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A comparison of the effects of selective metabotropic glutamate receptor agonists on synaptically evoked whole cell currents of rat spinal ventral horn neurones *in vitro*

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1 Whole cell synaptic currents were recorded under voltage clamp from a total of 54 ventral horn neurones held near to their resting potential by the patch clamp technique in immature rat spinal cord preparations *in vitro*. Twenty eight neurones were identified, by antidromic invasion from ventral roots, as motoneurones. Excitatory postsynaptic currents (e.p.s.cs) of peak amplitude -480 pA $\pm$ 66 s.e.mean and -829 ± 124 pA were evoked respectively from the unidentified ventral horn neurones and the motoneurones in response to maximal activation of the segmental dorsal root.

2 The e.p.s.cs were depressed reversibly by the metabotropic glutamate agonists 1S3S-1-aminocyclopentane-1,3-dicarboxylate (1S3S-ACPD) (EC_{50} 17.1 μM $\pm$ 0.3 s.e.mean, $n=14$) and L-2-amino-4-phosphonobutanoate (L-AP4) ($EC_{50}=2.19 \pm 0.19$ μM, $n=15$). Since both agonists independently produced more than 90% depression it is likely that the receptors that mediate their effects are present on the same presynaptic terminals.

3 When the Mg^{2+} concentration was raised from 0.75 mM to 2.75 mM together with the addition of 50 μM D-2-amino-5-phosphonopentanoate (AP5), a treatment which would increase the proportion of monosynaptic component in the e.p.s.c. the concentration-effect plots for both 1S3S-ACPD (EC_{50} 1.95 ± 0.4 μM, $n=8$) and L-AP4 (EC_{50} 0.55 ± 0.20 μM, $n=7$) were shifted to the left, suggesting that monosynaptic e.p.cs of primary afferents to ventral horn neurones are more susceptible to L-AP4 and 1S3S-ACPD than are other synapses in polysynaptic pathways.

4 1S3S-ACPD (20 and 50 μM) also caused mean sustained inward currents of 95 ± 31 pA ($n=6$) and 248 ± 49 pA ($n=10$) respectively. In the combined presence of AP5 (50 μM) and Mg^{2+} (2.75 mM) the mean response to 50 μM 1S3S-ACPD was reduced to 106 ± 18 pA ($n=4$). In the presence of tetrodotoxin (1 μM) the corresponding value was 48 ± 6 pA ($n=4$). Similar sustained inward currents produced by N-methyl-D-aspartate (NMDA) were almost abolished to <10 pA in the presence of AP5 and 2.75 mM Mg^{2+}. In the presence of tetrodotoxin the maximum inward current produced by NMDA was undiminished. Thus a large component of the excitatory action of 1S3S-ACPD was mediated at non-NMDA receptors both directly at the patch-clamped neurones and indirectly by synaptic relay.

Keywords: Spinal neurones; metabotropic; glutamate receptor

Introduction

Interest in the presynaptic depressant action of the phosphonic analogue of L-glutamate, L-2-amino-4-phosphonobutanoate (L-AP4), arises from the observation of the potent selective depressant action which L-AP4 has on synaptic transmission via the lateral perforant pathway in the hippocampal slice preparation (Koerner & Cotman, 1981). In spinal cord preparations L-AP4 was shown from extracellular recordings to depress spinal segmental transmission in the absence of post-junctional antagonism of the effects of excitatory amino acids. It was suggested from this observation that L-AP4 might be a selective agonist for presynaptic glutamate autoreceptors (Evans *et al.*, 1982). Intracellular recordings from pre- and post-synaptic cultured hippocampal neurones have shown that the presynaptic depressant action of L-AP4 is mimicked by L-glutamate (Forsythe & Clements, 1990).

Combined expression cloning and electrophysiological techniques have indicated that L-AP4 activates a subtype of metabotropic glutamate receptor (mGluR4) (Tanabe *et al.*, 1993), although this is unlikely to be the receptor involved in the presynaptic depressant action (Jane *et al.*, 1994). The mGluR agonist (1S, 3S)-1-aminocyclopentane-1,3-dicarboxylate (1S3S-ACPD) has been found also to have a potent L-AP4-like depressant action on segmental reflexes of hemisected spinal cord preparations (Kemp *et al.*, 1993) which is less sensitive to antagonism by α-methyl-AP4 than is the depression produced by L-AP4 (Jane *et al.*, 1994). Thus, whilst there is no direct evidence for the selectivity of these agonists for different mGluRs in intact tissues, it is possible that there may be at least two distinct mGluRs that operate as separate presynaptic control elements activated by glutamate. The present study was carried out in order to investigate possible roles of these L-AP4- and 1S3S-ACPD-sensitive receptors in segmental synaptic transmission in the spinal cord.

