6Dt = r^2$. The concentration of glutamate at the *mean* peak amplitude of the PSP was calculated as $2{\cdot}7 \times 10^{-4}$ mol l^{-1}, assuming a

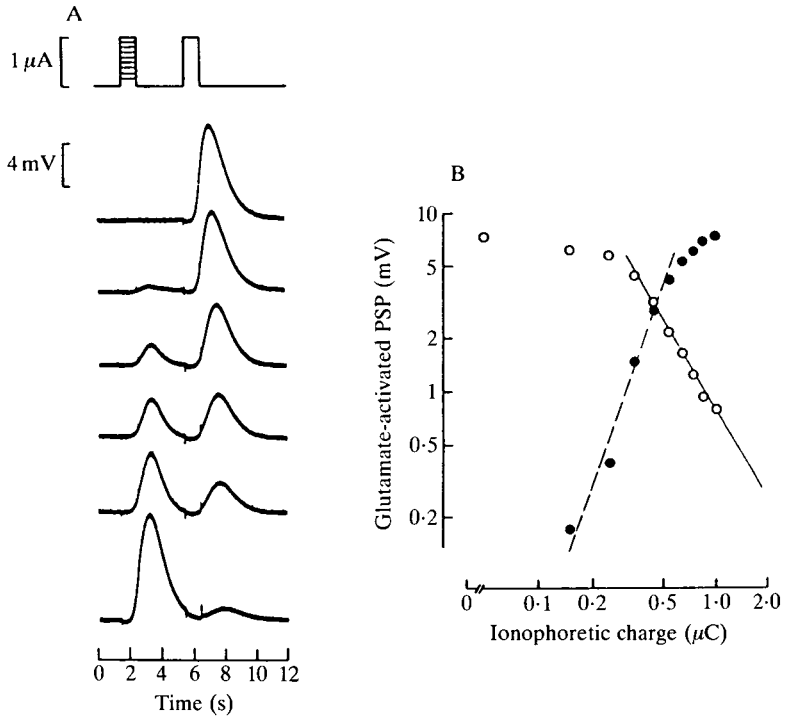

Fig. 4. Concentration-dependence of glutamate-activated responses. (A) Records of depolarizing potentials in response to ionophoretic application of L-glutamate to the postsynaptic membrane. Upper trace: ionophoretic pulse paradigm – prime and test ionophoretic pulses (1 μA, 1 s) were separated by a 4 s interval. Membrane potential −66 mV; temperature 18°C. (B) Double logarithmic plot of the amplitude of the glutamate potential, obtained in response to test (○) and prime (●) ionophoretic pulses, as a function of the ionophoretic charge of the prime pulse. Least-squares fit to the data points (dashed and filled lines) give slopes of 1·7 and 2·2, respectively.

transport number of 0·126 and diffusion coefficient of $7{\cdot}6\times10^{-6}\,cm^2\,s^{-1}$ for glutamate (see Onodera & Takeuchi, 1976, 1980).

Desensitization of glutamate receptors

Desensitization of glutamate receptors to repeated, or continuous, application of glutamate has been shown at the squid postsynaptic axon (Miledi, 1967; Kelly & Gage, 1969; Stanley, 1984). Desensitization of the postsynaptic membrane response to ionophoretic application of L-glutamate is shown in Fig. 4A. Increasing the ionophoretic dose of glutamate applied prior to a test pulse of glutamate progressively reduced the amplitude of the glutamate-induced postsynaptic potential. Similarly, bath application of sea water containing $10\,mmol\,l^{-1}$ sodium glutamate inhibited the postsynaptic response to test pulses of L-glutamate. The reversible block of the neurally evoked EPSP occurred after 35–40 min of bath perfusion with $10\,mmol\,l^{-1}$ sodium glutamate. The time course of desensitization

