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Metabotropic glutamate receptor subtypes mediating slow inward tail current (I_{ADP}) induction and inhibition of synaptic transmission in olfactory cortical neurones

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1 The pharmacological features of the pre- and postsynaptic metabotropic glutamate receptors (mGluRs) present in the guinea-pig olfactory cortex, were examined in brain slices *in vitro* by use of a conventional intracellular current clamp/voltage clamp recording technique.

2 Bath-application of *trans*-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD) (50 μM) produced a sustained membrane depolarization, increase in cell excitability and induction of a post-stimulus inward (afterdepolarizing) tail current (I_{ADP}) (measured under 'hybrid' voltage clamp) similar to those evoked by the muscarinic receptor agonist oxotremorine-M (OXO-M, 2 μM).

3 L-Glutamate (0.25–1 mM, in the presence of 20 μM 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) and 100 μM DL-amino-5-phosphono valeric acid (DL-APV)) or the broad spectrum mGluR agonists 1**S**,3**R**-aminocyclopentane-1,3-dicarboxylic acid (1**S**,3**R**-ACPD, 10 μM), 1**S**,3**S**-ACPD (50 μM), ibotenate (Ibo; 25 μM, in the presence of 100 μM DL-APV), the selective mGluR I agonists (**S**)-3,5-dihydroxyphenylglycine ((**S**)-3,5-DHPG, 10 μM), (**S**)-3-hydroxyphenylglycine ((**S**)-3HPG, 50 μM), or quisqualate (10 μM, in the presence of 20 μM CNQX), but not the mGluR II agonist 2**S**,1′**S**,2′**S**-2-(2′-carboxycyclopropyl)-glycine (L-CCGI, 1 μM) or mGluR III agonist L(+)-2-amino-4-phosphonobutyric acid (L-AP4, 1 mM), were all effective in producing membrane depolarization and inducing a post-stimulus I_{ADP}. Unexpectedly, the proposed mGluR II-selective agonist (2**S**,1′**R**,2′**R**,3′**R**)-2-(2′,3′-dicarboxycyclopropyl)-glycine (DCG-IV, 10 μM, in the presence of 100 μM DL-APV) was also active.

4 The excitatory effects induced by 10 μM 1**S**,3**R**-ACPD were reversibly antagonized by the mGluR I/II antagonist (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG, 0.5–1 mM), as well as the selective mGluR I antagonists (**S**)-4-carboxyphenylglycine ((**S**)-4CPG) and (**S**)-4-carboxy-3-hydroxyphenyl glycine ((**S**)-4C3HPG) (both at 1 mM), but not the nonselective mGluR antagonist L(+)-2-amino-3-phosphonopropionic acid (L-AP3, 1 mM) or the selective mGluR III antagonist (**S**)-α-methyl-L-AP4 (MAP4, 1 mM).

5 The excitatory postsynaptic potentials (e.p.s.ps), induced by single focal stimulation of cortical excitatory fibre tracts, were markedly reduced by 1**S**,3**R**-ACPD or L-AP4 (both at 10 μM), and by the selective mGluR II agonists (mGluR I antagonists) (**S**)-4CPG or (**S**)-4C3HPG (both at 1 mM) but not (**S**)-3,5-DHPG or (**S**)-3HPG (both at 100 μM).

6 The inhibitory effects of 1**S**-3**R**-ACPD, but not L-AP4, were reversibly blocked by (+)-MCPG (1 mM), whereas those produced by L-AP4, but not 1**S**,3**R**-ACPD, were blocked by the selective mGluR III antagonist MAP4 (1 mM).

7 It is concluded that a group I mGluR is most likely involved in mediating excitatory postsynaptic effects, whereas two distinct mGluRs (e.g. group II and III) might serve as presynaptic inhibitory autoreceptors in the guinea-pig olfactory cortex.

Keywords: Metabotropic glutamate receptors (mGluRs); 1**S**,3**R**-ACPD; L-AP4; depolarization; slow inward tail current (I_{ADP}); synaptic transmission; olfactory cortex; intracellular recording

Introduction

Metabotropic glutamate receptors (mGluRs) are a large family of G-protein-linked receptors with diverse properties in terms of intracellular transduction coupling, pharmacology, and central neuronal distribution. They are activated by glutamate, quisqualate (QA) and ibotenate (Ibo) but not N-methyl-D-aspartate (NMDA), kainate (KA) or α-amino-3-hydroxy-5-methyl-isoxazole-4-propionate (AMPA) (Schoepp & Johnson, 1988; Nakaniski, 1992; Schoepp & Conn, 1993; Pin & Duvoisin, 1995).

At least eight different mGluR subtypes (mGluR1-mGluR8) have now been cloned, which can be divided into three subgroups according to their sequence similarity, agonist selectivity and intracellular effector mechanisms: mGluR1/mGluR5 (Group I mGluRs), mGluR2/mGluR3 (Group II mGluRs) and mGluR4/mGluR6/mGluR7/mGluR8 (Group III mGluRs) (for review see Pin & Duvoisin, 1995). Group I mGluRs are coupled to the stimulation of phosphoinositide (PI) hydrolysis and intracellular Ca^{2+} mobilization, and are potently activated by L-quisqualic acid (QA), the cyclic glutamate analogue 1**S**,3**R**-1-aminocyclopentane-1,3-dicarboxylic acid (1**S**,3**R**-ACPD), its racemic form (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) or the *cis*-isomer 1**S**,3**S**-aminocyclopentane-1,3-dicarboxylic acid (1**S**,3**S**-ACPD), but are insensitive to L-2-amino-4-phosphonobutyrate (L-AP4). Group II mGluRs (negatively coupled to adenyl cyclase and 3′:5′-cyclic monophosphate (cyclic AMP) formation) are also potently activated by *trans*-ACPD or the 1**S**,3**R**- and 1**S**,3**S**-isomers, but are insensitive to QA and L-AP4. Finally, Group III mGluRs (also negative linked to adenyl cyclase activity) are activated by L-AP4 but are in-

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sensitive to QA, *trans*-ACPD or 1**S**,3**R**- and 1**S**, 3**S**-ACPD (for references see Schoepp, 1994; Pin & Duvoisin, 1995). The lack of specific agonists and antagonists for mGluRs in the past, has limited the precise characterization of the various receptor populations until the recent development of more selective phenylglycine derivatives (Eaton *et al*., 1993; Birse *et al*., 1993; Jane *et al*., 1993). Thus, (**S**)-3-hydroxyphenylglycine ((**S**)-3HPG) and (**S**)-3,5-dihydroxyphenylglycine ((**S**)-3,5-DHPG) act as partial agonists at group I mGluRs whereas 2**S**,1′**S**,2′**S**-2-(2′-carboxycyclopropyl)-glycine (L-CCGI) and (2**S**,1′**R**,2′**R**,3′**R**)-2-(2′,3′-dicarboxycyclopropyl)-glycine (DCG-IV) potently activate group II mGluRs (Birse *et al*., 1993; Hayashi *et al*., 1994; Watkins & Collingridge, 1994).

Metabotropic glutamate receptors are believed to be involved in a variety of neuromodulatory mechanisms such as long-term potentiation (LTP) (Bashir *et al*., 1993; Bortolotto & Collingridge, 1995) or long-term depression (LTD) of synaptic transmission (Liu *et al*., 1993; Calabresi *et al*., 1993) and the presynaptic control of glutamate release (Schoepp, 1994; Collins, 1993; 1994). In addition a role for mGluRs in neurodegenerative processes has also been proposed (Schoepp & Conn, 1993). Recent studies in our laboratory have shown that mGluRs in the guinea-pig olfactory cortex play an important role in mediating a prolonged neuronal excitability increase (Constanti & Libri, 1992; Libri *et al*., 1996). Application of *trans*-ACPD evoked a sequence of excitatory events (membrane depolarization, sustained repetitive neuronal discharge) similar to those produced by muscarinic receptor (mAChR) activation (Constanti & Libri, 1992), and in particular, induced the appearance of a slow post-stimulus afterdepolarization (sADP) (Constanti & Bagetta, 1991; Constanti & Libri, 1992; Constanti *et al*., 1993; Libri *et al*., 1994), believed to be mediated by a novel Ca^{2+}-sensitive K^+ current (I_{ADP}; Constanti *et al*., 1993); this current was thought to be important in governing long-term neuronal excitability as well as cortical epileptogenesis.

Although activation of presynaptic mGluRs in the olfactory cortex leads to a depression in excitatory synaptic transmission, and may play a role in the induction of theta-burst high frequency stimulation-dependent LTP (Collins, 1993; 1994) and the formation and consolidation of olfactory memory processes (Kaba *et al*., 1994), it is still unclear what subtypes of mGluR mediate the pre- and postsynaptic effects of mGluR stimulation in this brain area. In the present study we aimed to explore the pharmacological profile of pre- and postsynaptic mGluRs in the olfactory cortex, with particular emphasis on the likely receptor type(s) involved in the induction of the novel I_{ADP} tail current in these cells. The effects of a variety of metabotropic receptor ligands, claimed to act as full agonists, partial agonists and/or antagonists were therefore examined in guinea-pig olfactory cortical slices *in vitro*, by use of a conventional intracellular current clamp/voltage clamp recording technique.

Methods

Preparation and incubation of the slices

The methods used for the electrophysiological experiments in guinea-pig olfactory cortex were essentially similar to those described previously (Constanti *et al*., 1993). Briefly, adult albino guinea-pigs (250–400 g; of either sex) were decapitated and the brain rapidly removed. A rectangular tissue block comprising the lateral olfactory tract (LOT) and surrounding olfactory cortex was stuck with cyanoacrylate glue to the cutting state of a Campden Vibroslice/M tissue cutter. Slices (~450 μm thick, sectioned perpendicular to the pial surface, and along the LOT axis) were cut in aerated ice-cold (4°C) Krebs solution, and stored in oxygenated Krebs at 32°C for 30 min before transferring to the recording chamber. The composition of the Krebs solution was (mM): NaCl 118, KCl 3, $CaCl_2$ 1.5, $NaHCO_3$ 25, $MgCl_2.6H_2O$ 1 and D-glucose 11 (bubbled with 95% O_2 : 5% CO_2, pH 7.4).