Methods

Hemisected spinal cords from immature (5 to 8 day old) rats were superfused with medium of the following composition (mM) maintained at room temperature (22 to 24°C): NaCl 118, KCl 3, $CaCl_2$ 1.5, $MgSO_4$ 0.75, dextrose 12, $NaHCO_3$ 24 gassed with 95%/5% O_2/CO_2. The blind patch method (Blanton *et al.*, 1989) was used to obtain gigaseals followed by whole cell recordings (Axopatch 1D amplifier and PClamp 5.5 software) via patch pipettes placed within 250 μm of the pial surface of the spinal cord. The Axopatch 1D was used in voltage clamp mode. For recordings of synaptic currents the low pass filter of the Axopatch 1D was set at 2 kHz and the sampling interval selected in the software varied upwards from a minimum of 80 μs. Shorter latencies were measured from records on digital analogue tape records sampled initially at a rate of 12 kHz. The fastest sampling rate (8 μs) was used to record charging curves of cell attached patches. The patch pipettes were introduced to the ventral grey matter through a

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hole about 100 μm in depth made by tearing the pia mater along the ventrolateral surface of the spinal cord with watchmaker's forceps.

The patch pipettes (resistance 3 to 6 megohms) were filled with the following solution (mM): potassium gluconate 130, KCl 10, EGTA 11, HEPES 10, $CaCl_2$ 1, $MgCl_2$ 2, adjusted to pH 7.4 with NaOH (final concentration of Na^+ approximately 30 mM).

The capacitance and series resistance compensation controls of the Axopatch 1D were not used. However, series resistances were estimated from the area under the charging curve of the cell in response to a voltage command of 10 mV or less applied within the first 5 min of whole cell recording. In whole cell mode uncompensated series resistances were never more than fourfold higher than the original resistance of the pipettes. Values of whole cell capacitance measured in some experiments, from the area under charging curves, ranged from 15 to 85 pF corresponding to apparent cell diameters from 22 to 52 μm. Presumably the latter values reflect the spatial extent of the voltage clamp over the neurone rather than the absolute size of the neurones. The large size of the neurones and the presence of largely potassium ions inside the pipettes contributed to poor spatial control of voltage clamp as reflected by all-or-none and sometimes double action currents in response to depolarizing voltage steps.

Dorsal or ventral roots were stimulated via glass suction electrodes with the cathode internal and similar electrodes were used to record the evoked compound action potential from the same ventral root (Agrawal & Evans, 1986).

The percentage depression of peak amplitude and of area under the curve of inward synaptic currents were measured following the addition of either single or cumulative increases in concentration of drugs. In the results section, values for percentage depression refer to peak amplitude unless stated otherwise. Ten to fifteen minutes was required to attain equilibrium at each concentration, as assessed from less than 4% change in consecutive synaptic responses displayed continuously on a penchart.

EC_{50} values for percentage depression of excitatory postsynaptic current (e.p.s.cs) were obtained for each neurone tested by a least squares fit to the relationship, % depression/100 = agonist concentration^P/($EC_{50}{}^P$ + agonist concentration^P) using the InPlot software package (GraphPad Software Inc.). For those cells on which only one or two concentrations of drug were tested the slope factor P was fixed at unity. The significance of differences between means was tested either by Student's *t* test or by rank sum using the Wilcoxon two sample test.

High performance liquid chromatographic analysis of stock solutions (10 and 50 mM) of the sodium salt of 1S3S-ACPD was carried out according to Maurs *et al.* (1988) following precolumn chiral derivatisation with *o*-phthalaldehyde and N-acetyl-L-cysteine.

L-AP4, 1S3S-ACPD, D-2-amino-5-phosphonopentanoate (AP5) and N-methyl-D-asparate (NMDA) were provided by Tocris Cookson Ltd.

Results

Whole cell synaptic currents were recorded from a total of 54 ventral horn neurones. Whole cell recording configurations were maintained for periods which ranged from 1 to 7 h. Each of the neurones recorded from was in a different spinal cord preparation.