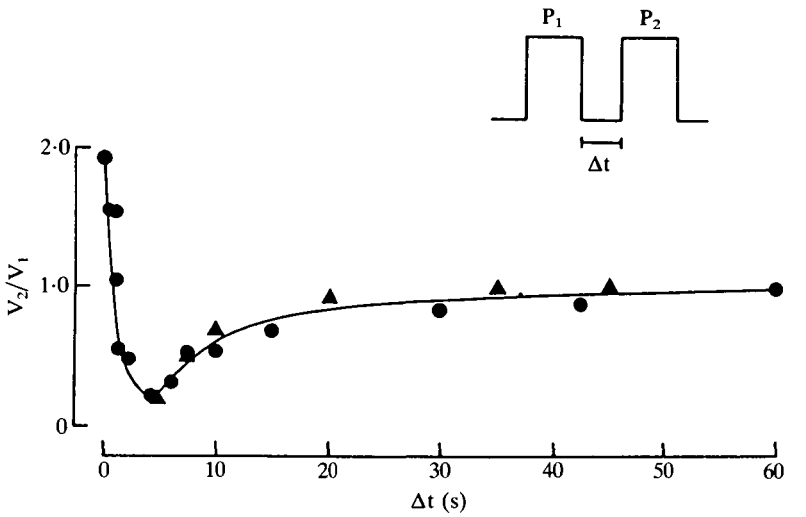

Fig. 5. Time course of the onset and recovery from desensitization. The ratio of the glutamate-induced depolarization (V_2/V_1) is plotted as a function of the interval (Δt) between the ionophoretic pulses (P_1, P_2). The decrease in the ratio V_2/V_1 is followed by a slow time-dependent recovery with an initial time constant of 8·5 s. Different symbols represent data from two experiments.

of the response to glutamate examined in two preparations using a two-pulse protocol is shown in Fig. 5. The interval between ionophoretic pulses of L-glutamate (1 μC) to the postsynaptic membrane was varied and the ratio of postsynaptic potentials (V_2/V_1) calculated. The rationale of the method is that the response to the test pulse indicates the fraction of the receptor population that has recovered from desensitization induced by the control dose. The initial decrease in the ratio V_2/V_1 was followed by a slow time-dependent recovery of the postsynaptic response. The onset of desensitization depended on the frequency and amplitude of the applied glutamate pulses. The apparent time constant for the onset of desensitization was 1·5 s and the EPSP amplitude was depressed to 80 % of control with 0·5 μC pulses of glutamate applied at 1 Hz. Under the given experimental conditions, the time-dependent recovery from desensitization over the initial 60 s could be fitted by a single exponential function with a time constant of 8·5 s.

Concanavalin A, a plant lectin which has been shown to inhibit desensitization of junctional and extrajunctional glutamate receptors in locust muscle (Mathers & Usherwood, 1978) and glutamate receptors in molluscan neurones (Kehoe, 1978), was tested on the glutamate-evoked response at the squid postsynaptic membrane. In three experiments, continuous perfusion of the squid giant synapse for 90–120 min with sea water containing 100–200 $\mu g\,ml^{-1}$ concanavalin A failed to abolish desensitization of the PSP evoked by repetitive ionophoretic application of L-glutamate. The prolonged exposure should have been sufficient to allow diffusion of concanavalin A to the postsynaptic membrane, although only an

examination of the distribution of labelled concanavalin A would have indicated if restricted access could have accounted for its lack of effect on the glutamate receptors.

Discussion

The present experiments describe the concentration-dependence and ionic mechanisms underlying the postsynaptic response of the squid giant synapse to the ionophoretic application of L-glutamate. The postsynaptic sensitivity to glutamate was highly localized at discrete sites along the synaptic junction (see Miledi, 1967) which may correspond to the intervals at which narrow branching collaterals of the postsynaptic axon pierce the sheath of glial cells separating axons to contact the presynaptic axon (Young, 1973; Pumplin & Reese, 1978; Martin & Miledi, 1986). The irregularities in the geometry of the postsynaptic element and spatial distribution of postsynaptic receptors with respect to the location of the ionophoretic pipette may contribute to the temporal dispersion in glutamate receptor activation (channel opening) and, hence, to the difference observed in the time course of the postsynaptic responses to neurally evoked transmitter release and glutamate ionophoresis.

The evidence supporting L-glutamate as an excitatory transmitter at the squid giant synapse includes depression (desensitization) of the neurally evoked EPSP and spontaneous miniature EPSP with bath application (Kelly & Gage, 1969; Augustine & Eckert, 1984) and irreversible blockade of the EPSP and glutamate-induced depolarization by a specific glutamate receptor antagonist, Joro spider toxin (JSTX; Kawai *et al.* 1983). Although L-glutamate is one of the most effective excitatory amino acids tested at the squid giant synapse (Miledi, 1967; DeSantis *et al.* 1978; Stanley, 1983; Eusebi *et al.* 1985), the observation that the depolarization evoked by glutamate has a different reversal potential from that of the EPSP suggests either that glutamate may not be the endogenous neurotransmitter (Miledi, 1969) or that there may be a difference in the ion selectivities of the postsynaptic receptor channels opened by glutamate and the endogenous transmitter. The reversal potential measurement of −36 mV obtained from extrapolation of the relationship between the amplitude of the glutamate-induced potential and the membrane potential is similiar to previous measurements of the 'glutamate' reversal potential (Miledi, 1969; Eusebi *et al.* 1985).

