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Characterization of metabotropic glutamate receptor-mediated facilitation of N-methyl-D-aspartate depolarization of neocortical neurones

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1 Facilitation of the N-methyl-D-aspartate (NMDA) receptor-mediated depolarization of cortical neurones induced by metabotropic glutamate receptor (mGluR) agonists in the presence of tetrodotoxin has been examined by use of grease-gap recording.

2 Quisqualate (1-2 μM) and 10 to 100 μM 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) facilitated the NMDA-, but not the kainate-induced depolarization with an EC_{50} of 16 μM for 1S,3R-ACPD. The facilitation induced by quisqualate was reduced, but not blocked, by 4 μM 6-cyano-7-nitroquinoxaline-2,3-dione.

3 D,L-2-Amino-3-phosphonopropionic acid and D,L-2-amino-4-phosphonobutyric acid antagonized the 1S,3R-ACPD facilitation in a non-competitive manner with IC_{50} values of 0.24 μM and 4.4 μM respectively.

4 Homologous desensitization of the 1S,3R-ACPD induced facilitation was not observed. The facilitation was not altered by 10 nM staurosporine or 3 μM phorbol diacetate.

5 Substitution of 20 μM 8-bromo-cyclic adenosine monophosphate, 20 μM 8-bromo-cyclic guanosine monophosphate, or 10 μM arachidonic acid for 1S,3R-ACPD did not induce facilitation of the NMDA response. However, the 1S,3R-ACPD facilitation was potentiated by 10 mM *myo*-inositol and exhibited heterologous desensitization following exposure to 100 μM 5-hydroxytryptamine.

6 The 1S,3R-ACPD-induced facilitation persisted in both 10 μM nifedipine and nominally Ca^{2+}-free medium and was only gradually eliminated following addition of 100 μM *bis*-(-o-aminophenoxy)-ethane-*N,N,N,N*-tetraacetic acid in Ca^{2+}-free medium. Facilitation of the NMDA response induced by carbachol, but not phenylephrine, was also observed in nominally Ca^{2+}-free medium. Perfusing 50 μM *bis*-(-aminophenoxy)-ethane-*N,N,N,N*-tetraacetic acid aminoethoxy eliminated the 1S,3R-ACPD facilitation.

7 These experiments have shown that mGluR agonists selectively facilitate the NMDA depolarization of cortical wedges, most likely by activating one or more mGluR subtypes that couple to phospholipase C. We conclude the facilitation results from a Ca^{2+}-sensitive mechanism dependent on activation of phospholipase C and release of internal Ca^{2+}. The facilitation is not contingent on activation of protein kinase C or entry of Ca^{2+} through nifedipine-sensitive Ca^{2+} channels.

Keywords: Transmitter interaction; NMDA; metabotropic glutamate receptors; desensitization; calcium; cortical neurones; phospholipase C

Introduction

Projection neurones (mainly pyramidal cells; Lorente de No, 1949) of the cerebral cortex are depolarized by N-methyl-D-aspartate (NMDA; Harrison & Simmonds, 1985) and this depolarization is facilitated following activation of 5-hydroxytryptamine (5-HT) 5-HT_{2A} receptors, muscarinic acetylcholine receptors (AChR), and α_1-adrenoceptors (Reynolds *et al.*, 1988; Nedergaard *et al.*, 1987; Neuman & Rahman, 1992; Rahman & Neuman, 1993a). All of these receptors couple positively to phospholipase C via guanine nucleotide binding proteins (G-proteins) whereby they stimulate phosphoinositide hydrolysis generating a second messenger cascade consisting of diacylglycerol and inositol 1,4,5 trisphosphate (Berridge, 1987). We have proposed that facilitation of the NMDA depolarization in cortical neurones is dependent on a rise in intracellular Ca^{2+} that follows stimulation of phospholipase C, but is independent of the activation of protein kinase C by diacylglycerol (Rahman & Neuman, 1993b,c,d). This proposal is consistent with observations of the NMDA receptor mediated facilitation induced by muscarinic AChR at CA1 neurones of the hippocampus (Markram & Segal, 1992).

The metabotropic glutamate receptor (mGluR) is another member of the G-protein receptor superfamily that contains receptor subtypes which couple positively to phospholipase C (Nakanishi, 1994; Bockaert *et al.*, 1993). Thus, the mGluR1 and the mGluR5 subtypes have been shown to activate phospholipase C (Abe *et al.*, 1992; Tanabe *et al.*, 1992). mGluR also exhibit positive coupling to guanylate cyclase and phospholipase A_2, as well as both positive and negative coupling to adenylate cyclase (Nakanishi, 1994; Schoepp & Conn, 1993). Agonists at mGluR facilitate NMDA receptor-mediated responses in hippocampal CA1 neurones (Aniksztejn *et al.*, 1991; 1992; Harvey *et al.*, 1991; Harvey & Collingridge, 1993), rat olfactory cortex (Collins, 1993), neonatal spinal motoneurones (Birse *et al.*, 1993), spinal dorsal horn neurones (Bleakman *et al.*, 1992), and in *Xenopus* oocytes expressing rat brain mRNA (Kelso *et al.*, 1992). However, the mechanism underlying the facilitation is not uniform across preparations. Thus, facilitation of the NMDA response is mediated by protein kinase C in *Xenopus* oocytes (Kelso *et al.*, 1992), whereas at hippocampal neurones there is evidence for and against involvement of protein kinase C (Aniksztejn *et al.*, 1991; 1992; Harvey &

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Collingridge, 1993). Moreover, the facilitation is selective for NMDA responses in the hippocampus, whereas α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate responses are also facilitated at dorsal horn neurones (Bleakman *et al.*, 1992). In the present study, facilitation of the NMDA depolarization induced by mGluR agonists was investigated in neocortical neurones along with possible mechanisms underlying the facilitation. Receptor desensitization and Ca^{2+} sensitivity of the mGluR facilitation were also examined. A preliminary report has been communicated (Neuman & Rahman, 1995).

