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Inflammation alters the effects of mGlu receptor agonists on spinal nociceptive neurones

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Abstract

Several types of metabotropic glutamate receptor are known to be located in the spinal cord. This study examined the effects of the metabotropic glutamate receptor agonists (1S,3R)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3R)-ACPD), (*S*)-3,5-dihydroxyphenylglycine ((*S*)-3,5-DHPG) and (1S,3S)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3S)-ACPD) on the electrically evoked responses of dorsal horn neurones recorded in normal animals and in animals 3 h after the induction of carrageenan inflammation. The group I and II agonist (1S,3R)-ACPD produced facilitations of the noxious evoked neuronal responses in normal animals, but inhibited these responses following carrageenan inflammation. The group II agonist (1S,3S)-ACPD also produced inhibitions in the carrageenan animals, in contrast to the mixed effects seen in normal animals. The group I agonist (*S*)-3,5-DHPG produced mixed effects (inhibitions and facilitations) in both normal and carrageenan animals. This in vivo study shows that the effects of metabotropic glutamate receptor agonists are more complex than in vitro studies have suggested to date. © 1998 Elsevier Science B.V.

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1. Introduction

Glutamate is the main excitatory transmitter in the dorsal horn of the spinal cord, the first modulatory site in the relay of sensory information to the brain. The discovery of metabotropic receptors for glutamate in addition to the well established ionotropic receptors such as the α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) and *N*-methyl-D-aspartic acid (NMDA) receptors opened up new possibilities for the modulation of nociceptive messages at the level of the spinal cord.

To date, eight genes coding for metabotropic receptors have been cloned, ($mGlu_{1-8}$), from which more receptors can be generated owing to the formation of splice variants from $mGlu_1$, $mGlu_4$ and $mGlu_5$ (Pin and Duvoisin, 1995). These receptors can be classified into 3 groups (groups I–III), on the basis of their sequence homology, transduction mechanism and pharmacology. $mGlu_1$ and $mGlu_5$ receptors form group I, and when expressed, activation of these receptors by glutamate or agonists such as (1S,3R)-ACPD leads to stimulation of phospholipase C. Group II ($mGlu_2$ and $mGlu_3$) and group III ($mGlu_{4,6-8}$) metabotropic glutamate receptors are negatively coupled to adenylyl cyclase in expression systems. The situation regarding transduction mechanisms is not so clear cut when native metabotropic glutamate receptors are considered, with a number of other transduction systems including a direct G protein-mediated inhibition of voltage sensitive Ca^{2+} channels and regulation of K^+ channels being reported, in addition to actions via phospholipase C and adenylyl cyclase (see Pin and Duvoisin, 1995).

Of these mGlu receptors, high levels of staining for $mGlu_1$, $mGlu_5$ and $mGlu_7$ receptors are found in lamina I and II of the dorsal horn of the spinal cord in rats (Shigemoto et al., 1992; Vidnyánszky et al., 1994; Ohishi et al., 1995b), with the $mGlu_5$ receptor being located on the soma and dendrites of dorsal horn neurones, possibly post-synaptic to the terminals of C-fibre primary afferents (Vidnyánszky et al., 1994), whereas a proportion of $mGlu_7$ receptors are found on C-fibre terminals themselves (Ohishi et al., 1995b). Levels of mRNA coding for $mGlu_2$ and $mGlu_4$ receptors are extremely low in the spinal cord (Ohishi et al., 1993a, 1995a), with only a moderate signal for $mGlu_3$ receptors in the dorsal horn, with much of this

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staining being on glia (Ohishi et al., 1993b). No mRNA coding for $mGlu_6$ or $mGlu_8$ receptors has been detected in the rat spinal cord (Valerio et al., 1997). Thus, group II and III mGlu receptors appear to be poorly represented on intrinsic neurones in the spinal cord in normal animals. However, electrophysiological studies have suggested that in many areas of the central nervous system these receptors have a predominantly presynaptic location (see Pin and Duvoisin, 1995). If this is the case in the spinal cord, as is seen with $mGlu_7$ receptors (Ohishi et al., 1995b; Valerio et al., 1997), then mRNA coding for these receptors would not be expected to be located in the dorsal horn of the spinal cord. Antibody studies have yet to demonstrate whether primary afferent neurones entering the dorsal horn of the spinal cord do indeed show staining for these other group II and III mGlu receptors.