Recording and stimulation

Conventional intracellular current-clamp/voltage-clamp recordings were made from neurones in the periamygdaloid area of the slice with glass microelectrodes filled with 4 M potassium acetate (tip resistances 50–70 MΩ), connected to a Dagan 8100 sample-and-hold preamplifier (2–3 kHz switching frequency,; 25% duty cycle). By use of transverse fibre optic illumination, cell layers were discerned under a conventional stereomicroscope (Nikon SMZ-1) as a translucent area running parallel to the pial surface; electrodes were positioned within the olfactory pyramidal cell layers II–III. Membrane resistance and firing properties were measured by passing negative or positive current pulses of increasing intensities (~0.5–3.5 nA, 160 ms duration) and recording the resultant electrotonic potentials. Unless otherwise stated, cells were systematically depolarized to −70 mV (near firing threshold) by applying constant injection of depolarizing current.

Orthodromic stimulation was delivered through a bipolar nichrome wire electrode (50 μm diameter, insulated except at the tip) placed in cortical layer I, a few mm away from the recording electrode, in order to activate LOT afferent and association fibres projecting to layer II–III neurones. Excitatory postsynaptic potentials (e.p.s.ps) were evoked in response to stimuli of increasing intensities (15–50 V) and constant duration (200 μs), delivered by a Digitimer isolated DS2 stimulator. Stimulus strength was adjusted so that the synaptic response was just sub-threshold for evoking orthodromic action potentials. All measurements were performed before, during and after bath-application of pharmacological agents so that each neurone served as its own control.

Sampled membrane current and voltage signals were fed to a Digidata 1200 analogue-to-digital interface (Axon Instruments, Foster City, CA, U.S.A.) coupled to a PC computer (4DX2-66 Viglen Ltd., U.K.), to acquire data and perform off-line analyses with pCLAMP 6.0.1 software (Axon Instruments). Data were also recorded on a Gould ink-jet chart recorder. Most recordings were stable for periods of 1–5 h. In some experiments, microelectrodes were filled with 2% neurobiotin (Vector-Laboratories, CA; freshly dissolved in 2 M potassium acetate) for intracellular staining of recorded neurones. The neurotracer was injected intracellularly by passing depolarizing current pulses (1–2 nA, 300 ms, 1–2 Hz) through the recording electrode for at least 15–20 min before drug application. At the end of the experiment, slices were fixed in 4% paraformaldehyde, and injected neurones visualized under conventional light microscopy by incubation of the slices with an avidin-biotinylated HRP reagent (Vectastain ABC; Vector Laboratories, CA) followed by reaction with diaminobenzidine and H_2O_2 as previously described in detail (Libri *et al*., 1994). Data are expressed as mean ± s.e.mean. Where appropriate, differences between data groups are presented as percentage (%) change versus control. EC_{50} values were estimated from least squares fitted curves drawn according to a simple sigmoidal model: $y/y_{max} = a^n/(a^n + EC_{50}{}^n)$, by use of SigmaPlot software (version 4, Jandel Scientific; CA). Statistical significance between data groups was assessed by a Wilcoxon signed rank test.

Drugs

Drugs used in this study included *trans*-ACPD, 1**S**,3**R**-ACPD, 1**S**,3**S**-ACPD, 1**R**,3**S**-ACPD, 2**S**,1′**S**,2′**S**-2-(2′-carboxycyclopropyl)-glycine (L-CCGI), (**S**)-3-hydroxyphenylglycine ((**S**)-3HPG), (**S**)-3,5-dihydroxyphenylglycine ((**S**)-3,5-DHPG), Ibo, QA, L-glutamic acid (L-glutamate), L-AP4, (**S**)-α-methyl-L-AP4 (MAP4), α-methyl-4-carboxyphenylglycine ((+)-MCPG), (**S**)-4-carboxyphenylglycine ((**S**)-4CPG), (**S**)-4-carboxy-3-hydroxyphenylglycine ((**S**)-4C3HPG), L-AP3, DL-amino-5-phosphono valeric acid (DL-APV), and 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX) (all from Tocris Neuramin, Essex, U.K., with some agonists also kindly provided by Prof J.C. Watkins, University of Bristol, U.K.). (2**S**,1′**R**,2′**R**,3′**R**)-2-(2′,3′-dicarb-

oxycyclopropyl)-glycine (DCG-IV) was kindly provided by Dr H. Shinozaki, The Tokyo Metropolitan Institute of Medical Science, Japan; OXO-M was obtained from Semat Ltd., U.K., and L-aspartic acid-β-hydroxamate from Sigma Ltd. Except when noted, drugs were prepared in Krebs solution and bath-applied by superfusion (bath-exchange time $\sim$30 s). Since DCG-IV is a potent agonist at group II mGluRs and NMDA receptors (Hayashi *et al*., 1993), the experiments with this agent were performed in the presence of 100 μM DL-APV to prevent activation of ionotropic-glutamate receptors. Likewise, experiments with L-glutamate were performed in the presence of 20 μM CNQX and 100 μM DL-APV, whereas the respective NMDA and non-NMDA receptor agonists Ibo and QA where bath-applied in combination with 100 μM DL-APV or 20 μM CNQX, respectively. CNQX was pre-dissolved in dimethylsulphoxide (DMSO) to give a 10 mM stock solution, whereas all the other metabotropic agonist/antagonist compounds were dissolved in 1 : 1 equivalent of 100 mM sodium hydroxide (NaOH) solution; they were then frozen in 10 mM stock aliquots and subsequently diluted in Krebs solution immediately before use. Final bath concentrations of DMSO (up to 0.5%) or NaOH (up to 0.1%) had no deleterious effects on cell membrane properties or muscarinic/metabotropic responsiveness.

Results

Intracellular data were obtained from 96 guinea-pig olfactory cortical neurones showing a mean resting membrane potential of -83.2 ± 0.8 mV, input resistance not less than 30 MΩ (mean 31.8 ± 2.4 MΩ) and action potential amplitude >100 mV (mean 102.7 ± 0.9 mV); their electrophysiological properties and firing behaviour were typical for pyramidal cells in rodent cerebral cortex (Connors & Gutnick, 1990). In addition, when a prolonged depolarizing stimulus (1.6 s) was applied to the recorded neurones, a post-stimulus slow afterhyperpolarization (sAHP) was usually observed. Only neurones showing electrophysiological features typical of deep layer II–III olfactory cortical neurones (Libri *et al*., 1994) were used in the present experiments. Cells electrophysiologically identified as superficial pyramidal neurones were regularly discarded, since they are known to show little or no sensitivity to mGluR (or muscarinic AChR) activation (Constanti & Libri, 1992; Constanti *et al*., 1993; Libri *et al*., 1994).

Effects of trans-*ACPD on neuronal excitability*

Initial experiments were conducted with the broad spectrum agonist *trans*-ACPD, that is a 1 : 1 racemic mixture of 1**S**,3**R**-

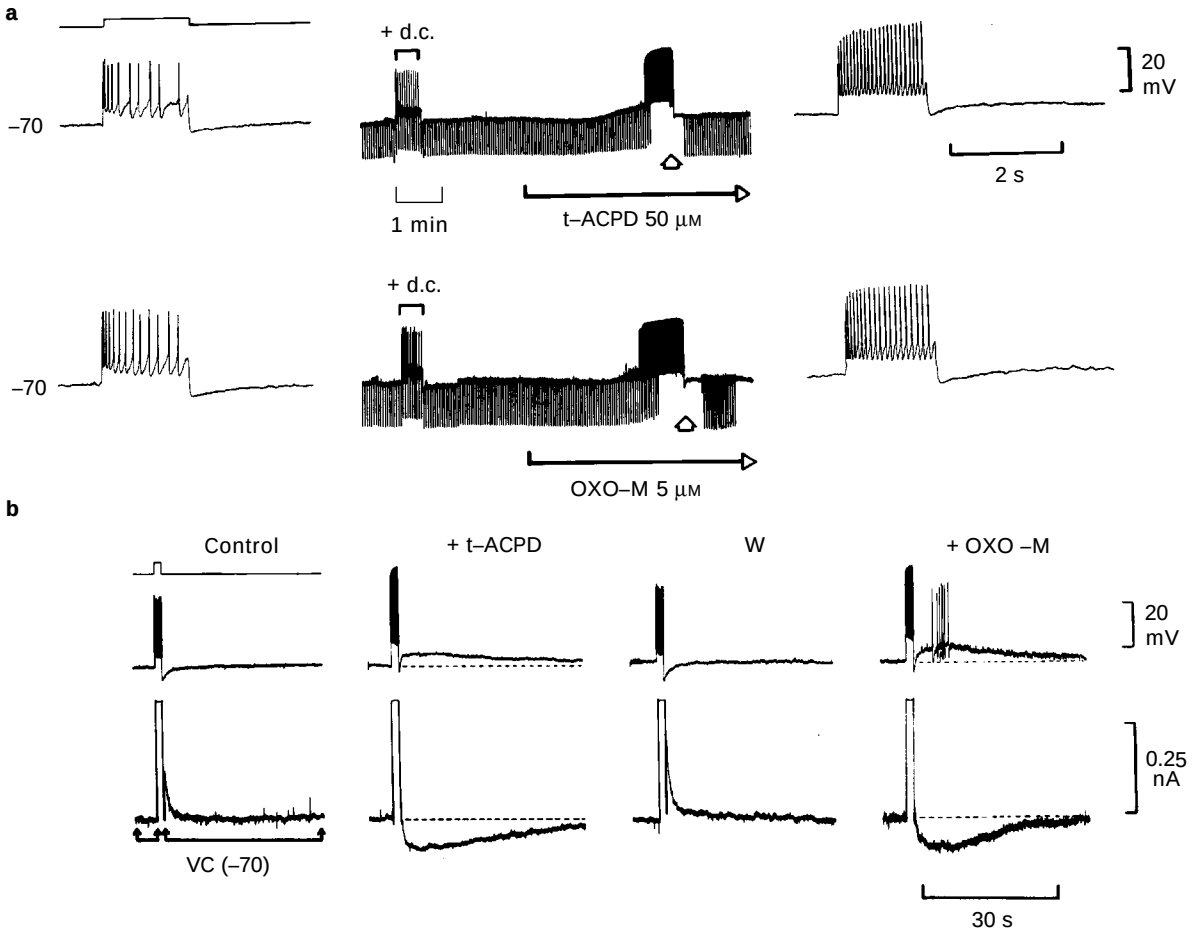

Figure 1 Metabotropic-glutamate and muscarinic responses of a guinea-pig olfactory cortical neurone maintained at -70 mV (near firing threshold) by positive current injection. (a) Left: electrotonic potentials with superimposed action potentials evoked in control solution in response to injected depolarizing current pulses (0.6 nA; 160 ms); middle: membrane depolarization and strong repetitive firing induced by a 2 min bath-application of 50 μM *trans*-ACPD (upper trace) or 5 μM oxotremorine-M (OXO-M) (lower trace). Open arrows indicate potential re-adjustment back to -70 mV to monitor changes in membrane input resistance; right: increased number of spikes (reduced accommodation) evoked during bath-application of *trans*-ACPD (upper trace) or OXO-M (lower trace). (b) From left to right, responses of same cell to injection of a 1.6 s depolarizing current pulse (1.2 nA) in control solution, in the presence of *trans*-ACPD, after 20 min drug washout and in the presence of OXO-M. Note that the post-stimulus afterhyperpolarization (AHP) evoked in control was replaced by a post-stimulus slow afterdepolarization (sADP) in the presence of *trans*-ACPD and OXO-M. Also the sADP evoked in OXO-M (but not in *trans*-ACPD) attained firing threshold and triggered repetitive action potentials. Bottom traces of each panel illustrate the underlying outward tail current (I_{AHF}, in control) or slow inward tail current (I_{ADP}, in *trans*-ACPD and OXO-M) revealed with a 'hybrid' voltage clamp command.