Methods

Cortical wedge preparation and recording

Wedges from dorsal sensorimotor cortex were prepared as described by Harrison & Simmonds (1985) and Rahman & Neuman (1993a). Briefly, male Sprague-Dawley rats (Charles River, Montreal; 100–300 g) were anaesthetized with ether and killed with a heavy blow. The brain was rapidly removed and 500 μm coronal slices cut at 0–4°C in modified artificial cerebrospinal fluid (ACSF). Slices were allowed to recover 30 min at room temperature (20–24°C) in modified ACSF before replacing the medium with normal ACSF. Normal ACSF consisted of (mM): NaCl 126, KCl 3.5, $CaCl_2$ 2, $MgCl_2$ 1.3, NaH_2PO_4 1.2, $NaHCO_3$ 25 and glucose 11. Gassed with O_2/CO_2 (95%/5%) the pH was 7.3. In modified ACSF the NaCl was replaced by iso-osmotic sucrose (252 mM; Aghajanian & Rasmussen, 1989). After an additional 45 min recovery in normal ACSF, a wedge of cortex was cut from the slice and mounted in a two compartment recording chamber (Harrison & Simmonds, 1985). Each compartment was perfused at 2 ml min^{-1}. Agonist-induced depolarization of the cell bodies was recorded with respect to the corpus callosum with Ag/AgCl electrodes embedded in 3% agar in saline.

Drugs were dissolved in ACSF and applied via a three way tap system to the cell body containing compartment. Tetrodotoxin (TTX; 0.1 μM) was perfused throughout to minimize indirect effects (Rahman & Neuman, 1993a). NMDA (50 μM) was applied for 2 min every 20 min unless otherwise indicated. Phorbol diacetate was perfused for 20 min and staurosporine, D,L-2-amino-3-phosphonopropionic acid (D,L-AP3) and D,L-2-amino-4-phosphonobutyric acid (D,L-AP4), *bis*-(o-aminophenoxy)-ethane-*N,N,N,N*, tetraacetic acid aminoethoxy (BAPTA-AM), *bis*-(o-aminophenoxy)-ethane-*N,N,N,N*, tetraacetic acid (BAPTA), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) were perfused 40 min before addition of agonists.

Data analysis

To quantitate and normalize drug responses, depolarization amplitude was converted to a percentage of control [(Treatment/Control) × 100] and the log normal value computed (Gaddum, 1945). Repeated measures were analyzed by paired *t* tests. Multiple comparisons were analyzed by one-way analysis of variance followed by the Bonferroni test (Instat, GraphPad Software). Data are presented as the antilog of the geometric mean ± s.e.mean. The standard error of the mean for transformed data is not symmetrical so the larger value is presented. Data were fitted using Table Curve (Jandel Scientific) from which EC_{50} and IC_{50} values were determined from the logistic dose-response curves. In a number of experiments comparisons were made between control and treatment conditions using separate sets of wedges for each condition. These experiments are identified in the appropriate legends.

Drugs and chemicals

The following drugs and chemicals were used: 1S,3R-1-aminocyclopentane-1,3-dicarboxylic acid (Tocris Neuramin), DL-2-amino-3-phosphonopropionic acid, D,L-2-amino-4-phosphonobutyric acid, and *bis*-(o-aminophenoxy)-ethane-*N,N,N,N*, tetraacetic acid aminoethoxy (Calbiochem), arachidonic acid, *bis*-(o-aminophenoxy)-ethane-*N,N,N,N*, tetraacetic acid (BAPTA), 8-bromoadenosine 3′:5′ cyclic monophosphate (8-bromo-cyclic AMP), 8-bromoguanosine 3′:5′ cyclic monophosphate (8-bromo-cyclic GMP), glycine, 5-hydroxytryptamine bimaleate, kainic acid, myo-inositol, nifedipine, N-methyl-D-aspartate (NMDA), phenylephrine, phorbol diacetate, quisqualate, staurosporine and tetrodotoxin (Sigma), carbamylcholine chloride (carbachol; Mann Research Laboratories), 6-cyano-7-nitroquinoxaline-2,3-dione (gift from Ferrosan). Phorbol diacetate and staurosporine were dissolved in dimethyl sulphoxide (100%) and diluted in ACSF. Equivalent dilutions of dimethyl sulphoxide did not alter the NMDA depolarization. Drug concentrations were calculated as the salt. Stock solutions were kept frozen until use.

Results

mGluR receptor agonists selectively facilitate the NMDA depolarization

Quisqualate is an agonist at both the mGluR and the α-amino-3-hydroxy-5-methyl-isoxazole proprionic acid (AMPA) receptor (Murphy & Miller, 1988; Schoepp *et al.*, 1990a). Neurones in cortical wedges are depolarized by 10 μM quisqualate (Harrison & Simmonds, 1985; Rahman & Neuman, 1993a), probably reflecting activation of AMPA receptors. However, no depolarization of cortical wedges was observed when 1 μM quisqualate was perfused (data not shown). Nevertheless, 1 μM quisqualate reversibly facilitated the NMDA depolarization (192 ± 13%, $n=6$; $P<0.01$, Figure 1). Blockade of AMPA and kainate receptors with 4 μM CNQX in the presence of 20 μM glycine did not prevent facilitation by 2 μM quisqualate (158 ± 6%, $n=4$, $P<0.05$), although the NMDA depolarization alone was significantly reduced by this treatment (65 ± 6%, $n=5$, $P<0.01$). The smaller NMDA response may