Compared with the ionotropic (AMPA and NMDA) receptors for glutamate, the role of the metabotropic receptors for glutamate in the spinal transmission of nociception is poorly understood. Activation of metabotropic glutamate receptors has been shown to potentiate ionotropic glutamate receptor mediated responses in the dorsal horn of the spinal cord (Bleakman et al., 1992; Cerne and Randic, 1992; Bond and Lodge, 1995), although this may be due to an enhancement of neuronal excitability rather than a specific interaction with ionotropic glutamate receptors (Jones and Headley, 1995), suggesting that metabotropic glutamate receptors may play a modulatory role in spinal nociceptive transmission. Coactivation of spinal AMPA and metabotropic glutamate receptors in the rat produces mechanical hyperalgesia (Meller et al., 1996), with higher spinal doses of the metabotropic glutamate receptor agonist (±)-1-aminocyclopentane-*trans*-1,3-dicarboxylic acid (*trans*-ACPD) or the selective group I agonist (*RS*)-3,5-dihydroxyphenylglycine ((*RS*)-3,5-DHPG) producing behaviour indicative of nociception (Fisher and Coderre, 1996a; Meller et al., 1996). In a model of acute inflammatory nociception, spinal administration of the metabotropic glutamate receptor agonists *trans*-ACPD, (*RS*)-3,5-DHPG (group I) and (1S,3S)-1-aminocyclopentane-1,3-dicarboxylic acid ((1S,3S)-ACPD) (group II) has been shown to potentiate the behavioural response to formalin (Coderre and Melzack, 1992; Fisher and Coderre, 1996b). The second phase of this behavioural response to formalin has been shown to be reduced by antagonists acting at group I metabotropic glutamate receptors (Fisher and Coderre, 1996b), as has the noxious evoked response of neurones rendered hyperexcitable by the induction of acute inflammation of the knee joint (Neugebauer et al., 1994). These studies point to a potential role of metabotropic glutamate receptors in spinal nociceptive processing.

The present study has used (1S,3R)-ACPD (an agonist at group I and II metabotropic glutamate receptors), (*S*)-3,5-DHPG (the active enantiomer of (*RS*)-3,5-DHPG) (a group I receptor agonist) and (1S,3S)-ACPD (predominantly an agonist at group II receptors), administered intrathecally in both normal animals, and in animals following the development of carrageenan inflammation to try to establish what role, if any, activation of these metabotropic glutamate receptors may have in the transmission and modulation of nociceptive messages in the spinal cord under different conditions.

2. Materials and methods

The methods are those described previously (Stanfa et al., 1992). Briefly, male Sprague Dawley rats (200–250 g) were anaesthetized with 3% halothane in a 33% O_2, 66% N_2O mixture and a tracheal cannula inserted, at which point the level of halothane anaesthesia was reduced to 2% for the remainder of the surgery. The rats were placed in a stereotaxic frame and a laminectomy performed (vertebrae L1–L3) to expose the spinal cord. The cord was held in metal clamps placed rostral and caudal to the laminectomy to provide stability during the electrophysiological recording. Prior to commencement of the recording, the level of halothane anaesthesia was reduced to 1.8%, which was sufficient to maintain complete areflexia.

Single unit extracellular recordings were made with a parylene-coated tungsten electrode from convergent dorsal horn neurones located in the deep (500–1000 μm) dorsal horn of the spinal cord. These neurones responded to innocuous stimuli such as brush and prod, and noxious stimuli such as pinch applied to their receptive fields, located on the toes of the ipsilateral hind paw. Electrical stimulation (2 ms wide pulse, 0.5 Hz), applied via two needles inserted into the centre of the receptive field, was used as the test stimulus for the experiment. Tests were conducted at ten minute intervals and consisted of trials of 16 stimuli given at 3 times the threshold current required for activation of each particular neurone. The neuronal responses were displayed against post-stimulus latency as a post-stimulus time histogram, from which the responses of the neurone evoked by the different fibre types could be separated on the basis of latency and quantified.

The metabotropic glutamate receptor agonists used in this study were dissolved in 0.9% saline and the pH of the solutions adjusted to pH 6.8–8.4 with NaOH. These drugs were applied to the exposed spinal cord in a volume of 50 μl following three stable control trials (less than 10% variation in the C-fibre-evoked response) and the evoked response of the neurone followed for 1 h. Saline controls were performed in some animals. In the carrageenan experiments, inflammation was induced by the injection of 100 μl of 2% carrageenan into the plantar surface of the ipsilateral hind paw. The evoked response of the neurone was followed for 3 h after the injection of carrageenan prior to the administration of drugs to the surface of the spinal cord. In these carrageenan experiments, the three controls immediately before the administration of drugs were used as controls for the subsequent drug effect.

The results are expressed as a percentage of the control response or as a % change from control, irrespective of the direction of change, in cases where the drug produced a bidirectional change in the evoked response (see results). Nonparametric statistics (Mann–Whitney test or Friedman repeated measures test with Dunn's multiple comparison test) were used to test for significance of results, with a P value ≤ 0.05 regarded as significant.