Twenty eight of the neurones were identified as motor neurones from the presence of antidromically evoked action currents following ventral root stimulation (Figure 1). The mean peak amplitude (±s.e.mean) of the antidromically evoked action currents was −4.20 ± 0.38 nA. The resting potentials and the amplitude of currents evoked by depolarizing steps were significantly different ($P<0.005$) between the motoneurones and the 26 unidentified ventral horn neurones. The

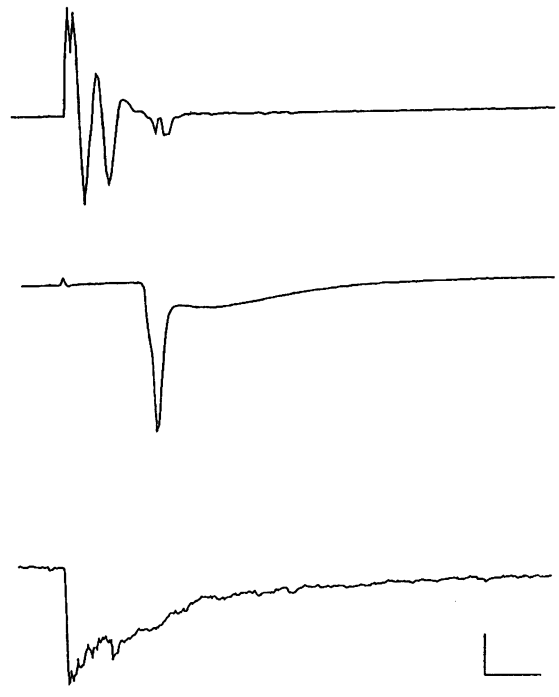

Figure 1 Characterization of ventral horn neurones. The upper trace is the compound action potential recorded extracellularly in the ventral root in response to a single shock applied to the ventral root 5 mm distal to the spinal cord. The resulting whole cell current is shown synchronously in the centre trace in which can be seen the stimulus artifact for both traces (scale bar 2 mV upper, 2 nA centre and 8 ms both traces). The bottom trace is a whole cell response of the same neurone following a single shock applied to the dorsal root (scale bar 0.2 nA, 100 ms).

mean resting potentials of motoneurones and the unidentified ventral horn neurones were −69.4 ± 1.0 and −63.7 ± 1.6 mV respectively. Reflecting poor spatial control of intracellular voltage, depolarizing voltage steps evoked all-or-none action currents of mean amplitude −4.28 ± 0.39 nA in the motoneurones and −2.89 ± 0.36 nA in the other neurones. The former value is very close to the value reported above for currents evoked antidromically. In the neurones held near to their resting potentials (−60 to −75 mV at I = 0) complex inward synaptic currents (e.p.s.cs) were evoked by supramaximal stimulation of a dorsal root (8 to 16 times threshold) (Figure 1). The mean latencies of e.p.s.cs of 7.5 ± 1.3 ms for the motoneurones and 7.4 ± 0.7 ms for the unidentified ventral horn cells were not significantly different. The mean time from stimulus artifact to peak amplitude of the e.p.s.cs recorded from the motoneurones was 13.2 ± 1.6 ms. In animals of this age at room temperature the conduction velocity of all dorsal root fibres is less than 1 m s^{-1}. Thus at least 5 ms of the above latency is accounted for by conduction delay. The mean peak amplitude of the e.p.s.cs recorded from the motoneurones (−829 ± 124 pA) was significantly ($P<0.05$) higher than that from the other neurones (−480 ± 66 pA).

The peak amplitudes of e.p.s.cs were depressed reversibly by 1S3S-ACPD (EC_{50} = 17.1 ± 0.3 μM, n = 14) (Figure 2) and L-AP4 (EC_{50} = 2.19 ± 0.19 μM, n = 15) (Figure 3). The corresponding EC_{50} values for depression of the area under the curve of e.p.s.cs were not significantly different ($P>0.1$) from the values above, being 18.8 ± 1.6 μM for 1S3S-ACPD and 1.65 ± 1.22 μM for L-AP4, indicating that there was no preferential depression of later or earlier components of the e.p.s.cs produced by either of the drugs. As indicated by Figures 2c and 3c e.p.s.cs were almost abolished at the highest concentrations of agonist tested. Thus the extrapolated mean maximal depressant action of 1S3S-ACPD on peak amplitude of e.p.s.cs was to 4 ± 2% of control levels and the corre-

sponding value for L-AP4 was to $-9 \pm 6\%$ of control levels. Comparison of EC_{50} values for depression of peak amplitude of e.p.s.cs in motoneurones and the unidentified ventral horn cells revealed no significant differences between them. Therefore the mean values above were pooled from all the neurones. The pooled values plotted in Figures 2c and 3c gave significantly different ($P<0.0005$) mean slope factors (Hill coefficients) for the concentration-effect plots, of 0.93 ± 0.02 and 0.66 ± 0.04 for 1S3S-ACPD (Figure 2c) and L-AP4 (Figure 3c) respectively.

