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Actions of agonists of metabotropic glutamate receptors on synaptic transmission and transmitter release in the olfactory cortex

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1 The effects of agonists of metabotropic glutamate receptors on the evoked N-wave complex in slices of mouse olfactory cortex have been studied: most experiments were carried out using slices perfused with Mg^{2+}-free solution to which 10 µM of either 6,7-dinitroquinoxaline-2,3-dione or 6-cyano-7-nitroquinoxaline-2,3-dione was applied.

2 Following agonist washout, a slowly developing, long lasting potentiation of the complex occurred which was confined to the N-methyl-D-aspartate (NMDA) receptor-mediated component of the potential. The relative agonist potencies were 1S,3**R**-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3**R**-ACPD, 5–250 µM) = quisqualate (5–50 µM) > 1**RS**,3**RS**-*cis*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD, 25–1000 µM) > L-glutamate (0.25–2.5 mM); NMDA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and L-aspartate were inactive.

3 Potentiation of the NMDA receptor-mediated component by 1S,3**R**-ACPD (0.1 mM) was non-competitively antagonised by S-(+)- but not **R**-(−)-2-amino-3-phosphonopropionate (AP3, 0.125 mM), equally by D-(−) and L-(+)-2-amino-4-phosphonobutyrate (0.25 mM) and also by the protein kinase C inhibitors sphingosine, (25 µM), sangivamycin (25 µM) and 5-(isoquinolinylsulphonyl)-3-methylpiperazine (50 µM).

4 In a series of input-output experiments, 1S,3**R**-ACPD (0.1 mM) reversibly reduced the latency to peak of the NMDA receptor-mediated component at submaximal stimulus intensities, an effect blocked by S-(+)-AP3 (0.125 mM). On agonist washout, there was an increase in the area of the NMDA receptor-mediated component over all stimulus intensities, an effect blocked by the inhibitors of protein kinase C and by S-(+)-AP3 (0.125 mM). 4-β-Phorbol-12,13-diacetate (2.5 µM) also potentiated the component, an action inhibited by protein kinase C inhibitors but not by S-(+)-AP3.

5 1S,3**R**-ACPD (0.1 mM) had no significant effect on postsynaptic responses evoked by NMDA, AMPA and kainate, but significantly reversed a partial antagonism of NMDA responses produced by 7-chlorokynurenate (2.5 µM).

6 The K^+-evoked release of glycine was selectively and significantly increased in the presence of 0.1 mM 1S,3**R**-ACPD (antagonized by 0.125 mM S-(+)-AP3) whereas following agonist washout, release of glycine fell to control levels but there was a significant increase in release of aspartate (antagonized by 25 µM sangivamycin and 0.125 mM S-(+)-AP3).

7 It is concluded that metabotropic glutamate receptors mediate (i) a reduction in the latency of the NMDA receptor-mediated component of potentials by a mechanism that is independent of protein kinase C but which may depend on increased glycine release and (ii) a long lasting increase in the total area of the potential by increasing transmitter (possibly aspartate) release by a mechanism that is protein kinase C-dependent.

Keywords: Metabotropic glutamate receptors; amino acid transmitters, NMDA receptors; olfactory cortex

Introduction

The excitatory neurotransmitter, glutamate, mediates its central effects by activation of two major classes of amino acid receptor, the co-called ionotropic and metabotropic receptors (Collingridge & Lester, 1989; Monaghan *et al.*, 1989). The ionotropic receptors, which are gated ion channels, are named after their selective agonists and include the N-methyl-D-aspartate (NMDA) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) subtypes. In contrast, metabotropic glutamate receptors, for which 1S,3**R**-1-aminocyclopentane-1,3-dicarboxylic acid (1S,3**R**-ACPD) is a selective agonist (Irving *et al.*, 1990; Schoepp *et al.*, 1991), are linked by guanine nucleotide binding proteins to their effectors. At least in some cases, activation of the receptors triggers the formation of the second messengers *myo*-inositol-1,4,5-trisphosphate (Challis *et al.*, 1988; Baird *et al.*, 1991) and, presumbly, diacylglycerol which, in turn, mobilises intracellular Ca^{2+} (Murphy & Miller, 1989; 1990) and activates protein kinase C (Manzoni *et al.*, 1990), respectively.

Although the functions of metabotropic glutamate receptors are poorly understood, there is growing evidence suggesting an important role in modulating neurotransmission. Presynaptically localized receptors in the hippocampus (Baskys & Malenka, 1991) and striatum (Lovinger, 1991) inhibit transmitter release when activated by 1**RS**,3**RS**-*cis*-1-aminocyclopentane-1,3,-dicarboxylic acid (ACPD). Metabotropic glutamate receptors are also found postsynaptically, for ACPD directly potentiates excitatory responses of hippocampal pyramidal cells (Desai & Conn, 1991), probably by inhibition of a Ca^{2+}-activated K^+ conductance (Stratton *et al.*, 1989; 1990). In the cerebellum, ACPD causes a transient depolarization of Purkinje cells superimposed on a long last-

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ing depression of transmission (Crepel *et al.*, 1991) and a role for metabotropic glutamate receptors in hippocampal long term potentiation has also been proposed (McGuiness *et al.*, 1991a; Otani & Ben-Ari, 1991; Zheng & Gallagher, 1992).

In spite of this growing list there are few studies which define whether the effects of metabotropic glutamate receptor activation are mediated primarily by release of intracellular Ca^{2+} or by activation of protein kinase C. Charpak *et al.* (1990) showed that inhibition of hippocampal K^+ conductances by agonists of metabotropic glutamate receptors was independent of any changes in intracellular Ca^{2+}, suggesting a primary rôle for protein kinase C. In contrast, the oscillatory currents evoked by activation of metabotropic receptors in *Xenopus* oocytes injected with rat brain mRNA (Sugiyama *et al.*, 1987) are typical of responses caused by inositol phosphate-evoked Ca^{2+} release; a similar mechanism may underlie the enhancement of hippocampal long term potentiation by ACPD, a phenomenon which is unaffected by co-application of the protein kinase C inhibitor, sphingosine (McGuiness *et al.*, 1991b). The study presented here was undertaken to investigate the roles and mechanism by which metabotropic glutamate receptors modulate excitatory amino acid-mediated transmission in the mouse olfactory cortex. A preliminary account of this work has been published elsewhere (Collins, 1992).