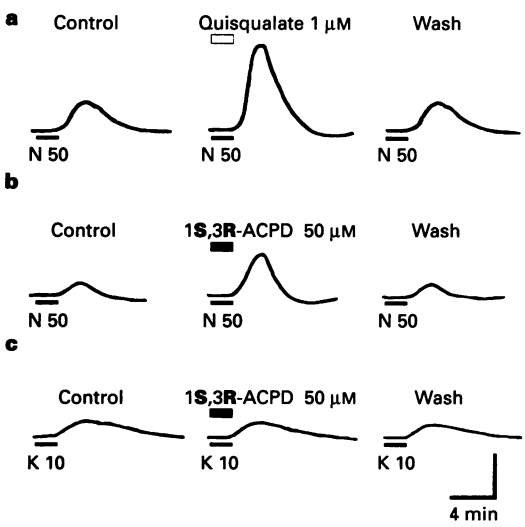

Figure 1 Metabotropic glutamate receptor agonists facilitate the depolarization of cortical neurones induced by NMDA: (a) Co-application of quisqualate (1 μM) with NMDA (N) enhances the NMDA depolarization. (b) 1S,3R-ACPD (50 μM) also facilitates the depolarization induced by NMDA. (c) The depolarization induced by kainate (K) is not facilitated by 1S,3R-ACPD (50 μM). The traces in this and subsequent figures were digitized from the chart recordings using a scanner. The duration of drug application is indicated by the bars above and below the traces. Drug concentrations are in μM. Data from 3 wedges. See text for abbreviations. The voltage calibration is 2 mV for (a) and (b) and 1 mV for (c).

have resulted from incomplete protection of the NMDA receptor by glycine or an action of glycine at strychnine-sensitive glycine receptors (Thomson, 1990).

Unlike 10 μM quisqualate, perfusion of 10 to 200 μM 1S,3R-ACPD did not result in a depolarization; however, 1S,3R-ACPD reversibly enhanced the NMDA depolarization (Figure 1). Substituting 10 μM kainic acid for NMDA did not result in facilitation by 50 μM 1S,3R-CPD (101±2%, $n=5$; Figure 1). As shown in Figure 2, facilitation of the NMDA response by 1S,3R-ACPD varied in a concentration-dependent manner with an EC_{50} of 16 μM.

In contrast to the facilitation induced by activation of 5-HT_{2A} receptors (Rahman & Neuman, 1993a), a second exposure to 1S,3R-ACPD 40 min after the first exposure did not reveal homologous desensitization (Figure 2). Absence of desensitization allowed a close examination of the facilitation produced by 1S,3R-ACPD. Concentration-response curves for NMDA were obtained with and without 1S,3R-ACPD. To allow ready comparison between wedges, the data from each wedge were normalized to the maximum response, i.e. the depolarization induced by 50 μM 1S,3R-ACPD plus 50 μM NMDA. When the facilitation was examined in this manner, 1S,3R-ACPD shifted the NMDA concentration-response curve to the left (Figure 3). Dose-ratios for 25 and 50 μM 1S,3R-ACPD were 0.83 and 0.63 respectively and were linear for the concentration range employed (0 to 50 μM ACPD; $r^2=0.998$; F statistic=456; Figure 3 insert). Changes in the maximum response were not ascertained due to irreversible loss of the NMDA response following perfusion with elevated concentrations of NMDA (data not shown; see also Harrison & Simmonds, 1985).

Antagonism of the 1S,3R-ACPD facilitation

D,L-AP3 and D,L-AP4 antagonize metabotropic glutamate receptor-mediated phosphoinositide hydrolysis (Schoepp *et al.*, 1990a,b). Bath application of D,L-AP3 (0.1 to 50 μM) and D,L-AP4 (1 to 100 μM) did not alter the NMDA depolarization (98±2%, $n=10$). However, both agents reduced 1S,3R-ACPD facilitation of the NMDA response in a concentration-dependent manner (Figure 4a). The IC_{50} values for D,L-AP3 and D,L-AP4 were 0.24 and 4.4 μM respectively. Varying the concentration of 1S,3R-ACPD in the presence of D,L-AP3 (Figure 4b) revealed that the antagonism was not competitive. Similar observations were made with AP4 (data not shown).

Heterologous desensitization of 1S,3R-ACPD facilitation

Prolonged perfusion with 3 μM phorbol diacetate, an activator of protein kinase C (Agopyan & Agopyan, 1991), did not significantly depress the 1S,3R-ACPD facilitation (Table 1) compared to the same treatment in the presence of 10 nM staurosporine, a nonselective inhibitor of protein kinase C (Ruegg & Burgess, 1989; Tamaoki *et al.*, 1986).

Facilitation of the NMDA response induced by carbachol and phenylephrine exhibits heterologous desensitization following exposure to 100 μM 5-HT (Rahman & Neuman, 1993b,c). This probably reflects depletion of substrate for phosphoinositide hydrolysis (Fain & Berridge, 1979; Rahman & Neuman, 1993c). As shown in Table 2, the 1S,3R-ACPD induced facilitation was also eliminated by exposure to 100 μM 5-HT. On the other hand, perfusion of wedges with 10 mM *myo*-inositol, which enhances availability of the substrate for agonist-induced phosphoinositide hydrolysis (Fain & Berridge, 1979; Pontzer *et al.*, 1992; Rahman & Neuman, 1993c), resulted in a significant potentiation of the 1S,3R-ACPD-induced facilitation (Table 2).

Besides exhibiting coupling to phospholipase C, mGluRs also couple to phospholipase A_2, adenylate cyclase, and guanylate cyclase (Nakanishi, 1994; Aramori & Nakanishi, 1992). Moreover, arachidonic acid and cyclic AMP have been shown to enhance NMDA responses (Miller *et al.*, 1992; Cerne *et al.*, 1993). However, application of arachidonic acid, 8-bromo-cyclic AMP or 8-bromo-cyclic GMP, permeable analogues of cyclic AMP and cyclic GMP, failed to facilitate the NMDA depolarization (Table 3).

