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Induction of long-term potentiation of C fibre-evoked spinal field potentials requires recruitment of group I, but not group II/III metabotropic glutamate receptors

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Abstract

In superficial layers of the lumbar spinal dorsal horn, *N*-methyl-D-aspartate-dependent long-term potentiation (LTP) of C fibre-evoked field potentials, a synaptic model of central sensitisation and hyperalgesia, ensues the application of electrical high-frequency, high-intensity conditioning stimulation to the sciatic nerve. In order to investigate the putative involvement of the G protein-coupled metabotropic glutamate receptors (mGluRs) in the induction of this form of LTP, we applied a series of mGluR antagonists exhibiting distinct group-specific activity profiles to the spinal lumbar enlargement, prior to conditioning stimulation. The group I (mGluR1/5) and group II (mGluR2/3) mGluR antagonist (*S*)-α-methyl-4-carboxyphenylglycine or the selective mGluR1/5 antagonist (*S*)-4-carboxyphenylglycine consistently impaired the development of spinal LTP. However, potentiation occurred in the presence of the inactive enantiomer (*R*)-α-methyl-4-carboxyphenylglycine. LTP proved insensitive to the selective mGluR2/3 antagonists (2*S*)-α-ethylglutamic acid and LY341495, either spinally or intravenously delivered. LTP could also be induced in the presence of the selective group III (mGluR4/mGluR6–mGluR8) mGluR antagonist (*RS*)-α-methylserine-*O*-phosphate. However, none of the mGluR-active compounds alone noticeably altered the amplitudes of C fibre-evoked field potentials in the absence of conditioning stimulation. These findings suggest that the induction of LTP of C fibre-evoked field potentials in the spinal dorsal horn by high-frequency, high-intensity stimulation of afferent C fibres requires a group-specific mGluR recruitment, activation of mGluR1/5 but not that of mGluR4/6–8 and mGluR2/3 being a requisite step.

Keywords: Glutamate receptors; Synaptic plasticity; Spinal cord; Nociception; Excitatory amino acids; Electrophysiology

1. Introduction

A major neurotransmitter role for glutamate or aspartate in the primary afferent system is well established. Glutamate acts through two broad classes of receptors, namely those of the ionotropic type, which are ligand-gated cation channels and include α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA), kainate and *N*-methyl-D-aspartate (NMDA) receptors, and the G protein-coupled metabotropic glutamate receptors (mGluRs), which modulate the levels of multiple second messengers and regulate ionotropic glutamate receptor-operated excitatory and inhibitory synaptic transmission (Anwyl, 1999; Conn and Pin, 1997; Nakanishi et al., 1998). The eight mGluR subtypes so far cloned have been classified into groups I–III on the basis of sequence similarity, pharmacological properties and intracellular transduction mechanisms. Thus, group I comprises

Abbreviations: mGluR, metabotropic glutamate receptor; L-AP4, L-2-amino-4-phosphonobutanoate; AMPA, α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid; NMDA, *N*-methyl-D-aspartate; LTD, long-term depression; LTP, long-term potentiation; PKC, protein kinase C; PLC, phospholipase C; aCSF, artificial cerebrospinal fluid; cAMP, cyclic adenosine monophosphate; (*S*)-MCPG, (*S*)-α-methyl-4-carboxyphenyl glycine; (*R*)-MCPG, (*R*)-α-methyl-4-carboxyphenylglycine; (*S*)-4CPG, (*S*)-4-carboxyphenylglycine; MSOP, (*RS*)-α-methylserine-*O*-phosphate; EGLU, (2*S*)-α-ethylglutamic acid; (1*S*,3*R*)-ACPD, (1*S*,3*R*)-1-aminocyclo pentane-1,3-dicarboxylic acid.

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mGluR1 and mGluR5, which increase intracellular phosphoinositide turnover via phospholipase C activation. Groups II (mGluR2,3) and III (mGluR4,6–8) mGluRs inhibit stimulated formation of cyclic adenosine monophosphate (cAMP). Group III mGluRs are potently activated by L-2-amino-4-phosphonobutanoate (L-AP4).

