



The Effects of L-AP4 and L-serine-*O*-phosphate on Inhibition in Primary Somatosensory Cortex of the Adult Rat *In Vivo*

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Summary—The effects of two iontophoretically applied Group III mGluR agonists were studied on the inhibition in neocortex produced by natural stimulation of vibrissae. The agonists L-AP4 and L-serine-*O*-phosphate (L-SOP) were shown to produce qualitatively similar effects on the inhibition. Forty-four percent of neurones (total $n = 57$) displayed disinhibition during application of the agonists. The disinhibitory effects often outlasted the offset of the agonist application by at least 10 min. Concurrent application of the mGluR antagonist (+)- α -methyl-4-carboxyphenylglycine ((+)-MCPG) appeared to reverse the disinhibitory effects of L-AP4 and L-SOP in 3 out of 5 neurones tested. However (+)-MCPG itself was found to have disinhibitory effects in some neurones. Some neurones ($n = 7$) showed increases in inhibition during either L-AP4 or L-SOP application. These appeared most pronounced in those neurones where the initial (pre-drug) inhibition was minimal, perhaps suggesting that the agonists were disinhibiting a local disinhibition. The data obtained in the experiments suggest that the disinhibitory effects are mediated by a heteroreceptor on inhibitory terminals, action at which depresses the release of inhibitory transmitter. The possible role of the modulation of inhibition by presynaptic mGluRs is discussed.

Keywords—Metabotropic glutamate receptors (mGluRs), MCPG, excitatory amino acids, barrel cortex, disinhibition, iontophoresis.

The activation of metabotropic glutamate receptors (mGluRs) in the brain is known to produce profound effects on neurotransmission (Schoepp and Conn, 1993). Our understanding of the possible roles of the three different groups of mGluRs in neocortex is still at an early stage. Some actions of mGluRs appear to involve presynaptic modulation of transmitter release. An early study showed that micromolar concentrations of L-AP4,

a close structural analogue of L-glutamic acid, potently and selectively blocked transmission at lateral perforant path synapses on cells of the dentate gyrus (Koerner and Cotman, 1981). This action was later interpreted as a presynaptic reduction in excitatory transmitter release. A similar presynaptic action of L-AP4 was seen on monosynaptic and polysynaptic responses of cat dorsal horn neurones *in vivo* (Davies and Watkins, 1982). The depression occurred in most neurones studied (22 of 30), and L-AP4 appeared to be more potent than D-AP4. This result appeared to support the contemporary pharmacological studies being done in the isolated spinal cord preparation of the immature rat (Evans *et al.*, 1982). Since then numerous studies have showed a presynaptic depressant effect of L-AP4 on excitatory transmission (e.g. in the hippocampus (Baskys and Malenka, 1991); in piriform cortex (Hasselmo and Bower, 1991). The effects appear to be most pronounced in slices taken from young animals. Little or no effect of L-AP4 was seen on excitatory transmission in adult preparations of the thalamus (Salt and Eaton, 1995); Salt, personal communication), the striatum (Lovinger, 1991), and the neocortex (Cahusac, 1994).

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Abbreviations: 1S,3R-ACPD, (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid; 1S,3S-ACPD, (1S,3S)-1-aminocyclopentane-1,3-dicarboxylic acid; *trans*-ACPD, a 1:1 mixture of 1S,3R-ACPD and 1R,3S-ACPD; L-AP4, L-2-amino-4-phosphonobutyric acid; C, conditioning stimulus; CRF, centre receptive field; C-T, condition-test; IPSC, inhibitory postsynaptic current; L-CCG-I, (2S,3S,4R)- α -(carboxycyclopropyl)-glycine; (+)-MCPG, (+)- α -methyl-4-carboxyphenylglycine; mGluR, metabotropic glutamate receptor type; L-SOP, L-serine-*O*-phosphate; SI, primary somatosensory cortex; SRF, surround receptive field; T, test stimulus; Tc, test stimulus preceded by conditioning stimulus.

Several mGluR agonists (including L-AP4, (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (1*S*,3*R*-ACPD), 1*S*,3*S*-ACPD and *trans*-ACPD) have been shown to produce presynaptic depression of inhibitory transmission in the striatum (Calabresi *et al.*, 1992), olfactory bulb (Hayashi *et al.*, 1993), hippocampus (Desai *et al.*, 1992; Poncer and Miles, 1994), neocortex (Burke and Hablitz, 1994) and ventrobasal thalamus (Salt and Eaton, 1995). In addition, 1*S*,3*R*-ACPD and *trans*-ACPD have been shown to produce presynaptic depression of excitatory transmission in the neocortex of rats aged over 14 days (including adults) (Sladeczek *et al.*, 1993; Cahusac, 1994).

In the neocortex inhibition is mediated by local interneurons, and is important in controlling the temporal and spatial integration of excitatory activity. The present study was carried out to investigate the actions of L-AP4 on the inhibition of the response to a deflected vibrissa in which a preceding deflection had been applied to an adjacent vibrissa within the neurone's receptive field. Such inhibition would be normal during the whisking of vibrissae over an object, as commonly observed in the awake animal. The actions of L-serine-*O*-phosphate (L-SOP) were also studied since it has been shown to act at the same mGluR subtypes as L-AP4 (Tanabe *et al.*, 1993; Okamoto *et al.*, 1994), and has also been proposed as a possible endogenous agonist (Watkins *et al.*, 1990). The actions of (+)- α -methyl-4-carboxyphenylglycine ((+)-MCPG), an mGluR antagonist, were tested on the effects of these agonists.