Methods

Field potential experiments

Surface slices of olfactory cortex, nominal thickness of 300 μm, were prepared from freshly killed, adult male white mice and preincubated and perfused at room temperature in a solution which was continuously gassed with 95% O_2 and 5% CO_2 and which contained (mM): NaCl 118, $NaHCO_3$ 25, D-glucose 11, $CaCl_2$ 2.5, KCl 2.1 and KH_2PO_4 0.9 (Mg^{2+}-free solution). In some experiments, $MgSO_4$ (1 mM) was present. Surface, extracellular field potentials were evoked by stimulation of the lateral olfactory tracts of slices (0.2 Hz, 100 μs, various voltages) and recorded with techniques described elsewhere (Pickles & Simmonds, 1976; Collins, 1991). At the end of each experiment, a mixture of 25 μM D-(−)-2-amino-5-phosphonopentanoate (AP5) and either 10 μM 6,7-dinitroquinoxaline-2,3-dione (DNQX) or 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) was applied to block NMDA and AMPA/kainate receptor-mediated potentials, respectively. The residual waveform was subtracted from the potential under investigation using a Gould 260 digital waveform processor and the resultant potential usually quantified by measuring its total area. Agonist drug solutions were applied to the pial surface of slices at a rate of 1 drop every min for a sufficient time period for a plateau effect to be produced whereas antagonist drugs were applied throughout an experiment. Most agonist drug effects have been expressed as percentage changes in the area of potentials measured 15 min after agonist washout.

In some experiments, an analysis of the differential effects of 1S,3R-ACPD on the NMDA and AMPA/kainate receptor mediated components of field potentials was made (Collins, 1991). Recordings were made until the potentials were of constant area and peak amplitude. Drugs were then applied in the sequence: AP5 (25 μM for 6 min), drug-free solution (30–40 min for recovery), 1S,3R-ACPD (0.1 mM for 15 min), drug-free solution for 15 min, AP5 (25 μM for 6 min) followed immediately by the simultaneous application of CNQX or DNQX (10 μM for 15 min) and the NMDA and AMPA/kainate components isolated by the digital subtraction procedures described by Collins (1991). The experimental design assumed that the residual potential recorded in the presence of AP5 plus CNQX/DNQX was unchanged over the 100 min of a typical experiment; in control experiments, the area of this potential at the end of 100 min perfusion was 97.8 ± 2.8% (mean ± s.e.mean; $n = 4$) of that recorded at the beginning.

Input-output studies

Drug effects on the relationship between the stimulus input and evoked output of the NMDA receptor-mediated components of potentials were investigated in slices perfused with Mg^{2+}-free solution containing 25 μM picrotoxin and to which 10 μM CNQX was applied. Briefly, slices were stimulated with a range of voltages (see Collins & Richards, 1990 for details) and graphs plotted of (i) stimulus voltage *versus* amplitude of tract action potential, (ii) action potential amplitude *versus* area of potential and (iii) area of the potential *versus* latency to peak. The procedure was repeated 30 min later and if the graphs derived from the 2 runs could not be superimposed, the slice was discarded. The procedure was then repeated after application of 1S,3R-ACPD (15 min) or 4-β-phorbol-12,13-diacetate (PDAc, 30 min) and after drug washout for 15 min. When PDAc or sphingosine were tested, slices were perfused throughout with the respective solvents, dimethylsulphoxide (3.45 mM) and ethanol (18 mM). The effects of sangivamycin on adenosine receptors were abolished by including 0.3 mM theophylline in all solutions (Collins & Richards, 1990). The relationship between action potential amplitude and area of the NMDA receptor-mediated component of potentials was quantified by measuring the area under the curve of the graph, the upper limit of the action potential amplitude being defined by a vertical line crossed by all the curves.

Excitatory amino acid-evoked depolarizations

A series of experiments was carried out to ascertain the effect of 1S,3R-ACPD on responses evoked by single, submaximal concentrations of NMDA (50 μM), AMPA (5 μM) and kainate (50 μM). Slices were preincubated in Mg^{2+}-free solution and the d.c. potential across each slice monitored with extracellular electrodes (method of Brown & Galvan, 1979, modified by Collins & Surtees, 1986). Agonists were applied for 1 min every 30 min and depolarizations quantified by measuring peak deflections on a chart recorder. Responses were measured once they had stabilized, again at the end of a 10 min application of 1S,3R-ACPD (0.1 mM) and finally after perfusion of drug-free solution for 30 min.

Release experiments

The K^+-evoked release of endogenous aspartate, glutamate, glutamine, glycine and γ-aminobutyric acid (GABA) from cubes of olfactory cortex was monitored and assayed as described previously (Clark & Collins, 1976). Briefly, 40–50 mg wet wt. of 0.5 × 0.5 mm cubes of olfactory cortical tissues were perfused with Mg^{2+}-free solution at 35°C. Following perfusion for 15 min, 5 min samples were collected, the first 3 to monitor resting levels of amino acid release and a further 3 during which the tissue was continuously challenged with 50 mM KCl. The following protocols were used in 3 series of experiments: (1) 1S,3R-ACPD (0.1 mM) with or without S-(+)-2-amino-3-phosphonopropionate (S-(+)-AP3, 0.125 mM) present during the 15 min prior to sample collection; (2) 1S,3R-ACPD (0.1 mM) with or without S-(+)-AP3 (0.125 mM) present throughout the K^+ challenge; (3) S-(+)-AP3 (0.125 mM) alone present throughout the K^+; challenge. The amino acid contents of 5 μl aloquots of the samples were estimated by a double isotope microdansylation assay, full details of which are given by Clark & Collins (1976). The mean amino acid content of the 3 pre-K^+ samples was subtracted from each of the contents of the K^+ challenged samples and the differences summed to give the total increase in release.