Plastic changes in spinal sensory information processing that ensue peripheral nerve injury or inflammation underlie some pain perception abnormalities observed in clinic (Ji and Woolf, 2001; Melzack et al., 2001; Sandkühler, 2000; Zimmermann, 2001). One such phenomenon is the long-term potentiation (LTP) of field potentials evoked in the spinal dorsal horn, i.e. an enduring enhancement of synaptic efficacy between primary afferents and spinal neurones that is inducible by high-frequency stimulation of peripheral nerves both in vivo and in vitro (Ikeda et al., 2003; Liu et al., 1998; Liu and Sandkühler, 1995, 1997; Miletic and Miletic, 2000; Randić et al., 1993; Thompson et al., 1994). LTP of C fibre-evoked field potentials, which can be induced both by supramaximal electrical activation of C fibres or peripheral nerve insult (Sandkühler and Liu, 1998), is likely related to some forms of altered nociception (Lozier and Kendig, 1995; Randić et al., 1993; Sandkühler, 2000; Willis, 2002).

In the spinal cord, both facilitating and impeding effects on synaptic transmission have been attributed to mGluRs in electrophysiological and behavioural models. Thus, whereas activation of spinal mGluRs may enhance nociceptive neuronal responses (Neugebauer et al., 1994; Young et al., 1995), recruitment of mGluRs of groups I and II is required for the induction of a homosynaptic form of long-term depression (LTD) in vitro (Chen and Sandkühler, 2000). In spite of evidence implicating mGluRs in spinal mechanisms of nociception and antinociception, a possible role for this receptor family in the LTP of responses evoked by nociceptive, peripheral C fibres in vivo has not yet been explored.

We aimed at delineating the involvement of each of the three groups of mGluRs in the induction of LTP of C fibre-evoked spinal field potentials by electrical high-frequency, high-intensity stimulation of primary afferent fibres, by applying group-preferring mGluR-ligands prior to conditioning stimulation.

2. Methods

Animal experiments were performed according to the European Communities Council Directive (86/609/ECC) on adult male *Sprague–Dawley* rats (250–350 g; OF1, Iffa-Credo, L'Arbresle, France, and Charles River Deutschland, Sultzfeld, Germany). The protocols for animal care and use were approved by the institutional Animal Care Committee, Regierungspräsidium Karlsruhe, Germany, and the appropriate committee at the Basque Country University. Anaesthesia was induced by intraperitoneal urethane (1.5 g/kg) and lasted for the entire experiment. Surgery was initiated upon full removal of corneal and cutaneous reflexes and adequacy of anaesthesia was verified by complete areflexia and stable haemodynamic parameters during noxious stimulation. A tracheotomy was performed to maintain an open, low-resistance airway. Cannulae were inserted into the left common carotid artery and the right jugular vein for arterial blood pressure monitoring (80–100 mm Hg) and continuous infusion of Tyrode's solution (in mM: 137 NaCl, 2.7 KCl, 1.4 $CaCl_2$, 1 $MgCl_2$, 6 $NaHCO_3$, 2.1 NaH_2PO_4, 6.5 *D*-(+)-glucose; pH 7.4) at 0.8–1 ml/h, respectively. Colorectal temperature was continuously monitored and euthermia (37–38 °C) was maintained via a feedback-controlled underbody heating pad for the duration of the experimental procedure. The left sciatic nerve was exposed and placed onto platinum hook-electrodes for bipolar electrical stimulation and the vertebral column was immobilised to a rigid frame. Bilateral dorsal laminectomies were performed at vertebrae T13-L1 and the dura mater overlaying lumbosacral spinal segments was carefully removed. The exposed spinal tissue was covered with warm paraffin oil, except for the spinal segments to be superfused.

2.1. Nerve stimulation and electrophysiological recording

Tungsten microelectrodes (impedance 5 MΩ) were inserted at a depth of 50–300 μm from the dorsal surface of spinal cord by an electrically controlled microdrive. Single monophasic, square-wave pulses of 0.5 ms duration were delivered to the sciatic trunk at a mid-thigh level at an intensity of 20–25 V on a per-minute basis, and the elicited spinal field potentials were amplified (analogue band-pass set at 1–550 Hz), displayed on an oscilloscope, and digitised to a PC-based computer at a sampling rate of 10 kHz via an A/D converter card (DT281-F-16SE). A pulse amplitude of 10 V was calculated to equal a current intensity of 1.5 mA in our experimental montage.

Following a 40 min baseline recording period of stable, C fibre-evoked field potentials, four high-frequency (100 Hz) trains of 100 pulses of 0.5 ms duration were delivered at an intensity of 30–40 V to the sciatic nerve, 10 s apart. Recordings lasted for at least 3 h from the application of tetanic stimulation and the rats were killed by an overdose of pentobarbital at the end of the experimental protocol.