Some of these data were published in an abstract (Wan and Cahusac, 1995).

METHODS

Adult male Wistar rats ($n = 13$, mean weight 597 g, range 484–787 g, all of age less than 1 yr) were anaesthetized by i.p. injection of urethane (25% solution, up to 2 g/kg), and were prepared for electrophysiological recording by tracheal and jugular vein cannulation. Level of anaesthesia was assessed by the absence of withdrawal responses to firm pinch of the hind limb, by the absence of whisker movements, and by continuous electrocardiogram monitoring on an oscilloscope throughout the duration of the experiment (6–12 hr) (where increased heart rate indicated decreased level of anaesthesia). Body temperature of each animal was maintained at 37°C with a homeostatically controlled heating blanket. The vibrissae on the contralateral side of the hemisphere used for recording were trimmed to 10–15 mm length from the face in preparation for stimulation. A rectangular section of skull was removed from bregma 0–5 mm posterior and 4–7 mm lateral from the midline. Electrode penetrations were made normal to the surface of the brain, and warm agar (2% in phosphate-buffered saline) used to cover the exposed brain. As described previously (Cahusac, 1994), concentric seven-barrelled pipettes made from boro-

silicate glass, with the central barrel filled with a carbon fibre for recording extracellular action potentials, were filled with combinations of the following substances: monosodium L-glutamate (0.5 M, pH 8.5), L-AP4 (0.1 M, pH 8), L-SOP (0.1 M, pH 8.4), (+)-MCPG (0.05 M, pH 9.0), pontamine sky blue dye (2% in 0.5 M Na acetate) and 1 M NaCl (for current balancing and current controls). All drugs were obtained from Tocris Cookson, Bristol. L-SOP was made up fresh on the day of each experiment.

Electrical activity was amplified and filtered (100–10 kHz) using an Axoprobe preamplifier and Neurolog equipment. Activity was continuously monitored visually on an oscilloscope and a filtered audio signal on a loudspeaker. Discriminated spikes were displayed on a second oscilloscope using a delay line, and triggered events including iontophoretic events and currents and stimulus events were collected on a computer hard disk using a CED1401plus interface. Iontophoretic ejection of glutamate (15–40 nA) and vibrissal deflections (using a sable hair paint brush) were used to search for single neurone activity. Synaptic responses were evoked by two piezoelectric bimorph (Vernitron) stimulators positioned on two selected vibrissae. Usually one of the selected vibrissae represented the centre receptive field (CRF) while a second neighbouring vibrissa was also selected and represented the surround receptive field (SRF). The CRF stimulus evoked the largest response with the shortest latencies (typically 6–12 msec) while the SRF stimulus evoked a weaker and longer latency response (Armstrong-James and Fox, 1987). In some experiments both stimuli evoked similar responses, and no CRF/SRF distinction could be made.

A condition-test (C-T) protocol was used in which a conditioning stimulus (C) and test stimulus (T) were presented sequentially, with 3 sec delay between each stimulus. In addition the C stimulus preceded another test stimulus by 50 msec, and this latter stimulus was designated Tc. The C and T (and Tc) stimuli were randomly allocated to different whiskers (of CRF and SRF whiskers), and for a given experiment the chosen stimulus-whisker allocation remained in place throughout the experiment. Analyses of the following 3 different responses was possible. (1) The response to the Tc stimulus, which was inhibited by the preceding C stimulus. (2) The normal response to the T stimulus presented alone. (3) The response to the C stimulus (which immediately preceded the Tc stimulus). Spikes were counted for each of these responses in a 45 msec window starting 5 msec after the stimulus onset (the 5 msec offset was used to remove any stimulus artefacts). An index of inhibition expressing the proportion of change of responses to the Tc stimulus from responses to the T stimulus was calculated using the formula:

$$\% \text{ inhibition} = \left(1 - \frac{T_c}{T}\right) \times 100$$

Agonists were applied for between 2 and 10 min. Recovery from the agonist effects were tested between 5 and 20 min after the end of the agonist application. Each neurone tested with the antagonist (+)-MCPG had previously been shown to display statistically significant disinhibition during L-AP4 or L-SOP application (using the statistical testing procedure described below). The antagonist was then applied for at least 3 min before the agonist application. Then the agonist was applied at the same current and duration as that initially used to produce disinhibition. The antagonist remained on during and for at least 3 min following the agonist application. The antagonist was then switched off, and after a minimum of 5 min the agonist tested once more alone to observe a recovery of disinhibition similar to that seen before the antagonist. Only those experiments where there was significant disinhibition before and after the antagonist application were used in analyses. Experiments were selected as showing an effect of the antagonist against the agonist disinhibitory action where statistically significant differences were not observed during the antagonist application (using the statistical testing procedure described below).

Statistical testing procedure. The data from each experiment was subjected to a two-way factorial ANOVA, in which each cell of the analysis contained data from 30 trials. The interaction between stimulus type (T and Tc) with time of testing (before and during drug application) was examined graphically by plotting the means, and was tested statistically. Individual *t*-test comparisons were then made between responses to the T stimulus before and during drug application, and between responses to the Tc stimulus before and during drug application. Final selection of experiments in which a drug effect was deemed to have occurred was based upon these two final *t*-tests, in which at least one of the *P* values had to be less than 0.05.