Investigation of the effect of changes in the ionic composition of the extracellular solution on the glutamate-activated response indicates that the postsynaptic depolarization is due to an increase in the sodium, potassium and calcium permeabilities of the postsynaptic membrane, similar to that described for the neurally evoked EPSP (Manalis, 1973; Llinas *et al.* 1974; Kusano *et al.* 1975). The negligible effect of nominally Ca^{2+}-free sea water on the glutamate potential is consistent with the small contribution of calcium ions (approximately 2% in normal sea water) to the glutamate-activated current calculated from the glutamate-induced rise in intracellular Ca^{2+} concentration measured by the

calcium indicators aequorin and arsenazo III in the squid postsynaptic axon (Eusebi *et al.* 1985). However, removal of extracellular Ca^{2+} from a Na^+-free bathing solution, which alone reduced the glutamate-activated PSP by approximately 50%, reversibly abolished the glutamate potential. This suggested either that the cation permeability of the glutamate receptor channel may be modified by the external Na^+ concentation or that the sodium substitute, $Tris^+$, blocks the glutamate receptor channel (see Anwyl, 1977). The apparent shift of the reversal potential to more positive potentials in the absence of external Na^+ is consistent with a change in either the potassium or the calcium permeability of the glutamate-activated channel. A class of glutamate receptors in mammalian spinal cord and hippocampal neurones activated by *N*-methyl-D-aspartate (NMDA) has recently been shown to permit a significant Ca^{2+} influx (MacDermott *et al.* 1986; Jahr & Stevens, 1987) and is blocked by extracellular Mg^{2+} (Nowak *et al.* 1984). However, the lack of effect of reducing the external Mg^{2+} concentration on the glutamate-evoked PSP amplitude and the relative potencies of the glutamate analogues kainate, quisqualate and NMDA on activation of the postsynaptic receptor channels at the squid giant synapse (Stanley, 1983; Eusebi *et al.* 1985) suggest that the excitatory amino acid receptor subtype is unlikely to be an NMDA receptor.

The dose–response characteristics for glutamate at the squid postsynaptic axon indicate that the glutamate-sensitivity of the postsynaptic membrane is an order of magnitude lower than that reported for junctional glutamate receptor channels in locust muscle (Cull-Candy, 1978), where an apparent dissociation constant of 300–500 μmol l^{-1} was obtained for the glutamate–receptor complex (Cull-Candy *et al.* 1981). This apparent difference in glutamate-sensitivity may reflect the ionic conditions under which glutamate receptor activation was studied rather than the concentration-dependence of the rate constants for the glutamate–receptor binding steps. The approximately second-power relationship between the glutamate-activated PSP and coulomb dose (glutamate concentration) suggests that at least two molecules of glutamate are required to activate the receptor channel. The value of the limiting slope of the coulomb dose–response relationship is similar to that obtained for glutamate receptor activation in locust muscle (Cull-Candy, 1978; Clark *et al.* 1979; Cull-Candy *et al.* 1981). However, interpretation of dose–response and desensitization properties may be complicated by changes in the ion concentration in the synaptic cleft caused by the prolonged postsynaptic depolarization and removal of glutamate from the vicinity of the receptors.

The kinetics of desensitization of glutamate receptors at the squid giant synapse examined here are qualitatively similar to those described for junctional glutamate receptors (D-responses) in locust muscle (Cull-Candy, 1978; Clark *et al.* 1979). Although the recovery time constant measured with the two-pulse method may contain some contribution from a time-dependent change in the distribution of receptors available for activation (Clark *et al.* 1979), the highly localized distribution of glutamate-sensitive sites and the similar time-to-peak of the control and test responses suggests that both doses of glutamate activate the same area of postsynaptic membrane. However, the extent and rates of desensitization of

glutamate receptors are likely to be underestimated due to spatial inhomogeneity and diffusion delays associated with the squid giant synapse preparation. Recent measurements of rapid receptor desensitization (millisecond time constant) by glutamate at excised patches of synaptic membrane of crayfish muscle (Franke *et al.* 1987) suggest that the experimental conditions may account for the slower rates of desensitization of glutamate receptors obtained at the squid giant synapse.

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