Drugs and chemicals

Sphingosine, 5-(isoquinolinylsulphonyl)-3-methylpiperazine, AMPA, NMDA, kainate and PDAc were purchased from Sigma whilst ACPD, 1S,3**R**-ACPD, DNQX, CNQX, D-(−)- and L-(+)-2-amino-4-phosphonobutyrate and S-(+)- and **R**-(−)-AP3 were from Tocris. Sangivamycin was a gift from the Natural Products Branch, National Cancer Institute, U.S.A. Sphingosine was dissolved in absolute ethanol, DNQX and CNQX in dimethylsulphoxide and PDAc in 25% v/v dimethylsulphoxide and diluted with the perfusion medium to give the working solutions; appropriate solvent controls were carried out as necessary. All other drugs were directly dissolved in the perfusion solution. Radiochemicals used in the assay for endogenous amino acid release were purchased from Amersham International plc and included L-[2,3-^{3}H]-aspartic acid (707 TBq mmol^{-1}), L-[G-^{3}H]-glutamic acid (1.85 TBq mmol^{-1}), L-[G-^{3}H]-glutamine (1.59 TBq mmol^{-1}), L-[G-^{3}H]-glycine (610 GBq mmol^{-1}), 4-amino-*n*-[2,3-^{3}H]-butyric acid (2.26 TBq mmol^{-1}) and [N-methyl-^{14}C]-dansyl chloride (4.12 GBq mmol^{-1}).

Data analysis

All data are presented as means ± s.e.mean for *n* experiments. In the agonist concentration-effect studies, EC$_{25}$ values (agonist concentration causing a 25% increase in the area of the NMDA receptor-mediated component of the N-wave complex) were determined by non-linear regression analysis and comparison of the values was carried out by analysis of variance followed by Dunnett's *t* test. Comparison of other mean values was carried out using either a paired or unpaired Student *t* test as appropriate. The significance level was set at $P < 0.05$.

Results

Supramaximal stimulation of the lateral olfactory tract of slices perfused with Mg^{2+}-containing solution evoked a characteristic surface potential, the N-wave complex. When components insensitive to AP5 and DNQX/CNQX were subtracted, the complex consisted of a short latency peak, which reflected monosynaptic excitation of a population of pyramidal cells (Gilbey & Wooster, 1979; Haberly, 1985) and which was largely mediated by AMPA/kainate receptors (Figure 1a and Collins, 1991) followed by a longer duration, low amplitude potential which reflected a disynaptic excitation of other pyramidal cells (Haberly, 1985) and to which NMDA receptors made a major contribution (Figure 1a). In many slices, a positive-going population spike was superimposed on the complex (Figure 1a and Pickles & Simmonds, 1978). Polysynaptic events were abolished by the stimulus parameters employed (Collins, 1991).

During application of neither ACPD (0.5 mM, 5 slices) nor 1S,3**R**-ACPD (0.1 mM, 5 slices) was there any consistent change in the form of the complex. Following washout, there was a progressive increase in the total area of the complex which required 15 min fully to develop and was confined to the NMDA receptor-mediated component (Figure 1a). In slices perfused with Mg^{2+}-free solution, the NMDA receptor-mediated component was augmented, presumably due to relief of the Mg^{2+} block of the ion channel (Nowak *et al.*, 1984; Mayer & Westbrook, 1987). Both ACPD (0.5 mM, 4 slices) and 1S,3**R**-ACPD (0.1 mM, 5 slices) selectively enhanced the NMDA receptor-mediated component although, as before, this only occurred following drug washout (Figure 1b). The maximum increase was achieved only with a drug contact time of 15 min followed by application of drug-free solution for a further 15 min although the effect persisted for at least 1 h (not shown). Because of these findings, agonist contact and washout times of 15 min were used in most subsequent experiments.