3. Results

3.1. Effects of (1S,3R)-ACPD on the evoked responses of neurones recorded in normal and carrageenan animals

The effects of 1, 5 and 50 μg of the group I and II metabotropic glutamate receptor agonist (1S,3R)-ACPD, administered intrathecally, were tested on the evoked responses of 6 neurones recorded in normal animals and 6 neurones recorded in animals 3 h after the induction of carrageenan inflammation. (1S,3R)-ACPD produced markedly differing effects in the two groups of animals (Table 1 and Fig. 1). In normal animals, (1S,3R)-ACPD produced modest, although significant, facilitations of the C-fibre-evoked response of the dorsal horn neurones (Friedman statistic, Fr = 14.6, $P = 0.002$), and a greater, but variable (128–302% of control) enhancement of the post-discharge of the neurones (Fr = 9.72, $P = 0.021$). The A β-fibre-evoked response of the neurones was not affected by 1 or 5 μg of (1S,3R)-ACPD, although 50 μg of the drug produced a modest inhibition of this response to $87.8 \pm 5.0\%$ of control ($n = 5$). The effects of these doses of (1S,3R)-ACPD on the noxious evoked responses of dorsal horn neurones recorded in normal animals did not appear to be strongly dose-dependent. The neuronal responses were slightly enhanced after 1 μg of (1S,3R)-ACPD, whereas 5 and 50 μg of the drug produced greater but equal facilitations of the evoked responses, suggesting a plateau of effect had been reached.

In contrast to the actions of (1S,3R)-ACPD in normal animals, following 3 h of carrageenan inflammation, the same doses of (1S,3R)-ACPD (1–50 μg) produced significant inhibitions of the C-fibre-evoked response (Friedman statistic, Fr = 13.4, $P = 0.0038$) and post-discharge (Fr = 9.0, $P = 0.029$) of the dorsal horn neurones, with a greater effect of the drug seen on the post-discharge of the neurones than on the C-fibre-evoked response (Table 1 and Fig. 1). These doses of (1S,3R)-ACPD did not have any effect on the A β-fibre-evoked responses of the neurones recorded in the carrageenan animals (A β-fibre-evoked response $91.3 \pm 6.8\%$ of control with 50 μg, $n = 5$). As in the normal animals, the effects of (1S,3R)-ACPD were not strongly dose-dependent in the carrageenan animals, although 50 μg of (1S,3R)-ACPD tended to produce a greater inhibition of the post-discharge than 5 μg of the drug in these animals.

Thus, the actions of the group I and II metabotropic glutamate receptor agonist (1S,3R)-ACPD are not fixed, but are altered by the development of peripheral inflammation, such that activation of these metabotropic receptors produces facilitations of the C-fibre-evoked response and post-discharge of the neurones in normal animals, but inhibitions of these responses following inflammation.

3.2. Effects of intrathecal (S)-3,5-DHPG on the evoked responses of dorsal horn neurones recorded in normal animals and in animals 3 h after the induction of carrageenan inflammation

The effects of 1, 5 and 50 μg of the group I metabotropic glutamate receptor agonist (*S*)-3,5-DHPG, administered intrathecally, were tested on the evoked responses of 7 neurones recorded in normal animals and 5 neurones recorded in animals 3 h after the induction of carrageenan inflammation. Unlike the group I and II metabotropic glutamate receptor agonist (1S,3R)-ACPD, (*S*)-3,5-DHPG produced mixed effects on the evoked responses of neurones recorded in both normal animals and in animals 3 h post-carrageenan (Fig. 2).

In normal animals, (*S*)-3,5-DHPG facilitated the C-fibre-evoked response and post-discharge of some neurones, whilst inhibiting these responses in other neurones. The post-discharge of the neurones was affected to a greater degree (range 9–359% of control with 50 μg) than the C-fibre-evoked response (range 46–131% of control) by (*S*)-3,5-DHPG. The A β-fibre-evoked response of the

Table 1
Effects of intrathecal 1S,3R-ACPD on the noxious evoked responses of dorsal horn neurones recorded in normal animals and in animals 3 h after the injection of carrageenan

	C-fibres		Post-discharge	
	Normal, $n = 6$	Carrageenan, $n = 6$	Normal, $n = 5$	Carrageenan, $n = 5$
1 μg 1S,3R-ACPD	$112.8 \pm 5.3\%$	$84.3 \pm 2.8\%$	$183.6 \pm 40.6\%$	$67.2 \pm 5.8\%$
5 μg 1S,3R-ACPD	$127.0 \pm 6.0\%^{**}$	$82.2 \pm 3.0\%^{*}$	$219.4 \pm 34.9\%^{*}$	$73.0 \pm 9.9\%$
50 μg 1S,3R-ACPD	$123.3 \pm 3.7\%$	$79.2 \pm 4.4\%^{**}$	$209.4 \pm 31.9\%$	$47.6 \pm 4.7\%^{*}$

All data are expressed as a percentage of control responses $\pm$ S.E.
$^{*}P < 0.05$, $^{**}P < 0.01$ compared with control (Dunn's multiple comparison test).