ACPD and 1**R**,3**S**-ACPD. As previously shown (Constanti & Libri, 1992), bath-application of *trans*-ACPD (50 μM; 2 min; $n=28$) evoked persistent excitatory effects, consisting of a slow membrane depolarization (9.7 ± 1.2 mV, observed in 17/28 cells; see below) an increase in input resistance ($14.8\pm1.6\%$ measured at -70 mV resting potential), an intense neuronal discharge (~10 Hz frequency) and a reduction in firing accommodation, as revealed by the increased number of spikes evoked during a 160 ms depolarizing current pulse ($144\pm28\%$ relative to control) (Figure 1a). The membrane depolarization was often preceded (10/17 cells) by an initial brief hyperpolarization (1.9 ± 0.8 mV amplitude, measured to the peak; 39.4 ± 3.1 s duration) associated with a slight decrease in input resistance ($<1\%$). In addition, during the action of *trans*-ACPD, the sAHP evoked in control in response to a large (1.6 s) depolarizing stimulus, was replaced by a prominent slow after-depolarization (sADP) (10.7 ± 2.8 mV) (Figure 1b). The slow inward tail current (I_{ADP}) underlying the sADP could be conveniently revealed by a 'hybrid' voltage clamp protocol, in which a manual switch to voltage-clamp at -70 mV holding potential, was made immediately after a 1.6 s depolarizing stimulus delivered in current-clamp. The slowly decaying I_{ADP} (0.21 ± 0.03 nA amplitude, 39.6 ± 4.1 s duration; $n=28$) became progressively more activated by depolarizing stimuli of increasing intensity (from 0.25 to 1.5 nA) and it was decreased (and eventually disappeared) as the post-stimulus holding potential was clamped from -70 to -100 mV; tetrodotoxin (1 μM) had little effect on the induced I_{ADP} amplitude or duration (c.f. Constanti *et al*., 1993).

The I_{ADP} amplitude evoked by a fixed depolarizating stimulus was also dependent on the concentration of *trans*-ACPD applied (25–100 μM; $n=5$–28), although only a small increment was observed between the size and time-course of tail currents induced by 50 or 100 μM doses (Table 1). The half-maximal effective concentration (EC_{50}) of *trans*-ACPD (estimated from a least-squares sigmoidal curve fit to the averaged data) was 35 μM. This I_{ADP} induction effect was fully developed after 2–5 min of bath-application and remained relatively stable as long as the metabotropic agonist was present, although occassionally, the evoked I_{ADP} amplitude declined after 15–20 min of continuous superfusion. In addition, the neuronal effects of *trans*-ACPD were completely reversible on washout (15–20 min) and could be reliably reproduced following repeated bath-applications of this agonist (every 30–45 min). However, a substantial variability in neuronal responsiveness was observed in response to 50 μM *trans*-ACPD in different cells. In accordance with our previous findings with the muscarinic receptor agonist OXO-M (Constanti & Libri, 1992; Constanti *et al*., 1993; Libri *et al*., 1994), only $\sim50\%$ of the recorded neurones (28 out of 52 cells tested; termed type 1) generated a prominent sADP in response to metabotropic activation and were consequently capable of undergoing a prolonged neuronal excitation following a brief bath application of this agonist. In contrast, the remaining 24 cells (termed type 2), although indistinguishable electrophysiologically from type 1 responding cells (similar membrane properties and firing patterns) gave an analogous, though somewhat weaker discharge in response to 50 μM *trans*-ACPD (or 2–5 μM OXO-M; see below) and failed to generate a prominent (<5 mV) sADP upon stimulation. The excitatory metabotropic response profile was confirmed in 8 neurobiotin-labelled neurones. Five out of eight stained cells showed response properties characteristic of type 1 neurones and were morphologically identified as deep pyramidal neurones located in cortical layer III, whereas three behaved as type 2 responding neurones (located in layer III or layer II–III border) and had variable, non-pyramidal morphology (Libri *et al*., 1994).

Comparison of metabotropic and muscarinic agonist effects

The slow neuronal excitatory effects induced by *trans*-ACPD (50 μM) were comparable with those produced by OXO-M (2–5 μM) (Figure 1a). In addition, the slow inward tail current (I_{ADP}) revealed under 'hybrid' voltage clamp was similar, both in amplitude and duration. Thus, 5 μM OXO-M ($n=21$) produced a slightly more pronounced excitatory effect and I_{ADP} relative to that observed with 50 μM *trans*-ACPD (mean depolarization $=11.2\pm0.5$ mV; $I_{ADP}=0.30\pm0.04$ nA amplitude, 42.3 ± 3.8 s duration) (Figure 1b; Table 1). However, the muscarinic effects were more prolonged than those produced by activation of mGluRs, although the concentration of OXO-M used (5 μM) was 10 times lower than the concentration of *trans*-ACPD (50 μM) (2 min application times) (Figure 1b). On lowering the concentration of OXO-M to 2 μM ($n=6$) a more matched excitatory effect and I_{ADP} to that observed with 50 μM *trans*-ACPD was produced (depolarization $=9.2\pm0.6$ mV; $I_{ADP}=0.18\pm0.05$ nA amplitude, 38.4 ± 3.9 s duration; estimated $EC_{50}=3.5$ μM) (Table 1). In cells showing I_{ADP} decline in the continued presence of 50 μM *trans*-ACPD, the co-application of 2–5 μM OXO-M always induced a larger I_{ADP}, suggesting a desensitization of the metabotropic receptor rather than the I_{ADP} induction mechanism. Other basic biophysical properties of the I_{ADP} tail current induced by OXO-M

Table 1 Comparison of metabotropic and muscarinic agonist effects on olfactory cortical neurones

Drug	*Conc.* (μM)	n	*Depolarization* (mV)[a]	*Increase in* R_{in} *(% change)*[b]	*Increase in no. of spikes (% change)*[c]	I_{ADP} *amplitude* (nA)[d]
trans-ACPD	25	5	4.2 ± 0.5	7.3 ± 1.4	82 ± 20	0.06 ± 0.03
	50	28	9.7 ± 1.2*	14.8 ± 1.6	144 ± 28	0.21 ± 0.03
	100	5	10.3 ± 1.4	15.3 ± 2.1	151 ± 23	0.28 ± 0.05
1**S**,3**R**-ACPD	1	3	$\leqslant2$	$\leqslant2.5$	$\leqslant5$	0.06 ± 0.04
	5	5	5.3 ± 1.0	6.8 ± 1.5	90 ± 22	0.11 ± 0.03
	10	44	10.1 ± 0.9	12.9 ± 2.6	150 ± 46	0.20 ± 0.02
	50	8	12.1 ± 0.7	18.4 ± 2.6	151 ± 44	0.30 ± 0.06
Oxotremorine-M	2	6	9.2 ± 0.6	14.1 ± 1.6	139 ± 31	0.18 ± 0.05
	5	21	11.2 ± 0.5	19.8 ± 2.4	157 ± 35	0.30 ± 0.04
	10	15	14.5 ± 1.3	26.8 ± 3.2	185 ± 56	0.36 ± 0.06

Data are given as mean$\pm$s.e.mean. Each neurone served as its own control. [a] Following a 2 min bath-application. [b] Neuronal input resistance (R_{in}) was tested in response to standard ($\leqslant20$ mV) hyperpolarizing current pulses (160 ms, 0.25 nA). [c] During drug-superfusion and in response to standard depolarizing current pulses (160 ms, 0.25 nA). [d] In response to a standard control stimulus of approximately 0.5 nA and 1.6 s, following a 'hybrid' voltage-clamp protocol. All measurements were performed at -70 mV holding membrane potential (near firing threshold) maintained by positive current injection. Estimated EC_{50} values for *trans*-ACPD, 1**S**,3**R**-ACPD and oxotremorine-M were 35, 9 and 3.5 μM, respectively, determined from least squares regression fits to averaged data (SigmaPlot software) according to the relation: $r=r_{max}(a^{n_H})/(a^{a_H}+K^{n_H})$, where $r=I_{ADP}$ amplitude, $r_{max}=$maximal I_{ADP} amplitude, $a=$agonist concentration, $K=EC_{50}$ and $n_H=$ slope factor. *Measured in 17/28 cells that responded with an initial slow depolarization (see text).

(dependence on external Ca^{2+} or K^+ concentration, voltage sensitivity) (Constanti *et al*., 1993) were generally indistinguishable from those of the I_{ADP} induced by metabotropic activation (data not shown). Likewise, after washout of the two drugs, the depolarization and repetitive firing were sustained for a prolonged period of time. Thus, recovery was typically slow (~30 min) after 2 min superfusion of the slice with 2 (or 5 μM) OXO-M, and in some experiments more than 45 min of washing was required before the discharges ceased (Constanti *et al*., 1993), whereas it was invariably faster (15–20 min) after washout of 50 μM *trans*-ACPD.