2.2. Data analysis and statistics

Only the wave-form component of the elicited spinal field potential considered to be driven by C fibre activation (Liu and Sandkühler, 1997) was selected for analysis, i.e. the negative trace segment whose latencies of 90–130 ms corresponded to conduction velocities below 1.2 m/s. The amplitudes of C fibre-evoked field potentials, as determined by the distance from the baseline trace to the nadir of

the negative deflection, were analysed off-line (Datawave System, Longmont, CO, USA).

The statistical significance of changes underwent by C fibre-evoked field potentials following high-frequency stimulation was assessed by comparing mean amplitudes of potentials recorded 15 min prior to conditioning stimulation to those obtained 60 min after high-frequency stimulation. The effect of pharmacological treatment with mGluR-ligand solutions per se on evoked field potentials was examined by comparing mean amplitudes preceding drug delivery, i.e. with the cord superfused with artificial cerebrospinal fluid (aCSF, see below), to those obtained immediately before tetanic stimulation. The non-parametric Kruskal–Wallis test was used for statistical analysis (SAS Institute, Cary, NC, USA) and an α value of 0.05 was used as criterion of significance.

2.3. Drug preparation and delivery

Drugs used for spinal application included (*S*)-α-methyl-4-carboxyphenylglycine ((*S*)-MCPG; 500 μM), (*R*)-α-methyl-4-carboxyphenylglycine ((*R*)-MCPG; 500 μM), (*S*)-4-carboxyphenylglycine ((*S*)-4CPG; 200 μM), (*RS*)-α-methylserine-*O*-phosphate (MSOP; 200 μM), (2*S*)-α-ethyl-glutamic acid (EGLU; 200 μM) and LY341495 (10 μM), all six from Tocris (Bristol, UK). Working solutions were prepared in aCSF (in mM: 130 NaCl, 3.5 KCl, 1.25 NaH_2PO_4, 24 $NaHCO_3$, 1.2 $CaCl_2$, 1.2 $MgSO_4$, 10 *D*-(+)-glucose; pH 7.4) immediately before delivery, and applied by controlled spinal superfusion (Beck et al., 1995) 10 min prior to conditioning stimulation. Each mGluR-ligand was tested in five rats, and five control animals received only aCSF superfusion. In four experiments, LY341495 was administered intravenously at 1 mg/kg in 250 μl isotonic saline 10 min prior to conditioning stimulation.

3. Results

Conditioning high-frequency stimulation of the sciatic nerve consistently induced an enduring and significant enhancement of the amplitudes of C fibre-evoked potentials in the superficial spinal dorsal horn to 202 ± 24% (mean ± SEM 1 h after tetanic stimulation; $P < 0.05$, $n = 5$) of the mean control values in aCSF-superfused rats (Fig. 1). LTP lasted until termination of the experimental protocol.

Spinal superfusion with (*S*)-MCPG alone did not influence dorsal horn potentials elicited by C fibre stimulation. However, LTP failed to develop in the presence of (*S*)-MCPG in the dorsal horn milieu ($n = 5$; Fig. 2). LTP could be induced to 192 ± 34% of control ($P < 0.05$, $n = 5$; Fig. 2) under the effects of an equimolar dose of the inactive enantiomer (*R*)-MCPG, suggesting a receptor-specific action of (*S*)-MCPG. Likewise, the selective group I mGluR antagonist (*S*)-4CPG consistently prevented the induction of LTP ($n = 5$; Fig. 3), yet showing no noticeable influence on the amplitudes of C fibre-evoked field potentials prior to tetanic stimulation.

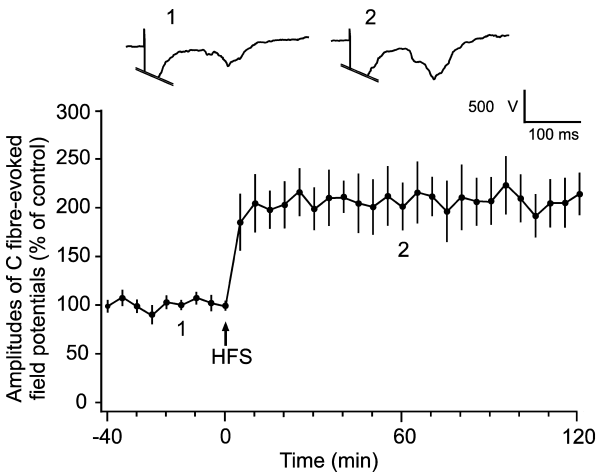

Fig. 1. Time course of LTP of C fibre-evoked field potentials in the spinal dorsal horn of control, aCSF-superfused rats, following high-frequency, high-intensity conditioning stimulation (HFS) of the sciatic nerve. Each plot represents the mean of five consecutive recordings, from five animals each, and error bars represent SEM. Individual, example field potentials are shown at the top from minutes −15 (1) and 60 (2) relative to conditioning stimulation.