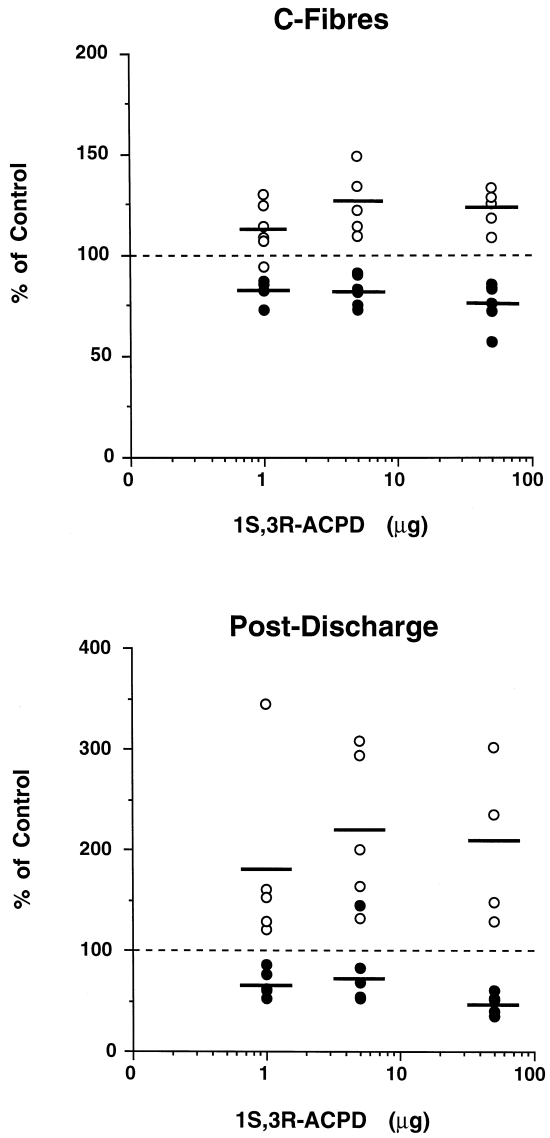

Fig. 1. Effects of intrathecally administered (1S,3R)-ACPD on the electrically evoked C-fibre-evoked response and post-discharge of dorsal horn neurones recorded in normal animals (open circles) and in animals 3 h post-carrageenan (filled circles). The horizontal bars represent the mean effect of (1S,3R)-ACPD on the responses in each group (normal/carrageenan) of animals. In normal animals, (1S,3R)-ACPD facilitated the noxious evoked responses of the neurones, whereas these responses were inhibited by (1S,3R)-ACPD following carrageenan. $n = 5–6$ neurones/group.

neurones did not appear to be significantly affected by any of the doses of (*S*)-3,5-DHPG tested. The mixed effects seen on the noxious evoked responses of the neurones recorded in normal animals, with a greater effect on the post-discharge of the neurones were also seen in the carrageenan animals.

As a result of the bidirectional change in the neuronal responses produced by (*S*)-3,5-DHPG, when all the neurones are considered together, it appears that (*S*)-3,5-DHPG did not have a significant overall effect on the noxious evoked neuronal responses. However, as Fig. 2 shows, this is clearly not the case, as the variability produced in the noxious evoked neuronal response following (*S*)-3,5-DHPG in normal animals and in animals after carrageenan inflammation, is much greater than that seen after the administration of saline alone. Thus, as Table 2 shows, all doses in normal animals and 5 and 50 μg of (*S*)-3,5-DHPG in the carrageenan animals, produced a significantly greater change in the noxious evoked responses (regardless of the direction of change) than saline alone. Furthermore, irrespective of the direction of the change in the evoked neuronal response produced by (*S*)-3,5-DHPG, the magni-