At the end of some experiments two dye marks or microlesions were made, one at the end of the track and one more superficial. Following an overdose of anaesthetic, the brain was removed and placed in 10% formaldehyde in phosphate buffered saline for at least one week. Transverse 150 μ m thick sections were cut on a freezing microtome and sites were plotted under the microscope using a drawing tube.

RESULTS

The analyses are based upon data obtained from a total of 57 neurones. In the case of 10 neurones both L-AP4 and L-SOP were tested separately on the same neurones, with the result that a total of 67 experiments were performed. The analyses (e.g. means) presented below will usually represent those obtained from the total number of experiments (rather than the number of neurones), and those analyses relating to numbers of neurones will be identified specifically as such.

The C–T protocol used resulted in significant inhibition

to the test stimulus when it was preceded by the conditioning stimulus (mean 66.6%, SD 20.8%, $n = 67$). An example of inhibition so produced is shown in Fig. 1. In all except 3 experiments (see below) this inhibition was more significant than at the $P < 0.01$ level using a *t*-test.

The effects of iontophoretically applied L-AP4 (median current = 35 nA, range 18–200 nA) on the inhibition were studied in 57 neurones. In 12 neurones, 10 of which had been studied with L-AP4 plus a further 2 neurones, the effects of iontophoretically applied L-SOP (median current = 71 nA, range 50–150 nA) was studied. The mean inhibition for all experiments during the application of the agonists was 56.5% (SD 29.1%), and the change in inhibition during agonist application compared with the mean inhibition before application was small but statistically significant (mean change in inhibition = –10.1%, $t = 3.27$, $df = 66$, $P = 0.0017$). The statistically significant decrease in inhibition during drug application indicates that the general effect of the agonists was to produce disinhibition, since under the null hypothesis of no drug effect the mean percentage change in inhibition would be at 0.

The distribution of percentage changes in inhibition produced by the agonists was examined in detail. The changes were clearly not normally distributed as determined by the Shapiro–Wilk test for normality (in which the percentage changes were correlated with their normal scores, $r = 0.9257$, $n = 67$, $P < 0.01$). The lack of fit with the normal distribution was due to an abundance of

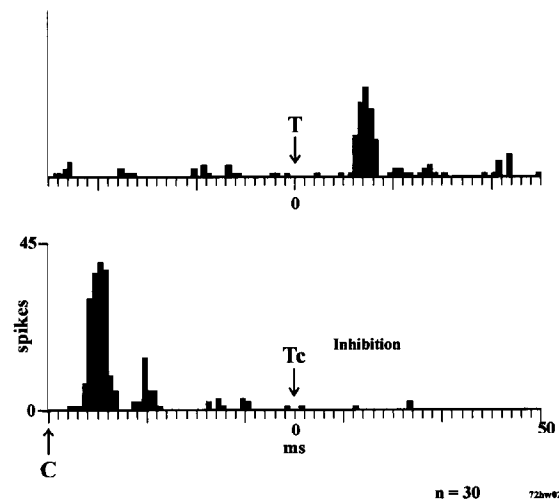


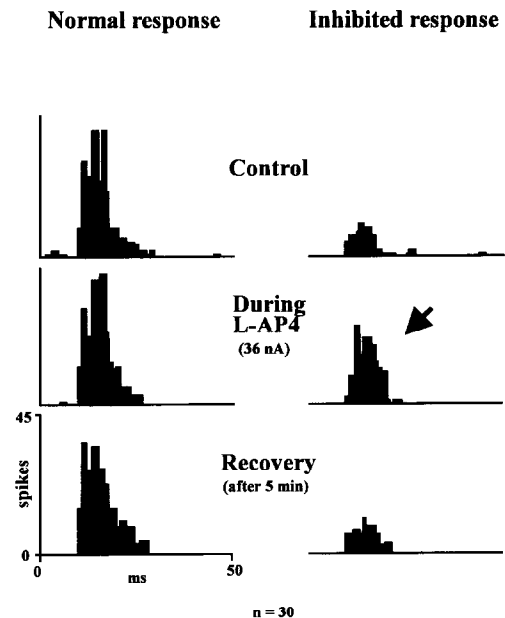
Fig. 1. Peristimulus time histogram illustrating an example of inhibition produced by the C–T protocol in a single SI cortical neurone. The top histogram shows the normal response to stimulus T, whose onset is shown by the labelled arrow at time 0. The histogram below shows the response to the conditioning stimulus C (onset indicated by labelled arrow to the left) and the inhibited response to the following stimulus T (denoted Tc, onset indicated by labelled arrow at time 0). The position of the inhibited response is labelled with “Inhibition”. Each histogram is composed of 30 trials. The vertical axis represents the number of spikes, and the horizontal axis time in msec.

extreme values at both ends of the distribution (i.e. the distribution was leptokurtic). Rather than use arbitrary cut-off points on this distribution the statistical testing procedure described in the Methods section was used. Using this procedure the expected type I error rate would be approx 7 experiments (that is, if there were no effect then in 7 experiments an erroneous conclusion of a significant effect would be made ($2 \times 0.05 \times 67 = 6.7$)). Out of the 67 experiments 33 were found to be statistically significant using the procedure, and this was highly statistically significantly different from the expected number of 6.7 under the null hypothesis ($\chi^2 = 114.7$, $df = 1$, $P < 0.0001$).

Experiments in which there were statistically significant decreases in inhibition, significant increases in inhibition, and those which were not statistically significant were analysed separately. The classification consisted of those neurones that displayed disinhibition during the agonist application, those where the inhibition was increased, and those that were unaffected, respectively.