Another important difference between muscarinic and metabotropic responses was that the baseline current 'noise' that was typically evident in OXO-M under voltage clamp, and previously described as a tetrodotoxin (TTX)-sensitive background component of synaptic origin (Constanti *et al*., 1993), was characteristically absent during superfusion with *trans*-ACPD (Figure 1b). Finally, in any given experiment, application of OXO-M (2 or 5 μM) always produced a clear membrane depolarization associated with induction of the post-stimulus sADP (depending on the type of neurone recorded i.e. type 1 or 2). In contrast, following superfusion with *trans*-ACPD (50 μM), a substantial intercell variability in the depolarizing response was observed even in cells that generated a large amplitude sADP upon stimulation. The most common metabotropic-agonist response observed (17 out of 28 neurones) was of the type illustrated in Figure 1. However, in 11/28 of the recorded cells, little or no depolarization was observed during agonist application, although a sustained neuronal discharge could surprisingly be induced by injecting a single brief depolarizing stimulus and a prominent sADP could be generated following a larger depolarizing current pulse; this suggests that membrane depolarization is not an invariant requirement for sADP induction following cortical mGluR activation.

As previously shown (Constanti & Libri, 1992; Constanti *et al*., 1993), the muscarinic AChR antagonist atropine inhibited the slow depolarization and I_{ADP} current induced by a subsequent exposure to 10 μM OXO-M, although it failed to reduce the duration and intensity of the slow neuronal excitation or the I_{ADP} tail current induced by 50 μM *trans*-ACPD. Likewise, a similar muscarinic- (but not metabotropic) response block was produced by 15 min pretreatment with the same excitatory phenomena and sADP induction could be triggered by two pharmacologically distinct receptor systems in these neurones.

Activity of various metabotropic receptor agonists

In an attempt to explore the pharmacological profile of the mGluR subtype(s) in guinea-pig olfactory cortical neurones and thus to obtain (indirectly) some information on the likely intracellular transduction mechanisms linked to I_{ADP} induction, the potencies of several putative group selective mGluR agonists were examined. Subsequent experiments were therefore performed with the more potent 1**S**,3**R** enantiomer of ACPD (10 μM, $n=44$) that produced cellular responses indistinguishable from those elicited by 50 μM *trans*-ACPD (Table 1). As observed with *trans*-ACPD, the membrane depolarization induced by 1**S**,3**R**-ACPD was often (26 out of 44 cells tested) preceded by a transient hyperpolarization (2.7 ± 0.2 mV amplitude; 47.4 ± 2.6 s duration) associated with a small (<1%) decrease in input resistance. The active dose range for 1**S**,3**R**-ACPD was within 1–50 μM ($n=3-44$), giving an esti-

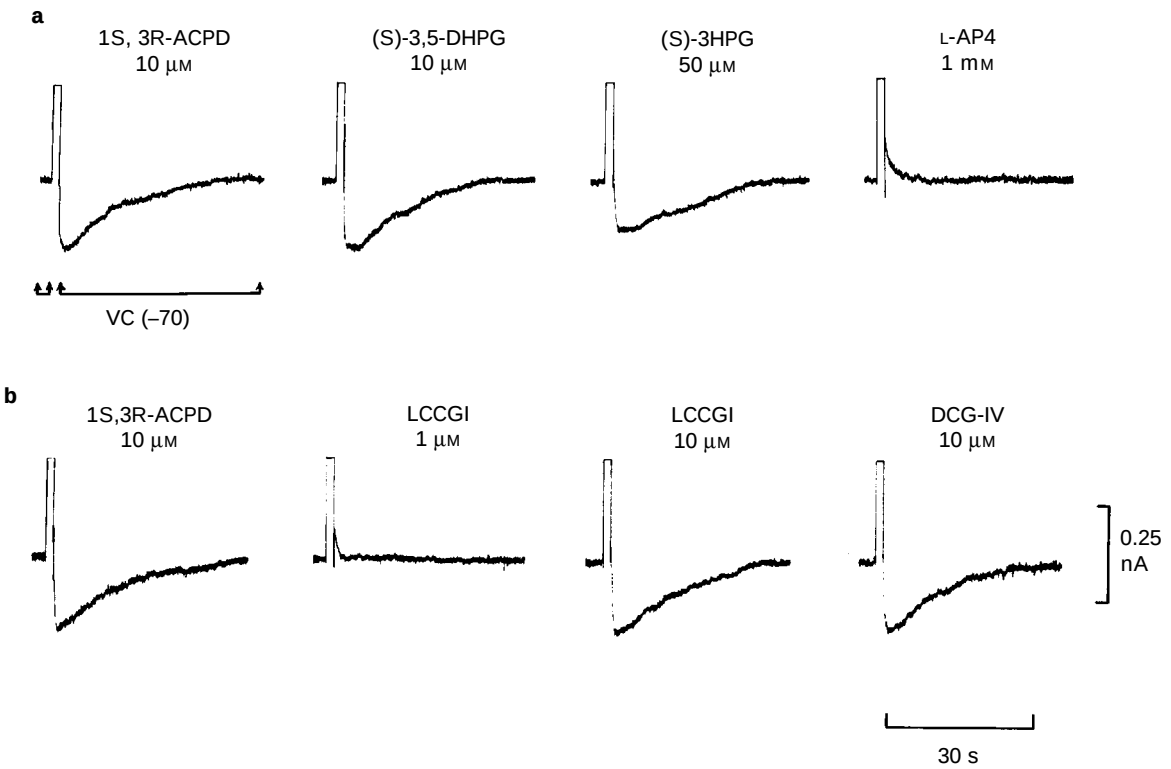

Figure 2 Comparison of I_{ADP} tail currents evoked at −70 mV in the presence of various group selective mGluR agonists, following a 'hybrid' voltage clamp protocol. (a) Only the selective group I mGluR agonists (**S**)-3,5-DHPG (10 μM) and (**S**)-3HPG (50 μM) were effective in generating a post-stimulus I_{ADP} whereas the selective group III mGluR agonist L-AP4 (1 mM) was inactive. (b) In a different cell, bath-application of the proposed mGluR II selective agonists L-CCGI (at 10 μM but not at 1 μM) or DCG-IV (10 μM, in the presence of 100 μM DL-APV) induced the appearance of a post-stimulus I_{ADP} similar to that elicited by 1**S**,3**R**-ACPD (10 μM). The preparation was returned to normal Krebs solution after termination of each agonist application and a 30 min washout period was allowed between each drug superfusion; a set-current stimulus of 0.5 nA, 1.6 s was used throughout. Scale bars apply to all traces.

mated EC_{50} of 9 μM (least squares sigmoidal curve fit to averaged data) (Table 1). Therefore, a dose of 10 μM was considered as a near half-maximally effective concentration and used routinely throughout this study. The activity of the various agonists was assessed by testing their ability to depolarize the recorded neurones and/or to generate a post-stimulus I_{ADP} studied by use of a 'hybrid' voltage-clamp approach. As a convenient measure of relative agonist potency in any one experiment, the amplitude of the I_{ADP} induced in the presence of a given agonist was normalized with the respect to the I_{ADP} amplitude induced initially by a standard 10 μM dose of 1**S**,3**R**-ACPD, so that each neurone served as its own control; a set stimulus intensity of approximately 0.5 nA, 1.6 s was used throughout, being adjusted at the onset of each experiment to evoke 15–20 spikes during the spike train.

The broad spectrum mGluR agonist 1**S**,3**R**-ACPD (50 μM; $n=4$), or the selective group I mGluR agonists (**S**)-3,5-DHPG (10 μM; $n=5$) and (**S**)-3HPG (50 μM; $n=3$) (Birse *et al*., 1993; Hayashi *et al*., 1994; Davies *et al*., 1995) caused a prolonged neuronal excitability increase (e.g. membrane depolarization, reduction in firing accomodation, increase in input resistance and post-stimulus I_{ADP} generation) that was comparable to that evoked by 10 μM 1**S**,3**R**-ACPD, with (**S**)-3,5-DHPG being approximately equivalent in potency and (**S**)-3HPG being about one-fifth as potent. By contrast, the isomer 1**R**,3**S**-ACPD (100 μM; $n=4$), the selective group II mGluR agonist L-CCGI (1 μM; $n=3$) (Hayashi *et al*., 1992) or the selective group III mGluR agonist L-AP4 (up to 1 mM; $n=5$) (Schoepp, 1994; Pin & Duvoisin, 1995) failed to affect the passive membrane properties or to induce an I_{ADP}. However, when the concentration of L-CCGI was increased up to 10 μM ($n=9$), a depolarizating action (including a post-stimulus I_{ADP}) similar to that elicited by 10 μM 1**S**,3**R**-ACPD was observed (Figure 2). This confirms that L-CCGI is a broad spectrum mGluR agonist at concentrations higher than 1 μM (Hayashi *et al*., 1992; Schoepp *et al*., 1994; Davies *et al*., 1995). Surprisingly, similar 1**S**,3**R**-ACPD-like excitatory effects could also be detected on applying the proposed group II mGluR selective agonist DCG-IV (10μM, in the presence of 100 μM DL-APV; $n=3$) (Hayashi *et al*., 1993) (Figure 2; experiments performed in the presence of 100 μM DL-APV to prevent ionotropic-glutamate responses; Ishida *et al*., 1993). The most likely explanation for such an unexpected result is that DCG-IV, like L-CCGI, is a selective agonist for group II mGluRs at low concentrations but also activates group I mGluRs at higher doses (see Discussion).