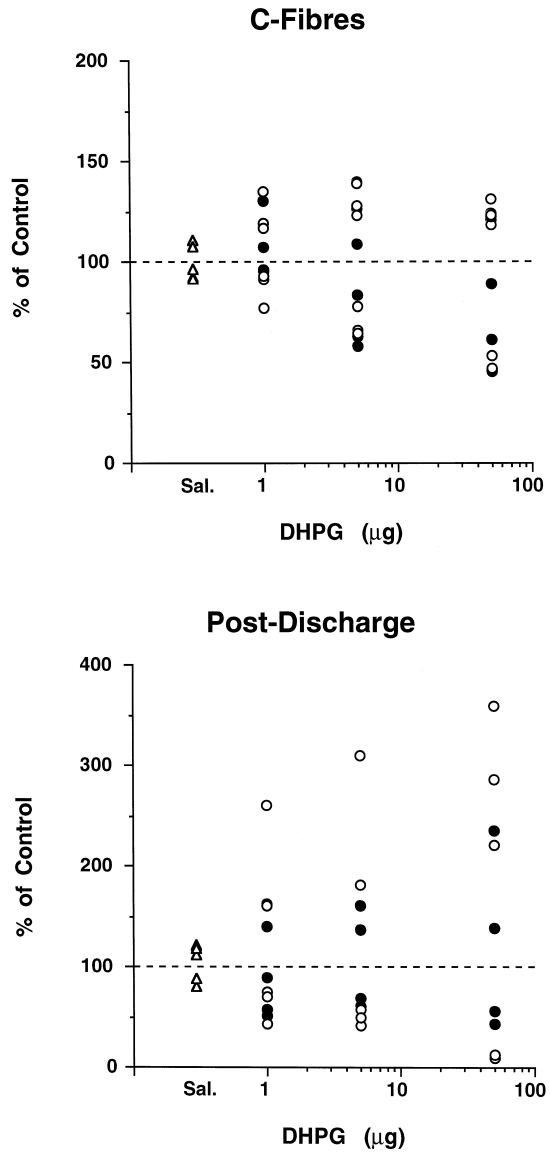

Fig. 2. Effects of intrathecally administered (*S*)-3, 5-DHPG on the electrically evoked C-fibre-evoked response and post-discharge of dorsal horn neurones recorded in normal animals (open circles) and in animals 3 h post-carrageenan (filled circles). The open triangles represent the effects of intrathecal administration of saline (sal.) on the C-fibre-evoked response and post-discharge of a similar population of neurones. $n = 5–7$ neurones/group.

Table 2
Magnitude of the change in the noxious evoked response of neurones (regardless of direction of change) evoked by intrathecal (*S*)-3, 5-DHPG or saline in normal animals and in animals 3 h after the injection of carrageenan

	C-fibres		Post-discharge	
	Normal, $n = 7$	Carrageenan, $n = 5$	Normal, $n = 5$	Carrageenan, $n = 5$
1 μg DHPG	$18.4 \pm 3.5\%^{*}$	$16.2 \pm 6.7\%$	$66.8 \pm 24.5\%^{**}$	$41.0 \pm 8.4\%$
5 μg DHPG	$31.7 \pm 2.8\%^{**}$	$26.2 \pm 6.1\%^{*}$	$88.6 \pm 31.0\%^{**}$	$44.0 \pm 5.3\%^{**}$
50 μg DHPG	$35.4 \pm 5.9\%^{**}$	$30.0 \pm 7.7\%^{**}$	$148.6 \pm 32.7\%^{**}$	$73.0 \pm 18.0\%^{**}$
Saline	$7.6 \pm 1.0\%$, $n = 5$		$16.2 \pm 1.8\%$, $n = 5$	

Data are presented as mean percentage change from control ± S.E.
$^{*}P < 0.05$, $^{**}P < 0.01$ compared with magnitude of change in response induced by saline alone (Mann–Whitney test).

tude of this change tended to increase with increasing doses of the drug, indicating a dose-related drug effect, with 50 μg of (*S*)-3,5-DHPG producing a significantly greater magnitude of change in the C-fibre-evoked response ($P = 0.038$) and post-discharge of the neurones ($P = 0.05$) than 1 μg of (*S*)-3,5-DHPG in normal animals. This trend was also seen in the carrageenan animals, although the effects of (*S*)-3,5-DHPG on the post-discharge tended to be smaller in the carrageenan animals than in the normal animals.

Thus the group I metabotropic glutamate receptor agonist (*S*)-3,5-DHPG produced mixed effects (both facilitations and inhibitions) on the C-fibre-evoked response and post-discharge of the dorsal horn neurones recorded in both normal animals, and in animals 3 h after the injection of carrageenan into the paw. Unlike the effects of the group I and II metabotropic glutamate receptor agonist (1S,3R)-ACPD, the effects of (*S*)-3,5-DHPG were not dramatically altered by the development of peripheral inflammation, although as Fig. 2 shows, there was a tendency for (*S*)-3,5-DHPG to produce smaller facilitations following the development of inflammation.