Disinhibition produced by L-AP4 and L-SOP

In 39% of experiments significant disinhibition was observed during agonist applications. In the case of L-AP4, disinhibition was observed in 21 of 55 experiments, and the mean change in percentage inhibition was $-32.3 \pm 6.0\%$ (\pm SEM). The data from one neurone that displayed disinhibition during L-AP4 is presented in Fig. 2. Disinhibition using L-SOP was observed in 5 of 12 experiments, and the mean change in inhibition was $-19.7 \pm 3.5\%$. The data from experiments in which disinhibition was observed is presented in Table 1 and Table 2. In Fig. 3 a bar diagram displays the mean % change of responses to Tc, T and C stimuli from respective control (before agonist) responses. The disinhibitory effects produced by the two agonists were qualitatively similar and were seen in a similar proportion of experiments, although L-AP4 produced greater disinhibition with lower iontophoretic currents. The analyses of experiments obtained from 10 neurones in which both L-AP4 and L-SOP were studied was examined. In 3 neurones neither agonist produced any effect, and in one neurone disinhibition was produced by both agonists. In 3 neurones L-SOP alone produced disinhibition while L-AP4 had no effect. In 1 neurone L-AP4 alone increased the inhibition, while in the remaining 2 neurones L-SOP alone increased the inhibition. Although in some neurones the effects of the agonists were similar, or there



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Fig. 2. Peristimulus time histograms presenting data from a single SI cortical neurone that displayed disinhibition during the iontophoretic application of L-AP4. The histograms on the left side show the normal responses to the T stimulus, and those on the right show the inhibited responses that result when the T stimulus was preceded by the C stimulus (Tc stimulus). The top pair of histograms show the control responses before agonist application. The middle pair of histograms show the responses during L-AP4 applied with a current of 36 nA. The arrow in this histogram indicates the disinhibition of the response to Tc. The bottom pair of histograms show the recovery of responses back to control levels, 5 min after the offset of L-AP4. Note that the same level of inhibition is apparent as in the control. The level of inhibition for this neurone was decreased by 39% during L-AP4.

was an effect with one compound but not the other, there were no cases of disinhibition caused by one agonist and increased inhibition caused by the other. In many experiments both L-AP4 and L-SOP increased spontaneous activity during their application (see Table 2). In some experiments L-AP4 and L-SOP depressed normal synaptic responses (see mean responses for T and C in Table 2), though in some cases responses were enhanced. As expected there was a significant positive correlation between the effects of the agonists on T and on C stimuli ($r = 0.401$, $n = 67$, $P < 0.002$). These effects were in addition to the observed disinhibitory effects. None of

Table 1. Disinhibitory effects of L-AP4 and L-SOP

	Before agonist (% inhibition)	During agonist (% inhibition)	After agonist (% inhibition)	Agonist current (nA)	<i>n</i>
L-AP4	68.6 ± 4.0	36.4 ± 7.8	51.9 ± 12.2	32 (18–200)	21
L-SOP	63.6 ± 8.5	43.9 ± 10.1	52.3 ± 11.2	80 (50–150)	5

For each agonist the mean % inhibition \pm SEM is shown before, during, and after (recovery) iontophoretic application. The next column shows the median (with range) iontophoretic currents used. The final column gives the numbers of neurones. The disinhibitory effect of L-AP4 appeared to be greater than that of L-SOP, and lower currents were required.

Table 2. Mean firing rates in spikes/sec (\pm SEM) for before, during, and after iontophoretic application of L-AP4 and L-SOP, in those experiments in which disinhibition occurred

		Before agonist spikes/sec	During agonist spikes/sec	After agonist spikes/sec	Number of experiments
L-AP4	Spont.	12.0 \pm 3.7	13.7 \pm 4.9	11.4 \pm 3.6	21
	T	105.3 \pm 12.6	90.9 \pm 11.2	94.9 \pm 13.4	
	Tc	33.8 \pm 5.6	53.2 \pm 7.2	38.6 \pm 6.8	
	C	111.8 \pm 17.8	109.3 \pm 15.2	114.1 \pm 18.1	
L-SOP	Spont.	6.8 \pm 1.1	10.8 \pm 3.8	8.3 \pm 1.1	5
	T	146.6 \pm 27.1	135.0 \pm 28.7	145.1 \pm 33.1	
	Tc	50.2 \pm 14.1	69.6 \pm 14.8	64.4 \pm 15.9	
	C	131.2 \pm 18.7	138.4 \pm 24.2	138.0 \pm 21.5	

Spontaneous activity (Spont.), response to the test stimulus alone (T), response to the test stimulus when preceded by the conditioning stimulus (Tc), and response to the conditioning stimulus (C), are given in 4 different rows for each agonist. Number of experiments is given in the right column.

these effects outlasted the offset of the agonist application, since the responses after agonist application (in recovery) were back to those observed before agonist application. This contrasted with the normally long lasting disinhibitory effects of both the agonists (see Table 1 and Table 2). In the protocol used the recovery of inhibition was usually assessed 5–10 min after the end of the agonist application. In some cases the disinhibition was still apparent 20 min after the end of the agonist application, which was the longest recovery period used. The actual duration of the disinhibitory effects was therefore not determined in this study.

