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# The contribution of metabotropic glutamate receptors to models of persistent and chronic pain

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Abstract

The possible involvement of spinal metabotropic glutamate receptors (mGluRs) were examined in animal models of persistent and chronic pain. In Study 1, it was shown that spinal administration of relatively selective group I mGluR antagonists, or a selective group III mGluR agonist, but not a non-selective mGluR antagonist, slightly, but significantly reduced nociceptive scores in the rat formalin test. Also, spinal administration of a non-selective mGluR agonist, or a selective group I mGluR agonist, but not a relatively selective group II agonist, enhanced formalin-induced nociception. The pro-nociceptive effects of these agents were reversed by a non-selective mGluR antagonist or by an *N*-methyl-D-aspartate receptor (NMDAR) antagonist. In Study 2, it was shown that intrathecal administration of two non-selective mGluR agonists or a selective group I mGluR agonist, but not a selective group II or group III mGluR agonist, produced spontaneous nociceptive behaviours (SNBs) in rats. Also, the SNBs induced by these agents were reduced by a non-selective mGluR antagonist or by an NMDAR antagonist. In Study 3, it was shown that intrathecal administration of a selective group I mGluR agonist produced persistent mechanical allodynia, mechanical hyperalgesia and heat hyperalgesia in rats. In Study 4, it was shown that early, but not late intrathecal administration of a relatively selective group I mGluR antagonist reduced nociceptive behaviours in a model of neuropathic pain. In Study 5, it was shown that intrathecal administration of a selective group I mGluR antagonist reduced mechanical allodynia and cold hyperalgesia, while a selective group II mGluR agonist and a selective group III mGluR agonist only reduced mechanical allodynia and cold hyperalgesia, respectively, in

the neuropathic pain model. Results from these studies first suggest that spinal group I mGluRs may be more critically involved in the development of chronic nociceptive behaviours, compared to persistent nociceptive behaviours. Second, spinal administration of group II or group III mGluR agonists appear to be more effective in reducing certain types of chronic nociceptive behaviours, than reducing persistent nociception. Third, the development of nociceptive behaviours in models of pain may involve a group I mGluR/NMDAR interaction.

Résumé

Nous avons étudié, sur des modèles animaux, le rôle que pourraient jouer les récepteurs métabotropiques du glutamate de la moelle épinière (mGluRs) dans la douleur chronique et persistante. La première étude a permis de démontrer que l'administration spinale d'antagonistes des mGluRs relativement sélectifs du groupe I, ou d'un agoniste sélectif du mGluR du groupe III, mais non d'un antagoniste non sélectif des mGluRs, se traduit par une réduction légère mais significative des scores nociceptifs de rats soumis au test du formaldéhyde. De plus, l'administration dans la moelle épinière d'un agoniste non sélectif des mGluRs ou d'un agoniste sélectif du groupe I de ce récepteur, mais non d'un agoniste relativement sélectif du groupe II, a accru la nociception induite par le formaldéhyde. L'administration d'un agoniste non sélectif des mGluRs ou d'un agoniste du récepteur *N*-méthyl-D-aspartae (NMDAR) a inversé les effets pro-nociceptifs de ces agents. Dans le cadre de la deuxième étude, nous avons démontré que l'administration intrathécale, à des rats, de deux agonistes non sélectifs de mGluR ou d'un agoniste sélectif dugroupe I, mais non d'un agoniste sélectif du groupe II ou d'un agoniste du groupe III, provoque des comportements nociceptifs spontanés (CNS). De plus, l'administration d'un agoniste non sélectif des mGluRs ou d'un antagoniste du NMDA a atténué les CNS provoqués par ces agents. Une troisième étude, également réalisée sur des rats, a permis de démontrer que l'administration intrathécale d'un agoniste sélectif du groupe I des mGluRs produit une allodynie mécanique, une hyperalgie mécanique et une hyperalgie à la chaleur. Dans le cadre d'une quatrième étude, nous avons démontré que l'administration intrathécale précoce mais non tardive d'un agoniste relativement sélectif des mGluRs atténue les comportements nociceptifs

observés dans un modèle de douleur névropathique. Une cinquième étude a permis de démontrer que l'administration intrathécale d'un agoniste sélectif du groupe I des mGluRs atténue l'allodynie mécanique et l'hyperalgésie au froid, alors que l'administration d'un agoniste sélectif du groupe II ou d'un agoniste sélectif du groupe III n'atténue respectivement que l'allodynie mécanique et l'hyperalgésie au froid observées avec le modèle de la douleur névropathique. Les résultats de ces études semblent indiquer que les mGluRs du groupe I spinaux pourraient jouer un rôle plus critique dans l'apparition de comportements nociceptifs chroniques que dans l'apparition de comportements nociceptifs persistants. De plus, l'administration dans la moelle épinière d'agonistes des mGluRs du groupe II ou III semble atténuer davantage certains types de comportements nociceptifs chroniques que la nociception persistante. Enfin, l'apparition de comportements nociceptifs observés dans des modèles de douleur pourrait être due en partie à une interaction entre les mGluRs du groupe I et les NMDARs.

Table of Contents

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## Statement of Original Contributions

Since 1991, a large number of studies have been published, which clearly demonstrate that ionotropic glutamate receptors (iGluRs) play a critical role in a number of different animal models of pain. However, when I started my Ph.D. training with Dr. Coderre, in September of 1994, little was known about the contribution of metabotropic glutamate receptors (mGluRs) to persistent and chronic nociception. Although the results from two recently published electrophysiological studies implicated the involvement of mGluRs in inflammation-related nociception (Neugebauer et al. 1994; Young et al. 1994), no behavioural studies had yet been published. Therefore, under the direction of Dr. Coderre, I carried out a series of experiments to investigate the possible contribution of mGluRs to persistent and chronic models of pain.

Study 1 represents the first attempt to investigate the possible contribution of mGluRs to nociceptive responses in a model of pain, using relatively selective mGluR compounds. Although the formalin test has been used extensively to demonstrate the role of iGluRs in nociception, Study 1 is the first demonstration that spinal group I mGluRs play a role in the formalin model, and that activation of spinal group III mGluRs produces antinocicepton in this model. This study also provides the first evidence that selective activation of group I mGluRs enhances *N*-methyl-D-aspartate receptor (NMDAR) function in the spinal cord, which mediates the facilitation of chemically-induced nociception in rats.

Although Meller and colleagues (1993) first showed that spinal administration of a non-selective mGluR compound produces nociception in rats, Study 2 of my thesis represents the first attempt to determine specifically which mGluR subtype(s) is(are) pro-

nociceptive. Although a number of investigators have previously characterized the spontaneous nociceptive behaviours (SNBs) induced by iGluR agonists, this study is the first characterization of mGluR agonist-related SNBs. Also, this is the first demonstration that selective activation of spinal group I mGluRs produces persistent nociception in rats, and that a group I mGluR/NMDAR interaction mediates the development of persistent nociception.

Although Meller and colleagues (1993) also showed that spinal co-administration of AMPA and a *non-selective* mGluR agonist produces *acute* mechanical hyperalgesia, Study 3 represents the first attempt to thoroughly characterize the types of nociceptive behaviours induced by spinal administration of a *selective* mGluR agonist. This is the first demonstration that *selective* activation of spinal group I mGluRs produces *persistent* mechanical allodynia, mechanical hyperalgesia and cold hyperalgesia in rats.

Study 4 represents the first attempt to determine the possible antinociceptive effects of a spinally administered mGluR compound in a model of neuropathic pain. Although Mao, Price, Hayes, Lu and Mayer (1992) had previously shown that iGluR subtypes differentially influence the development and maintenance of heat hyperalgesia associated with chronic constriction injury (CCI) of the sciatic nerve, Study 4 is the first attempt to determine whether an mGluR compound influences the development and/or maintenance of mechanical allodynia and cold hyperalgesia in the CCI model. This study represents the first evidence that group I mGluRs contribute to the development, but not the maintenance of CCI-related nociceptive responses.

Study 5 represents the first attempt to determine whether *selective* blockade of spinal group I mGluRs, or *selective* activation of spinal group II or group III mGluRs

produces antinociception in the CCI model. Results from this study not only confirm that spinal group I mGluRs contribute to the development of mechanical allodynia and cold hyperalgesia in the neuropathic pain model, but it is also the first demonstration that activation of spinal group II and group III mGluRs differentially modulate the development of these nociceptive behaviours.

Together, these studies have made a significant contribution to our understanding of how mGluRs in the spinal cord contribute to persistent and chronic nociception.

List of Tables

Page

List of Figures

Page

List of Appendices

| | |
|---|---|
| 4C3HPG | 4-carboxy-3-hydroxyphenylglycine |
| 4CPG | 4-carboxyphenylglycine |
| 7CK | 7-chlorokynurenate |
| ACPD | 1-aminocyclopentane-1,3-dicarboxylic acid |
| ADA | azetidine-2,4-dicarboxylic acid |
| ADPD | (2*S*,4*S*)-2-amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-dioic acid |
| AIDA | 1-aminoindan-1,5-dicarboxylic acid |
| AP4 | 2-amino-4-phosphonobutyric acid |
| AP5 | 2-amino-5-phosphonopropionic acid |
| AP7 | 2-amino-7-phosphonopropionic acid |
| APDC | 4-aminopyrrolidine-2,4-dicarboxylate |
| AMPA | α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid |
| AMPAR | α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor |
| Asp | aspartate |
| $Ca^{2+}$ | calcium |
| $[Ca^{2+}]_I$ | intracellular calcium concentration |
| cAMP | cyclic adenosine 3',5'-monophosphate |
| CCG-I | (2*S*,1'*S*,2'*S*)-2-(carboxycyclopropyl)-glycine |
| CNS | central nervous system |
| CNQX | 6-cyano-7-nitro-quinoxalinedione |
| CPP | 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid |
| CTZ | cyclothiazide |
| DAG | diacylglycerol |
| DCG-IV | (2*S*,1'*R*,2'*R*,3'*R*)-2-(2,3-dicarboxycyclopropyl)glycine |
| DGG | γ-D-glutamyglycine |
| DHPG | 3,5-dihydroxyphenylglycine |
| DNQX | 6,7-dinitro-quinoxalinedione |
| DTNB | 5,5'-dithiobis-2-nitro-benzoic acid |
| DTT | dithothreitol |
| EAAs | excitatory amino acids |
| EGlu | ethyl glutamate |
| EPSCs | excitatory postsynaptic currents |
| EPSPs | excitatory postsynaptic potentials |
| Glu | L-glutamate |
| GluR | glutamate receptor |
| G-proteins | GTP-binding proteins |
| GTP | guanosine 5'-triphosphate |
| $IP_3$ | inositol trisphosphate |
| $K^+$ | potassium |
| KA | kainate |
| KAR | kainate receptor |

| | |
|---|---|
| LTP | long-term potentiation |
| MAP4 | (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid |
| MCCG | (2*S*,3*S*,4*S*)-methyl-2-(2-carboxycyclopropyl)glycine |
| MCPG | α-methyl-4-carboxyphenylglycine |
| $Mg^{2+}$ | magnesium |
| MK-801 | dizocilpine maleate |
| MSPG | α-methyl-4-sulfono-phenylglycine |
| MSOPPE | (*RS*)-α-methylserine-O-phosphate monophenyl-phoryl ester |
| MTPG | α-methyl-4-tetrazoylphenylglycine |
| $Na^{+}$ | sodium |
| NBQX | 2,3-dihydroxy-6-nitro7-sulfamoyl-benzo(f)quinoxaline |
| NMDA | *N*-methyl-D-aspartate |
| NMDAR | *N*-methyl-D-aspartate receptor |
| NS | nervous system |
| NS-102 | 5-nitro-6,7,8,9-tetrahydrobenzo[g]indol-2,3-dione-3-oxime |
| PCP | phencyclidine |
| PI | phosphatidylinositol |
| $PIP_2$ | phosphatidylinositol-4,5-bisphosphate |
| PLC | phospholipase C |
| Quis | quisqualate |
| SOP | serine-*O*-phosphate |
| VGCC | voltage-gated calcium channels |

# CHAPTER 1: THESIS INTRODUCTION

## Classification, Pharmacology, Signal Transduction Mechanisms, and Localization of Glutamate Receptors (GluRs)

More than 30 years ago, neuroscientists discovered that application of the excitatory amino acids (EAAs), L-glutamate (Glu) or L-aspartate (Asp), to various regions of the mammalian central nervous system (CNS) strongly depolarizes the neuronal membrane potential (Curtis & Watkins, 1963; Krnjevic & Phillis, 1963; McLennan, Huffman & Marshall, 1968). Since these first studies, research has made remarkable progress in elucidating the mechanisms that mediate the action of these two major excitatory neurotransmitters in the CNS. The use of novel electrophysiological and neuroanatomical techniques, in conjunction with recently developed selective pharmacological tools, has significantly contributed to our understanding of the diverse functions of EAAs in the nervous system (NS).

In an early review, Watkins and Evans (1981) cited evidence for the existence of three distinct glutamate receptor (GluR) subtypes in the mammalian CNS. The GluR subtypes, *N*-methyl-D-aspartate (NMDA), quisqualate (Quis), and kainate (KA) were classified based on their preferred exogenous agonists. In 1984, Foster and Fagg noted that a fourth GluR, which responds to L-2-amino-4-phosphonobutyric acid (L-AP4), had been identified, and a few years later, Monaghan, Bridges and Cotman (1989) reported that a fifth receptor, which was selectively activated by *trans*-1-aminocyclopenantane-1,3-dicarboxylic acid (*trans*-ACPD or (1*S*,3*R*)-ACPD), had been added to the GluR family.

Since the late 1980's, the GluR classification scheme has been modified considerably. First, the term 'quisqualate' has been replaced with α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA). This revision is based on the demonstration that AMPA is a more selective agonist at this receptor subtype, since Quis also stimulates the production of inositol triphosphate ($IP_3$) via the *trans*-ACPD-sensitive receptor (see Lodge & Collingridge, 1991). Second, the iGluRs, AMPA and KA, are often collectively referred to as non-NMDARs, since the agonists, AMPA and KA display some activity at each other's receptor (Watkins, Krogsgaard-Larsen & Honoré, 1991). Third, the most current classification system distinguishes between ionotropic glutamate receptors (iGluRs), which form ion channels, and metabotropic glutamate receptors (mGluRs), which are linked through guanosine 5'-triphosphate (GTP)-binding proteins (G-proteins) to the production or inhibition of intracellular messengers (Watkins & Collingridge, 1994).

Ionotropic Glutamate Receptors (iGluRs)

The *N*-Methyl-D-Aspartate Receptor (NMDAR). The NMDAR mediates the slow component of excitatory postsynaptic currents (EPSCs) in the mammalian CNS (Forsythe & Westbrook, 1988; MacDonald & Wojtowicz, 1980; Trussell & Fischbach, 1989; Trussell, Thio, Zorumski & Fischbach, 1988). This iGluR subtype is characterized by a voltage-dependent magnesium ($Mg^{2+}$) block (Mayer, Westbrook & Guthrie, 1984; Nowak, Bregestovski, Ascher, Herbet & Prochiantz, 1984), and by a high permeability to calcium ($Ca^{2+}$) (MacDermott, Mayer, Westbrook, Smith & Barker, 1986; Mayer & Westbrook, 1987).

The binding of ligands to different sites on the NMDAR complex negatively or positively modulates NMDAR channel activity. The NMDAR complex is comprised of the following binding sites:

1. The Glu recognition site: In addition to Glu, several other agonists, such as NMDA and ibotenate bind at this site, as well as the competitive antagonists, D-2-amino-5-phosphonopropionic acid (D-AP5), D,L-2-amino-7-phosphonopropionic acid (D,L-AP7) and 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP). The binding of Glu at this site opens the channel if it is not blocked by $Mg^{2+}$.

2. The strychnine-insensitive glycine binding site: Binding of the natural ligand, glycine, or of the selective agonists, D-serine or D-alanine, at this site, is mandatory for activation of the NMDAR channel. Also, binding of ligands to this site facilitates the action of ligands at the Glu recognition site. The action of glycine at this site is selectively antagonized by 7-chlorokynurenate (7CK).

3. The voltage-dependent ion channel: This channel is blocked by extracellular $Mg^{2+}$ when the neuronal membrane is at resting potential. The release of Glu into the synaptic cleft rapidly activates non-NMDARs, which depolarizes the postsynaptic membrane and removes the voltage-dependent $Mg^{2+}$ blockade, allowing full activation of the NMDAR.

4. The phencyclidine (PCP) binding site: The binding of the high affinity non-competitive antagonists, PCP and dizocilpine maleate (MK-801), as well as the low affinity non-competitive antagonists, ketamine, memantine, dextromethorphan and dextrorphan to this site inhibits NMDAR activity.

5. The polyamine binding site: Several polyamines, such as spermidine and spermine, act at this site and enhance the binding of ligands to the PCP site. Antagonists at this site include ifenprodil and eliprodil.

6. The binding site for zinc and other cations: The binding of several cations to this site inhibits NMDAR activity.

7. The redox modulatory site: Thiol-oxidizing (i.e. 5,5'-dithiobis-(2-nitro-benzoic acid (DTNB) and nitric oxide) or disulfide-reducing (i.e. dithothreitol (DTT), cysteine, and homocysteine) agents act at this site and have been shown to negatively or positively modulate NMDAR activity, respectively.

8. The proton sensitive site: Protons acting at this site or near the face of the receptor inhibit NMDAR activity.

(see Hollman & Heineman, 1994; Kemp & Leeson, 1993; Lodge & Johnson, 1991; Scott, Sutton & Dolphin, 1993; Sucher, Awobuluyi, Choi & Lipton, 1996 for reviews).

Although several NMDAR subunits have been cloned (NR1 (including eight splice variants), NR2A-NR2D) (Ikeda et al. 1992; Ishii et al. 1993; Kutsuwada et al. 1992; Meguro et al. 1992; Monyer et al. 1992), NMDARs expressing different combinations of these subunits are not yet pharmacologically distinguishable. However, it is of general consensus that multiple types of NMDARs with slightly different functional properties exist in the mammalian NS, and these properties are determined by various combinations of subunits comprising the receptor. *In situ* hybridization studies have determined the mRNA expression patterns for each NMDAR subunit in the rat CNS. These studies have demonstrated that although NR1 subunit localization is ubiquitous in the brain (Moriyoshi, Masu, Ishii, Shigemoto, Mizuno & Nakanishi, 1991;

Shigemoto, Nakanishi & Mizuno, 1992), each NR2 subunit is differentially expressed. For example, high levels of the NR2A subunit are found in the cerebrocortex, cerebellar cortex, hippocampus and olfactory bulb, whereas, NR2B, NR2C and NR2D mRNA are most prominently expressed in the telencephalon, cerebellar cortex and diencephalon/lower brain stem regions, respectively (Kutsuwada et al. 1992; Monyer et al. 1992; Monyer, Burnashev, Laurie, Sakmann & Seeburg, 1994; Moriyoshi et al. 1991; Watanabe, Inoe, Sakimura & Mishina, 1992). Details relating to the spinal cord distribution of NMDAR subunits are discussed below (see Localization of EAAs and GluRs in the Dorsal Horn and Primary Afferent Neurons).

Accumulating knowledge about NMDAR function has generated much excitement in the neuroscience community. Evidence suggests that this iGluR subtype mediates neurotoxicity, as well as several types of neuronal plasticity in the mammalian CNS. For example, the NMDAR has been shown to play a critical role in the induction of hippocampal long-term potentiation (LTP), the neural model of learning and memory (Collingridge, Kehl & McLennan, 1983; Harris, Ganong & Cotman, 1984; Morris, Anderson, Lynch & Baudry, 1986); the formation of kindling, the experimental model of epilepsy (Bowyer, 1982; Bowyer, Albertson, Winters & Baselt, 1983; Gilbert & Mack, 1990; Morimoto, Katayama, Inoue & Sato, 1991; see Löscher, 1998 for review); and the initiation of "wind-up", the experimental model for injury-related sensitization of spinal cord neurons (Davies & Lodge, 1987; Dickenson & Sullivan, 1987a). Moreover, evidence suggests that excessive NMDAR activity may play a role in the etiology of neuronal degenerative disorders (see Meldrum & Garthwaite, 1990, for review).

The non-NMDARs. The α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid receptor (AMPAR) and kainate receptor (KAR) mediate the fast component of EPSCs in the mammalian CNS (Tang, Dichter & Morad, 1989; Trussell et al. 1988). The ion channels formed by these iGluR subtypes are permeable to sodium ($Na^+$) and potassium ($K^+$) (Jahr & Stevens, 1987). Unlike KARs, most AMPARs in the NS are impermeable to $Ca^{2+}$ (Geiger et al. 1995; Jonas & Burnashev, 1995; Köhler, Burnashev, Sakmann & Seeburg, 1993).

In addition to Glu, agonists that act at the non-NMDARs include Quis, AMPA and KA. Although AMPA and KA are agonists at each of the non-NMDAR subtypes, autoradiography studies have demonstrated that the distribution of high affinity [$^3$H] KA binding is distinct from that of [$^3$H] AMPA in the mammalian CNS (see Monaghan et al. 1989 for review). With the advance of molecular biological techniques, a number of non-NMDAR subunits have been cloned and categorized according to agonist selectivity. These studies demonstrate that GluR1-GluR4 display a higher affinity for AMPA over KA, whereas GluR5-GluR7, KA1 and KA2 show more selectivity for KA over AMPA (Boulter et al. 1990; Hollmann, O'Shea-Greanfield, Rogers & Heinemann, 1989; Keinänen et al. 1990). Therefore, combinations of GluR1-GluR4 subunits are thought to form native AMPA-preferring receptors, whereas native KA-preferring receptors are formed by combinations of the GluR5-GluR7, KA1 and KA2 subunits. Evidence suggests that the GluR2 subunit determines the $Ca^{2+}$ permeability of the ion channel, since an AMPAR complex that includes this subunit displays very low permeability to this cation. In contrast, AMPAR complexes, composed of GluR1, GluR3 and GluR4, form ion

channels that are permeable to $Ca^{2+}$ (Boutler et al. 1990; Hume, Dingledine & Heinemann, 1991; Nakanishi, Schneider & Axel, 1990). Thus, it is believed that, in the mammalian NS, most of the AMPA-preferring receptors include the GluR2 subunit, since most native AMPARs are impermeable to $Ca^{2+}$.

The compounds, 6-cyano-7-nitro-quinoxalinedione (CNQX), 6,7-dinitro-quinoxalinedione (DNQX) and 2,3-dihydroxy-6-nitro7-sulfamoyl-benzo(f)quinoxaline (NBQX) are competitive antagonists at both the AMPAR and KAR (Fletcher, Martin, Aram, Honoré & Honoré, 1988; Sheardown, Neilsen, Hansen, Jacobsen & Honoré, 1990). However, GYKI 52466 (Donevan & Rogawski, 1993) and GYKI 53655 (Paternain, Morales & Lerma, 1995) have been used to discriminate between these two non-NMDAR subtypes, since these compounds are selective, non-competitive antagonists at the AMPAR. Also, evidence suggests that 5-nitro-6,7,8,9-tetrahydrobenzo[g]indol-2,3-dione-3-oxime (NS-102) is a selective antagonist for the KAR (Johansen, Drejer, Watjen & Nielsen, 1993; Lerma, Paternain, Naranjo & Mellstrim, 1993).

*In situ* hybridization studies have determined the expression patterns of the mRNAs for each AMPAR subunit in the rat CNS (Boutler et al. 1990; Keinänen et al. 1990; Sommer et al. 1990). These studies have demonstrated that GluR1-GluR4 are highly expressed in various cell populations in the hippocampus and cerebellum, as well as in different layers of the cerebrocortex. However, the expression pattern of each AMPAR subunit varies across different cell populations in each of these brain areas. *In situ* hybridization studies, examining the expression patterns of the mRNAs for each KAR subunit, show that they are differentially distributed throughout the rat CNS (Bahn,

Volk & Wisden, 1994; Bettler et al. 1990, 1992; Wisden & Seeburg, 1993). For example, KAR subunits are strongly expressed in the dentate gyrus (GluR6, KA1 and KA2) and CA3 region (GluR6, KA1, and KA2) of the hippocampus, as well as in the stellate/basket (GluR7), granule (GluR6) and Purkinje (GluR5) cells of the cerebellum. Also, two KAR subunits are also strongly expressed in certain layers of the cingulate (GluR5,GluR7) and piriform cortex (GluR5), as well as in the septum (GluR5), caudate-putamen, and reticular thalamus (GluR7). Details relating to the spinal cord distribution of AMPAR and KAR subunits are discussed below (see Localization of EAAs and GluRs in the Dorsal Horn and Primary Afferent Neurons).

Evidence suggests that excessive non-NMDAR activity is involved in pathological conditions involving neuronal degeneration. For example, a number of studies have demonstrated that non-NMDAR antagonists reduce hippocampal neuronal cell loss in animal models of ischemia (Buchan, Li, Cho & Pulsinelli, 1991; Diemer, Jøorensen, Johansen, Sheardown, & Honoré, 1992; Pulsinelli, Sarokin & Buchan, 1993; Sheardown et al. 1990). Interestingly, it has recently been reported that following global ischemia, the expression of GluR2 mRNA is reduced in hippocampal neurons (Pellegrini-Giampietro, Pulsinelli & Zukin, 1994; Pellegrini-Giampietro, Zukin, Bennett, Con & Pulsinelli, 1992). Since AMPARs without the GluR2 subunit form ion channels that are permeable to $Ca^{2+}$, it has been suggested that a change in the AMPAR subunit composition contributes to the Glu-induced excessive $Ca^{2+}$ influx, which is known to mediate ischemia-related cell death (Pellegrini-Giampietro, Gorter, Bennett & Zukin, 1997).

Metabotropic Glutamate Receptors (mGluRs)

Group I mGluRs. The group I mGluRs, mGluR1 (cloned splice variants include a, b and c) and mGluR5 (cloned splice variants include a and b) are linked by a G-protein to phosphatidylinositol (PI) hydrolysis (Houamed et al. 1991; Joly, Gomeza, Brabet, Curry, Bockaert & Pin, 1995; Masu, Tanabe, Tsuchida, Shigemoto & Nakanishi, 1991; Pickering et al. 1993; Pin, Waeber, Prezeau, Bockaert & Heinemann, 1992). It is thought that the binding of Glu to group I mGluRs activates a stimulatory G-protein, which in turn activates the enzyme, phospholipase C (PLC). Following activation, this enzyme cleaves PI- 4,5-bisphosphate ($PIP_2$) into two second messengers, $IP_3$ and diacylglycerol (DAG). $IP_3$ diffuses into the cytoplasm, binds to a receptor on the endoplasmic reticulum and releases $Ca^{2+}$ from internal stores. DAG remains in the plasma membrane, where it has the potential to activate protein kinase C (PKC) or release arachidonic acid from the cellular membrane. Studies have provided evidence that activation of *native* group I mGluRs in various regions of the mammalian CNS leads to PI hydrolysis (Aronica, Condorelli, Nicoletti, Dell'Albani, Amico & Balazs, 1993; Bedingfield, Kemp, Jane, Tse, Roberts & Watkins, 1995; Birse et al. 1993; Brammer et al. 1991; Casabona et al. 1997; Eaton et al. 1993; Klein, Reymann & Riedel, 1997; Littman et al. 1995; Littman, Munir, Flagg & Robinson, 1992; Littman & Robinson, 1994; Lorenzini, Bisso, Fortuna & Michalek, 1996; Manzoni et al. 1991; Manzoni, Fagni, Pin, Rassendren, Poulet, Sladeczek & Bockaert, 1990; Mistry & Challiss, 1996; Mistry, Prabhu, Godwin & Challiss, 1996; Neil, Kendall & Alexander, 1996; Patel, Moore, Thompson, Keith & Salama, 1990; Pellegrini-Giampietro, Torregrossa & Moroni, 1996; Schoepp,

Goldsworthy, Johnson, Salhoff & Baker, 1994; Schoepp, Johnson, Salhoff, McDonald & Johnston, 1991; Sladeczek et al. 1992; Suzdak, Sheardown & Honoré, 1993; Toms, Jane, Tse & Roberts, 1995; Weiss, Ellis, Hendley & Lenox, 1989), the mobilization of intracellular $Ca^{2+}$ (Brammer et al. 1991; Carmant, Woodhall, Ouardouz, Robitaille & Lacaille, 1997; Courtney, Lambert & Nicholls, 1990; Guiramand, Vignes & Recasens, 1991; Llano, Dreesen, Kano & Konnerty, 1991; Murphy & Miller, 1988, 1989; Netzeband, Parsons, Sweeney & Gruol, 1997; Phenna, Jane & Chad, 1995), the translocation and/or activation of PKC (Angenstein, Hirschfelder & Staak, 1997; Fukunaga, Soderling, & Miyamoto, 1992; Manzoni, Finiels-Marlier, Sassetti, Bockaert, le Peuch & Sladczek, 1990; Vaccarino, Liljequist & Tallman, 1991), the production of nitric oxide (Bhardwaj, Northington, Martin, Hanley, Traystman & Koehler, 1997; Okada, 1992), and the release of arachidonic acid (Dumuis et al. 1993; Sladeczek et al. 1992).

The pharmacological profiles for the group I mGluR subtypes have been well characterized. Table 1.1 shows the *median* half-maximum effective concentrations of various agonists to induce PI hydrolysis ($EC_{50}$), and the *median* half-maximum effective concentrations of various antagonists to block agonist-induced PI hydrolysis ($IC_{50}$) in cells lines expressing cloned rat group I mGluRs. These values were calculated from the $EC_{50}$ and $IC_{50}$ values reported in the cited studies. Table 1.1 also shows the potency rank of each compound, which is based on its median $EC_{50}$ or $IC_{50}$ value.

Table 1.1: Pharmacology of cloned rat mGluRs

| mGluR compound | mGluR1a | mGluR1b | mGluR1c | mGluR5a | mGluR5b | mGluR2 | mGluR3 | mGluR4a | mGluR6 | mGluR7 | mGluR8 |
|---|---|---|---|---|---|---|---|---|---|---|---|
| glutamate | **AG2**$_{(12)}$ | **AG3**$_{(56)}$ | **AG2**$_{(13)}$ | **AG4**$_{(10)}$ | **AG3**$_{(8)}$ | **AG5**$_{(12)}$ | **AG3**$_{(4)}$ | **AG2**$_{(4)}$ | **AG3**$_{(16)}$ | **AG3**$_{(1150)}$ | **AG3**$_{(1)}$ |
| ibotenate | **AG3**$_{(32)}$ | **AG2**$_{(44)}$ | **AG3**$_{(60)}$ | **AG3**$_{(6)}$ | **AG2**$_{(2)}$ | **AG7**$_{(35)}$ | **AG5**$_{(12)}$ | **AG3**$_{(50)}$ | **AG4**$_{(>300)}$ | -- | -- |
| quisqualate | **AG1**$_{(0.4)}$ | **AG1**$_{(3)}$ | **AG1**$_{(0.8)}$ | **AG1**$_{(0.2)}$ | **AG1**$_{(0.06)}$ | **AG9**$_{(>1000)}$ | **AG7**$_{(40)}$ | NE | **AG4**$_{(>300)}$ | NE | -- |
| ACPD[a] | **AG5**$_{(43)}$ | **AG4**$_{(105)}$ | **AG4**$_{(130)}$ | **AG5**$_{(16)}$ | **AG4**$_{(10)}$ | **AG4**$_{(8)}$ | **AG4**$_{(8)}$ | NE | NE | NE | **AG4**$_{(47)}$ |
| (*RS*)-DHPG | **AG4**$_{(33)}$ | -- | -- | **AG2**$_{(2)}$ | -- | NE | -- | NE | -- | NE | NE |
| *trans*-ADA | **AG7**$_{(189)}$ | -- | -- | **AG6**$_{(32)}$ | -- | -- | -- | -- | -- | -- | -- |
| (*RS*)-AIDA | **ANT3**$_{(110)}$ | -- | -- | **ANT**$_{(>1000)}$ | -- | **AG**$_{(>1000)}$ | -- | -- | -- | -- | -- |
| 4C3HPG[b] | **ANT1**$_{(40)}$ | -- | -- | **AG**$_{(>300)}$ | **AG**$_{(?)}$ | **AG6**$_{(34)}$ | -- | NE | -- | -- | -- |
| (*S*)-4CPG | **ANT2**$_{(52)}$ | -- | -- | NE | NE | **AG8**$_{(533)}$ | -- | NE | -- | -- | -- |
| MCPG[c] | **ANT4**$_{(112)}$ | -- | -- | NE | NE | **ANT**$_{(370)}$ | -- | NE | -- | NE | **ANT2**$_{(320)}$ |
| (1*S*,3*S*)-ACPD | **AG8**$_{(300)}$ | -- | -- | **AG7**$_{(>300)}$ | **AG5**$_{(>300)}$ | -- | **AG6**$_{(30)}$ | -- | -- | -- | -- |
| L-CCG-I | **AG6**$_{(50)}$ | -- | -- | -- | -- | **AG2**$_{(0.5)}$ | **AG2**$_{(1)}$ | **AG3**$_{(50)}$ | -- | -- | **AG2**$_{(0.6)}$ |
| DCG-IV | NE | -- | -- | NE | -- | **AG1**$_{(0.3)}$ | **AG1**$_{(0.2)}$ | NE | -- | NE- | NE |
| (2*R*,4*R*)-APDC[d] | NE | -- | -- | -- | -- | **AG3**$_{(1.5)}$ | -- | -- | -- | -- | -- |
| MCCG | NE | -- | -- | -- | -- | **ANT**$_{(?)}$ | -- | -- | NE | -- | -- |
| MSPG | NE | -- | -- | -- | -- | **ANT2**$_{(250)}$ | -- | **ANT**$_{(>1000)}$ | -- | -- | -- |
| MTPG | **ANT**$_{(>1000)}$ | -- | -- | NE | -- | **ANT3**$_{(450)}$ | -- | NE | -- | -- | -- |
| ADPD | NE | -- | -- | NE | -- | **ANT1**$_{(18)}$ | -- | -- | -- | -- | -- |
| L-AP4 | NE | -- | NE | NE | -- | NE | NE | **AG1**$_{(0.9)}$ | **AG1**$_{(0.9)}$ | **AG2**$_{(330)}$ | **AG1**$_{(0.5)}$ |
| L-SOP | NE | -- | -- | -- | -- | -- | -- | **AG2**$_{(4)}$ | **AG2**$_{(3)}$ | **AG1**$_{(160)}$ | -- |
| MAP4 | NE | -- | -- | -- | -- | **ANT**$_{(?)}$ | -- | **ANT**$_{(130)}$ | **AG**$_{(?)}$ | -- | **ANT1**$_{(<50)}$ |
| References | 1,2,3,4,5,6,7,8,9, 10,11,12,13,14,15, 16,32,33 | 17 | 18 | 10,11,14, 15,19,20, 32,33 | 14 | 4,5,6,7,8,9, 10,19,21, 22,32,33 | 6,23,24 | 6,7,8,9,12, 19,23,25, 26,32,33 | 4,27 | 19,28,29 | 30,31 |

AG#, relative potency rank of agonist; ANT#, relative potency rank of antagonist; numbers inside brackets are the median $IC_{50}/EC_{50}$ values (in μM) calculated from the cited references; ?, $IC_{50}/EC_{50}$ values were not cited in reference; NE, compound was not effective at the maximum concentration tested; --, the compound was not tested; [a] (1S,3R)-ACPD); [b] ((S)-4C3HPG or (RS)-4C3HPG); [c] ((+)-MCPG or (RS)-MCPG); [d] ($IC_{50}/EC_{50}$ values were obtained from cloned human mGluRs); References: 1 (Houamed et al. 1991); 2 (Masu et al. 1991);3 (Thomsen and Suzdak, 1993a); 4 (Sekiyama et al. 1996); 5 (Schoepp et al. 1995); 6 (Hayashi et al. 1993); 7 (Hayashi et al. 1994);8 (Hayashi et al. 1992); 9 (Thomsen et al. 1994); 10 (Moroni et al. 1997); 11 (Manuhan-Vaughan et al. 1996);12 (Thomsen and Suzdak, 1993b);13 (Thomsen et al. 1993); 14 (Joly et al. 1995); 15 (Brabet et al. 1995) ; 16 (Ito et al. 1992); 17 (Pickering et al. 1993);18 (Pin et al. 1992).; 19 (Gereau and Conn, 1995); 20 (Abe et al. 1992); 21 (Tanabe et al. 1992); 22 (Flor et al. 1995); 23 (Tanabe et al. 1993); 24 (Pin et al. 1994); 25 (Thomsen et al. 1992); 26 (Johansen and Robinson, 1995); 27 (Nakajima et al. 1993); 28 (Saugstad et al. 1994); 29 (Okamoto et al. 1994); 30 (Saugstad et al. 1997); 31 (Duvoisin et al. 1995); 32 (Gomeza et al. 1996); 33 (Wermuth et al. 1996)

The rank order of agonist potency for mGluR1a is the following: Quis > Glu > ibotenate > (*RS*)-3,5-dihydroxyphenylglycine ((*RS*)-DHPG) > (1*S*,3*R*)-ACPD > (2*S*,1'*S*,2'*S*)-2-(carboxycyclopropyl)-glycine (L-CCG-I) > *trans*-azetidine-2,4-dicarboxylic acid (*trans*-ADA) > (1*S*,3*S*)-ACPD (Brabet, Bockaert & Pin, 1995; Hayashi et al. 1992; Houamed et al. 1991; Ito et al. 1992; Joly et al. 1995; Manahan-Vaughan et al. 1996; Masu et al. 1991; Thomsen, Mulvihill, Haldeman, Pickering, Hampson & Suzdak, 1993; Thomsen & Suzdak, 1993a). Although the agonist profile for mGluR1a is best characterized, the rank order of potency, for the agonists that were tested, is similar for all three splice variants (Pickering et al. 1993; Pin et al. 1992). The rank order of agonist potency for mGluR5a is the following: Quis > (*RS*)-DHPG > ibotenate > Glu > (1*S*,3*R*)-ACPD > *trans*-ADA > (1*S*,3*S*)-ACPD (Abe, Sugihara, Nawa, Shigemoto, Mizuno & Nakanishi, 1992; Brabet et al. 1995; Joly et al. 1995; Manahan-Vaughan et al. 1996). The mGluR5b pharmacological profile, for the agonists that were tested, is identical to that of mGluR5a (Joly et al. 1995).

Group I mGluRs are characterized by their high sensitivity to Quis; however, (*RS*)-DHPG and *trans*-ADA appear to be the most selective agonists for this group of mGluRs, given that these agonists have no effect at group II or group III mGluRs (Gereau & Conn, 1995a; Manahan-Vaughan et al. 1996; Saugstad, Kinzie, Shinohara, Segerson & Westbrook, 1997). Interestingly, (*RS*)-DHPG and *trans*-ADA have been shown to activate mGluR5a with a higher potency, compared to mGluR1a (Brabet et al. 1995; Manahan-Vaughan et al. 1996). It should be noted that, although (1*S*,3*S*)-ACPD and L-CCG-I display some activity at group I mGluRs (Hayashi et al. 1992; Joly et al. 1995), many investigators have used these compounds as selective group II mGluR agonists.

The rank order of antagonist potency for mGluR1a is as follows: (*S*)- or (*RS*)-4-carboxy-3-hydroxyphenylglycine ((*S*)- or (*RS*)-4C3HPG) > (*S*)-4-carboxyphenylglycine ((*S*)-4CPG) > (*RS*)-1-aminoindan-1,5-dicarboxylic acid ((*RS*)-AIDA) > (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG) or (*RS*)-MCPG (Hayashi et al. 1994; Moroni et al. 1997; Thomsen, Boel & Suzdak, 1994; Thomsen & Suzdak, 1993a). The antagonist profiles have not yet been determined for mGluR1b and mGluR1c. Interestingly, recent evidence suggests that several phenylglycine derivatives may discriminate between mGluR1a and mGluR5a. For example, two studies have shown that (+)-MCPG and (*S*)-4CPG display no activity at mGluR5a, and (*S*)-4C3HPG, at high concentrations, acts as a partial agonist at this receptor subtype (Brabet et al. 1995; Joly et al. 1995). The most selective group I mGluR antagonist currently available is (*RS*)-AIDA (Pellicciari et al. 1995); however, it should be noted that, recently, this compound has been shown to activate group II mGluRs at high concentrations (1000 μM) (Moroni et al. 1997).

A number of studies have examined the distribution of the group I mGluRs within the rat CNS, using antibodies against, or mRNAs for, each receptor subtype. In the rat brain, several studies have demonstrated that the highest distribution of mGluR1a is found in the olfactory bulb, area CA1 and the dentate gyrus of the hippocampus, neostriatum, globus pallidus, substantia nigra, a number of nuclei in the thalamus and hypothalamus, Purkinje cells of the cerebellum, superior colliculus and on interneurons of the neocortex (Casabona et al. 1997; Fotuhi et al. 1993; Martin, Blackstone, Huganir & Price, 1992; Petralia et al. 1997; Testa, Friberg, Weiss & Standaert, 1998; Testa, Standaert, Young & Penny, 1994). Two studies, comparing the expression patterns of

each splice variant of mGluR1, reported some differences in the regional distribution within the cerebellum, olfactory bulb and striatum (Grandes, Mateos, Rüegg, Kuhn, & Knöpfel, 1994; Pin et al. 1992). Ultrastructural examination has revealed that mGluR1a is primarily localized on postsynaptic membranes (Martin et al. 1992; Petralia et al. 1997; Shigemoto et al. 1997; Van den Pol, 1994), often in the periphery of the synapse (Baude, Nusser, Roberts, Mulvihill, McIlhinney & Somegyi, 1993; Lujan, Nusser, Roberts, Shigemoto & Somogyi, 1996; Nusser, Mulvihill, Streit & Somogyi, 1994; Petralia et al. 1997). It has been suggested that the perisynaptic localization of this mGluR would permit activation only during periods in which Glu concentrations were sufficiently high to diffuse away from the release site (Baude et al. 1993). It is also of interest that mGluR1a, as well as mGluR1b, have been shown to be localized on presynaptic terminals in the striatum (Fotuhi et al. 1993).

Studies have demonstrated that mGluR5a is highly expressed in the olfactory tubercle, anterior olfactory nucleus, caudate/putamen, nucleus accumbens, lateral septum, area CA1 and dentate gyrus of the hippocampus, inferior colliculus and in regions of the cerebrocortex (Casabona et al. 1997; Romano, Sesma, McDonald, O'Malley, Van den Pol & Olney, 1995; Shigemoto, Nomura, Ohishi, Sugihara, Nakanishi & Mizuno, 1993). Although Shigemoto et al. (1993) reported that mGluR5a is predominantly located in postsynaptic membranes in the rat brain, Romano et al. (1995) found that this receptor subtype shows some presynaptic localization in the CA1 region and dentate gyrus of the hippocampus, as well as in regions of the cerebrocortex. It has been suggested that presynaptic group I mGluRs may function as autoreceptors, regulating transmitter release in certain regions of the rat brain (Romano et al. 1995). Details relating to the spinal cord

distribution of group I mGluRs are discussed below (see Localization of EAAs and GluRs in the Dorsal Horn and Primary Afferent Neurons).

Group II mGluRs. Activation of the group II mGluRs, mGluR2 and mGluR3, which are negatively linked through a G-protein to adenylate cyclase, leads to the inhibition of cyclic adenosine 3',5'-monophosphate (cAMP) accumulation (Tanabe, Masu, Ishii, Shigemoto & Nakanishi, 1992; Tanabe, Nomura, Masu, Shigemoto, Mizuno & Nakanishi, 1993).

The pharmacological profiles for the group II mGluR subtypes have been well characterized. Table 1.1 shows the *median* half-maximum effective concentrations of various agonists to inhibit forskolin-stimulated cAMP ($IC_{50}$), and the *median* half-maximum effective concentrations of various antagonists to block agonist-induced inhibition of forskolin-stimulated cAMP ($IC_{50}$) in cells lines expressing cloned rat group II mGluRs. The rank order of agonist potency for mGluR2 is the following: (2*S*,1'*R*,2'*R*,3'*R*)-2-(2,3-dicarboxycyclopropyl)glycine (DCG-IV) ≥ L-CCG-I > (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate ((2*R*,4*R*)-APDC) > (1*S*,3*R*)-ACPD > Glu > (*S*)- or (*RS*)-4C3HPG > ibotenate > (*S*)-4CPG > Quis (Flor et al. 1995; Hayashi et al. 1992, 1993, 1994; Schoepp et al. 1995; Tanabe et al. 1992; Thomsen et al. 1994). The mGluR3 pharmacological profile, for the agonists that were tested, is similar to that of mGluR2 (Hayashi et al. 1993; Pin, Joly, Heinemann & Bockaert, 1994; Tanabe et al. 1993). Although DCG-IV and L-CCG-I are the most potent agonists at group II mGluRs, (2*R*,4*R*)-APDC is the most selective agonist currently available. It should be noted that

DCG-IV also activates the NMDAR at high concentrations (Hayashi et al. 1992), and that L-CCG-I is also an agonist at mGluR1a, mGluR4a and mGluR8 (Saugstad et al. 1997).

For several years, (+)- or (*RS*)-MCPG was the only group II mGluR antagonist available (Hayashi et al. 1994; Thomsen et al. 1994). More recently, however, several antagonists, such as (2*S*,4*S*)-2-amino-4-(4,4-diphenylbut-1-yl)-pentane-1,5-dioic acid (ADPD) (Wermuth et al. 1996), α-methyl-4-sulfono-phenylglycine (MSPG), α-methyl-4-tetrazoylphenylglycine (MTPG)), and (2*S*,3*S*,4*S*)-methyl-2-(2-carboxycyclopropyl) glycine (MCCG) (Gomeza, Brabet, Parmentier, Restituito, Bockaert & Pin, 1996) have been synthesized and shown to be selective for cloned group II mGluRs (see Table 1.1).

A number of studies have examined the distribution of the group II mGluRs within the rat CNS, using antibodies against, or mRNAs for, the receptor subtypes. Evidence suggests that mGluR2 and mGluR3 have overlapping, but distinct expression patterns in the rat brain. The highest expression of mGluR2 is found in golgi cells of the cerebellum, whereas mGluR3 is most prominently expressed in the reticular nucleus of the thalamus (Ohishi, Shigemoto, Nakanishi & Mizuno, 1993a). Although mGluR3 is also expressed in cerebellar golgi cells, but with moderate intensity, mGluR2 and mGluR3 show distinct regional distribution patterns in other cerebellar cells, as well as in neurons of the olfactory bulb and in different nuclei of the thalamus (Ohishi et al. 1993a). A number of studies have demonstrated that both group II mGluR subtypes are localized pre- and postsynaptically in the hippocampus, olfactory bulb, cerebellum and cerebrocortex (Neki, Ohishi, Kaneko, Shigemoto, Nakanishi & Mizuno, 1996; Ohishi, Ogawa-Meguro, Shigemoto, Kaneko, Nakanishi & Mizuno, 1994; Petralia, Wang,

Niedzielski & Wenthold, 1996; Shigemoto et al. 1997). The presynaptic localization of group II mGluRs suggest that these receptor subtypes function as autoreceptors, modulating transmitter release in the rat brain. Details relating to the spinal cord distribution of group II mGluRs are discussed below (see Localization of EAAs and GluRs in the Dorsal Horn and Primary Afferent Neurons).

Group III mGluRs. The group III mGluRs, mGluR4 (cloned splice variants include a and b), mGluR6, mGluR7 and mGluR8, are also linked to the inhibition of cAMP formation (Duvoisin, Zhang & Ramonell, 1995; Nakajima et al. 1993; Okamoto et al. 1994; Saugstad, Kinzie, Mulvihill, Segerson & Westbrook, 1994; Tanabe et al. 1992, 1993; Thomsen, Pekhletski, Haldeman, Gilbert, O'Hara & Hampson, 1997), but they are pharmacologically distinct from group II mGluRs. Specifically, group III mGluRs are insensitive to (1*S*,3*R*)-ACPD (with the exception of mGluR8), and are preferentially activated by L-AP4. The rank order of agonist potency for mGluR4 and mGluR6 are the following: L-AP4 > L-serine-*O*-phosphate (L-SOP) = Glu > L-CCG-I (Hayashi et al. 1992; Tanabe et al. 1993; Thomsen, Kristensen, Mulvihill, Haldeman & Suzdak, 1992; Thomsen & Suzdak, 1993b) and L-AP4 > L-SOP > Glu > Quis = ibotenate (Nakajima et al. 1993), respectively. mGluR7 has been reported to have a slightly different pharmacological profile, such that it has a lower affinity for L-AP4 and L-SOP, compared to mGluR4 and mGluR6 (Okamoto et al. 1994; Saugstad et al. 1994). mGluR8 is also different from the other group III mGluRs, since it has been shown to be more sensitive to L-CCG-I than the other group III mGluRs, and it is activated by (1*S*,3*R*)-ACPD (Saugstad et al. 1997). The rank order of agonist potency for mGluR7 and mGluR8 are the following: L-SOP > L-AP4 > Glu (Okamoto et al. 1994; Saugstad et al. 1994) and L-

AP4 > L-CCG-I > Glu > (1*S*,3*R*)-ACPD (Duvoisin et al. 1995; Saugstad et al. 1997), respectively. See Table 1.1 for the calculated median $IC_{50}$ value for inhibiting forskolin-stimulated cAMP and potency rank for each agonist.