3.3. Effects of intrathecal (1S,3S)-ACPD on the evoked responses of dorsal horn neurones recorded in normal animals and in animals 3 h after the induction of carrageenan inflammation

The effects of 1, 5 and 50 μg of the group II metabotropic glutamate receptor agonist (1S,3S)-ACPD, administered intrathecally, were tested on the evoked responses of 6 neurones recorded in normal animals and 6 neurones recorded in animals 3 h after the induction of carrageenan inflammation. The effects of (1S,3S)-ACPD on the C-fibre-evoked response and post-discharge of dorsal horn neurones recorded in normal animals were not uniform, with the response of some neurones being facilitated by (1S,3S)-ACPD, whilst the same responses of other neurones were inhibited by (1S,3S)-ACPD (Fig. 3). As a result of the mixed effects of (1S,3S)-ACPD on the evoked neuronal responses, there is no overall change in either the C-fibre-evoked response or post-discharge of the neurones recorded in normal animals. The magnitude of these changes, irrespective of the direction of change, produced by (1S,3S)-ACPD in the C-fibre-evoked response (5 and 50 μg) and post-discharge (1 and 5 μg) were significantly greater than those produced by the intrathecal administra-

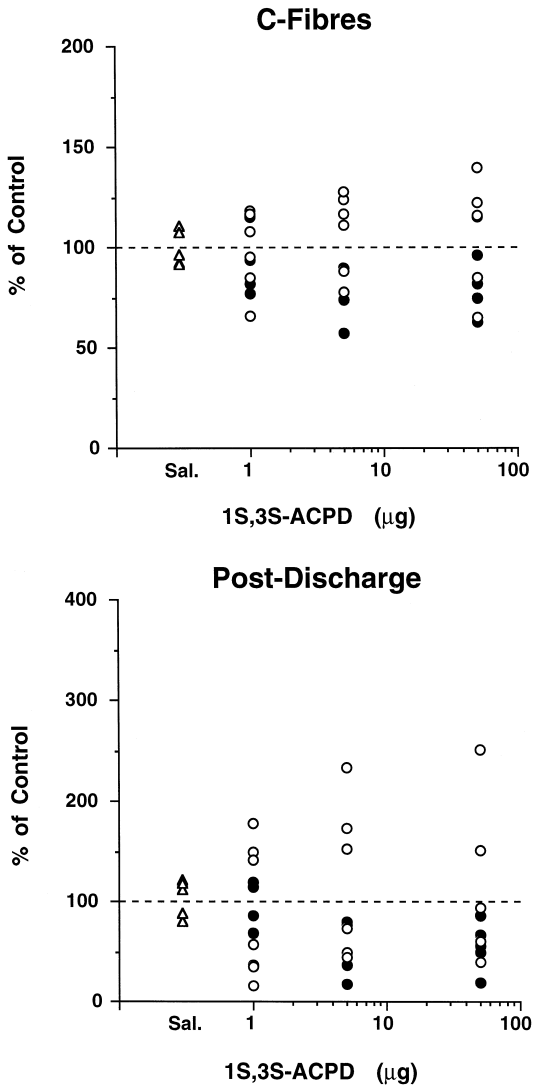

Fig. 3. Effects of intrathecally administered (1S,3S)-ACPD on the electrically evoked C-fibre-evoked response and post-discharge of dorsal horn neurones recorded in normal animals (open circles) and in animals 3 h post-carrageenan (filled circles). The open triangles represent the effects of intrathecal administration of saline (sal.) on the C-fibre-evoked response and post-discharge of a similar population of neurones. $n = 5$–6 neurones/group.

tion of saline alone ($P < 0.01$), however, they did not appear to be strongly dose-related.

In contrast to the effects of (1S,3S)-ACPD on the evoked responses of dorsal horn neurones recorded in normal animals, the effects of this drug on the evoked response of the neurones were predominantly inhibitory following 3 h of carrageenan inflammation, with (1S,3S)-ACPD producing a significant inhibition of the C-fibre-evoked response ($Fr = 8.28$, $P = 0.04$) and post-discharge of the neurones ($Fr = 7.65$, $P = 0.05$) (Fig. 3). As in the normal animals, the post-discharge of the neurones was more sensitive to the effects of (1S,3S)-ACPD than the C-fibre-evoked response of the neurones, with 50 μg of (1S,3S)-ACPD producing an inhibition of the post-discharge of the neurones to $55.2 \pm 10.9\%$ of control compared with the modest inhibition of the C-fibre response to $86.2 \pm 9.0\%$ of control ($n = 5$) in the carrageenan animals. In the carrageenan, but not the normal animals, the Aβ-fibre-evoked response of the neurones was modestly inhibited by (1S,3S)-ACPD, with 50 μg of (1S,3S)-ACPD inhibiting this response to $84.0 \pm 1.6\%$ of control.