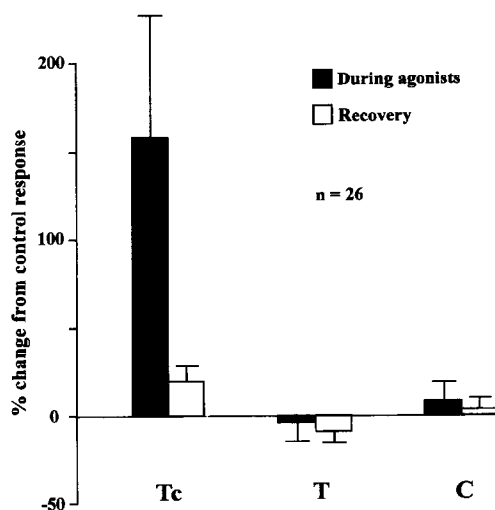


Fig. 3. Bar diagram of data from the 26 experiments that displayed disinhibition during L-AP4 or L-SOP. The height of the bars represents the mean % change from the control responses. The bars on the left represent the data from responses to the Tc stimulus. The mean % change in the response during agonist application (dark bar) was increased by over 150% compared with responses to the Tc control stimulus. The recovery of responses (open bar) to the Tc stimulus following agonist offset showed a small residual increased response. The mean % change in responses to the other stimuli, T and C, during and after (recovery) agonist application were small compared with the mean change seen to Tc. Standard errors of each mean are indicated on the bars.

Increases in inhibition produced by L-AP4 and L-SOP

In a few experiments ($n = 7$, of 67) statistically significant increases in inhibition, rather than disinhibition, were observed (see Table 3 and Table 4). Such increased inhibition was seen with both L-AP4 ($n = 5$) and L-SOP ($n = 2$). It is notable that the greatest increases in inhibition (mean level of inhibition during agonists $60.7 \pm 14.7\%$) were seen in those 3 experiments (of 67) that displayed the weakest inhibition before agonist application (mean $13.7 \pm 16.1\%$). In these 3 experiments a similar change in inhibition due to the agonists was observed (mean percentage change in the inhibition was $47.0 \pm 1.4\%$). In two of these experiments the inhibition before agonist application was not significant using a t -test at the 0.05 significance level, while in the third the inhibition was marginally significant ($P = 0.039$). As stated above the inhibition produced by the C–T protocol in all the other experiments was more significant than at the 0.01 level. A comparison between percentage inhibition before agonist application for experiments which subsequently showed increased inhibition with those that showed disinhibition revealed a statistically significant difference (mean difference = -27.7% , $t = 3.06$, $df = 31$, $P = 0.0045$). Furthermore there was a statistically significant correlation between the change in inhibition and the amount of inhibition before drug application for those experiments in which agonist effects (either disinhibition or increased inhibition) were observed ($r = -0.373$, $n = 33$, $P = 0.032$). The results of these analyses suggest that the direction of the effects of the agonists is conditional upon the level of inhibition prior to agonist application.

Actions of (+)-MCPG

The disinhibition produced by the agonists could be reversibly blocked by the mGluR antagonist (+)-MCPG (180–195 nA) in 3 of 5 neurones tested (2 of 4 used L-AP4, and 1 of 1 used L-SOP as the agonist). The combined data obtained from the 3 neurones in which blockade occurred are shown in Fig. 4. In each of the neurones disinhibition of a similar level to that initially observed with

Table 3. Increased inhibition observed in 5 neurones during L-AP4 ($n = 5$) and L-SOP ($n = 2$) application

Before agonist (% inhibition)	During agonist (% inhibition)	After agonist % (inhibition)	Agonist current (nA)	<i>n</i>
39.9 ± 11.7	67.7 ± 7.9	58.3 ± 7.6	55 (20–85)	7

The same categories of information are presented as those in Table 1, figures given \pm SEM. Note that the degree of inhibition before agonist application is lower than the corresponding means given in Table 1.

the agonist was seen when the antagonist application was terminated (after at least 5 min). This shows that the blockade of the disinhibitory effect of the agonists was reversible.

However (+)-MCPG itself had a significant disinhibitory effect in 3 of the neurones, but in none of these was the disinhibition enhanced during concurrent agonist application. In two of these neurones (+)-MCPG did not reverse the agonist induced disinhibition, however in the third neurone the disinhibition was reversed. Given these results the apparent antagonism by (+)-MCPG should be interpreted with caution.

Other analyses

Further analyses were performed on the data in order to determine any factors that might be predictive of a disinhibitory action of the agonists, and also to obtain clues about the location of the receptors mediating the disinhibition. No predictive factors were discovered from the following: the current of agonist application, the age of the animals used, the magnitude of the conditioning stimulus relative to that of the test stimulus. Analysis of the latter factor indicates that disinhibition does not depend upon the CRF or SRF nature of the C and of the T stimuli.

To provide clues about the location of the receptors the various actions of the agonists were compared across neurones that had been classified according to spike width. Neurones could be classified as narrow spike units if their extracellularly recorded action potential widths were less than 0.7 msec. Neurones with spike widths that exceeded 1 msec were classified as wide spike units. These two classes of units are believed to correspond to inhibitory interneurons and to excitatory pyramidal neurones (Simons, 1978; Connors and Gutnick, 1990). Not all recordings from neurones could be readily classified (e.g. some spike widths were intermediate between 0.7 and 1 msec). Both narrow spike ($n = 5$) and