In the present preparations the shortest latency component of synaptic transmission is monosynaptic from low threshold primary afferents and is not considered to involve NMDA receptors (Jahr & Yoshioka, 1986). In the present study 1S3S-ACPD and L-AP4 were tested on this component of synaptic activity in the presence of 2.75 mM $MgSO_4$ and the NMDA receptor antagonist D-AP5 (50 μM). Comparison of the lower trace in Figure 3a and the upper in Figure 3b shows that the NMDA antagonist mixture depressed all phases of e.p.s.cs producing a less complex profile. Application of 50 μM D-AP5 and 2.75 mM Mg^{2+} caused $48 \pm 5\%$ ($n=13$) depression of the mean peak amplitude of e.p.s.cs recorded from motoneurones. As anticipated there was a greater effect of the NMDA blocking mixture on the mean area under the curve of e.p.s.cs which was depressed by $86 \pm 2\%$. The mean latency of e.p.s.cs produced in the presence of the NMDA blocking mixture was increased, by 0.26 ± 0.06 ms to 7.74 ms ($P<0.005$) and the mean time to peak amplitude was shortened significantly ($P<0.05$), by 2.9 ± 1.1 ms, to 10.3 ms ($n=13$). Under these conditions the mean EC_{50} values for depression of the peak amplitude of e.p.s.cs were decreased several fold to 1.95 ± 0.4 μM ($n=8$, $P<0.0005$) for 1S3S-ACPD and to 0.55 ± 0.20 ($n=7$, $P<0.0005$) for L-AP4. The slope of the concentration-effect plot for 1S3S-ACPD (0.62 ± 0.07), but not that for L-AP4 (0.51 ± 0.11), was diminished significantly ($P<0.0005$) in the presence of the NMDA blocking mixture so that there was no significant difference between the slopes of the plots for 1S3S-ACPD and L-AP4 (filled circles in Figures 2c and 3c).

At levels that produced synaptic depression, 1S3S-ACPD also evoked a sustained inward current (Figure 4a,b) and increased the frequency of spontaneous synaptic currents (Figure 5a). 1S3S-ACPD at 20 and 50 μM caused mean sustained inward currents of 95 ± 31 pA ($n=6$) and 248 ± 49 pA ($n=14$), respectively. L-AP4, unlike 1S3S-ACPD, had no excitatory actions at concentrations that produced marked synaptic depression.

The 1R3R form of ACPD is a potent NMDA receptor agonist (Curry *et al.*, 1988; Sunter *et al.*, 1991) which could have been responsible for the excitatory effects of ACPD in the present experiments. High performance liquid chromatographic analysis of the 1S3S-ACPD solutions applied to the preparation in the present study indicated that no more than 1.6% of any of the other three forms of ACPD could have been present in the sample. Nevertheless the effects of 1S3S-ACPD were compared with the effects of NMDA. At 2, 5 and

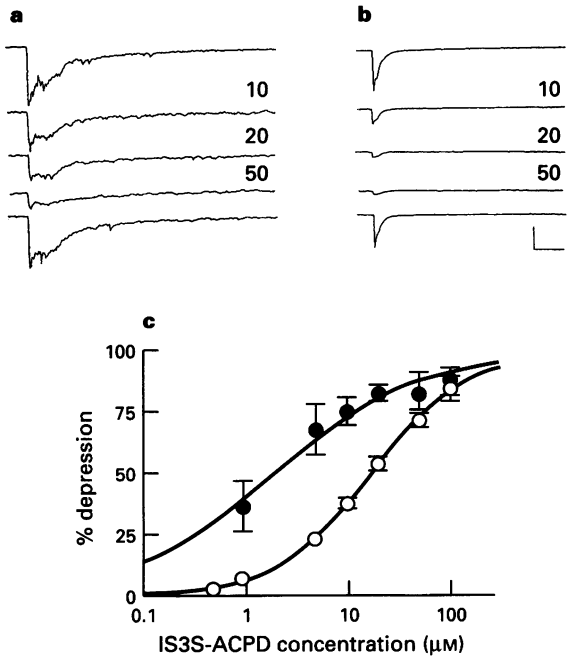

Figure 2 Depressant effect of 1S3S-ACPD on whole cell synaptic responses evoked by a single shock applied to the dorsal root. (a) Shows depression of whole cell synaptic response of a motoneurone by 1S3S-ACPD at the micromolar concentrations indicated at the end of each trace. (b) Is a similar series to (a) in a different motoneurone except that D-AP5 (50 μM) and 2.75 mM Mg^{2+} were present throughout in order to block NMDA receptor-mediated effects. In (a) and (b) the first traces were recorded 2 min before application of 1S3S-ACPD and the last traces were recorded 30 and 20 min respectively following removal of 1S3S-ACPD. Scale bar 100 pA and 200 ms in (a), 100 ms in (b). (c) Plots of log concentration 1S3S-ACPD versus effect: (○) means from 14 neurones treated as in (a); (●) means from eight neurones tested as in (b). Values are mean ± s.e.mean. Curves fitted by least squares.