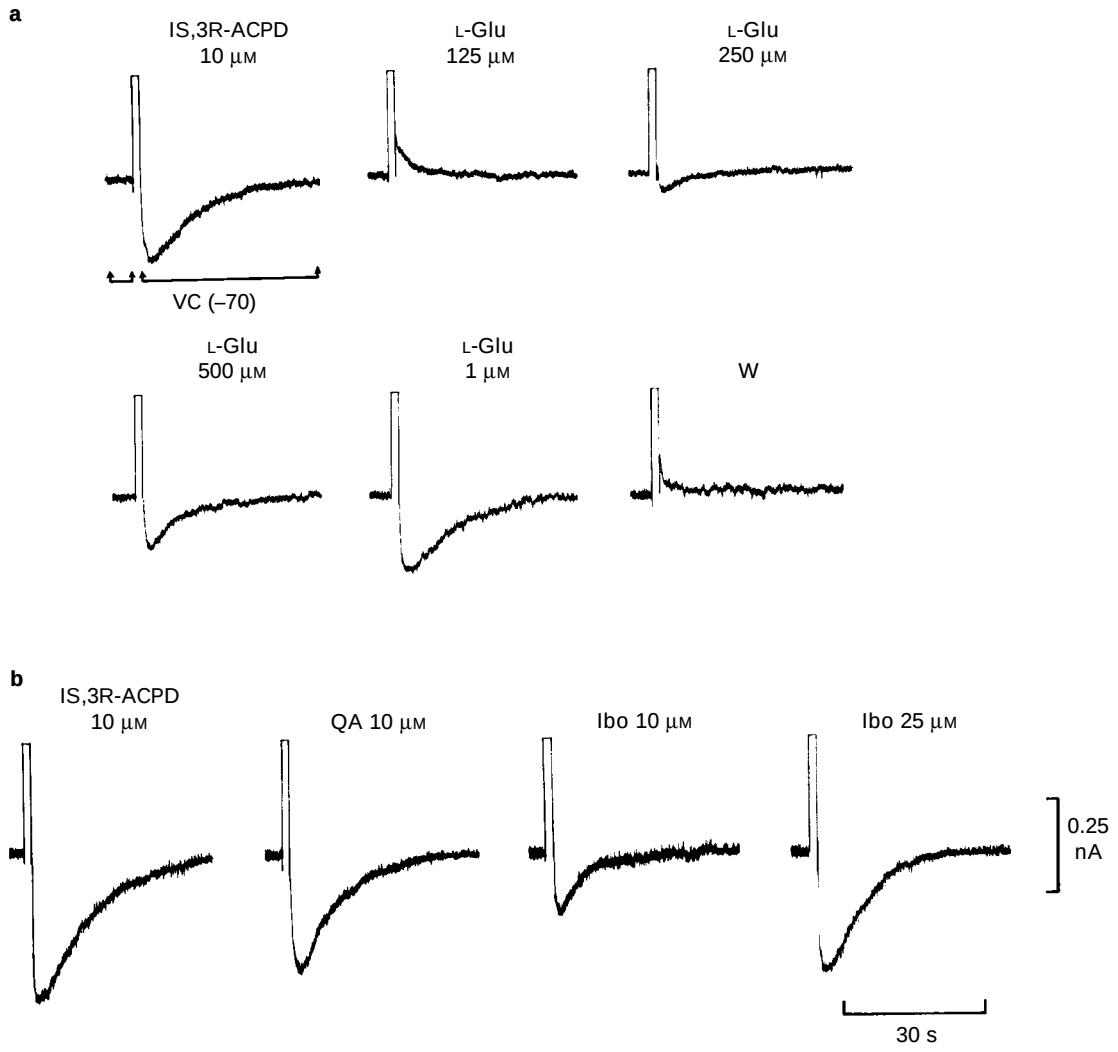

Figure 3 Comparison of I_{ADP} tail currents evoked at −70 mV in response to a standard depolarizing stimulus (0.5 nA, 1.6 s), with a 'hybrid' voltage clamp protocol. (a) In the presence of 1**S**,3**R**-ACPD (10 μM) or increasing concentrations of L-glutamate (0.25–1 mM, in 20 μM CNQX and 100 μM DL-APV); note the progessive increase in I_{ADP} amplitude. W shows recovery after washout of 1 mM glutamate. (b) Different cell: I_{ADP}s induced in the presence of 1**S**,3**R**-ACPD (10 μM), quisqualate (QA: 10 μM, in the presence of 20 μM CNQX) or increasing concentrations of ibotenate (Ibo; 10–25 μM, in 100 μM DL-APV). The preparation was returned to normal Krebs solution after termination of each agonist application and a 30 min washout period was allowed between each drug superfusion. Scale bars apply to all traces.

Table 2 Comparison of activities of various mGluR agonists in inducing an I_{ADP} tail current

mGluR agonists	*Conc.* (μM)	n	I_{ADP} *amplitude* (nA)	*Normalized* I_{ADP}[a]
1**S**,3**R**-ACPD	10	44	0.20±0.02	1
1**S**,3**S**-ACPD	10	3	0.06±0.01	0.26±0.03
	50	4	0.20±0.08	0.88±0.02
(**S**)-3,5-DHPG	10	5	0.21±0.03	0.93±0.03
(**S**)-3HPG	10	2	0.02±0.01	0.09±0.01
	50	3	0.18±0.05	0.78±0.04
L-CCGI	10	9	0.22±0.02	0.94±0.04
DCG-IV[c]	10	3	0.21±0.05	0.93±0.05
L-Glutamate[b]	250	3	0.07±0.03	0.30±0.02
	500	3	0.14±0.07	0.61±0.04
	1000	3	0.22±0.08	0.95±0.03
Ibotenate[c]	10	2	0.09±0.03	0.39±0.05
	25	3	0.20±0.07	0.89±0.08
QA[d]	10	5	0.21±0.01	0.92±0.06

[a] The I_{ADP} amplitude (under 'hybrid' clamp) produced by each agonist was normalized in the same neurone with the respect to the I_{ADP} induced by 10 μM 1**S**,3**R**-ACPD; a set-current stimulus of 0.5 nA, 1.6 s was used throughout. [b] In the presence of 20 μM CNQX and 100 μM DL-APV. [c] In the presence of 100 μM DL-APV. [d] In the presence of 20 μM CNQX. 1**R**,3**S**-ACPD (100 μM; $n=4$), L-AP4 (1 mM; $n=5$) and L-CCGI (1 μM; $n=3$) showed no postsynaptic activity. Data are means±s.e.mean.

The slow neuronal excitatory effects induced by 1**S**,3**R**-ACPD were also compared with those produced by the endogenous neurotransmitter glutamate and the respective NMDA and non-NMDA receptor agonists Ibo and QA, during the continuous presence of 20 μM CNQX and/or 100 μM DL-APV to prevent activation of ionotropic-glutamate receptors. These concentrations of CNQX and DL-APV alone had no effect on membrane properties or action potential duration. Bath-application of L-glutamate (0.25–1 mM, in 20 μM CNQX and 100 μM DL-APV; $n=3$) mimicked the increase in neuronal excitability produced by 1**S**,3**R**-ACPD, in a dose-dependent manner (Figure 3). In contrast, in two neurones, glutamate did not cause any change of the membrane potential even at the highest concentration used (1 mM), and only a small I_{ADP} could be generated upon stimulation. However, in such cells following a 15 min pre-incubation with the selective glutamate-uptake blocker L-aspartic acid-β-hydroxamate (100 μM) (Roberts & Watkins, 1975), the subsequent exposure to 1 mM glutamate was capable of generating a membrane depolarization and post-stimulus I_{ADP}. Finally, superfusion of the slice with 10 μM QA (in the presence of 20 μM CNQX; $n=5$) evoked a membrane depolarization and slow inward tail current comparable to those produced by 10 μM 1**S**,3**R**-ACPD (Figure 3b) whereas 25 μM ibotenic acid (Ibo) (in the presence of 100 μM DL-APV; $n=3$) was required to evoke a similar response. These results are summarized in Table 2.

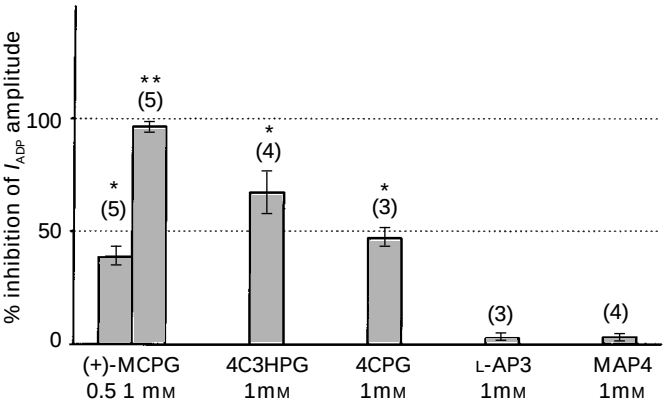

Figure 4 Effects of various group selective mGluR antagonists (15 min preincubation) on the post-stimulus I_{ADP} amplitude induced by a subsequent exposure to a standard 10 μM dose of 1**S**,3**R**-ACPD. Data are expressed as mean percentage inhibition (±s.e.mean) of the peak I_{ADP} amplitude induced by 1**S**,3**R**-ACPD. Numbers in parentheses above each bar indicate number of cells for each experiment. Asterisks indicate significant differences relative to control I_{ADP} amplitude in 1**S**,3**R**-ACPD; (*$P<0.05$; **$P<0.01$, by Wilcoxon signed rank test). The antagonist effects were reversible within 10 min of drug-washout. All measurements were performed at a holding membrane potential of −70 mV following a 'hybrid' voltage clamp protocol; a set-current stimulus of 0.5 nA, 1.6 s was used throughout.

Activity of various metabotropic receptor antagonists

In order to define the relative agonist selectivity across group I and II mGluR subtypes, the phenylglycine derivatives (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG), (**S**)-4-carboxyphenylglycine ((**S**)-4CPG) and (**S**)-4-carboxy-3-hydroxyphenyl glycine ((**S**)-4C3HPG) (Eaton *et al*., 1993; Birse *et al*., 1993; Jane *et al*., 1993; Watkins & Collingridge, 1994) were tested on excitatory responses elicited by 10 μM 1**S**,3**R**-ACPD. In addition, we also examined the effectiveness of the non-selective mGluR antagonist L(+)-2-amino-3-phosphonopropionic acid (L-AP4) (Schoepp, 1994; Pin & Duvoisin, 1995) or the selective group III mGluR antagonist (**S**)-α-methyl-L-AP4 (MAP4) (Salt & Eaton, 1995). To estimate the relative potencies of the various mGluR antagonists tested, we determined in each experiment the percentage depression of the peak I_{ADP} amplitude induced by a standard 10 μM dose of 1**S**,3**R**-ACPD. None of the antagonists applied alone showed any effect on membrane properties or spike frequency adaptation, nor did they induce the appearance of an I_{ADP} upon stimulation. However, all phenylglycine derivatives tested (15 min preincubation) significantly inhibited the slow depolarization and I_{ADP} current induced by a subsequent exposure to 10 μM 1**S**,3**R**-ACPD ($P<0.05$; Wilcoxon signed rank test), without affecting the slow neuronal excitation and I_{ADP} induced by 10 μM OXO-M. (**S**)-4CPG behaved as the weakest antagonist (1 mM; $n=3$) whereas (**S**)-4C3HPG (1 mM; $n=4$) and (+)-MCPG (0.5 and 1 mM; $n=5$ at each concentration) showed a higher antagonist potency, with (+)-MCPG being the most effective (Figure 4). By contrast, L-AP3 (1 mM; $n=3$) and MAP4 (1 mM; $n=4$) were ineffective in antagonizing the 1**S**,3**R**-ACPD-induced excitatory responses (15 min pre-application).