wide spike ($n = 16$) neurones displayed disinhibition to the agonists (5 were unclassified). Five neurones displaying increased inhibition were wide spike and 2 were narrow spike neurones. Irrespective of the agonist effects category, the agonists had no greater effect on responses to the T stimulus in narrow spike units ($89.3 \pm 7.6\%$ of before) compared with those responses in wide spike units ($96.8 \pm 4.0\%$). Similarly there were no differences between effects on responses to the C stimulus in narrow ($119.1 \pm 19.9\%$) compared with wide ($101.1 \pm 5.9\%$) spike neurones. Analysis of those experiments categorized as showing disinhibition indicated possible (though statistically non-significant) differences between narrow (T stimulus: $82.1 \pm 15.0\%$; C stimulus: $122.4 \pm 31.0\%$) and wide (T stimulus: $88.6 \pm 6.0\%$; C stimulus: $106.1 \pm 12.0\%$) spike neurones. For this category of neurone the disinhibition produced in narrow spike units was not significantly greater than that observed in wide spike units (changes of $-45.6 \pm 19\%$ and $-25.8 \pm 5.3\%$ respectively, $t = 1.43$, $df = 19$, $P = 0.17$). Further, in narrow spike units only, there appeared to be a correlation between the amount of disinhibition produced and the amount of depression of responses to the T stimulus ($r = 0.975$, $n = 5$, $P = 0.005$). Finally, in these 5 neurones the agonists had no effect on spontaneous firing (24.8 spikes/sec before application and 22.7 spikes/sec during application, $t = -0.26$, $n = 5$, $P = 0.81$). Those categorized as showing increased inhibition tended to have enhanced responses to the T stimulus during drug application ($114.6 \pm 9.9\%$) and to the C stimulus ($122.2 \pm 17.3\%$), though neither of these effects were statistically significantly different from 100%. In summary these results show that both presumed inhibitory and presumed excitatory neurones display disinhibition to the application of the agonists. The disinhibition displayed by presumed inhibitory neurones was not significantly different to that displayed by presumed excitatory neurones. Also for these neurones there was a positive association between the degree of disinhibition

Table 4. Mean firing rates in spikes/sec (\pm SEM) for before, during, and after iontophoretic application of L-AP4 and L-SOP, in those experiments in which increased inhibition occurred. The same categories of information are presented as those in Table 2

		Before agonist spikes/sec	During agonist spikes/sec	After agonist spikes/sec	Number of experiments
L-AP4 & L-SOP	Spont.	14.5 ± 4.4	15.8 ± 5.9	18.0 ± 6.1	7
	T	99.1 ± 21.2	113.4 ± 28.5	136.2 ± 25.7	
	Tc	48.5 ± 6.8	30.2 ± 9.3	47.5 ± 7.5	
	C	107.6 ± 22.5	130.4 ± 34.8	128.2 ± 22.2	

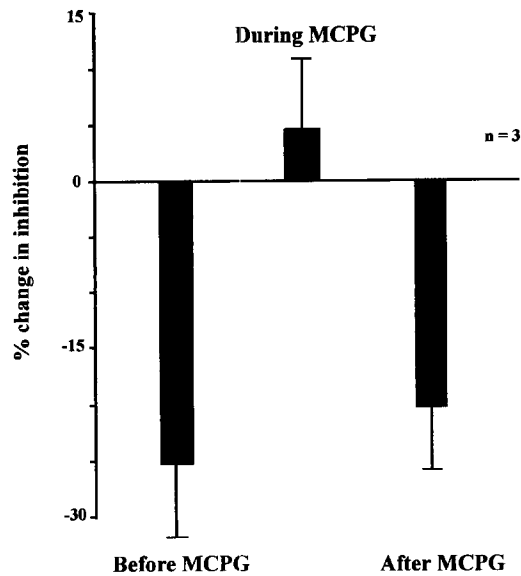


Fig. 4. Bar diagram illustrating the antagonism by (+)-MCPG of disinhibition produced by L-AP4 and L-SOP. The left bar represents the mean % change in inhibition produced by the agonists before the antagonist application. The disinhibition is shown by the decreased level (by 25%) of inhibition. The middle bar represents the mean % change in inhibition during agonist application concurrent with (+)-MCPG (180–190 nA). This shows a block of the disinhibition (the mean % inhibition is actually slightly increased). The right hand bar represents the recovery of disinhibition produced by the agonists, showing that the antagonism was reversible. Antagonism was seen in 3 of the 5 cells tested. Standard errors of the means are indicated on the bars.

produced and the degree of depression of responses to the T stimulus alone.

Recording sites in SI cortex

Figure 5 shows the positions of recording sites made in SI cortex at which disinhibition and increased inhibition were observed. There did not appear to be any layer-specific preferences for disinhibition, as disinhibition was observed in all layers (II–VI) sampled. The sites at which increased inhibition was observed were all in layers V–VI, and microdrive readings from the remaining (unplotted) cells suggest that sites were located between layers IV–VI.

DISCUSSION

Inhibition was produced in single SI cortical neurones using a C–T protocol in which stimulation of one vibrissa preceded the stimulation of a second vibrissa by 50 msec. The inhibition so produced was significantly reduced in about 44% of neurones studied using iontophoretically applied L-AP4 and L-SOP. Both compounds produced qualitatively similar effects, although L-AP4 tended to produce greater disinhibition with lower iontophoretic currents. The results are consistent with an action at the same type of receptor within the Group III class of mGluRs (Tanabe *et al.*, 1993; Okamoto *et al.*, 1994). The

