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SYNAPTIC EXCITATION IN CULTURES OF MOUSE SPINAL CORD NEURONES: RECEPTOR PHARMACOLOGY AND BEHAVIOUR OF SYNAPTIC CURRENTS

BY P. G. NELSON, R. Y. K. PUN* AND G. L. WESTBROOK

From the Laboratory of Developmental Neurobiology, IRP, NICHD, Bldg. 36, Room 2A-21, National Institutes of Health, Bethesda, MD 20892, U.S.A.

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SUMMARY

1. Fast monosynaptic excitatory post-synaptic potentials between spinal cord neurones in cell culture (s.c.–s.c. e.p.s.p.s) were studied with current-clamp and two-electrode voltage-clamp methods. The reversal potential, response to acidic amino acid antagonists, and behaviour of the synaptic current were examined.

2. The amplitude of the e.p.s.p. increased with membrane potential hyperpolarization and decreased with depolarization. The reversal potential of the e.p.s.p. was $+3{\cdot}8 \pm 2{\cdot}5$ mV (mean ± S.E. of mean). The reversal potential for responses to ionophoretically applied L-glutamate and L-aspartate was also near 0 mV.

3. The acidic amino acid antagonist, cis-2,3-piperidine dicarboxylic acid (PDA, 0·25–1·0 mM) reversibly antagonized the monosynaptic e.p.s.p.s as well as responses to kainate (KA) or quisqualate (QA).

4. The selective *N*-methyl-D-aspartate antagonist, (±) 2-amino-5-phosphonovaleric acid (APV), had little effect on either the monosynaptic e.p.s.p.s or responses to QA or KA at concentrations that abolished responses to L-aspartate.

5. Under voltage clamp, the peak synaptic current (e.p.s.c.) was linearly related to the membrane potential, increasing in amplitude with hyperpolarization and decreasing with depolarization from the resting potential.

6. The decay of a somatic e.p.s.c. was well fitted by a single exponential function with a time constant of 0·6 ms at 25 °C. E.p.s.c.s which had proximal dendritic locations had decay time constants of 1–2 ms.

7. The decay time constant was voltage-insensitive between −80 and +10 mV.

8. We suggest that an acidic amino acid receptor other than that for NMDA mediates excitatory transmission at the s.c.–s.c. synapse; and that the underlying conductance mechanism is voltage insensitive with an estimated mean channel lifetime of less than 1 ms.

* Present address: Department of Physiology and Biophysics, University of Cincinnati College of Medicine, Cincinnati, OH 45267, U.S.A.

INTRODUCTION

The chemical basis of synaptic transmission is now well established in the mammalian central nervous system. At a number of synaptic pathways in the brain and spinal cord, evidence is accumulating that the excitatory amino acids L-glutamate and L-aspartate or their analogues act as neurotransmitters (Puil, 1981; Fonnum, 1984). On the basis of studies with antagonists, it has been proposed that there are at least three separate excitatory amino acid receptors in the spinal cord, activated selectively by *N*-methyl-D-aspartate (NMDA), kainate (KA) and quisqualate (QA), which may participate in synaptic transmission (McLennan, 1981; Watkins, 1981). For example, extracellular studies *in vivo* have revealed that synaptic excitation of dorsal horn neurones by dorsal root stimulation is antagonized by the selective NMDA antagonist, 2-amino-5-phosphonovalerate (APV). Polysynaptic excitation was more sensitive to APV than monosynaptic excitation, thus suggesting that NMDA receptors may mediate synaptic excitation at intrinsic pathways within the spinal cord (Davies & Watkins, 1982).

Recently it has become clear that the conductance mechanisms associated with different acidic amino acid receptors can also be distinguished on the basis of their voltage sensitivity. NMDA activates a highly voltage-sensitive conductance in cultures of spinal cord neurones (MacDonald, Porietis & Wojtowicz, 1982), which results from a voltage-dependent channel block by physiological concentrations of Mg^{2+} (Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984; Mayer & Westbrook, 1985). KA and QA activate relatively voltage-insensitive conductances (MacDonald & Porietis, 1982) whereas L-glutamate and several of its analogues may act as mixed agonists that activate conductances associated with both NMDA and non-NMDA receptors (Mayer & Westbrook, 1984).

Previous studies of the fast monosynaptic excitatory post-synaptic potential (e.p.s.p.) between spinal cord neurones in primary dissociated cultures (s.c.–s.c. e.p.s.p.) have established that these synapses have the morphological and physiological properties characteristic of chemical synapses (Neale, Nelson, Macdonald, Christian & Bowers, 1983; Nelson, Marshall, Pun, Christian, Sheriff, Macdonald & Neale, 1983*a*). However, the identity of the transmitter and the properties of the underlying conductance mechanism have not been determined. Although excitatory amino acids have been considered to be the leading transmitter candidates, differences between the reversal potentials of monosynaptic e.p.s.p.s and exogenously applied L-glutamate have been reported (Ransom, Bullock & Nelson, 1977*a*; Brookes, 1978). We report here the effect of acidic amino acid antagonists on monosynaptic s.c.–s.c. e.p.s.p.s; and the behaviour of the excitatory synaptic current (e.p.s.c.) under two-electrode voltage clamp. Our results suggest that not the NMDA receptor, but rather either KA or QA receptors are likely to mediate the fast monosynaptic e.p.s.p. between spinal cord neurones in culture. The synaptic current underlying the fast monosynaptic e.p.s.p. is voltage insensitive with a decay time constant of 1 ms or less (25 °C). Preliminary results of some of these experiments have been presented (Pun, Westbrook & Nelson, 1982; Nelson, Pun & Westbrook, 1983*b*).

METHODS

Cell cultures

Primary dissociated cultures of whole spinal cord or cultures of the ventral half of the spinal cord were used for electrophysiological experiments. The methods for preparing these cultures have been previously described (Ransom, Neale, Henkart, Bullock & Nelson, 1977*b*; Guthrie & Brenneman, 1982). Briefly, spinal cords were dissected from 12–14 day embryonic mouse embryos (C57BL/6J), dissociated and plated in 35 mm collagen-coated dishes (Falcon) at a density of $5–8 \times 10^5$ cells/ml. Growth medium contained either 90% Minimal Essential Medium (MEM, Gibco, U.S.A.) with 10% horse serum; or 95% MEM/5% horse serum with an added nutrient supplement (Romijn, Habets, Mud & Wolters, 1982). Background cells were suppressed by exposure to a mixture of 5′-fluoro-2-deoxyuridine and uridine (15 and 35 μg/ml, respectively) for 48 h between days 7 and 9 in culture. Cultures were grown for 3–6 weeks before being used for electrophysiological experiments.

Electrophysiology

Recordings were made at room temperature (25–28 °C) on an inverted microscope equipped with phase contrast optics. The usual magnification was 200. The standard recording solution contained (mM): Na^+, 140; K^+, 4·5; Cl^-, 165; HEPES (*N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulphonic acid), 10; glucose, 10; Ca^{2+}, 5; Mg^{2+}, 5. The Mg^{2+} concentration reduced spontaneous polysynaptic activity and thus facilitated study of monosynaptic connexions; the elevated Ca^{2+} concentration was used to stabilize two-electrode impalements. In some experiments with acidic amino acid antagonists, Mg^{2+} was omitted from the recording solution to avoid masking possible synaptically-activated NMDA receptors. The pH was adjusted to 7·3 with NaOH and monitored with Phenol red (10 mg/l). Sucrose was added to match the osmolarity of the growth solutions (320–325 mosM).

Current clamp

Recordings were made with conventional bridge-circuit amplifiers. Micro-electrodes filled with K acetate (KAc, 3 M, pH 7·0) were used to impale two separate spinal cord neurones within the same microscope field. Micro-electrodes were made from fibre-filled borosilicate tubing and had d.c. resistances of 50–80 MΩ. Action potentials were evoked in both cells to test if the cell pair was synaptically connected. Only monosynaptic e.p.s.p.s were studied. Criteria for a monosynaptic e.p.s.p. included: (1) an e.p.s.p. which followed the presynaptic action potential with a latency of less than 4 ms (usually within 2 ms), and (2) no variation in latency with high frequency stimulation (> 10 Hz). Dye-filling of presynaptic neurones in these cultures has demonstrated that e.p.s.p.s identified by these criteria are monosynaptic (Neale, Macdonald & Nelson, 1978; Neale *et al.* 1983). Membrane potential was monitored on a Brush recorder and a digital voltmeter. Evoked monosynaptic responses were photographed on the oscilloscope screen and, in later experiments, stored on a PDP 11/34 laboratory computer for later analysis.

For measurement of the synaptic reversal potentials, a second micro-electrode was inserted in the post-synaptic neurone after identification of a monosynaptic connexion. The second electrode (CsCl, 1 M, pH 7·0) was used for injection of Cs ions (usually 1–3 nA × 5 min) and for passage of current during membrane polarization. The use of Cs, a K channel blocker, effectively reduced outward rectification of the membrane. Despite the reduction of outward rectification by Cs^+, there was only a small decrease (usually 10 mV or less) in the resting potential after Cs^+ injection; this contrasts with whole-cell patch recording with Cs^+ electrodes (140 mM-CsCl) where the resting potential declines to near 0 mV (Fenwick, Marty & Neher, 1982). This suggests that the internal Cs^+ concentration was relatively low in our experiments. The post-synaptic neurone was then polarized between −100 and +20 mV with steady current injection while recording the membrane potential and evoked synaptic potential with the KAc micro-electrode.

Pharmacological studies

The antagonists (±) 2-amino-5-phosphonvalerate (APV), *cis*-2,3-piperidine dicarboxylic acid (PDA) and γ-D-glutamylglycine (DGG) were dissolved in recording medium and the pH of the solution readjusted to pH 7·3. All antagonists were applied by pressure application from pipettes (5–10 μm tip) placed near ($\leqslant$ 100 μm) the post-synaptic neurone. Since drug application was by

leakage or by slight positive pressure to the back of the pipette (1–2 lbf/in^2), drug pipettes were kept distant from the cell pair during control periods. Perfusion with control medium occasionally caused a 1–2 mV hyperpolarization from the resting potential but had no effect on evoked synaptic responses or ionophoretic responses.