The compound, (*S*)-2-amino-2-methyl-4-phosphonobutanoic acid (MAP4), is the most selective group III mGluR antagonist currently available (Johansen & Robinson, 1995; Saugstad et al. 1997). It should be noted, however, that this compound displays partial agonist activity at mGluR6 (Sekiyama et al. 1996). It is interesting to note that, although (+)-MCPG produces no effect at mGluR4a (Hayashi et al. 1994; Thomsen et al. 1994), this phenylglycine derivative has recently been shown to be an antagonist at mGluR8 (Saugstad et al. 1997).

A number of studies have examined the distribution of the group III mGluRs within the rat CNS, using antibodies against, or mRNAs for, the receptor subtypes. Evidence suggests that the group III mGluR subtypes have distinct distribution patterns in the rat brain. Interestingly, the expression of mGluR6 is restricted to the inner nuclear layer of the retina (Nakajima et al. 1993). Studies have demonstrated that the expression of mGluR8 is concentrated in the main and accessory olfactory bulb (Duvoisin et al. 1995; Kinoshita, Ohishi, Nomura, Shigemoto, Nakanishi & Mizuno, 1996), whereas mGluR4 and mGluR7 are expressed widely, but differentially, throughout the rat brain (Bradley, Levey, Hersch & Conn, 1996; Ohishi, Akazawa, Shigemoto, Nakanishi & Mizuno, 1995). Specifically, the highest expression of mGluR4 is found in the granule cells of the cerebellum and olfactory bulb, whereas mGluR7 expression is most prominent in tufted and mitral cells of the main olfactory bulb, mitral cells of the accessory olfactory bulb, medial septal nucleus and neurons in the locus coeruleus

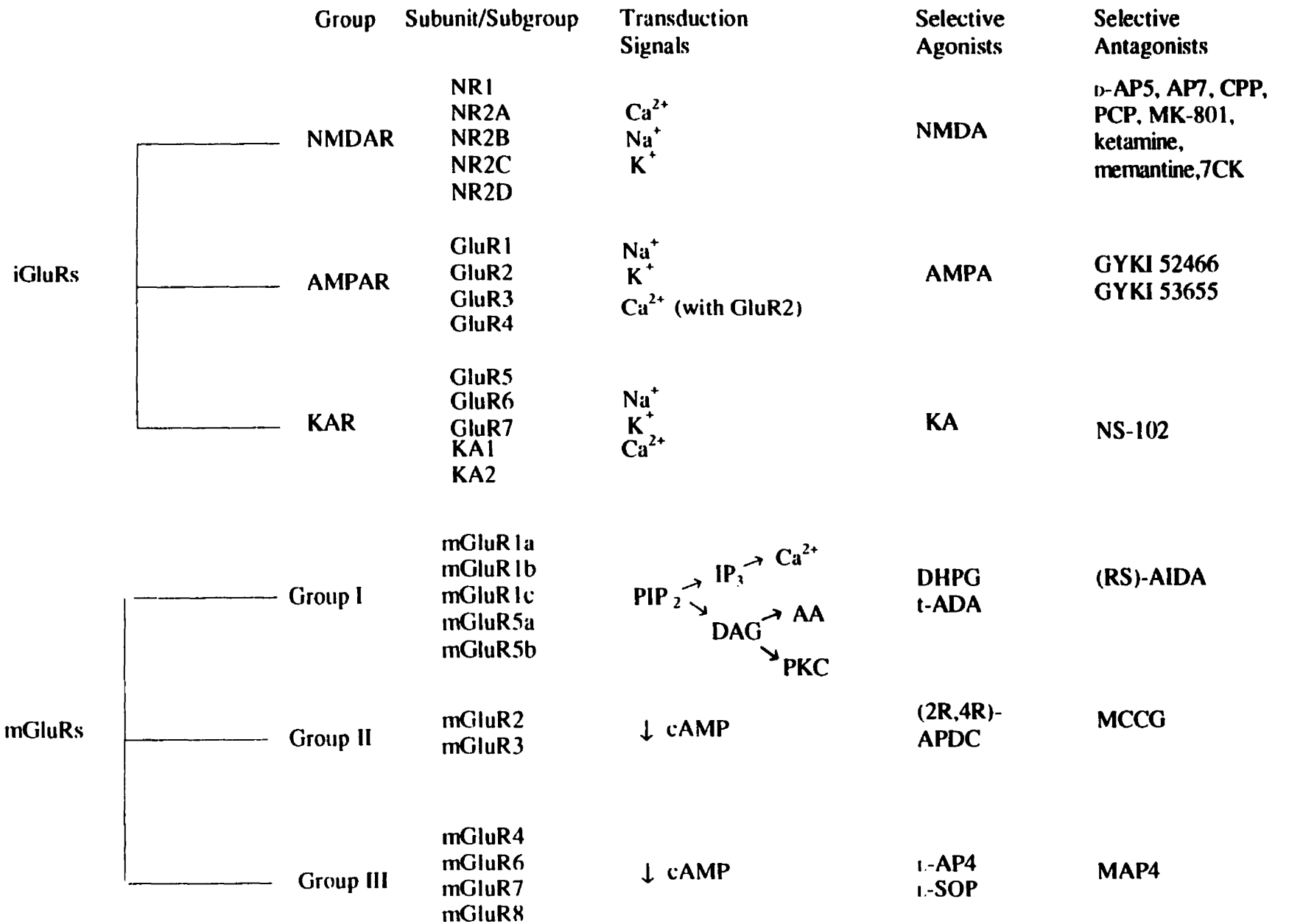
(Ohishi, Akazawa, Shigemoto, Nakanishi & Mizuno, 1995). Furthermore, mGluR7 is expressed with moderate intensity in most regions in the rat brain, including the neocortex, basal ganglia, amygdala, preoptic region, hypothalamus, thalamus and lower brainstem (Ohishi, Akazawa, Shigemoto, Nakanishi & Mizuno, 1995). Studies have provided convincing evidence that mGluR7 and mGluR8 are predominantly localized presynaptically, whereas mGluR4 is found both pre- and postsynaptically in the rat CNS (Bradley et al. 1996; Duvoisin et al. 1995; Kinoshita, Ohishi, Neki, Nomura, Shigemoto, Takada, Nakanishi & Mizuno, 1996; Kinoshita, Ohishi, Nomura, Shigemoto, Nakanishi & Mizuno, 1996; Shigemoto et al. 1996, 1997). These studies suggest that presynaptic group III mGluRs also act as autoreceptors, modulating the release of transmitters. Details relating to the spinal cord distribution of group III mGluRs are discussed below (see Localization of EAAs and GluRs in the Dorsal Horn and Primary Afferent Neurons).

Glutamate Receptors: An Overview

After 35 years of research, and with the application of molecular biological technology, neuroscientists now understand that the diverse and complex actions of Glu in the mammalian are subserved by different members of a large family of GluRs. Twenty-two genes have been identified, 14 are iGluR subunits (NR1, NR2A-NR2D (NMDAR subunits), GluR1-GluR4 (AMPAR subunits), GluR5-GluR7, KA1, and KA2 (KAR subunits)) and eight are mGluR subtypes (mGluR1, mGluR5 (group I), mGluR2, mGluR3 (group II), mGluR4, mGluR6-mGluR8 (group III)). Furthermore, a number of splice variants have also been included in the GluR family. It is likely that combinations of subunits produce subtypes of the NMDA, AMPA and KA receptors in the mammalian CNS; however, they are not yet pharmacologically distinguishable. The availability of

selective NMDAR antagonists, and the more recent synthesis of antagonists that discriminate between the AMPA or KA receptors have allowed neuroscientists to explore the functions of iGluRs in the mammalian NS. Only very recently, however, have compounds been developed that are selective for each of the three groups of mGluRs. Unfortunately, most of the currently available agonists and antagonists act at more than one group, therefore, neuroscientists have had to speculate about the involvement of a specific group of mGluRs in the CNS, based on the differences or similarities between the effects of two or more compounds. For example, the effects of (*S*)-4CPG (the group I mGluR antagonist/group II mGluR agonist) may be compared to the effects of (+)-MCPG (the group I and II mGluR antagonist). See Figure 1.1, which summarizes the details relating to the classification, pharmacology, and signal transduction mechanisms of GluRs.

Fig. 1.1


Group
Subunit/Subgroup
Transduction Signals
Selective Agonists
Selective Antagonists
iGluRs
NMDAR
NR1
NR2A
NR2B
NR2C
NR2D
Ca2+
Na+
K+
NMDA
D-AP5, AP7, CPP, PCP, MK-801, ketamine, memantine,7CK
AMPAR
GluR1
GluR2
GluR3
GluR4
Na+
K+
Ca2+ (with GluR2)
AMPA
GYKI 52466
GYKI 53655
KAR
GluR5
GluR6
GluR7
KA1
KA2
Na+
K+
Ca2+
KA
NS-102
mGluRs
Group I
mGluR1a
mGluR1b
mGluR1c
mGluR5a
mGluR5b
PIP2
IP3
Ca2+
DAG
AA
PKC
DHPG
t-ADA
(RS)-AIDA
Group II
mGluR2
mGluR3
↓ cAMP
(2R,4R)-APDC
MCCG
Group III
mGluR4
mGluR6
mGluR7
mGluR8
↓ cAMP
L-AP4
L-SOP
MAP4

## Physiological Roles of mGluRs

### Excitatory effects

Early evidence that suggested mGluRs contribute to excitatory synaptic transmission was provided by the demonstration that application of (1*S*,3*R*)-ACPD or Quis (but not AMPA) to the rat hippocampus depolarizes neurons, reduces the afterhyperpolarization that follows a train of action potentials, and decreases the accommodation of cell firing, *in vitro* (Charpak, Gahwiler, Do & Knöpfel, 1990; Stratton, Worley & Baraban, 1989). Since these early reports, studies have shown that Quis or (1*S*,3*R*)-ACPD produces a variety of excitatory responses in numerous regions of the mammalian brain, including slow depolarization of neurons in the guinea-pig olfactory cortex (Libri, Constanti, Zibetti & Postlethwaite, 1997), and in the rat ventrobasal thalamus (Eaton et al. 1993; Jane, Jones, Pook, Salt, Sunter & Watkins, 1993; Salt & Eaton, 1995a), dorsolateral septal nucleus (Zheng & Gallagher, 1992; Zheng, Gallagher & Connor, 1996; Zheng, Hasuo & Gallagher, 1995), hippocampus (Desai, Smith & Conn, 1992) nucleus of the solitary tract (Glaum & Miller, 1992), ventral tegmental area (Shen & Johnson, 1997), and cerebellum (Glaum, Slater, Rossi, & Miller, 1992). Also, others have reported that Quis or (1*S*,3*R*)-ACPD application induces a slow afterdepolarization followed by an increase in the frequency of spontaneous action potentials or miniature excitatory postsynaptic potentials (EPSPs) in neurons from the guinea-pig olfactory cortex (Libri et al. 1997) or from the rat hippocampus (Ito et al. 1992), neocortex (Burke & Hablitz, 1996; Greene, Schwindt, & Crill, 1992, 1994), dorsolateral septal nucleus (Zheng & Gallagher, 1992), subthalamic nucleus (Abbott, Wigmore & Lacey, 1997), cerebellum (Netzeband et al. 1997), olfactory bulb (Schoppa & Westbrook, 1997), and

hypothalamus (Schrader & Tasker, 1997), *in vitro*. Furthermore, application of (1*S*,3*R*)-ACPD to the hippocampus (Bortolotto & Collingridge, 1992, 1993, 1995; Breakwell, Rowan & Anwyl, 1996; Chinestra, Diabira, Urban, Barrioneuvo & Ben-Ari, 1994; Collins, Scollon, Russell & Davies, 1995; O'Connor, Rowan & Anwyl, 1995) or intracerebroventricular administration of (1*S*,3*R*)-ACPD (Manahan-Vaughan & Reymann, 1995) produces a slow-onset, long-lasting potentiation of hippocampal field EPSPs in the rat (*in vivo*).

In the spinal cord, (1*S*,3*R*)-ACPD also produces excitatory responses. For example, (1*S*,3*R*)-ACPD application depolarizes rat motoneurons in spinal cord slices (Eaton et al. 1993; Jane et al. 1993; Thompson, Jones & Kilpatrick, 1995) and increases the excitability of deep dorsal horn neurons in rat (Morisset & Nagy, 1996) and turtle (Russo, Nagy & Hounsgaard, 1997) spinal cord slices, *in vitro*. In rat sympathetic preganglion neurons, application of Quis or (1*S*,3*R*)-ACPD depolarizes the neurons and increases the width of the action potential, *in vitro* (Spanswick, Pickering, Gibson & Logan, 1995).

It is likely that Quis or (1*S*,3*R*)-ACPD increases neuronal excitability by activating group I mGluRs, given that the selective group I mGluR agonist, (*RS*)-DHPG, mimics the effects of the less selective mGluR compounds (Abbott et al. 1997; Ito et al. 1992; Libri et al. 1997; Netzeband et al. 1997; Russo et al. 1997; Schoppa & Westbrook, 1997; Schrader & Tasker, 1997). Also, application of (*S*)-DHPG to the rat hippocampus (O'Leary & O'Connor, 1997) or intracerebroventricular administration of (*RS*)-DHPG (Davis & Laroche, 1996; Manahan-Vaughan & Reymann, 1996) or *trans*-ADA (Manahan-Vaughan et al. 1996; Manahan-Vaughan & Reymann, 1996; Riedel, Manahan-

Vaughan, Kozikowski & Reyman, 1995), produces the slow-onset, long-lasting potentiation of hippocampal field EPSPs that is characteristic of (1*S*,3*R*)-ACPD.

The mGluR agonist-related increases in neuronal excitability may be mediated by a number of transduction signals. For example, evidence suggests that the excitatory effects of Quis, (1*S*,3*R*)-ACPD or selective group I mGluR agonists in the rat hippocampus (Baskys, Bernstein, Barolet & Carlen, 1990; Charpak et al. 1990; Charpak & Gahwiler, 1991; Chinestra et al. 1994; Guerineau, Gahwiler & Gerber, 1994; Ito et al. 1992; Luthi, Gahwiler & Gerber, 1997; Stratton et al. 1989), neocortex (Greene et al. 1994), dorsolateral geniculate nucleus (McCormick & von Krosigk, 1992) and sympathetic preganglionic neurons (Spanswick et al. 1995) are associated with an inhibition of $K^+$ currents. Also, mGluR agonists may influence neuronal excitability by regulating other cation channels. For example, studies have demonstrated that Quis, (1*S*,3*R*)-ACPD or selective group I mGluR agonists increase intracellular $Ca^{2+}$ concentrations ($[Ca^{2+}]_I$) by activating voltage-gated $Ca^{2+}$ channels (VGCCs) in the rat cerebellum (Chavis, Fagni, Bockaert & Lansman, 1995; Chavis, Nooney, Bockaert, Fagni, Feltz & Bossu, 1995), dorsolateral septal nucleus (Zheng et al. 1996), hippocampus (Jaffe & Brown, 1994) and dorsal horn of the spinal cord (Bleakman, Rusin, Chard, Glaum & Miller, 1992). Studies have also reported an mGluR agonist-related activation of a $Ca^{2+}$-dependent nonspecific cation current in the rat hippocampus (Congar, Leinekugel, Ben-Ari & Crepel, 1997; Crepel, Aniksztejn, Ben-Ari & Hammond, 1994).

Inhibitory effects

A number of studies have demonstrated that activation of group II or group III mGluRs inhibit excitatory transmission in the rat CNS. For example, application of (1*S*,3*R*)-ACPD, the group II mGluR agonists (DCG-IV, L-CCG-I, (1*S*,3*S*)-ACPD or (2*R*,4*R*)-APDC) or the group III mGluR agonists (L-AP4 or L-SOP) reduces excitatory transmission in cultured olfactory bulb mitral cells (Schoppa & Westbrook, 1997), magnocellular neurons of the hypothalamic supraoptic nucleus (Schrader & Tasker, 1997), in neurons in the locus coeruleus (Dube & Marshall, 1997), nucleus accumbens (Manzoni, Michel & Bockaert, 1997), striatum (Lovinger & McCool, 1995; Pisani, Calabresi, Centonze & Bernardi, 1997a; Tyler & Lovinger, 1995), hippocampus (Baskys & Malenka, 1991; Gereau & Conn, 1995b; Jouvenceau, Dutar & Billard, 1995; Kamiya, Shinozaki & Yamamoto, 1996; Macek, Winder, Gereau, Ladd & Conn, 1996; Vignes et al. 1995) and ventral spinal cord (Cao, Evans, Headley & Udvarhelyi, 1995; Jane, Jones, Pook, Tse & Watkins, 1994; Jane, Pittway, Sunter, Thomas & Watkins, 1995; Jane, Thomas, Tse & Watkins, 1996; Thomas, Jane, Tse & Watkins, 1996).

The group II mGluR agonist-related inhibitory effects were shown to be preferentially blocked by application of the antagonists, (*RS*)-MCPG (Dube & Marshall, 1997; Lovinger & McCool, 1995), (+)-MCPG (Kamiya et al. 1996; Vignes et al. 1995), ethyl Glu (EGlu) (Dube & Marshall, 1997; Jane et al. 1996), MCCG (Jane et al. 1994; Macek et al. 1996; Vignes et al. 1995), (*RS*)-α-methylserine-O-phosphate monophenyl-phoryl ester (MSOPPE) (Thomas et al. 1996), or MTPG (Jane et al. 1995). In contrast, the inhibitory effects of group III mGluR agonists are preferentially blocked by the

antagonists, MAP4 (Dube & Marshall, 1997; Macek et al. 1996; Pisani et al. 1997a; Vignes et al. 1995) as well as by the novel group III mGluR antagonists, MPPG and MSOP (Cao et al. 1995; Jane et al. 1994, 1995). Together, these studies provide convincing evidence that mGluR-mediated depression of excitatory synaptic transmission in the CNS is attributable to activation of two pharmacologically distinct groups of receptors. The authors of these studies have proposed that presynaptic group II and group III mGluRs act as 'autoreceptors' that modulate excitatory synaptic transmission by decreasing the release of EAAs.

Several studies have investigated the possible transduction signals that mediate the group II and group III mGluR agonist-related depression of synaptic transmission. Evidence suggests that activation of these two groups of mGluRs reduce $[Ca^{2+}]_i$ by inhibiting VGCCs. For example, studies have demonstrated that application of (1*S*,3*R*)-ACPD or the group II mGluR agonists, L-CCG-I or (*S*)-4C3HPG, reduces $Ca^{2+}$ conductance through VGCCs in the rat cerebellum (Chavis, Fagni, Bockaert & Lansman, 1995; Chavis, Shinoaki, Bockaert & Fagni, 1994) and striatum (Stefani, Pisani, Mercuri, Bernardi & Calabrasi, 1994). Also, application of the group III mGluR agonist, L-AP4, to the rat cerebrocortex (Herrero, Vázquez, Miras-Portugal & Sánchez-Prieto, 1996; Stefani, Spadoni & Bernardi, 1996; Vázquez, Budd, Herrero, Nicholls & Sánchez-Prieto, 1995; Vázquez, Herrero, Miras-Portugal & Sánchez-Prieto, 1995), hippocampus (Sahara & Westbrook, 1993; Swartz & Bean, 1992), or olfactory bulb (Trombley & Westbrook, 1992) has also been shown to produce the same inhibitory effect on VGCCs.

mGluR-Mediated Regulation of EAA Release

Evidence suggests that activation of mGluRs positively regulates the release of EAAs in the rat CNS. For example, application of (1*S*,3*R*)-ACPD to cerebrocortical slices (Lombardi, Leonardi & Moroni, 1996; Lombardi, Pellegrini-Giampietro, Leonard, Cherici, Pellicciari & Moroni, 1994; Moroni et al. 1997) potentiates depolarization-induced output of [$^3$H]-Asp and/or [$^3$H]-Glu. Also, perfusion of cerebrocortical (Herrero, Castro, Miras-Portugal & Sánchez-Prieto, 1996; Herrero, Miras-Portugal & Sánchez-Prieto, 1992a; Herrero, Miras-Portugal & Sánchez-Prieto, 1992b; Herrero, Miras-Portugal & Sánchez-Prieto, 1994; Vázquez, Budd, Herrero, Nicholls & Sánchez-Prieto, 1995; Vázquez, Herrero, Miras-Portugal & Sánchez-Prieto, 1994; Vázquez, Herrero, Miras-Portugal & Sánchez-Prieto, 1995) or hippocampal (McGahon & Lynch, 1994) synaptosomes with (1*S*,3*R*)-ACPD, in the presence of arachidonic acid, potentiates the release of Glu. Moreover, Jones, Lawrence and Beart (1998) demonstrated that micro-dialysis of (1*S*,3*R*)-ACPD into the rat nucleus of the solitary tract increases the basal release of Glu and Asp, *in vivo*.

The notion that group I mGluRs are responsible for these effects is first supported by the demonstration that the (1*S*,3*R*)-ACPD-related increase in EAA release is attenuated by the selective group I mGluR antagonist, (*RS*)-AIDA (Moroni et al. 1997) or by the phenylglycine derivatives, (+)-MCPG or (*S*)-4CPG (Jones et al. 1998). Second, perfusion of synaptosomes with the PKC activator, 4β-phorbol dibutyrate, in the presence of arachidonic acid, mimics the effects of (1*S*,3*R*)-ACPD (Herrero et al. 1992a,b; Coffey,

Herrero, Sihra, Sánchez-Prieto & Nicholls, 1994). Third, perfusion of synaptosomes with the PKC inhibitor, staurosporine, blocks the effects of (1*S*,3*R*)-ACPD (Vázquez, Herrero, Miras-Portugal & Sánchez-Prieto, 1995).

Studies have also provided evidence that activation of mGluRs *inhibits* the release of EAAs in the rat CNS. For example, application of (1*S*,3*R*)-ACPD to striatal slices reduces the depolarization-induced output of [$^3$H]-Asp and/or [$^3$H]-Glu (Lombardi et al. 1994, 1996; Lombardi, Alesiani, Leonardi, Cherici, Pellicciari & Moroni, 1993). The proposal that the inhibitory effects of the non-selective mGluR agonist on EAA release are attributable to activation of group II mGluRs is supported by the demonstration that systemic (Battaglia, Monn & Schoepp, 1997) or local administration (Cozzi et al. 1997) of more selective group II mGluR agonists to rats reduces veratridine-evoked or tonic Glu release, respectively, *in vivo*. Also, application of the group II mGluR agonist, L-CCG-I, to rat striatal slices (Lombardi et al. 1993, 1994) or perfusion of rat striatal synaptosomes with the group II mGluR agonist, (1*S*,3*S*)-ACPD (East, Hill & Brotchie, 1995), reduces depolarization- or 4-aminopyridine-induced EAA release. Although East et al. (1995) provided evidence that activation of group III mGluRs inhibits the release of Glu in the striatum, others have demonstrated that application of L-AP4 to rat striatal slices does not modulate the release of EAAs (Lombardi et al. 1993, 1994). Evidence suggests that group II and group III mGluRs regulate the release of EAAs in the rat cerebrocortex. For example, application of L-AP4 to cerebrocortical slices (Lombardi et al. 1994), or perfusion of cerebrocortical synaptosomes with (1*S*,3*S*)-ACPD (Attwell et al. 1995) or L-AP4 (Herrero, Vázquez, Miras-Portugal & Sánchez-Prieto, 1996; Vázquez, Budd,

Herrero, Nicholls & Sánchez-Prieto, 1995; Vázquez, Herrero, Miras-Portugal & Sánchez-Prieto, 1995) reduces depolarization- or 4-aminopyridine-induced EAA release.

It is of particular interest that neuroanatomical, electrophysiological and EAA release studies have provided evidence that a subset of each group of mGluRs function as autoreceptors. Specifically, activation of presynaptic group I mGluRs may increase neuronal excitability in various regions of the mammalian NS by facilitating the release of EAAs, while activation of group II or group III mGluRs likely decrease excitatory synaptic transmission by attenuating the release of EAAs. This concept has attracted much attention within the scientific community. It is likely that novel therapeutic strategies will be developed to target presynaptic mGluRs in an effort to prevent or reduce pathological changes in neuronal function, which are associated with excessive Glu release. This mechanism is thought to contribute to various conditions in the clinical population (i.e. epilepsy, stroke and neuronal degenerative disorders).

mGluR-Mediated Regulation of iGluR Function

A number of studies have also demonstrated that activation of mGluRs enhance iGluR function in the rat CNS. For example, application of the non-selective mGluR compound, (1*S*,3*R*)-ACPD, potentiates NMDAR- and/or non-NMDAR-mediated responses in the hippocampus (Aniksztejn, Bregestovski & Ben-Ari, 1991; Fitzjohn, Irving, Palmer, Harvey, Lodge & Collingridge, 1996; O'Connor et al. 1995; O'Connor, Rowan & Anwyl, 1994), neocortex (Rahman & Neuman, 1996), nucleus of the solitary tract (Glaum & Miller, 1993; Glaum, Sunter, Udvarhelyi, Watkins & Miller, 1993), cerebellum (Rossi, D'Angelo & Taglietti, 1996), and in the ventral (Ugolini, Corsi & Bordi, 1997) or dorsal (Bleakman et al. 1992; Bond & Lodge, 1995; Cerne & Randic,

1992; Jones & Headley, 1995) spinal cord. It is likely that (1*S*,3*R*)-ACPD potentiates iGluR function by activating group I mGluRs, since selective group I mGluR agonists mimic the effects of the less selective mGluR agonist in the rat hippocampus (Doherty, Palmer, Henley, Collingridge, & Jane, 1997; Fitzjohn et al. 1996), striatum (Pisani, Calabresi, Centonze & Bernardi, 1997b) and spinal cord (Bond & Lodge, 1995; Jones & Headley, 1995; Ugolini et al. 1997). Also, the excitatory effects of (*RS*)-DHPG or (1*S*,3*R*)-ACPD are blocked by application of phenylglycine derivatives (Bond & Lodge, 1995; Fitzjohn et al. 1996; Glaum et al. 1993; Harvey, Palmer, Irving, Clarke & Collingridge, 1996; O'Connor et al. 1994; Rossi et al. 1996; Ugolini et al. 1997) or by PKC inhibitors (Aniksztejn et al. 1991; O'Connor et al. 1995; Ugolini et al. 1997).

Other evidence suggesting that mGluRs interact with iGluRs has been provided by the demonstration that (1*S*,3*R*)-ACPD-induced LTP of hippocampal field EPSPs is blocked by application of NMDAR (Breakwell et al. 1996; Collins et al. 1995) or non-NMDAR (Bortolotto & Collingridge, 1995) antagonists. Also, it has been reported that application of selective group I mGluR agonists potentiate NMDA-induced neuronal degeneration in cultured murine cortical neurons (Bruno, Copani, Knöpfel, Kuhn, Casabona, Dell'Albani, Condorelli & Nicoletti, 1995).

Studies have also demonstrated that activation of mGluRs reduce iGluR function in the rat CNS. For example, in the CA1 region of the neonatal rat hippocampus, application of (1*S*,3*R*)-ACPD or the selective group III mGluR agonist, L-AP4, depresses the NMDA-, but not the AMPA-mediated component of the EPSC induced by electrical stimulation (Baskys & Malenka, 1991). Also, in the rat striatum, application of (1*S*,3*R*)-ACPD depresses the NMDAR- or AMPAR-mediated components of the EPSPs

(Lovinger, 1991). It has been suggested that activation of presynaptic group II and group III mGluRs are responsible for the inhibition of iGluR-mediated synaptic transmission (Baskys & Malenka, 1991; Lovinger, 1991). Additional evidence that mGluRs modulate iGluR function has been provided with the demonstration that application of the group II mGluR agonists, DCG-IV (Bruno et al. 1997; Bruno, Battaglia, Copani, Giffard, Raciti, Raffaele, Shinozaki & Nicoletti, 1995; Bruno, Copani, Battaglia, Raffaele, Shinozaki & Nicoletti, 1994; Buisson, Yu & Choi 1996), L-CCG-I (Bruno et al. 1994, 1997) or (*S*)-4C3HPG (Bruno et al. 1997; Buisson & Choi, 1995), or application of group III mGluR agonists, L-AP4 or L-SOP (Bruno et al. 1996), attenuates NMDA-induced excitotoxicity in cultured neurons.

## GluR-Mediated Transmission of Sensory and Nociceptive Information in the Rat Spinal Cord

Several lines of research support the notion that EAAs mediate synaptic transmission in the rat spinal cord. First, Glu and Asp, as well as the GluR subtypes are localized within the spinal sensory/nociceptive pathways. Second, application of GluR antagonists to the rat spinal cord reduces dorsal horn neuronal responses to primary afferent stimulation and to cutaneous innocuous/noxious stimulation. Third, application of EAAs to the spinal cord produces an increase in the spontaneous activity of dorsal horn neurons, as well as dorsal horn neuronal responses to cutaneous innocuous/noxious stimulation. Fourth, intrathecal administration of EAAs produces acute nociception in rats. Fifth, treatment with GluR antagonists reduce nociception in animal models of persistent and chronic pain. These points will be discussed in detail below.

### Localization of EAAs and GluRs in the Dorsal Horn and Primary Afferent Neurons

A number of studies have provided neuroanatomical evidence that GluRs mediate synaptic transmission in the rat spinal cord. This notion is first supported by the demonstrated that high levels of Glu and Asp are found in large- and small- diameter primary afferent fibers, as well as in dorsal horn interneurons and projection neurons (De Biasi & Rustioni, 1988; Lekan & Carlton, 1995; Miller, Clements, Larson & Beitz, 1988; Tracey, De Biasi, Phend & Rustioni, 1991; Wanaka et al. 1987; Westlund, McNeill, Coggeshall, 1989; Westlund, McNeil, Patterson, Coggeshall, 1989).

Second, a number of studies have also demonstrated that multiple GluR subtypes are localized within the sensory/nociceptive pathways of the rat spinal cord. For example, several of these studies have examined the expression patterns of the mRNAs for the iGluR subunits in the dorsal horn of the rat spinal cord. For example, Furuyama et al. (1993) found that the NMDAR subunit, NR1, is moderately and evenly distributed throughout the laminae of the dorsal horn. Others have examined the distribution of the subunits NR2A-NR2D. One report showed that NR2A is expressed in every laminae in the dorsal horn, except lamina II, whereas NR2B is expressed only in lamina II in the dorsal horn of the mouse spinal cord (Watanabe, Mishina & Inoue, 1994). Another study showed that NR2C is expressed with low intensity and restricted to lamina II, whereas NR2D is expressed with low intensity diffusely throughout the dorsal horn of the rat spinal cord (Tölle, Berthele, Zieglgänsberger, Seeburg & Wisden, 1993). Furuyama et al. (1993) demonstrated that the mRNAs for the AMPAR subunits each show slightly different expression patterns in the rat. For example, high expression of GluR2 is found in lamina II and the superficial part of lamina III. GluR3 expression is somewhat concentrated in these laminae, but labeling is not as intense as for GluR2. Also, the

expression of GluR3 is dispersed throughout laminae II-VI with moderate intensity, whereas the expression of GluR4 is very low in the dorsal horn. These investigators also found that the KAR subunit, KA1, is weakly and diffusely expressed throughout the dorsal horn. Tölle et al. (1993) also examined the expression patterns of the mRNAs for the KAR subunits, GluR5-GluR7, KA1 and KA2. These investigators found that GluR5 is only expressed with weak intensity in lamina I, whereas low levels of GluR7 are found throughout the dorsal horn. GluR6 is not expressed in the rat spinal cord, and contrary to the finding of Furuyama et al. (1993), Tölle et al. (1993) did not find that KA1 is expressed in the dorsal horn. Tölle et al. (1993) also reported that KA2 is moderately expressed in lamina II. In summary, since the mRNAs for the iGluR subunits display different expression patterns in the dorsal horn, it is likely that distinct NMDAR, AMPAR and KAR subtypes mediate dorsal horn neuronal responses to different types of primary afferent input.

A few studies have also examined the expression patterns of the mRNAs for the mGluR subtypes in the dorsal horn of the rat spinal cord. The group I mGluR, mGluR5, shows the strongest expression in the dorsal horn compared to all other mGluR subtypes (Boxall et al. 1998). These investigators reported that this receptor subtype shows the highest expression in laminae I-IV. Vidnayánszky et al. (1994) demonstrated that intense labeling of an anti-mGluR5a antibody was concentrated in laminae I and II. Interestingly, at the electron microscopy level, these investigators showed that the antibody staining was localized on postsynaptic cell bodies and dendrites, and that the immunoreactive dendrites appeared to be targeted by presumed primary afferent C-fiber terminals. The group III mGluR, mGluR7, is the second most strongly expressed mGluR in the dorsal

horn (Boxall et al. 1998). Evidence suggests that mGluR7 is most prominently expressed in the superficial laminae of the rat dorsal horn (Boxall et al. 1998; Li et al. 1997; Ohishi, Akazawa, Shigemoto, Nakanishi and Mizuno, 1995; Ohishi, Nomura, Ding, Shigemoto, Wada, Kinoshita, Li, Neki, Nakanishi & Mizuno, 1995. Interestingly, at the electron microscopic level, Ohishi, Nomura, Ding, Shigemoto, Wada, Kinoshita, Li, Neki, Nakanishi and Mizuno (1995) reported that an anti-mGluR7 antibody intensely labels axon terminals of presumed primary afferent fibers. Boxall et al. (1998) demonstrated that mRNA for the group I mGluR, mGluR1 is mainly distributed in the deeper laminae (III-VII) and lower levels are found in laminae I and II. Evidence suggests that the group II mGluR, mGluR3, is expressed with low to moderate intensity in laminae II-V (Boxall et al. 1998; Ohishi et al. 1993a). Moreover, Ohishi et al. (1993a) reported that mGluR3 is expressed on cell bodies in the dorsal horn. Boxall et al. (1998) also found low expression of the group III mGluR, mGluR4, in laminae I and II, as well as dispersed, intense expression in the rest of the grey matter of the spinal cord, especially in the interneurons of the dorsal horn. Several studies have reported that the group II mGluR, mGluR2, and the group III mGluRs, mGluR6 and mGluR8, are undetectable in the rat spinal cord (Boxall et al. 1998; Nakajima et al. 1993).

In summary, several mGluR subtypes (mGluR1, mGluR3, mGluR4 and mGluR5) are localized on postsynaptic neurons in laminae I and II of the dorsal horn. These data suggest that these mGluRs may either enhance (mGluR1 and mGluR5) or inhibit (mGluR3 and mGluR4) primary afferent nociceptive transmission, since Aδ- and C-fibers are known to preferentially terminate in lamina I and lamina II, respectively. Also,

evidence that mGluR7 is localized presynaptically on primary afferent terminals suggests that it may act as an autoreceptor and modulate Aδ- and C-fiber/dorsal horn neuronal transmission by reducing the release of neurotransmitters (Ohishi, Nomura, Ding, Shigemoto, Wada, Kinoshita, Li, Neki, Nakanishi and Mizuno, 1995). Studies have also demonstrated that several mGluR subtypes (mGluR1 and mGluR3) are localized on postsynaptic neurons in deeper lamina of the dorsal horn (laminae III-IV), which suggests that these mGluR subtypes may enhance (mGluR1) or inhibit (mGluR3) Aβ primary afferent transmission, since these fibers are known to preferentially terminate in the deeper layers of the dorsal horn.

GluR-Mediated Synaptic Transmission in the Spinal Cord

In 1991, Kangra and Randic demonstrated that selective electrical activation of low-threshold (Aβ) or high-threshold (Aδ + C) primary afferent fibers increases the release of Glu and Asp in the dorsal horn of the rat spinal cord. This report, in conjunction with electrophysiological studies, demonstrating that GluR antagonists block primary afferent-dorsal horn neuronal responses, provides evidence that GluRs mediate the transmission of tactile and nociceptive information in the rat spinal cord. More specifically, results from these electrophysiological studies suggest that non-NMDARs mediate dorsal horn neuronal responses to acute innocuous and noxious cutaneous stimulation, whereas the NMDAR appears to be activated only after prolonged noxious stimulation of the cutaneous receptive field. This proposal is supported by the demonstration that application of the non-selective iGluR antagonist, γ-D-glutamyglycine (DGG), but not the selective NMDAR antagonist, D-AP5, to the rat spinal cord, reduces

dorsal horn neuronal responses to brief Aβ- and C-fiber stimulation (Dickenson & Sullivan, 1990). Also, application of the competitive non-NMDA antagonist, NBQX or CNQX, or the non-competitive AMPA antagonists, GYKI 52466 or GYKI 53655, to the rat spinal cord reduces dorsal horn neuronal responses to noxious cutaneous thermal stimulation (Cumberbatch, Herrero & Headley, 1994), or to innocuous (Budai & Larson, 1994; Cumberbatch et al. 1994; King & Lopez-Garcia, 1993) and noxious (Budai & Larson, 1994; King & Lopez-Garcia, 1993) mechanical stimulation. Neugebauer, Lücke & Schaible (1993) reported that iontophoretic application of the non-NMDAR antagonist, CNQX, but not the NMDAR antagonists, ketamine or D,L-AP5, reliably reduces dorsal horn neuronal responses to innocuous pressure of the rat knee joint. King and Lopez-Garcia (1993) also found that application of D-AP5, reduces the EPSPs, but does not affect the neuronal firing evoked by cutaneous innocuous and noxious stimulation.

In contrast, evidence suggests that the NMDAR mediates spinal cord neuronal responses elicited by repeated C-fiber stimulation. Repeated C-fiber stimulation produces a progressive increase in post-stimulus action potentials in dorsal (Mendell, 1966) and ventral (Woolf & Swett, 1984) horn neurons. This phenomenon, called "wind-up", is a simple model of injury-induced sensitization of spinal cord neurons. The proposal that the NMDAR plays a critical role in wind-up, is supported by the demonstration that application of the NMDAR antagonists, D-AP5, ketamine, or 7CK to the rat spinal cord blocks the C-fiber evoked increases in dorsal horn neuronal activity (Chapman & Dickenson, 1992; Chizh, Cumberbatch, Herrero, Stirk & Headley, 1997; Davies & Lodge, 1987; Dickenson & Aydar, 1991; Dickenson & Sullivan, 1987a, 1990).

Comparatively few studies have examined the role of mGluRs in excitatory synaptic transmission in the mammalian spinal dorsal horn. Young et al. (1997) demonstrated that application of the group I mGluR antagonist, cyclothiazide (CTZ), to the rat spinal cord does not reduce dorsal horn neuronal activity evoked by innocuous cutaneous mechanical stimulation. In contrast, Budai and Larson (1998) showed that iontophoretic application of the group 1 mGluR antagonist/group 2 mGluR agonist, (*S*)-4C3HPG, blocks C-fiber-evoked potentiation of the dorsal horn neuronal responses. These data and data from other studies (Young et al. 1994, 1995, 1997) suggest that group I mGluRs are not involved in the processing of acute innocuous and noxious cutaneous information, but may mediate dorsal horn neuronal responses following prolonged noxious stimulation of the cutaneous field. Moreover, Boxall, Thompson, Dray, Dickenson and Urban (1996) showed that superfusion with (+)-MCPG reduces the amplitude of ventral root responses evoked by low frequency high intensity stimulation of the dorsal root, and blocked ventral root depolarization evoked by capsaicin application, which suggests that mGluRs contribute to nociceptive reflex activity evoked by high-intensity stimulation.

Dorsal Horn Neuronal Responses to the Application of GluR Agonists

Several studies have examined the effects of iGluR agonists on spontaneous and evoked spinal cord neuronal activity. For example, intrathecal administration of NMDA (Sher & Mitchell, 1990), iontophoretic application of NMDA, AMPA or KA to the spinal cord (Cumberbatch et al. 1994), or intraspinal injection of Quis (Yezierski & Park, 1993) increases the background firing rate of rat dorsal horn neurons. Also, intrathecal administration of NMDA (Sher & Mitchell, 1990), iontophoretic application of NMDA,

AMPA (Aanonsen, Lei, Wilcox, 1990; Cumberbatch et al. 1994) or KA (Cumberbatch et al. 1994), or intraspinal injection of Quis (Yezierski & Park, 1993) increases dorsal horn neuronal responses to innocuous and noxious cutaneous mechanical stimulation. In the monkey, Dougherty and Willis (1991) demonstrated that iontophoretic application of NMDA or Quis to the spinal cord increases spinothalamic tract neuronal responses to innocuous cutaneous mechanical stimulation. Although, Aanonsen et al. (1990) reported that NMDA, but not AMPA or KA application to the rat spinal cord facilitates neuronal responses to noxious cutaneous thermal stimulation, Cumberbatch et al. (1994) found that all three iGluR agonists equally enhanced dorsal horn neuronal responses to this type of stimulation.

Comparatively few studies have examined the effects of mGluR compounds on spinal cord neuronal activity. In 1994, Palecek, Palecková, Dougherty and Willis demonstrated that iontophoretic application of low doses of (1*S*,3*R*)-ACPD, to the primate spinal cord increases spinothalamic tract neuronal responses to innocuous mechanical stimulation. Recently, Budai and Larson (1998) demonstrated that iontophoretic application of the non-selective mGluR agonist, (1*S*,3*R*)-ACPD, to the rat spinal cord significantly increases dorsal horn neuronal responses to innocuous mechanical stimulation and to high frequency C-fiber stimulation, and that pretreatment with (*S*)-4C3HPG blocks these responses, *in vitro*. These data suggest that group 1 mGluRs are involved in the processing of nociceptive information in the dorsal horn and that activation of this group of mGluRs increases the sensitivity of dorsal horn neurons to tactile stimulation. Also, Boxall et al. (1996) demonstrated that superfusion of the rat spinal cord with (1*S*,3*R*)-ACPD induces ventral root depolarization, which is attenuated

by the non-selective mGluR antagonist, (+)-MCPG, *in vitro*. These data suggest that mGluRs contribute to the nociceptive reflex in rats.

Behavioural Nociceptive Responses to Intrathecal Administration of GluR Agonists

A number of studies have demonstrated that spinal administration of GluR agonists induces various types of acute nociceptive behaviours in rodents. For example, intrathecal treatment with the iGluR agonists, NMDA, AMPA or KA, the non-NMDAR/mGluR agonist, Quis (Aanonsen & Wilcox, 1987, 1989; Brambilla, Prudentino, Grippa & Borsini, 1996; Kolhekar & Gebhart, 1994; Kolhekar, Meller & Gebhart, 1993; Mjellem-Joly, Lund, Berge & Hole, 1991; Okano, Kuraishi & Satoh, 1995; Raigorodsky & Urca, 1987, 1990; Sun & Larson, 1991), or the non-selective mGluR agonist, (1*S*,3*R*)-ACPD (Meller, Dystra & Gebhart, 1996), produces spontaneous nociceptive behaviours in rats or mice. Also, low doses of intrathecal NMDA produce acute thermal hyperalgesia (Aanonsen & Wilcox, 1987; Advokat, Ghorpade & Wolfe, 1994; Kolhekar et al. 1993; Malmberg & Yaksh, 1993; Meller et al. 1996; Meller, Gebhart, Maves, 1992; Raigorodsky & Urca, 1987), mechanical hyperalgesia (Ferreira & Lorenzetti, 1994) and touch-evoked allodynia (Raigorodsky & Urca, 1987). Furthermore, Ma and Woolf (1995) showed that intrathecal NMDA administration enhances the flexion withdrawal reflex to innocuous and noxious mechanical stimulation in the decerebrate-spinal rats, suggesting that the agonist produces touch-evoked allodynia and mechanical hyperalgesia in these animals.

Interestingly, two reports have suggested that intrathecal treatment with AMPA, KA, Quis, or (1*S*,3*R*)-ACPD, does not induce heat hyperalgesia in rodents (Aanonsen & Wilcox, 1987; Meller et al. 1996). However, intrathecal administration of AMPA

(Brambilla et al. 1996; Ferreira & Lorenzetti, 1994), Quis (Meller, Dystra & Gebhart, 1993) or co-administration of AMPA and *trans*-ACPD (Meller et al. 1993) produces acute mechanical hyperalgesia in rats, suggesting that activation of the AMPAR or co-activation of the AMPAR and mGluRs may contribute to the spinal mechanisms mediating the development of mechanical hyperalgesia in persistent and chronic pain conditions.

GluR-Mediated Spinal Cord Plasticity and its Role in Persistent and Chronic Pain

Several lines of research support the notion that GluRs mediate injury-related spinal cord plasticity, as well as the nociceptive responses of rats in models of persistent and chronic pain. First, studies have demonstrated an increase in the release of EAAs in models of persistent and chronic pain. Second, GluR antagonists reduce injury-related increases in dorsal horn neuronal responses. Third, GluR antagonists attenuate nociceptive responses of rats in models of persistent and chronic nociception. These points will be discussed in more detail below.

GluR-Mediated Spinal Cord Plasticity

Peripheral tissue or nerve injury produces an initial barrage of primary afferent fiber activity, followed by a persistent, less intense level of activity. The consequential release of primary afferent fiber neurochemicals, including EAAs, induce and contribute to the maintenance of prolonged increases in dorsal horn neuronal sensitivity, thought to contribute to persistent and chronic pain conditions. Tissue inflammation- or nerve injury-induced central sensitization in the spinal cord may be characterized by an increase in spontaneous activity; an increase in responsiveness, or a decrease in response

threshold, to cutaneous stimulation or to primary afferent electrical stimulation; and an expansion of cutaneous receptive fields.

The hypothesis that peripheral tissue or nerve injury produces a persistent activity of primary afferent fibers and a subsequent release of EAAs is supported by the demonstration that EAA levels in the dorsal horn are increased following peripheral inflammation (Sluka & Westlund, 1992), nerve injury (Kawamata & Omote, 1996), and chemical-induced injury (Skilling, Smullin, Beitz & Larson, 1988) in animal models. Also, electrophysiological studies have demonstrated that persistent spinal cord neuronal sensitization occurs in response to tissue inflammation produced by ultraviolet (UV) irradiation of the hindpaw (Chapman & Dickenson, 1994), intradermal injection of complete Freund's adjuvant (Calvino, Villanueva & Le Bars, 1987; Hylden, Nahin, Traub & Dubner, 1989; Menétrey & Besson, 1982; Ren, Hylden, Williams, Ruda & Dubner, 1992) or capsaicin (Dougherty, Palecek, Palecková, Sorkin & Willis, 1992), intra-articular injection of kaolin and carrageenan (Neugebauer, Lücke & Schaible, 1993; Neugebauer, Lücke & Schaible, 1994), or cutaneous application of mustard oil (Munro, Fleetwood-Walker & Mitchell, 1994; Woolf & Thompson, 1991), as well as chemically-induced injury (Chapman & Dickenson, 1995; Dickenson & Aydar, 1991; Dickenson & Sullivan, 1987a, Haley, Sullivan & Dickenson, 1990), nerve injury (Devor & Wall, 1978; Laird & Bennett, 1993; Palecek et al. 1992; Sotgui, Biella & Riva, 1994, 1995; Takaishi, Eisele & Carstens, 1996) and thermal injury (Kenshalo, Leonard, Chung & Willis, 1982; Price, Hayes, Ruda & Dubner, 1978; Woolf, 1983).

Evidence suggests that injury-related increases in dorsal horn neuronal sensitivity are mediated, in part, by GluRs. For example, a number of studies have shown that

iGluRs contribute significantly to chemically-induced sensitization of spinal cord neurons in the rat. Specifically, the hyperexcitability of dorsal horn neurons induced by a subcutaneous injection of formalin is reduced by intrathecal administration of the NMDAR antagonists, AP5 (Haley et al. 1990) or 7CK (Chapman & Dickenson, 1995; Dickenson & Aydar, 1991), intravenous administration of the NMDAR antagonists, MK-801 or ketamine (Haley et al. 1990), or intrathecal administration of the non-NMDAR antagonist, CNQX (Chapman & Dickenson, 1995). Studies have shown that iGluRs contribute significantly to inflammation-related sensitization of spinal cord neurons in the rat. Also, iontophoretic application of AP5, ketamine, or CNQX (Neugebauer et al. 1994; Neugebauer, Lücke & Schaible, 1993) to the spinal cord, or intravenous administration of the non-competitive NMDAR antagonist, memantine (Neugebauer, Kornhuber, Lücke & Schaible, 1993) reduces the enhanced dorsal horn neuronal responses to innocuous and noxious cutaneous mechanical stimulation in rats with knee joint inflammation. Furthermore, intravenous administration of the NMDAR antagonist, MK-801, prevents the expansion of peripheral receptive fields, reduces the spontaneous activity of dorsal horn neurons, as well as the enhanced responses to noxious cutaneous thermal and mechanical following an intradermal injection of complete Freund's adjuvant (Ren, Hylden, Williams, Ruda & Dubner, 1992). Chapman and Dickenson (1994) demonstrated that intrathecal administration of 7CK significantly reduces the spontaneous activity of dorsal horn neurons, as well as the enhanced responses to Aβ- and C-fiber stimulation following UV irradiation of the hindpaw.