disinhibitory effects were strongly reduced by the mGluR antagonist (+)-MCPG, suggesting that the effects were mediated by mGluRs. This antagonist has been shown to block the presynaptic depressant effects of L-AP4 on neonatal rat motoneurons (Kemp *et al.*, 1994), and was shown to block the reduction of miniature IPSC frequency induced by *trans*-ACPD (Poncer and Miles, 1994). However, in the present study (+)-MCPG itself was found to produce disinhibition. This suggests that it may be acting as an agonist in some capacity but at a different receptor to L-AP4 and L-SOP, since the disinhibitory effects of (+)-MCPG were not additive to those of L-AP4 and L-SOP (and in one case the disinhibition so produced was blocked by (+)-MCPG). Although (+)-MCPG has been shown to be an antagonist at mGluR1 and mGluR2, it has no actions at mGluR4 (Hayashi *et al.*, 1994). To our knowledge the spectrum of actions of (+)-MCPG at other mGluRs has not been studied. Of Group III mGluRs, mGluR6 is not found in the cortex, while mGluR4 has a restricted distribution there (Tanabe *et al.*, 1993). In contrast mGluR7 is seen throughout cortex (Okamoto *et al.*, 1994), and therefore is a candidate for the mediation of disinhibitory effects observed in SI cortex in the present study. It is possible that additional receptor types were involved in producing the other effects sometimes observed in this study, such as the increases in inhibition and the depression of excitatory transmission.

Presynaptic depression of neurotransmission produced by various mGluR agonists was often described as “L-AP4-like”, and many reports appeared to equate the

- Disinhibition
- Increase in inhibition

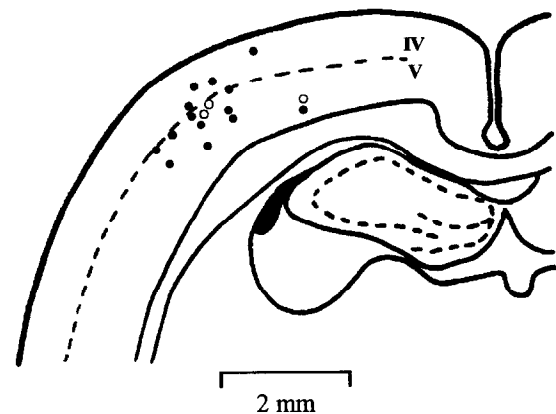


Fig. 5. The recording sites of 14 neurones in which disinhibition (●) and increased inhibition (○) was observed to agonist applications. The sites are plotted on a diagram of a transverse section through SI cortex at 2.5 mm posterior to bregma. The dotted line shown through the cortex represents the border between layers IV and V. Disinhibition was observed in all layers, from layers II–VI.

actions of the ACPD isomers with the same receptor as that at which L-AP4 acts (Pook *et al.*, 1992). However, closer reading of the earlier literature supports the notion for different presynaptic receptors. For example L-AP4 at 100 μ M had no effect on excitatory input to striatal cells, but the excitatory input was depressed by *trans*-ACPD (Lovinger, 1991); and the effects of L-AP4 and *trans*-ACPD were additive (Rainnie and Shinnick-Gallagher, 1992). A study in SI cortex (Cahusac, 1994) found the actions of L-AP4 to be qualitatively quite different to the presynaptic depressant effects of 1*S*,3*R*-ACPD on excitatory synaptic responses tested on the same neurones. Recently, with the advent of new selective antagonists it has been shown that the presynaptic actions (on excitatory and inhibitory transmission) of L-AP4 can be discriminated from those of 1*S*,3*R*-ACPD, 1*S*,3*S*-ACPD and (2*S*,3*S*,4*R*)- α -(carboxycyclopropyl)-glycine (L-CCG-I) (Jane *et al.*, 1994; Salt and Eaton, 1995). These data would suggest that L-AP4 presynaptic actions are mediated by mGluR4/7 and that 1*S*,3*R*-ACPD, 1*S*,3*S*-ACPD and L-CCG-I actions are mediated by mGluR2/3.

In a recent study (Salt and Eaton, 1995) the disinhibitory actions of L-AP4 in thalamus were interpreted as an action on inhibitory terminals, since inhibitory cell bodies and dendrites are remotely situated in the reticular thalamic nucleus. In the cortex the interpretation is complicated by the fact that inhibitory neurones are intrinsic to cortex. There are at least three possible locations for the mGluRs responsible for the disinhibitory effects observed in the present study. The most likely locations of these mGluRs are: (1) on the terminals of local inhibitory interneurons (as a heteroreceptor), their activation leading to a reduction in the release of GABA; (2) on excitatory terminals made onto inhibitory interneurons (as an autoreceptor), their activation leading to a reduction in the release of excitatory transmitter; (3) on inhibitory interneuron dendrites and cell bodies, their activation leading to a depression in firing and hence decrease in inhibitory transmission to postsynaptic targets. These possibilities are not necessarily mutually exclusive. The first two possibilities require further consideration, while the third possibility may be discounted since the spontaneous firing of narrow spike neurones (presumed inhibitory) generally was unaffected by the agonists. If (2) was true then excitatory transmission onto narrow spike neurones would tend to be reduced. In general this was not found, since the responses of these neurones to the T stimulus alone during drug application were 89.3% of control (before drug), and their responses to the C stimulus were 119.1% of control. Both these figures were not significantly different from the expected 100%, and their mean across T and C was 104.2%. However, the data obtained from those narrow spike neurones that displayed disinhibition indicated that their responses to the T alone stimulus was reduced in proportion to the magnitude of disinhibition. This intriguing result suggests that disinhibition in inhibitory neurones may result, in part at least, from the selective

depression of excitatory input to these neurones (and thus reduce feedback inhibition to themselves). However, in general, the results from this study provide support for (1) since disinhibition occurred with little or no change to either spontaneous firing or to excitatory transmission onto presumed excitatory and inhibitory neurones. The evidence obtained in the present study is therefore most consistent with a presynaptic location on inhibitory interneurons.