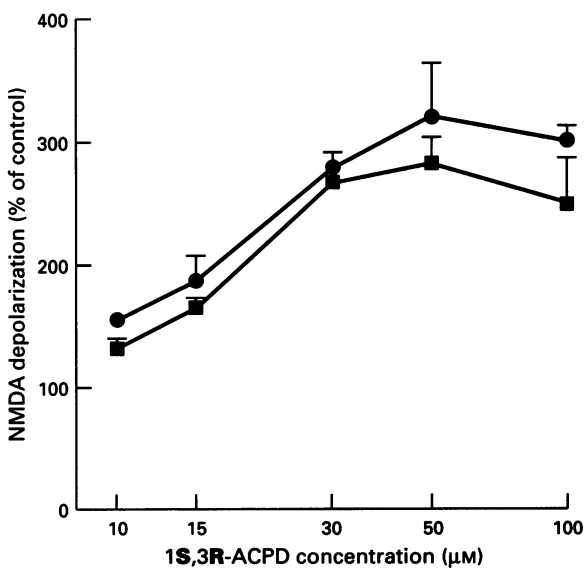

Figure 2 Concentration-dependent facilitation of the NMDA depolarization induced by 1S,3R-ACPD. Each wedge was exposed twice to the same concentration of 1S,3R-ACPD plus 50 μM NMDA separated by a 20 min interval: (●) and (■) represent the mean± s.e.mean for the first and the second exposure respectively. Note the small, nonsignificant reduction in the facilitation on second exposure; 5 to 8 wedges were used at each concentration.

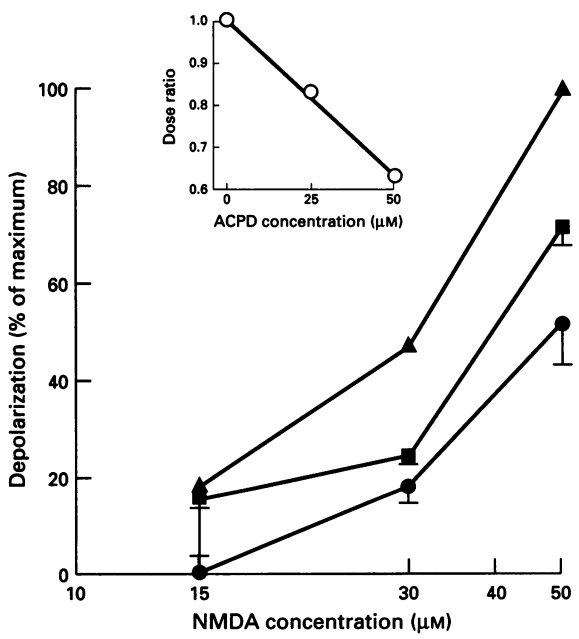

Figure 3 1S,3R-ACPD shifts the NMDA concentration-response curve to the left. Concentration-response curves are shown for NMDA alone (●) and in the presence of 25 μM (■), or 50 μM (▲) 1S,3R-ACPD. Responses for each wedge were normalized to the largest response (50 μM NMDA plus 50 μM 1S,3R-ACPD). Values are the mean±s.e.mean from 3 separate experiments. Insert illustrates the linear relationship between the dose-ratio, calculated at 50% of the maximum response, and the 1S,3R-ACPD concentration.

Ca^{2+}-dependence of the 1S,3R-ACPD mediated facilitation

A rise in intracellular Ca^{2+} has been proposed to underlie facilitation of the NMDA response induced by 5-HT_{2A} receptor activation in cortical neurones (Rahman & Neuman, 1993d; Neuman & Rahman, 1993) and by muscarinic AChR activation in hippocampal neurones (Markram & Segal, 1992). To investigate the requirement for external Ca^{2+}, 1S,3R-ACPD was applied following perfusion (40–60 min) with nominally Ca^{2+}-free ACSF, i.e. Ca^{2+} was not added to the ACSF. This treatment did not reduce the 1S,3R-ACPD or carbachol-induced facilitation of the NMDA response (Figure 5 and Table 4) or the NMDA response itself. However, the phenylephrine-induced facilitation was abolished by such treatment (Figure 5 and Table 4). Addition of 100 μM BAPTA to nominally Ca^{2+}-free ACSF induced run-down of the NMDA response and eliminated the 1S,3R-ACPD-mediated facilitation (75±7%, $n=4$, $P<0.05$; Figure 6a) over the course of 1 h.

Ca^{2+} influx through nifedipine-sensitive Ca^{2+} channels is necessary for the facilitation induced by 5-HT and thapsigargin (Rahman & Neuman, 1993d; Neuman & Rahman, 1993). Although 10 μM nifedipine significantly reduced the NMDA response (73±15%, $n=6$; $P<0.05$), there remained a significant facilitation induced by 30 μM 1S,3R-ACPD (221±8%, $n=4$; $P<0.05$; Figure 6b).

Finally, the importance of intracellular Ca^{2+} to the facilitation was examined by perfusion with 50 μM BAPTA-AM. BAPTA-AM chelates intracellular Ca^{2+} following its conversion to BAPTA by intracellular enzymes, but not extracellular Ca^{2+} (Niesen *et al.*, 1991). Perfusion of BAPTA-AM in nominally Ca^{2+}-free ACSF eliminated the 1S,3R-ACPD-induced facilitation (Figure 6c) but did not alter the NMDA-induced depolarization (97±2.5%, $n=5$).