In contrast, LTP induction was insensitive to spinal superfusion with the selective group II antagonists EGLU (LTP to 205 ± 33%, $n = 5$; $P < 0.05$) and LY341495 (LTP to 191 ± 35%, $n = 5$; $P < 0.05$) (Fig. 4A and B), and intravenous administration of LY341495 proved equally ineffective (LTP to 203 ± 31%, $n = 5$; $P < 0.05$)

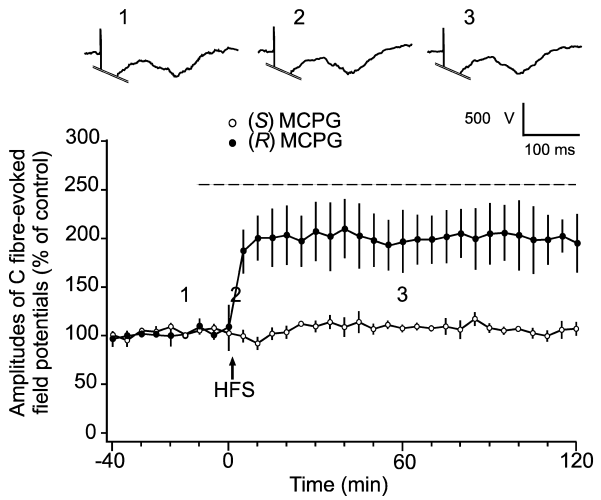

Fig. 2. MCPG stereospecifically prevents the generation of LTP of C fibre-evoked field potentials. Spinal superfusion with the group I–II mGluR antagonist (*S*)-MCPG (500 μM) blocks the induction of LTP. The dashed line reflects the onset and duration of drug superfusion. Individual field potentials from a representative experiment are shown at the top. LTP develops in the presence of the inactive enantiomer (*R*)-MCPG at equimolar concentrations. Neither drug alone altered the amplitudes of evoked field potentials (see (1) vs (2)). Individual field potentials at the top belong to the recording with (*S*)-MCPG and were obtained 15 min before- (1), immediately before- (2) and 60 min after (3) application of conditioning stimulation.

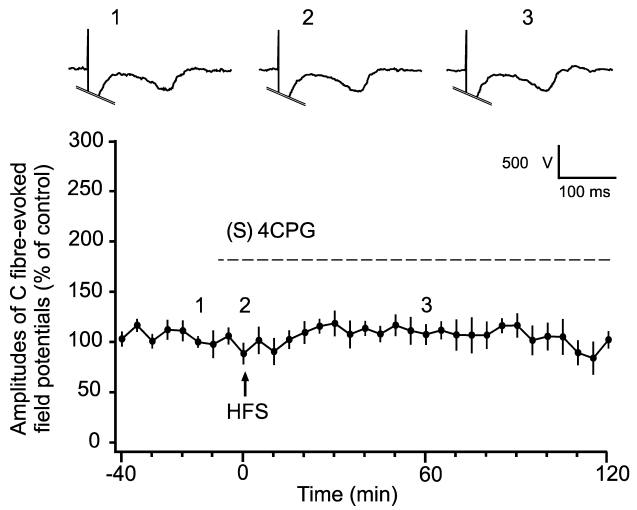

Fig. 3. Blockade of LTP induction of C fibre-evoked field potentials (3) results from pre-treatment with the group I mGluR antagonist (*S*)-4CPG (200 μM) starting 10 min (dashed line) prior to conditioning stimulation. However, the amplitudes of recorded potentials do not vary in the presence of (*S*)-4CPG prior to conditioning stimulation (2).

(Fig. 4C). Neither drug alone altered evoked field potentials (Fig. 4A–C).