Effects of mGluR agonists and antagonists on synaptic transmission

By means of comparison, we also tested the effectiveness of some mGluR agonists and antagonists on olfactory cortical synaptic transmission. In all recorded neurones ($n=22$), local subthreshold stimulation of cortical excitatory fibre tracts

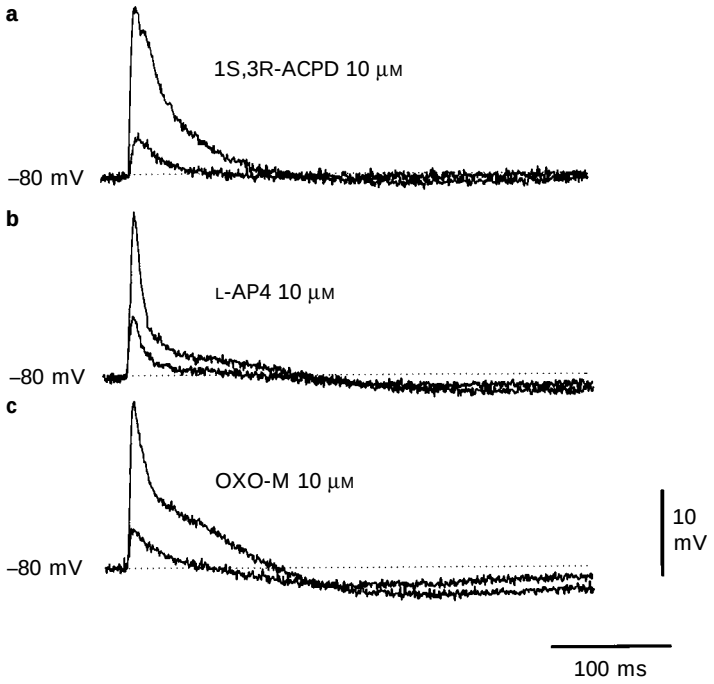

Figure 5 Postsynaptic potentials (p.s.ps) recorded intracellularly at −80 mV membrane potential, in response to orthodromic stimuli (25 V, 0.2 ms) delivered to the lateral olfactory tract (LOT)/association fibre terminals; stimulus strength was just subthreshold for evoking action potentials. P.s.ps evoked during the control period are superimposed to those evoked during a 2 min bath application of 10 μM 1**S**,3**R**-ACPD (a), 10 μM L-AP4 (b) or 10 μM oxotremorine-M (OXO-M,c). The preparation was returned to normal Krebs solution after termination of each agonist application and a 30 min washout period was allowed between each drug superfusion. Scale bars apply to all traces.

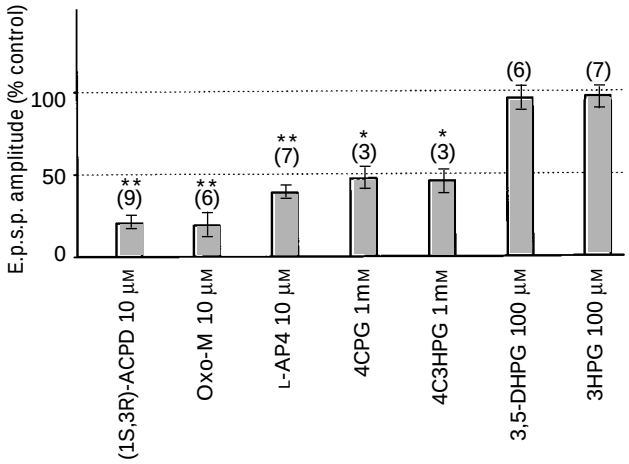

Figure 6 Depression of stimulus-evoked e.p.s.ps in the presence of various group selective mGluR agonists or the muscarinic AChR agonist oxotremorine-M (OXO-M). Data are expressed as mean percentage depression (±s.e.mean) of e.p.s.p. amplitude elicited at resting membrane potential (−80 mV) in response to local subthreshold stimulation of LOT/association fibre terminals (28 V, 0.2 ms). Numbers above each column indicate number of cells for each experiment. Asterisks indicate significant differences relative to control e.p.s.p. amplitude recorded in normal solution; (*$P<0.05$, **$P<0.01$, by Wilcoxon signed rank test). Note that lack of effects of the group I mGluR selective agonists (**S**)-3,5-DHPG or (**S**)-3HPG, even at concentrations higher than those capable of producing a strong membrane depolarization and post-stimulus I_{ADP} (c.f. Figure 2). E.p.s.ps returned to their control amplitudes upon drug washout and a 30 min washout period was allowed between each drug superfusion.

produced a characteristic excitatory/inhibitory postsynaptic potential (e.p.s.p./i.p.s.p.) sequence (Malcangio *et al*., 1995).

The peak e.p.s.p. amplitude evoked at −80 mV membrane potential (18.3±9.2 mV peak amplitude, 28.2±6.2 ms duration) was significantly reduced in the presence of 10 μM 1**S**,3**R**-ACPD (79.6±4.7%; $n=9$) or 10 μM OXO-M (81.1±7.4%; $n=6$) ($P<0.05$; Wilcoxon signed rank tests) (Figure 5; corrected for change in membrane potential). No desensitization of the synaptic metabotropic-glutamate or muscarinic mediated effects was observed during application periods up to 30 min, and evoked e.p.s.ps returned to their control amplitudes upon washout of either agonist (5–10 and 20–30 min, respectively). Likewise, evoked e.p.s.ps were significantly (and reversibly) depressed by exposure to 10 μM L-AP4 (61.2±3.8%; $n=7$; $P<0.05$, Wilcoxon test), without an effect on neuronal membrane properties (Figure 5).

Bath-application of the selective group I mGluR agonists (**S**)-3,5-DHPG ($n=5$) or (**S**)-3HPG ($n=3$) (both at 100 μM), failed to affect the amplitude of the stimulus-evoked synaptic responses. By contrast, the selective mGluR II agonists (mGluR I antagonists) (**S**)-4CPG (1 mM; $n=3$) or (**S**)-4C3HPG (1 mM; $n=3$), caused a significant and reversible reduction of stimulus-evoked e.p.s.ps (>50% inhibition; $P<0.05$, Wilcoxon test) (Figure 6). The depressant activity shown by 1**S**,3**R**-ACPD (0.1–50 μM), L-AP4 (0.1–100 μM) or (**S**)-4C3HPG (0.1–1 mM) was dose-dependent and reproducible, providing an adequate period (>20 min) of intermediate washout was allowed (estimated EC_{50} values; 2, 6 and 86 μM, respectively, by least squares regression; Figure 7).

In the continuous (15 min) presence of the group I/group II mGluR antagonist (+)-MCPG (1 mM), the inhibitory effect of 1**S**,3**R**-ACPD (10 μM; $n=3$) was invariably abolished, whereas

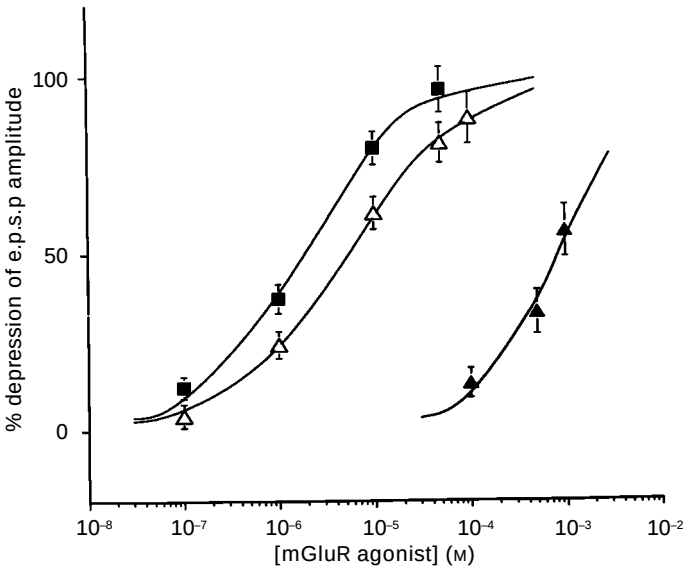

Figure 7 Pooled log dose-response relations for mGluR agonist-induced depression of olfactory cortical synaptic transmission. Data are expressed as mean percentage depression (±s.e.mean; $n=3$ slices at each concentration) of e.p.s.p. amplitude elicited at resting membrane potential in response to subthreshold stimulation of cortical excitatory fibre tracts (25–30 V, 0.2 ms). 1**S**,3**R**-ACPD (0.1–50 μM) (■); L-AP4 (0.1–50 μM) (△); (**S**)-4C3HPG (0.1–1 mM) (▲). E.p.s.ps returned to their control amplitudes upon drug washout and a 30 min washout period was allowed between each drug superfusion. Lines are least squares regression fits to averaged data (SigmaPlot software) according to the relation: % depression = 100 $a^{n_H}/(a^{n_H}+K^{n_H})$, where a = agonist concentration, $K=EC_{50}$ and n_H = slope factor. Estimated EC_{50} values for 1**S**,3**R**-ACPD, L-AP4 and 4C3HPG were 2, 6 and 86 μM, respectively.

L-AP4 (10 μM; $n=3$) was still effective in reducing the amplitude of the stimulus-evoked e.p.s.ps (57.6±4.4% inhibition, not significantly different from control; $P>0.03$, Wilcoxon test). The opposite result was obtained in the presence of the selective group III mGluR antagonist MAP4 (1 mM), with the inhibitory effect of L-AP4 (10 μM; $n=3$) being blocked, and the effect of 1**S**,3**R**-ACPD (10 μM; $n=3$) remaining unaffected (77.3±7.1% inhibition, NS (not significantly) different from control; $P<0.03$). (+)-MCPG or MAP4 alone had no effect on the e.p.s.p. amplitude (Figure 8). Finally, none of the mGluR antagonists tested influenced the synaptic depressant activity induced by 10 μM OXO-M ($n=4$).