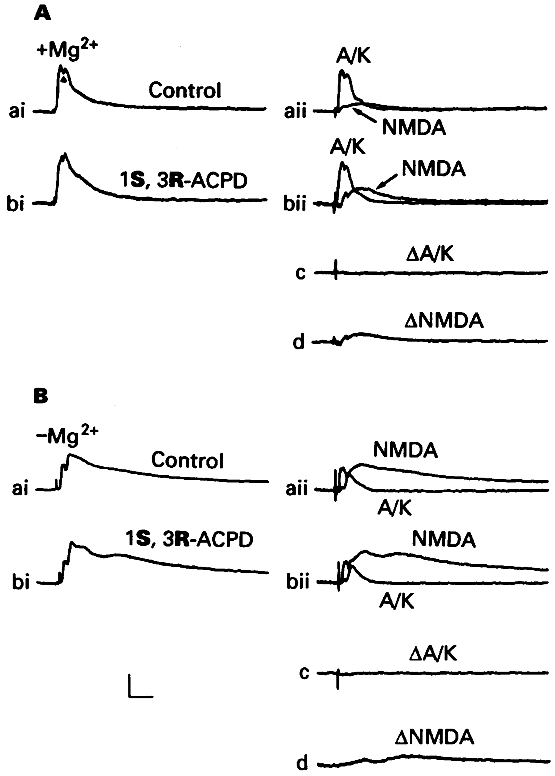

Figure 1 1S,3**R**-1-aminocyclopentane-1,3-dicarboxylate (1S,3**R**-ACPD; 0.1 mM) selectively enhances the N-methyl-D-aspartate (NMDA) receptor-mediated components of potentials evoked on supramaximal stimulation of the lateral olfactory tract of olfactory cortical slices. The results are from one slice perfused with solution containing 1 mM Mg^{2+} (A) and a second with Mg^{2+}-free solution (B). Each tracing is an average of 4 sweeps. Tracings labelled (ai) (Control) illustrate the pre-drug controls (solid arrow head indicates population spike) whilst (bi) (1S,3**R**-ACPD) shows the potentials evoked at the end of a 15 min washout period following application of 1S,3**R**-ACPD for 15 min. The NMDA and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate/kainate (A/K) components of the control and post 1S,3**R**-ACPD potentials are shown in (aii) and (bii). Subtraction of the individual components in (aii) from those in (bii) gives the 1S,3**R**-ACPD induced changes in the A/K (c; ΔA/K) and NMDA (d; ΔNMDA) components of the potential. Calibration bars 0.4 mV, 20 ms.

The ability of various excitatory amino acids to potentiate the NMDA receptor-mediated components of the N-wave complex was studied in slices perfused with Mg^{2+}-free solution and to which 10 μM DNQX or CNQX was applied (Figure 2). The relative potencies (μM concentrations producing a 25% increase in the area of the potential in parentheses) were 1S,3**R**-ACPD (8.0 ± 1.6, $n = 4$) = quisqualate (11.2 ± 2.7, $n = 7$) > ACPD (82.3 ± 7.7, $n = 6$) > L-glutamate (1120 ± 207, $n = 4$) and the corresponding maximum percentage increases in the area of the component were 65.1 ± 4.2, 38.2 ± 3.8, 44.2 ± 3.7 and 26.9 ± 1.9, respectively (means ± s.e.mean). Note that kainate, NMDA and L-aspartate were inactive. The presence or otherwise of Mg^{2+}, DNQX or picrotoxin had no significant effect on the action of ACPD (Table 1; 1S,3**R**-ACPD not tested). The increase caused by 1S,3**R**-ACPD occurred equally at short and long latencies and was unaffected by co-application of glycine (50 μM; Table 1). 1S,3**R**-ACPD applied to 4 slices perfused with AP5 (25 μM), followed by washout of both drugs, caused a 56.7 ± 6.9% increase in the area of the potential, the μM concentration causing a 25% increase being 9.7 ± 1.1 (means ± s.e.means). Finally, the effects of repeated applications of a single concentration of 10 μM 1S,3**R**-ACPD were not additive; the increase in area following washout of the first dose (28.6 ± 3.6%) was not significantly different following

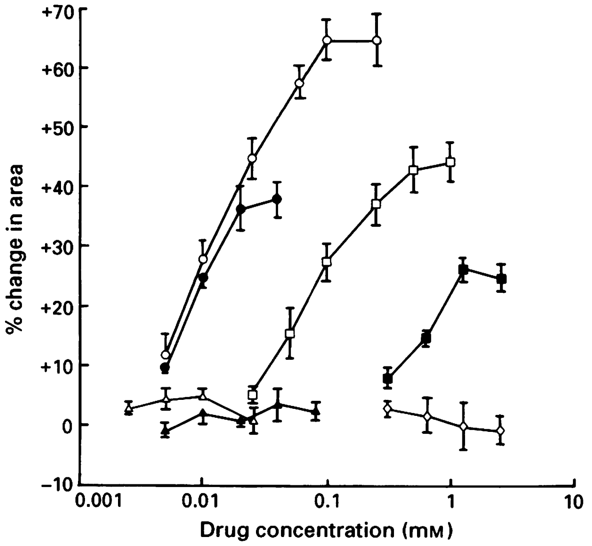

Figure 2 Concentration-effect curves for 1S,3R-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD, ○), quisqualate (Q, ●), 1RS,3RS-1-aminocyclopentane-1,3-dicarboxylate (ACPD, □), L-glutamate (Glu, ■), N-methyl-D-aspartate (NMDA, △), α-amino-3-hydroxy-5-methyl-4-ioxazolepropionate (AMPA, ▲) and L-aspartate (Asp, ◇) showing the percentage increase in the area of the NMDA receptor mediated component of potentials evoked in slices perfused with Mg^{2+}-free solution and to which 10 μM 6,7-dinitroquinoxaline-2,3-dione was applied throughout. The vertical lines represent the s.e.mean and the points are the means of 5–8 values.

the second (27.4 ± 2.7%; means ± s.e.means, $n = 4$).

Of the potential antagonists tested, RS-(+)-AP3 antagonized the effects of ACPD and quisqualate (Figure 3a, b) on the NMDA receptor-mediated component of potentials and 0.125 mM S-(+)-AP3 antagonized the effect of 1S,3R-ACPD, the R-(−)-isomer being essentially inactive (Figure 3c). S-(+)-AP3 alone had no effect on the potential and was ineffective if applied after the effect of 1S,3R-ACPD had developed (4 slices, not shown). In contrast, AP4 caused a reversible reduction in the amplitude and area of the potential (see Anson & Collins, 1987) and its enantiomers were equipotent antagonists of the effects of 1S,3R-ACPD (Figure 3d). The protein kinase C inhibitors, 5-(isoquinolinylsulphonyl)-3-methylpiperazine, sangivamycin and sphingosine (Hidaka *et al.*, 1984; Loomis & Bell, 1988), all blocked the effect of 1S,3R-ACPD on the NMDA receptor-mediated component of the N-wave complex (Table 1).