The acidic amino acids L-aspartate (0·2 M, pH 8·5), L-glutamate (0·2 M, pH 8·5), KA and QA (10 mM in 150 mM-NaCl, pH 7·4) were applied by ionophoresis. Ionophoretic electrodes were fabricated in the same manner as recording micro-electrodes. Retaining currents of +5 to +25 nA and ejection currents of −5 to −100 nA were used. An automatic current balancing circuit kept the net current flowing through the bath at zero. All drugs were purchased from Cambridge Research Biochemicals (U.K.) or Sigma (U.S.A.).

Voltage clamp

The post-synaptic neurone was voltage clamped using methods similar to those previously described (Mayer & Westbrook, 1983). The post-synaptic neurone was initially impaled with a Cs_2SO_4 micro-electrode for identification of a monosynaptic e.p.s.p. A second CsCl micro-electrode was then inserted. Prior to switching to voltage clamp, a synaptic potential and a hyperpolarizing voltage transient to a current pulse were stored on a PDP 11/23 laboratory computer for later analysis of e.p.s.p. shape indices (see Rall, Burke, Smith, Nelson & Frank, 1967; Jack, Miller, Porter & Redman, 1971). Equalizing time constants of the hyperpolarizing voltage transient were obtained by the method of Rall (1969). Electrotonic length (L) was estimated from $L = \pi[\tau_m/\tau_1 - 1]^{-1/2}$ where τ_m is the membrane time constant and τ_1 is the first equalizing time constant.

To reduce capacitative coupling between electrodes, the depth of the recording medium was reduced to 1 mm. In addition a driven shield surrounded the voltage electrode to within 100–200 μm of the tip. The shield was made by painting the shank of the electrode with silver, and then covering the paint with insulating material (Q-dope, GC Electronics, U.S.A.). The top of the silver was left exposed and made electrical contact with a metal tube surrounding the barrel of the electrode above the surface of the bath. Using 50 MΩ micro-electrodes the usual settling time of the capacitative current to a 10 mV voltage step was about 0·5 ms. This was presumably due in large part to charging of dendritic membranes since the membrane voltage at the soma reached a steady value more rapidly (within 0·2–0·4 ms). However, we did not use voltage jumps in the analysis of the synaptic current (see below) and thus the gain–band width product of the clamp was sufficient to resolve the time course of the synaptic current. Membrane current was measured with a current-to-voltage converter (3 dB point, 1·6 kHz). The presynaptic stimulating electrode was covered with a grounded shield to the surface of the bath.

The post-synaptic neurone was initially clamped at the resting potential and a series of e.p.s.c.s were evoked at 1–10 Hz. Averages of eight to thirty-two trials were collected. The holding potential was then changed by injection of steady current, and averaged synaptic currents were recorded at a series of membrane potentials between −100 and +20. Signals of the presynaptic action potential, post-synaptic current and post-synaptic voltage were amplified, filtered at 2 kHz (one-pole), digitized at 10–15 kHz and then stored on the computer for later analysis.

Following completion of the study, the presynaptic neurone was filled with the fluorescent dye Lucifer Yellow (4%, Sigma) using a separate intracellular micro-electrode. The microscope field containing the cell pair was then viewed and photographed with phase contrast and fluorescent optics using a Zeiss Photomicroscope II. In judging whether a synaptic input was located on the soma or on dendrites, i.e. proximal or distal, all points of close contact were considered as potential synaptic contacts whether axosomatic, axodendritic or dendrodendritic since no attempt was made to identify synaptic structures by electron microscopy.

RESULTS

Reversal potential of the e.p.s.p.

One of the criteria for transmitter identification is coincidence of the equilibrium potential of the synaptic potential ($E_{e.p.s.p.}$) with that of the exogenously applied transmitter (Werman, 1980). However, accurate measurement of synaptic reversal potentials can be difficult for several reasons (for review, see Redman, 1979). In

previous studies, reversal of s.c.–s.c. e.p.s.p.s in culture could not always be obtained even after injection of tetraethylammonium ions into the post-synaptic neurone (Macdonald, Pun, Neale & Nelson, 1983). In addition, comparisons of the extrapolated e.p.s.p. reversal potential with the extrapolated reversal for L-glutamate have shown marked differences (Ransom *et al.* 1977*a*; Brookes, 1978).

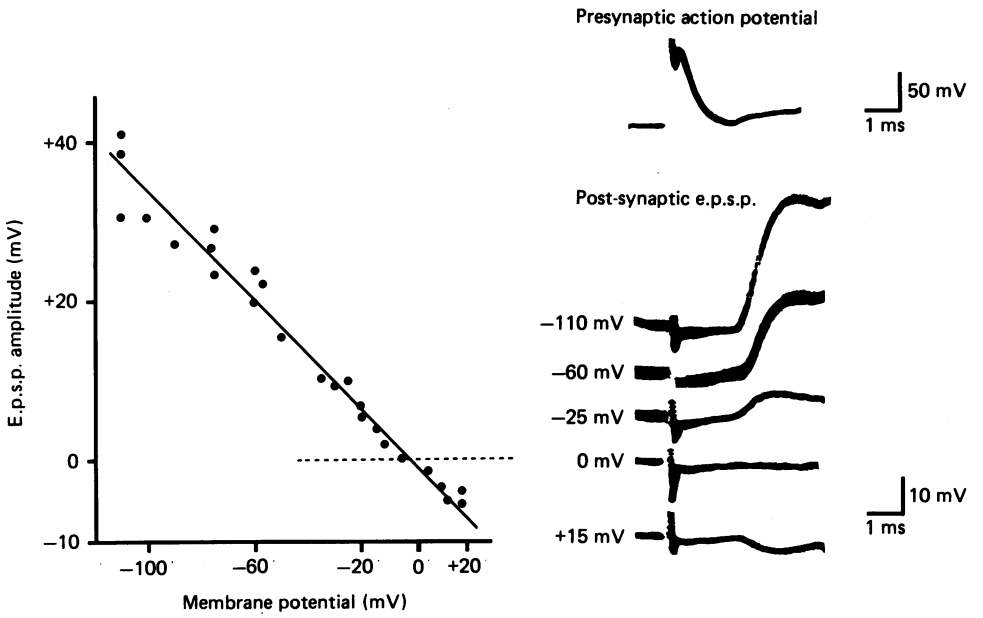

Fig. 1. Reversal potential of the monosynaptic e.p.s.p. Oscilloscope tracings of a monosynaptic e.p.s.p. evoked 1·9 ms after a presynaptic action potential are shown at right. The membrane potential was polarized with steady current through a Cs_2SO_4 micro-electrode while recording the membrane potential through a KAc micro-electrode. Averages of three to five trials were plotted as a function of membrane potential and the reversal potential obtained by interpolation from the regression line ($r = 0{\cdot}99$). Dotted line indicates reversal potential for this neurone (−1 mV). Cell pair 2 in Table 1.

We have re-examined this issue using a two-electrode current clamp after injection of Cs ions into the post-synaptic neurone. Monosynaptic e.p.s.p.s reached a peak amplitude within 2 ms and had amplitudes of 2–20 mV at the resting potential. When the post-synaptic membrane was polarized with steady direct current, the e.p.s.p. amplitude increased with hyperpolarization and decreased with depolarization (Fig. 1). Reversal of the e.p.s.p. was obtained in all cases where the membrane could be polarized to at least +20 mV (Table 1). The average reversal potential, obtained by interpolation of regression lines, was $+3{\cdot}8 \pm 2{\cdot}5$ mV (mean ± S.E. of mean). Due to scatter of e.p.s.p. amplitudes at hyperpolarized membrane potentials, extrapolated reversal potentials (obtained using e.p.s.p. amplitudes at membrane potentials between −50 and −100 mV) were not well fitted by linear regression and thus proved to be unreliable in estimating the actual reversal potential (see Table 1).

TABLE 1. Reversal potential for the s.c.–s.c. e.p.s.p.

Cell pair	$E_{e.p.s.p.}$ (mV)	Extrapolated $E_{e.p.s.p.}$ (mV)
1	−1	−14
2	−1	−13
3	+9	+33
4	+13	+39
5	+2	−16
6	−1	—
7	+19	+44
8	−9	—
9	+2	—
10	+5	+50
Mean ± S.E.	+3·8 ± 2·5	

The reversal potentials ($E_{e.p.s.p.}$) were obtained by interpolation from the regression line ($r > 0{\cdot}95$, $n = 10$) as shown in Fig. 1. The extrapolated $E_{e.p.s.p.}$ was obtained from linear regression of the e.p.s.p. amplitudes between membrane potentials of −50 and −100 mV, then extrapolating to the null potential ($r = 0{\cdot}75$–$0{\cdot}98$, $n = 7$).

Reversal potential of L-*glutamate and* L-*aspartate responses*

The reversal potential (E_r) for the responses evoked by the excitatory amino acids L-glutamate and L-aspartate were examined under identical conditions to those for the e.p.s.p. except that measurements were made under two-electrode voltage clamp. In this way we could obtain values for the reversal potential as well as determine the voltage dependence of both agonists under these conditions. In cultures of spinal cord neurones, L-aspartate is highly selective for the NMDA receptor and thus shows voltage-dependent behaviour (MacDonald *et al.* 1982; Mayer & Westbrook, 1984); L-glutamate appears to act on both NMDA and voltage-insensitive non-NMDA receptors and thus shows an intermediate voltage dependence (Mayer & Westbrook, 1984). The current–voltage relationship of currents evoked by ionophoresis of L-glutamate and L-aspartate on a spinal cord neurone is shown in Fig. 2. Although the two agonists differ in their voltage sensitivity, a clear reversal of current flow was seen with both agonists when the membrane potential was held at +10 mV. The reversal potential for L-glutamate was 0 ± 1 mV and for L-aspartate was -2 ± 2 mV (mean ± S.E. of mean, $n = 7$). These values were not significantly different from the $E_{e.p.s.p.}$ (two-sample t test).