Comparatively few studies have examined the role of mGluRs in injury-related spinal cord plasticity. These studies have provided evidence suggesting that mGluRs contribute to inflammation-related enhancement of spinal cord neuronal function. For example, iontophoretic application of the non-selective mGluR antagonists, L-AP3, or (*RS*)-4C3HPG attenuates the dorsal horn neuronal responses to repeated cutaneous application of mustard oil (Young et al. 1994, 1995) and to innocuous and noxious mechanical stimulation following inflammation of the knee joint (Neugebauer et al. 1994) in the rat. Recently, Young et al. (1997) provided evidence that group I mGluRs are involved in the inflammation-related dorsal horn sensitization with the demonstration that application of the group I mGluR antagonist, CTZ, reduced the increases in dorsal horn neuronal activity evoked by cutaneous application of mustard oil.

GluR-Mediated Nociception in Models of Pain

It has been well established that iGluRs contribute significantly to chemically-induced nociception. For example, in the formalin model, intrathecal, intraperitoneal or subcutaneous treatment with the non-competitive NMDAR antagonists, MK-801 (Coderre, 1993; Coderre & Melzack, 1992a; Yamamoto & Yaksh, 1992a), dextromethorphan (Elliott, Brodsky, Hynansky, Foley & Inturrisi, 1995), ketamine (Millan & Sequin, 1994; Nässtrom, Karlsson & Post, 1992) or memantine (Eisenberg, Vos & Strassman, 1993; Millan & Sequin, 1994), or intrathecal treatment with the competitive NMDAR antagonists, AP5 (Coderre, 1993; Coderre & Melzack, 1992a; Nässtrom et al. 1992), AP7 (Nässtrom et al. 1992), CGS 19755 (Hunter & Singh, 1994), CPP (Kristensen, Karlsten, Gordh & Berg, 1994; Nässtrom et al. 1992) to rats or mice, significantly reduces nociceptive scores. Interestingly, Chaplan, Malmberg and Yaksh,

(1997) reported that different NMDAR antagonists are not equally effective in this test. These investigators showed that intrathecal administration of the NMDAR antagonists produced the following rank order of potency for attenuating formalin-induced nociception: AP5 > memantine ≥ MK-801 > dextrorphan > dextromethorphan > ketamine. Evidence also suggests that the non-NMDAR contributes to nociceptive responses in the formalin model. For example, studies have demonstrated that intraperitoneal or intrathecal administration of the non-NMDAR antagonists, NBQX (Hunter & Singh, 1994), DNQX or CNQX (Näsström et al. 1992) significantly attenuates formalin-induced nociception in rats or mice.

It has also been well established that iGluRs contribute significantly to inflammation-related nociception. For example, heat hyperalgesia associated with a subcutaneous injection of carrageenan into the rat hindpaw is significantly reduced by intrathecal treatment with MK-801 (Ren, Hylden, Williams, Ruda & Dubner, 1992; Ren, Williams, Hylden, Ruda & Dubner, 1992; Yamamoto, Shimoyana & Mizuguchi, 1993a), ketamine (Klimscha, Hováth, Szikszay, Dobos & Benedek, 1998; Ren, Williams, Hylden, Ruda & Dubner, 1992), (+/-)-AP5 or CPP (Ren, Williams, Hylden, Ruda & Dubner, 1992). Also, intraperitoneal administration of L-687,414 or L-701,324, which are antagonists at the NMDAR glycine modulatory site, have also been shown to attenuate carrageenan-induced mechanical hyperalgesia in rats (Laird, Mason, Webb, Hill & Hargreaves, 1996). Mechanical hyperalgesia associated with a subcutaneous injection of complete Freund's adjuvant into the rat hindpaw is also reduced by intrathecal treatment with (+/-)-AP5 or MK-801 (Ren & Dubner, 1993). The involvement of non-NMDARs

have also been implicated to play a role in inflammation-related nociception. This is supported by the demonstration that intrathecal administration of CNQX to rats reduces thermal hyperalgesia associated with subcutaneous carrageenan injection (Ren, Williams, Hylden, Ruda & Dubner, 1992).

Only one recent study has examined the role of mGluRs in inflammation-related nociception. Specifically, Young et al. (1997) reported that intrathecal administration of (*S*)-4C3HPG reduces mechanical and thermal hyperalgesia in rats with carrageenan-induced inflammation.

It has been well established that the NMDAR contributes significantly to models of neuropathic pain. For example, the earliest studies demonstrated that intraperitoneal or intrathecal administration of the non-competitive NMDAR antagonists, MK-801 (Davar, Hama, Deykin, Vos & Maciewicz, 1991; Mao, Price, Hayes, Lu & Mayer, 1992; Mao Price, Mayer, Lu & Hayes, 1992; Yamamoto & Yaksh, 1992b), dextrorphan (Mao, Hayes, Lu, Mayer & Frenk, 1993; Tal & Bennett, 1993) or ketamine (Mao et al. 1993) attenuates heat hyperalgesia associated with loose ligation of the rats' sciatic nerve. In 1994, Tal and Bennett reported that heat hyperalgesia, but not mechanical allodynia, is sensitive to treatment with dextrorphan in this model. This finding, in part, led to the proposal that heat hyperalgesia, but not mechanical allodynia in models of pain is mediated by the NMDAR (Meller, 1994). Since then, however, it has been demonstrated that intrathecal administration of ketamine attenuates mechanical hyperalgesia and mechanical allodynia in the sciatic nerve constriction injury model (Hartrick, Wise & Patterson, 1997). Also, in another neuropathic pain model, Kim, Na, Yoon, Han, Ko and Hong (1997) reported that intraperitoneal administration of MK-801 to rats reduces both

mechanical allodynia and thermal allodynia associated with unilateral transection of the inferior caudal trunk.

Others have demonstrated that a number of different types of neuropathic pain-related behaviours are sensitive to NMDAR antagonist treatment in a model that involves tight ligation of the rats' $5^{th}$ and $6^{th}$ lumbar spinal nerves. Specifically, intraperitoneal administration of ketamine or memantine to rats reduces mechanical hyperalgesia and mechanical allodynia (Carlton & Hargett, 1995; Qian, Brown & Carlton, 1996), as well as cold allodynia and spontaneous nociceptive behaviours (Qian et al. 1996) in this model. Interestingly, Chaplan et al. (1997) reported that different types of NMDAR antagonists are not equally effective in reducing mechanical allodynia associated with ligation of the two lumbar spinal nerves. These investigators showed that, although all of the NMDAR antagonists that were tested attenuated mechanical allodynia in the spinal nerve-injured rats, intrathecal administration of the compounds produced the following rank order of antinociceptive potency: memantine = D-AP5 > dextrorphan = dextromethorphan > MK-801 > ketamine. In summary, these studies clearly demonstrate that the NMDAR makes a significant contribution to different types of neuropathic pain-related behaviours.

Only one study has shown that non-NMDAR play a role in neuropathic pain models. Specifically, Mao, Hayes, Lu and Mayer (1992) showed that intrathecal administration of the AMPA/kainate antagonist, CNQX, attenuates heat hyperalgesia in rats following sciatic nerve constriction injury.

I have reviewed the large body of literature that clearly demonstrates that iGluRs play a critical role in persistent and chronic nociceptive processes in the spinal cord. In contrast, little is known about the contribution of spinal mGluRs to nociception; therefore, I carried out a series of experiments to explore the possible role(s) of mGluRs in animal models of persistent and chronic pain. Linking chapters, which precede each of the five studies, provide a more detailed discussion of the rationale for each experiment that was carried out.

# CHAPTER 2: THE ROLE OF mGluRs IN THE FORMALIN MODEL

The Formalin Test

In 1977, Dubuisson and Dennis characterized the formalin test and described a number of advantages to using this model compared to other nociceptive tests that were commonly used at the time (i.e. tail-flick and hot-plate tests). For example, the formalin test produces prolonged nociceptive responses in animals, which creates a more valid model for continuous pain in the clinical population. Also, in contrast to other tests, the formalin model requires no restraint during the testing period and does not restrict the behavioural responses of the animals (Dubuisson & Dennis, 1977).

In addition to the above mentioned advantages, the formalin paradigm includes an objective method for measuring the intensity of nociceptive behaviours, which was developed by Dubuisson and Dennis (1977). Following a subcutanteous injection of dilute formalin into the forepaw of rats, each animal is scored according to a four point scale. The categories of the scale include the following criteria: 0, full weight on injected paw; 1, reduced weight on injected paw; 2, elevation of injected paw; 3, licking, biting and shaking of injected paw. A weighted average nociceptive intensity score, based on the time spent in each behavioural category, is calculated for each observation interval according to the following formula:

$$[T1 + (2)T2 + (3)T3]/\text{total T}$$

T1, T2, and T3 is the time (seconds) spent exhibiting behaviours in categories 1, 2, and 3; total T is the total time for the observation interval. This weighted score method has since been validated as an accurate measure of pain intensity (Coderre, Fundytus, McKenna, Dalal & Melzack, 1993). One revision of the 1977 methodology requires injecting the hindpaw with dilute formalin instead of the forepaw, since licking of the hindpaw rarely

occurs during normal grooming behaviour. This revision reduces the possibility that grooming will be incorrectly included as a nociceptive behaviour.

A characteristic feature of the formalin test is a biphasic nociceptive response. Immediately following the formalin injection, the animal expresses intense nociceptive behaviours (i.e. licking, biting and shaking of the hindpaw) for a three to five minute period (early phase). Subsequently, the rat shows few or no nociceptive behaviours for the next 10 to 15 minutes (intermediate phase), and then shows moderately intense nociceptive behaviours for the next 20 to 60 minutes (late phase).

Investigators have suggested that the early and late phases have distinct mechanisms. The early phase appears to be mediated by dorsal horn neuronal responses evoked by primary afferent C-fiber activity, since agents that inhibit C-fiber activity, reduce first phase neuronal responses (Dickenson & Sullivan, 1987a,b). It has also been suggested that the occurrence of the first phase is essential for the development of the later tonic phase. This is supported by the demonstration that second phase nociceptive responses are reduced if lidocaine is administered, intrathecally, prior to, but not after the first phase is over (Coderre, Vaccarino & Melzack, 1990). It has been suggested that increases in C-fiber activity evoked by subcutaneous formalin produces a sensitization of dorsal horn neurons, which is mediated by the NMDAR and significantly contributes to late phase nociceptive responses. This proposal is supported by the demonstration that intrathecal pre-treatment with NMDAR antagonists reduces second, but not first phase dorsal horn neuronal (Haley et al. 1990; Chapman & Dickenson, 1995) and nociceptive behavioural (Coderre & Melzack, 1992a; Yamamoto & Yaksh, 1992a; Hunter & Singh, 1994) responses in rats.

Rationale for Experiments

Although it had been clearly established that iGluRs play a critical role in the development and/or maintenance of persistent and chronic nociception, prior to the publication of this manuscript little was known about the possible involvement of mGluRs in models of pain. This was likely due to the fact that compounds, selective for the mGluR subtypes, were just becoming available.

In 1992, Coderre and Melzack used two non-selective mGluR compounds to explore the possible role of mGluRs in formalin-induced nociception. Although these investigators did not find that intrathecal administration of the non-selective mGluR antagonist, L-AP3, attenuates formalin-induced nociception, they found that intrathecal administration of the non-selective mGluR agonist, (1*S*,3*R*)-ACPD is pro-nociceptive in this model. Unfortunately, more selective mGluR compounds were not available at the time, which prevented a more detailed investigation.

By 1995, relatively selective mGluR antagonists and agonists had become available, and results from three electrophysiological studies strongly suggested that mGluRs (likely group I mGluRs) are involved in models of inflammation-related nociception (Neugebauer et al. 1994; Young et al. 1994, 1995). However, we were not aware of any behavioural studies demonstrating the antinociceptive effects of mGluR compounds in models of pain. Thus, we performed a series of experiments in an effort to thoroughly investigate the effects of the most recently developed mGluR compounds. For this investigation, we specifically chose the formalin model, since this test has been used extensively to implicate EAAs and GluRs in nociception.

Given that the literature had extensively demonstrated that activation of group I mGluRs increases neuronal excitability and that activation of group II or group III mGluRs decreases neuronal excitability in the CNS, we explored the possibility that group I mGluRs may contribute to the development of nociceptive behaviours and that activation of group II or group III mGluRs may attenuate nociceptive behaviours in models of pain. Therefore, in the first experiment, the following mGluR compounds were administered intrathecally to rats 10 min prior to a subcutaneous injection of dilute formalin (2.5% (50 μl)) into the plantar surface of the rat hindpaw: ((+)-MCPG) (non-selective mGluR antagonist), (*S*)-4CPG, (*S*)-4C3HPG (group I mGluR antagonist/ group II mGluR agonists), (L-AP4) (group III mGluR agonist). Preliminary experiments indicated that intrathecal administration of the group I mGluR agonist, (*RS*)-DHPG or the group II mGluR agonists, (1*S*,3*S*)-ACPD (≥ 25 nmol) and DCG-IV (> 2.0 nmol) produced excitatory responses in the rats (i.e. produced spontaneous nociceptive behaviours). Therefore, we explored the possibility that lower doses of these compounds may produce pro-nociceptive effects in the formalin test.

The main findings from this study are the following:

1. Intrathecal pretreatment with L-AP4, (*S*)-4CPG, or (*S*)-4C3HPG, but not (+)-MCPG slightly reduces second phase formalin-induced nociception, suggesting that group I mGluRs play a minor role in formalin-induced nociception and that activation of group II or group III mGluRs produces a slight reduction in nociceptive responses in this model.

2. Intrathecal pretreatment with (*RS*)-DHPG (1*S*,3*S*)-ACPD, but not DCG-IV facilitates second phase formalin nociceptive scores, suggesting that group I mGluRs are localized within the nociceptive pathways mediating formalin-induced nociception. Also, it is likely that the pro-nociceptive effects of (1*S*,3*S*)-ACPD are attributable to activation of spinal group I mGluRs, since it has been demonstrated that this agonist displays some activity at this group of mGluRs (Joly et al. 1995).

3. Intrathecal administration of the NMDAR antagonist, D-AP5, or the mGluR compounds, (*S*)-4CPG or (+)-MCPG, attenuates the mGluR agonist-related facilitation of formalin nociceptive scores, verifying that the pro-nociceptive effects are mediated by mGluRs (likely, group I mGluRs), and suggesting that the agonist-induced facilitation of formalin nociceptive scores involves a group I mGluR/NMDAR interaction.

STUDY 1

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# The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception

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Summary

The present study examined the role of mGluRs in nociceptive responses of male Long-Evans rats following a 50 µl subcutaneous (s.c.) injection of 1% (30 µl) or 2.5% (50 µl) formalin to the plantar surface of the hindpaw. Intrathecal (i.t.) administration of the mGluR4/mGluR6-mGluR8 agonist, L(+)-2-amino-4-phosphonobutyric acid (L-AP4), the mGluR1/mGluR5 antagonists, (*S*)-4-carboxyphenylglycine ((*S*)-4CPG) or (*S*)-4-carboxy-3-hydroxyphenylglycine ((*S*)-4C3HPG), but not the non-selective antagonist, (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG), to the lumbar spinal cord slightly reduced second phase nociceptive responses. An i.t. injection of the mGluR1/mGluR5 agonist, (*RS*)-3,5-dihydroxyphenylglycine ((*RS*)-DHPG) or the mGluR2/mGluR3 agonist, (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic acid ((1*S*,3*S*)-ACPD), but not (2*S*,1'*R*,2'*R*,3'*R*)-2-(2'3'-dicarboxy-cyclopropyl)-glycine (DCG-IV), dose-dependently enhanced formalin-induced nociception in the second phase. In addition, the facilitation of nociceptive responses induced by (1*S*,3*S*)-ACPD or (*RS*)-DHPG was reduced by prior i.t. administration of the mGluR antagonists, (+)-MCPG or (*S*)-4C3HPG, respectively, as well as by the *N*-methyl-D-aspartate (NMDA) receptor antagonist, D(-)-2-amino-5-phosphonopentanoic acid (D-AP5). These results indicate that although mGluRs may play a minor role in formalin-induced nociception, mGluR agonist-related facilitation of formalin scores may reflect an interaction with the NMDA receptor.

Key words: Nociception; Metabotropic glutamate receptors; mGluR; Excitatory amino acids; *N*-methyl-D-aspartate; Formalin test

The formalin test has been used as a model of persistent pain and central nervous system (CNS) plasticity (Coderre et al. 1990). A subcutaneous (s.c.) injection of formalin into the hindpaw of the rat produces a biphasic nociceptive response. Formalin-induced nociception includes a 5 min early phase of intense nociceptive behaviour, followed by a 10-15 min period of reduced nociceptive activity. The animal subsequently expresses a late phase of moderate nociceptive activity for a duration of 20-60 min post injection (Dubuisson and Dennis 1977). Dickenson and Sullivan (1987b) reported a biphasic increase in neuronal excitation in the dorsal horn of the lumbar spinal cord that parallels the behavioural response in the formalin test. It has been proposed that nociceptor activation during the early phase produces dorsal horn neuronal sensitization, which contributes to nociception in the late phase of the formalin test (Dickenson and Sullivan 1987c; Coderre et al. 1990).

Recent evidence suggests that excitatory amino acids (EAAs), such as glutamate and aspartate, play a role in various types of spinal cord plasticity that have been proposed to mediate nociception. For example, iontophoretic application of EAAs increases cutaneous receptive field size (Zieglgänsberger and Herz 1971) and produces 'windup' (King et al. 1988; Gerber and Randic 1989), a form of central sensitization that is expressed as a cumulative increase in postsynaptic depolarization and a consequent prolongation of dorsal horn neuronal discharge (Mendell 1966; Schouenbourg and Dickenson 1985). In addition, EAA antagonists reduce windup in both dorsal (Davies and Lodge 1987; Dickenson and Sullivan 1987a) and ventral (Woolf and Tompson 1991) horn neurons, and prevent sensitization of dorsal horn neurons elicited by noxious stimulation (Dougherty et al. 1992).

Investigators have also demonstrated the involvement of EAAs in formalin-induced nociception. For example, Skilling et al. (1988) reported that a s.c. injection of formalin produces an increase in glutamate and aspartate release in dorsal horn neurons. In addition, Haley et al. (1990) showed that pretreatment with D(-)-2-amino-5-

phosphonopentanoic acid (D-AP5) (intrathecal (i.t.)) or intravenous (i.v.) MK-801 attenuates formalin-induced changes in dorsal horn neuronal activity in the late phase. The late phase behavioural response is also reduced by *N*-methyl-D-aspartate (NMDA) antagonist pretreatment. For example, in the rat, i.t. administration of MK-801 (Coderre and Melzack 1992; Yamamoto and Yaksh 1992), CPP (Kristensen et al. 1994) or D-AP5 (Coderre and Melzack 1992) effectively reduces nociceptive scores. In the mouse, administration of the NMDA antagonists, D-AP5, 2-amino-7-phosphonopropionic acid (AP7), CPP (Näsström et al. 1992) and DL-AP5, but not the kainate antagonist, urethane (Murray et al. 1991), had antinociceptive effects in the formalin test. Furthermore, Coderre and Melzack (1992) found that an i.t. injection of the agonists, L-glutamate, L-aspartate or NMDA enhances formalin-induced nociceptive behaviour during the intermediate and late phases of the test. Others have also suggested the involvement of the α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA) receptor in formalin-induced nociception (Näsström et al. 1992; Hunter and Singh 1994). Thus, Näsström et al. (1992) demonstrated that i.t. pretreatment with the AMPA antagonists, 6,7-dinitro-quinoxalinedione (DNQX) or 6-cyano-7-nitro-quinoxalinedione (CNQX), attenuates late phase nociceptive scores of mice in the formalin test. Also, Hunter and Singh (1994) found that an i.p. injection of the AMPA antagonist, 6-nitro-7-sulphamoyl-benzo(f)- quinoxaline-2,3-dione (NBQX), reduces early and late phase formalin scores of rats. However, others have found that the AMPA receptor does not play a significant role in formalin-induced nociception (Coderre and Melzack 1992).

A group of metabotropic glutamate receptors (mGluR1 to mGluR8) that are linked to intracellular second messenger systems have recently been identified and distinguished from the ionotropic glutamate receptors (iGluRs), NMDA, AMPA and kainate. For example, activation of mGluR1 or mGluR5 leads to phosphoinositide hydrolysis (Abe et al. 1992), increases in cyclic adenosine 3',5'-monophosphate (cAMP) accumulation (Aramori and Nakanishi 1992), and intracellular $Ca^{2+}$ mobilization (Masu

et al. 1991; Aramori and Nakanishi 1992). The remaining mGluRs are linked to cAMP inhibition, but are distinguished by a differential agonist and antagonist selectivity (Jane et al. 1994). For example, (1*S*,3*S*)-ACPD stimulates mGluR2 and mGluR3 (Watkins and Collingridge 1994), while mGluR4, mGluR6-mGluR8 are activated selectively by L-AP4 (Nakanishi et al. 1992; Thomsen et al. 1992; Nakajima et al. 1993; Tanabe et al. 1993; Okamoto et al. 1994).

Although application of the mGluR antagonist, L-2-amino-3-phosphonopropionic acid (L-AP3), has been shown to attenuate rat dorsal horn neuronal activity associated with repeated mustard oil application (Young et al. 1995) and knee joint inflammation (Neugebauer et al. 1994), the compound did not influence nociceptive scores in the formalin test (Coderre and Melzack 1992). However, the non-selective mGluR agonist, *trans*-ACPD, has been shown to facilitate formalin-induced nociception (Coderre and Melzack 1992). In addition, a combination of *trans*-ACPD and NMDA produced a greater increase in formalin scores than administration of either agonist alone, suggesting a possible interactive effect between the two receptors. It is of interest that several laboratories have provided evidence that mGluR activity may potentiate AMPA or NMDA responses (Bleakman et al. 1992; Cerne and Randic 1992; Bond and Lodge 1995).

In the present study, the contribution of mGluRs in formalin-induced nociception was further assessed by examining the effects of pretreatment with the following compounds: the non-selective mGluR antagonist, (+)-α-methyl-4-carboxyphenylglycine ((+)-MCPG) (Hayashi et al. 1994; Kemp et al. 1994); the relatively selective mGluR1/mGluR5 agonist, (*RS*)-3,5-dihydroxyphenylglycine ((*RS*)-DHPG) (Ito et al. 1992; Schoepp et al. 1994) and antagonists, (*S*)-4-carboxy-3-hydroxyphenylglycine ((*S*)-4C3HPG) and (*S*)-4-carboxyphenylglycine ((*S*)-4CPG) (Thomsen and Suzdak 1993; Hayashi et al. 1994); and the selective mGluR2/mGluR3 agonists, (2*S*,1'*R*,2'*R*,3'*R*)-2-(2'3'-dicarboxy-cyclopropyl)-glycine (DCG-IV) (Ishida et al. 1993; Ohfune et al. 1993)

and (1*S*,3*S*)-1-aminocyclopentane-1,3-dicarboxylic ((1*S*,3*S*)-ACPD) (Jane et al. 1994; Watkins and Collingridge 1994) and the mGluR4/mGluR6-mGluR8 agonist, L(+)-2-amino-4-phosphonobutyric acid L-AP4 (Nakanishi 1992; Thomsen et al. 1992; Nakajima et al. 1993; Tanabe et al. 1993; Okamoto et al. 1994) (drug actions are summarized in Table I). In addition, the present study further examined the possibility of a NMDA/mGluR interaction in the formalin test by administering the NMDA antagonist, D-AP5, prior to the mGluR agonists, (1*S*,3*S*)-ACPD or (*RS*)-DHPG.

Methods

Long-Evans male rats (220-457 g; Charles River) were randomly assigned to one of 21 groups. In the first experiment, 56 animals received one of the following drug treatments: vehicle, L-AP4, (+)-MCPG, (*S*)-4CPG or (*S*)-4C3HPG. Ten minutes following an i.t. injection of either vehicle or one of the mGluR compounds (100 or 500 nmol), each rat was given a s.c. injection of 2.5% formalin (50 µl) into the plantar surface of one hindpaw and immediately observed for nociceptive testing. The formalin test was performed in a 30 x 30 x 30 cm Plexiglas® box, under which a mirror was placed at a 45° angle to allow full view of the hindpaws. At 5 min intervals during a 50 min period, a weighted nociceptive score was calculated based on the time spent in each of the following behavioural categories: 0 = full weight on injected paw; 1 = reduced weight on injected paw; 2 = elevation of injected paw; 3 = licking, biting and shaking of injected paw. A weighted average nociceptive score was calculated by multiplying the total time spent in each category by its category weight, summing these products and dividing the total time for each block (see Coderre, Fundytus, McKenna, Dalal & Melzack, 1993 for a validation of this method).

Since preliminary studies indicated that the agonists, (1*S*,3*S*)-ACPD and (*RS*)-DHPG, enhanced formalin scores, 1.0% formalin (30 µl) was used in the second experiment to avoid a ceiling effect of nociceptive scores. Forty-four animals were tested

according to the above protocol 10 min after an i.t. injection of either vehicle or one dose (0.2, 2.0 or 20 nmol) of (1*S*,3*S*)-ACPD or (*RS*)-DHPG. In addition, several rats were treated with the highest dose of each agonist, without the formalin injection, and although some tail licking was observed, no nociceptive behaviours involving the hindpaws were evident. A single dose of DCG-IV (0.2 nmol) was administered to a group of eight rats; the higher doses were not used since they produced nociceptive effects that interfered with the formalin test.

In the last experiment, 13 rats were treated with the NMDA receptor antagonist, D-AP5 (5 nmol), 10 min prior to a 20 nmol injection of either (1*S*,3*S*)-ACPD or (*RS*)-DHPG. In addition, 13 animals received 100 nmol of either (+)-MCPG or (*S*)-4C3HPG, 10 min before an injection of (1*S*,3*S*)-ACPD or (*RS*)-DHPG, respectively. Each rat was then injected with 1.0% formalin (s.c.; 30 µl) to the hindpaw 10 min after the agonist injection, and was immediately scored for nociceptive responses. D-AP5 (5 nmol) was selected, since a pilot study indicated that this dose does not influence formalin scores. Also, nociceptive behaviours are not significantly reduced by 100 nmol of (+)-MCPG or (*S*)-4C3HPG (Fig. 1B, 1D).

All drugs and vehicle (dilute NaOH/saline solution) were administered i.t. by lumbar puncture, in a volume of 30 µl, between the L2 and L3 vertebrae, while rats were under brief halothane anesthesia. The compounds were dissolved in either 0.9% saline (D-AP5, (*RS*)-DHPG, DCG-IV) or a dilute NaOH/saline solution (L-AP4, (+)-MCPG, (*S*)-4C3HPG, (*S*)-4CPG, (1*S*,3*S*)-ACPD). All drugs were obtained from Tocris Cookson, St. Louis, MO, USA, except D-AP5 (Research Biochemicals Int., Natick, MA, USA).

Results

Fig. 1A-D illustrate formalin nociceptive scores for rats pretreated with 100 or 500 nmol of L-AP4, (+)-MCPG, (*S*)-4CPG, and (*S*)-4C3HPG, respectively, in comparison to vehicle animals.

Analysis of variance (ANOVA) for experiment 1 revealed a significant main effect of Time ($F(9,423) = 61.52$, $P<0.001$) and Drug x Time interaction ($F(72,423) = 1.67$, $P<0.001$). The main effect of Drug ($F(8,47) = 1.52$, $P>0.05$), however, was not statistically significant. Post hoc analyses (Dunnett's *t*-test) for the Drug x Time interaction indicated that the 100 nmol dose of L-AP4 significantly decreased scores between 10-15 and 40-50 min post formalin injection, and animals treated with the higher dose (500 nmol) showed reduced responses between 15-20, 30-35 and 40-45 min compared to vehicle controls. The lower 100 nmol dose of (*S*)-4CPG also reduced nociceptive scores between 10-20 and 40-50 min of the test, although 500 nmol of the compound decreased nociception in the last 5 min only. In addition, rats treated with 100 nmol of (*S*)-4C3HPG had lower scores only in the last 5 min compared to vehicle animals, while administration of 500 nmol of the drug decreased nociceptive scores during the last 5 min of the test. (*S*)-4C3HPG (500 nmol), however, produced an unexpected increase in nociceptive responses between 15-20 min following the formalin injection. In contrast, (+)-MCPG did not significantly influence formalin-induced nociception.

Fig. 2 illustrates the dose-response curves for pretreatment with the agonists, (1*S*,3*S*)-ACPD and (*RS*)-DHPG in the early (A) and late (B) phases of the formalin test. The graphs also include data for the single dose of DCG-IV (0.2 nmol).

Although there was no significant main effect of Drug ($F(6,37) = 1.91$, $P>0.05$) in the early phase, ANOVA revealed significant main effects of Drug ($F(7,44) = 5.15$, $P<0.001$) and Time ($F(8,352) = 11.17$, $P<0.001$) in the late phase. The Drug x Time interaction ($F(56,352) = 0.98$, $P>0.05$), however, was not significant. Dunnett's *t*-tests for the main effect of Drug indicated that administration of 20 and 2, but not 0.2 nmol of (1*S*,3*S*)-ACPD and (*RS*)-DHPG significantly enhanced formalin-induced nociception compared to the vehicle control group. Also, rats treated with DCG-IV (0.2 nmol) did not significantly differ in nociception from vehicle control rats.

Fig. 3A illustrates formalin nociceptive responses for animals that received vehicle or an antagonist treatment ((+)-MCPG or D-AP5) prior to an injection of (1*S*,3*S*)-ACPD. Fig. 3B illustrates formalin nociceptive scores for rats injected with vehicle or an antagonist treatment ((*S*)-4C3HPG or D-AP5) prior to an injection of (*RS*)-DHPG. In experiment 3, ANOVA revealed significant main effects of Drug ($F(6,43) = 7.47$, $P<0.001$) and Time ($F(9,387) = 11.59$, $P<0.001$), but no significant Drug x Time interaction ($F(54,387) = 1.29$, $P>0.05$). Dunnett's post hoc tests for the main effect of Drug indicated that, although the (1*S*,3*S*)-ACPD- and (*RS*)-DHPG-treated rats showed enhanced nociceptive responses, the rats given agonist treatments preceded by mGluR and NMDA antagonist pretreatment did not differ significantly from vehicle.

Discussion

Although it has been well established that NMDA receptors are involved in formalin-induced nociception (Haley et al. 1990; Coderre and Melzack 1992; Näsström et al. 1992; Yamamoto and Yaksh 1992; Kristensen et al. 1994), little is known about the role of mGluRs. The present study examined the contribution of mGluRs in the formalin

model, since i.t. administration of the mGluR agonist, *trans*-ACPD, has been found to facilitate formalin-induced nociception (Coderre and Melzack 1992), and iontophoretic application of mGluR antagonists inhibit thalamic (Eaton et al. 1993) and dorsal horn (Young et al. 1994, 1995) neuronal excitation induced by noxious stimulation. In addition, the mGluR antagonist, L-AP3, attenuates spinal cord neuronal hyperexcitability associated with acute inflammation of the knee joint in the rat (Neugebauer et al. 1994). Furthermore, the involvement of mGluRs has been demonstrated in various animal models of plasticity (Aronica et al. 1991; Izumi et al. 1991; Daniel et al. 1992; Bortolotto and Collingridge 1993; Iversen et al. 1994; Riedel et al. 1994).

The most significant finding obtained in the first experiment was that the mGluR4/mGluR6-mGluR8 agonist, L-AP4, significantly decreased responses both early and late in the second phase of the formalin test. Although somewhat limited, the consistent antinociceptive effects of L-AP4 compare with its ability to induce presynaptic-mediated depression of CNS responses (Baskys and Malenka 1991; Jane et al. 1994; Kemp et al. 1994; Jane et al. 1995) and inhibit $Ca^{2+}$-dependent glutamate release in synaptosomes (Vázquez et al. 1995). These effects of L-AP4 may influence formalin-induced nociception, since s.c. formalin elicits both an increase in glutamate release (Skilling et al. 1988), and a biphasic increase in dorsal horn neuron activity that parallels the behavioural response (Dickenson and Sullivan 1987b).

In contrast to the consistent antinociceptive effects of the agonist, L-AP4, the present study showed that administration of several mGluR antagonists produced either no effect, or minor inconsistent reductions, in nociceptive scores in the formalin test. Although antinociceptive effects of (+)-MCPG have been demonstrated in other models

(Eaton et al. 1993; Young et al. 1994), the non-selective mGluR antagonist did not influence nociceptive scores in this study. Evidence indicates that (+)-MCPG is an antagonist at several mGluR subtypes (Pook et al. 1993; Hayashi et al. 1994; Kemp et al. 1994), therefore, it is possible that a potential attenuation of the formalin response via the mGluR1/mGluR5 antagonism may have been negated by excitatory effects produced by antagonism of L-AP4-type receptors negatively linked to cAMP.

Both (*S*)-4CPG and (*S*)-4C3HPG, which are potent mGluR1/mGluR5 antagonists and mGluR2/mGluR3 agonists (Hayashi et al. 1994), decreased nociceptive responses later in the second phase of the formalin test. Also, (*S*)-4CPG significantly reduced formalin scores early in the second phase of the test. Interestingly, a greater degree of antinociception was produced by the lower dose of (*S*)-4CPG, with the higher dose producing little antinociceptive activity. Since (*S*)-4CPG is a relatively selective mGluR1/mGluR5 antagonist with a lower potency at mGluR2/mGluR3 (Hayashi et al. 1994), we propose that it may produce antinociception at low doses by antagonizing mGluR1/mGluR5, but that this effect is negated by mGluR2/mGluR3 agonism as the dose is increased. This hypothesis is further supported by the observation that (*S*)-4C3HPG, which is a less selective mGluR1/mGluR5 antagonist with relatively greater mGluR2/mGluR3 agonism than (*S*)-4CPG (Hayashi, et al. 1994), is a less effective antinociceptive agent even at low doses.

The second series of experiments demonstrated that administration of the agonists, (*RS*)-DHPG and (1*S*,3*S*)-ACPD dose-dependently enhanced nociceptive scores. It is not surprising that (*RS*)-DHPG increased nociceptive scores since administration of the agonist has been shown to produce excitatory effects in various neuronal systems

(Bond and Lodge 1995; Tizzano et al. 1995). Furthermore, (*RS*)-DHPG has been shown to increase phosphoinositide hydrolysis (Schoepp et al. 1994), which stimulates the production of the intracellular messengers, inositol triphosphate ($IP_3$) and diacylglycerol (DAG), and DAG, in turn, activates protein kinase C (PKC). PKC has been found to potentiate NMDA-induced currents (Chen and Huang 1992), and PKC activators increase the release of glutamate and aspartate in the spinal cord (Gerber et al. 1989). Furthermore, formalin injury has been shown to increase translocation and activation of PKC in dorsal horn neurons, and i.t. administration of PKC inhibitors attenuate formalin-induced nociception (Yashpal et al. 1995).

The effects of (1*S*,3*S*)-ACPD in the formalin test and its ability to produce spontaneous nociceptive behaviours at higher doses was unexpected, since the agonist has been shown to depress excitation in the rat spinal cord (Pook et al. 1992; Jane et al. 1994; Kemp et al. 1994). However, the hyperalgesic actions of (1*S*,3*S*)-ACPD are consistent with the poor antinociceptive activity of (*S*)-4C3HPG and (*S*)-4CPG, which as we previously suggested, may be related to their mGluR2/mGluR3 agonism. Alternatively, the observed excitatory actions of (1*S*,3*S*)-ACPD may be due to a non-selective effect at another mGluR subtype, since (1*S*,3*S*)-ACPD has been found to stimulate phosphoinositide hydrolysis in the guinea pig cerebral cortex (Cartmell et al. 1993; Jones and Roberts 1993).

Only one dose of DCG-IV was used in the study, since administration of higher doses produced side effects, such as freezing, occasional jumping and vocalizing, as well as other spontaneous nociceptive behaviours. Administration of the lowest dose also produced some nociceptive side effects (i.e. freezing) that may have interfered with the

formalin response, which would explain why DCG-IV-treated rats had slightly lower scores in the second phase compared to the other groups. Given that (1*S*,3*S*)-ACPD produced hyperalgesia in the formalin test and DCG-IV produced spontaneous nociceptive behaviours, it is possible that the spinal activation of mGluR2/mGluR3 produces unanticipated pro-nociceptive effects. This effect may be due to a presynaptically-mediated reduction in inhibition due to modulation of the GABAergic system, which has been shown in the thalamus following application of the mGluR2/mGluR3 agonist, (2*S*,3*S*,4*S*)α-(carboxycyclopropyl)-glycine (L-CCG-I; Salt and Eaton 1995). However, DCG-IV is also an agonist at NMDA receptors (Hayashi et al. 1993; Ishida et al. 1993), therefore, since i.t. administration of the agonist, NMDA, produces spontaneous nociceptive behaviours (Raigorodsky and Urca 1987; Aanonsen and Wilcox 1989; Kolhekar et al. 1993; Kolhekar and Gebhart 1994; Zochodne et al. 1994) it is possible that the DCG-IV-related side effects are due to this action.

The third series of experiments showed that when the mGluR antagonists, (*S*)-4C3HPG or (+)-MCPG, are injected prior to the agonists, (*RS*)-DHPG or (1*S*,3*S*)-ACPD, respectively, the facilitation of formalin-induced nociception is attenuated. These data provide evidence that the agonist-induced enhancement of formalin scores is mediated, at least in part, by mGluRs. In addition, the NMDA antagonist, D-AP5, reduced the facilitation of nociceptive scores by (*RS*)-DHPG or (1*S*,3*S*)-ACPD. These data are consistent with the demonstration that mGluRs influence the iGluR-mediated responses. For example, investigators have reported that perfusion with *trans*-ACPD, (1*S*,3*R*)-ACPD or (1*R*,3*S*)-ACPD potentiates responses elicited by AMPA or NMDA (Cerne and Randic

1992) and application of (1*S*,3*R*)-ACPD enhances AMPA- or NMDA-mediated increases in intracellular $Ca^{2+}$ levels (Bleakman et al. 1992) in rat dorsal horn neurons. Also, Bond and Lodge (1995) demonstrated that microelectrophoresis of mGluR1/mGluR5 ((*RS*)-DHPG, (*S*)-homoquisqualate, quisqualate) or mGluR2/mGluR3 (L-CCG-I, (1*S*,3*S*)-ACPD), but not mGluR4/mGluR6-mGluR8 (L-AP4, L-serine-*O*-phosphate (L-SOP)) agonists, enhance NMDA- and AMPA-mediated increases in dorsal horn neuronal excitability. In addition, these investigators found that (1*S*,3*R*)-ACPD-mediated responses are reduced by (+)-MCPG and (*S*)-4C3HPG, but not (*S*)-4CPG. In contrast, Baskys and Malenka (1991) showed that iontophoretic application of *trans*-ACPD or L-AP4 depresses NMDA- and AMPA-mediated responses in rat hippocampal slices. It is also of interest that modulation of NMDA or AMPA receptor activity via mGluRs may be involved in models of plasticity, such as long term potentiation (LTP; Bashir et al. 1993; Sergueeva et al. 1993) and neurotoxicity (Koh et al. 1991; Bruno et al. 1995).

In conclusion, although mGluRs play a minor role in formalin-induced nociception, as demonstrated by only a partial reduction in nociceptive scores with several mGluR compounds, the present study provides additional evidence for a possible pro-nociceptive effect of mGluR2/mGluR3 agonists, and an excitatory interaction between NMDA and mGluRs. The failure of (*S*)-4CPG and 4C3HPG to dramatically reduce formalin-induced nociception, combined with the pro-nociceptive effects of (1*S*,3*S*)-ACPD and DCG-IV, suggest that agonist activity at spinal mGluR2/mGluR3 may produce unexpected excitatory effects. In contrast, the consistent antinociceptive effects of L-AP4 suggests that agonist activity at presynaptic L-AP4-type receptors (mGluR4/mGluR6-mGluR8) produces a significant inhibitory influence. It is expected

that activation of L-AP4 receptors negatively linked to cAMP may produce a decrease in glutamate release and a consequent reduction of postsynaptic NMDA receptor activity. Finally, we propose that since (*RS*)-DHPG-induced facilitation of nociceptive scores is reduced by the NMDA antagonist, D-AP5, it is possible that stimulation of phosphoinositide hydrolysis via mGluR1/mGluR5 activation leads to increases in mGluR-mediated glutamate or aspartate release, which may consequently enhance postsynaptic NMDA receptor activity in the dorsal horn. However, the (*RS*)-DHPG-induced increase in formalin scores during the second phase may also reflect a direct enhancement of postsynaptic NMDA receptor activity.

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References

Aanonsen, L.M. and Wilcox, G.L., Muscimol, gamma-aminobutyric acidA receptors and excitatory amino acids in the mouse spinal cord, J. Pharmacol. Exp. Ther. 248 (1989) 1034-1038.

Abe, T., Sugihara, H., Nawa, H., Shigemoto, R., Mizuno, N. and Nakanishi, S., Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/$Ca^{2+}$ signal transduction, J. Biol. Chem. 267 (1992) 13361-13368.

Aramori I. and Nakanishi S., Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells, Neuron 8 (1992) 757-765.

Aronica E., Frey U., Wagner M., Schroeder H., Krug M., Ruthrich H., Catania MV., Nicoletti F. and Reymann K.G., Enhanced sensitivity of "metabotropic" glutamate receptors after induction of long-term potentiation in rat hippocampus, J. Neurochem. 57 (1991) 376-383.

Bashir Z.I., Bortolotto Z.A., Davies, C.H., Berretta N., Irving A.J., Seal A.J., Henley J.M., Jane D.E., Watkins J.C. and Collingridge G.L., Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors, Nature 363 (1993) 347-350.

Baskys A. and Malenka R.C., Agonists at metabotropic glutamate receptors presynaptically inhibit EPSCs in neonatal rat hippocampus, J. Physiol. 444 (1991) 687-701.

Bleakman D., Rusin K.I., Chard P.S., Glaum S.R. and Miller R.J., Metabotropic glutamate receptors potentiate ionotropic glutamate responses in the rat dorsal horn, Mol Pharmacol 42 (1992) 192-196.

Bond, A. and Lodge D., Pharmacology of metabotropic glutamate receptor-mediated enhancement of responses to excitatory and inhibitory amino acids on rat spinal neurones *in vivo*, Neuropharmacol. 34 (1995)1015-1023.

Bortolotto ZA. and Collingridge G.L., Characterization of LTP induced by the activation of glutamate metabotropic receptors in area CA1 of the hippocampus, Neuropharmacol. 32 (1993) 1-9.

Bruno V., Copani A., Knöpfel T., Kuhn R., Casabona G., Albani P.D. Condorelli D.F. and Nicoletti F., Activation of metabotropic glutamate receptors coupled to inositol

phospholipid hydrolysis amplifies NMDA-induced neuronal degeneration in cultured cortical cells, Neuropharmacol. 34 (1995) 1089-1098.

Cartmell J., Curtis A.R., Kemp J.A., Kendall D.A. and Alexander S.P., Subtypes of metabotropic excitatory amino acid receptor distinguished by stereoisomers of the rigid glutamate analogue, 1-aminocyclopentane-1,3-dicarboxylate, Neurosci. Lett. 153 (1993) 107-110.

Cerne R. and Randic M., Modulation of AMPA and NMDA responses in rat spinal dorsal horn neurons by trans-1-aminocyclopentante-1,3-dicarboxylic acid, Neurosci. Lett. 144 (1992) 180-184.

Chen L. and Huang L.-Y.M., Protein kinase C reduces $Mg^{2+}$ block of NMDA-receptor channels as a mechanism of modulation, Nature 356 (1992) 521-523.

Coderre T.J., Fundytus M.E., McKenna J.E., Dalal S. and Melzack R., The formalin test: a validation of the weighted scores method of behavioural pain rating, Pain 54 (1993) 43-50.

Coderre T.J. and Melzack R., The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury, J. Neurosci. 12 (1992) 3665-3670.

Coderre T.J., Vaccarino A.L. and Melzack R., Central nervous system plasticity in tonic pain response to subcutaneous formalin injection, Brain Res. 535 (1990) 155-158.

Daniel H., Hemart N., Jaillard D. and Crepel F., Coactivation of metabotropic glutamate receptors and of voltage-gated calcium channels induces long-term depression in cerebellar Purkinje cells *in vitro*, Brain Res 90(1992) 327-331.

Davies S.N. and Lodge D., Evidence for involvement of N-methylaspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat, Brain Res. 424 (1987) 402-406.

Dickenson A.H. and Sullivan A.F., Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation, Neuropharmacol. 26 (1987a) 1235-1238.

Dickenson A.H. and Sullivan A.F., Peripheral origins and central modulation of subcutaneous formalin-induced sensitivity of rat dorsal horn neurones, Neurosci. Lett. 83 (1987b) 207-211.

Dickenson A.H. and Sullivan A.F., Subcutaneous formalin-induced activity of dorsal horn neurons in the rat: differential response to an intrathecal opiate administered pre or post formalin, Pain 30 (1987c) 349-360.

Dougherty P.M., Palecek J., Palecková V., Sorkin L.S. and Willis, W.D. The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli, J. Neurosci. 12 (1992) 3025-3041.

Dubuisson D. and Dennis S.G., The formalin test: a quantitative study of the analgesic effect of morphine, meperidine, and brain stem stimulation in rats and cats, Pain 4 (1977) 161-174.

Eaton S.A., Jane D.E., Jones L.St.J., Porter R.H.P., Pook P.C.-K., Sunter D.C., Udvarhelyi P.M., Roberts P.J., Salt T.E. and Watkins J.C. Competitive antagonism at

metabotropic glutamate receptors by (*S*)-4-carboxyphenylglycine and (*RS*)-α-methyl-4-carboxyphenglycine, Eur. J. Pharmacol. 244 (1993) 195-197.

Gerber G., Kangrga I., Ryu P.D., Larew J.S.A. and Randic M., Multiple effects of phorbol esters in the rat spinal dorsal horn, J. Neurosci. 9 (1989) 3606-3617.

Gerber G. and Randic M., Participation of excitatory amino acid receptors in the slow excitatory synaptic transmission in the rat spinal cord *in vitro*, Neurosci. Lett. 106 (1989) 220-228.

Haley J.E., Sullivan A.F. and Dickenson, A.H., Evidence for spinal *N*-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat, Brain Res. 518 (1990) 218-226.

Hayashi Y., Momiyama A., Takahashi T., Ohishi H., Ogawa-Meguro R., Shigemoto R., Mizuno N. and Nakanishi S., Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb, Nature 366 (1993) 687-690.

Hayashi Y., Sekiyama N., Nakanishi S., Jane D.E., Sunter, D.C., Birse E.F., Udvarhelyi P.M. and Watkins J.C., Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes, J. Neurosci. 14 (1994) 3370-3377.

Hunter J.C. and Singh L., Role of excitatory amino acid receptors in the mediation of the nociceptive response to formalin in the rat, Neurosci. Lett. 174 (1994) 217-221.

Ishida M., Saitoh T., Shimamoto K., Ohfune Y. and Shinozaki H., A novel metabotropic glutamate receptor agonist: marked depression of monosynaptic excitation in the newborn rat isolated spinal cord, Br. J. Pharmacol. 109 (1993) 1169-1177.

Ito I., Kohda A., Tanabe S., Hirose E., Hayashi M., Mitsunaga S. and Sugiyama H., 3,5-dihydroxyphenylglycine: a potent agonist of metabotropic glutamate receptors, Neuropharmacol. Neurotoxicol. 3 (1992) 1013-1016.

Iversen L., Mulvihill E., Haldeman B., Diemer N.H., Kaiser F., Sheardown M. and Kristensen P., Changes in metabotropic glutamate receptor mRNA levels following global ischemia: increase of a putative presynaptic subtype (mGluR4) in highly vulnerable rat brain areas, J. Neurochem. 63 (1994) 625-633.

Izumi Y., Clifford D.B. and Zorumski C., 2-amino-3-phosphonopropionate blocks the induction and maintenance of long-term potentiation in rat hippocampal slices, Neurosci. Lett. 12 (1991) 187-190.

Jane D.E., Jones P.L.St.J., Pook P.C.-K., Tse H.-W. and Watkins J.C., Actions of two new antagonists showing selectivity for different sub-types of metabotropic glutamate receptor in the neonatal rat spinal cord, Br. J. Pharmacol. 112 (1994) 809-816.

Jane D.E., Pittaway K., Sunter D.C., Thomas K.N. and Watkins J.C., New phenylglycine derivatives with potent and selective antagonist activity at presynaptic glutamate receptors in neonatal rat spinal cord, Neuropharmacol. 34 (1995) 851-856.

Jones P.G. and Roberts P.J., Stimulation of phosphoinositide hydrolysis in the guinea pig cortex by isomers of 1-aminocyclo-pentane-1,3-dicarboxylate (ACPD), Mol. Neuropharmacol. 3 (1993) 17.

Kemp M., Roberts P., Pook P., Jane D., Jones A., Jones P., Sunter D., Advarhelyi P. and Watkins J., Antagonism of presynaptically mediated depressant responses and cyclic AMP-coupled metabotropic glutamate receptors, Eur. J. Pharmacol. 266 (1994) 187-192.