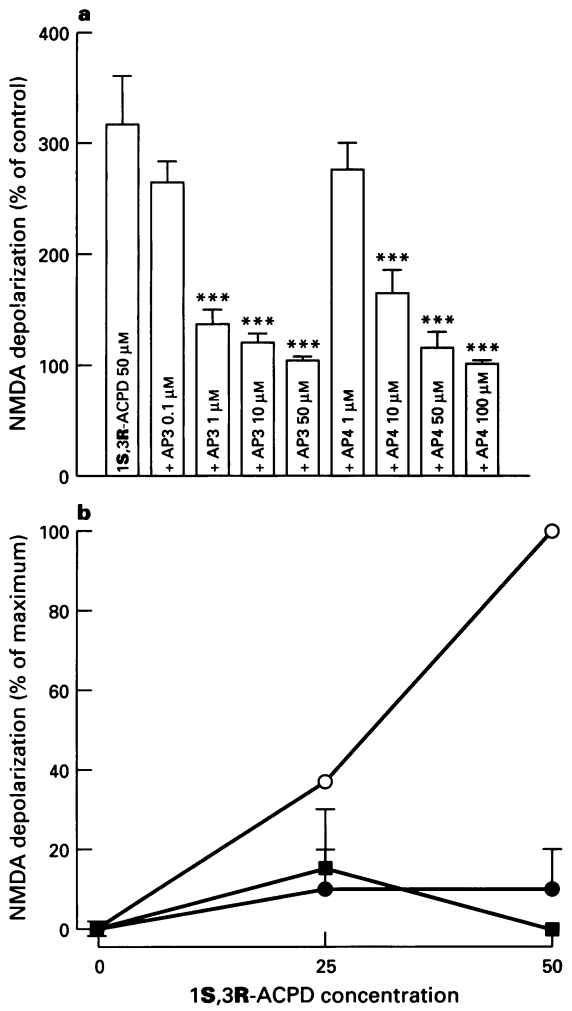

Figure 4 D,L-AP3 and D,L-AP4 antagonize the 1S,3R-ACPD facilitation: (a) D,L-AP3 and DL-AP4 reduce the 1S,3R-ACPD (50 μM) facilitation in a concentration-dependent manner. NMDA depolarization in the presence of each concentration of antagonist was used as control for that concentration; 4 to 6 wedges were used at each antagonist concentration. ***$P<0.001$, 1S,3R-ACPD vs 1S,3R-ACPD plus antagonist. (b) Concentration-response curves are shown for 1S,3R-ACPD in the absence (○) and presence of D,L-AP3 1 μM (●) and 10 μM (■). Values are the mean±s.e.mean from 5 separate experiments.

Discussion

The present experiments demonstrate that application of mGluR agonists facilitate the depolarization of cortical neurones induced by NMDA, but not by kainate. The facilitation was manifest as a 1S,3R-ACPD concentration-dependent shift of the NMDA concentration-response curve to the left. Moreover, the facilitation was observed at concentrations of quisqualate and 1S,3R-ACPD at which neither agonist produced an overt depolarization on its own. Although not ex-

Table 1 Phorbol diacetate and staurosporine fail to alter the 1S,3R-ACPD-induced facilitation

Treatment	*NMDA depolarization* (% of control)
1S,3R-ACPD (30 μM)	266±24% ($n=8$)
+Phorbol diacetate (3 μM)	228±21% ($n=5$)
+Staurosporine (10 nM)	271±19% ($n=5$)

No significant differences were found by analysis of variance. Control and treatments are from different groups of wedges.

Table 2 5-HT desensitizes and *myo*-inositol potentiates the 1S,3R-ACPD response

Treatment	*NMDA depolarization* (% of control)
1S,3R-ACPD (50 μM)	299±18% ($n=6$)
1S,3R-ACPD after 5-HT[a]	93±7% ($n=4$)
1S,3R-ACPD (50 μM)	298±19% ($n=5$)
+10 mM *myo*-inositol[b]	379±12% ($n=4$)[c]

[a]1S,3R-ACPD (50 μM) was applied 40 min after 5-HT 100 μM. [b]*Myo*-inositol was added to the ACSF and perfused throughout the experiment. [c]$P<0.05$. Separate sets of wedges were used for control and treatment conditions.

Table 3 Arachidonic acid, 8-bromo-cyclic AMP, and 8-bromo-cyclic GMP fail to mimic the facilitation induced by 1S,3R-ACPD

Treatment	*NMDA depolarization* (% of control)
Arachidonic acid 10 μM	98±2% ($n=5$)
8-bromo-cyclic AMP 20 μM	91±5% ($n=4$)
8-bromo-cyclic GMP 20 μM	98±16% ($n=4$)

Each agent was co-applied with 50 μM NMDA for 2 min. Separate wedges were used for each agent tested.

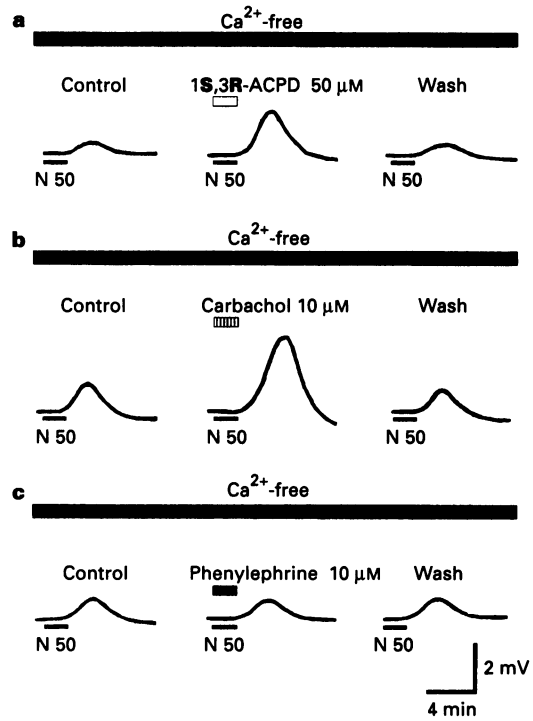

Figure 5 Effects of perfusing nominally Ca^{2+}-free ACSF on the facilitation induced by 1S,3R-ACPD, carbachol, and phenylephrine: (a) the facilitation induced by 1S,3R-ACPD is not reduced in Ca^{2+}-free ACSF; (b) the facilitation induced by carbachol is not reduced in Ca^{2+}-free ACSF; (c) the facilitation induced by phenylephrine is eliminated during perfusion with Ca^{2+}-free ACSF. Data from three wedges.