Discussion

The present results confirm our original observation (Constanti & Libri, 1992) that activation of postsynaptic mGluRs in guinea-pig olfactory cortex pyramidal neurones results in a prolonged depolarization, increase in cell excitability and induction of a post-stimulus inward tail current (I_{ADP}) similar to those evoked by muscarinic AChR activation. In previous studies, we proposed that the muscarinic I_{ADP} represents a novel Ca^{2+}-sensitive K^+ conductance expressed selectively in 'deep' (cortical layer III) olfactory cortical neurones (Constanti *et al*., 1993; Libri *et al*., 1994). Our present data suggest that a similar ionic mechanism might also underlie the mGluR-induced I_{ADP} in these cells. We have also shown that under some circumstances (e.g. baclofen-induced $GABA_B$ receptor down-regulation) activation of mGluRs (or muscarinic AChRs) may initiate atypical spontaneous cortical epileptiform burst discharges (Libri *et al*., 1995). Interestingly, the prolonged muscarinic and metabotropic-glutamate agonist-induced excitatory responses in guinea-pig olfactory cortical neurones, could be reversibly shortened by the broad spectrum anticonvulsant agent felbamate (Libri *et al*., 1996). These observations suggest that I_{ADP} induction following activation of mGluRs or muscarinic AChRs may well play a crucial role in the control of long-term cortical excitability as well as cortical epileptogenesis.

Comparison between muscarinic and metabotropic-glutamate responses and induced I_{ADP} currents

Although activation of either mGluRs or muscarinic AChRs produced similar persistent neuronal excitations and I_{ADP} tail currents, some notable differences between the metabotropic and muscarinic response profiles were also evident. Firstly, the muscarinic effects (of OXO-M) were more pronounced than those produced by mGluR activation, and recovery was typically slower after OXO-M washout. Secondly, in responding (type 1) cells, muscarinic I_{ADP} induction, was always associated with a clear membrane depolarization, whereas a substantial intercell variability was observed in the depolarizing response to a given dose of mGluR agonist; also, (unlike OXO-M), the metabotropic excitatory effects occassionally desensitized after 15–20 min of continuous agonist superfusion. Similar agonist-induced desensitization of mGluRs (coupled to PI hydrolysis) has been shown in other studies (Catania *et al*., 1991; Herrero *et al*., 1994). The actions of 1**S**,3**R**-ACPD and OXO-M were clearly mediated by activation of mGluRs, and muscarinic AChRs respectively, since the effects of OXO-M were unaffected by selective mGluR antagonists, whereas the actions of 1**S**,3**R**-ACPD were effectively blocked. Likewise, the effects of OXO-M were abolished by atropine or the selective M_1 muscarinic AChR antagonist, pirenzepine, whereas metabotropic-glutamate responses were unaffected by such treatments.

Similar excitatory postsynaptic responses to cholinergic and metabotropic-glutamate stimulants have also been recorded in neurones from several other regions of the mammalian brain (Hasuo *et al*., 1990; Desai & Conn, 1991; Womble & Moises, 1994; Guèrineau *et al*., 1994; 1995; Davies *et al*., 1995). In particular, similar membrane depolarization, loss of spike frequency accommodation, block of slow after-hyperpolarizations and induction of sADPs have been recorded from hippocampal, septal, amygdaloid and necortical neurones, following application of either muscarinic AChR (McCormick & Prince, 1986; Schwindt *et al*., 1988; Hasuo *et al*., 1990; Andrade, 1991; Womble & Moises, 1993) or mGluR agonists (Zheng & Gallagher, 1992; Caeser *et al*., 1993; Womble & Moises, 1994; Greene *et al*., 1994; Davies *et al*., 1995). Muscarinic AChR and mGluR agonists can also modulate a variety of neuronal K^+ or Ca^{2+}-dependent currents in a similar manner (Stratton *et al*., 1989; 1990; Charpak *et al*., 1990; Desai & Conn, 1991; Swartz & Bean, 1992; Trombley & Westbrook, 1992; Sayer *et al*., 1992; Crépel *et al*., 1994; Guèrineau *et al*., 1994; 1995), confirming that cross-talk between muscarinic and metabotropic-glutamate receptors can occur at multiple sites and suggesting that muscarinic AChR- or mGluR-dependent long-term changes in cortical neuronal excitability and sADP generation may be linked to a common intracellular transduction mechanism. It is well known that various muscarinic AChRs and mGluRs are coupled to the same second messenger system in several areas of the brain, e.g. the PI hydrolysis pathway (for references see North, 1989; Shoepp, 1994; Pin & Duvoisin, 1995) leading to the production of diaclyglycerol and inositol (1,4,5)-triphosphate (IP_3) and the release of Ca^{2+} from intracellular stores (Simpson *et al*., 1995).

Since both M_1-type muscarinic AChR and group I mGluRs are coupled to the stimulation of PI turnover and intracellular Ca^{2+} mobilization, it is not unreasonable to speculate that muscarinic AChR- or mGluR-induced sustained depolarization and sADP induction in guinea-pig olfactory cortical neurones may reflect similar mechanisms of PI turnover stimulation and Ca^{2+} mobilization/Ca^{2+}-conductance modulation, which maintain a steady-state of constant depolarization and increased excitability. In keeping with this, in these and

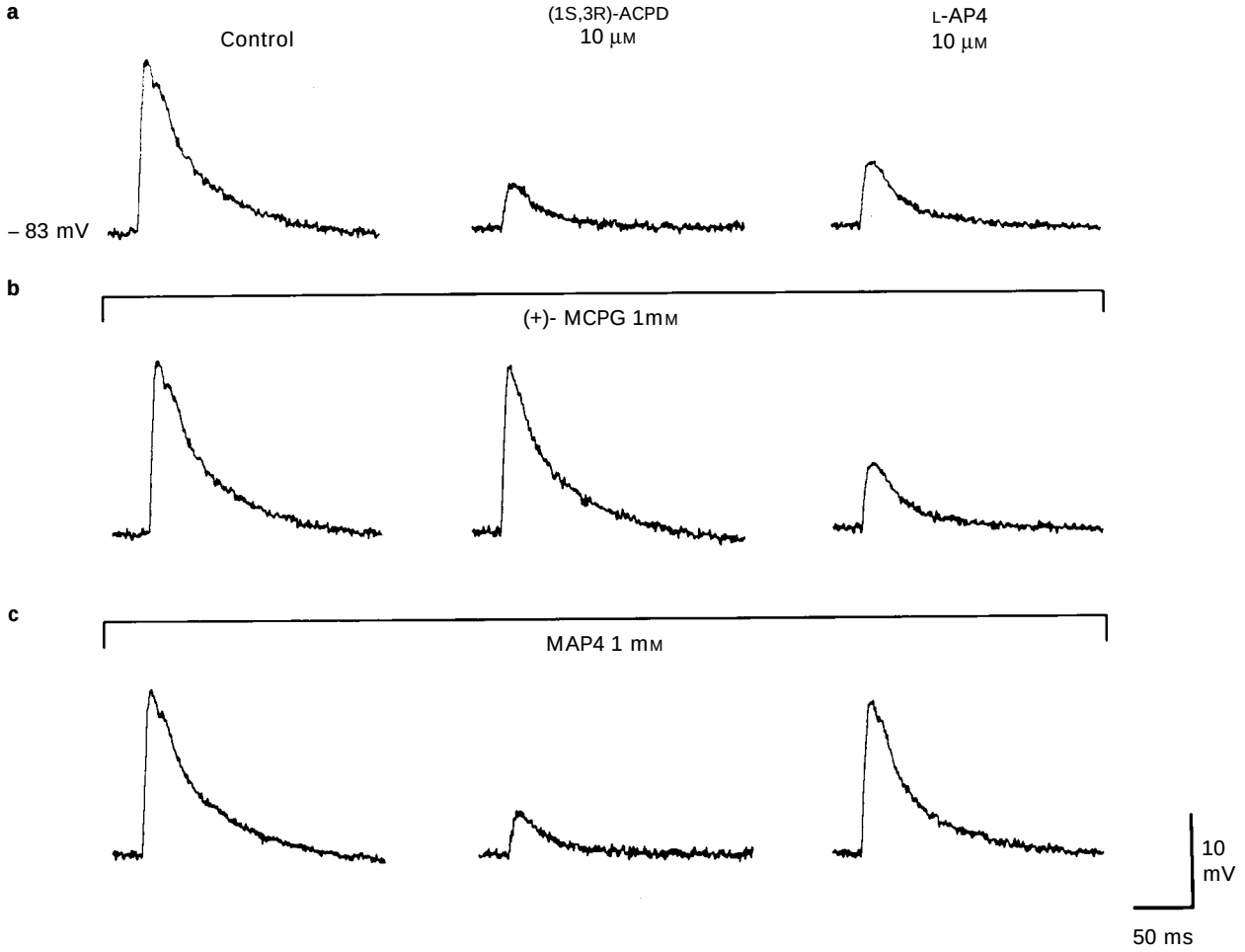

Figure 8 Effects of group selective mGluR antagonists on 1**S**,3**R**-ACPD or L-AP4-induced depression of synaptic transmission in a single olfactory cortical neurone. (a) In control, bath application (2 min) of 10 μM of 1**S**,3**R**-ACPD or L-AP4 markedly reduced the e.p.s.p. amplitude induced at resting membrane potential (−83 mV) by subthreshold cortical stimulation (30 V, 0.2 ms). (b) The inhibitory effect of 1**S**,3**R**-ACPD (but not L-AP4) was abolished in the continuous (15 min) presence of 1 mM (+)-MCPG, whereas in (c) the L-AP4 (but not 1**S**,3**R**-ACPD) effect was blocked in the presence of 1 mM MAP4. (+)-MCPG or MAP4 alone had no effect on the e.p.s.p. amplitude. A 30 min washout period was allowed between each drug superfusion. Scale bars apply to all traces.

previous experiments (Constanti *et al*., 1993) we found that the excitatory effects of OXO-M (including I_{ADP} generation) were reversibly abolished by nanomolar doses of the selective M_1-type muscarinic AChR antagonist pirenzepine. In addition, as discussed below, we found that the pharmacological profile of the mGluR linked to postsynaptic excitation in guinea-pig olfactory cortical neurones is compatible with activation of a group I (PI-coupled), rather than a group II or III (cyclic AMP-coupled) receptor subtype.