King A.E., Thompson S.W.N., Urban L. and Woolf C.J., An intracellular analysis of amino acid induced excitations of deep dorsal horn neurones in the rat spinal cord slice, Neurosci. Lett. 89 (1988) 286-292.

Koh J.-Y., Palmer E., and Cotman C.W., Activation of the metabotropic glutamate receptor attenuates *N*-methyl-D-aspartate neurotoxicity in cortical cultures, Proc. Natl. Acad. Sci. USA 88 (1991) 9431-9435.

Kolhekar R. and Gebhart G.F., NMDA and quisqualate modulation of visceral nociception in the rat, Brain Res. 651 (1994) 215-226.

Kolhekar R., Meller S.T. and Gebhart G.F., Characterization of the role of spinal *N*-methyl-D-aspartate receptors in thermal nociception in the rat, Neurosci. 57 (1993) 385-395.

Kristensen J.D., Karlsten R., Gordh T., and Berge O.-G., The NMDA antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) has antinociceptive effect after intrathecal injection in the rat, Pain 56 (1994) 59-67.

Masu M., Tanabe Y., Ysuchida K., Shigemoto R., and Nakanishi S., Sequence and expression of metabotropic glutamate receptor, Nature 349 (1991) 760-765.

Mendell L.M., Physiological properties of unmyelinated fiber projections to the spinal cord, Exp. Neurol. 16 (1966) 316-332.

Murray C.W., Cowan A. and Larson A.A., Neurokinin and NMDA antagonists (but not a kainic acid antagonist) are antinociceptive in the mouse formalin model, Pain, 44 (1991) 179-185.

Nakajima Y., Iwakabe H., Akawaza C., Nawa H., Shigemoto R., Mizuno N. and Nakanishi S., Molecular characterization of a novel retinal metabotropic glutamate

receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate, J. Biol. Chem. 268 (1993) 11868-11873.

Nakanishi S., Molecular diversity of glutamate receptors and implications for brain function, Science 258 (1992) 597-603.

Näsström J., Karlsson U. and Post C., Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice, Eur. J. Pharmacol. 212 (1992) 21-29.

Neugebauer V., Lücke T. and Schaible H.G., Requirement of metabotropic glutamate receptors for the generation of inflammation-evoked hyperexcitability in rat spinal cord neurons, Eur. J. Neurosci. 6 (1994) 1179-1186.

Ohfune Y., Shimamoto K., Ishida M. and Shinozaki H., Synthesis of L-2-(2,3-dicarboxycyclopropyl)glycine: novel conformationally restricted glutamate analog, Bioorg. Med. Chem. Lett. 3 (1993) 15-18.

Okamoto N., Hori S., Akazawa C., Hayashi Y., Shigemoto R., Mizuno N. and Nakanishi S. Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction, J. Biol. Chem .269 (1994) 1231-1236.

Pook P.C.-K., Sunter D.C., Udvarhelvi P.M. and Watkins J.C., Evidence for presynaptic depression of monosynaptic excitation in neonatal rat motoneurones by (1*S*,3*S*)- and (1*S*,3*R*)-ACPD, Exp. Physiol. 77 (1992) 529.

Pook P.C.-K., Birse E.F., Jane D.E., Jones A.W., Jones P.L.St.J., Jones K.N., Mewett K.N., Sunter, D.C., Udvarhelyi, D.C., Wharton, B. and Watkins J.C., Differential

actions of the metabotropic glutamate receptor antagonists 4C-PG and αM4C-PG at L-AP4-like receptors in neonatal rat spinal cord, Br. J. Pharmacol. 108 (1993) 87P.

Raigorodsky G. and Urca G., Intrathecal *N*-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems, Brain Res. 422 (1987) 158-162.

Riedel G., Wetzel W. and Reymann K.G., (*R*,*S*)-α-methyl-4-carboxyphenylglycine ((+)-MCPG) blocks spatial learning in rats and long-term potentiation in the dentate gyrus *in vivo*, Neurosci. Lett. 167 (1994) 141-144.

Salt T.E. and Eaton S.A., Distinct presynaptic metabotropic receptors for L-AP4 and CCGI on gabaergic terminals: pharmacological evidence using novel ∝-methyl derivative mGluR antagonists, MAP4 and MCCG, in the rat thalamus, *in vivo*, Neurosci. 65 (1995) 5-13.

Schoepp D.D., Novel functions for subtypes of metabotropic glutamate receptors, Neurochem. Int. 24 (1994) 439-449.

Schoepp D.D., Goldsworthy J., Johnson B.G., Salhoff C.R., and Baker S.R., 3,5-dihydroxyphenylglycine is a highly selective agonist for phosphoinositide-linked metabotropic glutamate receptors in the rat hippocampus, J. Neurochem. 63 (1994) 769-772.

Schouenbourg J. and Dickenson A.H., The effects of a distant noxious stimulation on A and C fibre evoked flexion reflexes and neuronal activity in the dorsal horn of the rat, Brain Res. 328 (1985) 23-32.

Sergueeva O.A., Nikolai B., Fedorov B. and Reymann K.G., An antagonist of glutamate metabotropic receptors, (*RS*)-α-methyl-4-carboxyphenylglycine, prevents the LTP-

related increase in postsynaptic AMPA sensitivity in hippocampal slices, Neuropharmacol. 32 (1993) 933-935.

Skilling S.R., Smullin D.H., Beitz A.J. and Larson A.A., Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation, J. Neurochem. 51 (1988) 127-132.

Tanabe Y., Nomura A., Masu M., Shigemoto R., Mizuno N. and Nakanishi S., Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4, J. Neurosci. 13 (1993) 1372-1378.

Thomsen C., Kristensen P., Mulvihill E., Haldeman B. and Suzdak S.D., L-2-amino-4-phosphonobutyrate (L-AP4) is an agonist at the type IV metabotropic glutamate receptor which is negatively coupled to adenylate cyclase, Eur. J. Pharmacol. 227 (1992) 361-362.

Thomsen C. and Suzdak P.D., 4-carboxy-3-hydroxyphenylglycine, an antagonist at type I metabotropic glutamate receptors, Eur. J. Pharmacol. 245 (1993) 299-301.

Tizzano J.P., Griffey K.I. and Schoepp D.D. Induction or protection of limbic seizures in mice by mGluR subtype selective agonists, Neuropharmacol. 34 (1995) 1063-1067.

Vázquez E., Budd D.C., Herrero I., Nicholls D.G. and Sánchez-Prieto J., Co-existence and interaction between facilitatory and inhibitory metabotropic glutamate receptors and the inhibitory adensosine A1 receptor in cerebrocortical nerve terminals, Neuropharmacol., 34 (1995) 919-927.

Watkins J. and Collingridge G., Phenylglycine derivatives as antagonists of metabotropic glutamate receptors, TiPS 15 (1994) 333-342.

Woolf C.J. and Thompson S.W.N., The induction and maintenance of central sensitization is dependent on N-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury hypersensitivity states, Pain 44 (1991) 293-299.

Yamamoto T. and Yaksh T.L., Comparison of the antinociceptive effects of pre- and posttreatment with intrathecal morphine and MK801, an NMDA antagonist, on the formalin test in the rat, Anesthesiol. 77 (1992) 757-763.

Yashpal K., Pitcher G.M., Parent A., Quirion R. and Coderre T.J., Noxious thermal and chemical stimulation induce increases in $^{3}$H-phorbol 12,13-dibutyrate binding in spinal cord dorsal horn as well as persistent pain and hyperalgesia, which is reduced by inhibition of protein kinase C, J. Neurosci. 15 (1995) 3263-3272.

Young M.R., Fleetwood-Walker S.M., Mitchell R. and Dickenson T., The involvement of metabotropic glutamate receptors and their intracellular signalling pathways in sustained nociceptive transmission in rat dorsal horn neurons, Neuropharmacol. 34 (1995) 1033-1041.

Young M.R., Fleetwood-Walker M., Mitchell, R. and Munro F.E., Evidence for a role of metabotropic glutamate receptors in sustained nociceptive inputs to rat dorsal horn neurons, Neuropharmacol. 33 (1994) 141-144.

Zieglgänsberger W. and Herz A., Changes of cutaneous receptive fields of spino-cervical-tract neurones and other dorsal horn neurones by microelectrophoretically administered amino acids, Exp. Brain Res. 13 (1971) 111-126.

Zochodne D.W., Murray M., Nag S. and Riopelle R.J., A segmental chronic pain syndrome in rats associated with intrathecal infusion of NMDA: evidence for selective action in the dorsal horn, Can. J. Neurol. Sci. 21 (1994) 24-28.

TABLE I

ACTION OF mGluR COMPOUNDS

| Compound | Mechanism of action |
|---|---|
| (*S*)-4CPG | mGluR1/5 antagonist<br>mGluR2/3 agonist |
| (*S*)-4C3HPG | mGluR1/5 antagonist<br>mGluR2/3 agonist |
| (+)-MCPG | nonselective mGluR antagonist |
| L-AP4 | mGluR4/6-8 agonist |
| (1*S*,3*S*)-ACPD | mGluR2/3 agonist |
| (*RS*)-DHPG | mGluR1/5 agonist |
| DCG-IV | mGluR2/3 agonist<br>NMDA agonist |

Figure Captions

Fig. 1. Nociceptive scores (±SEM) of rats treated with vehicle, 100 or 500 nmol of mGluR compounds (A, L-AP4; B, (+)-MCPG; C, (*S*)-4CPG; and D, (*S*)-4C3HPG) prior to 50 µl of 2.5% formalin. N = 6 for each group, except for 100 nmol of L-AP4 (N = 8). The asterisks indicate significant differences between the experimental and vehicle conditions (** $P<0.01$, * $P<0.05$, Dunnett's *t*-test).

Fig. 2. Nociceptive scores (±SEM) for early (A: 0-5 min) and late (B: 5-50 min) phases following 30 µl of 1.0% formalin in rats pre-treated with vehicle; 0.2 nmol of DCG-IV; 0.2, 2.0 or 20 nmol of (1*S*,3*S*)-ACPD or (*RS*)-DHPG. N = 6 for each group, except for vehicle and DCG-IV (N = 8). The asterisks indicate significant differences between the experimental and vehicle conditions (** $P<0.01$, * $P<0.05$, Dunnett's t test).

Fig. 3. Nociceptive scores (±SEM) in response to 30 µl of 1.0% formalin for the following drug conditions: A, (1*S*,3*S*)-ACPD (N = 10), (+)-MCPG + (1*S*,3*S*)-ACPD (N = 6), D-AP5 + (1*S*,3*S*)-ACPD (N = 7) or vehicle (N = 8); B, (*RS*)-DHPG (N = 6), (*S*)-4C3HPG + (*RS*)-DHPG (N = 7), D-AP5 + (*RS*)-DHPG (N = 6) or vehicle. Post hoc analyses (Dunnett's t) of the main effect of Drug indicate that there were significantly greater nociceptive scores in the groups that received (1*S*,3*S*)-ACPD or (*RS*)-DHPG, but not in groups that received prior treatment with (+)-MCPG, (*S*)-4C3HPG or D-AP5.

Fig. 1

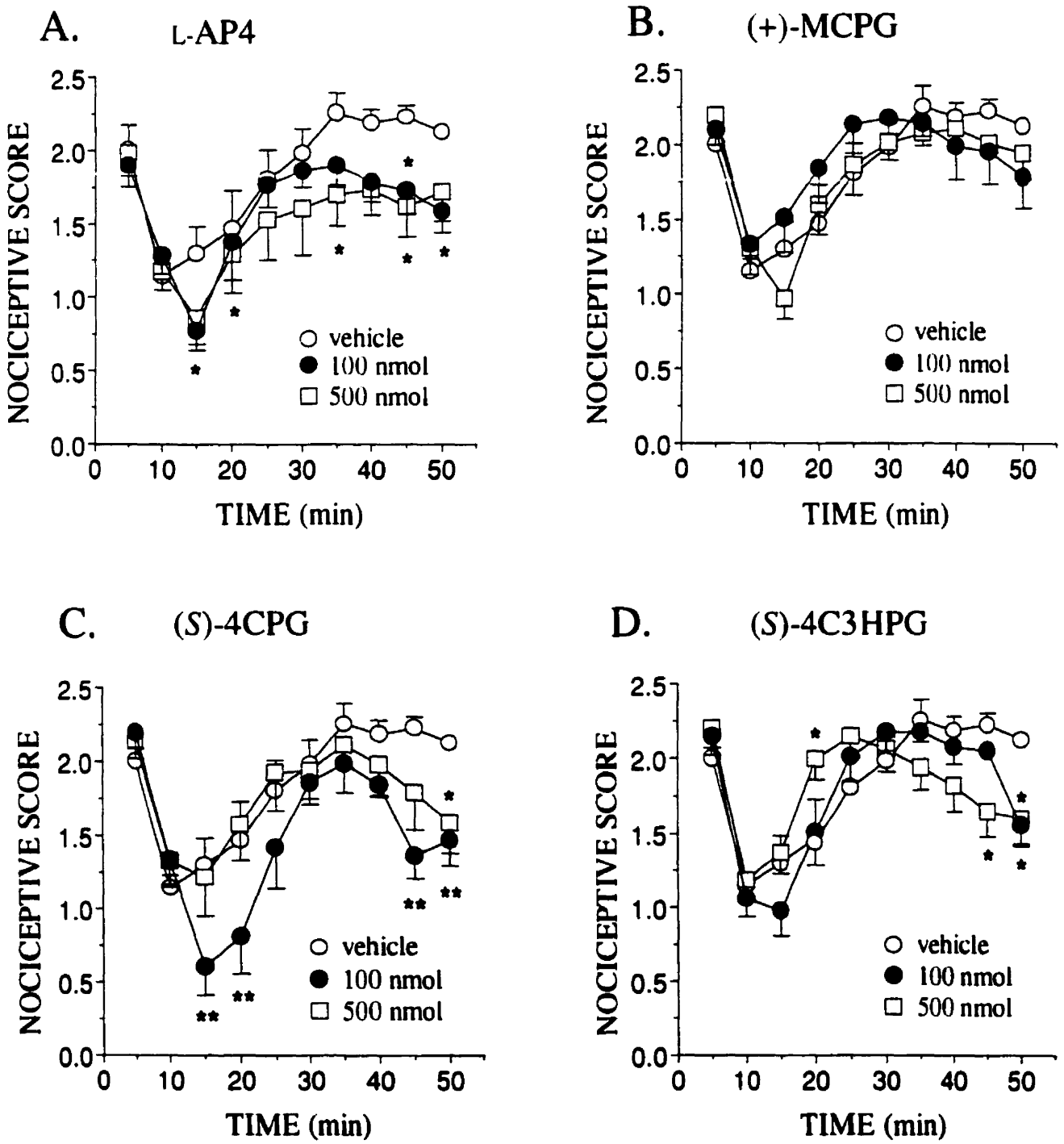

A.
L-AP4
B.
(+)-MCPG
C.
(S)-4CPG
D.
(S)-4C3HPG
NOCICEPTIVE SCORE
TIME (min)
vehicle
100 nmol
500 nmol
0.0
0.5
1.0
1.5
2.0
2.5
0
10
20
30
40
50

Fig. 2

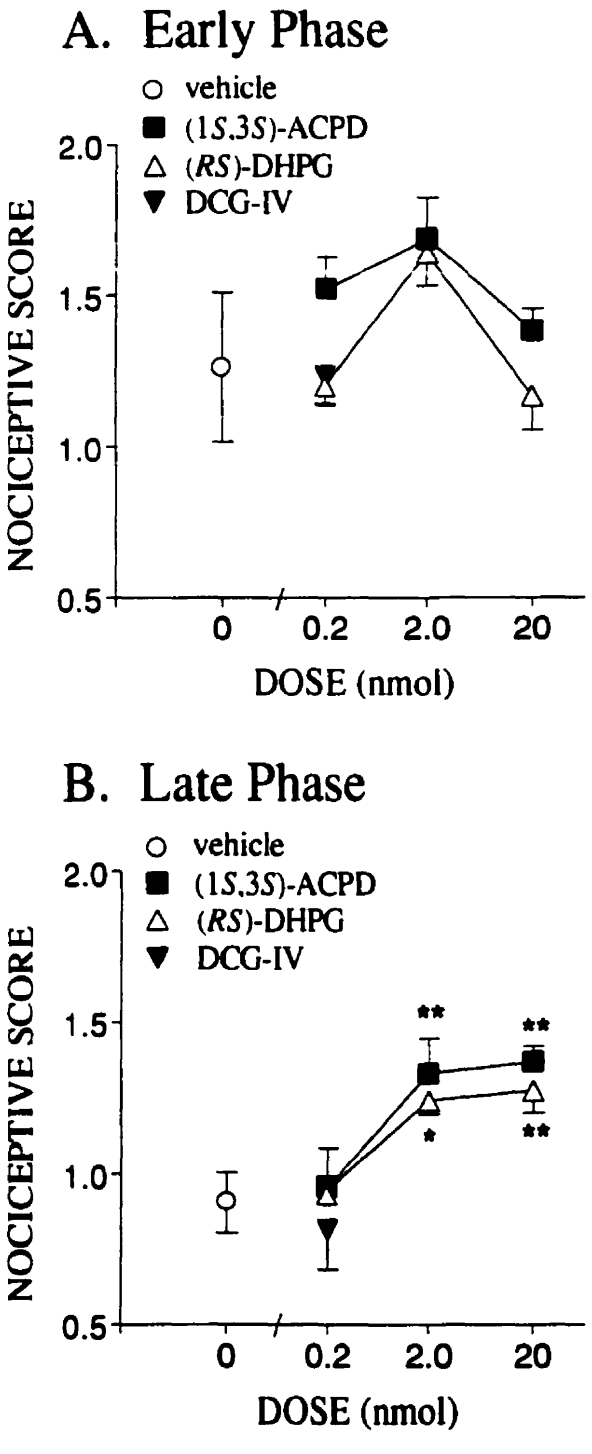
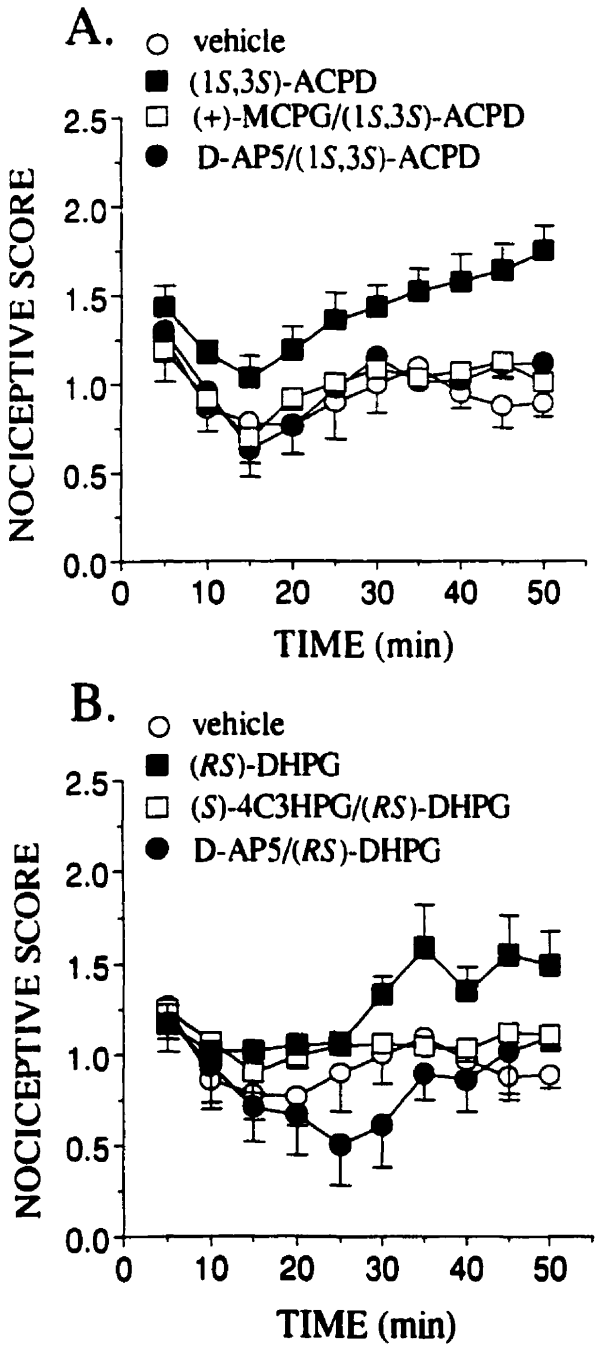

A. Early Phase
vehicle
(1S,3S)-ACPD
(RS)-DHPG
DCG-IV
NOCICEPTIVE SCORE
2.0
1.5
1.0
0.5
0
0.2
2.0
20
DOSE (nmol)
B. Late Phase
vehicle
(1S,3S)-ACPD
(RS)-DHPG
DCG-IV
NOCICEPTIVE SCORE
2.0
1.5
1.0
0.5
**
**
*
**
0
0.2
2.0
20
DOSE (nmol)

Fig. 3


A.
vehicle
(1S,3S)-ACPD
(+)-MCPG/(1S,3S)-ACPD
D-AP5/(1S,3S)-ACPD
NOCICEPTIVE SCORE
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
TIME (min)
B.
vehicle
(RS)-DHPG
(S)-4C3HPG/(RS)-DHPG
D-AP5/(RS)-DHPG
NOCICEPTIVE SCORE
2.5
2.0
1.5
1.0
0.5
0.0
0
10
20
30
40
50
TIME (min)

# CHAPTER 3: mGluR AGONIST-INDUCED SPONTANEOUS NOCICEPTIVE BEHAVIOURS

Rationale for Experiments

While performing preliminary experiments for Study 1, I discovered that intrathecal administration of (1*S*,3*R*)-ACPD (the non-selective mGluR agonist), (*RS*)-DHPG (the selective group I mGluR agonist) or (1*S*,3*S*)-ACPD (the presumed group II mGluR agonist), at doses of ≥ 25 nmol, reliably produces spontaneous behaviours that appear to be nociceptive in nature (i.e. elevation or licking of the hindpaws or tail). We presumed that the mGluR agonist-induced behaviours were nociceptive-related, since the same kinds of spontaneous behaviours are commonly observed in models of persistent and chronic pain.

The observation that spinal administration of mGluR agonists produces nociceptive behaviours was interesting, since it suggested that functional mGluRs are localized within certain spinal nociceptive pathways in the spinal cord, and that these mGluRs may play a role in the development of spontaneous nociceptive behaviours (SNBs) in models of pain. Therefore, we performed a series of experiments in order to fully characterize the mGluR agonist-induced SNBs in rats. Although we suspected that the (1*S*,3*R*)-ACPD- or (1*S*,3*S*)-ACPD-related behaviours were attributable to their activity at group I mGluRs, we couldn't be absolutely certain. Therefore, we performed a thorough investigation of the possible pro-nociceptive spinal mGluR subtype(s), by treating rats intrathecally with the following selective mGluR compounds, as well as the non-selective agonists (1S,3R)-ACPD and (1S,3S)-ACPD, and quantifying all mGluR agonist-related SNBs: (*RS*)-DHPG, *trans*-ADA (group I mGluR agonists), (2*R*,4*R*)-APDC (group II mGluR agonist) and L-AP4 (group III mGluR agonist).

The main findings from this study are the following:

1. Although intrathecal administration of (*RS*)-DHPG, (1*S*,3*R*)-ACPD or (1*S*,3*S*)-ACPD reliably produced SNBs, intrathecal administration with the selective group II mGluR agonist, (2*R*,4*R*)-APDC, or the selective group III mGluR agonist, L-AP4, did not, suggesting that (1*S*,3*R*)-ACPD- or (1*S*,3*S*)-ACPD-related SNBs are attributable to their activity at group I mGluRs.

2. The SNBs induced by intrathecal (*RS*)-DHPG were evident for a significantly longer duration (> 10 hrs) compared to those induced by an equivalent dose of (1*S*,3*R*)-ACPD or (1*S*,3*S*)-ACPD. These data suggest long-lasting nociceptive behaviours are produced only when spinal group I mGluRs are selectively activated, and suggest that these mGluRs may contribute to the development of nociceptive behaviours in models of chronic pain.

3. Intraperitoneal pretreatment with morphine significantly reduced the time that rats spent exhibiting the (*RS*)-DHPG-induced behaviours, which supports the notion that these behaviours are nociceptive in nature.

4. Intrathecal pretreatment with the NMDAR antagonist, D-AP5, significantly reduced the time that rats spent exhibiting the (*RS*)-DHPG-induced SNBs, suggesting that a group I mGluR/NMDAR interaction mediates the development of persistent nociception in rats.

It should be noted that, at the time, the pharmaceutical company, Tocris Cookson, listed *trans*-ADA as a selective mGluR5 agonist. Therefore, in the manuscript we concluded that mGluR1 mediates the mGluR agonist-induced SNBs, since intrathecal *trans*-ADA did not produce any of these behaviours. However, soon after the publication

of this manuscript, Manahan-Vaughan et al. 1996 demonstrated that *trans*-ADA also activates mGluR1, although with a lower potency than (*RS*)-DHPG (5.7 times lower). Considering the high dose of *trans*-ADA used in this study (up to 500 nmol), it is conceivable that the compound acted at mGluR1; therefore, it is unknown why no observable SNBs were observed in the animals. It should be noted, however, that Young and colleagues (1997) recently demonstrated that iontophoretic application of *trans*-ADA produces *minor* increases in dorsal horn neuronal activity relative to (*RS*)-DHPG, which is consistent with our behavioural data.

STUDY 2

**Fisher, K. & Coderre, T.J. (1996). Comparison of nociceptive effects produced by intrathecal administration of mGluR agonists.** NeuroReport, 7:2743-2747.

Comparison of nociceptive effects produced by intrathecal administration of mGluR agonists

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Running title: mGluR agonist-induced spontaneous nociception

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Abstract

The present study examined the mGluR subtypes involved in (1*S*,3*R*)-ACPD-induced spontaneous nociceptive behaviours (SNB) by administering i.t. the following selective agonists: (*RS*)-DHPG, *trans*-ADA (Group I; mGluR1/5 and mGluR5, respectively), (1*S*,3*S*)-ACPD, (2*R*,4*R*)-APDC (Group II), and L-AP4 (Group III). Results indicated that (*RS*)-DHPG administration induced SNB that were of significantly greater intensity and longer duration than those induced by an equal dose of (1*S*,3*R*)-ACPD. No other agonists produced SNB, except (1*S*,3*S*)-ACPD, which may be attributable to a non-selective action at mGluR1. Also, i.t. treatment with the mGluR antagonist, (+)-MCPG, or the NMDA antagonist, D-AP5, prior to (*RS*)-DHPG administration, dose-dependently reduced SNB. It is suggested that a possible interaction between NMDA and mGluR1 is a critical event in the maintenance of persistent pain.

Key words: Excitatory amino acids; mGluR; Metabotropic glutamate receptors; NMDA; Nociception

Introduction

Endogenous excitatory amino acids, such as glutamate and aspartate, activate the ionotropic glutamate receptors (iGluR), *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid (AMPA) and kainate, as well as the recently identified group of metabotropic glutamate receptors (mGluR1-8) that are linked to intracellular second messenger systems.

It is well known that iGluRs play a significant role in nociceptive processes. For example, intrathecal (i.t.) administration of NMDA antagonists attenuate nociceptive responses in the formalin test[1,2], and hyperalgesia associated with peripheral nerve ligation[3], as well as, with carrageenan[4]- and Freund's adjuvant[5]-induced inflammation. In addition, i.t. administration of NMDA to the lumbar spinal cord produces hyperalgesia and SNB in rats and mice[6,7].

Recent evidence suggests that mGluRs may also play a role in nociception. Specifically, research has demonstrated that iontophoretic application of the mGluR antagonist, L-2-amino-3-phosphonopropionic acid (L-AP3), attenuates rat dorsal horn neuronal activity associated with repeated mustard oil application[8] and knee joint inflammation[9]. In addition, i.t. administration of quisqualate, an agonist at both AMPA and mGluRs, produces mechanical[10] and visceral[11] hyperalgesia, as well as spontaneous nociceptive behaviours (SNB)[12]. The non-selective mGluR agonist, *trans*-1-aminocyclopentane-1,3-dicarboxylic acid (*trans*-ACPD) also produces SNB[13] and enhances formalin-induced nociception[1] when administered i.t.. Furthermore,

iontophoretic application of the agonist increases primate dorsal horn neuronal responses to innocuous and noxious mechanical stimuli[14].

An interaction between iGluRs and mGluRs has also been implicated in nociception. Specifically, i.t. coadministration of AMPA and *trans*-ACPD produces mechanical hyperalgesia[15]. In the formalin test, i.t. coadministration of NMDA and *trans*-ACPD, produces a greater increase in nociceptive scores than administration of either agonist alone[1], and i.t. pretreatment with the NMDA antagonist, D(-)-2-amino-5-phosphono-pentanoic acid (D-AP5), attenuates the enhancement of nociceptive scores produced by low doses of the mGluR agonists, (1*S*,3*S*)-ACPD and (*RS*)-3,5-dihydroxyphenyl-glycine ((*RS*)-DHPG)[16].

Since *trans*-ACPD is a non-selective mGluR agonist, it is not known which mGluR(s) is/are critical for its nociceptive effects. The present study examined the specific mGluRs involved in *trans*-ACPD-induced SNB by administering the following selective mGluR agonists: (*RS*)-DHPG, *trans*-ADA (Group I; mGluR1/5 and mGluR5, respectively), (1*S*,3*S*)-ACPD, (2*R*,4*R*)-APDC (Group II), and L-AP4 (Group III). Since (*RS*)-DHPG administration produced SNB that were of considerably greater intensity and longer duration compared to (1*S*,3*R*)-ACPD, the possible mechanisms underlying the action of (*RS*)-DHPG were investigated by pre-administering the mGluR antagonist, (+)-α-methyl-4-carboxyphenyl-glycine ((+)-MCPG), or the NMDA antagonist, D-AP5. Also, in order to verify that the observed behaviours associated with (*RS*)-DHPG administration are specifically nociceptive, the present study investigated whether morphine treatment reduces (*RS*)-DHPG-related behaviours.

Material and Methods

Long-Evans male rats (230-400g; Charles River) were randomly assigned to one of 19 groups. In the first experiment, 39 animals were injected i.t. with either 25 or 50 nmol (1*S*,3*S*)-ACPD, (1*S*,3*R*)-ACPD or (*RS*)-DHPG. Also, nine rats were injected i.t. with 50 nmol of *trans*-ADA, (2*R*,4*R*)-APDC, or L-AP4, with additional rats receiving up to 500 nmol of these agonists. Five min later, each rat was placed in a 30 x 30 x 30 cm Plexiglas® box and the following SNB were timed for two consecutive 30 min periods: elevating, shaking or stamping of the hindpaw (HE), licking or biting of the hindpaw (HL), elevating or whipping of the tail (TE) and licking or biting of the tail (TL). In the second experiment, 49 rats were administered i.t. 0.9% saline, dilute NaOH/saline vehicle (pH=8.0), (+)-MCPG (1, 10 or 100 nmol) or D-AP5 (1, 5 or 25 nmol), 10 min prior to (*RS*)-DHPG treatment (50 nmol). Each rat was then tested over a 60 min period using the same protocol described above. In the third experiment, 13 rats were administered subcutaneously (s.c.) 0.9% saline or morphine (2 mg/kg) immediately following i.t. (*RS*)-DHPG treatment (50 nmol). Each rat was then tested using the same protocol describe above.

Agonists and antagonists were administered i.t. in a volume of 30 and 50 µl, respectively, by lumbar puncture between L2 and L3 vertebrae while rats were under brief halothane anesthesia. Compounds were dissolved in either 0.9% saline (D-AP5, (*RS*)-DHPG, (2*R*,4*R*)-APDC) or a 42 mM NaOH/saline solution ((+)-MCPG, (1*S*,3*S*)-ACPD, (1*S*,3*R*)-ACPD, trans-ADA). All the mGluR compounds were obtained from

Tocris Cookson, St. Louis, MO, while D-AP5 was supplied by Research Biochemicals, Natick, MA.

Results

In experiment 1, only data from the (1*S*,3*S*)-ACPD, (1*S*,3*R*)-ACPD and (*RS*)-DHPG groups were included in the analyses, since administration of *trans*-ADA, (2*R*,4*R*)-APDC and L-AP4 did not produce any SNB, even with doses up to 500 nmol. A Kruskal-Wallis ANOVA for non-parametric data indicated a significant effect of drug treatment for time spent in three of the nociceptive categories (HE ($H(5,39)=24.1$, $P<0.01$), TE ($H(5,39)=10.9$, $P<0.05$) and TL ($H(5,39)=22.2$, $P<0.01$)) during the first 30 min of testing (Fig. 1A). During the second 30 min of testing, Kruskal-Wallis ANOVA also indicated a significant effect of drug treatment for HE ($H(5,39)=29.3$, $P<0.01$), TE ($H(5,39)=29.5$, $P<0.01$), HL ($H(5,39)=21.3$, $P<0.01$) and TL ($H(5,39)=24.6$, $P<0.01$) (Fig. 1B).

Post hoc comparisons (Mann-Whitney U test) revealed that animals that received 50 nmol of (*RS*)-DHPG exhibited significantly more HE during the first 30 min period and HE, TE and HL during the second 30 min period than did animals that received an equal dose of (1*S*,3*S*)-ACPD or (1*S*,3*R*)-ACPD. Administration of 50 nmol of (*RS*)-DHPG also resulted in more HE and TE during the second testing period than did 25 nmol of the drug. However, animals that received 25 nmol of (*RS*)-DHPG exhibited significantly more HE during the first 30 min, and HL during the second 30 min, than those that received an equal dose of (1*S*,3*S*)-ACPD or (1*S*,3*R*)-ACPD. Administration of 25 nmol of (*RS*)-DHPG also resulted in more TE during both the first and second 30 min periods than did 25 nmol of (1*S*,3*S*)-ACPD.

Administration of 25 or 50 nmol of (1*S*,3*R*)-ACPD to rats resulted in more HE during the first 30 min period, as well as HE and TE during the second 30 min period than did equal doses of (1*S*,3*S*)-ACPD. Also, administration of 25 nmol of (1*S*,3*R*)-ACPD to rats resulted in more TE compared to the same dose of (1*S*,3*S*)-ACPD.

Interestingly, animals that received 25 nmol of (*RS*)-DHPG spent more time TL than those that received a higher dose of the drug, or an equal dose of (1*S*,3*S*)-ACPD or (1*S*,3*R*)-ACPD during either testing period. In addition, animals that received 50 nmol of (1*S*,3*R*)-ACPD spent more time TL in the first 30 min period than rats that received 25 nmol of the drug, or 50 nmol of either (1*S*,3*S*)-ACPD or (*RS*)-DHPG. During the second 30 min period, administration of 50 nmol of (1*S*,3*R*)-ACPD resulted in more time TL than did 25 nmol of the drug. Lastly, animals that received a higher dose of (1*S*,3*S*)-ACPD spent significantly more time TL compared to those that received a lower dose of the drug.

In experiment 2 assessing the effects of (+)-MCPG and D-AP5 on (*RS*)-DHPG-induced behaviours, Kruskal-Wallis ANOVA indicated a significant dose effect of (+)-MCPG for HE ($H(3,26)=9.3$, $P<0.05$), TE ($H(3,26)=12.8$, $P<0.01$) and TL ($H(3,26)=8.9$, $P<0.05$). Post hoc comparisons (Mann-Whitney U test) revealed that rats pretreated with 10 or 100 nmol of (+)-MCPG exhibited significantly less HE or TE compared to the vehicle group (Fig. 2A). In contrast, animals treated with 1 or 10 nmol of the mGluR antagonist spent significantly more time TL than vehicle treated animals (Fig. 2B). Administration of (+)-MCPG of did not significantly influence the time spent HL.

The Kruskal-Wallis ANOVA also indicated a significant dose effect of D-AP5 for HE ($H(3,23)=14.2$, $P<0.01$) and TE ($H(3,23)=13.3$, $P<0.01$). Post hoc comparisons

revealed that rats pretreated with 5 or 25 nmol of D-AP5 exhibited significantly less HE and TE. Pretreatment with 1 nmol of D-AP5 also produced a significant reduction in TE (Fig. 2C). In contrast, there were no significant differences between the D-AP5 and vehicle groups for HL or TL (Fig. 2D).

The results from experiment 3 indicated that, compared to the saline treatment, morphine substantially reduced (*RS*)-DHPG-induced HE (median = 648.0 vs. 29.5 sec), TE (median = 669.0 vs. 12.0 sec) and HL (median = 337.0 vs. 7.0 sec), but did not influence TL (median = 18.0 vs 27.0 sec) of rats.

Discussion

First, the present study demonstrated that i.t. administration of the selective mGluR1/mGluR5 agonist, (*RS*)-DHPG, reliably produced intense SNB, which persisted for a remarkably longer duration than those following (1*S*,3*R*)-ACPD administration. No other agonist produced nociceptive effects, except (1*S*,3*S*)-ACPD. The SNB induced by (1*S*,3*S*)-ACPD- and (1*S*,3*R*)-ACPD administration decreased progressively over the 60 min, with very few behaviours exhibited in the second 30 min period. On the other hand, administration of the highest dose of (*RS*)-DHPG produced a striking increase in the expression of SNB, particularly HE, in the second testing period. In fact, by the end of the second testing period, most of these animals were persistently positioned with the hindpaws and tail elevated, and maintained this posture for several hours after the injection. The study also provides strong evidence that these behaviours are nociceptive in nature, since, s.c. administration of morphine almost completely abolished the (*RS*)-DHPG-induced effects.

Interestingly, HE and TE appeared to be indicative of greater nociceptive intensity since these behaviours occurred more frequently following administration of the higher dose of the agonists compared to the lower dose. Also, animals that received 50 nmol of (*RS*)-DHPG were more likely to express these nociceptive behaviours compared to those that received the same dose of (1*S*,3*S*)-ACPD or (1*S*,3*R*)-ACPD. In contrast, TL appeared to be indicative of milder intensity nociception, and was more likely to occur when animals spent less time HE and TE. For example, administration of the lower dose of (*RS*)-DHPG to rats produced more TL than the higher dose. However, the frequency of TL decreased if the animals showed few nociceptive behaviours in general during the testing period, as in the case of treatment with the lower dose of (1*S*,3*S*)-ACPD or (1*S*,3*R*)-ACPD. HL was not a particularly salient nociceptive behaviour, and occurred only during the second 30 min period in animals that received 25 or 50 nmol of (*RS*)-DHPG.

Second, the present study demonstrated that (*RS*)-DHPG-induced HE and TE were significantly reduced by a pretreatment with either the mGluR antagonist, (+)-MCPG, or the NMDA antagonist, D-AP5. Although an increase in TL occurred following administration of 1 or 10 nmol of (+)-MCPG, the highest dose (100 nmol) of the mGluR antagonist and each dose of D-AP5, effectively *reduced* this nociceptive behaviour. These data demonstrate a link between NMDA and mGluRs in response to i.t. (*RS*)-DHPG. This proposal is supported by the fact that NMDA receptors are not tonically active, since the dose of D-AP5 used in the present study does not produce analgesia in the absence of intense nociceptive stimuli[2]. Therefore, since D-AP5 attenuates (*RS*)-DHPG-induced SNB, it is suggested that the selective activation of mGluR1/5 leads to activity at NMDA

receptors, via an unknown mechanism. This proposal is consistent with research demonstrating that microelectrophoresis of (*RS*)-DHPG, as well as (1*S*,3*R*)-ACPD and (1*S*,3*S*)-ACPD[17] enhances NMDA-mediated increases in spinal cord neuronal excitability.

The data from this study provide evidence that activation of phosphoinositide hydrolysis-linked mGluRs are responsible for the nociceptive effects of the non-selective mGluR agonist, (1*S*,3*R*)-ACPD. It is known that (*RS*)-DHPG selectively and potently activates mGluR1 and mGluR5[18] and that this agonist is more potent than (1*S*,3*R*)-ACPD in stimulating phosphoinositide hydrolysis[19]. This may explain why administration of (*RS*)-DHPG produced much greater nociceptive effects than (1*S*,3*R*)-ACPD. Furthermore, although (1*S*,3*R*)-ACPD is also an agonist at negatively-linked cAMP mGluRs[20], the selective Group II and Group III agonists, (2*R*,4*R*)-ACPD and L-AP4, respectively, did not produce SNB even when 10 times the dose of (*RS*)-DHPG was administered. However, a transient excitatory action of the Group II agonist, (1*S*,3*S*)-ACPD, was observed in this study, which may be attributable to a non-selective effect at Group I receptors. This hypothesis is supported by the demonstration that application of (1*S*,3*S*)-ACPD stimulates phosphoinositide hydrolysis in the guinea pig cerebral cortex[21].

Results from the present study also provides evidence that the nociceptive effects of (1*S*,3*R*)-ACPD are attributable specifically to mGluR1 activation. This is supported by the fact that administration of the selective mGluR5 agonist, *trans*-ADA[22], did not produce any SNB, even when 10 times the dose of (*RS*)-DHPG was administered.

It is possible that stimulation of phosphoinositide hydrolysis via mGluR1 leads to an increase in either glutamate/aspartate release or NMDA channel activity, which may

produce a persistent facilitation of NMDA-mediated dorsal horn neuronal responses, and a resultant expression of SNB. It has been suggested that presynaptic phosphoinositide hydrolysis-linked mGluRs facilitate glutamate release[23]. Also, it is known that phosphoinositide hydrolysis stimulates production of the intracellular messengers, inositol triphosphate (IP3) and diacylglycerol (DAG) and DAG, in turn, activates protein kinase C (PKC). It has been demonstrated that PKC potentiates NMDA-induced currents[24] and PKC activators increase the release of glutamate and aspartate in the spinal cord[25].

Conclusion

Administration of the mGluR1/mGluR5 agonist, (*RS*)-DHPG, but not the more selective mGluR5 agonist, *trans*-ADA, produces long lasting SNB. These behaviours are not only reduced by pretreatment with the mGluR antagonist, (+)-MCPG, but also by the NMDA antagonist, D-AP5. Therefore, it is suggested that an interaction between the NMDA and mGluR1 may contribute to the maintenance of persistent pain phenomena, such as allodynia and spontaneous pain.

Acknowledgments

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References

1. Coderre TJ and Melzack R. *J. Neurosci.* 12, 3665-3670 (1992).

2. Coderre TJ and Van Empel I. *Pain.* 59, 345-352 (1994).

3. Davar G, Hama A, Deykin A et al. *Brain Res.* 553, 327-330 (1991).

4. Ren K, Williams GM, Hylden JLK et al. *Eur. J. Pharmacol.* 219, 235-243 (1992).

5. Ren K and Dubner R. *Neurosci. Lett.* 163, 22-26 (1993).

6. Raigorodsky G and Urca G. *Brain Res.* 422, 158-162 (1987).

7. Kolhekar R, Meller ST and Gebhart GF. *Neurosci.* 57, 385-395 (1993).

8. Young MR, Fleetwood-Walker SM, Mitchell R et al. *Neuropharmacol.* 34, 1033-1041 (1995).

9. Neugebauer V, Lücke T and Schaible HG. *Eur. J. Neurosci.* 6, 1179-1186 (1994).

10. Aanonsen LM, Lei S and Wilcox GL. *Pain* 41, 309-321 (1990).

11. Kolhekar R and Gebhart GF. *Brain Res.* 651, 215-226 (1994).

12. Raigorodsky G and Urca G. *Eur. J. Pharmacol.* 182, 37-47 (1990).

13. Meller ST, Dykstra C and Gebhart GF. *Neurosci.* 71, 327-335 (1996).

14. Palecek J, Palecková V, Dougherty PM et al. *Pain* 56, 261-269 (1994).

15. Meller ST, Dykstra CL and Gebhart GF. *NeuroReport.* 4, 879-882 (1993).

16. Fisher KF and Coderre TJ. The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception. *Pain*, In press.

17. Bond A and Lodge D. *Neuropharmacol.* 34, 1015-1023 (1995).

18. Brabet I, Bockaert MJ and Pin J-P. *Neuropharmacol.* 34, 895-903 (1995).

19. Schoepp DD, Goldsworthy J, Johnson BG et al. *J. Neurochem.* 63, 769-772 (1994).

20. Schoepp DD, Johnson BG and Monn JA. *J. Neurochem.* 58, 1184-1186 (1992).

21. Cartmell J, Curtis AR, Kemp JA et al. *Neurosci. Lett.* 153, 107-110 (1993).

22. Kozikowski AP, Tückmantel W, Liao Y et al. *Med. Cem.* 36, 2706-2708 (1993).

23. Herrero I, Miras-Portugal MT and Sánchez-Prieto J. *Nature* 360, 163-166 (1992).

24. Chen L and Huang L.-YM. *Nature* 356, 521-523 (1992).

25. Gerber G and Randic M. *Neurosci. Lett.* 106, 220-228 (1989).

Figure Captions

FIG.1. Median time (sec) spent by rats in the four nociceptive behaviours (HE, HL, TE, TL) from 5-35 (A) and 35-65 (B) min post i.t. treatment with 25 or 50 nmol of (1*S*,3*S*)-ACPD, (1*S*,3*R*)-ACPD or (*RS*)-DHPG. N=6 for each group, except for 25 nmol of (1*S*,3*S*)-ACPD (N=8) and (1*S*,3*R*)-ACPD (N=7).

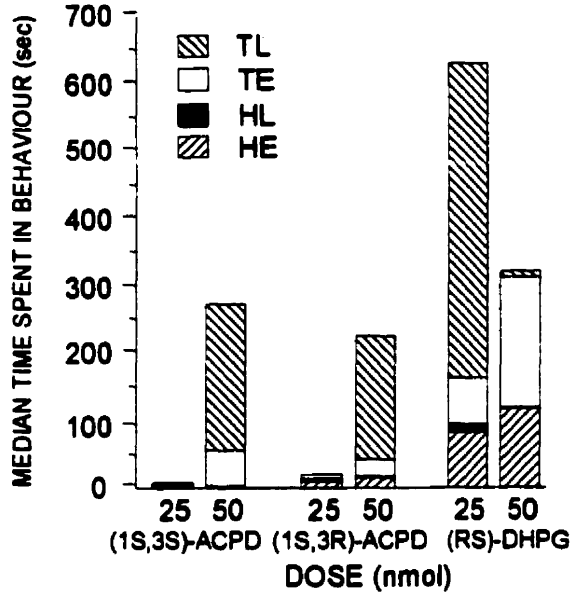
FIG.2. Median time (sec) spent in the four nociceptive behaviours (HE, TE, HL, TL), over a 60 min testing period, for rats pretreated i.t. with 0 (dilute sodium hydroxide (Na/OH) vehicle), 1, 10 or 100 nmol of (+)-MCPG (A,B) or 0 (saline vehicle), 1, 5 or 25 nmol of D-AP5 (C,D), 10 min prior to the (*RS*)-DHPG (50 nmol) injection. N=6 for each group, except for 100 nmol of (+)-MCPG (N=8) and 25 nmol of D-AP5 (N=5). The post hoc analyses (Mann-Whitney U tests) indicate significant differences between the vehicle and experimental conditions (** $P<0.01$, * $P<0.05$).

General summary

The present study examined the intensity and duration of spontaneous pain behaviours following spinal treatment with various metabotropic glutamate receptor agonists in rats. The possible mechanisms underlying the action of one of these agonists, (*RS*)-DHPG, were further investigated, since the pain producing effects of the compound were of considerably greater intensity and longer duration than those observed with the other agents tested. Results showed that blocking metabotropic glutamate or *N*-methyl-D-aspartate (NMDA) receptors with spinal (+)-MCPG or D-AP5 pretreatment, respectively, reduced the spontaneous pain behaviours produced by the (*RS*)-DHPG injection. These data suggest that an NMDA/metabotropic glutamate receptor interaction may contribute to the maintenance of persistent pain phenomenon, such as allodynia (noxious response to innocuous stimuli) and spontaneous pain.