Input-output experiments

Application of 0.1 mM 1S,3R-ACPD had no effect on the stimulus voltage-tract action potential relationship (5 slices, not shown) but increased the area under the graph of action potential *versus* area of potential (Figure 4a); this effect was only observed following washout of the 1S,3R-ACPD and was significantly antagonized by S-(+)-AP3 and the 3 protein kinase C inhibitors (Table 2). In the presence of 1S,3R-ACPD, the latency to peak of the NMDA receptor-mediated component was reduced at submaximal stimulus intensities, an effect readily reversed on washout (Figure 4c), mimicked by 0.5 mM D-serine (4 slices, not shown) but not antagonized by sangivamycin or 5-(isoquinolinylsulphonyl)-3-methylpiperazine (each tested on 4 slices at 25 μM, not shown). PDAc (2.5 μM) increased the area of the potential for a given amplitude of tract action potential, an effect blocked by protein kinase C inhibitors (Table 2) but not S-(+)-AP3. PDAc had no effect on the latency to peak of the potential (Figure 4d).

Excitatory amino acid-evoked depolarizations

Application of 1S,3R-ACPD alone (0.1 mM) did not evoke a cellular depolarization (6 slices) neither did it significantly affect the responses evoked by NMDA, AMPA and kainate (4 slices each, not shown). 7-Chlorokynurenate (2.5 μM) reduced the response to NMDA to 16.4 ± 2.9% of control whereas during co-perfusion of 1S,3R-ACPD (0.1 mM) responses to NMDA were reduced to only 40.7 ± 3.7% (means ± s.e.mean, $n = 8$; significant difference, $P < 0.05$).

K^+-evoked release of amino acids

Exposure of olfactory cortical slices to K^+ (50 mM) significantly increased release of aspartate and glutamate ($P < 0.05$) which was largely Ca^{2+}-dependent (Table 3). Co-perfusion with S-(+)-AP3 (0.125 mM) significantly reduced release of glycine only. 1S,3R-ACPD in the presence of K^+ caused a highly significant ($P < 0.001$) potentiation of glycine release and a smaller increase in aspartate release. Addition of S-(+)-AP3 significantly reduced the effect of 1S,3R-ACPD on glycine release. In experiments in which the K^+ challenge was given following washout of 1S,3R-ACPD, aspartate release was significantly increased ($P < 0.05$) whereas glycine release had returned to control levels; the presence of S-(+)-AP3 throughout completely prevented the increase in aspartate release caused by 1S,3R-ACPD. Sangivamycin (25 μM) had no effect on the potentiation of glycine release by 1S,3R-ACPD but significantly reduced the increase in release of aspartate (Table 3).

Discussion

1S,3R-ACPD had two distinct effects on the NMDA receptor-mediated component of the N-wave complex. First, it caused a long lasting increase in the area of the potential. That the effect was blocked by S-(+)-AP3, the active emantiomer in antagonizing metabotropic receptors (Irving *et al.*, 1990; Schoepp *et al.*, 1990a) and that the potencies of the agonists tested mirrored their potencies in stimulating phosphoinositide turnover (Sladeczek *et al.*, 1985; 1988; Schoepp *et al.*, 1990b) strongly suggests that the effect was mediated by metabotropic glutamate receptors. The potentiation was also antagonized by AP4, another potential antagonist of metabotropic glutamate receptors (Schoepp *et al.*, 1990b). However, unlike AP3, AP4 reversibly reduces excitatory transmission in the olfactory cortex (Anson & Collins, 1987) so that antagonism of the effects of 1S,3R-ACPD by AP4 cannot be used as a diagnostic test of a role for metabotropic receptors. The second effect was to reduce the latency to peak of the potential. This action occurred only at submaximal stimulus intensities, was readily reversible on drug washout and was also antagonized by S-(+)-AP3, again suggesting a role for metabotropic glutamate receptors. In addition to these electrophysiological actions, the K^+-evoked release of endogenous glycine was increased in the presence of 1S,3R-ACPD whereas following washout, there was a selective increase in aspartate release. Both these effects were also sensitive to S-(+)-AP3.

The results provide some evidence of the mechanisms by which 1S,3R-ACPD might increase the area of the potential. Activation of the NMDA receptor complex *per se* was unnecessary, for NMDA itself did not affect the potential and 1S,3R-ACPD potentiated the area of the potential even when applied to slices perfused with AP5. The inability of picrotoxin to block the effects of ACPD suggests that changes in GABA-mediated inhibition were not involved (Desai & Conn, 1991). One possibility is that the glycine released by the 1S,3R-ACPD could displace any DNQX/CNQX which might antagonize the strychnine-insensitive glycine site of the NMDA receptor complex (Johnson & Ascher, 1987; Birch *et al.*, 1988). This would seem an

Table 1 Some characteristics of the effect of 1**RS**, 3**RS**-*cis*-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) and 1S, 3**R**-ACPD on the NMDA receptor-mediated component of evoked potentials

Agonist	*Experimental conditions*	*n*	*Drug*	*Concentration which increased area by 25% (μM)*[a]	*P*	*Maximum percentage increase in area*[a]	*P*
ACPD	$-Mg^{2+}$, + DNQX	6	–	82.3 ± 7.5	–	44.2 ± 3.7	–
(25 to 1000 μM)	$-Mg^{2+}$, + DNQX	4	–	96.5 ± 10.5	NS[b]	40.5 ± 7.2	NS[b]
	$-Mg^{2+}$, − DNQX	4	–	81.7 ± 6.7	NS[b]	38.6 ± 3.6	NS[b]
	$-Mg^{2+}$, + DNQX	4	Picrotoxin	80.4 ± 6.4	NS[b]	32.7 ± 6.4	NS[b]
1S,3**R**-ACPD	$-Mg^{2+}$, + CNQX	4	–				
(5–250 μM)	total			8.0 ± 1.6	<0.001[b]	65.1 ± 4.2	<0.05[b]
	0–40 ms			7.2 ± 1.4	NS[c]	67.0 ± 2.9	NS[c]
	40–450 ms			10.1 ± 2.7	NS[c]	66.1 ± 6.3	NS[c]
	$-Mg^{2+}$, + CNQX	4	Glycine	10.2 ± 1.8	NS[c]	58.2 ± 6.6	NS[c]
	$-Mg^{2+}$, + CNQX	4	Sangivamycin	>250	–	19.3 ± 1.2	<0.001
	$-Mg^{2+}$, + CNQX	5	5-Isoquinolinyl-sulphonyl)-3-methyl-piperazine	>250	–	13.6 ± 2.6	<0.001
	$-Mg^{2+}$, + CNQX + ethanol	4	–	12.3 ± 2.8	NS[c]	44.2 ± 6.8	NS[c]
	$-Mg^{2+}$, + CNQX + ethanol	4	Sphingosine	>250	–	12.0 ± 0.24	<0.005[d]