Table 4 Ca^{2+}-dependence of the 1S,3R-ACPD, carbachol and phenylephrine-induced facilitation

Treatment	*NMDA depolarization* (% of control) Ca^{2+}	Ca^{2+}-*free*
1S,3R-ACPD (50 μM)	266 ± 24%	252 ± 23% (n = 6)
Carbachol (10 μM)	260 ± 11%	266 ± 24% (n = 4)
Phenylephrine (10 μM)	230 ± 7%	97 ± 4% (n = 4)***

***$P < 0.001$, (unpaired t test), Ca^{2+} vs Ca^{2+}-free treatment.

amined in detail, the order of potency with respect to inducing facilitation of the NMDA response appears to be quisqualate > 1S,3R-ACPD. 1S,3R-ACPD is a selective agonist at mGluR (Irving *et al.*, 1990; Palmer *et al.*, 1989), whereas quisqualate is an agonist at both AMPA and mGluR (Murphy & Miller, 1988). However, in the presence of the AMPA/kainate antagonist CNQX (Honore *et al.*, 1987; Neuman *et al.*, 1988), we observed that quisqualate remained effective in facilitating the NMDA response. The smaller than expected quisqualate-induced facilitation present during perfusion of CNQX doubtless reflects loss of a small contribution from the AMPA receptor activation. Thus, quisqualate enhances formation of inositol polyphosphates along with Ca^{2+} entry and mobilization, in part, through its action on AMPA receptors (Baird *et al.*, 1991; Murphy & Miller, 1988).

The facilitatory response induced by 1S,3R-ACPD was antagonized noncompetitively by both D,L-AP3 and D,L-AP4. Neither antagonist altered the depolarization induced by NMDA in the absence of 1S,3R-ACPD indicating that, under the conditions of the present experiments, there was insufficient glutamate/aspartate release in the wedges to activate mGluR and facilitate the NMDA response. This is not the case for

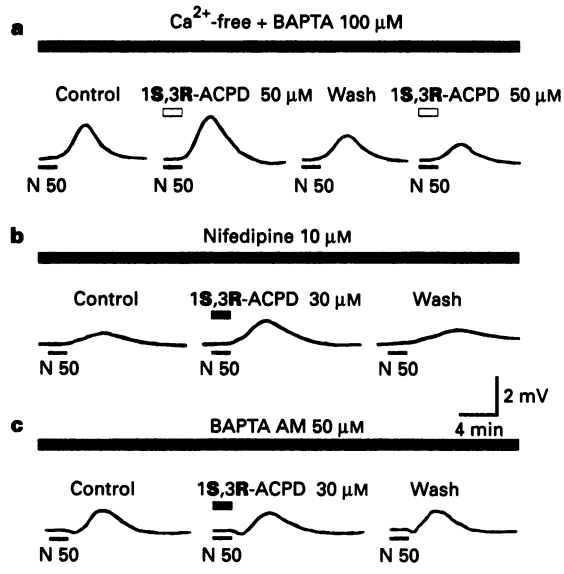

Figure 6 Effect of Ca^{2+}-free ACSF plus BAPTA, nifedipine, and BAPTA-AM on the 1S,3R-ACPD facilitation: (a) perfusion of 100 μM BAPTA in nominally Ca^{2+}-free ACSF reduced then eliminated the 1S,3R-ACPD (50 μM)-induced facilitation. Note, the NMDA depolarization was also reduced by this treatment. (b) Nifedipine (10 μM) did not block the 1S.3R-ACPD (30 μM)-induced facilitation. (c) Perfusion of 50 μM BAPTA-AM eliminated the 1S,3R-ACPD (50 μM)-induced facilitation. Data from three wedges.

other G-protein coupled receptors in cortical wedges which induce facilitation. Thus, scopolamine and prazosin, but not ritanserin, significantly reduce the amplitude of the NMDA depolarization (Rahman & Neuman, 1993a). Surprisingly, the IC_{50} values for D,L-AP3 and D,L-AP4 antagonism of the 1S,3R-ACPD facilitation are over two orders of magnitude lower than previously reported for mGluR mediated responses (Schoepp *et al.*, 1990a,b; Schoepp & Conn, 1993). Whether this reflects the nature of the response examined, i.e. phosphoinositide hydrolysis versus facilitation of the NMDA depolarization, is not clear. D,L-AP3 and D,L-AP4 are reportedly weak partial agonists (Schoepp *et al.*, 1990a,b; Schoepp & Conn, 1993). However, within the concentration-range of D,L-AP3 and D,L-AP4 examined, no partial agonism was observed with respect to facilitating the NMDA response.

In keeping with observations on hippocampal neurones (Aniksztejn *et al.*, 1991; 1992; Harvey *et al.*, 1991; Harvey & Collingridge, 1993), spinal neurones (Bleakman *et al.*, 1992) and *Xenopus* oocytes (Kelso *et al.*, 1992), the above observations are compatible with quisqualate and 1S,3R-ACPD acting at mGluR to facilitate the NMDA depolarization. Since the facilitation is present in nominally Ca^{2+}-free ACSF containing TTX, presumably the mGluR mediating the facilitation are located on projection neurones, i.e. neurones with axons that pass through the grease seal (Rahman & Neuman, 1993a; Rahman *et al.*, 1995). MGluR couple through G-proteins to ion channels and a variety of second messenger systems including phospholipase C and D, adenylate cyclase (both positive and negative coupling), guanylate cyclase and phospholipase A_2 (Nakanishi, 1994; Schoepp & Conn, 1993). Activation of phospholipase C and adenylate cyclase as well as application of arachidonic acid has been shown to result in facilitation of the NMDA response in different brain regions (Markram & Segal, 1992; Rahman & Neuman, 1993b; Cerne *et al.*, 1993; Miller *et al.*, 1992). However, our observations demonstrate that in cortical wedges arachidonic acid, 8-bromo-cyclic AMP, and 8-bromo-cyclic GMP are unable to substitute for 1S,3R-ACPD in evoking facilitation. These findings are in agreement with findings from CA1 hippocampal neurones

(Harvey & Collingridge, 1993). Involvement of phospholipase D is unlikely, since L-AP3 is a full agonist at mGluR which couple to phospholipase D (Boss & Conn, 1992).