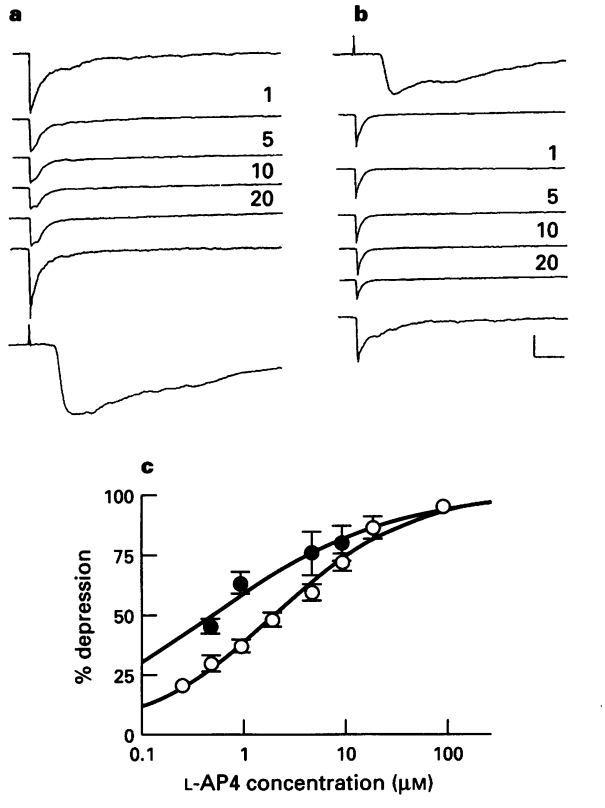

Figure 3 Depressant effect of L-AP4 on whole cell synaptic responses of a motoneurone. (a) Shows depression of whole cell synaptic response of a motoneurone by L-AP4 at the micromolar concentrations indicated at the end of each trace. In (a) the first trace was recorded 2 min before application of L-AP4 and the last two traces 30 min following its removal. In (b) the first two traces were recorded 15 min following introduction of AP5 and 2.75 mM Mg^{2+} and 2 min before application of L-AP4. Scale bar 400 pA and 100 ms. The bottom trace in (a) and the top trace in (b) are displayed at a higher sampling rate in order to show the initial component of the synaptic current in detail, horizontal scale bar 5.6 ms. (c) Log concentration effect plot for L-AP4: (○) mean values from 15 neurones treated as in (a); (●) corresponding mean values from seven neurones treated as in (b). Other details as for Figure 2.

10 μM, NMDA produced mean sustained inward currents of 40 ($n=1$), 136±29 ($n=3$) and 317±91 pA ($n=3$) respectively as illustrated for one neurone in Figure 4c. As found with 1S3S-ACPD, the sustained inward current induced by NMDA was associated with an increase in the frequency of spontaneous synaptic currents (Figure 5b). NMDA also had a depressant action on evoked currents (Figure 4c). However, the depressant action of NMDA, unlike that of 1S3S-ACPD, was always preceded by a sustained inward current (Figure 4c).

In order to block NMDA receptor-mediated currents induced by agonists, preparations were bathed in 50 μM D-AP5 and the concentration of Mg^{2+} was raised to 2.75 mM, as for the recordings of e.p.s.cs above. In the NMDA receptor blocking medium, NMDA (10 μM) induced sustained inward currents of less than 10 pA (Figure 4d) and produced no significant depression of the dorsal root evoked e.p.s.cs. In the presence of the blocking medium the mean 1S3S-ACPD-induced inward currents (20 and 50 μM) were lower than control values at 65±10 pA ($n=4$) and 106±18 pA ($n=4$) respectively (Figure 4b). However the latter values are not significantly different from the control levels obtained in the absence of the NMDA blocking mixture.

In further experiments, preparations were treated with tetrodotoxin in order to show whether the inward current induced by 1S3S-ACPD was either relayed synaptically, perhaps as a consequence of disinhibition, or was through a direct pre- or postsynaptic action of 1S3S-ACPD at the recording site. As illustrated in Figure 6a and b, NMDA ($n=3$) and 1S3S-ACPD ($n=4$) still produced inward current when synaptic transmission was blocked in the presence of tetrodotoxin (1 μM). However, the excitatory potencies of NMDA and 1S3S-ACPD were decreased as illustrated by the rightward shifts in the concentration-effect plots in Figure 6c. The inward current induced by 50 μM 1S3S-ACPD was depressed to 48.0±6 pA ($n=4$) by tetrodotoxin and in three other neurones tested with concentrations up to 1 mM, 1S3S-ACPD produced inward