Fig. 1

A. 5 - 35 Minutes Post Injection




B. 35 - 65 Minutes Post Injection

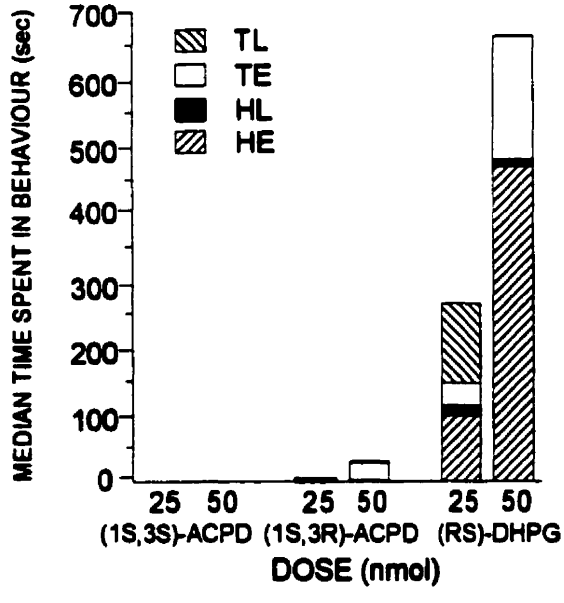
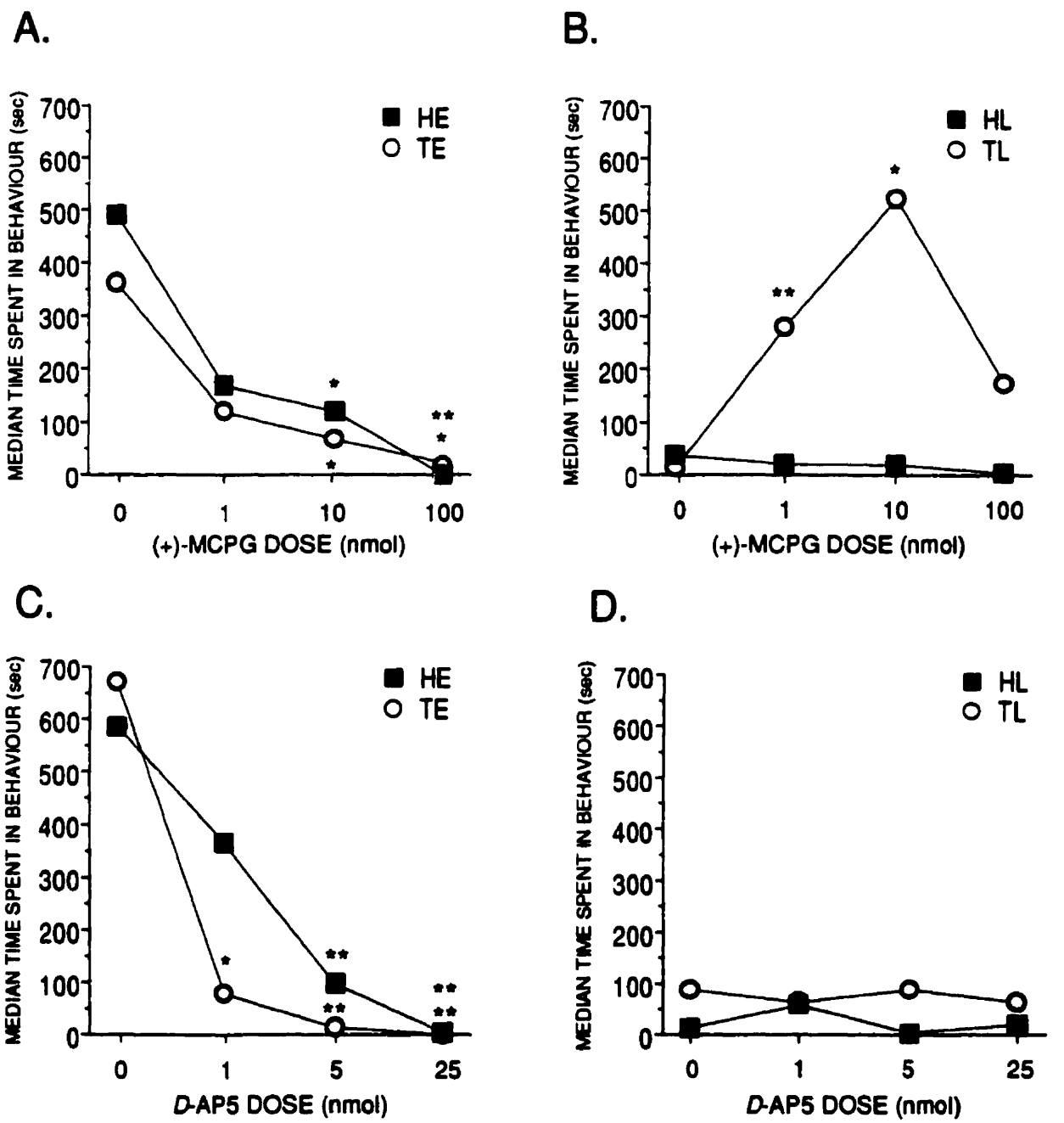


Fig. 2


A.
B.
C.
D.
MEDIAN TIME SPENT IN BEHAVIOUR (sec)
700
600
500
400
300
200
100
0
HE
TE
HL
TL
0
1
10
100
5
25
(+)-MCPG DOSE (nmol)
D-AP5 DOSE (nmol)
*
**

# CHAPTER 4: (*RS*)-DHPG-INDUCED HYPERALGESIA AND ALLODYNIA

Rationale for Experiments

Prior to the publication of this manuscript, only two behavioural studies had shown that intrathecal administration of an mGluR agonist produces nociceptive behaviours (Meller et al. 1993, 1996), yet a relatively large number of studies had clearly demonstrated that intrathecal administration of iGluRs to rats or mice produces hyperalgesia and allodynia in rodents (Aanonsen & Wilcox, 1987; Raigorodsky & Urca, 1987; Malmberg & Yaksh, 1992, 1993; Meller et al. 1992; Kolhekar et al. 1993; Advokat et al. 1994; Ferreira & Lorenzetti, 1994; Brambilla et al. 1996; Meller et al. 1996). Given that Meller et al. (1993, 1996) had examined the behavioural effects of a non-selective mGluR agonist ((1*S*,3*R*)-ACPD) only, and that we had previously shown that intrathecal administration of the selective group I mGluR agonist, (*RS*)-DHPG, is pro-nociceptive in rats (Studies 1 and 2), we decided to perform a series of experiments to fully characterize all nociceptive behaviours associated with intrathecal (*RS*)-DHPG.

The main finding from this study is that intrathecal administration of (*RS*)-DHPG produces persistent heat hyperalgesia, mechanical allodynia and mechanical hyperalgesia in rats, suggesting that group I mGluRs may contribute to the development of these types of nociceptive behaviours in models of pain. These findings are in contrast to those obtained by Meller and colleagues (1993, 1996). These investigators reported that intrathecal administration of (1*S*,3*R*)-ACPD does not produce heat hyperalgesia and that (1*S*,3*R*)-ACPD must be co-administered with AMPA in order to produce mechanical hyperalgesia. However, it should be noted that, although (1*S*,3*R*)-ACPD is an agonist at group I mGluRs, it is also a potent agonist at group II mGluRs. Therefore, it is difficult to compare the effects of (*RS*)-DHPG with (1*S*,3*R*)-ACPD especially since we later found

that intrathecal administration of a selective group II mGluR agonist is antinociceptive in a model of chronic pain (Study 5).

We also found that rats treated intrathecally with (*RS*)-DHPG showed an increased sensitivity in the cold water test. However, the differences between the experimental and control animals were not statistically significant. This may be attributable to an overlap in the scores between the two groups, since several (*RS*)-DHPG-treated animals showed a tendency to freeze during the post-injection testing periods, and, consequently, exhibited few behaviours that fit the response criteria. It is likely that intrathecal administration of (*RS*)-DHPG produces cold hyperalgesia, since piloerection was observed in the majority of (*RS*)-DHPG-, but in none of the saline-treated animals. Since piloerection and freezing behaviour are indicative of increased stress levels in the rat, it may be that this test was too noxious for many of the (*RS*)-DHPG-treated animals

STUDY 3

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# Hyperalgesia and allodynia induced by intrathecal (*RS*)-dihydroxyphenylglycine in rats

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Running title: (*RS*)-DHPG-induced nociception

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Abstract

To investigate the role of Group I mGluRs in allodynia and hyperalgesia, we examined the behavioural responses of rats to noxious and non-noxious mechanical and thermal stimuli following intrathecal (i.t.) treatment (25 nmol) with the selective $mGluR_{1/5}$ agonist, (*RS*)-dihydroxyphenylglycine ((*RS*)-DHPG). (*RS*)-DHPG administration produced: a persistent decrease in response latency on a 48°C hotplate, a reduction in the 50% response threshold to von Frey hairs, and an increase in responses to a tail pinch. These data suggest that activation of spinal $mGluR_{1/5}$ receptors plays a role in the development of persistent allodynia and hyperalgesia associated with tissue or nerve injury.

*Key words:* Allodynia; Dihydroxyphenylglycine; DHPG; Hyperalgesia; Metabotropic glutamate receptors; mGluR;. Nociception

Introduction

Endogenous excitatory amino acids in the central nervous system, such as glutamate and aspartate, activate the ionotropic glutamate receptors (iGluRs), for NMDA, AMPA, and kainate, as well as a group of recently identified metabotropic glutamate receptors ($mGluR_1$-$mGluR_8$). Although it is well established that the iGluRs are involved in nociceptive processes[1-5], little is known about the role of metabotropic glutamate receptors (mGluRs). We have previously reported that i.t. administration of the non-selective mGluR agonist, *trans*-D,L-1-amino-1,3-cyclopentane-dicarboxylic acid (*trans*-ACPD), or the selective $mGluR_{1/5}$ agonist (*RS*)-dihydroxyphenylglycine ((*RS*)-DHPG), produces spontaneous nociceptive behaviours in rats[6]. Coadministration of *trans*-ACPD and AMPA by the i.t. route also produces mechanical hyperalgesia in rats[7]. In addition, iontophoretic application of *trans*-ACPD to the spinal cord facilitates spinal thalamic tract neuronal responses to innocuous mechanical stimuli in monkeys[8]. Furthermore, iontophoretic application of the mGluR antagonists, L-1-amino-3-phosphonopropanoic acid (L-AP3) or (*RS*)-4-carboxy-3-hydroxyphenylglycine ((*RS*)-4C3HPG), reduces dorsal horn neuronal responses associated with repeated mustard oil application[9] or knee joint inflammation[10] in rats.

The aim of the present study was to investigate the possible role of $mGluR_{1/5}$ receptors in hyperalgesia and allodynia, by examining the behavioural responses of rats to noxious and non-noxious mechanical and thermal (hot and cold) stimuli following i.t. (*RS*)-DHPG treatment.

Materials and Methods

*Drug administration*: Male Long-Evans rats (225-325 g; Charles River) were injected i.t with either 25 nmol (30 µl) of (*RS*)-DHPG (Tocris Cookson, St. Louis, MO), or an equivalent volume of vehicle (0.9% saline). Injections were performed by lumbar puncture, rostral to the L3 vertebra, while rats were under brief halothane anaesthesia.

*Behavioural testing*: Prior to baseline testing for mechanical allodynia, each rat was habituated to a testing box (27 x 16 x 21 cm) with a wire-mesh grid floor, for 1 h. For baseline and post injection testing, a series of von Frey hairs (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.10 g) were applied through the grid floor to the ventral surface of the left hindpaw of each rat. Each hair was applied for 7 s or until the animal withdrew the hindpaw without ambulating. During each testing trial, the series of hairs were presented following an up-down procedure, described and validated by Chaplan et al. (1994)[11], and the 50% response threshold was calculated for each rat. Following two baseline trials, eight (*RS*)-DHPG- and eight saline-treated animals were tested 30, 60, 90, 120, 150, 180 and 210 min post injection.

Each testing trial for mechanical hyperalgesia involved applying a clip, with a 300 g force, to the tail of the rat (6.0 cm from the tip) for 5 s. The quality of the response (i.e. vocalization, tail licking) elicited by the animal was recorded for each trial. After the baseline trial was performed for each rat, 10 DHPG-treated and five saline-treated animals were tested 30, 60, 90 and 120 min post injection

Prior to baseline testing for heat hyperalgesia, each rat was habituated to a hotplate at room temperature for 15 - 30 min. For baseline and post-injection testing, each

rat was placed on the hotplate at 48°C and the latency was recorded until the rat licked, shook or showed a prolonged lift of one of the hindpaws. The trial was terminated after 25 s if no response was observed. Following a single baseline trial, 12 (*RS*)-DHPG- and 11 saline-treated animals were tested 90, 180, 270, 360 and 450 min post-injection.

Prior to baseline testing for cold hyperalgesia, each rat was habituated to standing in a room temperature, 1 cm deep, water bath for 15 - 30 min. For baseline and post-injection testing, each rat was placed in a 1 cm deep, 1°C water bath for 75 s, during which it was noted if the rat licked, shook, or showed a prolonged lift of one of the hindpaws when not ambulating. In addition to the first response latency, the frequency of responses for both hindpaws, and the cumulative duration of all responses were recorded. If the animal jumped before making a specific response with one of the hindpaws, the jump was considered the first response and the latency was recorded. Following a baseline trial, 14 (*RS*)-DHPG- and seven saline-treated animals were tested 90, 180 and 270 min post-injection.

Results

*Mechanical allodynia*: Figure 1A shows the mean 50% von Frey withdrawal thresholds for (*RS*)-DHPG- and saline-treated rats, at baseline and the post-injection time intervals. Friedman analysis of variance (ANOVA) for repeated, non-parametric data indicated a significant main effect of Time ($X^2$ (7) = 22.63, $P$<0.01) for the (*RS*)-DHPG, but not the saline condition. *Post hoc* analyses with the Wilcoxon rank sum test revealed that (*RS*)-DHPG-treated rats had significantly lower 50 % von Frey withdrawal thresholds, 30, 60, 90, 120, 150 and 180 min post-injection, compared to baseline. Analyses with the Mann-

Whitney U-test indicated that compared to saline-treated rats, (*RS*)-DHPG-treated animals had significantly lower 50 % von Frey withdrawal thresholds at the 30, 60, 90, 120, 150 and 180 min time intervals.

*Mechanical hyperalgesia*: Prior to (*RS*)-DHPG treatment, a tail pinch elicited only orienting behaviours (not nociceptive responses) in the rats, but at 30, 60, 90 and 120 min post-injection, a tail pinch elicited vigorous nociceptive responses in 90%, 80%, 70% and 70% of the animals, respectively (see Table I). Responses included vocalization, licking the tail or testicles and/or attempting to bite the tail clip. These behaviours were not observed in the saline-treated animals at any time interval, with the exception of one rat that licked its tail, following the tail pinch, at the 90 min interval only.

*Heat hyperalgesia*: Figure 1B shows the mean response hotplate latencies for (*RS*)-DHPG- and saline-treated rats, at baseline and the post-injection time intervals. ANOVA for repeated parametric data revealed significant main effects of drug ($F(1,21) = 6.45$, $P<0.05$) and time ($F(5,105) = 5.61$, $P<0.001$), as well as a significant drug x time interaction ($F(5,105) = 4.66$, $P<0.001$). *Post hoc* analyses (Tukey HSD) revealed that (*RS*)-DHPG-treated animals had significantly shorter response latencies 90, 180, 270 and 360 min post-injection, compared to baseline. Also, (*RS*)-DHPG-treated animals had significantly shorter response latencies, than did saline-treated animals at 90 and 180 min.

*Cold hyperalgesia*: Figure 1C shows the mean response frequencies for (*RS*)-DHPG- and saline-treated rats, at baseline and the post-injection time intervals. Analyses with the Wilcoxon rank sum test revealed that, compared to baseline, (*RS*)-DHPG-, but not saline-treated animals, responded more frequently at 90 and 180 min post-injection. However, analyses with the Mann-Whitney U-test indicated that the differences in response

frequency between the (*RS*)-DHPG- and saline-treated groups were not statistically significant. ANOVA of the response latency and response duration indicated that there were no significant differences.

Discussion

We have shown previously that i.t. treatment with 50 nmol (*RS*)-DHPG, the potent and selective $mGluR_{1/5}$ agonist[12], consistently produces spontaneous nociceptive behaviours in rats, which have been observed up to 10 h after injection[6]. In the present study we have demonstrated that i.t. administration of half the dose (25 nmol) of (*RS*)-DHPG produces heat hyperalgesia, mechanical allodynia and mechanical hyperalgesia in rats for at least 6, 3 and 2 h post-injection, respectively, without producing spontaneous nociceptive behaviours that interfere with nociceptive testing. Thus, there is a dose-dependent production of first thermal and mechanical hyperalgesia and mechanical allodynia, and with higher doses spontaneous behaviours, associated with intrathecal (*RS*)-DHPG administration. As for cold sensitivity, although (*RS*)-DHPG-treated rats exhibited a significant increase in response frequency in the cold water test, the differences between the experimental and control animals were not statistically significant.

The magnitude and duration of the (*RS*)-DHPG-induced effects are of considerable interest. We suggest that intrathecally administered (*RS*)-DHPG produces a prolonged activation of both presynaptic $mGluR_{1/5}$ receptors localized on primary afferent fibers and postsynaptic $mGluR_{1/5}$ receptors localized on spinal cord dorsal horn neurons. Although it is currently unknown whether presynaptic $mGluR_{1/5}$ receptors enhance the release of glutamate from primary afferent fibers, this possibility is suggested since recent studies have shown that presynaptic $mGluR_{1/5}$ receptors facilitate the release of glutamate

from rat cerebrocortical[13] and hippocampal[14] synaptosomes. On the other hand, (*RS*)-DHPG-induced hyperalgesia and allodynia may also reflect a postsynaptic $mGluR_{1/5}$-mediated increase in dorsal horn neuronal responses to C- and/or Aβ-fiber stimulation, respectively. This hypothesis is supported by the findings that iontophoretic application of $mGluR_{1/5}$ agonists potentiates dorsal horn neuronal responses to the iGluR agonists, NMDA and AMPA[15,16]. In addition, we have previously demonstrated that an iGluR-mGluR interaction plays a role in nociceptive processing. Thus, i.t. administration of non-analgesic doses of the NMDA antagonist, D(-)-2-amino-5-phosphonopentanoic acid (D-AP5), reduces (*RS*)-DHPG-induced spontaneous nociceptive behaviours[6], as well as (*RS*)-DHPG-induced facilitation of formalin nociceptive scores[17].

Conclusion

Intrathecal administration of the potent and selective $mGluR_{1/5}$ agonist (*RS*)-DHPG, produces prolonged mechanical allodynia and hyperalgesia, as well as thermal hyperalgesia in rats. The persistence of (*RS*)-DHPG-induced nociception may be explained by an $mGluR_{1/5}$-mediated increase in spinal glutamate release and/or a postsynaptic $mGluR_{1/5}$-mediated facilitation of dorsal horn neuronal responses to noxious and non-noxious stimuli.

General Summary

The authors have examined the contribution of a subtype of metabotropic glutamate receptors (mGluRs) in nociceptive or pain processing in the spinal cord of rats. Spinal injection of a Group I mGluR agonist, (*RS*)-dihydroxyphenylglycine produced a prolonged increase in sensitivity to noxious thermal and mechanical stimuli

(hyperalgesia), as well as nociceptive, or painful, responses to non-noxious mechanical stimuli. The results suggest that activation of spinal Group I mGluRs plays a role in the development of increased pain sensitivity associated with tissue or nerve injury.

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References

1. Raigorodsky G and Urca G. *Brain Res* 422, 158-162 (1987).

2. Kolhekar R, Meller ST and Gebhart GF. *Neuroscience* 57, 385-395 (1994).

3. Davar G, Hama A, Deykin A et al. *Brain Res* 553, 327-330 (1991).

4. Mao J, Price DD, Mayer DJ et al. *Brain Res* 576, 254-262 (1992).

5. Ren K, Hylden JLK, Williams GM et al. *Pain* 50, 331-344 (1992).

6. Fisher K, Coderre TJ. *NeuroReport* 7, 2743-2747 (1996).

7. Meller ST, Dykstra CL and Gebhart GF. *NeuroReport* 4, 879-882 (1993).

8. Palecek J, Palecková V, Dougherty PM et al. *Pain* 56, 261-269 (1994).

9. Young MR, Fleetwood-Walker SM, Mitchell R et al. *Neuropharmacology* 33, 141-144 (1994).

10. Neugebauer V, Lücke T and Schaible HG. *Eur J Neurosci* 6, 1179-1186 (1994).

11. Chaplan SR, Bach FW, Pogrel JW et al. *J Neurosci Methods* 53, 55-63 (1994).

12. Schoepp DD, Goldsworthy J, Johnson BG et al. *J Neurochem* 63, 769-772 (1994).

13. Herrero I, Miras-Portugal MT and Sánchez-Prieto J. *J Neurochem* 59, 1574-1577 (1992).

14. Herrero I, Miras-Portugal MT and Sánchez-Prieto J. *Nature* 360, 163-166 (1992).

15. Bleakman D, Rusin KI, Chard PS et al. *Mol Pharmacol* 42, 192-196 (1992).

16. Bond A and Lodge D. *Neuropharmacol* 34, 1015-1023 (1995).

17. Fisher K and Coderre TJ. *Pain* 68, 255-263 (1996).

Table 1. Number of responders/non-responders to a tail pinch, at baseline and 30, 60, 90 and 120 min following i.t. saline or (*RS*)-DHPG.

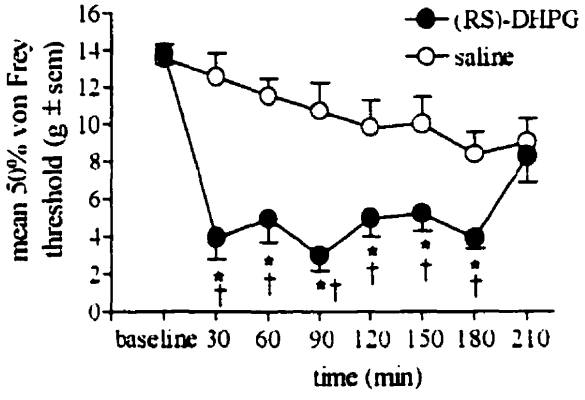
| Treatment | Baseline | 30 min | 60 min | 90 min | 120 min |
|---|---|---|---|---|---|
| Saline | 0/5 | 0/5 | 0/5 | 1/4 | 0/5 |
| (*RS*)-DHPG | 0/10 | 9/1 | 8/2 | 7/3 | 7/3 |

Figure Captions

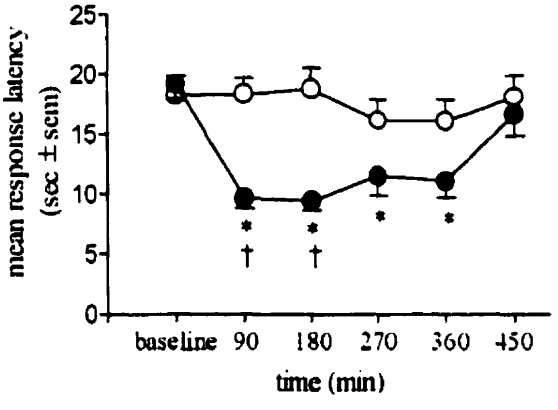
Figure 1. (A) Mean (± s.e.m.) 50% von Frey withdrawal thresholds (g) for rats, prior to (baseline) and 30, 60, 90, 120, 150, 180 and 210 min after i.t. (*RS*)-DHPG or saline treatment. *Post hoc* analyses indicate significant differences between the (*RS*)-DHPG and saline conditions (†$P<0.05$; Mann-Whitney U-tests) and between baseline and post-injection withdrawal thresholds in the (*RS*)-DHPG condition (*$P<0.05$; Wilcoxon rank sum tests). (B) The mean (± s.e.m) hotplate response latencies (sec) for rats, prior to (baseline) and 90, 180, 270, 360 and 450 min after i.t. (*RS*)-DHPG or saline treatment. Post hoc analyses of the drug x trial interaction indicate significant differences between the (*RS*)-DHPG and saline conditions (†$P<0.05$) and between baseline and post-injection response latencies (*$P<0.05$; Tukey HSD tests). (C) Mean (± s.e.m.) cold water response frequencies during a 75 sec testing period, prior to (baseline) and 90, 180 and 270 min after i.t. (*RS*)-DHPG or saline treatment. *Post hoc* analyses indicate significant differences between baseline and post-injection response frequencies for the (*RS*)-DHPG condition (*$P<0.05$; Wilcoxon rank sum tests).

Fig. 1

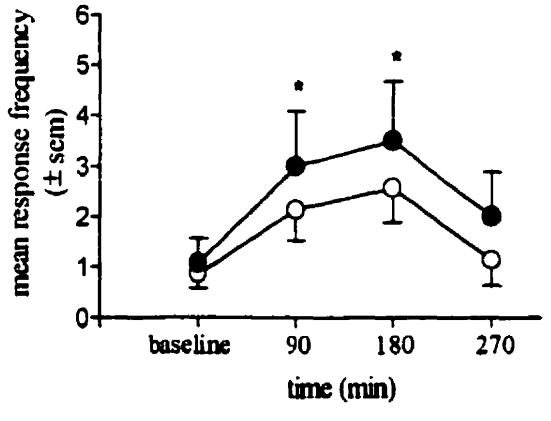
**A.**


(RS)-DHPG
saline
mean 50% von Frey threshold (g ± sem)
16
14
12
10
8
6
4
2
0
baseline 30 60 90 120 150 180 210
time (min)

**B.**


mean response latency (sec ± sem)
25
20
15
10
5
0
baseline 90 180 270 360 450
time (min)

**C.**


mean response frequency (± sem)
6
5
4
3
2
1
0
baseline 90 180 270
time (min)

# CHAPTER 5: (*S*)-4CPG-RELATED ANTINOCICEPTION IN A MODEL OF NEUROPATHIC PAIN

The Sciatic Nerve Chronic Constriction Injury (CCI) Model

The most common mononeuropathic pain model used in recent years is the one developed and characterized by Bennett and Xie in (1988). This procedure requires tying four chromic gut ligatures loosely around the rat's sciatic nerve. Within 24 hours post-surgery, the rat shows behaviours, such as lifting, licking and shaking of the hindpaw on the operated side, which have been interpreted to be spontaneous nociceptive behaviours (Attal, Jazat, Kayser & Guilbaud, 1990; Bennett & Xie 1988). Rats with sciatic nerve injury also exhibit hyperalgesia and allodynia of the hindpaw. Evidence for hyperalgesia is extrapolated from the observed characteristics of the rat's response to noxious cold, hot or mechanical stimuli. For example, rats with CCI will show a decrease in the post-operative response latencies compared to pre-operative latencies, an increase in the response duration, and an increase in the incidence of an exaggerated response to noxious stimuli i.e. licking or shaking of the hindpaw. Evidence for allodynia is extrapolated from the observed characteristics of the rat's response to innocuous mechanical and thermal stimuli. For example, rats with CCI will exhibit a decrease in the detection threshold and an exaggerated response to von Frey stimulation, post-surgery. Also following surgery, the animals will exhibit exaggerated responses to innocuous cold and warm stimuli (Attal et al. 1990; Bennett & Xie 1988)

Research has elucidated a number of peripheral and central neural mechanisms mediating the development and maintenance of the CCI-induced nociceptive behaviours. CCI of the sciatic nerve initially produces a degeneration of large and small myelinated axons, and to a lesser extent, unmyelinated axons in the sciatic nerve (Basbaum, Gautron, Jazat, Mayes & Guilbaud, 1991; Coggeshall, Dougherty, Pover & Carlton, 1993;

Guilbaud, Gautron, Jazat, Ratinahirana, Hassig & Hauw, 1993; Kajander & Bennett 1992; Mosconi & Kruger 1996). Damaged primary afferent Aβ-, Aδ-, and later, C-fibers begin to discharge spontaneously, and this abnormal activity is thought to mediate the spontaneous pain-related behaviours in the animals (Kajander & Bennett 1992; Kajander, Wakisaka & Bennett, 1992; Study & Kral 1996; Tal & Eliav 1996). It is of general consensus that the increase in spontaneous C-fiber activity, and the consequential increase in release of EAAs, as well as other neurochemicals (i.e. substance P), contributes to the development of central sensitization (for review, see Coderre, Katz, Vaccarino & Melzack, 1993). Many have proposed that changes in dorsal horn neuronal excitability contribute to the development and maintenance of abnormal nociceptive behaviours exhibited by rats with CCI, and that these changes are mediated, at least in part, by the NMDAR (Davar et al. 1991; Mao et al. 1993; Yamamoto and Yaksh 1992b). Interestingly, Mao, Price, Hayes, Lu and Mayer (1992) found that the NMDAR contributes to the development *and* the maintenance of CCI-induced thermal hyperalgesia, whereas non-NMDARs contribute only to the development of this nociceptive behaviour.

Rationale for Experiments

The discovery that selective activation of spinal group I mGluRs produces persistent pain-related behaviours, such as spontaneous nociceptive behaviours (Study 2), allodynia and hyperalgesia (Study 3), behaviours that are characteristically exhibited by rats with nerve injury, led us to speculate that this group of mGluRs may contribute to the development of these behaviours in models of neuropathic pain. Also contributing to this

hypothesis was the observed duration of the (*RS*)-DHPG-induced behaviours exhibited by the animals, which was striking given that intrathecal administration of iGluR agonists, particularly NMDA, typically produces *acute* nociceptive behaviours in animals (Raigorodsky & Urca 1987; Coderre & Melzack 1991; Meller et al. 1996), and application of iGluR agonists to the rat spinal cord produces only *transient* increases in dorsal horn neuronal excitability to noxious and non-noxious stimuli (Cumberbatch et al. 1994). These data, as well as the finding that D-AP5 pretreatment reduces (*RS*)-DHPG-induced SNBs, suggest that group I mGluRs may contribute to the development of nociceptive behaviours in models of chronic pain by facilitating NMDAR-mediated alterations in dorsal horn neuronal sensitivity. It should be noted that the duration of the intrathecal effects of (RS)-DHPG might have been attributable one of several factors such as, group I mGluR-mediated neuroplasticity, group I mGluR-induced neurotoxicty as well as the pharmacokinetics of (RS)-DHPG.

In order to test the hypothesis that group I mGluRs contribute to models of neuropathic pain, we performed a series of experiments to examine the potential antinociceptive effects of intrathecal (*S*)-4CPG in rats with CCI. (*S*)-4CPG was specifically chosen since it was the most selective group I mGluR antagonist at the time; however, it was also known to activate group II mGluRs with a weaker potency. Since Mao, Price, Hayes, Lu and Mayer (1992) reported that the development and maintenance of CCI-related nociceptive behaviours are mediated by different, but overlapping spinal mechanisms, we explored the possibility that (*S*)-4CPG treatment might differentially influence these time-related components of neuropathic nociceptive behaviours.

The main finding from this study is that early intrathecal treatment with (*S*)-4CPG (twice daily injections on post-operative days 0-3), but not late treatment (twice daily injections on post-operative days 8-11) attenuates mechanical allodynia and cold hyperalgesia in rats with CCI. These results suggest that group I mGluRs are involved in the development of CCI-related mechanical allodynia and cold hyperalgesia, but not in the maintenance of these behaviours.

STUDY 4

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# Intrathecal administration of the mGluR compound, (*S*)-4CPG, attenuates hyperalgesia and allodynia associated with sciatic nerve constriction injury in rats.

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Abstract

The present study examined the effects of intrathecal (i.t.) treatment (twice daily injections on post-operative (PO) days 0-8) with the metabotropic glutamate receptor (mGluR) compound, (*S*)-4-carboxyphenylglycine ((*S*)-4CPG), or the non-competitive *N*-methyl-D-aspartate (NMDA) antagonist, dizocilipine maleate (MK-801), on mechanical allodynia and cold hyperalgesia associated with chronic constriction injury (CCI) of the sciatic nerve in rats. Also, the effects of *early* (twice daily injections on days 0-3) or *late* (twice daily injections on days 8-11) (*S*)-4CPG treatment on the injury-related mechanical allodynia and cold hyperalgesia were assessed in CCI rats. Results demonstrated that 8 day (*S*)-4CPG or MK-801 treatment attenuated mechanical allodynia (up to PO days 12 or 16, respectively) and cold hyperalgesia (up to PO day 8 or 16, respectively). Results also demonstrated that *early* (*S*)-4CPG treatment significantly attenuated the development of mechanical allodynia (90 and 270 nmol) and cold hyperalgesia (270 nmol). However, *late* treatment with (*S*)-4CPG did not reduce the nociceptive behaviours in either behavioural task. These data not only confirm that the NMDA receptor plays a role in chronic nociception, but also suggest that Group I mGluRs are more critically involved in the development, and not the maintenance, of mechanical allodynia and cold hyperalgesia associated with CCI in rats.

*Keywords:* Hyperalgesia; Allodynia; Nociception; Metabotropic glutamate receptors; mGluR; Neuropathic pain

1. Introduction

Chronic constriction of the rat's sciatic nerve with 4 loose chromic gut ligatures (Bennett and Xie 1988) is commonly used to investigate the mechanisms mediating neuropathic pain. Constriction of the sciatic nerve produces persistent hyperalgesia to noxious radiant heat and mechanical stimuli, allodynia to non-noxious warm and cold stimuli, and spontaneous nociceptive behaviours in rats (Bennett and Xie 1988; Attal et al. 1990). Recently, in an effort to standardize the degree of nerve injury and reduce inter-animal variability, Mosconi and Kruger (1996) developed a model that utilizes a cuff consisting of a 2 mm length of polyethylene tubing to produce a chronic constriction injury (CCI) of the sciatic nerve in rats. The resulting morphological and behavioural changes are similar to those that follow sciatic nerve ligation, but the degree of nerve injury and behavioural hyperalgesia produced by the cuff is less variable (Mosconi and Kruger 1996).

A number of laboratories have demonstrated that ionotropic glutamate receptors (iGluRs), such as *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazoleproprionic acid (AMPA), and/or kainate contribute to the development and/or maintenance of persistent nociceptive behaviours induced by CCI. Furthermore, it has been demonstrated that various classes of glutamate receptors may be differentially involved in either the development or maintenance of neuropathic pain behaviours. For example, systemic or intrathecal (i.t.) treatment with non-competitive NMDA antagonists prior to and/or early after surgery, attenuates the development of thermal hyperalgesia in nerve injured rats (Mao et al. 1992a; 1992b; 1993). Also, a single i.t. injection of the non-

competitive NMDA antagonist MK-801, administered several days post surgery, after the neuropathy is well developed, reduces thermal hyperalgesia associated with nerve constriction injury (Davar et al. 1991; Mao et al. 1992a; 1992b; 1993; Yamamoto and Yaksh 1992). In contrast, the iGluRs, AMPA and/or kainate, have been shown to be involved in the development, but not the maintenance, of persistent neuropathic pain behaviours, since i.t. treatment with the AMPA/kainate antagonist, 6-cyano-7-nitro-quinoxalinedione (CNQX), administered pre-, but not post-surgery, attenuates thermal hyperalgesia in nerve injured rats (Mao et al. 1992a).

Recently, with the synthesis of selective compounds, research has shown that metabotropic glutamate receptors (mGluRs) are also involved in nociception. The family of mGluRs are linked through G-proteins to intracellular second messenger systems. Activation of Group I mGluRs (mGluR1 and mGluR5) produces phosphoinositide hydrolysis, while activation of Group II (mGluR2 and mGluR3) or Group III (mGluR4, mGluR6-8) mGluRs inhibits cAMP production (Nakanishi 1992; Hayashi et al. 1994; Duvoisin et al. 1995). The suggestion that mGluRs play a role in nociception is supported by studies demonstrating that iontophoretic application of mGluR antagonists attenuates rat dorsal horn neuronal responses associated with repeated mustard oil application (Young et al. 1994; Young et al. 1995) and knee joint inflammation (Neugebauer et al. 1994). Furthermore, i.t. administration of the mGluR/AMPA agonist, quisqualate, produces mechanical (Aanonsen et al. 1990) and thermal (Kolhekar and Gebhart 1994) hyperalgesia, and spontaneous nociceptive behaviours (Sun and Larson 1991) in rats or mice. Also, i.t. administration of the selective Group I agonist, (*RS*)-dihydroxyphenylglycine ((*RS*)-DHPG), produces persistent thermal hyperalgesia,

mechanical allodynia/hyperalgesia (Fisher and Coderre 1997), and spontaneous nociceptive behaviours (Fisher and Coderre 1996a) in rats.

Since administration of (*RS*)-DHPG produces nociceptive behaviours that are similar to those observed in neuropathic pain models, it is suggested that mGluRs, particularly Group I mGluRs, may play a role in the pathogenesis of neuropathic pain. Thus, the present study examined the effects of the Group I antagonist, (*S*)-4-carboxyphenylglycine ((*S*)-4CPG) (Hayashi et al. 1994; Kingston et al. 1995), on mechanical allodynia and cold hyperalgesia in the CCI model developed by Mosconi and Kruger (1996). First, the effects of 8 day i.t. treatment with (*S*)-4CPG were compared with the non-competitive NMDA antagonist, dizocilipine maleate (MK-801), on mechanical allodynia and cold hyperalgesia. Second, since evidence suggests that different classes of glutamate receptors contribute differentially to the development and maintenance of neuropathic pain, we examined the effects of *early* vs *late* i.t. treatment with (*S*)-4CPG on nociceptive behaviours in rats with CCI.

## 2. Methods

### *2.1 Animals*

These experiments were performed on 76 male, Long-Evans hooded rats (Charles River) weighing 280-415 g. They were maintained under controlled lighting conditions with food and water available ad libitum. In all cases, the guidelines described in Care and Use of Experimental Animals of the Canadian Council of Animal Care (Vols. I and II) were strictly followed. All experiments were approved by the animal care committee at the Clinical Research Institute of Montreal.

*2.2 Chronic constriction injury*

Prior to surgery, all rats were anaesthetized with sodium pentobarbital (65 mg/kg; i.p.). A chronic constriction injury was produced by placing a 2 mm length of polyethylene tubing (PE-90) around the sciatic nerve of each rat, according to the method of Mosconi and Kruger (1996). The cuff was placed after exposing the left sciatic nerve mid-thigh and isolating the nerve from surrounding tissue. Surgery for the sham condition involved exposing the left sciatic nerve, but the nerve was not manipulated or cuffed.

*2.3 Drug administration*

MK-801 (Research Biochemicals Int., Natick, MA, USA), (*S*)-4CPG (Tocris Cookson, St. Louis, MO, USA) and vehicle were administered i.t. by lumbar puncture, in a volume of 30 µl, between the L2 and L3 vertebrae, while rats were under brief halothane anesthesia. MK-801 and (*S*)-4CPG were dissolved in 0.9% saline and NaOH (pH 8), respectively. The highest doses of (*S*)-4CPG and MK-801 used were 270 nmol and 30 nmol, respectively. Although the 270 nmol dose of (*S*)-4CPG was high, the mGluR compound was well tolerated and none of the animals exhibited motor disturbances at any time during the study. Equivalent doses of MK-801 and (*S*)-4CPG were not used since treatment with such high doses of MK-801 produces significant motor problems in rats (Coderre and Van Empel 1994). In this study, it was noted that administration of the 30 nmol dose of MK-801 produced some mild stereotypical alterations in motor activity (i.e., hyperlocomotion and balance loss) in the animals for a short period of time following injection (up to 30 min post-injection). However, at the time of nociceptive testing, none of these animals exhibited motor problems of any kind.

*2.3.1 Experiment 1*

Rats were randomly assigned to one of the following eight groups: (*S*)-4CPG (90 nmol)/CCI; MK-801 (30 nmol)/CCI; NaOH vehicle/CCI; saline vehicle/CCI; (*S*)-4CPG (90 nmol)/sham; MK-801 (30 nmol)/sham; NaOH vehicle/sham; saline vehicle/sham. Each rat was injected i.t. with vehicle, MK-801 or (*S*)-4CPG, 15 min prior to surgery and then every 12 hours for 9 days. On PO days 4 and 8, nociceptive testing began 2 hrs after the i.t. injection.

*2.3.2 Experiment 2*

Rats were randomly assigned to one of the following six groups: (*S*)-4CPG (30 nmol)/CCI; (*S*)-4CPG (90 nmol)/CCI; (*S*)-4CPG (270 nmol)/CCI; NaOH vehicle/CCI; (*S*)-4CPG (270 nmol)/sham; NaOH vehicle/sham. Each animal was injected i.t. with vehicle or one of the three doses of (*S*)-4CPG, 15 min prior to surgery and then every 12 hours for 4 days.

*2.3.3 Experiment 3*

Nociceptive testing was performed on rats prior to surgery (baseline) and then on PO days 4 and 8. Rats were then randomly assigned to one of two groups: (*S*)-4CPG (270 nmol)/CCI; NaOH vehicle/CCI. Each animal was injected i.t. with vehicle or (*S*)-4CPG, immediately after testing on PO day 8, and then every 12 hrs for 4 days.

*2.4 Nociceptive testing*

*2.4.1 Mechanical allodynia testing.*

Prior to baseline testing, each rat was habituated to a testing box (27 x 16 x 21 cm) with a wire-mesh grid floor, for a one hour period. For baseline and post-operative (PO) testing, a series of von Frey hairs (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.10

g) were applied through the grid floor to the ventral surface of the left hindpaw of each rat. Each hair was applied for a 7 second period or until the animal withdrew the hindpaw without ambulating. During each testing trial, the series of hairs were presented following an up-down procedure, described and validated by Chaplan et al. (1994), and the 50% response threshold was calculated for each rat.

*2.4.2 Cold hyperalgesia testing.*

For baseline and PO testing, each rat was placed in a 1 cm deep, 1°C water bath with a metal floor, for a 75 second period. During the testing period, a response was counted if the rat licked, shook, or showed a prolonged lift of the hindpaw when not ambulating. In addition to the latency of the first response, the frequency of responses, and the cumulative duration of all responses were recorded for the left neuropathic hindpaw.

Mechanical allodynia and cold hyperalgesia testing was performed 1-2 days prior to surgery to obtain baseline values, and then on PO days 4, 8, 12 and 16.

3. Results

*3.1 Experiment 1*

The effects of 8 day i.t. treatment with vehicle, (*S*)-4CPG or MK-801 on mechanical allodynia and cold hyperalgesia were analyzed. No differences were observed between the NaOH- or saline-treated animals, therefore, these animals were included in one vehicle control group for all statistical analyses.

*3.1.1 Mechanical allodynia*

The 50% von Frey response threshold for the neuropathic hindpaw was first analyzed with Friedman ANOVA for repeated non-parametric data. Results indicated that the main effect of Time for the vehicle- ( $X^2(4) = 11.11$, $P<0.05$) and (*S*)-4CPG- ($X^2(4) = 9.58$, $P<0.05$), but not for the MK-801-( $X^2(4) = 3.27$, $P>0.05$) treated CCI animals was statistically significant. *Post hoc* comparisons (Wilcoxon Signed-Rank test) revealed that, compared to baseline, vehicle-treated rats had significantly lower response thresholds on all PO testing days (Fig. 1A), demonstrating the development of a prolonged mechanical allodynia. However, (*S*)-4CPG-treated animals exhibited significantly lower response thresholds on PO day 16 only, compared to baseline (Fig. 1A), suggesting that the (*S*)-4CPG treatment reduced the development of mechanical allodynia up to at least day 12, 4 days after the treatment had ended. However, since the thresholds of (*S*)-4CPG-treated rats were reduced slightly, no statistically significant differences were found between the (*S*)-4CPG and vehicle groups. The lack of a significant time effect for MK-801 suggests that the MK-801 treatment reduced the development of mechanical allodynia throughout the entire testing period. Further analyses with the Mann-Whitney U-test for independent-groups data indicated that, compared to vehicle-, MK-801-treated animals had significantly higher response thresholds on PO day 8 (Fig. 1A).

*3.1.2 Cold hyperalgesia*

Although three dependent variables were used to assess the degree of cold hyperalgesia in this experiment, response frequency was the most reliable measure for detecting changes in sensitivity over time and for detecting differences between the experimental and control groups. Friedman ANOVA for the response frequency indicated

that the main effect of Time for the vehicle- ($X^2(4) = 15.771$, $P<0.01$), but not for the (*S*)-4CPG- ($X^2(4) = 6.20$, $P>0.05$) or MK-801- ($X^2(4) = 5.67$, $P>0.05$) treated CCI animals was statistically significant. *Post hoc* comparisons (Wilcoxon Signed-Rank test) revealed that, compared to baseline, vehicle-treated rats exhibited a significant increase in response frequency during all of the PO testing periods (Fig. 1B), demonstrating the development of a prolonged cold hyperalgesia. The lack of a significant time effect for the (*S*)-4CPG and MK-801 groups suggest that the treatments reduced the development of cold hyperalgesia throughout the entire testing period. Furthermore, analyses with the Mann-Whitney U-test indicated that, compared to vehicle-, (*S*)-4CPG-treated animals exhibited fewer responses on PO days 4 and 8, while MK-801-treated animals exhibited fewer responses on PO days 8, 12 and 16 (Fig. 1B).

Treatment with (*S*)-4CPG or MK-801 to sham animals, on PO days 0-8, did not produce analgesia in the mechanical allodynia or cold hyperalgesia tests (Figs. 1C and D). Although the von Frey thresholds of the sham MK-801-treated rats appear to be reduced, they are not significantly reduced compared to their own baseline level, which is somewhat lower than other groups. Regardless, this lowered threshold in sham animals can not account for the effects of MK-801 in neuropathic rats where the thresholds are higher than in neuropathic control rats.

*3.2 Experiment 2*

The effects of *early* (*S*)-4CPG treatment on mechanical allodynia and cold hyperalgesia were analyzed.

*3.2.1 Mechanical allodynia*

Friedman ANOVA for the 50% von Frey response threshold, for the neuropathic hindpaw, indicated that the main effect of Time was statistically significant for the vehicle ($X^2(4) = 13.73$, $P<0.01$), and the 30 nmol ($X^2(4) = 13.03$, $P<0.01$), 90 nmol ($X^2(4) = 12.27$, $P<0.05$), and 270 nmol ( $X^2(4) = 11.70$, $P<0.05$) (*S*)-4CPG dose groups in the CCI condition. *Post hoc* comparisons (Wilcoxon Signed-Rank test) revealed that, compared to baseline, animals treated with the 0 or 30 nmol doses of (*S*)-4CPG had significantly lower response thresholds on all of the PO testing days, demonstrating the development of a prolonged mechanical allodynia in these animals. However, animals treated with the 90 or 270 nmol doses exhibited significantly lower response thresholds than baseline only on PO days 12 and 16 (Fig. 2A), demonstrating that treatment with the higher doses of (*S*)-4CPG reduced the development of mechanical allodynia up to at least day 8, 5 days after the treatment had ended. Kruskal-Wallis ANOVA for multiple independent-groups data indicated that the main effect of Drug was statistically significant on PO days 4 ($H(3) = 13.01$, $P<0.005$) and 8 ($H(3) = 12.77$, $P<0.005$). *Post hoc* comparisons (Mann-Whitney U-test) for the significant Kruskal-Wallis ANOVAs revealed that, compared to vehicle-treated animals, rats treated with the 30 or 90 nmol doses had significantly higher response thresholds on PO day 8, while rats treated with the 270 nmol dose had significantly higher response thresholds on PO days 4 and 8 (Fig. 2A).

*3.2.2 Cold hyperalgesia*

Friedman ANOVA for the response frequency indicated that the main effect of Time for the vehicle ($X^2(4) = 15.00$, $P<0.005$), and the 30 nmol ($X^2(4) = 10.57$, $P<0.05$), and 90 nmol ($X^2(4) = 14.67$, $P<0.005$) (*S*)-4CPG dose groups were statistically significant for the CCI condition. However, the main effect of time was not statistically significant for the 270 nmol (*S*)-4CPG dose group ( $X^2(4) = 9.10$, $P>0.05$). *Post hoc* comparisons (Wilcoxon Signed-Rank test) revealed that, compared to baseline, animals treated with the 0 or 30 nmol doses of (*S*)-4CPG exhibited significantly more responses on all of the PO testing days, while animals treated with 90 nmol of (*S*)-4CPG exhibited significantly more responses on all of the PO testing days, except day 4 (Fig. 2B). The lack of a significant time effect for the 270 nmol dose of (*S*)-4CPG demonstrates that treatment with the highest dose of the mGluR compound reduced cold hyperalgesia throughout the entire testing period. Kruskal-Wallis ANOVA indicated that the main effect of Drug was statistically significant on PO day 8 ($H(3) = 11.10$, $P<0.01$), and subsequent p*ost hoc* comparisons (Mann-Whitney U test) revealed that animals treated with the 270 nmol dose responded less frequently than vehicle-treated animals on that day (Fig. 2B).

Treatment with 270 nmol of (*S*)-4CPG to sham animals on PO days 0-3 did not produce analgesia in the mechanical allodynia or cold hyperalgesia tests (Figs 2C and D).

*3.3 Experiment 3*

The effects of *late* (*S*)-4CPG treatment on mechanical allodynia and cold hyperalgesia were analyzed.

*3.3.1 Mechanical allodynia*

Friedman ANOVA for the 50% von Frey response threshold indicated that the main effect of Time was statistically significant for vehicle- ($X^2(4) = 15.33$, $P<0.01$) and (*S*)-4CPG- ($X^2(4) = 15.43$, $P<0.01$) treated CCI animals. *Post hoc* comparisons (Wilcoxon Signed-Rank test) revealed that, compared to baseline, vehicle- and (*S*)-4CPG-treated rats had significantly lower response thresholds on all of the PO testing days (Fig. 3A). Furthermore, analyses with the Mann-Whitney U-test indicated that there were no significant differences between the vehicle and (*S*)-4CPG response thresholds on any of the testing days (Fig. 3A). These results demonstrate that *late* treatment with (*S*)-4CPG did not reduce mechanical allodynia.