When present, Mg^{2+} was at a concentration of 1 mM, CNQX/DNQX 10 μM, picrotoxin 25 μM, glycine 50 μM, sangivamycin 25 μM, 5-isoquinolinylsulphonyl-3-methylpiperazine 50 μM, and sphingosine 25 μM.

[a] Measured 15 min after agonist washout (see Methods).

[b] Compared to value for ACPD ($-Mg^{2+}$, + DNQX).

[c] Compared to value for the effect of 1S,3**R**-ACPD ($-Mg^{2+}$, + CNQX) on the total potential.

[d] Compared to ethanol control.

All values are means ± s.e.means. Statistical analysis was performed using Dunnett's *t* test. NS, not significant.

unlikely basis for the potentiation in that; (i) potentiation by 1S,3R-ACPD occurred on co-application of exogenous glycine and in the absence of DNQX, (ii) release of endogenous glycine only occurred *during* application of 1S,3R-ACPD whereas potentiation of the potential was maintained at times *after* glycine release had returned to control levels and (iii) the effects of 1S,3R-ACPD on the potential, but not on glycine release, were sensitive to inhibitors of protein kinase C. In contrast, the reduction in the latency to peak of the NMDA receptor-mediated component of the potential seems to have a fundamentally different mechanism in that the effect was recorded only in the presence of the agonist and was not antagonized by inhibitors of protein kinase C or mimicked by PDAc, an activator of the enzyme (Castagna *et al.*, 1982); indeed, its time course and pharmacology closely paralleled those of the