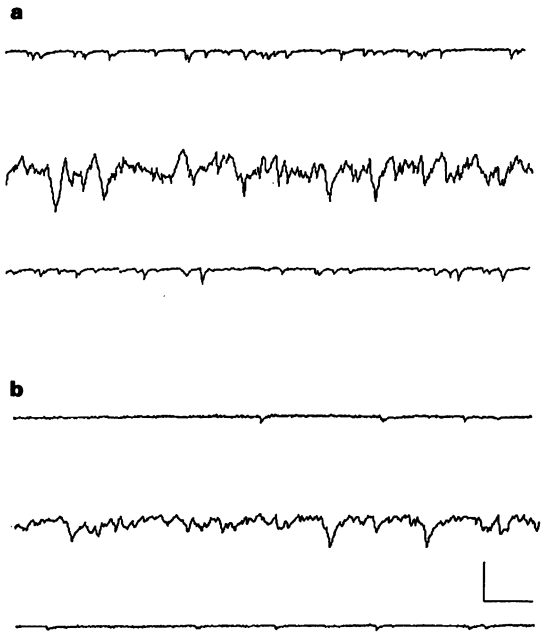

Figure 5 Profile of oscillatory spontaneous inward currents produced by (a) 1S3S-ACPD (50 μM) and (b) NMDA (5 μM). The traces are shown in sequence, from above downwards, 2 min before addition, in the presence of and 25 min after washout of the respective agonist. Scale bar 100 pA, horizontal 200 ms.

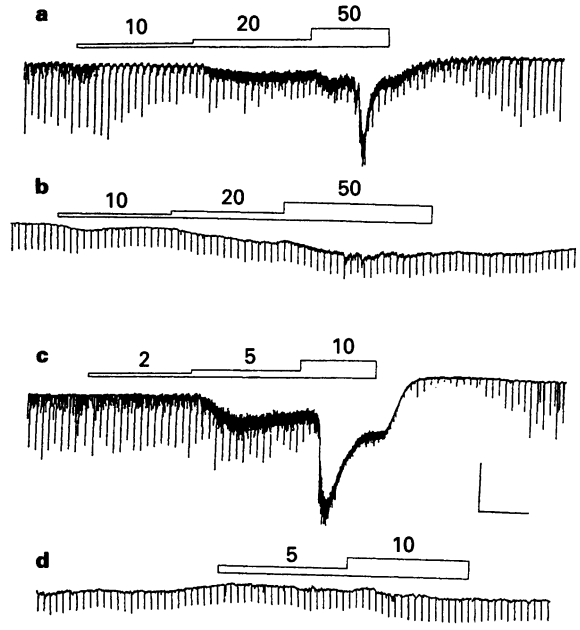

Figure 4 Pen chart recordings showing inward whole cell currents evoked by 1S3S-ACPD and NMDA. The vertical deflections are the synaptic responses (as illustrated by Figures 2 and 3a and b) to dorsal root stimulation applied at 40 s intervals. (a) Increasing concentrations (μM) of 1S3S-ACPD were applied as indicated above the trace. (b) Is a similar recording to (a) except that D-AP5 (50 μM) and Mg^{2+} (2.75 mM) were present throughout. (c) and (d) are similar to (a) and (b) respectively except that, instead of 1S3S-ACPD, NMDA was applied. It can be seen that the inward current induced by NMDA was abolished by the mixture of antagonists whereas 1S3S-ACPD had an excitatory action that was resistant to the blockers. Scale bar 0.2 nA, 5 min.