The present study revealed that during agonist application approx 44% of 57 neurones displayed disinhibition and 12% displayed increases in inhibition. There was evidence that the direction of change in inhibition was conditional upon the initial level of inhibition produced by the C-T protocol. These results are in contrast to those obtained in the thalamus (Salt and Eaton, 1995) where apparently all neurones displayed disinhibition to iontophoretically applied L-AP4 using a similar C-T protocol to the present study. In addition the level of disinhibition caused by the agonists was lower in cortex than in thalamus [for L-AP4 the mean change in inhibition was -30% for cortex and -56% for thalamus (Salt and Eaton, 1995)]. The disinhibition observed in cortex was often found to outlast the application of agonist by at least several minutes (sometimes for over 20 min), again in contrast to the limited temporal effects of L-AP4 observed in thalamus (Salt and Eaton, 1995; Salt, personal communication). These findings further demonstrate the differences between cortex and thalamus in mGluR mediated effects. In a previous study in SI cortex the mGluR agonist *trans*-ACPD (and 1*S*,3*R*-ACPD) was found to have excitatory and depressant effects that depended upon the cortical layer (Cahusac, 1994). In a recent study using the same C-T protocol as the present study the results obtained clearly demonstrated that 1*S*,3*R*-ACPD had actions on neurotransmission that were generally distinct from those produced by L-AP4 (Wan and Cahusac, 1995a). Thus in most cells studied 1*S*,3*R*-ACPD depressed excitatory synaptic transmission without affecting inhibitory transmission. In only a few cells (4 of 23) did 1*S*,3*R*-ACPD reduce inhibition as well as strongly reducing excitatory responses to T and C stimuli. However the likelihood was that this reduction in inhibition was as a direct result of the reduced excitatory input to the recorded and neighbouring neurones. The results obtained with 1*S*,3*R*-ACPD (and *trans*-ACPD) are consistent with previous studies demonstrating depressant effects on excitatory transmission in neocortex (Sladeczek *et al.*, 1993; Cahusac, 1994). The presynaptic depressant effect of 1*S*,3*R*-ACPD on excitatory transmission in cortex is in contrast to the uniformly excitatory postsynaptic effects of this compound seen in the thalamus (Salt and Eaton, 1991). Such regional differences in mGluR mediated effects are not surprising given the distinct anatomical and functional characteristics of these brain areas (Sherman and Koch, 1990; Douglas and Martin, 1990).

This study also found preliminary evidence for the

agonists *increasing* the level of inhibition when the neurone under study was subject to weak inhibition using the C–T protocol. One interpretation of the increased inhibition is that the agonists disinhibit local disinhibition. Local disinhibition would appear to have been the case for this category of neurone since they showed significantly less (in 2 cases not significant) inhibition to the C–T protocol prior to agonist application. The iontophoretically applied agonists could then have a preferential effect on inhibitory terminals made onto inhibitory neurones that in turn then synapse onto the recorded neurone. The disinhibition of an inhibitory circuit (that was itself disinhibited by the C–T protocol) thus results in increased inhibition observed in the recorded neurone. The agonists could produce a differential effect by acting only on inhibitory terminals made on local inhibitory interneurons in the vicinity of the recorded neurone. These disinhibited inhibitory neurones would then inhibit the recorded neurone by more distant contacts (perhaps some distance up the apical dendrite) made onto the recorded neurone, and these contact sites would be too distant for the agonist to reach by diffusion.

Physiological activation of a heteroreceptor on inhibitory neurone terminals by excitatory transmitter (such as glutamate) could occur in cortex if inhibitory and excitatory terminals are sufficiently close to each other. No presynaptic contacts onto inhibitory (or excitatory) terminals have been identified in neocortex. It is of interest that in primary visual cortex some 26% of all GABAergic synapses are made onto dendritic spines, in close proximity to excitatory synapses (Beaulieu and Somogyi, 1990), and thus may be accessible to normal or high release of excitatory neurotransmitter. A similar apposition of inhibitory and excitatory terminals is likely to exist in SI cortex, allowing glutamate (released in excess) to act on extrasynaptic mGluRs on inhibitory terminals. It has also been proposed that L-SOP may be the endogenous agonist for L-AP4 receptors, instead of glutamate (Watkins *et al.*, 1990), but the cellular localization and site of release of L-SOP remain to be established. If these receptors are of physiological importance some consideration must be given to their specific role in cortex. These receptors would provide a mechanism by which inhibition can be modulated. This would be important in controlling the precise temporal and spatial integration of neural firing in SI cortex. Specifically, the modulation would tend to enhance strong excitatory inputs to a neurone by reducing the inhibition that would normally attend such high activity, in particular feedback inhibition but also lateral inhibition from neighbouring neurone assemblies or barrels (as apparent in this work). The signal-to-noise ratio of strongly excited neurones compared with weakly excited neurones would be increased. An alternative way of viewing this situation is to say that the competition between differentially active neurones is enhanced, and so serves as a contrast enhancement mechanism between single neurones or between assemblies of neurones. The apparently long-lasting

disinhibition seen in this study (more than 10 min after the end of agonist applications) suggests that these mGluRs have some role in activity-dependent plasticity, and this aspect deserves further investigation.

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