It is possible that the values of the equilibrium potentials for the e.p.s.p. or for responses to the excitatory amino acids could be altered by internal Cs^+ if Cs is impermeant and present in high concentrations. However, previous experiments have suggested that Cs^+ permeates channels gated by excitatory amino acids (Nowak *et al.* 1984; Mayer & Westbrook, 1984). Cs also permeates frog end-plate channels (Adams, Dwyer & Hille, 1980), but not glutamate-activated channels at the insect neuromuscular junction (Anwyl, 1977). In addition the internal concentration of Cs^+ in our experiments was probably low (see Methods); therefore it seems unlikely that Cs^+ had a major effect on $E_{e.p.s.p.}$.

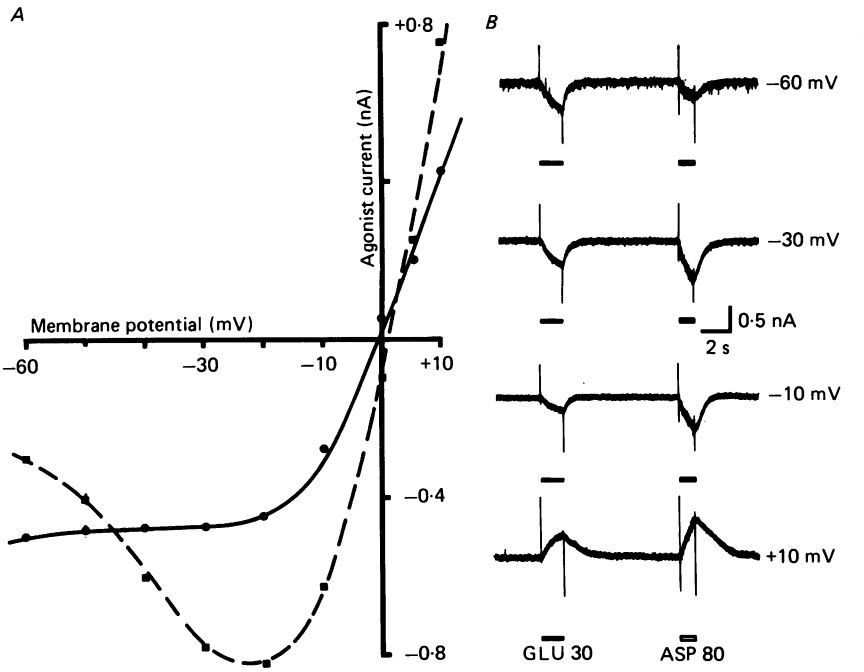

Fig. 2. Reversal potential for L-glutamate and L-aspartate. A spinal cord neurone was voltage clamped with 2 CsCl micro-electrodes. Responses to ionophoresis of L-glutamate (30 nC) and L-aspartate (80 nC) applied near the soma were measured while varying the holding potential (*B*). Current–voltage relationship for responses to ionophoretically applied L-glutamate (continuous line) and L-aspartate (dashed line) is shown in *A*. The reversal potential of the amino acid evoked current was 0 mV for L-glutamate and +2 mV for L-aspartate for this neurone. Bars indicate duration of the ionophoretic pulse. Bath contained 1 μM-tetrodotoxin.

Effects of acidic amino acid antagonists

The effects of PDA and APV were examined on both monosynaptic e.p.s.p.s and responses to ionophoretically-applied excitatory amino acids. PDA has been shown to block responses to ionophoretic application of the selective agonists KA, QA and NMDA, but not acetylcholine in the spinal cord (Davies, Evans, Francis, Jones & Watkins, 1981) whereas APV is a selective antagonist at the NMDA receptor (Davies & Watkins, 1982). L-Glutamate was not used in these experiments due to its mixed agonist action at both NMDA and non-NMDA receptors whereas L-aspartate was considered to act as a selective NMDA receptor agonist on these neurones (Mayer & Westbrook, 1984). Since the 5 mM-Mg^{2+} in the standard recording solution virtually abolishes NMDA responses near the resting potential (Mayer & Westbrook, 1985), antagonist experiments were also performed in Mg^{2+}-free solution. Results were comparable in both solutions although e.p.s.p. amplitudes were generally larger in Mg^{2+}-free medium.

Both in Mg^{2+}-containing medium and in Mg^{2+}-free medium, PDA (0·25–1·0 mM) when applied by local perfusion markedly reduced the amplitude of the monosynaptic e.p.s.p. whereas APV (0·25–1·0 mM) had little or no effect (Fig. 3). At these

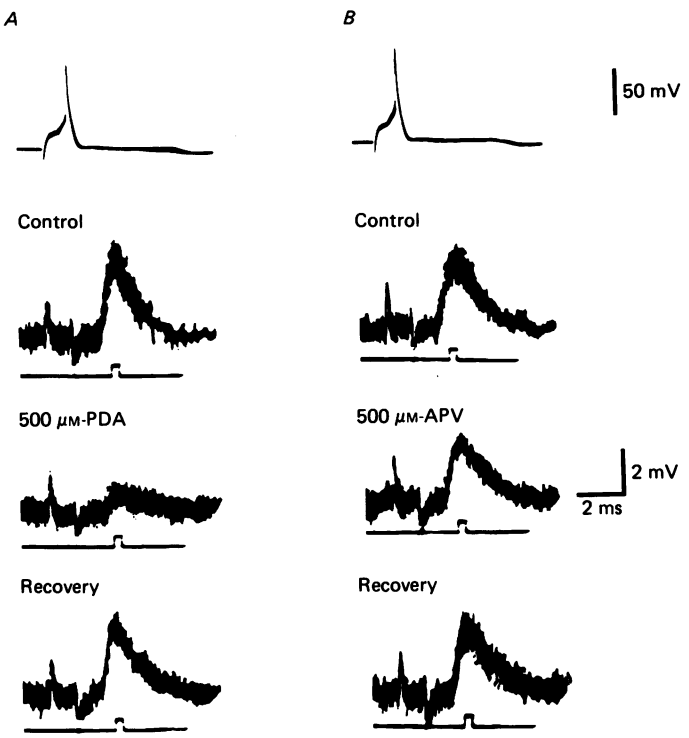

Fig. 3. PDA reduced the monosynaptic e.p.s.p. A monosynaptic e.p.s.p. was evoked at the resting potential of −59 mV in Mg^{2+}-free recording medium. The top trace in *A* and *B* shows the presynaptic action potential; bottom three pairs of traces show evoked e.p.s.p.s before, during and after antagonist application. PDA (500 μM) and APV (500 μM) were applied by leakage from 10 μm pipettes positioned near the post-synaptic neurone. PDA reversibly reduced the amplitude of the e.p.s.p. to 25% of control while APV had little effect. The degree of antagonism was expressed as a percentage of the control response. E.p.s.p. amplitudes were obtained by averaging 150–250 consecutive trials. The trace under each e.p.s.p. shows the time window used for sampling (sampling rate 5 kHz).

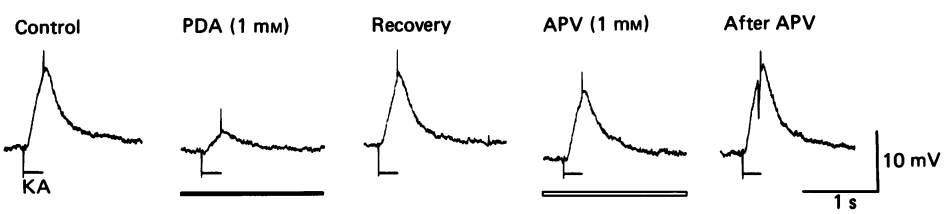

Fig. 4. The effect of PDA and APV on responses to kainic acid. Kainic acid, applied by ionophoresis (14 nC) at regular intervals, evoked a 15 mV depolarization from the resting potential. PDA (1 mM, filled bar) and APV (1 mM, open bar) were applied by leakage from matched 10 μm pipettes positioned near the soma. PDA markedly reduced the KA response while APV had little effect. The resting potential was unchanged by application of the antagonists. The short bar marks the duration of the ionophoretic pulse (200 ms). Spinal cord neurone recorded in bath containing 5 mM-Ca^{2+} and 5 mM-Mg^{2+} with a KAc micro-electrode.

concentrations PDA was effective in reducing responses evoked by ionophoretic application of either KA (Fig. 4) or QA (Fig. 6). The reduction of the e.p.s.p. by PDA was due to a post-synaptic action since quantal analysis using the coefficient of variation method for the cell pair shown in Fig. 3 revealed a decrease in quantal

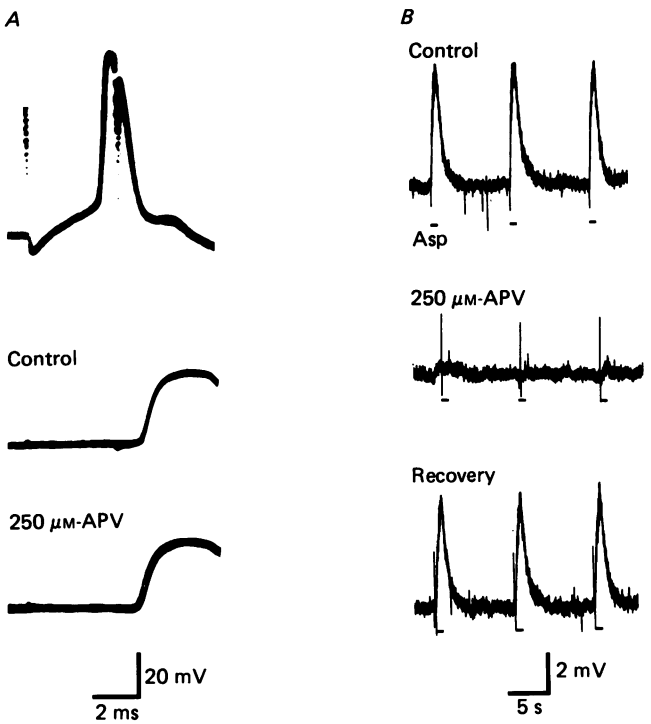

Fig. 5. The effect of APV on the s.c.–s.c. e.p.s.p. and responses to L-aspartate. *A*, a monosynaptic e.p.s.p. was evoked in a Mg^{2+}-free recording solution. The NMDA antagonist, APV (250 μM), was applied by leak from a nearby 10 μm pipette. Upper trace, presynaptic action potential; middle trace, control e.p.s.p.; lower trace, e.p.s.p. in the presence of APV. Record of e.p.s.p. was truncated to exclude long latency polysynaptic activity. *B*, although APV was without effect on the e.p.s.p., the response to ionophoresis of L-aspartate (50 nC) on the same neurone was completely abolished. Short bar indicates duration of ionophoretic pulse. Resting potential −68 mV.

amplitude without an effect on quantal content (not shown). The method of quantal analysis used has been previously described in detail (Nelson *et al.* 1983*a*).