Pharmacological profile of postsynaptic olfactory cortical mGluRs: effects of mGluR agonists

Our results indicate that the excitatory effects of mGluR activation in olfactory neurones, including the induction of a post-burst sADP, are most likely mediated by a group I mGluR (mGluR 1 and/or mGluR 5 subtype) or a closely related receptor. This conclusion is based on the observed effectiveness of the agonists tested and the ability of the selective group I mGluR antagonists to prevent the actions of 10 μM 1**S**,3**R**-ACPD. The present findings are also consistent with the proposed distribution of mGluRs in the piriform cortex, where the mRNAs coding for the mGluR 1 and mGluR 5 subtypes predominate over the mGluR 2 and mGluR 3 subtype mRNAs (Shigemoto *et al*., 1992; Ohishi *et al*., 1993).

With regard to mGluR agonist pharmacology, we found that with the exception DCG-IV, only compounds that act selectively at group I mGluRs (e.g. (**S**)-3,5-DHPG, (**S**)-3HPG, QA) and broad spectrum mGluR agonists (e.g. *trans*-ACPD, 1**S**,3**R**-ACPD, 1**S**,3**S**-ACPD, Ibo) were effective in producing a prolonged neuronal excitability increase and a post-stimulus sADP. In contrast, selective group II (e.g. L-CCGI at 1 μM concentration) or group III (e.g. L-AP4, up to 1 mM) mGluR agonists failed to affect the neuronal membrane properties and/or to generate an I_{ADP} tail current upon stimulation. However, on increasing the concentration of L-CCGI up to 10 μM, similar 1**S**,3**R**-ACPD-like postsynaptic effects could also be detected. This is in agreement with previous studies showing that L-CCGI acts as a selective agonist for group II mGluRs at concentrations $\leqslant 1$ μM (Hayashi *et al*., 1992) and as a broad spectrum (groups II and III) mGluR agonist at higher doses (Hayashi *et al*., 1992; Schoepp *et al*., 1994; Davies *et al*., 1995). Unexpectedly, a strong membrane depolarization and the appearance of an I_{ADP} could also be produced on applying the selective group II mGluR agonist DCG-IV. Although this compound potently activates group II mGluR subtypes at low concentrations (with low activity at group III and no activity at group I mGluRs; Hayashi *et al*., 1993), it can also act as an NMDA receptor agonist at concentrations higher than 10 μM (Hayashi *et al*., 1993) and may enhance the stimulation of PI hydrolysis induced in rat hippocampal slices by either QA or submaximal concentrations of Ibo or 1**S**,3**R**-ACPD (Nicoletti *et al*., 1993; Genazzani *et al*., 1994). Interestingly, Schoepp *et*

al. (1996) have shown that activation of group II mGluRs may act synergistically with group I to stimulate 1**S**,3**R**-ACPD-induced increases in cyclic AMP formation in the neonatal rat hippocampus. In the present investigation, the excitatory effects of DCG-IV (10 μM) were insensitive to the NMDA receptor antagonist DL-APV (100 μM), suggesting that they were not of ionotropic origin. Therefore, the question arises whether both mGluR I and II subtypes are involved in the mediation of postsynaptic excitation in olfactory neurones, or whether DCG-IV, like L-CCGI, is a selective agonist for group II mGluRs at low concentrations but also activates group I mGluRs at higher doses. The former possibility seems unlikely since it would imply that the observed metabotropic response is mediated by both an increase in PI hydrolysis (group I mGluRs) and inhibition of cyclic AMP formation (group II mGluRs). However, this cannot completely be excluded, since activation of multiple mGluR subtypes (either PI- or cyclic AMP-coupled) may both mediate Ca^{2+}-channel inhibition in rat cortical neurones (Choi & Lovinger, 1996). It is also possible that the mGluR present on our neurones and sensitive to DCG-IV, is a group I-like receptor (yet to be cloned) closely related in structure to either mGluR 1 or 5, but showing a slightly different pharmacological profile.

Effects of mGluR antagonists

When tested at a concentration of 1 mM, the group I/group II mGluR antagonist (+)-MCPG, and the selective group I mGluR antagonists (**S**)-4CPG and (**S**)-4C3HPG, clearly antagonized both the membrane depolarization and post-stimulus I_{ADP} induced by 10 μM 1**S**,3**R**-ACPD. The estimated order of effectiveness was: (+)-MCPG > (**S**)-4C3HPG > (**S**)-4CPG. In contrast, the unselective mGluR antagonist L-AP3 (see Schoepp, 1994) or the selective group III mGluR antagonist MAP4 failed to reduce 1**S**,3**R**-ACPD-induced responses; (see also Hu & Storm, 1992; Zheng & Gallagher, 1995a). Interestingly, neither (**S**)-4C3HPG nor (**S**)-4CPG, which can act as partial agonists at group II mGluRs (Hayashi *et al*., 1994; Watkins & Collingridge, 1994), altered the membrane properties or induced an I_{ADP}. It has recently been shown that (**S**)-4C3HPG and (**S**)-4CPG have differential effects on the two group I, PI-coupled mGluRs (e.g. mGluR 1 and mGluR 5), being potent antagonists at mGluR 1 but almost inactive at mGluR 5 (Brabet *et al*., 1995). On the above basis, we suggest an involvement of a group I (most likely mGluR 1), or a closely related receptor, in the mediation of metabotropic agonist-induced membrane depolarization and I_{ADP} induction in olfactory cortical cells.

Metabotropic effects of L-glutamate

In the presence of ionotropic-glutamate receptor antagonists, L-glutamate mimicked the increase in neuronal excitability and I_{ADP} induced by 1**S**,3**R**-ACPD, in a dose-dependent manner. Our data are thus consistent with those of Guèrineau *et al*. (1995) who described an inward membrane current activated by 1**S**,3**R**-ACPD, QA or glutamate, in hippocampal neurones. Likewise, a fast inward current (Q_m) evoked by the same agents was identified in 'nonburster' dorsolateral septal neurones (DLSN) (Zheng & Gallagher, 1995a), although glutamate was ineffective on DLSN 'spontaneous bursting' cells (Zheng & Gallagher, 1992). The latter was explained by the possible presence of glutamate-uptake mechanisms in the slice preparation. More recently, a novel type of glutamate-insensitive metabotropic receptor (responsible for inducing burst firing), was proposed to exist on these cells (Zheng & Gallagher, 1995b). Interestingly, in our experiments, high concentrations of glutamate (0.25–1 mM) were necessary to activate postsynaptic mGluRs and, occasionally, glutamate (1 mM) was only effective after a pre-incubation of the slice with the glutamate-uptake blocker L-aspartic acid-β-hydroxamate (Roberts & Watkins, 1975).

Pharmacological profile of presynaptic olfactory cortical mGluRs

Activation of mGluRs reduces synaptic transmission in a variety of brain regions (Jane *et al*., 1994; Vignes *et al*., 1995; Gereau & Conn, 1995; for reviews see Schoepp, 1994; Watkins & Collingridge, 1994; Pin & Duvoisin, 1995). Our findings are in accordance with such previous studies in showing that two pharmacologically distinct mGluR subtypes (e.g. group II and III mGluRs, negatively linked to cyclic AMP formation) might serve as presynaptic inhibitory autoreceptors in the olfactory cortex. Thus, agonists selective for these mGluR subtypes were the most potent in producing reversible depression of evoked synaptic potentials (i.e. the group I/group II mGluR agonist 1**S**,3**R**-ACPD, the selective group III mGluR agonist L-AP4 as well as the selective mGluR II agonists (mGluR I antagonists) (**S**)-4C3HPG and (**S**)-4CPG). The estimated order of agonist effectiveness was: 1**S**,3**R**-ACPD > L-AP4 > (**S**)-4C3HPG > (**S**)-4CPG. Similar synaptic depressant activity was shown by the muscarinic agonist OXO-M as previously demonstrated (Williams & Constanti, 1988; Bagetta & Constanti, 1990), but not by the group I mGluR selective agonists (**S**)-3,5-DHPG or (**S**)-3HPG, even at concentrations higher than those capable of producing a strong presynaptic effect. We also found that the group I/group II mGluR antagonist (+)-MCPG (1 mM) blocked the presynaptic inhibitory effects of 1**S**,3**R**-ACPD but not of L-AP4, while the group III selective mGluR antagonist MAP4 (1 mM) blocked the effects of L-AP4 but not those of 1**S**,3**R**-ACPD. These results are in broad agreement with previous studies on spinal, thalamic and hippocampal neurones (Jane *et al*., 1994; Vignes *et al*., 1995; Manzoni *et al*., 1995). In keeping with the above, we also found that bath-application of the adenylate cyclase activator forskolin (10 μM, $n=3$) increased the e.p.s.p. amplitude by almost 100%, whereas it failed to show any effect on neuronal membrane properties (Libri *et al*., unpublished observations; see also Hopkins & Johnston, 1988; Chavez-Noriega & Stevens, 1994). This supports the idea that changes in presynaptic intracellular cyclic AMP levels can modulate synaptic efficacy in the mammalian brain. It is worth noting that the muscarinic AChR subtype believed to be involved in the suppression of olfactory cortical synaptic transmission is a PI-coupled M_1-receptor rather than a cyclic AMP-coupled M_2-type muscarinic AChR (Bagetta & Constanti, 1990). This apparent discrepancy could be explained on the basis of recent evidence showing that multiple mGluR subtypes, coupled to either an increase in PI hydrolysis (group I mGluRs) or inhibition of cyclic AMP formation (group II and III mGluRs), may be involved in modulation of the same biological events (Gereau & Conn, 1995; Choi & Lovinger, 1996).

In conclusion, from our observed mGluR agonist/antagonist profiles, we propose that group I mGluRs (most likely linked to PI hydrolysis) are involved in the appearance of the prolonged metabotropic-glutamate agonist-induced excitatory postsynaptic response and post-stimulus sADP, in guinea-pig olfactory cortical neurones, whereas at least two pharmacologically distinct mGluRs (most likely group II and III, negatively linked to cyclic AMP formation) may be involved, as presynaptic inhibitory autoreceptors, in the modulation of olfactory cortical synaptic transmission.

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