We could consistently induce LTP of C fibre-evoked field potentials in spinal synapses (LTP to 204 ± 29%, $n = 5$; $P < 0.05$) in the presence of the selective group III antagonist MSOP (Fig. 5). MSOP also failed to influence the amplitudes of C fibre-evoked field potentials prior to conditioning stimulation (Fig. 5).

4. Discussion

Robust LTP of C fibre-evoked field potentials has been induced in superficial layers of the spinal dorsal horn following high-frequency, high-intensity electrical stimulation of the ipsilateral sciatic nerve. As reported previously, the recorded field potentials probably reflect spinal compound excitatory postsynaptic potentials (EPSPs) rather than postsynaptic action potentials (Liu and Sandkühler, 1995, 1997).

4.1. Induction of LTP necessitates group I mGluR-activation

Both mGluR1 and mGluR5 are prominently expressed in perikarya and neuronal processes in superficial laminae of the spinal dorsal horn (Alvarez et al., 2000; Jia et al., 1999; Vidnyanszky et al., 1994) and there is now substantial support for a general pro-nociceptive role for spinal group I mGluR-activation (Fundytus, 2001; Neugebauer, 2002). Pharmacological blockade of mGluR1/5 can attenuate nocifensive behaviour in the early phase of the formalin test (Karim et al., 2001), and reduction of mGluR1 expression in the spinal cord by antisense oligonucleotides is followed by thermal antinociception (Young et al., 1998).