A direct NMDA-like agonist action of PDA was not seen, as has been described in immature rat spinal cord (Davies *et al.* 1981) and on CA1 neurones in the hippocampus (Collingridge, Kehl & McLennan, 1983*a*). However, an agonist action of PDA was not observed in frog spinal cord (Davies *et al.* 1981). We are uncertain whether these differences in the agonist activity of PDA reflect differences in the concentration reaching the neuronal surface, or could be due to regional and species variations in the NMDA receptor.

As expected, the selective NMDA antagonist APV has little effect on responses

evoked by ionophoretic application of either KA (Fig. 4) or QA (Fig. 6). However, when applied on the same neurone in Mg^{2+}-free medium, APV abolished the response to ionophoretically-applied L-aspartate but had no effect on the monosynaptic e.p.s.p. (Fig. 5). The effects of the antagonists on both e.p.s.p.s and responses to ionophoretic application of the selective agonists are summarized in Fig. 6. It is apparent that PDA

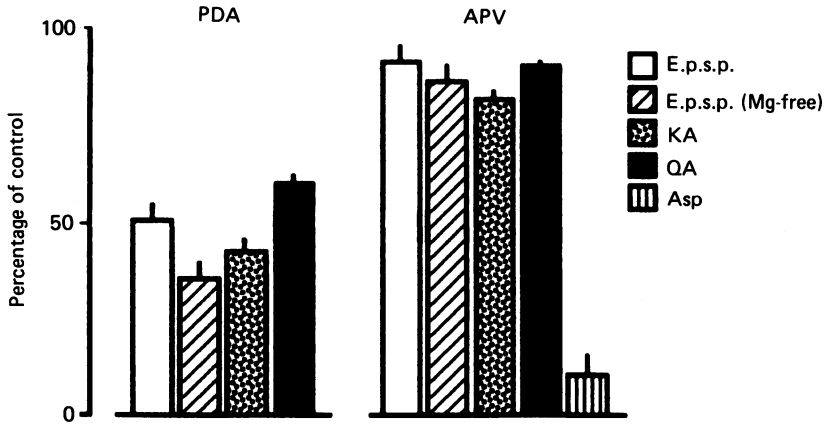

Fig. 6. The antagonism of the e.p.s.p. and responses to excitatory amino acids by PDA and APV. The reduction in the amplitude of monosynaptic e.p.s.p. in Mg^{2+}-containing medium ($n = 7$) and in Mg^{2+}-free medium ($n = 15$) were compared to the reduction of responses to KA ($n = 6$), QA ($n = 3$) and L-aspartate ($n = 9$). In all cases PDA (0·25–1·0 mM) and APV (0·25–1·0 mM) were applied by local perfusion of the neurone. Values shown are mean ± S.E. of mean as a percentage of the control responses. PDA antagonized both the e.p.s.p. (53 % of control in Mg^{2+}, 37 % of control in Mg^{2+}-free medium) and responses to KA (44 % of control) and QA (61 % of control). However, APV, even at these high concentrations, was only effective against the response to L-aspartate (11 % of control).

was about equally effective in antagonizing the monosynaptic e.p.s.p. and the responses evoked by KA and QA, whereas APV was only effective against responses evoked by L-aspartate. In a few neurones DGG (1 mM) also antagonized the e.p.s.p. (not shown) but we did not attempt to distinguish KA from QA receptors using this antagonist (see Davies & Watkins, 1981; McLennan & Liu, 1982).

The e.p.s.c.

The analysis of synaptic currents under voltage clamp has provided detailed kinetic information on synaptically-activated channels at the frog end-plate (Magleby & Stevens, 1972*a*, *b*); and on fast e.p.s.p.s in automatic ganglion cells (Kuba & Nishi, 1979; MacDermott, Connor, Dionne & Parsons, 1980; Rang, 1981). Recently this approach has been extended to central excitatory synapses (Finkel & Redman, 1983; Brown & Johnston, 1983). However, when a point voltage clamp is imposed at the soma of a post-synaptic neurone, the attenuation and distortion of the e.p.s.c. depends on the electrotonic location of the synaptic input and the frequency spectrum of the synaptic current (Rall, 1969; Johnston & Brown, 1983; Rall & Segev, 1985). This created severe limitations in the analysis of the rapid kinetics of the s.c.–s.c. e.p.s.c., since cultured spinal cord neurones have substantial dendritic arborizations

with electrotonic lengths which can vary between 0·5 and 2 (Ransom *et al.* 1977*b*; Guthrie & Westbrook, 1984). In addition excitatory synaptic inputs can be distributed over the entire neuronal surface (see Neale *et al.* 1983). Only eight of more than thirty monosynaptic inputs sampled had sufficiently rapid onsets, and decays uncontaminated by polysynaptic activity to be initially suitable for voltage clamp.

TABLE 2. S.c.–s.c. e.p.s.p.s

Cell pair	E.p.s.p. (mV)	t_{10-90}/τ_m	$t_{\frac{1}{2}\text{ width}}/\tau_m$	L	G_s(nS)	τ_d (ms)†
1	3·6	0·05	0·32	0·9	29	0·6
2	11	0·14	0·70	0·7	35	1·7
3	*	—	—	0·9	9	1·5
4	4·4	0·24	0·68	1·0	18	1·5
5	*	—	—	0·9	47	1·2
6	*	—	—	0·7	89	2·2
7	20	0·18	0·97	0·7	89	1·6
8	1·6	0·19	0·87	1·0	4	0·8

* Presynaptic stimulation evoked action potential at the resting potential.

† Values for the decay time constant (τ_d) are given for a holding potential of −60 mV.

The e.p.s.p. amplitude was measured at the resting membrane potential (−50 to −60 mV) before switching to voltage clamp. The membrane time constants (τ_m) were 12±2 ms (mean±S.E., $n = 8$). The electrotonic length (L) was 0·9±0·1 (mean±S.E., $n = 8$). The synaptic conductance (G_s) was obtained from the slope of the current–voltage (I–V) relationship for the e.p.s.c., $G_s = I/(V-V_r)$, where V was the reversal potential for the synaptic current. The shape parameters t_{10-90} and $t_{\frac{1}{2}\text{ width}}$ are the time between 10 and 90 % of the e.p.s.p. amplitude and the duration of the e.p.s.p. at 50 % of peak amplitude, respectively.

In order to assess the location of the synaptic input the normalized shape indices of the e.p.s.p. were determined (see Rall *et al.* 1967; Jack *et al.* 1971; Redman & Walmsley, 1983) before voltage clamp. Using the electrotonic length (L) derived for the post-synaptic neurone (see Table 2), an approximate synaptic location for a cable model of the neurone (Rall, 1977) was estimated (e.g. see Fig. 2, Finkel & Redman, 1983). After physiological study, the presynaptic neurone was filled with Lucifer Yellow to determine the distribution of presynaptic contacts on the post-synaptic neurone. In most cases the shape indices and dye injection indicated substantial non-somatic inputs. In only one of the eight synaptic pairs that were extensively studied (cell pair 1, Table 2) did the dye injection show exclusively somatic contacts where one axon branch terminated in a cluster of ten to twenty swellings on the soma of the post-synaptic neurone. Shape indices were also consistent with a somatic location for that synaptic input. In the other cell pairs, synaptic locations ranged from 0·2–0·4 space constants (λ) from the soma (Table 1 and Fig. 10).