On the positive side, *myo*-inositol potentiates the 1S,3R-ACPD facilitation, whereas prior exposure to 5-HT results in heterologous desensitization of the 1S,3R-ACPD-induced facilitation. Both observations are consistent with the hypothesis that activation of phospholipase C is critical for facilitating the NMDA response in cortical neurones (Rahman & Neuman, 1993a). *Myo*-inositol is a precursor of phosphoinositide 4,5-biphosphate, the substrate for phospholipase C (Berridge, 1987). Brain slices incubated in ACSF exhibit lower inositol levels than fresh brain tissue unless 10 mM *myo*-inositol is present (Sherman *et al.*, 1986). Enhancing substrate availability by perfusing hippocampal slices with *myo*-inositol results in greater inositol phosphate formation following carbachol-stimulated phosphoinositide hydrolysis (Pontzer *et al.*, 1992). As with the 1S,3R-ACPD facilitation, the facilitation mediated by 5-HT_{2A} receptors, α_1-adrenoceptors and muscarinic AChR, all of which employ phospholipase C for signal transduction at cortical neurones (Berridge, 1993), is potentiated by *myo*-inositol (Rahman & Neuman, 1993c). On the other hand, stimulation of phospholipase C with high concentrations of an agonist such as 5-HT, thereby reducing phosphoinositide substrate availability in the process (Fain & Berridge, 1979), results in heterologous desensitization of the carbachol and phenylephrine facilitation at cortical neurones (Rahman & Neuman, 1993b). This heterologous desensitization is prevented by addition of 10 mM *myo*-inositol to the perfusion medium (Rahman & Neuman, 1993c). Thus, the potentiation by *myo*-inositol and the heterologous desensitization induced by 5-HT are entirely consistent with the 1S,3R-ACPD facilitation being a consequence of stimulating phospholipase C. Moreover, the EC_{50} value for the 1S,3R-ACPD facilitation (16 μM) is in good agreement with the EC_{50} values reported for 1S,3R-ACPD-stimulated phosphoinositide hydrolysis in primary cerebrocortical cultures (15 μM; Birrell & Marcoux, 1993) and neonatal rat cerebral cortex slices (16 μM; Challiss *et al.*, 1994). Finally, D,L-AP3 and D,L-AP4 non-competitively inhibit mGluR induced phosphoinositide hydrolysis in cortical tissue (Schoepp *et al.*, 1990a,b) and it seems reasonable to conclude that these antagonists behave in a similar manner in the present study.

Of the mGluR so far identified, only the $mGluR_1$ and the $mGluR_5$ subtypes couple to phospholipase C (Abe *et al.*, 1992; Aramori & Nakanishi, 1992; Pin & Duvosin, 1995; Schoepp & Conn, 1993; Tanabe *et al.*, 1992). The $mGluR_5$ subtype is abundantly present in the cerebral cortex (Abe *et al.*, 1992; Minakami *et al.*, 1993; Shigemoto *et al.*, 1993), whereas the $mGluR_1$ subtype is found at a lower level (Shigemoto *et al.*, 1992). Agonist potency (quisqualate > 1S,3R-ACPD) for both receptors is similar to that observed in the present experiments (Abe *et al.*, 1992; Aramori & Nakanishi, 1992). Chinese hamster ovary cells transfected with $mGluR_1$ or $mGluR_5$ are insensitive to L-AP3, suggesting that L-AP3 acts at a site separate from the mGluR or that another mGluR, which remains to be identified, couples positively to phospholipase C. Since our observations cannot distinguish between these possibilities, further studies are required to characterize properly the mGluR mediating the facilitation.

Phosphoinositide specific phospholipase C plays a crucial role in initiating the surface mediated signal transduction by generating the second messenger molecules, diacylglycerol, and inositol 1,4,5-trisphosphate (Berridge, 1987; Wojcikiewicz *et al.*, 1993). Results accumulated during the last few years clearly indicate that there are a number of distinct variants of phospholipase C, which have been purified, cloned and sequenced (Rhee *et al.*, 1991). Carbachol, noradrenaline and 5-HT stimulated phosphoinositide hydrolysis in cortical slices exhibit varied requirements for external Ca^{2+}. Thus, the carbachol-evoked response is relatively insensitive to the concentration of external Ca^{2+} in contrast to the 5-HT and noradrenaline induced phosphoinositide hydrolysis (Kendall & Nahorski, 1984). Furthermore, 1S,3R-ACPD has been shown to stimulate phosphoinositide hydrolysis that is independent of extracellular Ca^{2+} (Birrell & Marcoux, 1993; Challis *et al.*, 1994). The distinct Ca^{2+} sensitivity associated with mGluR, 5-HT_{2A} receptors, α_1-adrenoceptors and muscarinic AChR with respect to the NMDA facilitation presumably indicates variability in the phospholipase C subtypes mediating signal transduction by these cell surface receptors rather than the Ca^{2+} requirements for the facilitation differing in a significant manner between receptors. Clearly, this remains to be established.

Depolarization of cortical pyramidal neurones with NMDA opens voltage-dependent Ca^{2+} channels (Markram & Sackman, 1994), including the L-type Ca^{2+} channel (Sayer *et al.*, 1992), thereby enhancing the concentration of intracellular Ca^{2+}. Nifedipine reduces the amplitude of the NMDA depolarization as does reduction of the concentration of external Ca^{2+} by perfusion of nominally Ca^{2+}-free ACSF along with BAPTA, suggesting that Ca^{2+} entry through L-type channels facilitates the NMDA response. However, the facilitation induced by 1S,3R-ACPD is not dependent on Ca^{2+} entry through L-type Ca^{2+} channels. 1S,3R-ACPD acting at mGluR results in blockade of L-type Ca^{2+} channels on cortical pyramidal neurones and occludes blockade of these channels by nifedipine (Sayer *et al.*, 1992). In keeping with these observations, nifedipine does not reduce the 1S,3R-ACPD facilitation. In this regard, the facilitation mediated by 5-HT_{2A} receptors is quite distinct in that the facilitation induced by 5-HT is reduced in a concentration-dependent manner by nifedipine (Rahman & Neuman, 1993d; Neuman & Rahman, 1993).