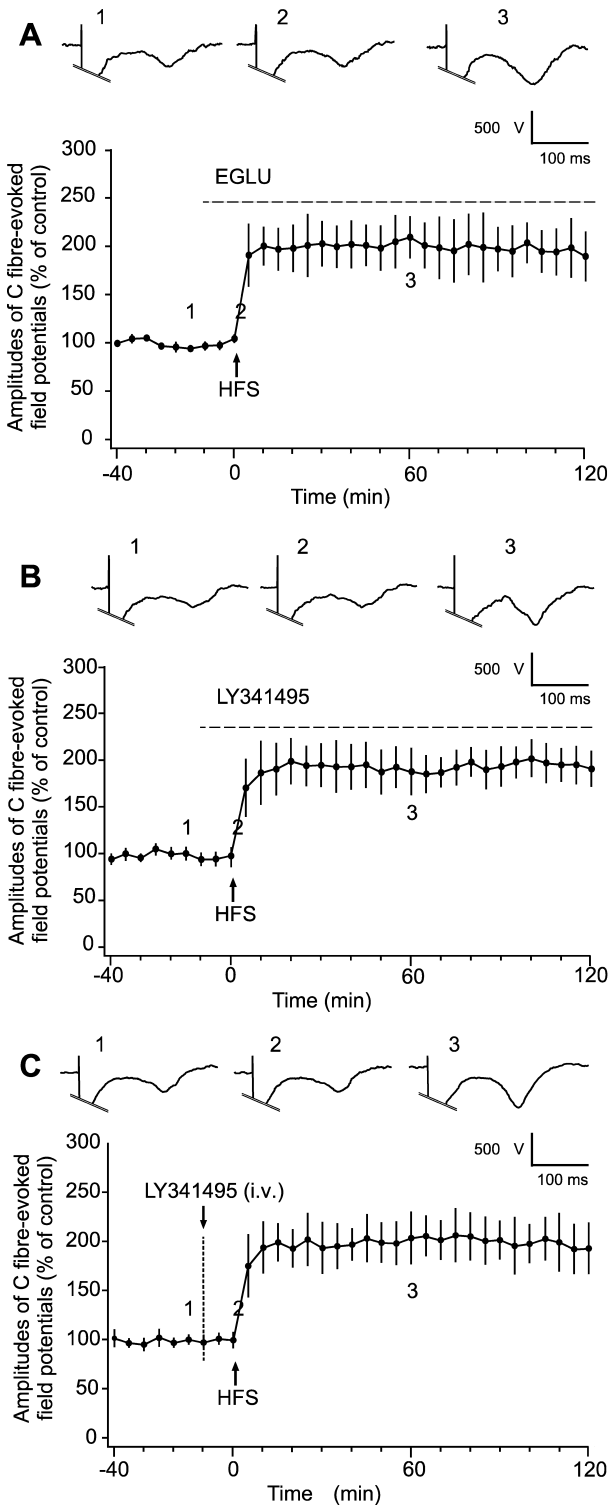

Fig. 4. The induction of LTP of C fibre-evoked field potentials is not influenced by blockade of group II mGluRs by the selective antagonists EGLU (200 μM) (A) or LY341495 (10 μM in B, 1 mg/kg i.v. in C): field potentials elicited by C fibre-activation undergo LTP in the presence of either drug. Individual, representative recordings from each set of experiments are shown above each diagram, recorded 15 min before- (1), immediately before- (2) and 60 min after (3) application of conditioning stimulus (HFS). Note the lack of effect of either mGluR-ligand per se ((2) vs (1)) on the amplitudes of evoked field potentials. The dashed lines indicate the onset and duration of controlled superfusion (A and B) or the time point of intravenous application (C).

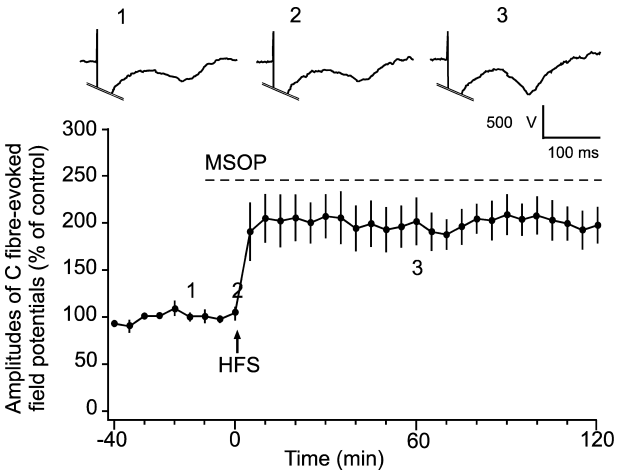

Fig. 5. Selective pharmacological blockade of group III mGluRs with MSOP fails to prevent the induction of LTP of C fibre-evoked spinal field potentials. Recorded potentials undergo LTP (3) in the presence of MSOP (200 μM). However, pharmacological blockade of group III mGluRs by MSOP does not alter the amplitudes of C fibre-evoked field potentials (2) prior to conditioning stimulation (HFS). Representative, individual recordings are shown at the top.

In the present study, amplitudes of C fibre-evoked field potentials were not altered by either (*S*)-MCPG or (*S*)-4CPG (Figs. 2 and 3), suggesting that the group I mGluR pathway may not be tonically active on spinal cord neurons driven by C fibre input. In contrast, considerable evidence implicates group I mGluRs in synaptic plasticity phenomena associated with prolonged and persistent pain, such as central sensitisation (Fundytus, 2001; Neugebauer, 2002). As shown here, the induction of LTP of C fibre-evoked field potentials by strong afferent stimulation is stereospecifically blocked by spinal antagonism of group I–II mGluRs by (*S*)-MCPG (Fig. 2). Potentiation also failed to develop in the presence of (*S*)-4CPG (Fig. 3), indicating that the observed MCPG-mediated blockade of LTP involves antagonism at group I mGluRs.

Group I mGluRs are thought to participate in the late expression and consolidation of LTP in vivo, as the induction of LTP in the hippocampus is followed by increased polyphosphoinositide hydrolysis in response to pharmacological agonism at mGluR sites (Aronica et al., 1991), and persistent LTP is contingent on group I mGluR-activation in freely moving rats (Manahan-Vaughan, 1997). Large surges of intracellular Ca^{2+} concentration are thought to lead to LTP by promoting kinase activity and protein phosphorylation, including phosphorylation of glutamate receptors (Barria et al., 1997; Nayak et al., 1996). In addition, intracellular metabolites linked to group I mGluR-activation such as arachidonic acid may contribute to the facilitation of glutamate release (Herrero et al., 1992). However, it is mechanistically relevant that activation of mGluR1/5 may not determine the direction of synaptic plasticity, since activation of group I mGluRs and some of their downstream signalling pathways including phospholipase C (PLC) are also a requisite step for the induction of LTD in spinal cord (Chen et al., 2000).

Out of the various intracellular effector systems that lie downstream of group I mGluRs, protein kinase C (PKC) and extracellular signal-regulated kinase (erk1/2) pathways seem to be involved in inflammatory and neuropathic pain. Activation of the erk1/2 pathway, which lies downstream of group I mGluRs and is critical for LTP and various forms of hippocampus-dependent learning (Sweatt, 2001), occurs in spinal neurones following noxious stimulation and reportedly mediates plasticity of nociceptive responses (Ji et al., 1999; Karim et al., 2001). PKC activity increases via mGluR1/5-activation (Karim et al., 2001) and is causally related to neuropathic pain (Malmberg et al., 1997). Moreover, spinal PKC activation is attenuated by antisense knockdown or pharmacological blockade of mGluR1 (Fundytus et al., 2001; Yashpal et al., 2001) in neuropathic rats.