An example of an excitatory synaptic current under voltage clamp is shown in Fig. 7*A*. A presynaptic action potential evoked a 20 mV e.p.s.p. at a membrane potential of −70 mV. When the post-synaptic neurone was voltage clamped a brief inward current rose rapidly to a peak of −5·75 nA, then decayed nearly back to the base line before the peak of the e.p.s.p. The time course of the e.p.s.c. was much more rapid than the e.p.s.p. since the membrane time constant of the post-synaptic neurone was long relative to the time course of the synaptic current. There was voltage escape during the rising phase of the e.p.s.c.; however, the voltage was well controlled during

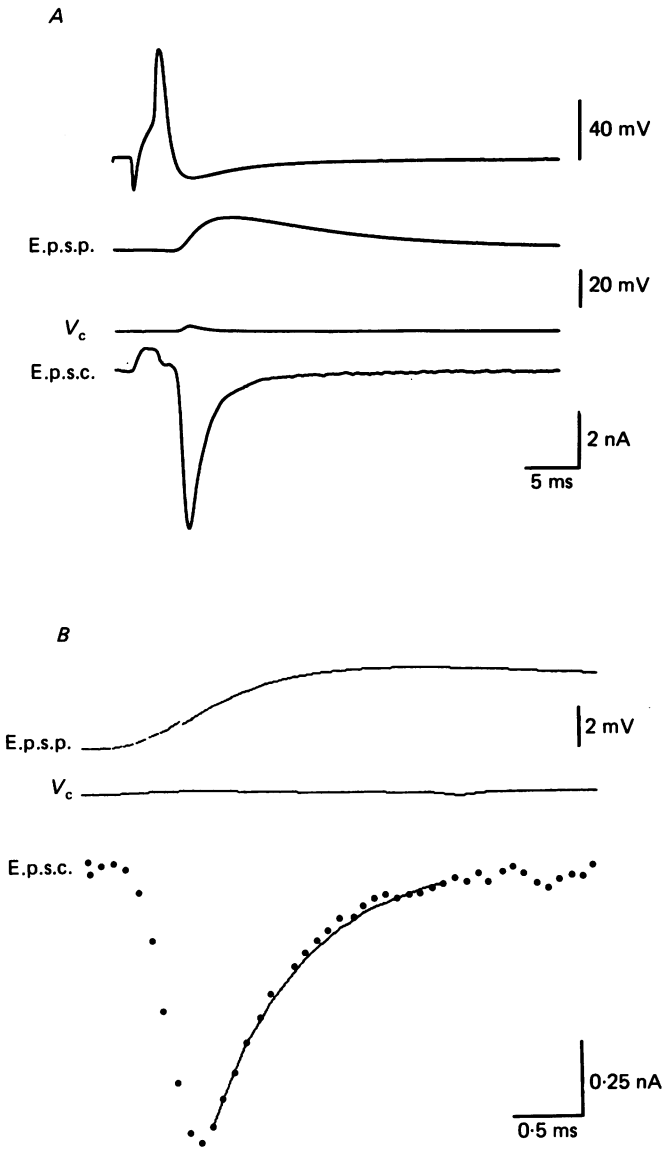

Fig. 7. S.c.–s.c. e.p.s.c. *A*, an action potential in the presynaptic neurone (top trace) elicited an e.p.s.p. (second trace from top) at a resting potential of −70 mV. The post-synaptic record is the average of thirty-two trials. Under two-electrode voltage clamp at a holding potential of −70 mV, the membrane voltage (V_c) and membrane current following the same presynaptic stimulation show the brief inward current underlying the e.p.s.p. The upward deflexion of the membrane current preceding the e.p.s.c. is due to capacitative coupling during the presynaptic stimulus. This synaptic input had a non-somatic component based on e.p.s.p. shape indices (Table 2) and dye injection of the presynaptic neurone (not shown). Cell pair 7 in Table 2. *B*, the e.p.s.p. and e.p.s.c. from another neurone where the synaptic input was somatic are shown on an expanded time-scale. The line shows a single exponential fitted by non-linear regression to the decay (τ_d) of the averaged e.p.s.c. ($n = 32$) at a holding potential of −60 mV. $\tau_d = 0{\cdot}6$ ms. The e.p.s.p. was also evoked from a membrane potential of −60 mV. Cell pair 1 in Table 2.

the decay of the current. Fluctuations in the amplitude of the e.p.s.c. due to the quantal nature of release at this synapse (Nelson *et al.* 1983*a*) were also easily apparent in individual trials (not shown).

An expanded sweep of the synaptic current of a somatic e.p.s.p. is shown in Fig. 7*B*. In this case there was essentially no voltage escape at the holding potential of −60 mV. The decay of the e.p.s.c. could be fitted by a single exponential function with a decay time constant of 0·6 ms (25 °C).

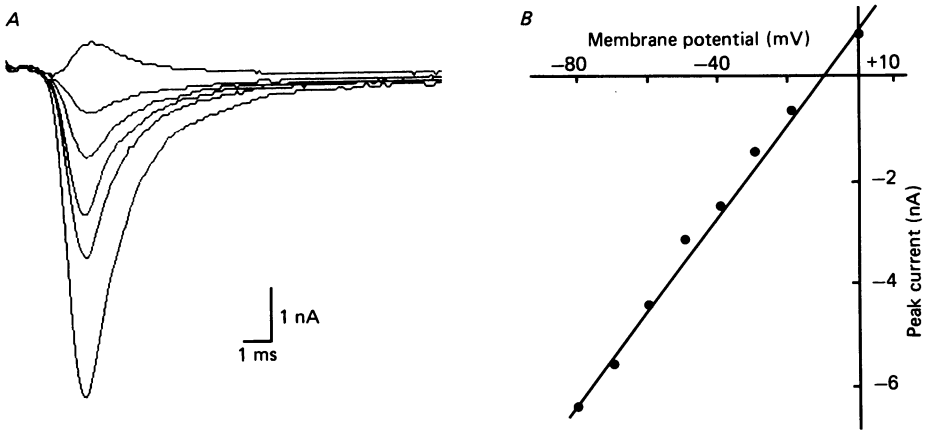

Fig. 8. Current–voltage relation of the peak e.p.s.c. A synaptic current was evoked at holding potentials from −80 mV to +10. *A*, membrane currents at holding potentials of −70, −50, −40, −30, −20, and 0 mV are superimposed after subtraction of the holding current. Due to voltage escape during the rising phase of the e.p.s.c. (see Fig. 7*A*, same neurone), the peak amplitude was partially attenuated; therefore, we estimated the peak current value by extrapolating the semilogarithmic plot of the e.p.s.c. decay to the time of the peak current. This value was taken as the peak amplitude and plotted as a function of membrane voltage in *B*. Line fit by linear regression ($r = > 0{\cdot}99$) had a slope of 89 nS. Voltage electrode, Cs_2SO_4; current electrode; CsCl. Cell pair 7 in Table 2.

Current–voltage relationship of the peak e.p.s.c.

A family of synaptic currents evoked at a series of holding potentials between −80 and 0 mV is shown in Fig. 8*A*. The amplitude of the e.p.s.c. increased at holding potentials negative to the rest potential and decreased at potentials positive to rest potential. The peak current amplitude plotted as a function of holding potential was linear (i.e. voltage insensitive) with a slope of 89 nS for this e.p.s.c. (Fig. 8*B*). This was the largest total conductance of the monosynaptic inputs studied. The wide range of synaptic conductances (4–89 nS, Table 2) is consistent with the large variation in the number of synaptic boutons observed in horseradish peroxidase filled presynaptic neurones of s.c.–s.c. e.p.s.p.s (Neale *et al.* 1983).

Decay of the synaptic current

The decays of the synaptic current (τ_d) were measured at holding potentials between −80 and +10 mV. Semilogarithmic plots of τ_d for two different e.p.s.c.s at

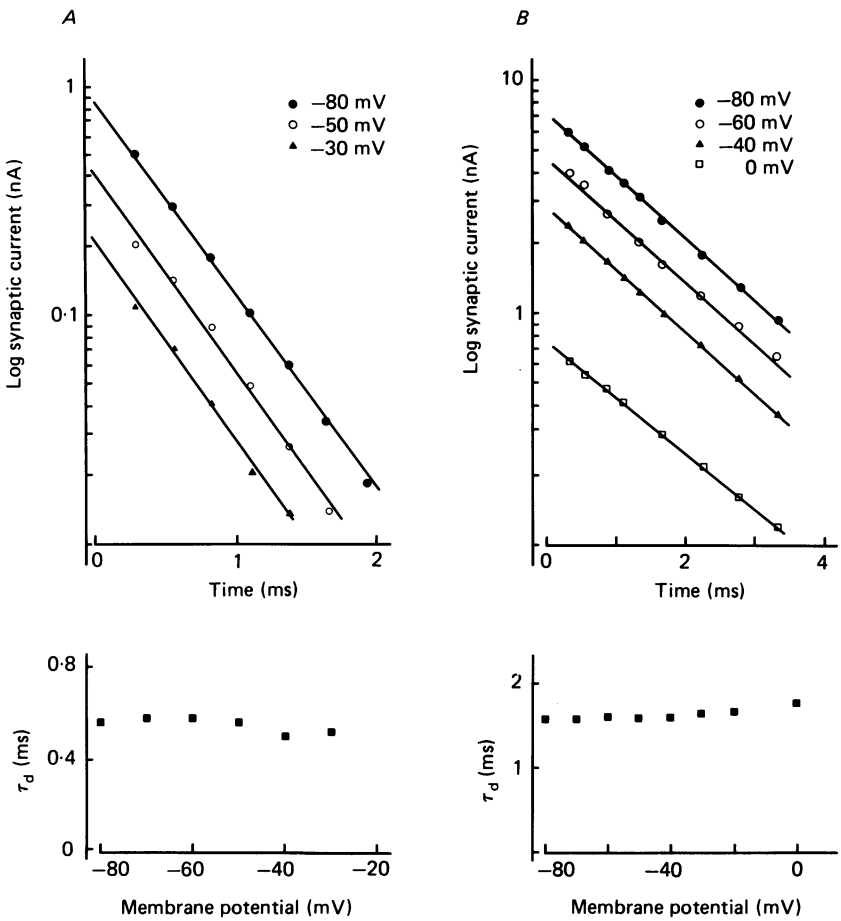

Fig. 9. Decay of the synaptic current. The decay of a somatic e.p.s.c. (*A*) and an e.p.s.c. with a non-somatic component (*B*) were plotted semilogarithmically for several different holding potentials. Decay time constants (τ_d) were obtained graphically and plotted as a function of membrane voltage (below). τ_d was $0{\cdot}57 \pm 0{\cdot}03$ ms (mean ± S.D.) for *A*, and $1{\cdot}6 \pm 0{\cdot}05$ ms (mean ± S.D.) for *B*. Slopes of the linear regression for the τ_d as a function of membrane potential were less than 0·005. *A*, cell pair 1 in Table 2. *B*, cell pair 7 in Table 2.

several membrane potentials are shown in Fig. 9. τ_d was independent of membrane voltage over a range of 80 and 50 mV, respectively (Fig. 9). Since not all cell pairs were tested over a full range of voltages, the ratio of τ_d at −60 mV to τ_d at −30 mV was used as a measure of the voltage sensitivity of the decay. For seven synaptic inputs this ratio was $1{\cdot}01 \pm 0{\cdot}07$ (mean ± S.D.), consistent with no measurable voltage dependence over this voltage range.