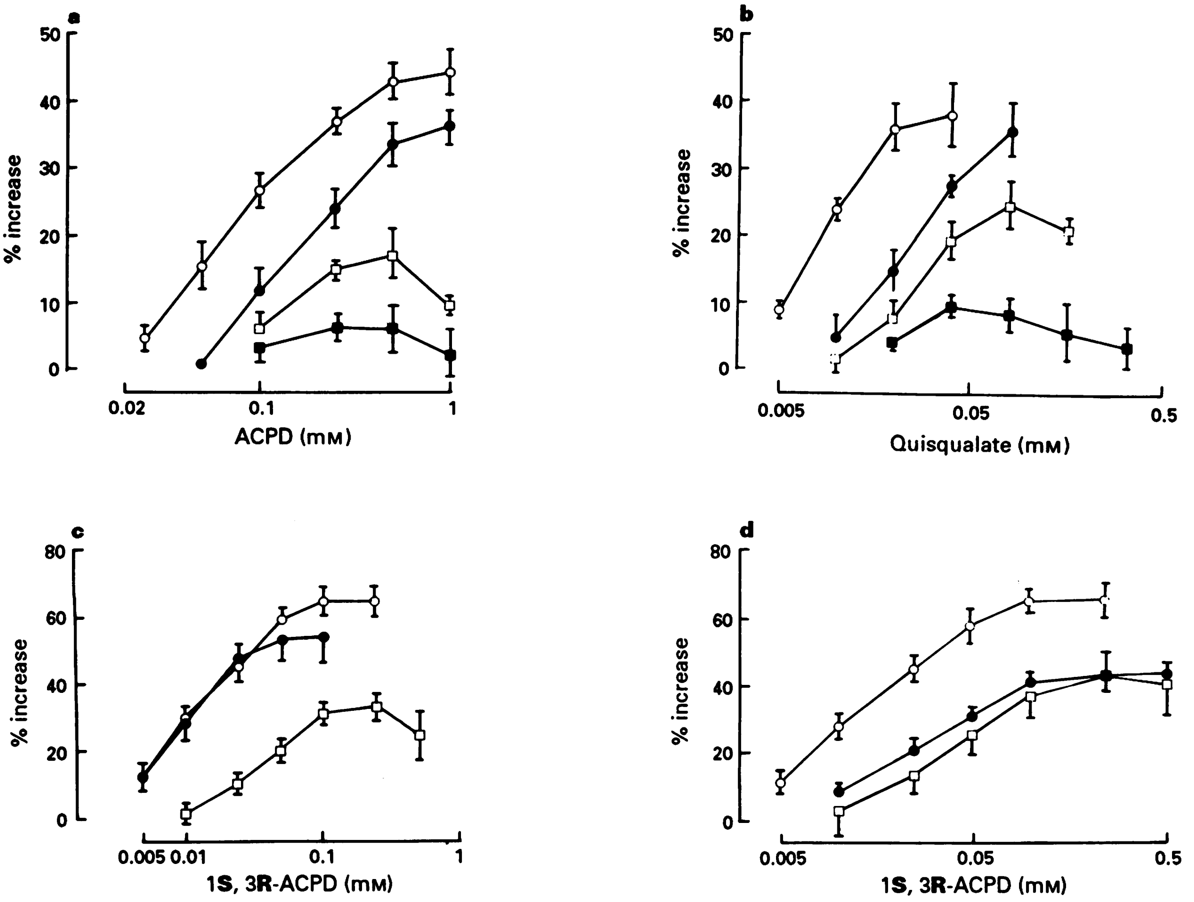

Figure 3 Effects of 2-amino-3-phosphonopropionate (AP3) and 2-amino-4-phosphonobutyrate (AP4) on the increase in the area of the N-methyl-D-aspartate receptor-mediated component of slices perfused in Mg^{2+}-free solution and to which 10 µM of either 6,7-dinitroquinoxaline-2,3-dione (a,b) or 6-cyano-7-nitroquinoxaline-2,3,-dione (c,d) was applied. Increasing concentrations of RS-(+)-AP3 (0.5 mM, ●; 1 mM, □; 2 mM, ■) antagonize the effects of (a) 1RS,3RS-aminocyclopentane-1,3-dicarboxylate (ACPD) and (b) quisqualate. (c) Effects of 0.125 mM R-(−)- (●) and S-(+)- (□) AP3 on the concentration effect curve to 1S,3R-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD; ○). (d) Effects of 0.25 mM D-(−) (●) and L-(+)- (□) AP4 on the concentration-effect curve to 1S,3R-ACPD (○). The vertical lines represent the s.e.mean and the points the mean of 4–7 values.

Table 2 Drug effects on the increase in the area of NMDA receptor-mediated potential by 1S,3R-1-amino-cyclopentane-1,3-dicarboxylic acid (1S,3R-ACPD) and 4-β-phorbol-12,13-diacetate (PDAc) in a series of input-output experiments

	% increase in area under the curve^a					
Drug	*1S,3R-ACPD* (0.1 mM)	n	P	*PDAc* (2.5 µM)	n	P
None (control)	58.4 ± 6.2	5	–	50.7 ± 5.2	5	–
S-(+)-AP3 (0.125 mM)	26.4 ± 1.9	5	<0.05	54.1 ± 6.1	5	NS
Sangivamycin (25 µM)	18.2 ± 3.6	5	<0.05	26.4 ± 3.8	5	<0.05
1-(5-Isoquinolinylsulphonyl)-3-methylpiperazine (50 µM)	29.7 ± 5.2	5	<0.05	38.0 ± 3.4	5	<0.05
Sphingosine solvent control	47.2 ± 4.9	4	–	46.4 ± 6.8	4	–
Sphingosine (25 µM)	16.0 ± 2.7	4	<0.01	24.2 ± 3.9	4	<0.05

All experiments were carried out using slices perfused with Mg^{2+}-free solution and to which CNQX (10 µM) and picrotoxin (25 µM) was applied.

^{a}Measured 15 min after washout of 1S,3R-ACPD or PDAc, as appropriate. Values are means ± s.e.means of the areas under the curve of action potential amplitude *versus* area of the NMDA receptor-mediated component. Statistical analysis was performed using an unpaired Student's *t* test, comparing each mean to the appropriate control. NS, not significant.

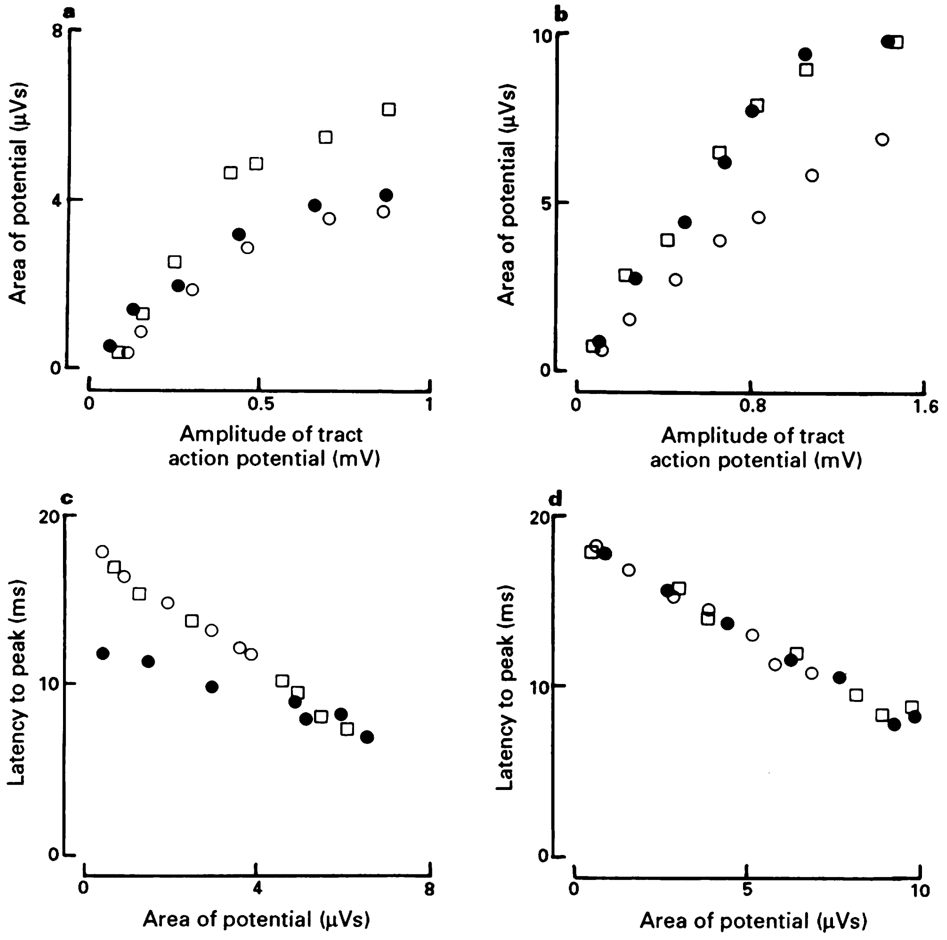

Figure 4 Effects of 0.1 mM 1S,3R-1-aminocyclopentane-1,3-dicarboxylate (1S,3R-ACPD; a and c) and 2.5 µM 4-β phorbol-12,13-diacetate (PDAc; b and d) in two stimulus input-evoked output experiments carried out in slices perfused in Mg^{2+}-free solution containing picrotoxin (25 µM) and to which 6-cyano-7-nitro-quinoxaline-2,3-dione (10 µM) was applied. Each slice was stimulated using a range of voltages before (○), during application of 1S,3R-ACPD for 15 min or PDAc for 30 min (●) and after perfusion with drug-free solution for 15 min (□). Mean results are given in Table 2.