4.2. Recruitment of spinal group II mGluRs is not required for LTP induction

We administered the selective group II mGluR antagonist LY341495 intravenously (1 mg/kg) prior to applying conditioning stimulation (Fig. 4C), but this pre-treatment did not block LTP induction. Similar doses significantly reverse attenuating effects by the group II mGluR agonist LY379268 on paw-licking behaviour in the second phase of the formalin test (Simmons et al., 2002). Spinal superfusion with LY341495 (Fig. 4B), did not prevent LTP either, thus providing no support for a role for group II mGluRs in the induction of LTP of C fibre-evoked field potentials. Moreover, the view that group II mGluR-activation is not required for the generation of spinal LTP is strengthened by the failure of the selective group II antagonist EGLU to impede potentiation (Fig. 4A).

The lack of involvement of mGluR2/3 in this form of synaptic plasticity is not surprising insofar as mGluR2/3 have been implicated primarily in the depression of excitatory and inhibitory responses in spinal in vitro preparations (Dong and Feldman, 1999; Gerber et al., 2000). The fact that input resistance and postsynaptic membrane potentials did not vary in these reports suggests a presynaptic mechanism of action and is consistent with a prominent occurrence of mGluR2/3 in presynaptic sites in superficial laminae of the spinal dorsal horn (Azkue et al., 2000; Carlton et al., 2001; Petralia et al., 1996). Moreover, extrasynaptic locations reported for mGluR2/3 in dorsal horn synapses (Azkue et al., 2000) support a *spillover* mechanism for mGluR2/3 activation similar to that suggested for spinal kainate receptors (Kerchner et al., 2001), whereby the receptor would be reached by diffusion of glutamate released from nearby synapses only under conditions of high glutamate release. An analogous mechanism of receptor activation by lateral glutamate diffusion has been proposed to operate in cerebellar glomeruli (Mitchell and Silver, 2000).

4.3. Group III mGluR-activation is not involved in spinal LTP induction

Hybridisation signal and immunoreactivity for mGluR4 and mGluR7 have been detected in the superficial dorsal horn and dorsal root ganglia (Azkue et al., 2001; Li et al., 1997; Ohishi et al., 1995), with a prominent expression of receptor protein in presynaptic sites of presumed small calibre, unmyelinated fibres (Azkue et al., 2001; Li et al., 1997). Subtype-selective agonism at mGluR8 by (*S*)-3,4-dicarboxyphenylglycine (DCPG) also has depressant effects on spinal responses evoked by primary afferents (Thomas et al., 2001), although the anatomy of spinal mGluR8 expression is not known. Evidence shows that a presynaptic mGluR is involved in the depression of synaptic responses in the CNS by the generic group III mGluR agonist L-AP4 (Anwyl, 1999; Conn and Pin, 1997). At the spinal cord level, activation of group III mGluRs by L-AP4 attenuates formalin-induced nociception in the second phase (Fisher and Coderre, 1996), and decreases the development of sensitisation ensuing peripheral nerve injury (Fisher et al., 2002). As suggested by the present data, the induction of LTP of C fibre-evoked field potentials does not require activation of group III mGluRs, which is in line with the view that group III mGluRs may act by attenuating rather than by facilitating spinal nociceptive responses. As opposed to group II mGluRs, which demonstrate no influence on normal nociceptive transmission, activation of group III mGluRs also attenuates responses to acute noxious and innocuous stimuli in the absence of central sensitisation (Gerber et al., 2000; Neugebauer et al., 2000). An inhibitory effect on acute transmission, however, does not seem to be tonically active, since spinal field potentials are not altered by spinally applied MSOP as shown here.

In summary, we provide evidence that activation of group I mGluRs is a requisite step for the induction of LTP of C fibre-evoked field potentials in the rat spinal dorsal horn, whereas activation of mGluR subtypes of groups II and III are not involved in this form of synaptic plasticity. This strengthens the view that glutamate receptors of the metabotropic type play a critical role in the development of plastic changes that may be relevant to pathological pain.

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