τ_d was significantly faster for the exclusively somatic input (Fig. 9*A*). The τ_d for the cell pairs with a non-somatic component was between 1 and 2 ms (Table 2). A simple explanation for the range of decays could be the spatial distortion of proximal dendritic synaptic inputs. To test the feasibility of this explanation we used a ten

compartment neurone model consisting of a soma and a single lumped process similar to that used by others (Rall, 1967; Johnston & Brown, 1983). The compartmental modelling program used has been previously described (Guthrie & Westbrook, 1984). A perfect voltage clamp was applied at the soma. The values assumed for the model were $L = 1$, $\tau_m = 15$ ms ($C_m = 1{\cdot}5\ \mu F/cm^2$, $R_m = 10000\ \Omega\ cm^2$), and $\rho = 5$. R_m and

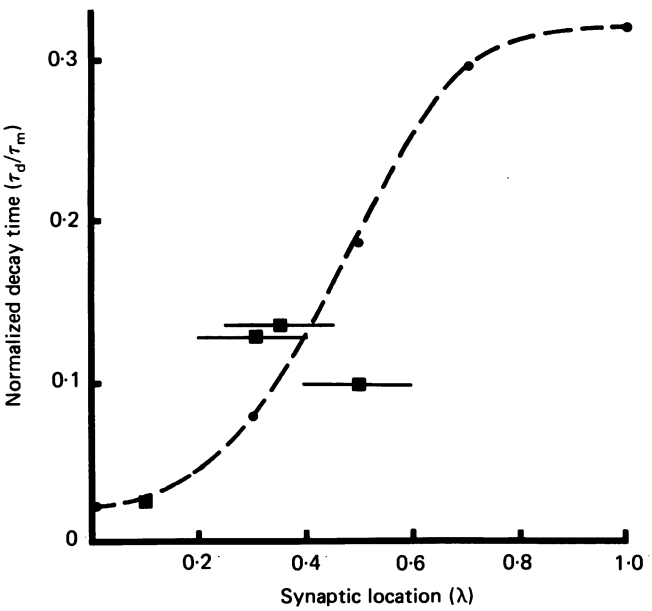

Fig. 10. Spatial distortion of a synaptic current. A ten compartment model was used to determine the change in the e.p.s.c. wave form as a function of synaptic location (see text for assumed values of the model). The time course of a model synaptic conductance with a peak of 10 pS was chosen to match the time course of the recorded somatic e.p.s.c. A small value was chosen for the conductance so that the effects of synaptic location could be determined without the effects of non-linear summation. For these values, the maximum voltage escape for the model was 2 mV for an input in compartment 10. (Cell pair 1 in Table 2 and Fig. 7*B*). The dotted line indicates the single exponential curve fitted to the decay of the somatically recorded e.p.s.c. as the synaptic location is moved further from the soma. The squares indicate experimental values of estimated synaptic locations and τ_d for four e.p.s.c.s. Synaptic locations were estimated using the normalized shape indices and values of electrotonic length, L, for each post-synaptic neurone. The bars around each experimental point indicate that site was estimated to the nearest 0·2 λ (space constant).

C_m are the specific membrane resistivity and capacitance, and ρ is the dendritic–soma conductance ratio. These values are similar to the neurones in Table 2 and those previously reported for cultured spinal cord neurones (Ransom *et al.* 1977*b*; Guthrie & Westbrook, 1984). A voltage-insensitive synaptic conductance (g_{psc}) of the form,

$$g_{psc} = kt\ e^{-\alpha t/\tau_m},$$

where t is time, k is a constant and $\alpha = 60$, was applied at each compartment. The decay of the currents, as measured at the soma, were then fitted to a single

exponential by non-linear regression. The normalized decay (τ_d/τ_m) of the modelled current as a function of synaptic input site is shown as the dotted line in Fig. 10. Each compartment was 0·1 space constants (λ) in length. A similar approach has been used by Johnston & Brown (1983) for hippocampal neurones. The model current, as measured at the soma, had a $\tau_d = 0{\cdot}5$ ms at $\lambda = 0{\cdot}1$ and 4·75 ms at $\lambda = 1$. The estimated synaptic locations and normalized decay time constants for one somatic and three non-somatic synaptic inputs are also plotted. The clustering of the non-somatic inputs near the predicted curve suggests that spatial distortion of a uniform synaptic conductance with a τ_d near 0·5 ms could be a sufficient explanation for the range of decay time constants observed.

DISCUSSION

Relatively little is known about the role of various acidic amino acid receptors and their underlying conductance mechanisms at putative excitatory amino acid mediated synapses in the central nervous system. In this paper we have attempted to characterize the post-synaptic pharmacology and conductance mechanism at one such synapse, the fast monosynaptic e.p.s.p. between cultured spinal cord neurones; and to compare our results with the expanding knowledge of the behaviour of excitatory amino acids.

Comparison of $E_{e.p.s.p.}$ with E_r for acidic amino acids

Experiments in the CA1 region and dentate gyrus of the hippocampus, where excitatory amino acids may act as transmitters, have shown good agreement between the equilibrium potentials for L-glutamate and for the e.p.s.p. (Hablitz & Langmoen, 1982; Crunelli, Forda & Kelly, 1984). Our values of +3 mV for the reversal of the e.p.s.p. and 0 mV for L-glutamate are also in close agreement, and compatible with a common mechanism. However, the reversal potential for conductances linked to QA, KA and NMDA receptors are similar for cultured spinal cord neurones (Mayer & Westbrook, 1984), and thus the equilibrium potential cannot distinguish between receptor subtypes in a medium of normal ionic composition.

Post-synaptic receptor mechanism

Refinements in the available acidic amino acid antagonists now permit the separation of responses due to activation of NMDA receptors from activation of KA and QA receptors. Our results are in accord with results from the spinal cord *in vivo* in that PDA is a relatively non-selective acidic amino acid antagonist that antagonizes dorsal root evoked synaptic excitation of motoneurones and dorsal horn neurones as well as responses to NMDA, KA and QA (Davies *et al.* 1981). APV, on the other hand, is highly selective for the NMDA receptor (Davies & Watkins, 1982). Thus the antagonism of the s.c.–s.c. e.p.s.p. by PDA and the lack of effect of APV at concentrations that abolish responses evoked by ionophoretic application of NMDA or L-aspartate suggest strongly that the post-synaptic receptor is an excitatory amino acid receptor, but of the 'non-NMDA' type. Similar conclusions have recently been reached for the monosynaptic e.p.s.p. between dorsal root ganglion explants and cultured dorsal horn neurones using kynurenic acid and APV as antagonists (Jahr

& Jessell, 1985). However, Peet, Leah & Curtis (1983) using extracellular recording *in vivo* were unable to antagonize monosynaptic responses with the nonselective blocker PDA.

Conductance mechanism

The rapid kinetics of the s.c.–s.c. e.p.s.p. and the non-somatic contribution of many of the synaptic inputs were limiting factors in the experiments that we could perform with the protocol used here. Therefore our analysis of the e.p.s.c. constitutes an initial description. We will limit our discussion to two general questions: (1) what is the upper limit of the mean open time for the synaptic channel; (2) could a highly voltage-sensitive conductance (i.e. the NMDA-mediated conductance) mediate the s.c.–s.c. e.p.s.p.?

Assuming a two-state synaptic channel, τ_d would equal the mean channel lifetime. However, the decay of the synaptic current can be influenced by a number of factors including the time course of transmitter release, receptor binding, diffusion, re-uptake, hydrolysis as well as the mean channel lifetime (Magleby & Stevens, 1972*b*). The process with the longest time course will be the limiting factor in determining τ_d. The relative importance of these factors for the s.c.–s.c. e.p.s.p. are not known. For example, removal of excitatory amino acids is thought to be by re-uptake (cf. Fonnum, 1984). Both neurones and glia possess re-uptake mechanisms for L-glutamate and L-aspartate (Balcar & Johnston, 1972; Henn, Goldstein & Hamberger, 1974). However, we made no attempt to test the validity of these assumptions required to relate the τ_d to the mean channel lifetime. Nonetheless using the τ_d of the somatic e.p.s.c., the mean lifetime of the synaptic channel should be no greater than about 0·6 ms (25 °C). It will be interesting to compare this estimate to fluctuation and single-channel analyses of channels activated by excitatory amino acids of the non-NMDA type.

NMDA receptors are linked to a highly voltage-sensitive conductance (MacDonald *et al.* 1982). This has recently been shown to be due to the effects of Mg^{2+} as a channel blocker (Nowak *et al.* 1984; Mayer & Westbrook, 1985). Since this channel block is highly voltage-dependent with the steady-state conductance increasing about e-fold per 25 mV depolarization (Mayer & Westbrook, 1985), this might be expected to influence either the peak e.p.s.c. or the decay time course of an NMDA-mediated e.p.s.c. in a voltage-dependent manner. Such effects of cholinergic channel blockers on synaptic currents have been examined in detail at the frog end-plate (Adams, 1976, 1977) and on e.p.s.c.s of rat submandibular ganglion cells (Rang, 1982; Gurney & Rang, 1984). Our experiments were performed in a high concentration of the channel blocker (5 mM-Mg^{2+}), but we did not see non-linear behaviour of the current–voltage relation of the peak synaptic current or changes in τ_d for the somatic e.p.s.c., despite the marked non-linearity of L-aspartate evoked currents under the same conditions (see Fig. 2). This finding, in combination with the lack of effect of APV on the s.c.–s.c. e.p.s.p., is inconsistent with a role for voltage-sensitive (i.e. NMDA-activated) channels in monosynaptic transmission at this synapse.