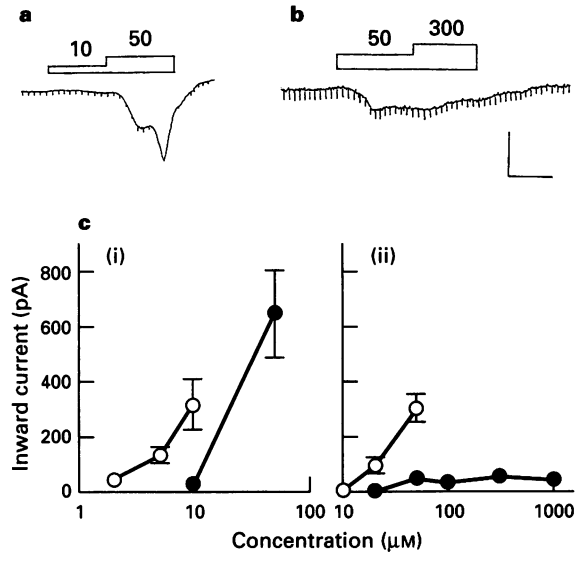

Figure 6 Traces (a) and (b) are pen recordings from two separate neurones obtained in the presence of tetrodotoxin (1 μM). This level of tetrodotoxin abolished synaptic currents. The small vertical deflections are dorsal root stimulus related artifacts. In (a) NMDA was introduced at 10 and 50 μM and in (b) 1S3S-ACPD was introduced at 50 and 300 μM as indicated by the bars above. Scale bar 400 pA and 5 min in (a) and 100 pA and 5.5 min in (b). (c) Comparison of peak inward currents produced by NMDA (i) and 1S3S-ACPD (ii) in the absence of tetrodotoxin (○, mean values ±s.e.mean taken from the text) and in the presence of tetrodotoxin (●). The error bar for the rightmost point at 50 μM 1S3S-ACPD ($n=4$) (in (ii)) falls within the diameter of the symbol. The other (●) in (ii) are single values from different neurones.

currents of approximately 50 pA. In the presence of tetrodotoxin the maximum effect of NMDA was undiminished within the range of concentrations tested (Figure 6c).

Discussion

The e.p.s.cs in these experiments were obtained from neurones under imperfect conditions of voltage clamp as indicated by the all-or-none action currents in response to voltage steps. Thus all parts of the membrane at which excitatory transmitter was released would not have been clamped at the holding potential. Therefore the duration of e.p.s.cs was likely to have been shortened due to activation of voltage-activated potassium conductances. Because of rapid inactivation, the contribution of voltage-activated sodium current is likely to have been less important. These deficiencies are unlikely to have distorted the pharmacological data since the e.p.s.cs measured were treated simply as synaptic responses of single neurones which resulted from dorsal root stimulation.

In the present experiments no attempt was made to show whether the depressant action of either L-AP4 or 1S3S-ACPD was produced postjunctionally. Previous experiments with spinal cord (Evans *et al.*, 1982) and cultured hippocampal neurones (Forsythe & Clements, 1990) have shown that at depressant concentrations L-AP4 has no significant postjunctional actions. Similarly 1S3S-ACPD has been found to produce no significant postjunctional antagonism of responses to excitatory amino acids at depressant concentrations (Pook *et al.*, 1992). In the present experiments, therefore, the depressant effects of L-AP4 and 1S3S-ACPD are likely to have been mediated presynaptically at terminals of interneurones in polysynaptic pathways as well as terminals synapsing with the neurones from which recordings were made. In the presence of either L-AP4 or 1S3S-ACPD, dorsal root evoked synaptic responses of single postsynaptic neurones were depressed to 10% or less of control levels.

The major part of polysynaptic activation would have been absent when a large component of NMDA receptor-mediated transmission was blocked by the presence of D-AP5 and raised Mg^{2+} (Evans *et al.*, 1982; Jahr & Joshioka, 1986). However, under that condition both drugs still produced near maximal depression (Figures 2 and 3) suggesting that there are receptors for both L-AP4 and 1S3S-ACPD on the same presynaptic terminals which monosynaptically activated the neurones from which recordings were made. If receptors for L-AP4 and 1S3S-ACPD were differentially distributed between afferent terminals it would be necessary to add the two drugs in combination in order to achieve near maximal blockade of transmission.

The present observations do not show that 1S3S-ACPD and L-AP4 act at different receptors. The studies of Jane *et al.* (1994) suggest this to be so because they found that the depressant action of L-AP4, but not that of 1S3S-ACPD, was reversed by the α-methyl analogue of L-AP4. It would be interesting to examine the depressant action of (1'S,2'S,2S) carboxycyclopropyl-glycine (L-CCG1) (Ishida *et al.*, 1993) under similar circumstances since this compound is considered to activate yet another presynaptic glutamate autoreceptor subtype different from those activated by either L-AP4 or 1S3S-ACPD (Jane *et al.*, 1994).

The leftward shifts in Figures 2 and 3 indicate increased potency of L-AP4 and 1S3S-ACPD in the presence of the NMDA blocking mixture. This might suggest that the drugs are more efficacious in depressing transmission from monosynaptic afferents onto ventral horn neurones. If this is so then later components of the e.p.s.cs, which are considered to reflect polysynaptic activity, should have been less sensitive to the drugs. In the present study, under control conditions, there was no significant difference between EC_{50} values for depression of e.p.s.cs estimated from either peak or area under the curve, the latter reflecting largely polysynaptic activity. However, the mean latency to peak as well as the amplitude of later components of the e.p.s.cs were decreased significantly in the presence of D-AP5 and raised Mg^{2+}. Thus the mean peak (maximum amplitude) of the complex e.p.s.cs obtained under control conditions was probably not monosynaptic, explaining why there was no difference in sensitivity to the drugs between peak amplitude and area under the curve of e.p.s.cs. The addition of AP5 plus raised Mg^{2+}, by depressing polysynaptic activity and thereby increasing the proportion of the e.p.s.cs contributed by monosynaptic inputs, would have caused the peak of e.p.s.cs to be always monosynaptic. It is curious that the situation with the $GABA_B$ agonist, baclofen, appears to be the reverse of that found for L-AP4 and 1S3S-ACPD in the present study. Extracellular recordings from ventral roots showed a five fold lower potency of baclofen in depressing monosynaptic compared to polysynaptic reflexes (Siarey *et al.*, 1992).