*3.3.2 Cold hyperalgesia*

Friedman ANOVA for the response frequency indicated that the main effect of Time was statistically significant for vehicle- ($X^2(4) = 15.03$, $P<0.01$) and (*S*)-4CPG- ($X^2(4) = 16.67$, $P<0.01$) treated animals. *Post hoc* comparisons (Wilcoxon Signed-Rank test) revealed that, compared to baseline, vehicle- and (*S*)-4CPG-treated rats exhibited a significant increase in response frequency on all of the PO testing days (Fig. 3B). Furthermore, analyses with the Mann-Whitney U-test indicated that there were no significant differences between the vehicle and (*S*)-4CPG response frequencies on any of the testing days (Fig. 3B). These results demonstrate that *late* treatment with (*S*)-4CPG did not reduce cold hyperalgesia.

4. Discussion

Although a number of laboratories have demonstrated that administration of NMDA antagonists attenuate persistent nociceptive behaviours in models of neuropathic pain (Davar et al. 1991; Carlton and Hargett 1995; Eisenberg, LaCross, & Strassman, 1995; Qian et al. 1996), the effects of mGluR compounds have not yet been explored in these models. The aim of the present study was to assess the possible antinociceptive effects of the mGluR compound, (*S*)-4CPG, using a recently developed CCI model (Mosconi and Kruger 1996).

First, the present study confirmed that placing a polyethylene tubing cuff around the sciatic nerve induces an injury that reliably produces persistent hyperalgesia in the operated hindpaw of vehicle control rats. It was found that these animals exhibited an increased sensitivity to von Frey hair stimulation and to the cold water bath as early as PO day 4, with mechanical allodynia and cold hyperalgesia peaking between PO days 8 to 16. This observation is in general agreement with Mosconi and Kruger (1996), who reported that 'pain-related' responses were maximal between PO days 10 to 14.

Second, the present study demonstrated that 8-day, twice daily, i.t. treatment with the NMDA antagonist, MK-801 (30 nmol), or the mGluR compound, (*S*)-4CPG (90 nmol), attenuated mechanical allodynia and cold hyperalgesia in CCI rats, but did not increase response thresholds in sham-operated controls. It should be noted that, although rats treated with MK-801 appeared to be sensitive to the cold water bath on PO day 4, these animals showed a consistent decrease in response frequency on subsequent testing days.

Third, the present study demonstrated that when the mGluR compound was administered to rats on PO days 0-3, (*S*)-4CPG treatment significantly attenuated the development of the injury-induced nociceptive behaviours. Administration of the highest and most effective dose of (*S*)-4CPG (270 nmol) reduced mechanical allodynia and cold hyperalgesia up to PO days 8 and 16, respectively, in CCI rats, but did not increase response thresholds in sham-operated controls. However, when the 270 nmol dose was administered on PO days 8-11, (*S*)-4CPG treatment did not reduce the well-established mechanical allodynia or cold hyperalgesia. In contrast, NMDA antagonists effectively reduce neuropathic pain behaviours when administered early *or* late post surgery (Davar et al. 1991; Mao et al. 1992a, 1992b; 1993; Yamamoto and Yaksh 1992; Carlton and Hargett 1995; Eisenberg et al. 1995).

In summary, this study demonstrated, for the first time, that treatment with an mGluR compound reduces nociceptive behaviours in a model of neuropathic pain. Specifically, i.t. (*S*)-4CPG administration attenuates the development of mechanical allodynia and cold hyperalgesia, but does not influence the maintenance of these nociceptive behaviours. Since (*S*)-4CPG is a potent Group I mGluR antagonist (Hayashi et al. 1994; Sekiyama et al. 1996) (with a greater selectivity for mGluR1α (Brabet et al. 1995; Kingston et al. 1995)), this study provides evidence that phosphoinositide hydrolysis-linked mGluRs contribute to spinal cord mechanisms that mediate the development of mechanical allodynia and cold hyperalgesia in CCI rats. This proposal is consistent with the demonstration that iontophoretic application of compounds known to be mGluR Group I antagonists reduces sensitization of the dorsal horn spinal cord

neurons induced by repeated cutaneous application of mustard oil (Young et al. 1994, 1995) or intra-articular inflammation (Neugebauer et al. 1994).

Activation of phosphoinositide hydrolysis-linked mGluRs leads to the translocation/activation of protein kinase C (PKC) (Manzoni et al. 1990), formation of cyclic adenosine 3'5'-monophosphate (cAMP) (Aramori and Nakanishi 1992; Winder and Conn 1992), mobilization of intracellular $Ca^{2+}$ (Berridge and Galione 1988; Murphy and Miller 1988; Irving et al. 1990; Masu et al. 1991; Abe et al. 1992), and release of arachidonic acid (Dumuis et al. 1990). One or several of these intracellular second messengers may contribute to injury-induced spinal cord plasticity, since studies have demonstrated that second messenger systems, like those activated by Group I mGluRs, play an important role in persistent and chronic nociception (Coderre 1992; Mao et al. 1992c; Coderre and Yashpal 1994; Igwe and Ning 1994; Munro et al. 1994; Yashpal et al. 1995; Young et al. 1995; Kawamata and Omote 1996).

It is also possible that Group I mGluRs contribute to persistent and chronic nociception by facilitating ionotropic glutamate receptor function in the spinal cord. Although the exact mechanism(s) is/are presently unknown, studies have demonstrated that activation of phosphoinositide hydrolysis-linked mGluRs enhance NMDA- and AMPA-mediated responses in the rat dorsal horn (Bleakman et al. 1992; Cerne and Randic 1992; Bond and Lodge 1995). Furthermore, evidence suggests that an mGluR/iGluR interaction mediates persistent nociceptive responses in rats (Fisher and Coderre 1996a,b).

It should be noted that, since (*S*)-4CPG is also a weak agonist at Group II mGluRs (Hayashi et al. 1994), we cannot rule out the possibility that this mechanism contributed

to the observed antinociceptive effects of the mGluR compound, particularly at the highest dose. Studies have shown that activation of Group II mGluRs produces a presynaptic-mediated depression of monosynaptic excitation of rat spinal motoneurons (Pook et al. 1992; Ishida et al. 1993; Jane et al. 1994; Jane et al. 1995).

In conclusion, this study has demonstrated that i.t. administration of the mGluR compound, (*S*)-4CPG, attenuates the development, but not the maintenance, of mechanical allodynia and cold hyperalgesia associated with CCI in rats. The effects of (*S*)-4CPG treatment are likely due to an antagonism of Group I mGluRs, since activation of these mGluRs leads to the production of second messenger systems known to play an important role in persistent and chronic nociception.

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References

Abe T., Sugihara H., Nawa H., Shigemoto R., Mizuno N. and Nakanishi S., Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/$Ca^{2+}$ signal transduction, J. Biol. Chem., 267 (1992) 13361-13368.

Aanonsen L.M., Lei S. and Wilcox G.L., Excitatory amino acid receptors and nociceptive neurotransmission in rat spinal cord, Pain, 41 (1990) 309-321.

Aramori I. and Nakanishi S., Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells, Neuron, 8 (1992) 757-765.

Attal N., Jazat F., Kayser V. and Guilbaud G., Further evidence for 'pain-related' behaviours in a model of unilateral peripheral mononeuropathy, Pain, 41 (1990) 235-251.

Bennett G.J. and Xie Y.-K., A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man, Pain, 33 (1988) 87-107.

Berridge M.J. and Galione A., Cytosolic calcium oscillators, FASEB J., 2 (1988) 3074-3083.

Bleakman D., Rusin K.I., Chard P.S., Glaum S.R. and Miller R.J., Metabotropic glutamate receptors potentiate ionotropic glutamate responses in the rat dorsal horn, Mol. Pharmacol., 42 (1992) 192-196.

Bond, A. and Lodge D., Pharmacology of metabotropic glutamate receptor-mediated enhancement of responses to excitatory and inhibitory amino acids on rat spinal neurones *in vivo*, Neuropharmacol., 34 (1995) 1015-1023.

Brabet I., Bockaert M.J. and Pin J.-P., Phenylglycine derivatives discriminate between mGluR1- and mGluR5-mediated responses, Neuropharmacol., 34 (1995) 895-903.

Carlton S.M. and Hargett G.L., Treatment with the NMDA antagonist memantine attenuates nociceptive responses to mechanical stimulation in neuropathic rats, Neurosci. Lett., 198 (1995) 115-118.

Cerne R. and Randic M., Modulation of AMPA and NMDA responses in rat spinal dorsal horn neurons by trans-1-aminocyclopentante-1,3-dicarboxylic acid, Neurosci. Lett., 144 (1992) 180-184.

Chaplan S.R., Bach F.W., Pogrel J.W., Chung J.M. and Yaksh T.L., Quantitative assessment of tactile allodynia in the rat paw, J. Neurosci. Meth., 53 (1994) 55-63.

Coderre T.J., Contribution of protein kinase C to central sensitization and persistent pain following tissue injury, Neurosci. Lett., 140 (1992) 181-184.

Coderre T.J., Fisher K, Fundytus ME., The role of ionotropic and metabotropic glutamate receptors in persistent nociception. In: T.S. Jensen, J.A. Turner, Z. Wiesenfeld- Mullin (Eds.), Progress in Pain Research and Management, Vol. 8, Proc. VIII World Congress on Pain, IASP Press, Seattle, (1997), pp. 259-275.

Coderre T.J. and Van Empel I., The utility of excitatory amino acid (EAA) antagonists as analgesic agents. I. comparison of the antinociceptive activity of various classes of EAA antagonists in mechanical, thermal and chemical nociceptive tests, Pain, 59 (1994) 345-352.

Coderre T.J. and Yashpal K., Intracellular messengers contributing to persistent nociception and hyperalgesia induced by L-glutamate and substance P in the rat formalin pain model, Eur. J. Neurosci., 6 (1994) 1328-1334.

Davar G., Hama A., Deykin A., Vos B. and Maciewicz R., MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy, Brain Res., 553 (1991) 327-330.

Dumuis A., Pin J.-P., Oomagari K., Sebben M. and Bockaert J., Arachidonic acid released from striatal neurons by joint stimulation of ionotropic and metabotropic quisqualate receptors, Nature, 347 (1990) 182-184.

Duvoisin R.M., Zhang C. and Ramonell K., Glutamate receptor expressed in the retina and olfactory bulb, J. Neurosci., 15 (1995) 3075-3083.

Eisenberg E., LaCross S., Strassman A.M., The clinically tested *N*-methyl-D-aspartate receptor antagonist memantine blocks and reverses thermal hyperalgesia in a rat model of painful mononeuropathy, Neurosci. Lett., 187 (1995) 15-20.

Fisher K. and Coderre T.J., Comparison of nociceptive effects produced by intrathecal administration of mGluR agonists. NeuroReport, 7 (1996a) 2743-2747.

Fisher K. and Coderre T.J., The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception. Pain, 68 (1996b) 255-263.

Fisher K. and Coderre T.J. Hyperalgesia and allodynia induced by intrathecal (*RS*)-dihydroxyphenylgycine administration in rats. NeuroReport, 1997 (*submitted*).

Hayashi Y., Sekiyama N., Nakanishi S., Jane D.E., Sunter, D.C., Birse E.F., Udvarhelyi P.M. and Watkins J.C., Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes, J. Neurosci., 14 (1994) 3370-3377.

Igwe O.J. and Ning L., Regulation of the second-messenger systems in the rat spinal cord during prolonged peripheral inflammation, Pain, 58 (1994) 63-75.

Irving A.J., Schofield J.G., Watkins J.C., Sunter D.C. and Collingridge G.L., 1*S*,3*R*-ACPD stimulates and L-AP3 blocks $Ca^{2+}$ mobilization in rat cerebellar neurons, Eur. J. Pharmacol., 186 (1990) 363-365.

Ishida M., Saitoh T., Shimamoto K., Ohfune Y. and Shinozaki H., A novel metabotropic glutamate receptor agonist: marked depression of monosynaptic excitation in the newborn rat isolated spinal cord, Br. J. Pharmacol., 109 (1993) 1169-1177.

Jane D.E., Jones P.L.St.J., Pook P.C.-K., Tse H.-W. and Watkins J.C., Actions of two new antagonists showing selectivity for different sub-types of metabotropic glutamate receptor in the neonatal rat spinal cord, Br. J. Pharmacol., 112 (1994) 809-816.

Jane D.E., Pittaway K., Sunter D.C., Thomas K.N. and Watkins J.C., New phenylglycine derivatives with potent and selective antagonist activity at presynaptic glutamate receptors in neonatal rat spinal cord, Neuropharmacol., 34 (1995) 851-856.

Kawamata M. and Omote K., Involvement of increased excitatory amino acids and intracellular $Ca^{2+}$ concentrations in the spinal dorsal horn in an animal model of neuropathic pain, Pain, 68 (1996) 85-96.

Kingston A.E., Burnett J.P., Mayne N.G. and Lodge D., Pharmacological Analysis of 4-carboxyphenylglycine derivatives: comparison of effects on mGluR1α and mGluR5a subtypes, Neuropharmacol., 34 (1995) 887-894.

Kolhekar R. and Gebhart G.F., NMDA and quisqualate modulation of visceral nociception in the rat, Brain Res., 651 (1994) 215-226.

Manzoni O.J.J., Finiels-Marlier F., Sassetti I., Bockaert J., le Pench C. and Sladeczek F.A.J., The glutamate receptor of the Qp-type activates protein kinase C and is regulated by protein kinase C, Neurosci. Lett., 109 (1990) 146-151.

Mao J., Price D.D., Hayes R.L., Lu J., Mayer D.J. and Frenk H., Intrathecal treatment with dextrorphan or ketamine potently reduces pain-related behaviours in a rat model of peripheral mononeuropathy, Brain Res., 605 (1993) 164-168.

Mao J., Price D.D., Hayes R.L. and Mayer D.J., Differential role of NMDA and non-NMDA receptor activation in induction and maintenance of thermal hyperalgesia in rats with painful peripheral mononeuropathy, Brain Res., 598 (1992a) 271-278.

Mao J., Price D.D., Hayes R.L. and Mayer D.J., Intrathecal MK-801 and local nerve anesthesia synergistically reduce nociceptive behaviours in rats with experimental peripheral mononeuropathy, Brain Res., 576 (1992b) 254-262.

Mao J., Price D.D., Hayes R.L. and Mayer D.J., Pain-related increases in spinal cord membrane-bound protein kinase C following peripheral nerve injury, Brain Res., 588 (1992c) 144-149.

Masu M., Tanabe Y., Ysuchida K., Shigemoto R., and Nakanishi S., Sequence and expression of metabotropic glutamate receptor, Nature, 349 (1991) 760-765.

Mosconi T. and Kruger L., Fixed-diameter polyethylene cuffs applied to the rat sciatic nerve induce a painful neuropathy: ultrastructural morphometric analysis of axonal alterations, Pain, 64 (1996) 37-57.

Munro F.E., Fleetwood-Walker, S.M. and Mitchell, R. Evidence for a role of protein kinase C in the sustained activation of rat dorsal horn neurons evoked by cutaneous mustard oil application. Neurosci.Lett., 170 (1994) 199-202.

Murphy S.N. and Miller R.J., A glutamate receptor regulates $Ca^{2+}$ mobilization in hippocampal neurons, Proc. Natl. Acad. Sci. USA, 85 (1988) 8737-8741.

Nakanishi S., Molecular diversity of glutamate receptors and implications for brain function, Science, 258 (1992) 597-603.

Neugebauer V., Lücke T. and Schaible H.G., Requirement of metabotropic glutamate receptors for the generation of inflammation-evoked hyperexcitability in rat spinal cord neurons, Eur. J. Neurosci., 6 (1994) 1179-1186.

Pook P.C.-K., Sunter D.C., Udvarhelvi P.M. and Watkins J.C., Evidence for presynaptic depression of monosynaptic excitation in neonatal rat motoneurones by (1*S*,3*S*)- and (1*S*,3*R*)-ACPD, Exp. Physiol., 77 (1992) 529-532.

Qian J., Brown S.D. and Carlton S.M., Systemic ketamine attenuates nociceptive behaviours in a rat model of peripheral neuropathy, Brain Res., 715 (1996) 51-62.

Sekiyama N., Hayashi Y., Nakanishi S., Jane D.E., Tse H.-W., Birse E.F. and Watkins J.C., Structure-activity relationships of new agonists and antagonists of different metabotropic glutamate receptor subtypes, Br. J. Pharmacol., 117 (1996) 1493-1503.

Sun X. and Larson A.A., Behavioural sensitization to kainic acid and quisqualic acid in mice: comparison to NMDA and substance P responses, J. Neurosci., 11 (1991) 3111-3123.

Winder D.G. and Conn P.J., Activation of metabotropic glutamate receptors in the hippocampus increases cyclic AMP accumulation, J. Neurochem., 59 (1992) 375-378.

Yamamoto T. and Yaksh T.L., Spinal pharmacology of thermal hyperesthesia induced by constriction injury of sciatic nerve. Excitatory amino acid antagonists, Pain, 49 (1992) 121-128.

Yashpal K., Pitcher G.M., Parent A., Quirion R. and Coderre T.J., Noxious thermal and chemical stimulation induce increases in $^{3}$H-phorbol 12,13-dibutyrate binding in spinal cord dorsal horn as well as persistent pain and hyperalgesia, which is reduced by inhibition of protein kinase C, J. Neurosci., 15 (1995) 3263-3272.

Young M.R., Fleetwood-Walker S.M., Mitchell R. and Dickenson T., The involvement of metabotropic glutamate receptors and their intracellular signalling pathways in sustained nociceptive transmission in rat dorsal horn neurons, Neuropharmacol., 34 (1995) 1033-1041.

Young M.R., Fleetwood-Walker M., Mitchell, R. and Munro F.E., Evidence for a role of metabotropic glutamate receptors in sustained nociceptive inputs to rat dorsal horn neurons, Neuropharmacol., 33 (1994) 141-144.

Figure Legends

Fig. 1. Mean 50% von Frey response threshold and cold water response frequency for the operated hindpaw of CCI (A and B, respectively) and sham (C and D, respectively) animals at baseline and on PO days 4, 8, 12 and 16. CCI and sham animals were treated with vehicle, (*S*)-4CPG (90 nmol), or MK-801 (30 nmol) on PO days 0-8. The * indicates a significant difference between baseline and the PO means ($P<0.05$; Wilcoxon Signed-Rank test). The † indicates a significant difference between the experimental and vehicle/control conditions ($P<0.05$; Mann-Whitney U-test).

Fig. 2. Mean 50% von Frey response threshold and cold water response frequency for the operated hindpaw of CCI (A and B, respectively) and sham (C and D, respectively) animals at baseline and on PO days 4, 8, 12 and 16. CCI animals were treated with vehicle (0 nmol), or with 30 nmol, 90 nmol or 270 nmol of (*S*)-4CPG, while sham animals were treated with vehicle or 270 nmol of (*S*)-4CPG, on PO days 0-3. The * indicates a significant difference between baseline and the PO means ($P<0.05$, Wilcoxon Signed-Rank test). The † indicates a significant difference between the experimental and vehicle/control conditions (†† $P<0.01$, † $P<0.05$, Mann-Whitney U-test).

Fig. 3. Mean 50% von Frey response threshold (A) and cold water response frequency (B) at baseline and on PO days 4, 8, 12 and 16, for the operated hindpaw of animals treated with vehicle (0 nmol) or 270 nmol of (*S*)-4CPG on PO days 8-11.

Fig. 1

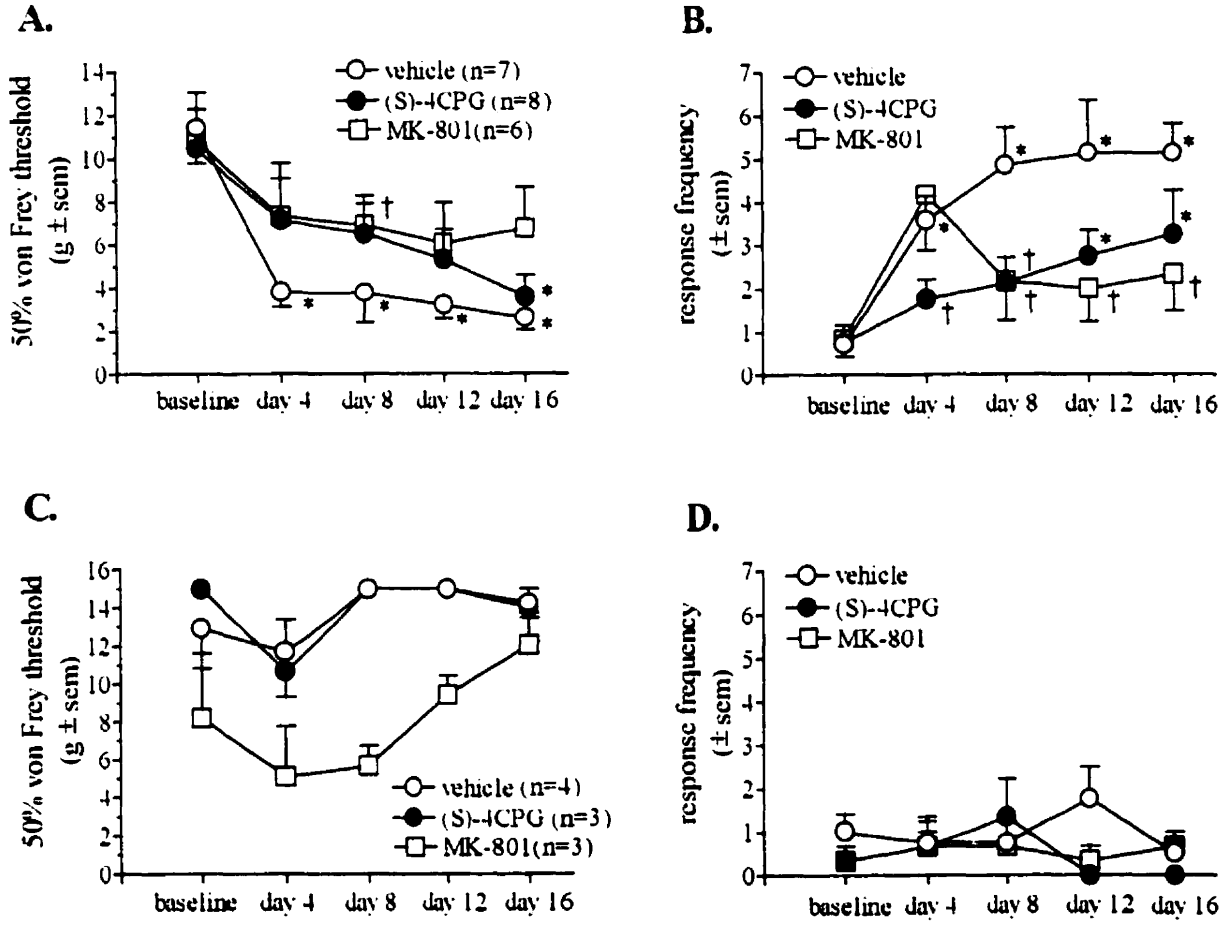

A.
50% von Frey threshold (g ± sem)
vehicle (n=7)
(S)-4CPG (n=8)
MK-801(n=6)
baseline
day 4
day 8
day 12
day 16
B.
response frequency (± sem)
vehicle
(S)-4CPG
MK-801
baseline
day 4
day 8
day 12
day 16
C.
50% von Frey threshold (g ± sem)
vehicle (n=4)
(S)-4CPG (n=3)
MK-801(n=3)
baseline
day 4
day 8
day 12
day 16
D.
response frequency (± sem)
vehicle
(S)-4CPG
MK-801
baseline
day 4
day 8
day 12
day 16

Fig. 2

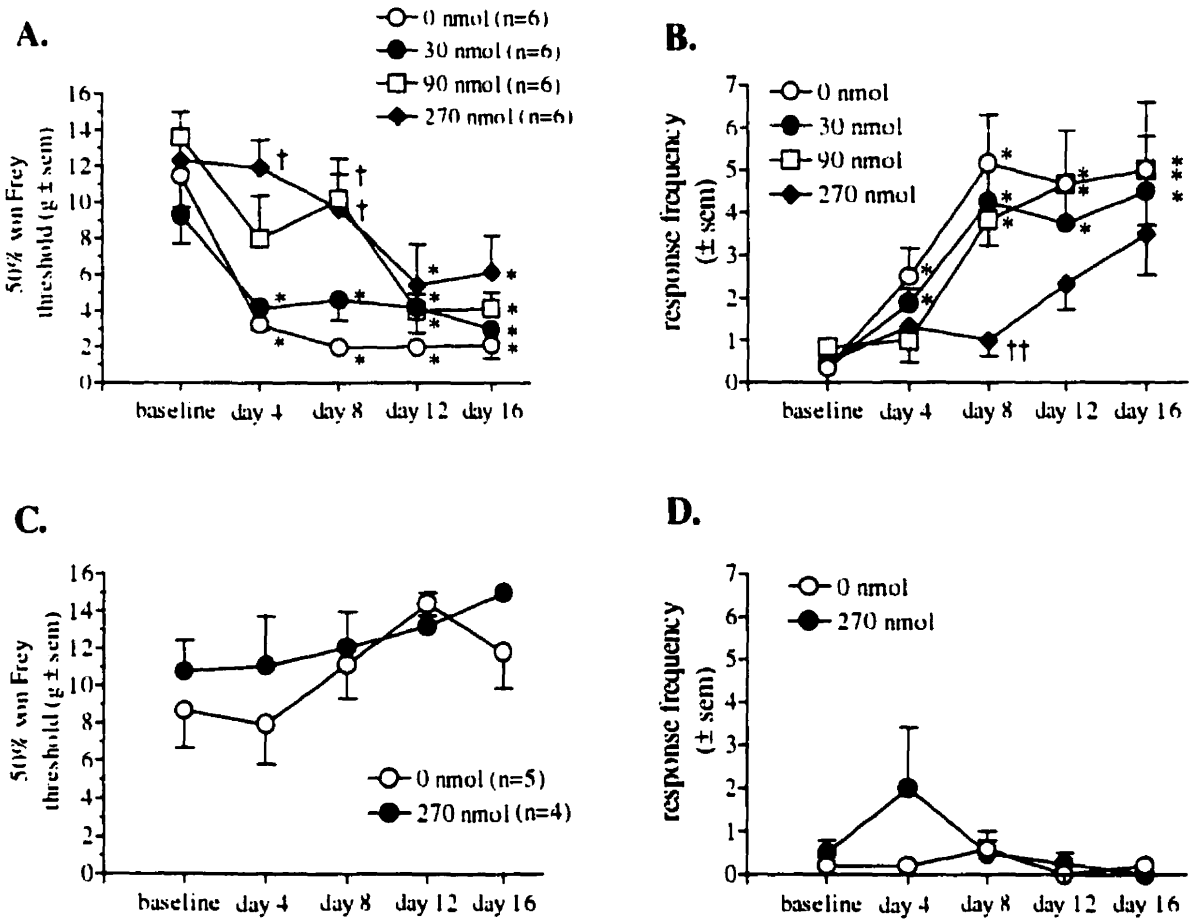
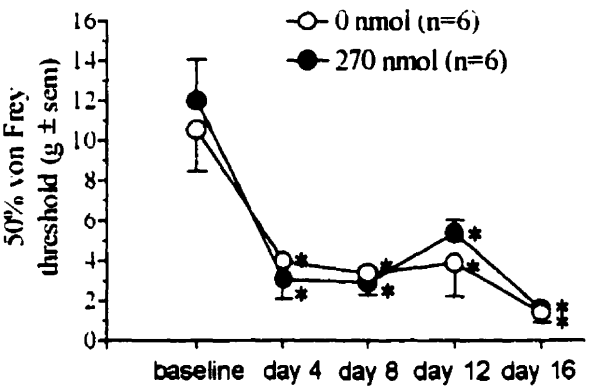
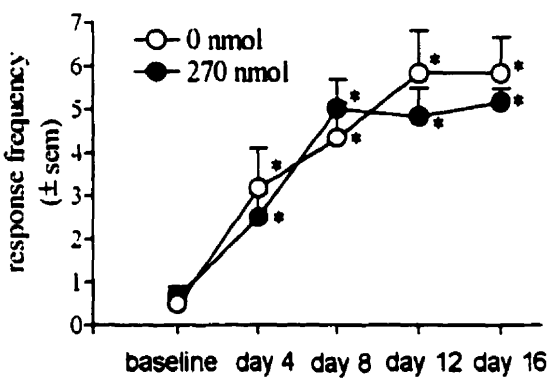

A.
0 nmol (n=6)
30 nmol (n=6)
90 nmol (n=6)
270 nmol (n=6)
50% von Frey threshold (g ± sem)
baseline
day 4
day 8
day 12
day 16
B.
0 nmol
30 nmol
90 nmol
270 nmol
response frequency (± sem)
C.
0 nmol (n=5)
270 nmol (n=4)
D.

Fig. 3

A.


0 nmol (n=6)
270 nmol (n=6)
50% von Frey threshold (g ± sem)
16
14
12
10
8
6
4
2
0
baseline
day 4
day 8
day 12
day 16

B.


0 nmol
270 nmol
response frequency (± sem)
7
6
5
4
3
2
1
0
baseline
day 4
day 8
day 12
day 16

# CHAPTER 6: THE ROLE OF mGluRs IN THE CCI MODEL

Rationale for Experiments

As reported in Study 4, we found that intrathecal administration of (*S*)-4CPG significantly reduces the development of mechanical allodynia and cold hyperalgesia in rats with CCI. Unfortunately, we could not state with absolute certainty that group I mGluRs are involved in the development of these nociceptive behaviours. It was possible that the compound's action at group II mGluRs contributed to its antinociceptive effects, particularly at the highest dose. Therefore, for the last study of my dissertation, we wanted to determine whether group I mGluR blockade and/or group II mGluR activation mediated the antinociceptive effects of (*S*)-4CPG in the CCI model. This study had become possible with the recent availability of (*RS*)-AIDA, a selective group I mGluR antagonist. To determine the possible contribution of group II mGluR activation we tested the effects of (2*R*,4*R*)-APDC. Also, we decided to explore the possible antinociceptive effects of the group III mGluR agonist, L-AP4, in the CCI model, given that this compound appeared to be the most effective compound for attenuating formalin-induced nociception (Study 1).

The main findings from this study are the following:

1. Intrathecal administration of (*RS*)-AIDA on post-operative days 0-3 significantly attenuated the CCI-related mechanical allodynia and cold hyperalgesia when animals were tested on post-operative day 4, suggesting that group I mGluRs are involved in the development of these nociceptive behaviours.

2. Intrathecal administration of the (2*R*,4*R*)-APDC or L-AP4, on post-operative days 0-3 significantly attenuated the CCI-related mechanical allodynia and cold hyperalgesia, respectively, when animals were tested on post-operative day 4. These data

demonstrate that activation of group II mGluRs attenuates the development of mechanical allodynia, while activation of group III mGluRs attenuates the development of cold hyperalgesia. The differential sensitivity of these types of CCI-related behaviours to group II or group III mGluR agonists may reflect differences in the localization of these mGluR subtypes within the spinal nociceptive pathways.

## STUDY 5

**Fisher, K. and Coderre, T.M. (1998). The effects of intrathecal administration of selective metabotropic glutamate receptor compounds in a rat model of neuropathic pain. Pain (*submitted*).**

# The effects of intrathecal administration of selective metabotropic glutamate receptor compounds in a rat model of neuropathic pain

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Running title: mGluRs and CCI-induced nociception

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Abstract

In the present study, we examined the effects of intrathecal treatment (twice daily injections on post-operative (PO) days 0-3) with the group I mGluR antagonist, (*RS*)-1-aminoindan-1,5-dicarboxylic acid ((*RS*)-AIDA), the group II mGluR agonist, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate ((2*R*,4*R*)-APDC), or the group III mGluR agonist, L-2-amino-4-phosphonobutyrate (L-AP4), on mechanical allodynia and cold hyperalgesia associated with chronic constriction injury (CCI) of the sciatic nerve in rats. Results demonstrated that, compared to vehicle, administration of (*RS*)-AIDA reduced both mechanical allodynia and cold hyperalgesia on PO day 4, but not on PO days 8, 12 or 16. However, administration of (2*R*,4*R*)-APDC or L-AP4 treatment reduced only mechanical allodynia or cold hyperalgesia, respectively, on PO day 4. These data not only provide evidence that group I mGluRs contribute to the development of mechanical allodynia and cold hyperalgesia associated with CCI in rats, but also suggest that the neuropathic pain-related behaviours are differentially sensitive to selective activation of group II or group III mGluRs.

*Key words:* Hyperalgesia; Allodynia; Chronic constriction injury; Metabotropic glutamate receptors; mGluR; Neuropathic pain

1. Introduction

The excitatory amino acids (EAAs), glutamate and aspartate, activate two classes of glutamate receptors in the central nervous system (CNS). Ionotropic glutamate receptors (iGluRs), such as *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazole-propionate (AMPA) and kainate, are coupled to cation channels, whereas, metabotropic glutamate receptors (mGluRs) are coupled to G proteins that modulate or activate intracellular messenger systems. Molecular cloning and pharmacological studies have demonstrated the existence of three groups of mGluRs. Specifically, group I mGluRs (mGluR1 and mGluR5) are linked to phosphoinositide hydrolysis (Abe et al. 1992; Aramori and Nakanishi 1992; Ito et al. 1992), whereas group II (mGluR2 and mGluR3) (Hayashi et al. 1993, 1994; Tanabe et al. 1993; Thomsen et al. 1994) and group III (mGluR4, mGluR6-8) (Nakajima et al. 1993; Tanabe et al. 1993; Thomsen et al. 1992; Okamoto et al. 1994; Saugstad et al. 1994) mGluRs are linked to the inhibition of cAMP.

It has been well established that EAAs are critically involved in the neural processes that mediate persistent and chronic nociception, and for the last decade much of the research has been focused on the role of iGluRs. For example, in neuropathic pain models, studies have demonstrated that systemic or intrathecal administration of NMDA receptor antagonists attenuates heat (Davar et al. 1991; Mao et al. 1992a,b, 1993; Yamamoto and Yaksh 1992; Tal and Bennett 1993, 1994; Eisenberg et al. 1995), cold (Fisher et al. 1998) and mechanical (Carlton and Hargett 1995; Qian et al. 1996; Hartrick et al. 1997) hyperalgesia, as well as thermal allodynia (Qian et al. 1996; Kim et al. 1997),

mechanical allodynia (Carlton and Hargett 1995; Qian et al. 1996; Chaplan et al. 1997; Hartrick et al. 1997; Kim et al. 1997; Fisher et al. 1998), and spontaneous nociceptive behaviours (Mao et al. 1992b, 1993; Qian et al. 1996) in neuropathic rats. Although several investigators have suggested that mechanical and thermal hyperalgesia are differentially sensitive to NMDA receptor antagonist treatment (Tal and Bennett 1994; Meller 1994), data from the above cited studies suggest that these neuropathic pain-related behaviours share an NMDA-mediated central mechanism. Studies have also provided evidence that the iGluR, AMPA/kainate, contributes to the development of thermal hyperalgesia following nerve injury in rats (Mao et al. 1992a).

With the recent synthesis of compounds that are selective for mGluRs, electrophysiological (Neugebauer et al. 1994; Young et al. 1994, 1995; Boxall et al. 1996; Budai and Larson 1998) and behavioural (Meller et al. 1993; Fisher and Coderre 1996a,b; Fisher and Coderre 1998a,b) studies have shown that mGluRs are also involved in spinal cord nociceptive processing in the rat. Recently, we provided the first piece of evidence that mGluRs contribute to the development of neuropathic pain-related behaviours with the demonstration that intrathecal treatment with the mGluR compound, (*S*)-4-carboxyphenylglycine ((*S*)-4CPG) attenuates mechanical allodynia and cold hyperalgesia in rats with chronic constriction injury (CCI) of the sciatic nerve (Fisher et al. 1998).

Since (*S*)-4CPG is a group I mGluR antagonist, as well as a group II agonist (Hayashi et al. 1994), it is unknown which mechanism contributed to the antinociceptive effects of the compound in the CCI model. Therefore, in the present study we examined the effects of intrathecal treatment with the selective group I mGluR antagonist, (*RS*)-1-

aminoindan-1,5-dicarboxylic acid ((*RS*)-AIDA) (Pellicciari et al. 1995; Moroni et al. 1997), or the selective group II mGluR agonist, (2*R*,4*R*)-4-aminopyrrolidine-2,4-dicarboxylate ((2*R*,4*R*)-APDC) (Schoepp et al. 1995), on the development of mechanical allodynia and cold hyperalgesia associated with CCI in rats. Since activation of group II or group III mGluRs appear to produce similar physiological effects in a number of regions in the rat CNS (Jane et al. 1994; Cao et al. 1995; Vignes et al. 1995), we also examined the intrathecal effects of the selective group III mGluR agonist, L-2-amino-4-phosphonobutyrate (L-AP4) (Thomsen et al. 1992) in this model.

## 2. Methods

### *2.1 Animals*

These experiments were performed on 102 male, Long-Evans hooded rats (Charles River) weighing 270-350 g. They were maintained under controlled lighting conditions with food and water available *ad libitum*. In all cases, the guidelines described in Care and Use of Experimental Animals of the Canadian Council of Animal Care (Vols. I and II) were strictly followed. All experiments were approved by the animal care committee at the Clinical Research Institute of Montreal.

### *2.2 Chronic constriction injury*

Prior to surgery, all rats were anaesthetized with sodium pentobarbital (65 mg/kg; i.p.). A chronic constriction injury was produced by placing a 2 mm length of polyethylene tubing (PE-90) around the sciatic nerve of each rat, according to the method of Mosconi and Kruger (1996). The cuff was placed after exposing the left sciatic nerve mid-thigh and isolating the nerve from surrounding tissue. Surgery for the sham condition involved exposing the left sciatic nerve, but the nerve was not manipulated or cuffed.

*2.3 Drug administration*

Rats were randomly assigned to one of the following groups: (*RS*)-AIDA (30, 90 or 270 nmol)/CCI; (*RS*)-AIDA (270 nmol)/sham; (2*R*,4*R*)-APDC (30, 90 or 270 nmol)/CCI; (2*R*,4*R*)-APDC (270 nmol)/sham; L-AP4 (30, 90 or 270 nmol)/CCI; L-AP4 (270 nmol)/sham; vehicle/CCI; vehicle/sham. Each animal was treated 15 min prior to surgery and then every 12 hours for 4 days (from post-operative (PO) day 0 to 3). All compounds were administered intrathecally by lumbar puncture, in a volume of 30 µl, between the L2 and L3 vertebrae, while rats were under brief halothane anesthesia. None of the animals exhibited motor disturbances at any time during the study. All compounds were obtained from Tocris Cookson, St. Louis, MO, USA (except (2*R*,4*R*)-APDC, which was graciously donated by Eli Lilly Laboratories, Indianapolis, IN) and dissolved in NaOH (pH 8).

*2.4 Sensory testing*

*2.4.1 Mechanical allodynia testing.*

Prior to baseline testing, each rat was habituated to a testing box (27 x 16 x 21 cm) with a wire-mesh grid floor, for a one hour period. For baseline and PO testing, a series of von Frey hairs (0.41, 0.70, 1.20, 2.00, 3.63, 5.50, 8.50, and 15.10 g) were applied through the grid floor to the ventral surface of the left hindpaw of each rat. Each hair was applied for a 7 second period or until the animal withdrew the hindpaw without ambulating. During each testing trial, the series of hairs were presented following an up-down procedure, described and validated by Chaplan et al. (1994), and the 50% response threshold was calculated for each rat.

*2.4.2 Cold hyperalgesia testing.*

For baseline and PO testing, each rat was placed in a 1 cm deep, 1°C water bath with a metal floor, for a 75 second period. During the testing period, a response was counted if the rat licked, shook, or showed a prolonged lift of the operated hindpaw when not ambulating.

Mechanical allodynia and cold hyperalgesia testing was performed 1-2 days prior to surgery to obtain baseline values, and then on PO days 4, 8, 12 and 16.

3. Results

The effects of four day intrathecal treatment with 0 (vehicle), 30, 90 or 270 nmol of the group I mGluR antagonist, (*RS*)-AIDA, on mechanical allodynia and cold hyperalgesia was assessed with Kruskal-Wallis ANOVA for multiple groups non-parametric data. Analyses of the 50% von Frey response thresholds recorded for the operated hindlimb of CCI rats, during the mechanical allodynia testing periods, indicated a significant main effect of treatment on PO day 4 ($\chi^2$ (3) = 7.71, $P$<0.05), but not on days 8 ($\chi^2$ (3) = 4.56, $P$>0.05), 12 ($\chi^2$ (3) = 2.33 $P$>0.05), or 16 ($\chi^2$ (3) = 6.74 $P$>0.05). Subsequent *post hoc* analyses (Mann-Whitney U test) revealed that, on PO day 4, animals treated with the 30, 90 or 270 nmol doses of (*RS*)-AIDA exhibited significantly higher 50% von Frey response thresholds than vehicle-treated animals (Fig. 1A), suggesting that all three doses of the group I mGluR antagonist were effective in reducing the development of mechanical allodynia in the CCI rats. Analyses of the cold water response frequencies recorded for the operated hindlimb of CCI rats indicated a significant main effect of treatment on PO day 4 ($\chi^2$ (3) = 10.0, $P$<0.05), but not on days 8 ($\chi^2$ (3) = 6.34

$P>0.05$), 12 ($\chi^2$ (3) = 1.67, $P>0.05$) or 16 ($\chi^2$ (3) = 5.04, $P>0.05$). Subsequent *post hoc* analyses (Mann-Whitney U test) revealed that, on PO day 4, animals treated with the 90 or 270 nmol doses, but not the 30 nmol dose of (*RS*)-AIDA responded less frequently than vehicle-treated animals during the 75 sec testing period (Fig. 1B), suggesting that the two highest doses of the group I mGluR antagonist were effective in reducing the development of cold hyperalgesia in the CCI rats.

The effects of four day intrathecal treatment with 0 (vehicle), 30, 90 or 270 nmol of the group II mGluR agonist, (2*R*,4*R*)-APDC, on mechanical allodynia and cold hyperalgesia were assessed. Kruskal-Wallis ANOVA, for the 50% von Frey response thresholds for the operated hindlimb of CCI rats, indicated a significant main effect of treatment on PO day 4 ($\chi^2$ (3) = 11.73, $P<0.01$), but not on days 8 ($\chi^2$ (3) = 5.51, $P>0.05$), 12 ($\chi^2$ (3) = 1.08 $P>0.05$), or 16 ($\chi^2$ (3) = 6.40, $P>0.05$). Subsequent *post hoc* analyses (Mann-Whitney U test) revealed that, on PO day 4, animals treated with 30, 90 or 270 nmol of (2*R*,4*R*)-APDC exhibited significantly higher 50% von Frey response thresholds than vehicle-treated animals (Fig. 2A), suggesting that all three doses of the group II mGluR agonist were effective in reducing the development of mechanical allodynia in the CCI rats. Kruskal-Wallis ANOVA, for the cold water response frequencies for the operated hindlimb of CCI rats, indicated that the main effect of treatment was not significant on PO days 4 ($\chi^2$ (3) = 3.58, $P>0.05$), 8 ($\chi^2$ (3) = 5.08 $P>0.05$), 12 ($\chi^2$ (3) = 3.85, $P>0.05$) or 16 ($\chi^2$ (3) = 3.58, $P>0.05$) (Fig. 2B), suggesting that intrathecal treatment with the group II mGluR agonist did not attenuate the development of cold hyperalgesia in CCI rats.

The effects of four day intrathecal treatment with 0 (vehicle), 30, 90 or 270 nmol of the group III mGluR agonist, L-AP4, on mechanical allodynia and cold hyperalgesia were assessed. Kruskal-Wallis ANOVA, for the 50% von Frey response thresholds for the operated hindlimb of CCI rats, indicated that the main effect of treatment was not significant on PO days 4 ($\chi^2$ (3) = 7.05, $P$>0.05), 8 ($\chi^2$ (3) = 3.84, $P$>0.05), 12 ($\chi^2$ (3) = 0.43 $P$>0.05), or 16 ($\chi^2$ (3) = 3.90 $P$>0.05) (Fig. 3A), suggesting that intrathecal treatment with the group III mGluR agonist did not attenuate the development of mechanical allodynia in the CCI rats. Kruskal-Wallis ANOVA, for the cold water response frequencies for the operated hindlimb of CCI rats, indicated a significant main effect of treatment on PO day 4 ($\chi^2$ (3) = 9.58, $P$<0.05), but not on days 8 ($\chi^2$ (3) = 4.34, $P$>0.05), 12 ($\chi^2$ (3) = 1.67, $P$>0.05) or 16 ($\chi^2$ (3) = 5.04, $P$>0.05). Subsequent *post hoc* analyses (Mann-Whitney U test) revealed that, on PO day 4, animals treated with the 90 and 270 nmol doses, but not 30 nmol dose of L-AP4 responded less frequently than the vehicle-treated animals during the 75 sec testing period (Fig. 3B), suggesting that the two highest doses of the group III mGluR agonist were effective in reducing the development of cold hyperalgesia in the CCI rats.

The effects of four day intrathecal treatment with vehicle or 270 nmol of (*RS*)-AIDA, (2*R*,4*R*)-APDC or L-AP4 were also assessed in sham animals. Kruskal-Wallis ANOVA, for the 50% von Frey response thresholds and the cold water response frequencies for the operated hindlimb of sham animals, indicated no significant main effect of treatment on any of the PO testing days. These results suggest that, although intrathecal treatment with a selective group I mGluR antagonist, group II mGluR agonist

or group III mGluR agonist attenuates the development of mechanical allodynia and/or cold hyperalgesia in CCI animals, administration of the highest dose of these compounds (270 nmol) does not significantly alter the sensory responses of sham animals.

4. Discussion

Recently, we provided evidence that mGluRs play a role in the development of neuropathic pain-related behaviours in the CCI model with the demonstration that intrathecal treatment with the mGluR compound, (*S*)-4CPG, on PO days 0-3, attenuates mechanical allodynia and cold hyperalgesia on PO days 4 and 8, in CCI rats (Fisher et al. 1998). Although (*S*)-4CPG is a potent antagonist at group I mGluRs, we could not rule out the possibility that the compound's agonism of group II mGluRs also contributed to the antinociceptive effects in the CCI model. Therefore, the aim of the present study was to determine the specific contribution of group I mGluRs to the development of mechanical allodynia and cold hyperalgesia associated with CCI, using the selective group I mGluR antagonist, (*RS*)-AIDA (Pellicciari et al. 1995; Moroni et al. 1997), and to assess the possible antinociceptive effects of the selective group II mGluR agonist, (2*R*,4*R*)-APDC (Schoepp et al. 1995) in the model. We also assessed the intrathecal effects of the selective group III mGluR agonist, L-AP4 (Thomsen et al. 1992), which we have previously demonstrated produces antinociceptive effects in the rat formalin test (Fisher and Coderre, 1996a).

In the present study, we demonstrated that intrathecal treatment with 30, 90 or 270 nmol of the selective group I mGluR antagonist, (*RS*)-AIDA, on PO days 0-3 significantly reduced mechanical allodynia and cold hyperalgesia on PO day 4. It should be noted that although Moroni et al. (1997) reported that intracerebroventricular

administration of (*RS*)-AIDA increased nociceptive thresholds in naive rats, we did not find that intrathecal (*RS*)-AIDA was analgesic in the sham animals. It is likely that the discrepancy between the two studies is attributable to differences in route of administration. Interestingly, in the present study we also found that the neuropathic pain-related behaviours exhibited by the CCI rats were differentially modulated by the group II and group III mGluR agonists. Specifically, we found that intrathecal treatment with (2*R*,4*R*)-APDC (30, 90 or 270 nmol) and L-AP4 (90 or 270 nmol), on PO days 0-3, significantly attenuated mechanical allodynia and cold hyperalgesia, respectively, on PO day 4 in CCI rats.

First, results from this study suggest that the antinociceptive effects of (*S*)-4CPG treatment in the CCI model (Fisher et al. 1998) are likely attributable to the compound's antagonism of group I mGluRs, as well as to its agonism of group II mGluRs. It is interesting that, according to the vehicle-control/experimental group comparisons within each study, (*S*)-4CPG treatment produced a more prolonged attenuation of the nociceptive behaviours, which was observed up to PO day 8, whereas treatment with the more selective compounds, (*RS*)-AIDA or (2*R*,4*R*)-APDC attenuated the nociceptive behaviours on PO day 4 only.

Second, results from the present study also suggest that group I mGluRs contribute to the spinal cord mechanisms that mediate the development of mechanical allodynia and cold hyperalgesia associated with CCI in rats. These data are consistent with neuroanatomical evidence demonstrating that this group of mGluRs are localized within the spinal somatosensory/nociceptive pathways. For example, Valerio, Paterlini, Boifava, Memo and Spano, (1997) have reported that mGluR1a, mGluR5a, mGluR5b,

but not mGluR1b mRNA, are highly expressed in the rat spinal cord. Other studies have demonstrated that mGluR5a is strongly expressed in laminae I and II and gradually decreases toward the deeper layers of the rat dorsal horn (Vidnyánszky et al. 1994; Valerio, Rizzonelli, Paterlini, Moretto, Knöpfel, Kuhn, Memo and Spano, 1997). Interestingly, Vidnyánszky et al. (1994) found that mGluR5a immunoreactive dendrites in laminae I and II are often targeted by synaptic boutons of presumed polymodal primary afferent C-fiber terminals.