Thus, the group II metabotropic glutamate receptor agonist (1S,3S)-ACPD produced mixed effects on the noxious evoked responses of dorsal horn neurones recorded in normal animals, yet, following 3 h of carrageenan-induced inflammation, this drug produced predominantly inhibitions of these responses.

4. Discussion

The different classes of metabotropic glutamate receptors have a variety of potential roles in the spinal cord due to their differing locations and effector mechanisms. The results of this study show that spinal administration of the classical group I and II metabotropic glutamate receptor agonist (1S,3R)-ACPD facilitates the noxious evoked responses of dorsal horn neurones recorded in normal animals, suggesting that activation of spinal metabotropic glutamate receptors by endogenous glutamate may contribute to spinal nociceptive transmission. This is in agreement with other in vivo studies where excitation of dorsal horn neurones has been seen following iontophoresis of (1S,3R)-ACPD in the spinal cord (Young et al., 1995) and the finding that high spinal doses of *trans*-ACPD produce caudally-directed biting and scratching in rats, indicative of nociceptive behaviour (Meller et al., 1996).

In vitro studies on the actions of metabotropic glutamate receptors suggest that this excitatory effect is most likely to be mediated through activation of group I metabotropic glutamate receptors (Pin and Duvoisin, 1995), a view supported by the finding that selective activation of these group I receptors has been shown to produce excitations in the spinal cord in vivo (Jones and Headley, 1995; Fisher and Coderre, 1996a). However, when (*S*)-3,5-DHPG, a selective agonist at group I metabotropic glutamate receptors, was administered to the spinal cord in the present study, a mixture of facilitations and inhibitions of the evoked neuronal responses were seen, suggesting that activation of group I metabotropic glutamate receptors on excitatory neurones alone is not sufficient to explain the actions of (1S,3R)-ACPD in these animals. The $mGlu_5$ receptor, one of the receptors of the group I class of mGlu receptors, is known to be located on post-synaptic neurones in the dorsal horn of the spinal cord, apposed to C-fibre terminals (Vidnyánszky et al., 1994). It is possible that some of these receptors are located on inhibitory interneurones within the spinal cord and the spinal circuitry is such that activation of these neurones could lead to a subsequent inhibition of the convergent dorsal horn neurones from which the present recordings were made. Furthermore, activation of metabotropic glutamate receptors has been shown to facilitate the inhibitory effects of γ-aminobutyric acid (GABA) and glycine in the spinal cord in vivo (Bond and Lodge, 1995). Thus a predominance of inhibitions over excitations may occur in some neurones in the present experiments, leading to an inhibition rather than a facilitation of the evoked responses.

The mixed effects of (*S*)-3,5-DHPG in these normal animals appears to contrast with other in vivo studies which suggest that activation of group I receptors in the spinal cord leads to neuronal excitation and a facilitation of neuronal transmission. Thus, in a similar in vivo study performed in adult animals, the group I receptor agonist (*RS*)-3,5-DHPG produced an increase in the spontaneous activity of spinal neurones, indicative of a facilitatory effect (Jones and Headley, 1995). However, the agonist was applied iontophoretically, hence the effects seen are most likely to result from actions of (*RS*)-3,5-DHPG on the individual neurone and its immediate environment, rather than on the wider spinal circuitry as might be the case in the present study. In a behavioural study, intrathecal (*RS*)-3,5-DHPG produced spontaneous nociceptive behaviours, however, these were less with the higher dose of (*RS*)-3,5-DHPG than with the lower dose, suggesting that effects other than direct excitation may be occurring (Fisher and Coderre, 1996a).

The actions of the metabotropic glutamate receptor agonist (1S,3R)-ACPD in the present study cannot be solely attributed to actions at group II receptors either, since administration of the group II metabotropic glutamate receptor agonist (1S,3S)-ACPD to the spinal cord also produced mixed effects on the dorsal horn neurones recorded in normal animals, facilitating the response of some neurones, whilst inhibiting the responses of other neurones. Many in vitro studies have shown that activation of group II metabotropic glutamate receptors by agonists such as (1S,3S)-ACPD leads to an inhibition of responses in the spinal cord, predominantly by an action at presynaptic receptors (Pook et al., 1992; Jane et al., 1994). However, these in vitro studies have been performed on spinal cords taken from immature animals, which are anatomi-

cally and physiologically distinct from adult animals (see Fitzgerald, 1995). In vivo studies in adult animals using (1S,3S)-ACPD have found that this agonist can potentiate the excitatory actions of ionotropic glutamate receptor agonists on dorsal horn neurones (Bond and Lodge, 1995), which may explain the finding that intrathecal (1S,3S)-ACPD produced spontaneous nociceptive behaviours in adult rats (Fisher and Coderre, 1996a). Thus, it appears that the actions of metabotropic glutamate receptor agonists in vivo are not always as simple as might be suggested by in vitro studies. In addition, the actions of (1S,3R)-ACPD, which has actions at both group I and II receptors may lead to receptor interactions so that the net result is not simply the additive effects of activation of group I and group II receptors alone.