The slope of the concentration-effect plot for 1S3S-ACPD, but not that for L-AP4, was decreased in the presence of D-AP5 and raised Mg^{2+}. This effect may be related to 1S3S-ACPD-mediated indirect excitation which was largely depressed in the presence of D-AP5 (see below).

The present slope factors are significantly lower than values of 1.15 and 1.8 for 1S3S-ACPD and 0.95 and 0.85 for L-AP4 which we have estimated from each of four individual experiments of Figure 6 from Jane *et al.* (1994). The latter values are from concentration-effect plots for depression of the motoneurone monosynaptic population spike which would be expected to have a steeper concentration-effect relationship than that for the underlying population excitatory postsynaptic potential which is analogous to the e.p.s.cs of the present experiments. The present slope values, being significantly less than one, suggest heterogeneity of release sites involved in the generation of e.p.s.cs such that some were more easily blocked than others. This could occur due to blockade of monosynaptic elements at lower concentrations followed by blockade of more resistant pathways. Such an explanation would be consistent with the leftward shift in concentration-effect plots produced by the NMDA blocking mixture.

NMDA also had a marked depressant action on dorsal root evoked and spontaneous e.p.s.cs. But the depressant action of NMDA, unlike that of 1S3S-ACPD, was always preceded by an inward current response. In the presence of D-AP5, NMDA produced no inward current or depression of e.p.s.cs. The most likely explanation of the depression induced by NMDA is the outward current due to calcium-activated potassium channels. In the present study no steps were taken to suppress potassium conductances.

An excitatory action of the 1S,3S form of ACPD, used in the present study, has been reported to be consistent with 1% contamination with the 1**R**,3**R** form. The 1**R**,3**R** form of ACPD is a potent NMDA agonist (Curry *et al.*, 1988) equipotent with NMDA (Sunter *et al.*, 1991). It can be calculated from the present data that 1S3S-ACPD was six fold less potent as an excitant than NMDA. Thus the presence of approximately 17% of the 1**R**,3**R** form would be necessary to explain the observed level of excitatory activity. Furthermore the excitatory action, unlike that caused by NMDA, had significant resistance to D-AP5. Such resistance could indicate mGluR-mediated postjunctional excitation such as that caused by the 1S3**R** form of ACPD. However, it can be estimated from the relative excitatory potencies of NMDA and 1S3R-ACPD (Jane *et al.*, 1993) that a proportion of the 1S3**R** form of ACPD even higher than 17% would have been required to explain even the residual excitatory action of 1S3S-ACPD observed in the presence of tetrodotoxin in the present experiments.

An excitatory action of 1S3S-ACPD was not detected in previous extracellular recordings from ventral roots, but in that study the highest concentration tested was 10 μM (Pook *et al.*, 1992) which is threshold for the inward current observed in the present study. The inward current produced by 1S3S-ACPD was attenuated, as was that due to NMDA, in the presence of tetrodotoxin (Figure 6) indicating that a large part

of the excitatory action was relayed through interneurones (Brugger *et al.*, 1990). The mean inward current responses produced by 1S3S-ACPD were also depressed (although the difference between means was not significant at the $P<0.05$ level) in the presence of D-AP5 and raised Mg^{2+}, as would be expected from an excitatory action relayed via interneurones. In the presence of tetrodotoxin the maximum inward current response from 1S3S-ACPD, unlike that from NMDA, was markedly diminished. This suggests that the 1S3S-ACPD induced current is unlikely to be via operation of ionotropic receptors because a maximal ionotropic response should still be available in the presence of tetrodotoxin, as occurred with NMDA. Thus the residual tetrodotoxin-resistant effect of 1S3S-ACPD was probably a direct metabotropic action on motoneurones. Further investigation is required to characterize this excitatory action which may be similar to the excitatory effect of the 1S3R form of ACPD which has more marked excitatory actions combined with a presynaptic depressant effect (Kemp *et al.*, 1994).

In conclusion the present experiments indicate that release sites onto single ventral horn neurones are sensitive to mGluR agonists considered to activate different receptor types.

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