The currents of synaptic inputs on proximal dendrites were also voltage insensitive. However, the voltage dependence of a synaptic conductance can be obscured for a synaptic input at a distance from a point voltage clamp (cf. Fig. 4, Magleby &

Stevens, 1972*a*) due to both attenuation of the clamp potential at the synaptic site (voltage error) and to attenuation and distortion of the synaptic current (space clamp error). We minimized the voltage error by using steady currents to change the holding potential of the post-synaptic neurone. Such steady-state voltage attenuation should be less than 10% at $\lambda = 0{\cdot}2$ as Johnston & Brown (1983) have shown for a compartmental neurone model. In addition, calculations using the neurone model of Fig. 10 with different synaptic time courses suggested that for synaptic inputs at $\lambda = 0{\cdot}2$, voltage-sensitive changes in τ_d on the order of the e-fold per 50 mV should have been detectable over the range of voltages tested. Thus while we cannot exclude a small voltage dependence of the decay for the non-somatic inputs, it seems reasonable to exclude a highly voltage-sensitive conductance such as that activated by NMDA.

It is interesting to compare the behaviour of the s.c.–s.c. e.p.s.c. with other putative excitatory amino acid-mediated synapses. The Ia primary afferent – motoneurone somatic e.p.s.c. has a τ_d of 0·3–0·4 ms (37 °C); τ_d decreased more than 10% in two of four cases with a 25 mV hyperpolarization (Finkel & Redman, 1983). Assuming $Q_{10} = 3$ (Magleby & Stevens, 1972*b*; MacDermott *et al.* 1980), the τ_d for the somatic s.c.–s.c. e.p.s.c. is very similar, but shows less voltage dependence. At the mossy fibre input to CA3 neurones Brown & Johnston (1983) have reported a τ_d of 3–4 ms which decreased with depolarization, suggesting a different conductance mechanism.

Number of receptors per release site

A mean quantal amplitude of 170 μV has been calculated for s.c.–s.c. e.p.s.p.s (Nelson *et al.* 1983*a*). This corresponds to approximately twenty release sites for cell pair no. 1 in Table 2 which had a peak synaptic conductance of 29 nS, or 1·5 nS per release site. Nowak & Ascher (1984) have suggested that KA and QA channels have a small unitary conductance. Cull-Candy & Ogden (1985) have reported a low conductance channel of 140 fS activated by L-glutamate on cultured rat cerebellar neurones, in addition to a 50 pS channel which is similar to the NMDA-activated channels reported by Nowak *et al.* (1984). Thus if we assume a single-channel conductance of no more than 5 pS for a non-NMDA channel, at least 300 channels would be activated per release site. This compares to estimates of 100 open channels at the peak of a miniature excitatory post-synaptic current (m.e.p.c.) of rat submandibular ganglion cells (Rang, 1981); and 1700 open channels per quantum of transmitter at the frog end-plate (Anderson & Stevens, 1973). From analysis of electron micrographs of s.c.–s.c. e.p.s.p.s, the estimated area of the synaptic junction is approximately 0·062 μm^2 (E. A. Neale, unpublished observation). Assuming the transmitter is limited to the junction (but see Faber, Funch & Korn, 1985), the receptor density would need to be at least 5000/μm^2. An even higher receptor density (30000/μm^2) has been measured for the post-synaptic dense membrane at the mouse neuromuscular junction (Fertuck & Salpeter, 1976).

NMDA receptors and synaptic transmission

In the hippocampus the monosynaptic e.p.s.p. also appears to be of the non-NMDA type (Collingridge, Kehl & McLennan, 1983*b*; Crunelli, Forda & Kelly, 1983), but long-term potentiation at the CA1 subregion is sensitive to the NMDA antagonist,

APV (Collingridge *et al.* 1983*b*; Wigstrom & Gustafsson, 1984). It thus seems likely that non-NMDA receptors may mediate monosynaptic excitation at a number of central and primary afferent synapses while NMDA receptors function in another role. However, an e.p.s.p. possibly mediated by NMDA receptors has recently been reported in rat neocortex (Thompson, West & Lodge, 1985), and in the spinal cord of *Xenopus* embryos (Dale & Roberts, 1985). In addition, NMDA binding sites have been identified in the post-synaptic densities of isolated rat brains in approximately equal numbers with QA binding sites (Fagg & Matus, 1984). Our results would seem to contradict evidence *in vivo* that NMDA receptors mediate intraneuronal, i.e. polysynaptic, excitation in the spinal cord (Davies & Watkins, 1982). It is possible that synapses mediated by NMDA receptors are not represented in culture, but nearly all cultured spinal cord neurones respond to NMDA (MacDonald & Porietis, 1982; Mayer & Westbrook, 1985). We favour the possibility that NMDA receptors could serve as 'boosters' of dendritic synaptic activity and thus affect polysynaptic activity indirectly. The voltage dependence of the NMDA-activated conductance makes it ideally suited for a role as a modulator, perhaps even in extrasynaptic locations.

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REFERENCES

ADAMS, D. J., DWYER, T. M. & HILLE, B. (1980). The permeability of endplate channels to monovalent and divalent metal cations. *Journal of General Physiology* **75**, 493–510.

ADAMS, P. R. (1976). Drug blockade of open end-plate channels. *Journal of Physiology* **260**, 531–552.

ADAMS, P. R. (1977). Voltage jump analysis of procaine action at frog end-plate. *Journal of Physiology* **268**, 291–318.

ANDERSON, C. R. & STEVENS, C. F. (1973). Voltage clamp analysis of acetylcholine produced end-plate current fluctuations at frog neuromuscular junction. *Journal of Physiology* **235**, 655–691.

ANWYL, R. (1977). The effect of foreign cations, pH and pharmacological agents on the ionic permeability of an excitatory glutamate synapse. *Journal of Physiology* **273**, 389–404.

BALCAR, V. J. & JOHNSTON, G. A. R. (1972). The structural specificity of the high affinity uptake of L-glutamate and L-aspartate by rat brain slices. *Journal of Neurochemistry* **19**, 2657–2666.

BROOKES, N. (1978). Actions of glutamate on dissociated spinal neurons *in vitro*. *Developmental Neuroscience* **1**, 203–215.

BROWN, T. H. & JOHNSTON, D. (1983). Voltage-clamp analysis of mossy fiber synaptic input to hippocampal neurons. *Journal of Neurophysiology* **50**, 487–507.

COLLINGRIDGE, G. L., KEHL, S. J. & MCLENNAN, H. (1983*a*). The antagonism of amino acid-induced excitations of rat hippocampal CA1 neurones *in vitro*. *Journal of Physiology* **334**, 19–31.

COLLINGRIDGE, G. L., KEHL, S. J. & MCLENNAN, H. (1983*b*). Excitatory amino acids in synaptic transmission in the Schaffer collateral – commissural pathway of the rat hippocampus. *Journal of Physiology* **334**, 33–46.

CRUNELLI, V., FORDA, S. & KELLY, J. S. (1983). Blockade of amino acid-induced depolarizations and inhibition of excitatory post-synaptic potentials in rat dentate gyrus. *Journal of Physiology* **341**, 627–640.

CRUNELLI, V., FORDA, S. & KELLY, J. S. (1984). The reversal potential of excitatory amino acid action on granule cells of the rat dentate gyrus. *Journal of Physiology* **351**, 327–342.

CULL-CANDY, S. G. & OGDEN, D. C. (1985). Ion channels activated by L-glutamate and GABA in cultured cerebellar neurons of the rat. *Proceedings of the Royal Society* B **224**, 367–373.

DALE, N. & ROBERTS, A. (1985). Dual-component amino acid-mediated synaptic potentials: excitatory drive for swimming in *Xenopus* embryos. *Journal of Physiology* **363**, 35–59.

DAVIES, J., EVANS, R. H., FRANCIS, A. A., JONES, A. W. & WATKINS, J. C. (1981). Antagonism of excitatory amino acid-induced and synaptic excitation of spinal neurones by *cis*-2,3-piperidine dicarboxylate. *Journal of Neurochemistry* **36**, 1305–1307.

DAVIES, J. & WATKINS, J. C. (1981). Differentiation of kainate and quisqualate receptors in the cat spinal cord by selective antagonism with g-D(and L)-glutamylglycine. *Brain Research* **206**, 172–177.

DAVIES, J. & WATKINS, J. C. (1982). Actions of D and L forms of 2-amino-5-phosphonovalerate and 2-amino-4-phosphonobutyrate in the cat spinal cord. *Brain Research* **235**, 378–386.

FABER, D. S., FUNCH, P. G. & KORN, H. (1985). Evidence that receptors mediating central synaptic potentials extend beyond the postsynaptic density. *Proceedings of the National Academy of Sciences of the United States of America* **82**, 3504–3508.

FAGG, G. E. & MATUS, A. (1984). Selective association of *N*-methyl aspartate and quisqualate types of L-glutamate receptor with brain postsynaptic densities. *Proceedings of the National Academy of Sciences of the United States of America* **81**, 6877–6880.

FENWICK, E. M., MARTY, A. & NEHER, E. (1982). A patch-clamp study of bovine chromaffin cells and of their sensitivity to acetylcholine. *Journal of Physiology* **331**, 577–599.

FERTUCK, H. C. & SALPETER, M. M. (1976). Quantitation of junctional and extrajunctional acetylcholine receptors by electron microscope autoradiography after ^{125}I-α-bungarotoxin binding at mouse neuromuscular junctions. *Journal of Cell Biology* **69**, 144–158.

FINKEL, A. S. & REDMAN, S. J. (1983). The synaptic current evoked in cat spinal motoneurones by impulses in single group 1a axons. *Journal of Physiology* **342**, 615–632.

FONNUM, F. (1984). Glutamate: A neurotransmitter in mammalian brain. *Journal of Neurochemistry* **42**, 1–11.

GURNEY, A. M. & RANG, H. P. (1984). The channel-blocking action of methonium compounds on rat submandibular ganglion cells. *British Journal of Pharmacology* **82**, 623–642.