Results from this study are also consistent with electrophysiological evidence suggesting that activation of spinal group I mGluRs enhances dorsal horn neuronal responses to C-fiber-evoked, as well as to tactile stimulation. For example, Budai and Larson (1998) demonstrated that iontophoretic application of the non-selective mGluR agonist, (1*S*,3*R*)-ACPD, to the rat spinal cord significantly increases dorsal horn neuronal responses to innocuous mechanical stimulation and to high frequency C-fiber stimulation. Further, these investigators also showed that iontophoretic application of the group I mGluR antagonist/group II mGluR agonist, (*S*)-4C3HPG, blocked the (1*S*,3*R*)-ACPD-evoked and the C-fiber-evoked potentiation of dorsal horn neuronal responses. Others have provided electrophysiological evidence suggesting that group I mGluRs contribute to inflammation-related enhancement of spinal cord neuronal function. For example, iontophoretic application of the non-selective mGluR antagonists, L-AP3, or (*RS*)-4C3HPG attenuates the dorsal horn neuronal responses to repeated cutaneous application of mustard oil (Young et al. 1994, 1995) and to innocuous and noxious mechanical stimulation following inflammation of the knee joint (Neugebauer et al. 1994) in the rat. Furthermore, we have demonstrated that selective activation of spinal group I mGluRs

produces persistent mechanical hyperalgesia, mechanical allodynia, and heat hyperalgesia in rats (Fisher and Coderre 1998a).

Together, neuroanatomical, electrophysiological and behavioural studies provide convincing evidence that group I mGluRs are not only involved in the transmission of somatosensory/nociceptive information within the spinal cord, but also contribute to the alterations in dorsal horn neuronal function mediating persistent and chronic nociception. The suggestion that group I mGluRs contribute to spinal cord plasticity is consistent with studies demonstrating that selective activation of this group of mGluRs enhances neuronal excitability in a number of regions in the rat CNS (Ito et al. 1992; Davis and Laroche 1996; Manahan-Vaughan and Reymann 1996; Abbott et al. 1997; Netzeband et al. 1997; O'Leary and O'Connor 1997; Schoppa and Westbrook 1997; Schrader and Tasker 1997). Also, activation of group I mGluRs has been proposed to potentiate NMDA- and AMPA-mediated responses in the rat dorsal horn (Bleakman et al. 1992; Cerne and Randic 1992; Bond and Lodge 1995). Given that selective activation of primary afferent Aβ-, Aδ- or C-fibers increases the outflow of glutamate and aspartate from rat dorsal horn slices (Kangrga and Randic 1991), we propose that the CCI-related spontaneous discharges of injured primary afferent Aβ-, Aδ- or C-fibers (Kajander and Bennett 1992), and the subsequent accumulation of glutamate and/or aspartate in the dorsal horn (Kawamata and Omote 1996) activates spinal group I mGluRs. Activation of group I mGluRs in the superficial laminae or in the deeper laminae may contribute to the persistent sensitization of dorsal horn neurons that respond to noxious cold stimulation or innocuous mechanical stimulation, respectively.

The finding from the present study that treatment with (2*R*,4*R*)-APDC or L-AP4 differentially attenuated mechanical allodynia and cold hyperalgesia in CCI rats was surprising. Group II and group III mGluRs appear to have similar physiological roles in the CNS, such that selective activation of group II or group III mGluRs depresses excitatory transmission in a number of regions in the brain (Baskys and Malenka 1991; Lovinger and McCool 1995; Manzoni et al. 1995; Vignes et al. 1995; Kamiya et al. 1996; Macek et al. 1996; Pisani et al. 1997) and ventral spinal cord (Jane et al. 1994, 1995, 1996; Kemp et al. 1994; Cao et al. 1995; Thomas et al. 1996). Also, activation of group II (Lombardi et al. 1994; East et al. 1995; Battaglia et al. 1997; Cozzi et al. 1997) or group III (East et al. 1995; Vázquez, Budd, Herrero, Nicholls and Sánchez-Prieto, 1995; Vázquez, Herrero, Miras-Portugal and Sánchez-Prieto, 1995) mGluRs decreases evoked EAA release in the rat CNS. Therefore, we propose that the differential effects of (2*R*,4*R*)-APDC and L-AP4 may be related to differences between group II and group III mGluR localization within the somatosensory/nociceptive spinal pathway.

Studies have demonstrated that group III mGluRs are intensely expressed in axon terminals of presumed nociceptive primary afferent fibers terminating in laminae I and II of the rat dorsal horn (mGluR7) (Ohishi, Nomura, Ding, Shigemoto, Wada, Kinoshita, Li, Neki, Nakanishi and Mizuno, 1995; Li et al. 1997) and in the cell bodies of neurons in the dorsal root ganglia (mGluR4 and mGluR7) (Ohishi, Akazawa, Shigemoto, Nakanishi & Mizuno, 1995; Li et al. 1996). In contrast, group II mGluRs have been shown to be localized on neuronal cell bodies in the dorsal horn and lamina X of the rat spinal cord (mGluR3) (Ohishi et al. 1993). Although electrophysiological studies have provided evidence that both group II and group III mGluRs are localized on primary afferent fibers

or on interneurons presynaptic to ventral horn neurons (Jane et al. 1994, 1995, 1996; Kemp et al. 1994; Cao et al. 1995; Thomas et al. 1996), to our knowledge, there is no evidence that group II mGluRs are expressed on primary afferent terminals presynaptic to dorsal horn neurons in the rat. Therefore, we speculate that activation of postsynaptic group II mGluRs may reduce the CCI-related sensitization dorsal horn neurons that respond to innocuous mechanical stimulation, and thus there is a reduction in mechanical allodynia after intrathecal treatment with a group II mGluR agonist. The hypothesis that group II mGluRs modulate spinal cord plasticity is consistent with the demonstration that selective activation of group II mGluRs inhibits hippocampal long-term potentiation in the rat (Holscher et al. 1997; Huang et al. 1997), a model of synaptic plasticity, and attenuates NMDA-induced excitotoxicity in the mouse CNS (Bruno et al. 1994, 1995; Buisson et al. 1996) *in vivo* or *in vitro*.

Evidence suggests that activity in primary afferent C-fibers contributes to the development of thermal hyperalgesia in models of neuropathic pain (Shir and Seltzer 1990; Meller et al. 1992), and that activation of presynaptic group III mGluRs depresses excitatory transmission in the brain (Manzoni et al. 1995; Vignes et al. 1995; Macek et al. 1996) and spinal cord (Jane et al. 1994; Kemp et al. 1994; Cao et al. 1995; Jane et al. 1995; Jane et al. 1996; Thomas et al. 1996) by reducing the release of EAAs. Thus, we propose that intrathecal L-AP4 may activate presynaptic group III mGluRs localized on primary afferent nociceptive fiber terminals and reduce the EAA output to the superficial laminae of the dorsal horn. As a result, a group III mGluR-mediated decrease in EAA release may attenuate the CCI-induced alterations in dorsal horn neuronal function

underlying thermal hyperalgesia in the rats and account for the reduction in cold hyperalgesia produced by a group III mGluR agonist.

In conclusion, we demonstrated that intrathecal treatment with the selective group I mGluR antagonist, (*RS*)-AIDA, attenuates mechanical allodynia and cold hyperalgesia associated with CCI in rats, which suggests that spinal group I mGluRs contribute to the development of these pain-related behaviours. Also, the finding that treatment with (2*R*,4*R*)-APDC, or L-AP4, attenuates the development of mechanical allodynia or cold hyperalgesia, respectively, suggests that these nociceptive behaviours can be differentially reduced by selective activation of group II or group III mGluRs. These results may be attributable to the localization of the two distinct groups of mGluRs within the spinal somatosensory/nociceptive pathways.

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References

Abbott, A., Wigmore, M.A., and Lacey, M.G., Excitation of rat subthalamic nucleus neurones *in vitro* by activation of a group I metabotropic glutamate receptor, Brain Res., 766 (1997) 162-167.

Abe, T., Sugihara, H., Nawa, H., Shigemoto, R., Mizuno, N. and Nakanishi, S., Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/$Ca^{2+}$ signal transduction, J. Biol. Chem., 267 (1992) 13361-13368.

Aramori, I., and Nakanishi, S., Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells, Neuron, 8 (1992) 757-765.

Baskys, A., and Malenka, R.C., Agonists at metabotropic glutamate receptors presynaptically inhibit EPSCs in neonatal rat hippocampus, J. Physiol. (Lond.), 444 (1991) 687-701.

Battaglia, G., Monn, J.A., and Schoepp, D.D. *In vivo* inhibition of veratridine-evoked release of striatal excitatory amino acids by the group II metabotropic glutamate receptor agonist LY354740 in rats, Neurosci. Lett., 229 (1997) 161-164.

Bleakman, D., Rusin, K.I., Chard, P.S., Glaum, S.R., and Miller, R.J., Metabotropic glutamate receptors potentiate ionotropic glutamate responses in the rat dorsal horn, Mol. Pharmacol., 42 (1992) 192-196.

Bond, A., and Lodge, D., Pharmacology of metabotropic glutamate receptor-mediated enhancement of responses to excitatory and inhibitory amino acids on rat spinal neurones *in vivo*, Neuropharmacol., 34 (1995) 1015-1023.

Boxall, S.J., Thompson, S.W.N., Dray, A., Dickenson, A.H., and Urban, L., Metabotropic glutamate receptor activation contributes to nociceptive reflex activity in the rat spinal cord *in vitro*, Neuroscience, 74 (1996) 13-20.

Bruno, V., Battaglia, G., Copani, A., Giffard, R.G., Raciti, G., Raffaele, R., Shinozaki, H., and Nicoletti, F., Activation of class II or class III metabotropic glutamate receptors prevents cultured cortical neurons against excitotoxicity degeneration, Eur. J. Pharmacol., 7 (1995) 1906-1913.

Bruno, V., Copani, A., Battaglia, G., Raffaele, R., Shinozaki, H., and Nicoletti, F., Protective effect of the metabotropic glutamate receptor agonist, DCG-IV, against excitotoxicity neuronal death, Eur. J. Pharmacol., 256 (1994) 109-112,

Budai, D., and Larson, A.A., The involvement of metabotropic glutamate receptors in sensory transmission in dorsal horn of the rat spinal cord, Neuroscience, 83 (1998) 571-580.

Buisson, A., Yu, S.P., and Choi, D.W., DCG-IV selectively attenuates rapidly triggered NMDA-induced neurotoxicity in cortical neurons, Eur. J. Neurosci., 8 (1996) 138-143.

Cao, C.Q., Evans, R.H., Headley, P.M., and Udvarhelyi, P.M., A comparison of the effects of selective metabotropic glutamate receptor agonist on synaptically evoked whole cell currents of rat spinal ventral horn neurones *in vivo*, Br. J. Pharmacol., 115 (1995) 1469-1474.

Carlton, S.M., and Hargett, G.L., Treatment with the NMDA antagonist memantine attenuates nociceptive responses to mechanical stimulation in neuropathic rats, Neurosci. Lett., 198 (1995) 115-118.

Cerne, R., and Randic, M., Modulation of AMPA and NMDA responses in rat spinal dorsal horn neurons by *trans*-1-aminocyclopentante-1,3-dicarboxylic acid, Neurosci. Lett., 144 (1992) 180-184.

Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M., and Yaksh, T.L., Quantitative assessment of tactile allodynia in the rat paw, J. Neurosci. Meth., 53 (1994) 55-63.

Chaplan, S.R., Malmberg, A.B., and Yaksh, T.L., Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat, J. Pharmacol. Exp. Ther., 280 (1997) 829-838.

Cozzi, A., Attucci, S., Peruginelli, F., Marinozzi, M., Luneia, R., Pellicciari, R., and Moroni, F. Type 3 metabotropic glutamate (mGlu) receptors tonically inhibit transmitter release in rat caudate nucleus: in vivo studies with (2*S*,1'*S*,2'*S*,3'*R*)-2-(2'-carboxy-3'-phenylcyclopropyl)-glycine, a new potent and selective antagonist, Eur. J. Neurosci., 9 (1997) 1350-1355.

Davar, G., Hama, A., Deykin, A., Vos, B., and Maciewicz, R., MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy, Brain Res., 553 (1991) 327-330.

Davis, S., and Laroche, S., Activation of metabotropic glutamate receptors induce differential effects on synaptic transmission in the dentate gyrus and CA1 of the hippocampus in the anaesthetized rat, Neuropharmacol., 35 (1996) 337-346.

East, S.J., Hill, M.P., and Brotchie, J.M. Metabotropic glutamate receptor agonists inhibit endogenous glutamate release from rat striatal synaptosomes, Eur. J. Pharmacol., 277 (1995) 117-121.

Eisenberg, E., LaCross, S., and Strassman, A.M., The clinically tested *N*-methyl-D-aspartate receptor antagonist memantine blocks and reverses thermal hyperalgesia in a rat model of painful mononeuropathy, Neurosci. Lett., 187 (1995) 15-20.

Fisher, K., and Coderre, T.J., The contribution of metabotropic glutamate receptors (mGluRs) to formalin-induced nociception, Pain, 68 (1996a) 255-263.

Fisher, K., and Coderre, T.J., Comparison of nociceptive effects produced by intrathecal administration of mGluR agonists, NeuroReport, 7 (1996b) 2743-2747.

Fisher, K., and Coderre, TJ., Hyperalgesia and allodynia induced by intrathecal (*RS*)-dihydroxyphenylgycine administration in rats, NeuroReport (1998a) *in press.*

Fisher, K., Fundytus, M.E., Cahill, C.M., and Coderre, T.J., Intrathecal administration of the mGluR compound, (*S*)-4CPG, attenuates hyperalgesia and allodynia associated with sciatic nerve constriction injury in rats, Pain (1998) *in press*.

Hartrick, C.T., Wise, J.J., and Pattterson, J.S., Preemptive intrathecal ketamine delays mechanical hyperalgesia in the neuropathic rat, Anesth. Analg. 86 (1997) 557-560.

Hayashi, Y., Momiyama, A., Takahashi, T., Ohishi, H., Ogawa-Meguro, R., Shigemoto, R., Mizuno, N., and Nakanishi, S., Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb, Nature, 366 (1993) 687-690.

Hayashi, Y., Sekiyama, N., Nakanishi, S., Jane, D.E., Sunter, D.C., Birse, E.F., Udvarhelyi, P.M., and Watkins, J.C., Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes, J. Neurosci., 14 (1994) 3370-3377.

Holscher, C., Anwyl, R., and Rowan, M.J., Activation of group II metabotropic glutamate receptors blocks induction of long-term potentiation and depotentiation in area CA1 of the rat *in vivo*, Eur. J. Pharmacol., 322 (1997) 155-163.

Huang, L., Breakwell, N.A., Rowan, M.J., and Anwyl, R., PCC-IV inhibits the induction of long-term potentiation in the dentate gyrus *in vitro*, Eur. J. Pharmacol., 332 (1997) 161-165.

Ito, I., Kohda, A., Tanabe, S., Hirose, E., Hayashi, E., Mitsunaga, S. and Sugiyama, H., 3,5-Dihydroxyphenylglycine: a potent agonist of metabotropic glutamate receptors, NeuroReport, 3 (1992) 1013-1016.

Jane, D.E., Jones, P.L.St.J., Pook, P.C.-K., Tse, H.-W. and Watkins, J.C., Actions of two new antagonists showing selectivity for different sub-types of metabotropic glutamate receptor in the neonatal rat spinal cord, Br. J. Pharmacol., 112 (1994) 809-816.

Jane, D.E., Pittaway, K., Sunter, D.C., Thomas, K.N., and Watkins, J.C., New phenylglycine derivatives with potent and selective antagonist activity at presynaptic glutamate receptors in neonatal rat spinal cord, Neuropharmacol., 34 (1995) 851-856.

Jane, D.E., Thomas, N.K., Tse, H.W., and Watkins, J.C., Potent antagonists at the L-AP4- and (1*S*,3*S*)-ACPD-sensitive presynaptic metabotropic glutamate receptors in the neonatal rat spinal cord, Neuropharmacol., 35 (1996) 1029-1035.

Kajander, K.C., and Bennett, G.J., Onset of a painful peripheral neuropathy in rat: a partial and differential deafferentation and spontaneous discharge in Aβ and Aδ primary afferent neurons, J. Neurophysiol., (1992) 734-744.

Kamiya, H., Shinozaki, H., and Yamamoto, C., Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses, J. Physiol., 493 (1996) 447-455.

Kangrga, I., and Randic, M., Outflow of endogenous aspartate and glutamate from the rat spinal dorsal horn *in vitro* by activation of low-and high-threshold primary afferent fibers. Modulation by μ-opioids, Brain Res., 553 (1991) 347-352.

Kawamata, M., and Omote, K., Involvement of increased excitatory amino acids and intracellular $Ca^{2+}$ concentrations in the spinal dorsal horn in an animal model of neuropathic pain, Pain, 68 (1996) 85-96.

Kemp, M., Roberts, P., Pook, P., Jane, D., Jones, A., Jones, P., Sunter, D., Udvarhelyi, P., and Watkins, J., Antagonism of presynaptically mediated depressant responses and cyclic AMP-coupled metabotropic glutamate receptors, Eur. J. Pharmacol., 266 (1994) 187-192.

Kim, Y.I., Na, H.S., Yoon, Y.W., Han, H.C., Ko, K.H., and Hong, S.K., NMDA receptors are important for both mechanical and thermal allodynia from peripheral nerve injury in rats, NeuroReport, 8 (1997) 2149-2153.

Li, H., Ohishi, H., Kinoshita, A., Shigemoto, R., Nomura, S. and Mizuno, N., Localization of a metabotropic glutamate receptor, mGluR7, in axon terminals of presumed nociceptive, primary afferent fibers in the superficial layers of the spinal dorsal horn: an electron microscope study in the rat, Neurosci. Lett., 223 (1997) 153-156.

Li, J.L., Ohishi, H., Kaneko, T., Shigemoto, R., Neki, A., Nakanishi, S., and Muzuno, N., Immunohistochemical localization of a metabotropic glutamate receptor, mGluR7, in ganglion neurons of the rat; with special reference to the presence of glutamatergic ganglion neurons, Neurosci. Lett., 204 (1996) 9-12.

Lombardi, G., Pellegrini-Giampietro, D.E., Leonard, P., Cherici, G., Pellicciari, R., and Moroni, F., The depolarization-induced outflow of D-[$^3$H]aspartate from rat brain slices is modulated by metabotropic glutamate receptors, Neurochem. Int., 24 (1994) 525-532.

Lovinger, D.M., and McCool, B.A., Metabotropic glutamate receptor-mediated presynaptic depression at corticostriatal synapses involves mGluR2 or 3, J. Neurophysiol., 73 (1995) 1076-1083.

Macek, T.A., Winder, D.G., Gereau, R.W. 4th., Ladd, C.O., and Conn, P.J., Differential involvement of group II and group III mGluRs as autoreceptors at lateral and medial perforant path synapses, J. Neurophysiol., 76, (1996) 3798-3806.

Manahan-Vaughan, D., and Reymann, K.G., Metabotropic glutamate receptor subtype agonists facilitate long-term potentiation within a distinct time window in the dentate gyrus *in vivo*, Neuroscience, 74 (1996) 723-731.

Manzoni, O.J., Castillo, P.E., and Nicoll, R.A., Pharmacology of metabotropic glutamate receptors at the mossy fiber synapses of the guinea pig hippocampus, Neuropharmacol., 34 (1995) 965-971.

Mao, J., Price, D.D., Hayes, R.L., Lu J., Mayer, D.J., and Frenk, H., Intrathecal treatment with dextrorphan or ketamine potently reduces pain-related behaviours in a rat model of peripheral mononeuropathy, Brain Res., 605 (1993) 164-168.

Mao, J., Price, D.D., Hayes, R.L., and Mayer, D.J., Differential role of NMDA and non-NMDA receptor activation in induction and maintenance of thermal hyperalgesia in rats with painful peripheral mononeuropathy, Brain Res., 598 (1992a) 271-278.

Mao, J., Price, D.D., Hayes, R.L. and Mayer, D.J., Intrathecal MK-801 and local nerve anesthesia synergistically reduce nociceptive behaviours in rats with experimental peripheral mononeuropathy, Brain Res., 576 (1992b) 254-262.

Meller, S.T., Thermal and mechanical hyperalgesia: a distinct role for different excitatory amino acid receptors and signal transduction pathways? APS J., 3 (1994) 215-231.

Meller, S.T., Dykstra, C.L., and Gebhart, G.F., Acute mechanical hyperalgesia is produced by coactivation of AMPA and metabotropic glutamate receptors, NeuroReport, 4 (1993) 879-882.

Meller, S.T., Gebhart, G.F., and Maves, T.J., Neonatal capsaicin treatment prevents the development of the thermal hyperalgesia produced in a model of neuropathic pain in the rat, Pain, 51 (1992) 317-321.

Moroni, F., Lombardi, G., Thomsen, C., Leonardi, P., Attucci, S., Peruginelli, F., Torregrossa, S.A., Pellegrini-Giampietro, D.E., Luneia, R., and Pellicciari, R., Pharmacological characterization of 1-aminoindan-1,5-dicarboxylic acid, a potent mGluR1 antagonist. J. Pharmacol. Exp. Ther., 281 (1997) 721-729.

Mosconi, T., and Kruger, L., Fixed-diameter polyethylene cuffs applied to the rat sciatic nerve induce a painful neuropathy: ultrastructural morphometric analysis of axonal alterations, Pain, 64 (1996) 37-57.

Nakajima, Y., Iwakabe, H., Akazawa, C., Nawa, H., Shigemoto, R., Mizuno, N. and Nakanishi, S., Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate, J. Biol. Chem., 268 (1993) 11868-11873.

Netzeband, J.G., Parsons, K.L., Sweeney, D.D., and Gruol, D.L., Metabotropic glutamate receptor agonists alter neuronal excitability and $Ca^{2+}$ levels via the phospholipase C transduction pathway in cultured Purkinje neurons, J. Neurophysiol., 78 (1997) 63-75.

Neugebauer, V., Lücke, T., and Schaible, H.G., Requirement of metabotropic glutamate receptors for the generation of inflammation-evoked hyperexcitability in rat spinal cord neurons, Eur. J. Neurosci., 6 (1994) 1179-1186.

Ohishi, H., Akazawa, C., Shigemoto, R., Nakanishi, S., and Mizuno, N., Distribution of the mRNAs for L-2-amino-4-phosphonobutyrate-sensitive metabotropic glutamate

receptors, mGluR4 and mGluR7, in the rat brain, J. Comp. Neurol., 360 (1995) 555-570.

Ohishi, H., Nomura, S., Ding, Y.Q., Shigemoto, R., Wada, E., Kinoshita, A., Li, J.L., Neki, A., Nakanishi, S., and Mizuno, N. Presynaptic localization of a metabotropic glutamate receptor, mGluR7, in the primary afferent neurons: an immunohistochemical study in the rat, Neurosci. Lett., 202 (1995) 85-88.

Ohishi, H., Shigemoto, R., Nakanishi, S., and Mizuno, N. Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: an *in situ* hybridization study, J. Comp. Neurol., 335 (1993) 252-266.

Okamoto, N., Hori, S., Akazawa, C., Hayashi, Y., Shigemoto, R., Mizuno, N., and Nakanishi, S., Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction, J. Biol. Chem., 269 (1994) 1231-1236.

O'Leary, D.M., and O'Connor, J.J., Potentiation of synaptic transmission the rat dentate gyrus *in vitro* by (*S*)-3,5-dihydroxyphenylglycine ((*S*)-DHPG), Neurosci. Lett., 229 (1997) 29-32.

Pellicciari, R., Luneia, R., Costantino, G., Marinozzi, M., Natalini, B., Jakobsen, P., Kanstrup, A., Lombardi, G., Moroni, F., and Thomsen, C., 1-aminoindan-1,5-dicarboxylic acid: a novel antagonist at phospholipase C-linked metabotropic glutamate receptors, J. Med. Chem., 38 (1995) 3717-3719.

Pisani, A., Calabresi, P., Centonze, D., and Bernardi, G., Activation of group III metabotropic glutamate receptors depresses glutamatergic transmission at corticostriatal synapse, Neuropharmacol., 36 (1997) 845-851.

Qian, J., Brown, S.D. and Carlton, S.M., Systemic ketamine attenuates nociceptive behaviours in a rat model of peripheral neuropathy, Brain Res., 715 (1996) 51-62.

Saugstad, J.A., Kinzie, J.M., Mulvihill, E.R., Segerson, T.P., and Westbrook, G.L., Cloning an expression of a new member of the L-2-amino-4-phosphonbutyric acid-class of metabotropic glutamate receptors, Mol. Pharmacol., 45 (1994) 367-372.

Schoepp, D.D., Johnson, B.G., Salhoff, C.R., Valli, M.J., Desai, M.A., Burnett, J.P., Mayne, N.G., and Monn, J.A., Selective inhibition of forskolin-stimulated cyclic AMP formation in rat hippocampus by a novel mGluR agonist, 2R,4R-aminopyrrolidine-2,4-dicarboxylate, Neuropharmacol., 34 (1995) 843-850.

Schoppa, N.E., and Westbrook, G.L., Modulation of mEPSCs in olfactory bulb mitral cells by metabotropic glutamate receptors, J. Neurophysiol., 78 (1997) 1468-1475.

Schrader, L.A., and Tasker, J.G., Presynaptic modulation by metabotropic glutamate receptors of excitatory and inhibitory synaptic inputs to hypothalamic magnocellular neurons, J. Neurophysiol., 77 (1997) 527-536.

Shir, Y., and Seltzer, Z., A-fibers mediate mechanical hyperesthesia and allodynia and C-fibers mediate thermal hyperalgesia in a new model of causalgiform pain disorders in rats, Neurosci. Lett., 115 (1990) 62-67.

Tal, M., and Bennett, G.J., Dextrorphan relieves neuropathic heat-evoked hyperalgesia in the rat, Neurosci. Lett., 151 (1993) 107-110.

Tal, M., and Bennett, G.J., Neuropathic pain sensations are differentially sensitive to dextrorphan, NeuroReport, 5 (1994) 1438-1440.

Tanabe, Y., Nomura, A., Masu, M., Shigemoto, R., Mizuno, N., and Nakanishi, S., Signal transduction, pharmacological properties, and expression patterns of two rat

metabotropic glutamate receptors, mGluR3 and mGluR4, J. Neurosci., 13 (1993) 1372-1378.

Thomas, N.K., Jane, D.E., Tse, H.W., and Watkins, J.C., alpha-methyl derivatives of serine-O-phosphate as novel, selective competitive metabotropic glutamate receptor antagonists, Neuropharmacol., 35 (1996) 637-642.

Thomsen, C., Boel, E., and Suzdak, P.D., Actions of phenylglycine analogs at subtypes of the metabotropic glutamate receptor family, Eur. J. Pharmacol., 267 (1994) 77-84.

Thomsen, C., Kristensen, P., Mulvihill, E., Haldeman, B., and Suzdak, P.D., L-2-amino-4-phosphonobutyrate (L-AP4) is an agonist at the type IV metabotropic glutamate receptor which is negatively coupled to adenylate cyclase, Eur. J. Pharmacol., 227 (1992) 361-362.

Valerio, A., Paterlini, M., Boifava, M., Memo, M., and Spano, P., Metabotropic glutamate receptor mRNA expression in rat spinal cord, NeuroReport, 8 (1997) 2695-2699.

Valerio, A., Rizzonelli, P., Paterlini, M., Moretto, G., Knöpfel, T., Kuhn, R., Memo, M., and Spano, P., mGluR5 metabotropic glutamate receptor distribution in rat and human spinal cord: a developmental study, Neurosci. Res., 28 (1997) 49-57.

Vázquez, E., Budd, D.C., Herrero, I., Nicholls, D.G., and Sánchez-Prieto, J., Co-existence and interaction between facilitatory and inhibitory metabotropic glutamate receptors and the inhibitory adenosine $A_1$ receptor in cerebrocortical nerve terminals, Neuropharmacol., 34 (1995) 919-927.

Vázquez, E., Herrero, I., Miras-Portugal, M., and Sánchez-Prieto, J., Developmental change from inhibition to facilitation in the presynaptic control of glutamate exocytosis by metabotropic glutamate receptors, Neuroscience, 68 (1995) 117-124.

Vidnyánszky, Z., Hamori, J., Negyessy, L., Rüegg, D., Knöpfel, T., Kuhn, R., and Gorcs, T.J., Cellular and subcellular localization of the mGluR5a metabotropic glutamate receptor in rat spinal cord, NeuroReport, 6 (1994) 209-213.

Vignes, M., Clarke, V.R.J., Davies, C.H., Chambers, A., Jane, D.E., Watkins, J.C., and Collingridge, G.L., Pharmacological evidence for an involvement of group II and group III mGluRs in the presynaptic regulation of excitatory synaptic responses in the CA1 region of rat hippocampal slices, Neuropharmacol., 34 (1995) 973-982.

Yamamoto, T., and Yaksh, T.L., Spinal pharmacology of thermal hyperesthesia induced by constriction injury of sciatic nerve. Excitatory amino acid antagonists, Pain, 49 (1992) 121-128.

Young, M.R., Fleetwood-Walker, S.M., Mitchell, R. and Dickenson, T., The involvement of metabotropic glutamate receptors and their intracellular signalling pathways in sustained nociceptive transmission in rat dorsal horn neurons, Neuropharmacol., 34 (1995) 1033-1041.

Young, M.R., Fleetwood-Walker, M., Mitchell, R., and Munro, F.E., Evidence for a role of metabotropic glutamate receptors in sustained nociceptive inputs to rat dorsal horn neurons, Neuropharmacol., 33 (1994) 141-144.

Figure Legends

Fig. 1. Mean 50% von Frey response threshold (A) and cold water response frequency (B) for the operated hindlimb of CCI animals at baseline and on PO days 4, 8, 12 and 16. Animals were treated with vehicle (0 nmol) or (*RS*)-AIDA (30, 90 or 270 nmol) on PO days 0-3. The * indicates a significant difference between the experimental and vehicle/control conditions ($P<0.05$; Mann-Whitney U-test).

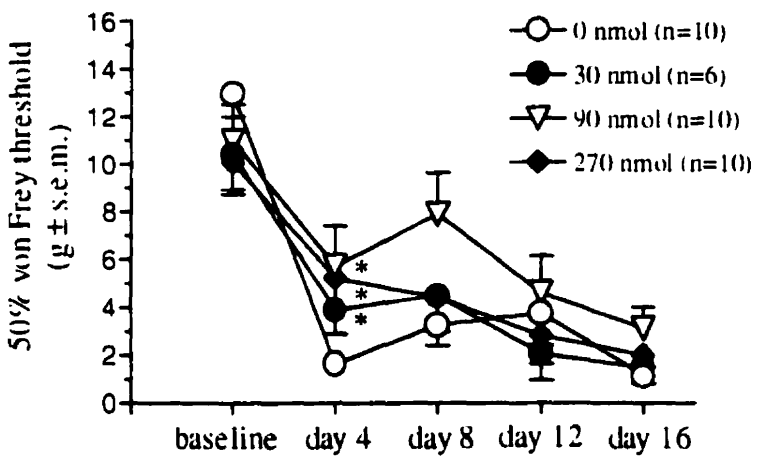
Fig. 2. Mean 50% von Frey response threshold (A) and cold water response frequency (B) for the operated hindlimb of CCI animals at baseline and on PO days 4, 8, 12 and 16. Animals were treated with vehicle (0 nmol) or (2*R*,4*R*)-APDC (30, 90 or 270 nmol) on PO days 0-3. The * indicates a significant difference between the experimental and vehicle/control conditions ($P<0.05$; Mann-Whitney U-test).

Fig. 3. Mean 50% von Frey response threshold (A) and cold water response frequency (B) for the operated hindlimb of CCI animals at baseline and on PO days 4, 8, 12 and 16. Animals were treated with vehicle (0 nmol) or L-AP4 (30, 90 or 270 nmol) on PO days 0-3. The * indicates a significant difference between the experimental and vehicle/control conditions ($P<0.05$; Mann-Whitney U-test).

Fig. 4. Mean 50% von Frey response threshold (A) and cold water response frequency (B) for the operated hindlimb of sham animals at baseline and on PO days 4, 8, 12 and 16. Animals were treated with vehicle (0 nmol), (*RS*)-AIDA (270 nmol), (2*R*,4*R*)-APDC (270 nmol) or L-AP4 (270 nmol) on PO days 0-3. Kruskal-Wallis indicated that the main effect of treatment was not significant.

Fig. 1

**A.**


50% von Frey threshold (g ± s.e.m.)
16
14
12
10
8
6
4
2
0
0 nmol (n=10)
30 nmol (n=6)
90 nmol (n=10)
270 nmol (n=10)
*
*
*
baseline
day 4
day 8
day 12
day 16

**B.**

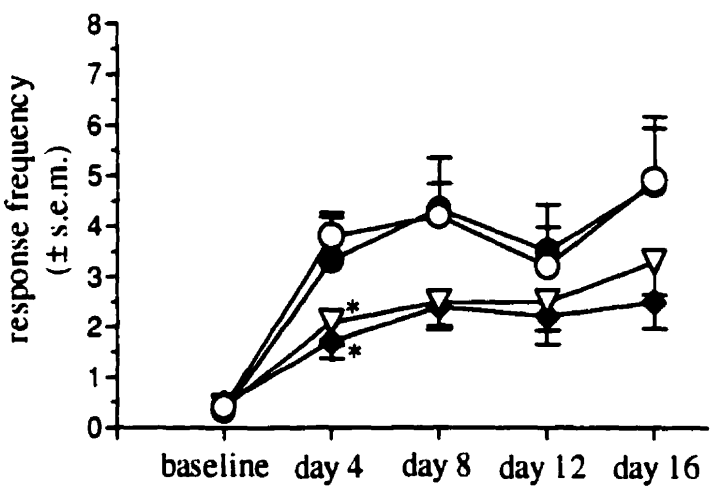
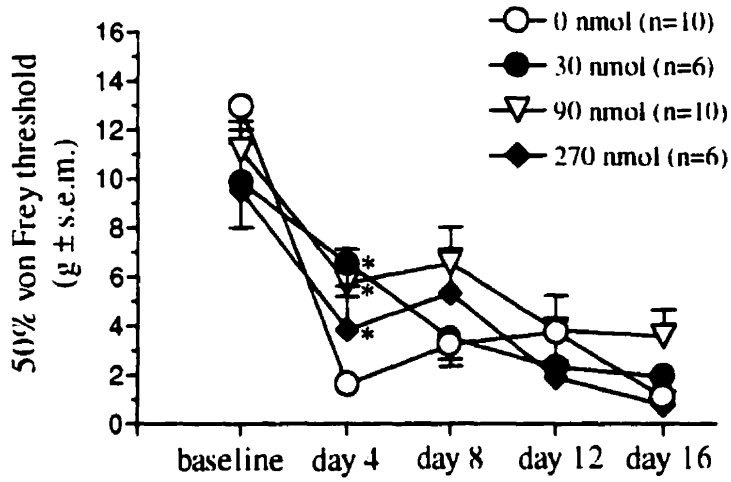

response frequency (± s.e.m.)
8
7
6
5
4
3
2
1
0
*
*
baseline
day 4
day 8
day 12
day 16

Fig. 2

**A.**


0 nmol (n=10)
30 nmol (n=6)
90 nmol (n=10)
270 nmol (n=6)
50% von Frey threshold
(g ± s.e.m.)
16
14
12
10
8
6
4
2
0
*
*
*
baseline
day 4
day 8
day 12
day 16

**B.**

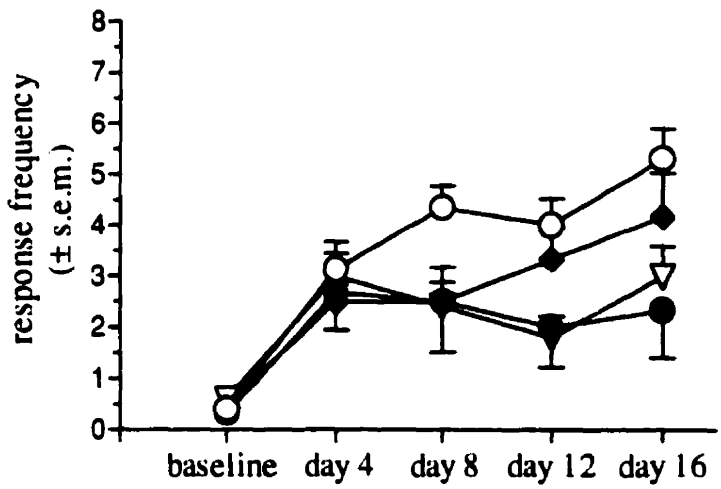
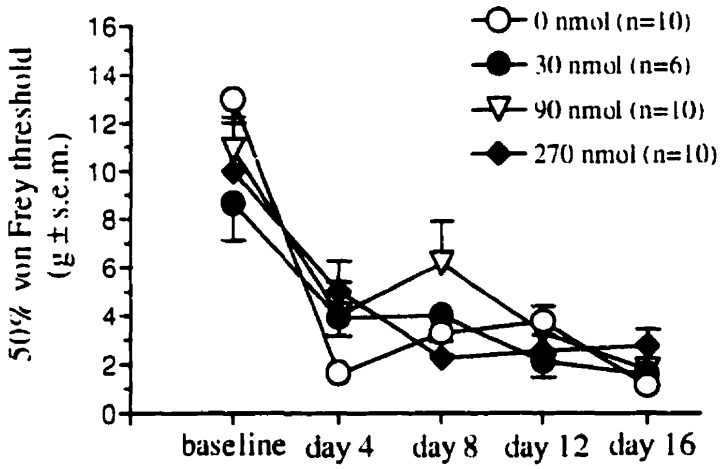

response frequency
(± s.e.m.)
8
7
6
5
4
3
2
1
0
baseline
day 4
day 8
day 12
day 16

Fig. 3

**A.**


0 nmol (n=10)
30 nmol (n=6)
90 nmol (n=10)
270 nmol (n=10)
50% von Frey threshold
(g ± s.e.m.)
0
2
4
6
8
10
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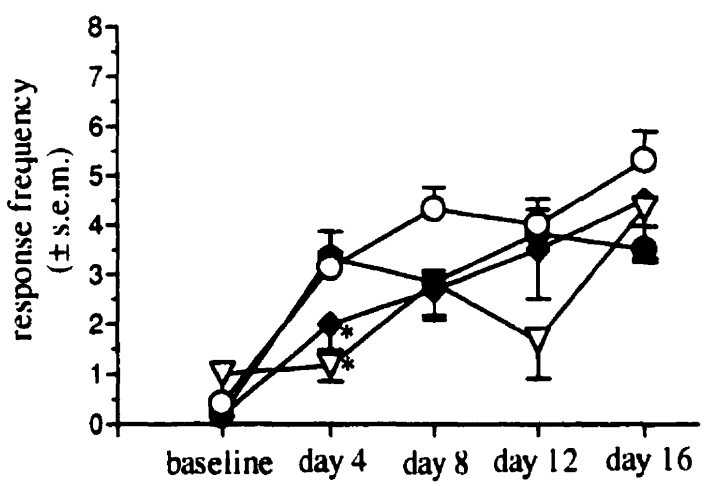
14
16
baseline
day 4
day 8
day 12
day 16

**B.**


response frequency
(± s.e.m.)
0
1
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3
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7
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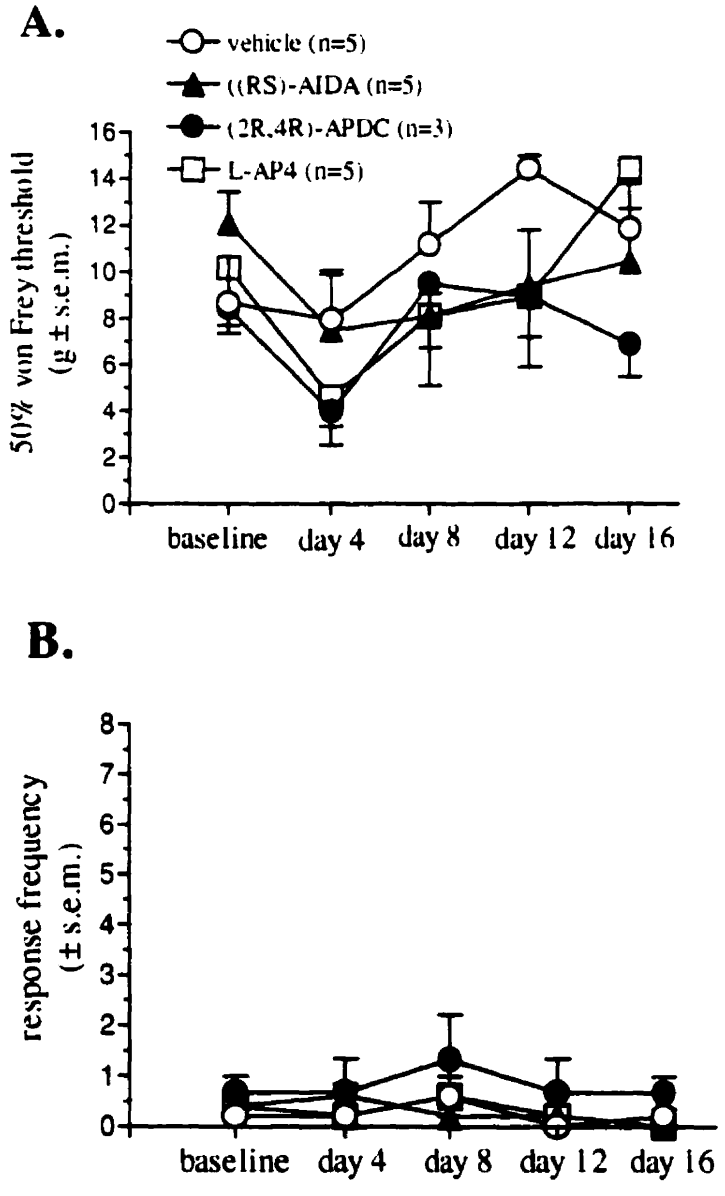
*
*
baseline
day 4
day 8
day 12
day 16

Fig. 4


A.
vehicle (n=5)
((RS)-AIDA (n=5)
(2R,4R)-APDC (n=3)
L-AP4 (n=5)
50% von Frey threshold (g ± s.e.m.)
0
2
4
6
8
10
12
14
16
baseline
day 4
day 8
day 12
day 16
B.
response frequency (± s.e.m.)
0
1
2
3
4
5
6
7
8
baseline
day 4
day 8
day 12
day 16

# CHAPTER 7: GENERAL DISCUSSION

A number of interesting findings have been obtained from the experiments described in this thesis. First, group I mGluRs appear to contribute more critically to the spinal processes mediating chronic versus persistent nociceptive states. Thus, although antagonists of group I mGluRs significantly reduced neuropathic pain in CCI rats (Studies 4 and 5), they only slightly reduced formalin-induced nociception (Study 1). This observation is in agreement with a recently published study from our laboratory demonstrating that intrathecal administration of antibodies against mGluR1 and mGluR5 attenuates cold hyperalgesia in the CCI model, but does not significantly reduce nociceptive scores in the formalin model (Fundytus, Fisher, Dray, Henry & Coderre, 1998). Also of interest from this previous study is the demonstration that intrathecal treatment with mGluR1 or mGluR5 antibodies does not influence the withdrawal responses of naive rats to radiant heat, suggesting that spinal group I mGluRs are also not involved in the processing of *acute* noxious stimuli. This is consistent with the findings described here indicating that group I mGluR antagonists did not influence thermal or mechanical sensitivity in sham-CCI rats (Studies 4 and 5). It should be noted that Young and colleagues (1997) have recently reported that intrathecal (*S*)-4C3HPG administration not only increases withdrawal thresholds to noxious thermal and mechanical stimulation in rats with carrageenan-induced inflammation, but also in *naïve* rats to a lesser degree. However, behavioural evidence from our laboratory, and electrophysiological evidence from a number of studies (Boxall et al. 1996; Budai & Larson, 1998; Neugebauer et al. 1994; Young et al. 1994, 1995), including the electrophysiological evidence from Young et al (1997), suggest that group I mGluRs contribute primarily to the injury-induced

increases in spinal cord neuronal sensitivity thought to mediate the development of long-lasting nociceptive behaviours.

The differential contribution of group I mGluRs to acute and persistent versus chronic spinal nociceptive processes may be explained, at least in part, by their neuronal localization. Several studies have shown that group I mGluRs are expressed peri- or extra-synaptically on neurons in the CNS (Baude et al. 1993; Nusser et al. 1994), including the dorsal horn of the spinal cord (Vidnyánszky et al. 1994). This finding has led investigators to speculate that group I mGluRs are activated only under conditions of excessive transmitter release, in which Glu accumulates in the synaptic cleft and spreads to the extrasynaptic regions (Baude et al. 1993; Nusser et al. 1994; Ottersen & Landsend, 1997; Vidnyánszky et al. 1994). It has been shown that an intraplantar injection of dilute formalin into the rat hindpaw induces biphasic primary afferent C-fiber activity (Puig & Sorkin, 1996), as well as an increase in the dorsal horn EAA concentration (Skilling et al. 1988). However, the probability of group I mGluRs being activated may be much greater, following the accumulation of EAAs released from damaged, continuously active primary afferent fibers in the CCI model (Kawamata & Omote, 1996) or from sensitized primary afferent fibers in chronic inflammatory models (Sluka & Westlund, 1992).

A second interesting finding is that group I mGluRs contribute to the development, but not the maintenance of nociceptive behaviours in a model of neuropathic pain (Study 4). Few studies have systematically compared the effects of various classes of compounds on the development versus the maintenance of different types of nociceptive behaviours in neuropathic pain models, thus, little is known about how the mechanisms, mediating each of these components, differ. However, it is known

that the NMDAR is involved in both the induction and the maintenance of CCI-related nociceptive behaviours (Mao, Price, Hayes, Lu & Mayer, 1992). Therefore, I propose that group I mGluRs may be required to initiate changes in dorsal horn neuronal sensitivity by enhancing NMDAR-mediated activity, but once the NMDAR-related intracellular events have been initiated, group I mGluRs may no longer be required to help maintain long-lasting changes in dorsal horn neuronal activity.

A third interesting finding is that mechanical allodynia is sensitive to spinal treatment with a group II mGluR agonists, while cold hyperalgesia is sensitive to treatment with a group III mGluR agonist in the CCI model (Study 5). Since group II and group III mGluR appear to be associated with similar transduction signals and have similar physiological roles in the CNS, I propose that the differential antinociceptive effects are related to their distinct localization patterns in the spinal cord. For example, evidence suggests that mGluR3 is expressed on postsynaptic neurons in layers II-V of the dorsal horn (Boxall et al. 1998; Ohishi et al. 1993a), whereas mGluR7 is strongly expressed on primary afferent fibers that terminate in laminae I and II (Li et al. 1997; Ohishi, Nomura, Ding, Shigemoto, Wada, Kinoshita, Li, Neki, Nakanaishi & Mizuno, 1995). Therefore, it may be that agonist-induced group II and group III mGluR activity separately reduces the injury-related increases in sensitivity of different dorsal horn neuronal populations. Thus, a group II mGluR agonist may reduce the sensitivity of deep dorsal horn neurons that respond to innocuous mechanical stimulation, while a group III mGluR agonist may reduce the sensitivity of superficial dorsal horn neurons that respond to noxious cold stimulation.

Aside from these more specific issues mentioned above, I would like to discuss in more detail the role of mGluRs in spinal nociceptive processing at this point. Specifically, I will propose a number of possible physiological/biochemical mechanisms to explain how group I mGluRs may contribute to the development of chronic nociceptive behaviours in models of pain, some of which may have been introduced in one or more of the manuscripts. I will also discuss several possible mechanisms that may mediate group II and group III mGluR agonist-related antinociception in models of pain. Figure 7.1 is a schematic diagram of the spinal dorsal horn, illustrating several possible mGluR-related mechanisms involved in the regulation of dorsal horn neuronal sensitivity (see page 209).

The Contribution of Group I mGluRs to Chronic Nociception: Possible Mechanisms

Enhancement of iGluR Channel Activity. Group I mGluRs may contribute to the development of chronic nociceptive behaviours by enhancing iGluR channel activity, which may lead to long-term changes in spinal cord neuronal sensitivity. These changes may be expressed as an increase in spontaneous dorsal horn neuronal activity and/or an increase in dorsal horn neuronal responses to cutaneous innocuous and noxious stimulation. Supporting the hypothesis that an mGluR/iGluR interaction contributes to the development of chronic nociceptive behaviours is evidence that group I mGluR activation potentiates the neuronal responses (Bleakman et al. 1992; Bond & Lodge, 1995; Budai & Larson, 1998; Cerne & Randic, 1992; Jones & Headley, 1995) and the increases in intracellular $Ca^{2+}$ concentration $[Ca^{2+}]_i$ (Bleakman et al. 1992) induced by NMDA or AMPA in the rat dorsal horn. Also, supporting this hypothesis is the demonstration that spinal group I mGluR-induced sustained SNBs are significantly reduced by NMDAR antagonism (Study 2).