Table 3 Effect of 1S,3**R**-1-aminocyclopentane-1,3-dicarboxylic acid (**1**S,3**R**-ACPD) on the K^+-evoked release of endogenous amino acids from cubes of mouse olfactory cortex

	K⁺*-evoked release* (pmol min⁻¹ mg⁻¹ wet weight)								
Experimental conditions	*Aspartate*	n	P	*Glutamate*	n	P	*Glycine*	n	P
K^+ (50 mM) control	27.2 ± 6.1	9	–	131 ± 19.7	12	–	11.4 ± 5.6	12	–
K^+ minus Ca^{2+}	6.3 ± 2.7	4	<0.05	26 ± 7.2	4	>0.05	4.8 ± 2.2	4	NS
K^+ plus S-(+)-AP3 (0.125 mM)	20.3 ± 4.6	4	NS	122 ± 13.8	6	NS	–24.6 ± 3.7	6	<0.05
K^+ in presence of 1S,3R-ACPD (0.1 mM)									
1S,3**R**-ACPD alone	55.4 ± 8.5	6	<0.05	126 ± 19.5	6	NS	87.6 ± 7.6	6	<0.001
plus S-(+)-AP3 (0.125 mM)	40.3 ± 7.7	5	<0.05	89.9 ± 9.4	5	NS	56.8 ± 6.2[a]	3	<0.05
plus sangivamycin (25 µM)	42.8 ± 6.2	3	NS	94.6 ± 12.1	3	NS	72.7 ± 9.4	3	NS
*K^+after washout of 1*S,3**R**-ACPD (0.1 mM)									
1S,3**R**-ACPD alone	53.0 ± 8.8	7	<0.05	144 ± 10.6	10	NS	1.19 ± 8	10	NS
plus S-(+)-AP3 (0.125 mM)	27.6 ± 6.6	6	NS	88.3 ± 10.2[a]	6	NS	– 16.4 ± 2.4[a]	6	<0.05
plus sangivamycin (25 µM)	30.2 ± 6.1	6	NS	131 ± 12.9	6	NS	10.1 ± 3.4	6	NS

All experiments were carried out using preparations perfused with Mg^{2+}-free solution.

Values are means ± s.e.means. Statistical analyses were performed by comparing values to the control using an unpaired Student's *t* test. NS, not significant.

[a] Significantly different from 1S,3**R**-ACPD alone ($P < 0.05$).

increase in glycine release.

It is proposed that the potentiation in the NMDA receptor-mediated component of potentials was caused by an increase in transmitter release from the terminals of the lateral olfactory tract which was triggered, and possibly maintained, by activation of protein kinase C. Evidence for a presynaptic locus is provided by the findings that; (i) 1S,3R-ACPD had no effect on the postsynaptic responses evoked by NMDA, (ii) the effects of 1S,3R-ACPD on the relationship between the tract action potential and evoked potential are best explained by an increase in transmitter release (Collins & Richards, 1990) and (iii) protein kinase C is located in the tract terminals and, when activated, increases transmitter release (Collins & Richards, 1990). It is tempting to speculate that the increased release of aspartate, a transmitter candidate of the lateral olfactory tract (Collins, 1986), plays a role in the phenomenon. The mechanisms by which 1S,3R-ACPD reduced the latency to peak of the potential is problematical for although an increase in postsynaptic excitability is consistent with the finding (see Constanti & Libri, 1992), 1S,3R-ACPD failed to potentiate postsynaptic responses to the excitants tested. It is possible that the increased levels of glycine acting on the strychnine-insensitive site on the NMDA receptor complex were involved for not only did D-serine, an agonist of the site (McBain *et al*., 1989) mimic the effect of 1S,3R-ACPD on the latency of the potential, but 1S,3R-ACPD significantly antagonized the reduction in NMDA responses caused by 7-chlorokynurenate, an antagonist of the strychnine insensitive glycine site (Kemp *et al*., 1988).

Metabotropic glutamate receptor-mediated increases in NMDA receptor-mediated responses have been reported by others (Aniksztejn *et al*., 1991; Harvey *et al*., 1991; Kinney & Slater, 1992) although increases in transmitter release were not thought to be involved. Indeed, with the exception of the present study, presynaptically located metabotropic glutamate receptors have been reported to inhibit transmitter release (Baskys & Malenka, 1991; Crepel *et al*., 1991; Lovinger, 1991). However, both ACPD and phorbol esters broaden the action potential of hippocampal neurones (Hu & Storm, 1992), effects which are compatible with a metabotropic glutamate receptor-induced increase in transmitter release mediated by protein kinase C.

In conclusion, it is proposed that metabotropic glutamate receptors in the olfactory cortex mediate a short term increase in pyramidal cell excitability, a long term and selective increase in NMDA receptor-mediated transmission, together with temporally similar increases in the release of glycine and aspartate, respectively. The long duration effects on transmission and aspartate release are dependent on activation of protein kinase C. There is no direct evidence that the electrophysiological and neurochemical events are related, neither is it clear that the early increase in excitability is related to the longer term changes. The results to do not preclude a role for metabotropic receptor-mediated changes in phosphoinositide turnover, cyclic AMP synthesis or release of arachidonic acid (Aramori & Nakanishi, 1992). Finally, it is likely that the reported potentiation of NMDA receptor-mediated events would affect long-term potentiation (Kanter & Haberly, 1990) and hence modulate the associative memory processes of the olfactory cortex (Haberly & Bower, 1989).

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