GUTHRIE, P. B. & BRENNEMAN, D. E. (1982). Mouse spinal cord in dissociated cell culture – separate culture of dorsal and ventral halves. *Society for Neuroscience Abstracts* **8**, 233.

GUTHRIE, P. B. & WESTBROOK, G. L. (1984). A.C. analysis of the electrical structure of mouse spinal cord in culture. *Biophysical Journal* **45**, 267a.

HABLITZ, J. J. & LANGMOEN, I. A. (1982). Excitation of hippocampal pyramidal cells by glutamate in the guinea-pig and rat. *Journal of Physiology* **325**, 317–331.

HENN, F. A., GOLDSTEIN, M. N. & HAMBERGER, A. (1974). Uptake of the neurotransmitter candidate glutamate by glia. *Nature* **249**, 663–664.

JACK, J. J. B., MILLER, S., PORTER, R. & REDMAN, S. J. (1971). The time course of minimal excitatory post-synaptic potentials evoked in spinal motoneurones by group 1a afferent fibres. *Journal of Physiology* **215**, 353–380.

JAHR, C. E. & JESSELL, T. M. (1985). Synaptic transmission between dorsal root ganglion and dorsal horn neurons in culture: antagonism of monosynaptic EPSPs and glutamate excitation by kynurenate. *Journal of Neuroscience* **5**, 2281–2289.

JOHNSTON, D. & BROWN, T. H. (1983). Interpretation of voltage-clamp measurements in hippocampal neurons. *Journal of Neurophysiology* **50**, 464–486.

KUBA, K. & NISHI, S. (1979). Characteristics of fast excitatory postsynaptic current in bullfrog sympathetic ganglion cells. *Pflügers Archiv* **378**, 205–212.

MACDERMOTT, A. B., CONNOR, E. A., DIONNE, V. E. & PARSONS, R. L. (1980). Voltage clamp study of fast excitatory synaptic currents in bullfrog sympathetic ganglion cells. *Journal of General Physiology* **75**, 39–80.

MACDONALD, J. F. & PORIETIS, A. V. (1982). DL-quisqualic and L-aspartic acids activate separate excitatory conductances in cultured spinal cord neurons. *Brain Research* **245**, 175–178.

MACDONALD, J. F., PORIETIS, A. V. & WOJTOWICZ, J. M. (1982). L-aspartic acid induces a region of negative slope conductance in the current–voltage relationship of cultured spinal cord neurons. *Brain Research* **237**, 248–253.

MACDONALD, R. L., PUN, R. Y. K., NEALE, E. A. & NELSON, P. G. (1983). Synaptic interactions between mammalian central neurons in cell culture. I. Reversal potential for exitatory post synaptic potentials. *Journal of Neurophysiology* **49**, 1428–1441.

MCLENNAN, H. (1980). On the nature of the receptors for various excitatory amino acids in the mammalian central nervous system. In *Glutamate as a Neurotransmitter*, ed. DICHIARA, G. & GESSA, G. L., pp. 253–262. New York: Raven Press.

McLENNAN, H. & LIU, J. (1982). The action of six antagonists of the excitatory amino acids on neurones of the rat spinal cord. *Experimental Brain Research* **45**, 151–156.

MAGLEBY, K. L. & STEVENS, C. F. (1972*a*). The effect of voltage on the time course of end-plate currents. *Journal of Physiology* **223**, 151–171.

MAGLEBY, K. L. & STEVENS, C. F. (1972*b*). A quantitative description of end-plate currents. *Journal of Physiology* **223**, 173–197.

MAYER, M. L. & WESTBROOK, G. L. (1983). A voltage-clamp analysis of inward (anomalous) rectification in mouse spinal sensory ganglion neurones. *Journal of Physiology* **340**, 19–45.

MAYER, M. L. & WESTBROOK, G. L. (1984). Mixed-agonist action of excitatory amino acids on mouse spinal cord neurones under voltage clamp. *Journal of Physiology* **354**, 29–53.

MAYER, M. L. & WESTBROOK, G. L. (1985). The action of *N*-methyl-D-aspartic acid on mouse spinal neurones in culture. *Journal of Physiology* **361**, 65–90.

NEALE, E. A., MACDONALD, R. L. & NELSON, P. G. (1978). Intracellular horseradish peroxidase injection for correlation of light and electron microscopic anatomy with synaptic physiology of cultured mouse spinal cord neurons. *Brain Research* **152**, 265–282.

NEALE, E. A., NELSON, P. G., MACDONALD, R. L., CHRISTIAN, C. N. & BOWERS, L. M. (1983). Synaptic interactions between mammalian central neurons in cell culture. III. Morphophysiological correlates of quantal synaptic transmission. *Journal of Neurophysiology* **49**, 1459–1468.

NELSON, P. G., MARSHALL, K. C., PUN, R. Y. K., CHRISTIAN, C. N., SHERIFF JR, W. H., MACDONALD, R. L. & NEALE, E. A. (1983*a*). Synaptic interactions between mammalian central neurons in cell culture. II. Quantal analysis of EPSPs. *Journal of Neurophysiology* **49**, 1442–1458.

NELSON, P. G., PUN, R. Y. K. & WESTBROOK, G. L. (1983*b*). Monosynaptic excitatory postsynaptic potentials and responses to putative amino acid neurotransmitters: A voltage clamp study. *Society for Neuroscience Abstracts* **9**, 1144.

NOWAK, L. M. & ASCHER, P. (1984). *N*-methyl-D-aspartic, kainic and quisqualic acid evoked currents in mammalian central neurones. *Society for Neuroscience Abstracts* **10**, 23.

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

PEET, M. J., LEAH, J. D. & CURTIS, D. R. (1983). Antagonists of synaptic and amino acid excitation of neurones in the cat spinal cord. *Brain Research* **266**, 83–95.

PUIL, E. (1981). S-Glutamate: Its interaction with spinal neurons. *Brain Research Reviews* **3**, 229–322.

PUN, R. Y. K., WESTBROOK, G. L. & NELSON, P. G. (1982). Effects of postsynaptic polarization on monosynaptic excitatory postsynaptic potentials (EPSPs) in spinal cord culture. *Society for Neuroscience Abstracts* **8**, 492.

RALL, W. (1967). Distinguishing theoretical synaptic potentials computed for different soma-dendritic distributions of synaptic input. *Journal of Neurophysiology* **30**, 1138–1168.

RALL, W. (1969). Time constants and electrotonic length of membrane cylinders and neurons. *Biophysical Journal* **9**, 1483–1508.

RALL, W. (1977). Core conductor theory of cable properties of neurons. In *Handbook of Physiology*, vol. 1, sect. 1, ed. KANDEL, E. R., pp. 39–97. Bethesda, Maryland: The American Physiological Society.

RALL, W., BURKE, R. E., SMITH, T. G., NELSON, P. G. & FRANK, K. (1967). Dendritic location of synapses and possible mechanisms for the monosynaptic e.p.s.p. in motoneurons. *Journal of Neurophysiology* **30**, 1169–1193.

RALL, W. & SEGEV, I. (1985). Space-clamp problems when voltage clamping branched neurons with intracellular microelectrodes. In *Voltage and Patch Clamping with Microelectrodes*, ed. SMITH JR, T. G., LECAR, H., REDMAN, S. J. & GAGE, P. W., pp. 191–216. Bethesda, Maryland: The American Physiological Society.

RANG, H. P. (1981). The characteristics of synaptic currents and responses to acetylcholine of rat submandibular ganglion cells. *Journal of Physiology* **311**, 23–55.

RANG, H. P. (1982). The action of ganglionic blocking drugs on the synaptic responses of rat submandibular ganglion cells. *British Journal of Pharmacology* **75**, 151–168.

RANSOM, B. R., BULLOCK, P. N. & NELSON, P. G. (1977*a*). Mouse spinal cord in cell culture. III. Neuronal chemosensitivity and its relationship to synaptic activity. *Journal of Neurophysiology* **40**, 1163–1177.

RANSOM, B. R., NEALE, E., HENKART, M., BULLOCK, P. N. & NELSON, P. G. (1977*b*). Mouse spinal

cord in cell culture. I. Morphology and intrinsic neuronal electrophysiological properties. *Journal of Neurophysiology* **40**, 1132–1150.

REDMAN, S. J. (1979). Junctional mechanisms at group Ia synapses. *Progress in Neurobiology* **12**, 33–83.

REDMAN, S. J. & WALMSLEY, B. (1983). The time course of synaptic potentials evoked in cat spinal motoneurones at identified group Ia synapses. *Journal of Physiology* **343**, 117–133.

ROMIJN, H. J., HABETS, A. M. M. C., MUD, M. T. & WOLTERS, P. S. (1982). Nerve outgrowth, synaptogenesis and bioelectric activity in fetal rat cerebral cortex tissue cultured in serum-free, chemically defined medium. *Developmental Brain Research*, **2**, 583–589.

THOMSON, A. M., WEST, D. C. & LODGE, D. (1985). An *N*-methylaspartate receptor-mediated synapse in rat cerebral cortex: a site of action of ketamine? *Nature* **313**, 479–481.

WATKINS, J. C. (1981). Pharmacology of excitatory amino acid neurotransmitters. In *Amino Acid Neurotransmitters*, ed. DEFEUDIS, F. V. & MANDEL, P., pp. 205–212. New York: Raven Press.

WERMAN, R. (1980). The reversal potential as a diagnostic tool in transmitter identification. In *Receptors for Neurotransmitters and Peptide Hormones*, ed. PEPEU, G., KUHAR, M. J. & ENNA, S. J., pp. 21–30. New York: Raven Press.

WIGSTROM, H. & GUSTAFSSON, B. (1984). A possible correlate of the postsynaptic condition for long-lasting potentiation in the guinea pig hippocampus *in vitro*. *Neuroscience Letters* **44**, 327–332.