This potential role of mGlu receptors in spinal nociceptive transmission is further complicated by the finding that in direct contrast to its actions in normal animals, the group I and II metabotropic glutamate receptor agonist (1S,3R)-ACPD produces inhibitions rather than facilitations of the noxious evoked response of dorsal horn neurones following 3 h of carrageenan inflammation. The results of the present study with selective group I and II metabotropic glutamate receptor agonists are unable to demonstrate conclusively which class of mGlu receptors are responsible for this change, although the group II metabotropic glutamate receptor agonist (1S,3S)-ACPD had predominantly inhibitory actions in the carrageenan animals, compared with its mixed effects in normal animals, suggesting that $mGlu_1$ and/or $mGlu_5$ receptors may be important in mediating the effects of (1S,3R)-ACPD in the carrageenan animals. Whilst the predominantly inhibitory actions of (1S,3S)-ACPD in the carrageenan animals in the present study are in keeping with the inhibitory role of group II metabotropic glutamate receptors suggested by in vitro studies (Pook et al., 1992; Jane et al., 1994), another in vivo study suggested a possible pronociceptive role for group II metabotropic glutamate receptors in inflammatory nociception, since spinal (1S,3S)-ACPD facilitates the second phase of the formalin response in rats, although this may have been due to nonselective actions at group I mGlu receptors (Fisher and Coderre, 1996b). The reason for this change to a predominantly inhibitory action following 3 h of carrageenan inflammation in the present study from mixed effects in normal animals is unclear. However, it may be the case that any facilitation of NMDA receptor mediated responses by (1S,3S)-ACPD (Bond and Lodge, 1995) following the development of inflammation may lead to compensatory inhibitory mechanisms such as those previously described in this model (Stanfa et al., 1992).

In contrast, the actions of the group I receptor agonist (*S*)-3,5-DHPG were little altered by the development of inflammation, although this agonist tended to produce smaller changes in the evoked response of the neurones following inflammation. $mGlu_5$ receptors, one of the receptors comprising group I metabotropic receptors, has been shown to have an extrasynaptic location in the spinal cord (Vidnyánszky et al., 1994) such that they might only be activated by endogenous glutamate in times of enhanced glutamate release, such as that produced by inflammation (Dray et al., 1994). It may be the case that following inflammation, there is a higher activation of this receptor by endogenous glutamate such that exogenous (*S*)-3,5-DHPG is able to have less of an effect of the evoked neuronal responses. Studies using selective antagonists of metabotropic glutamate receptors would help to clarify this point.

Some in vivo studies have been performed with metabotropic glutamate receptor antagonists to try to elucidate the potential roles of endogenous activation of these receptors in spinal nociceptive processing. The weak noncompetitive group I metabotropic glutamate receptor antagonist L-AP3 has been used by several groups to investigate the actions of endogenous glutamate at these receptors, and iontophoretic administration of this antagonist has been shown to inhibit the response of dorsal horn neurones to mustard oil (Young et al., 1994) and to mechanical pressure applied to the inflamed, but not normal knee joint (Neugebauer et al., 1994). Another study has used spinally administered (*S*)-4CPG and (*S*)-4C3HPG, which are group I metabotropic glutamate receptor antagonists, to show that activation of these receptors may play a modest role in the generation of the second phase of the formalin response (Fisher and Coderre, 1996b). However, these compounds are also agonists at group II metabotropic glutamate receptors, which may have a bearing on the modest and somewhat variable results obtained in this study.

The results of the present study, together with other in vivo studies, demonstrate that the potential actions of glutamate acting at metabotropic receptors in the spinal cord are complex, with the results in an intact and mature system being rather more diverse than in vitro studies in immature animals have suggested to date. One possibility is that nociceptive circuitry present in intact animals is arranged such that activation of the various metabotropic glutamate receptors can potentially produce excitations or inhibitions, either directly, or by producing disinhibitions or excitations of inhibitory interneurones. The lack of strong effects with metabotropic glutamate receptor agonists suggests that their role in spinal nociceptive transmission may be a predominantly modulatory one. However, further studies with selective antagonists at the various metabotropic receptor subtypes will further increase our knowledge of the role of these receptors.

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