The effectiveness of BAPTA-AM in eliminating the 1S,3R-ACPD facilitation suggests that an intracellular Ca^{2+}-dependent process is necessary for the facilitation. 1S,3R-ACPD induces a rise in the concentration of intracellular Ca^{2+} by generating inositol 1,4,5-trisphosphate, which then acts to release Ca^{2+} from the smooth endoplasmic reticulum (Berridge, 1993; Irving *et al.*, 1990). It is proposed that the inositol 1,4,5-trisphosphate-evoked release of Ca^{2+} is responsible for the facilitation and that BAPTA-AM acts to buffer this rise in Ca^{2+}, thus preventing the facilitation. BAPTA-AM also blocks facilitation of the NMDA response induced by thapsigargin (Rahman & Neuman, 1993d; Neuman & Rahman, 1993), which raises the concentration of intracellular Ca^{2+} by a mechanism independent of inositol trisphosphate formation (Thastrup *et al.*, 1990). Our observations are consistent with those from hippocampal neurones in which the muscarinic AChR-mediated facilitation is blocked by BAPTA applied intracellularly through the recording electrode (Markram & Segal, 1992).

No evidence was obtained in the present experiments that the facilitation of the NMDA depolarization involves the other arm of the phosphoinositide pathway, i.e. diacylglycerol-stimulated protein kinase C. Phorbol diacetate did not occlude the 1S,3R-ACPD facilitation and staurosporine, an inhibitor of protein kinase C (Ruegg & Burgess, 1989; Tamaoki *et al.*, 1986), did not block the facilitation. This is in keeping with the 1S,3R-ACPD-induced facilitation of the NMDA response in the cerebellum (Kinney & Slater, 1993), but differs from reports on the 1S,3R-ACPD facilitation in hippocampal neurones and *Xenopus* oocytes (Aniksztejn *et al.*, 1991; 1992; Kelso *et al.*, 1992; but see Harvey & Collingridge, 1993).

An interesting difference between the present findings and previous results with 5-HT_{2A}, α_1-adrenoceptor, and muscarinic AChR agonists is the apparent lack of homologous desensitization of mGluR (Rahman & Neuman, 1993b). Even at concentrations of 1S,3R-ACPD which induce a substantial facilitation, the magnitude of the facilitation was not reduced on second exposure. In the case of 5-HT, homologous desensitization results in part from activating protein kinase C (Rahman & Neuman, 1993b). Despite reports of protein kinase C regulating mGluR responses (Aniksztejn *et al.*, 1991; 1992; Harvey & Collingridge, 1993; Manzoni *et al.*, 1990; 1991), no evidence for potentiation of the facilitation by in-

hibition of protein kinase C was observed. Thus, at cortical neurones the feedback regulation of metabotropic receptors by protein kinase C appears to be absent, at least with respect to facilitation of the NMDA response.

In cortical wedges, limited substrate availability also contributes to homologous desensitization following activation of 5-HT_{2A} receptors, α_1-adrenoceptors and muscarinic AChR (Rahman & Neuman, 1993c). Thus, enhancing substrate availability by perfusing *myo*-inositol reduces homologous desensitization at 5-HT_{2A} receptors and eliminates homologous desensitization at α_1-adrenoceptors and muscarinic AChR. In contrast, the mGluR mediating facilitation of the NMDA response appears to be less sensitive to substrate concentration. In hippocampal slices, prolonged exposure to 10 μM 1S,3R-ACPD does not reduce the NMDA facilitation (Harvey & Collingridge, 1993), whereas muscarinic AChR-mediated responses, thought to involve phosphoinositide hydrolysis, undergo desensitization that is reduced by *myo*-inositol (Pontzer *et al.*, 1992). A number of factors including receptor density, receptor distribution, and the extent of coupling to ion channels and other second messengers could influence substrate utilization and therefore might account for the discrepancy with regard to homologous desensitization between 1S,3R-ACPD on the one hand and carbachol, phenylephrine and 5-HT on the other.

In many respects the present findings in cortical wedges are similar to observations on the 1S,3R-ACPD-induced facilitation in hippocampal slices obtained with comparable recording techniques by Harvey & Collingridge (1993). Thus, the facilitation in the hippocampus is insensitive to staurosporine, is not mimicked by arachidonic acid or 8-bromo cyclic AMP and does not exhibit homologous desensitization. In contrast to the present observations, however, the facilitation in the hippocampus is eliminated during perfusion with Ca^{2+}-free medium or phorbol diacetate. The apparent variance with regard to Ca^{2+} sensitivity may be accounted for by methodological differences. Harvey & Collingridge (1993) employed thinner tissue slices and prolonged perfusion of 1S,3R-ACPD, both of which could contribute to more rapid lowering of cellular Ca^{2+} during perfusion with Ca^{2+}-free medium. Moreover, the difference in Ca^{2+} sensitivity is not qualitative since lowering the Ca^{2+} concentration in cortical wedges by perfusing BAPTA also eliminates the 1S,3R-ACPD-induced facilitation. The sensitivity of the 1S,3R-ACPD facilitation to phorbol diacetate in the hippocampus may indicate differences between the cortex and the hippocampus with respect to receptor regulation or the mGluR subtype mediating the facilitation. The suggestion that the mGluR responsible for the facilitation in the hippocampus is regulated by feedback inhibition through protein kinase C (Harvey & Collingridge, 1993) appears unlikely in the absence of homologous desensitization or enhancement of the facilitation by staurosporine (see Rahman & Neuman, 1993b).

In conclusion, the present findings suggest that the 1S,3R-ACPD facilitation of the NMDA depolarization at cortical projection neurones is dependent on phospholipase C signal transduction and a rise in intracellular Ca^{2+}. In this regard, the mGluR-induced facilitation closely resembles facilitation of the NMDA response induced by other membrane surface receptors which couple positively to phospholipase C on these neurones.

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