Although the potential mechanisms mediating an mGluR/iGluR interaction in the dorsal horn have not yet been elucidated, Ugolini and colleagues (1997) recently demonstrated that a PKC-related mechanism mediates the group I mGluR/iGluR interaction in rat spinal ventral horn neurons. The proposal that group I mGluR-related PKC activity leads to an enhancement of iGluR function, and the consequential increase in dorsal horn neuronal sensitivity contributes to the development of chronic nociception, is supported, indirectly, by the following lines of evidence:

1. Activation of mGluRs are known to increase the translocation and activation of PKC in various regions of the CNS (Angenstein et al. 1997; Fukunago et al. 1992; Manzoni et al. 1990; Vaccarino et al. 1991).

2. The NMDAR subunit, NR1, is phosphorylated by PKC on a number of different sites, and phosphorylation of the NR1 subunit is thought to regulate NMDAR channel function (Tingley, Roche, Thompson & Huganir, 1993).

3. Activation of group I mGluR-mediated PKC potentiates NMDAR current in *Xenopus* oocytes (Kelso, Nelson & Leonard, 1992; Koga, Sakai, Tanaka & Saito, 1996).

4. Application of phorbol esters, which are activators of PKC (Gerber et al. 1989), or purified PKC (Chen & Huang, 1991, 1992) to the rat spinal cord potentiates NMDAR function, possibly by relieving the $Mg^{2+}$ blockade of the NMDAR channel and increasing the probability of the channel opening (Chen & Huang, 1992).

5. Application of (1*S*,3*R*)-ACPD or (*RS*)-DHPG produces a sensitization of rat dorsal horn neurons (Budai & Larson, 1998; Young et al. 1995, 1997), which is prevented by a PKC inhibitor (Young et al. 1995).

6. Significant increases in PKC translocation and activation in the spinal cord have been shown to be associated with models of pain (Mao, Price, Mayer & Hayes, 1992, 1993; Mao, Price, Phillips, Lu & Mayer, 1995; Yashpal, Pitcher, Parent, Quirion & Coderre, 1995).

7. Application of PKC inhibitors to the primate (Lin, Peng & Willis, 1996; Sluka et al. 1997) and rat (Munro et al. 1994) spinal cord, or spinal administration of PKC inhibitors to rats (Coderre, 1992; Coderre & Yashpal, 1994; Hayes et al. 1992; Sluka & Willis, 1997), reduces tissue injury-related sensitization of dorsal horn neurons or tissue injury-related nociceptive behaviours, respectively.

It has been suggested that the increased translocation and activation of PKC in models of pain are mediated, primarily, by the NMDAR (Mao, Price, Phillips, Lu & Mayer, 1995). However, our laboratory has recently found evidence to support the notion that, in addition to NMDARs, group I mGluRs contribute to injury-related increases in PKC activity, but only in a model of *chronic* pain (Yashpal, Fisher, Chabot & Coderre, 1998). Specifically, we found that intrathecal (*S*)-4CPG treatment significantly attenuates increases in PKC translocation and activation associated with the CCI model, but not with the formalin model. These data not only support the notion that group I mGluRs are more critically involved in models of *chronic* pain, but also suggest that the involvement of group I mGluRs in chronic nociception is mediated, at least in part, by a PKC-related mechanism.

Another possibility involved in nociception is that, activation of group I mGluRs, by an injury-related barrage of primary afferent activity, produces an increase in dorsal horn neuronal excitability, which may then lead to an indirect enhancement of iGluR

function. For example, group I mGluR activation might produce sustained changes in membrane potential which would first relieve, and then reduce the probability of the NMDAR voltage-dependent $Mg^{2+}$ blockade. Subsequently, enhanced NMDAR function may lead to further increases in dorsal horn neuronal sensitivity. The proposal that group I mGluR activation enhances spinal neuronal excitability is supported by the demonstration that perfusion of rat spinal cord slices with (1*S*,3*R*)-ACPD induces a progressive development of a plateau potential, as well as a high frequency accelerating discharge and a subsequent sustained afterdischarge of depolarized, lamina V dorsal horn neurons (Morisset & Nagy, 1996).

In this thesis, we have provided evidence that a group I mGluR/NMDAR interaction contributes to the development of persistent nociceptive behaviours (Study 2). Additional studies are needed to provide further evidence that a group I mGluR/NMDAR interaction significantly contributes to models of chronic pain, and to determine if and how a PKC-related mechanism mediates this interaction. This issue is of particular interest given that a number of different isoforms of PKC are known to be expressed in the rat spinal cord and may be differentially involved in various aspects of neuronal function (Akinori, 1998). Down-regulation of a specific group I mGluR-linked PKC isoform for the treatment of chronic pain may be a viable therapeutic strategy. This suggestion is supported by the demonstration that mice lacking PKCγ show normal responses to acute noxious stimuli, but do not develop pathological nociceptive behaviours associated with sciatic nerve injury (Malmberg, Chen, Tonegawa & Basbaum, 1997).

Increased Intracellular $Ca^{2+}$ Concentration $[Ca^{2+}]_i$. Another possible mechanism underlying group I mGluR-related enhancement of cellular excitability may be an increase in intracellular $Ca^{2+}$ concentration $[Ca^{2+}]_i$. It has been well-established that activation of group I mGluRs releases $Ca^{2+}$ from intracellular stores (Brammer et al. 1991; Carmant et al. 1997; Courtney et al. 1990; Guiramand et al. 1991; Llano et al. 1991; Murphy & Miller, 1988, 1989; Netzeband et al. 1997; Phenna et al. 1995). Evidence also suggests that group I mGluRs increase $Ca^{2+}$ conductance by enhancing voltage gated $Ca^{2+}$ channel (VGCC) activity in the CNS (Chavis, Fagni, Bockaert & Lansman, 1995; Chavis, Nooney, Bockaert, Fagni, Feltz & Bossu, 1995; Jaffe & Brown, 1994; Zheng et al. 1996). In the spinal cord, Morisset and Nagy (1996) found that the (1*S*,3*R*)-ACPD-induced increases in dorsal horn neuronal excitability are mediated by a progressive activation of L-type VGCC. This finding is consistent with the demonstration that (1*S*,3*R*)-ACPD-induced increases in $[Ca^{2+}]_i$ in laminae I-III dorsal horn neurons are reduced by an L-type VGCC antagonist (Bleakman et al. 1992).

Interestingly, Reichling and MacDermott (1993) showed that application of (1*S*,3*R*)-ACPD to rat dorsal horn neurons evokes a progressive, but prolonged, increase in $[Ca^{2+}]_i$, whereas application of NMDA evokes an immediate rise in the $[Ca^{2+}]_i$, which peaks for about 5 minutes, and then steadily decreases until the end of the testing period (20 minutes). Although NMDAR and group I mGluR agonists increase intracellular $Ca^{2+}$ in different ways, it is of interest to note that the pattern of NMDAR and mGluR agonist-induced changes in $[Ca^{2+}]_i$, *in vitro*, is similar to the pattern of SNBs induced by spinally administered (*RS*)-DHPG or NMDA, *in vivo*. For example, I have found that intrathecal administration of (*RS*)-DHPG produces a slow increase in the intensity of the nociceptive

behaviours, which peaks at about 1 hour post-injection (Study 2). In contrast, intrathecal administration of NMDA immediately produces intense nociceptive behaviours and the intensity gradually decreases until the behaviours disappear at about 30 minutes post-injection (unpublished observation; also see Skilling, Sun, Kurtz & Larson (1992) for evidence that the behavioural effect of intrathecal NMDA is generally short lived). These findings provide evidence that the development of sustained nociceptive behaviours is correlated with a group I mGluR-mediated increase in $[Ca^{2+}]_i$. This correlation is particularly interesting given that the link between $Ca^{2+}$ and nociception has been well-established in the literature (Chaplan, Pogrel & Yaksh, 1994; Calcutt & Chaplan, 1997; Chaplan et al. 1994; Coderre & Melzack, 1992b; Kawamata & Omote, 1996; Malmberg & Yaksh, 1994; Nebe, Vanegas, Neugebauer & Schaible, 1997). Future research is needed to explore the possibility that a group I mGluR-mediated increase in $[Ca^{2+}]_i$ contributes to the development of nociceptive behaviours in models of chronic pain.

Increased Release of EAAs. In manuscript #3, I suggested that activation of group I mGluRs, which may be localized on primary afferent C-fibers, produces sustained nociceptive responses in rats by increasing Glu and Asp release. This proposal is based on evidence suggesting that presynaptic group I mGluRs exist in various regions of the CNS (Fotuhi et al. 1993; Romano et al. 1995; Shigemoto et al. 1992), and that activation of group I mGluRs increases the release of Glu from synaptosomes (Herrero et al. 1992a, 1992b, 1994, 1996; McGahon & Lynch, 1994; Vázquez et al. 1994; Vázquez, Budd, Herrero, Nicholls & Sánchez-Prieto, 1995; Vázquez, Herrero, Miras-Portugal & Sánchez-Prieto, 1995). These data suggest that presynaptic group I mGluRs function as autoreceptors that facilitate Glu release in the CNS (Romano et al. 1995).

Recently, we tested the hypothesis that group I mGluR activation produces an increase in the release of Glu in the spinal cord. Not only did we find that perfusion of the rat spinal cord with (*RS*)-DHPG increases 4-aminopyridine-evoked release, but also that (*RS*)-DHPG-induced SNBs are reduced by pretreatment with Glu release inhibitors (Fisher, Lefebvre, Cahill & Coderre, unpublished observations). We also found that (*RS*)-DHPG-induced SNBs are significantly reduced in animals that have been treated neonatally with capsaicin, a neurotoxin that selectively destroys C-fibers. These findings support the notion that group I mGluR activation leads to an increase in Glu release, possibly from C-fibers, and that this mechanism contributes to the development of sustained nociceptive behaviours.

However, as far as I know, only one published study includes a detailed ultrastructural examination of spinal group I mGluRs, and these investigators found that mGluR5a is localized *postsynaptically* in the rat spinal cord (Vidnyánszky et al. 1994). Future studies may provide evidence that group I mGluRs are also localized presynaptically in the dorsal horn of the spinal cord. However, until then, it would be prudent to speculate how activation of *postsynaptic* group I mGluRs in the dorsal horn leads to the enhancement of Glu release. For this mechanism to play a role, it would be necessary to invoke the contribution of a retrograde messenger. Evidence from several studies suggest that group I mGluR activation leads to the production of the retrograde messenger nitric oxide (Bhardwaj et al. 1997; Okada, 1992; Yamada & Nabeshima, 1997). It is of general consensus that an increases in $[Ca^{2+}]_i$ leads to the activation of nitric oxide synthase, an enzyme that synthesizes nitric oxide. It is thought that nitric oxide diffuses across the postsynaptic neuronal membrane and acts at presynaptic nerve

terminals to produce an increased release of neurotransmitter via a cGMP-related mechanism (for reviews, see Hölscher, 1997; Lowenstein, Dinerman & Snyder, 1994). As far as I know, no one has examined whether *spinal* group I mGluR activation leads to nitric oxide production in the dorsal horn. However, the literature has clearly linked this retrograde messenger to nociception. For example, a number of studies have shown that increases in nitric oxide synthase expression in the dorsal horn occur in models of persistent and chronic pain (Gao & Qiao, 1998; Lam et al. 1996; Steel, Terenghi, Chung, Na, Carlton & Polak, 1994; Vizzard, Erdman & de Groat, 1995; Wu, Lin, Lu, Willis & Westlund, 1998; Yonehara et al. 1997). Also, others have shown that intrathecal administration of nitric oxide synthase inhibitors produces antinociception in various models of pain (Malmberg & Yaksh, 1993; Mao, Price, Zhu, Lu & Mayer, 1997; Meller, Cummings, Traub & Gebhart, 1994; Niedbala, Sanchez & Feria, 1995; Roche, Cook, Wilcox & Kajander, 1996; Sakurada et al. 1996; Yamamoto, Shimoyama & Mizuguchi, 1993b; Yonehara et al. 1997; Yoon, Sung & Chung, 1998).

It has been suggested that tissue injury-related increases in nitric oxide production are mediated, primarily, by the NMDAR (Gordh, Karlsten & Kristensen, 1995; Meller & Gebhart, 1993, 1994; Stanfa, Misra & Dickenson, 1996). However, future research is needed to explore the possibility that a group I mGluR-mediated increase in nitric oxide enhances the release of Glu in the spinal cord, and that this mechanism contributes to the development of nociceptive behaviours in models of chronic pain.

Antinociception Associated with Group II and Group III mGluR Agonism in Models of Pain: Possible Mechanisms

Group II mGluR Modulation of Voltage Gated Calcium Channels (VGCCs). In this thesis, we have provided evidence that activation of spinal group II mGluRs reduces nociceptive responses in models of pain. Since electrophysiological evidence suggests that activation of presynaptic group II mGluRs inhibits monosynaptic responses in motoneurones, by decreasing the release of EAAs (Cao et al. 1995; Jane et al. 1994, 1995, 1996; Thomas et al. 1996), it is tempting to speculate that presynaptic group II mGluRs, possibly localized on primary afferent fibers, also inhibit dorsal horn neuronal activity by reducing the release of EAAs. Unfortunately, there are few published details relating to the ultrastructural localization of group II mGluRs in the rat dorsal horn. Available evidence suggests that, although mGluR2 is not expressed in the rat spinal cord (Boxall et al. 1998; Ohishi et al. 1993b), mGluR3 is localized, *postsynaptically*, on dorsal horn neurons in laminae II-V (Boxall et al. 1998; Ohishi et al. 1993a).

Given that electrophysiological and neuroanatomical studies have not yet provided evidence that mGluR3 is localized on terminals presynaptic to dorsal horn neurons, it is necessary at this point to conclude that a group II mGluR agonist most likely attenuates nociceptive responses in models of pain by acting at postsynaptic mGluR3. I propose that activation of mGluR3 suppresses $Ca^{2+}$ conductance by inhibiting postsynaptic VGCC in the dorsal horn. This proposal is indirectly supported by the following lines of evidence:

1. Studies have demonstrated that group II mGluR activation suppresses $Ca^{2+}$ conductance by inhibiting N-type (Chavis et al. 1995: Choi & Lovinger, 1996; Glaum &

Miller, 1995; Ikeda, Lovinger, McCool & Lewis, 1995) or L-type (Chavis et al. 1994, 1995) VGCCs in the rat NS.

2. Antagonism of N-type VGCCs reduces dorsal horn neuronal sensitization induced by an intraplantar injection of formalin into the rat hindpaw (Diaz & Dickenson, 1997)

3. Intrathecal administration of L- and/or N-type VGCC antagonists to rats reduces nociceptive responses in the formalin model (Coderre & Melzack, 1992b; Malmberg & Yaksh, 1994) and intrathecal administration of an N-type VGCC antagonists to rats reduces mechanical allodynia in a model of neuropathic pain (Calcutt & Chaplan, 1997; Chaplan et al. 1994).

Our finding that spinal administration of a selective group II mGluR agonist produces antinociception in a model of neuropathic pain (Study 5), and the finding of Boxall and colleagues (1998) that mGluR3 expression in the dorsal horn is enhanced in a model of chronic inflammatory pain, will likely generate future research directed at exploring the functional role of spinal mGluR3 in nociceptive processes.

Group III mGluR Inhibition of EAA Release from Primary Afferent Fibers. Neuroanatomical studies have provided convincing evidence that mGluR7 is strongly expressed on primary afferent fibers, likely on C-fibers, terminating in laminae I and II of the rat dorsal horn (Li et al. 1997; Ohishi, Nomura, Ding, Shigemoto, Wada, Kinoshita, Li, Neki, Nakanishi & Mizuno, 1995). Moreover, evidence suggests that, in the rat CNS, mGluR7 localization is restricted to the presynaptic grid, which is the transmitter release site (Shigemoto et al. 1996). Electrophysiological studies have provided evidence that presynaptic group III mGluRs inhibit excitatory transmission in the spinal cord (Cao et al.

1995; Jane et al. 1994, 1995, 1996), by decreasing the release of EAAs. Release studies have confirmed that group III GluR activation leads to an inhibition of EAA release in rat brain synaptosomes (Herrero et al. 1996; Vázquez, Budd, Herrero, Nicholls & Sánchez-Prieto, 1995; Vázquez, Herrero, Miras-Portugal & Sánchez-Prieto, 1995). Considering these different lines of evidence, I propose that spinal administration of a group III mGluR agonist, acting at presynaptic mGluR7, may reduce the probability of EAAs released from the injured primary afferent fibers. This reduction in EAA release may attenuate the development of dorsal horn neuronal sensitization in laminae I and II.

How might spinal group III mGluRs be linked to the inhibition of EAA release? A few studies have examined the potential mechanisms that may mediate group III mGluR-related inhibition of EAA release in the rat brain. Evidence suggests that group III mGluR activity produces a suppression of $Ca^{2+}$ conductance through presynaptic VGCC (Takahashi, Forsythe, Tsujimoto, Barnes-Davies & Onedera, 1996). Specifically, studies have shown that L-AP4, the selective group III mGluR agonist, inhibits P/Q-type (Takahashi et al. 1996; Stefani, Spadoni & Bernardi, 1998) and N-type (Stefani et al. 1998) VGCC in the rat brain. Also, Vázquez and Sánchez-Prieto (1997) have shown that L-AP4 inhibits Glu release from cerebrocortical synaptosomes by suppressing N-type VGCCs. Future research is needed to explore the possibility that an mGluR7-related inhibition of presynaptic VGCCs mediates the antinociceptive effects of L-AP4 in models of pain.

General Conclusions

The results of the experiments included in my dissertation have clearly enhanced our understanding about the role of mGluRs in persistent and chronic nociceptive

conditions. The details outlined in my thesis also pose some interesting questions that will likely generate much research for the next few years. For example, why are mGluR compounds more effective in reducing nociceptive responses in models of chronic pain than in models of acute and persistent pain? Why do group I mGluRs contribute to the development, but not the maintenance of chronic nociceptive responses? Why do group II and group III mGluR agonists differentially influence nociceptive behaviours in a model of neuropathic pain? Which intracellular messengers generated by group I mGluRs play a role in the development of chronic nociception? Which intracellular messengers generated by group II and group III mGluRs are responsible for producing antinociception in models of pain? How do these intracellular messenger systems contribute to, or attenuate the development of nociceptive behaviours. Answers to these questions will not only enhance our understanding about how mGluRs regulate dorsal horn neuronal function, but may also stimulate the development of novel therapeutic strategies for the treatment of chronic pain in the clinical population.

Fig. 7.1

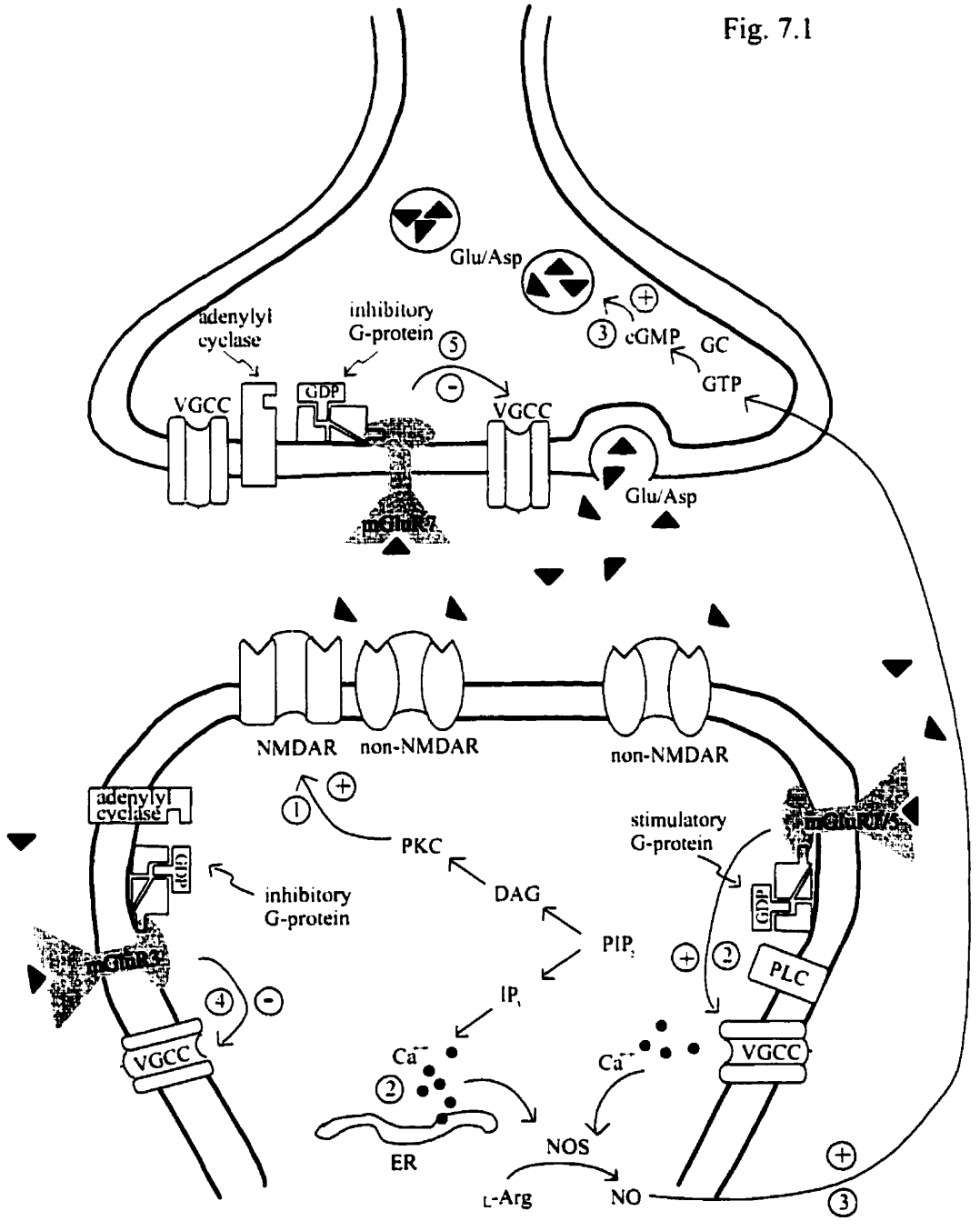

Glu/Asp
adenylyl cyclase
inhibitory G-protein
5
3
cGMP
GC
GTP
VGCC
GDP
VGCC
Glu/Asp
NMDAR
non-NMDAR
non-NMDAR
adenylyl cyclase
1
PKC
stimulatory G-protein
inhibitory G-protein
DAG
PIP2
2
PLC
IP3
4
VGCC
VGCC
Ca++
Ca++
2
ER
NOS
L-Arg
NO
3

Figure Legend

Fig. 7.1. A schematic diagram of the spinal dorsal horn, which illustrates several possible mGluR-related mechanisms involved in the regulation of dorsal horn neuronal sensitivity. Excessive amounts of the EAAs, glutamate (Glu) and aspartate (Asp), are released from damaged, continuously active primary afferent fibers in models of neuropathic pain or from sensitized primary afferent fibers in chronic inflammatory models. Released EAAs spread to the peri- and extra-synaptic regions in the extracellular space and bind to postsynaptic mGluR1/5. The binding of EAAs to mGluR1/5 activates a stimulatory G-protein, which in turn activates the enzyme phospholipase C (PLC). Following activation, this enzyme cleaves phosphatidylinositol (PI)-4,5-bisphosphate ($PIP_2$) into two second messengers, inositol trisphosphate ($IP_3$) and diacylglycerol (DAG). $IP_3$ diffuses into the cytoplasm, binds to a receptor on the endoplasmic reticulum (ER) and releases calcium ($Ca^{2+}$) from internal stores. DAG remains in the plasma membrane, where it activates protein kinase C (PKC). (1) The NMDAR subunit, NR1, may be phosphorylated by the PKC, which would enhance NMDAR channel function to facilitate NMDAR-mediated increases in dorsal horn neuronal sensitivity. (2) Activation of mGluR1/5 may also enhance dorsal horn neuronal excitability by increasing intracellular $Ca^{2+}$ concentration $[Ca^{2+}]_i$, by releasing $Ca^{2+}$ from intracellular stores (from the ER) or by enhancing voltage gated $Ca^{2+}$ channel (VGCC) function. (3) The mGluR1/5-related release of $Ca^{2+}$ from intracellular stores or increase in $[Ca^{2+}]_i$ through VGCCs activates nitric oxide synthase (NOS), which converts L-arginine (L-Arg) to nitric oxide (NO). NO may cross the postsynaptic dorsal horn neuronal membrane and act retrogradely at presynaptic nerve terminals to produce an increased release of EAAs by activating guanylyl cyclase (GC)

(GC converts guanosine 5'-trisphosphate (GTP) to cGMP). (4) In contrast, activation of postsynaptic mGluR3 may suppress $Ca^{2+}$ conductance by inhibiting VGCCs. An mGluR3-mediated inhibition of $Ca^{2+}$ influx through VGCCs may lead to a reduction in tissue injury-induced dorsal horn neuronal sensitization. (5) Activation of mGluR7, localized on primary afferent fibers terminating in laminae I and II of the dorsal horn, may suppress $Ca^{2+}$ conductance by inhibiting presynaptic VGCCs, thus, reducing the release of EAAs from the damaged, active primary afferent fibers. An mGluR7-mediated decrease in EAA release may lead to a reduction in tissue injury-induced dorsal horn neuronal sensitization.

References

Aanonsen, L.M. & Wilcox, G.L. (1987). Nociceptive action of excitatory amino acids in the mouse: effects of spinally administered opioids, phencyclidine and sigma agonists. Journal of Pharmacology & Experimental Therapeutics, 243, 9-19.

Aanonsen, L.M. & Wilcox, G.L. (1989). Muscimol, γ-aminobutyric $acid_A$ receptors and excitatory amino acids in the mouse spinal cord. Journal of Pharmacology and Experimental Therapeutics, 248, 1034-1038.

Aanonsen, L.M., Lei, S. & Wilcox, G.L. (1990). Excitatory amino acid receptors and nociceptive neurotransmission in rat spinal cord. Pain, 41, 309-321.

Abbott, A., Wigmore, M.A. & Lacey, M.G. (1997). Excitation of rat subthalamic nucleus neurones *in vitro* by activation of a group I metabotropic glutamate receptor. Brain Research, 766, 162-167.

Abe, T., Sugihara, H., Nawa, H., Shigemoto, R., Mizuno, N. & Nakanishi, S. (1992). Molecular characterization of a novel metabotropic glutamate receptor mGluR5 coupled to inositol phosphate/$Ca^{2+}$ signal transduction, Journal of Biological Chemistry, 267, 13361-13368.

Advokat, C., Ghorpade, A. & Wolfe, E. (1994). Intrathecal excitatory amino acid (EAA) agonists increase tail flick latencies (TFLs) of spinal rats. Pharmacology, Biochemistry & Behaviour, 48, 693-698.

Akinori, M. (1998). Subspecies of protein kinase C in the rat spinal cord. Progress in Neurobiology, 54, 499-530.

Angenstein, F., Hirschfelder, M. & Staak, S. (1997). Activation of metabotropic glutamate receptors increases endogenous protein kinase C substrate phosphorylation in adult hippocampal slices. Brain Research, 745, 46-54.

Aniksztejn, L., Bregestovski, P. & Ben-Ari, Y. (1991). Selective activation of quisqualate metabotropic receptor potentiates NMDA but not AMPA responses. European Journal of Pharmacology, 205, 327-328.

Aramori, I. & Nakanishi, S. (1992). Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. Neuron, 8, 757-765.

Aronica, E., Condorelli, D.F., Nicoletti, F., Dell'Albani, P., Amico, C. & Balazs, R. (1993). Metabotropic glutamate receptors in cultured cerebellar granule cells: developmental profile. Journal of Neurochemistry, 60, 559-565.

Aronica, E., Frey, U., Wagner, M., Schroeder, H., Krug, M., Ruthrich, H., Catania, M.V., Nicoletti, F. & Reymann, K.G. (1991). Enhanced sensitivity of "metabotropic" glutamate receptors after induction of long-term potentiation in rat hippocampus. Journal of Neurochemistry, 57, 376-383.

Attal, N., Jazat, F., Kayser, V. & Guilbaud, G. (1990). Further evidence for 'pain-related' behaviours in a model of unilateral peripheral mononeuropathy. Pain, 41, 235-251.

Attwell, P.J., Kaura, S., Sigala, G., Bradford, H.F., Croucher, M.J., Jane, D.E. & Watkins, J.C. (1995). Blockade of both epileptogenesis and glutamate release by (1*S*,3*S*)-ACPD, a presynaptic glutamate receptor agonist. Brain Research, 698, 155-162.

Bahn, S., Volk, B. & Wisden, W. (1994). Kainate receptor gene expression in the developing rat brain. Journal of Neuroscience, 14, 5525-5547.

Basbaum, A.I., Gautron, M., Jazat, F., Mayes, M. & Guilbaud, G. (1991). The spectrum of fiber loss in a model of neuropathic pain in the rat: an electron microscopic study. Pain, 47, 359-367.

Bashir, Z.I., Bortolotto, Z.A., Davies, C.H., Berretta, N., Irving, A.J., Seal, A.J., Henley, J.M., Jane, D.E., Watkins, J.C. & Collingridge, G.L. (1993). Induction of LTP in the hippocampus needs synaptic activation of glutamate metabotropic receptors. Nature, 363, 347-350.

Baskys, A., Bernstein, N.K., Barolet, A.W. & Carlen, P.L. (1990). NMDA and quisqualate reduce a Ca-dependent $K^+$ current by a protein kinase-mediated mechanism. Neuroscience Letters, 112, 76-81.

Baskys, A. & Malenka, R.C. (1991). Agonists at metabotropic glutamate receptors presynaptically inhibit EPSCs in neonatal rat hippocampus. Journal of Physiology, 444, 687-701.

Battaglia, G., Monn, J.A. & Schoepp, D.D. (1997). In vivo inhibition of veratridine-evoked release of striatal excitatory amino acids by the group II metabotropic glutamate receptor agonist LY354740 in rats. Neuroscience Letters, 229, 161-164.

Baude, A., Nusser, Z., Roberts, J.D.B., Mulvihill, E., McIlhinney, R.A.J. & Somegyi, P. (1993). The metabotropic glutamate receptor (mGluR1α) is concentrated at perisynaptic membrane of neuronal subpopulations as detected by immunogold reaction. Neuron, 11, 771-787.

Bedingfield, J.S., Kemp, M.C., Jane, D.E., Tse, H.-W., Roberts, P.J. & Watkins, J.C. (1995). Structure-activity relationships for a series of phenyglycine derivatives acting

at metabotropic glutamate receptors (mGluRs). British Journal of Pharmacology, 116, 3323-3329.

Bennett, G.J. & Xie, Y.-K. (1988). A peripheral mononeuropathy in rat that produces disorders of pain sensation like those seen in man. Pain, 33, 87-107.

Berridge, M.J. & Galione, A. (1988). Cytosolic calcium oscillators. FASEB Journal, 2, 3074-3083.

Bettler, B., Boulter, J., Hermans-Borgmeyer, I., O'Shea-Greenfield, A., Deneris, E.S., Moll, C., Borgmeyer, U., Hollman, M. & Heinemann, S. (1990). Cloning of a novel glutamate receptor subunit, GluR5: expression in the nervous system during development. Neuron, 5, 583-595.

Bettler, B., Egebjerg, J., Sharma, G., Pecht, G., Hermans-Borgmeyer, I., Moll, C., Stevens, C.F. & Heinemann, S. (1992). Cloning of a putative glutamate receptor: a low affinity kainate-binding subunit. Neuron, 8, 257-265.

Bhardwaj, A., Northington, F.J., Martin, L.J., Hanley, D.F., Traystman, R.J. & Koehler, R.C. (1997). Characterization of metabotropic glutamate receptor-mediated nitric oxide production in vivo. Journal of Cerebral Blood Flow and Metabolism, 17, 153-160.

Birse, E.F., Eaton, S.A., Jane, D.E., Jones, P.L.S.J., Porter, R.H.P., Pook, P.C.-K., Sunter, D.C., Udvarhelyi, P.M., Wharton, B., Roberts, P.J., Salt, T.E. & Watkins, J.C. (1993). Phenylglycine derivatives as new pharmacological tools for investigating the role of metabotropic glutamate receptors in the central nervous system. Neuroscience, 52, 481-488.

Bleakman, D., Rusin, K.I., Chard, P.S., Glaum, S.R. & Miller, R.J. (1992). Metabotropic glutamate receptors potentiate ionotropic glutamate responses in the rat dorsal horn. Molecular Pharmacology, 42, 192-196.

Bond, A. & Lodge, D. (1995). Pharmacology of metabotropic glutamate receptor-mediated enhancement of responses to excitatory and inhibitory amino acids on rat spinal neurones *in vivo*. Neuropharmacology, 34, 1015-1023.

Bortolotto, Z.A. & Collingridge, G.L. (1992). Activation of glutamate metabotropic receptors induces long-term potentiation. European Journal of Pharmacology, 214, 297-298.

Bortolotto, Z.A. & Collingridge, G.L. (1993). Characterization of LTP induced by the activation of glutamate metabotropic receptors in area CA1 of the hippocampus. Neuropharmacology, 32, 1-9.

Bortolotto, Z.A. & Collingridge, G.L. (1995). On the mechanism of long-term potentiation induced by (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid (ACPD) in rat hippocampal slices. Neuropharmacology, 34, 1003-1014.

Boulter, J., Hollmann, M., O'Shea-Greenfield, A., Hartley, M., Deneris, E., Maron C. & Heinemann, S. (1990). Molecular cloning and functional expression of glutamate receptor subunit genes. Science, 249, 1033-1037.

Bowyer, J.F. (1982). Phencyclidine inhibition of the rate of development of amygdaloid kindled seizures. Experimental Neurology, 75, 173-183.

Bowyer, J.F., Albertson, T.E., Winters, W.D. & Baselt, R.C. (1983). Ketamine-induced changes in kindled amygdaloid seizures. Neuropharmacology, 22, 887-894.

Boxall, S.J., Berthele, A., Laurie, D.J., Sommer, B., Zieglgänsberger, W., Urban, L. & Tölle, T.R. (1998). Enhanced expression of metabotropic glutamate receptor 3 messenger RNA in the rat spinal cord during ultraviolet irradiation induced peripheral inflammation. Neuroscience, 82, 591-602.

Boxall, S.J., Thompson, S.W.N., Dray, A., Dickenson, A.H. & Urban, L. (1996). Metabotropic glutamate receptor activation contributes to nociceptive reflex activity in the rat spinal cord *in vitro*. Neuroscience, 74, 13-20.

Brabet, I., Bockaert, M.J. & Pin, J.-P. (1995). Phenylglycine derivatives discriminate between mGluR1- and mGluR5-mediated responses. Neuropharmacology, 34, 895-903.

Bradley, S.R., Levey, A.I., Hersch, S.M. & Conn, P.J. (1996). Immunocytochemical localization of group III metabotropic glutamate receptors in the hippocampus with subtype-specific antibodies. Journal of Neuroscience, 16, 2044-2056.

Brambilla, A., Prudentino, A., Grippa, N. & Borsini, F. (1996). Pharmacological characterization of AMPA-induced biting behaviour in mice. European Journal of Pharmacology, 305, 115-117.

Brammer, M.J., Richmond, S., Xiang, J.Z., Adamson, P., Hajimohammadreza, I., Silva, M.A. & Campbell, I.C. (1991). Kainate and quisqualate effects on rat presynaptic cortical receptors are metabotropic and non-additive. Neuroscience Letters, 128, 231-234.

Breakwell, N.A., Rowan, M.J. & Anwyl, R. (1996). Metabotropic glutamate receptor dependent EPSP and EPSP-spike potentiation in area CA1 of the submerged rat hippocampal slice. Journal of Neurophysiology, 76, 3126-3135.

Bruno, V., Battaglia, G., Copani, A., Giffard, R.G., Raciti, G., Raffaele, R., Shinozaki, H. & Nicoletti, F. (1995). Activation of class II or III metabotropic glutamate receptors protects cultured cortical neurons against excitotoxic degeneration. European Journal of Neuroscience, 7, 1906-1913.

Bruno, V., Copani, A., Battaglia, G., Raffaele, R., Shinozaki, H. & Nicoletti, F. (1994). Protective effect of the metabotropic glutamate receptor agonist, DCG-IV, against excitotoxic neuronal death. European Journal of Pharmacology, 256, 109-112,

Bruno, V., Copani, A., Bonanno, L., Knöpfel, T., Kuhn, R., Roberts, P.J. & Nicoletti, F. (1996). Activation of group III metabotropic glutamate receptors is neuroprotective in cortical cultures. European Journal of Pharmacology, 310, 61-66.

Bruno, V., Copani, A., Knöpfel, T., Kuhn, R., Casabona, G., Dell'Albani, P., Condorelli, D.F. & Nicoletti, F. (1995). Activation of metabotropic glutamate receptors coupled to inositol phospholipid hydrolysis amplifies NMDA-induced neuronal degeneration in cultured cortical cells. Neuropharmacology, 34, 1089-1098.

Bruno, V., Sureda, F.X., Storto, M., Casabona, G., Caruso, A., Knöpfel, T., Kuhn, R. & Nicoletti, F. (1997). The neuroprotective activity of group II metabotropic glutamate receptors requires new protein synthesis and involves a glial-neuronal signaling. Journal of Neuroscience, 17, 1891-1897.

Buchan, A., Li, H., Cho, S. & Pulsinelli, W.A. (1991). Blockade of the AMPA receptor prevents CA1 hippocampal injury following severe but transient forebrain ischemia in adult rats. Neuroscience Letters, 132, 255-258.

Budai, D. & Larson, A.A. (1994). GYKI 52466 inhibits AMPA/kainate and peripheral mechanical sensory activity. NeuroReport, 5, 881-884.

Budai, D. & Larson, A.A. (1998). The involvement of metabotropic glutamate receptors in sensory transmission in dorsal horn of the rat spinal cord. Neuroscience, 83, 571-580.

Buisson, A. & Choi, D.W. (1995). The inhibitory mGluR agonist, s-4-carboxy-3-hydroxy-phenylglycine selectively attenuates NMDA neurotoxicity and oxygen-glucose deprivation-induced neuronal death. Neuropharmacology, 34, 1081-1087.

Buisson, A., Yu S.P. & Choi, D.W. (1996). DCG-IV selectively attenuates rapidly triggered NMDA-induced neurotoxicity in cortical neurons. European Journal of Neuroscience, 8, 138-143.

Burke, J.P. & Hablitz, J.J. (1996). G-protein activation by metabotropic glutamate receptors reduces spike frequency adaptation in neocortical neurons. Neuroscience, 75, 1996.

Calcutt, N.A. & Chaplan, S.R. (1997). Spinal pharmacology of tactile allodynia in diabetic rats. British Journal of Pharmacology, 122, 1478-1482.

Calvino, B., Villanueva, L. & Le Bars, D. (1987). Dorsal horn (convergent) neurones in the intact anaesthetized arthritic rat. I. Segmental excitatory influences. Pain, 28, 81-98.

Cao, C.Q., Evans, R.H., Headley, P.M. & Udvarhelyi, P.M. (1995). A comparison of the effects of selective metabotropic glutamate receptor agonists on synaptically evoked whole cell currents of rat spinal ventral horn neurones *in vitro*. British Journal of Pharmacology, 115, 1469-1474.

Carlton, S.M. & Hargett, G.L. (1995). Treatment with the NMDA antagonist memantine attenuates nociceptive responses to mechanical stimulation in neuropathic rats. Neuroscience Letters, 198, 115-118.

Carmant, L., Woodhall, G., Ouardouz, M., Robitaille, R. & Lacaille, J.-C. (1997). Interneuron-specific $Ca^{2+}$ responses linked to metabotropic and ionotropic glutamate receptors in rat hippocampal slices. European Journal of Neuroscience, 9, 1625-1635.

Cartmell, J., Curtis, A.R., Kemp, J.A., Kendall, D.A. & Alexander, S.P. (1993). Subtypes of metabotropic excitatory amino acid receptor distinguished by stereoisomers of the rigid glutamate analogue, 1-aminocyclopentane-1,3-dicarboxylate. Neuroscience Letters, 153, 107-110.

Casabona, G., Knöpfel, T., Kuhn, R., Gasparini, F., Baumann, P., Sortino, M.A., Copani, A. & Nicoletti, F. (1997). Expression and coupling to polyphosphoinositide hydrolysis of group I metabotropic glutamate receptors in early postnatal and adult brain. European Journal of Neuroscience, 9, 12-17.

Cerne, R. & Randic, M. (1992). Modulation of AMPA and NMDA responses in rat spinal cord dorsal horn neurons by *trans*-1-aminocyclopentane-1,3-dicarboxylic acid. Neuroscience Letters, 144, 180-184.

Chaplan, S.R., Bach, F.W., Pogrel, J.W., Chung, J.M. & Yaksh, T.L. (1994). Quantitative assessment of tactile allodynia in the rat paw. Journal of Neuroscience Methods, 53, 55-63.

Chaplan, S.R., Malmberg, A.B. & Yaksh, T.L. (1997). Efficacy of spinal NMDA receptor antagonism in formalin hyperalgesia and nerve injury evoked allodynia in the rat. Journal of Pharmacology and Experimental Therapeutics, 280, 829-838.

Chaplan, S.R., Progrel, J.W. & Yaksh, T.L. (1994). Role of voltage-dependent calcium channel subtypes in experimental tactile allodynia. Journal of Pharmacology & Experimental Therapeutics, 269, 1117-1123.

Chapman, V. & Dickenson, A.H. (1992). The combination of NMDA antagonism and morphine produces profound antinociception in the rat dorsal horn. Brain Research, 573, 321-323.

Chapman, V. & Dickenson, A.H. (1994). Enhanced responses of rat dorsal horn neurones after UV irradiation of the hindpaw; roles of the NMDA receptor. Neuroscience Letters, 176, 41-44.

Chapman, V. & Dickenson, A.H. (1995). Time-related roles of excitatory amino acid receptors during persistent noxiously evoked responses of rat dorsal horn neurones. Brain Research, 703, 45-50.

Charpak, S. & Gahwiler, B.H. (1991). Glutamate mediates a slow synaptic response in hippocampal slice cultures. Proceedings of the Royal Society of London - Series B: Biological Sciences, 243, 221-226.

Charpak, S., Gahwiler, B.H., Do, K.Q. & Knöpfel, T. (1990). Potassium conductances in hippocampal neurons blocked by excitatory amino-acid transmitters. Nature, 347, 765-767.

Chavis, P., Fagni, L., Bockaert, J. & Lansman, B. (1995). Modulation of calcium channels by metabotropic glutamate receptors in cerebellar granule cells. Neuropharmacology, 34, 929-937.

Chavis, P., Nooney, J.M., Bockaert, J., Fagni, L., Feltz, A. & Bossu, J.L. (1995). Facilitatory coupling between a glutamate metabotropic receptor and dihydropyridine-

sensitive calcium channels in cultured cerebellar granule cells. Journal of Neuroscience, 15, 135-143.

Chavis, P., Shinozaki, H., Bockaert, J. & Fagni, L. (1994). The metabotropic glutamate receptor types 2/3 inhibit L-type calcium channels via a pertussis toxin-sensitive G-protein in cultured cerebellar granule cells. Journal of Neuroscience, 14, 7067-7076.

Chen, L. & Huang, L.-Y.M. (1991). Sustained potentiation of NMDA receptor-mediated glutamate responses through activation of protein kinase C by a μ opioid. Neuron, 7, 319-326.

Chen, L. & Huang, L.-Y.M. (1992). Protein kinase C reduces $Mg^{2+}$ block of NMDA-receptor channels as a mechanism of modulation. Nature, 356, 521-523.

Chinestra, P., Diabira, D., Urban, N.N., Barrioneuvo, G. & Ben-Ari,Y. (1994). Major differences between long-term potentiation and ACPD-induced slow onset potentiation in hippocampus. Neuroscience Letters, 182, 177-180.

Chizh, B.A., Cumberbatch, M.J., Herrero, J.F., Stirk, G.C. & Headley, P.M. (1997). Stimulus intensity, cell excitation and the *N*-methyl-D-aspartate receptor component of sensory responses in the rat spinal cord in vivo. Neuroscience, 80, 251-265.

Choi, S. & Lovinger, D.M. (1996). Metabotropic glutamate receptor modulation of voltage-gated Ca2+ channels involves multiple receptor subtypes in cortical neurons. Journal of Neuroscience, 16, 36-45.

Coderre, T.J. (1992). Contribution of protein kinase C to central sensitization and persistent pain following tissue injury. Neuroscience Letters, 140, 181-184.

Coderre, T.J. (1993). Potent analgesia induced in rats by combined action at PCP and polyamine recognition sites of the NMDA receptor complex. European Journal of Neuroscience, 5, 390-393.

Coderre, T.J., Fundytus, M.E., McKenna, J.E., Dalal, S. & Melzack, R. (1993). The formalin test: a validation of the weighted scores method of behavioural pain rating. Pain, 54, 43-50.

Coderre, T.J., Katz, J., Vaccarino, A.L. & Melzack, R. (1993). Contribution of central neuroplasticity to pathological pain: review of clinical and experimental evidence. Pain, 52, 259-285.

Coderre, T.J. & Melzack, R. (1991). Central neural mediators of secondary hyperalgesia following heat injury in rats: neuropeptides and excitatory amino acids. Neuroscience Letters, 131, 71-74.

Coderre, T.J. & Melzack, R. (1992a). The contribution of excitatory amino acids to central sensitization and persistent nociception after formalin-induced tissue injury. Journal of Neuroscience, 12, 3665-3670.

Coderre, T.J. & Melzack, R. (1992b). The role of NMDA receptor-operated calcium channels in persistent nociception after formalin-induced tissue injury. Journal of Neuroscience, 12, 3671-3675.

Coderre, T.J., Vaccarino, A.L. & Melzack, R. (1990). Central nervous system plasticity in tonic pain response to subcutaneous formalin injection. Brain Research, 535, 155-158.

Coderre, T.J. & Van Empel, I. (1994). The utility of excitatory amino acid (EAA) antagonists as analgesic agents. I. Comparison of the antinociceptive activity of

various classes of EAA antagonists in mechanical, thermal and chemical nociceptive tests. Pain, 59, 345-352.

Coderre, T.J. & Yashpal, K. (1994). Intracellular messengers contributing to persistent nociception and hyperalgesia induced by L-glutamate and substance P in the rat formalin pain model. European Journal of Neuroscience, 6, 1328-1334.

Coffey, E.T., Herrero, I., Sihra, T.S., Sánchez-Prieto, J. & Nicholls, D.G. (1994). Glutamate exocytosis and MARCKS phosphorylation are enhanced by a metabotropic glutamate receptor coupled to a protein kinase C synergistically activated by diacylglycerol and arachidonic acid. Journal of Neurochemistry, 63, 1303-1310.

Coggeshall, R.E., Dougherty, P.M., Pover, C.M. & Carlton, S.M. (1993). Is large myelinated fiber loss associated with hyperalgesia in a model of experimental peripheral neuropathy in the rat? Pain, 52, 233-242.

Collingridge, G.L., Kehl, S.J. & McLennan, H. (1983). Excitatory amino acids in synaptic transmission in the Schaffer-commissural pathway of the rat hippocampus. Journal of Physiology, 334, 33-46.

Collins, D.R., Scollon, J.M., Russell, D.C. & Davies, S.N. (1995). Indirect potentiation of synaptic transmission by metabotropic glutamate receptors in the rat hippocampal slice. Brain Research, 684, 165-171.

Congar, P., Leinekugel, X., Ben-Ari, Y. & Crepel, V. (1997). A long-lasting calcium-activated non-selective cationic current is generated by synaptic stimulation or exogenous activation of group I metabotropic glutamate receptors in CA1 pyramidal neurons. Journal of Neuroscience, 17, 5366-5379.

Courtney, M.J., Lambert, J.J. & Nicholls, D.G. (1990). The interactions between plasma membrane depolarization and glutamate receptor activation in the regulation of cytoplasmic free calcium in cultured cerebellar granule cells. Journal of Neuroscience, 10, 3873-3879.

Cozzi, A., Attucci, S., Peruginelli, F., Marinozzi, M., Luneia, R., Pellicciari, R. & Moroni, F. (1997). Type 2 metabotropic glutamate (mGlu) receptors tonically inhibit transmitter release in rat caudate nucleus: *in vivo* studies with (2*S*,1'*S*,2'*S*,3'*R*)-2-(2'-carboxy-3'-phenylcyclopropyl)glycine, a new potent and selective antagonist. European Journal of Neuroscience, 9, 1350-1355.

Crepel, V., Aniksztejn, L., Ben-Ari, Y. & Hammond, C. (1994). Glutamate metabotropic receptors increase a $Ca^{2+}$-activated nonspecific cationic current in CA1 hippocampal neurons. Journal of Neurophysiology, 72, 1561-1569.

Cumberbatch, M.J., Herrero, J.F. & Headley, P.M. (1994). Exposure of rat spinal neurones to NMDA, AMPA and kainate produces only short-term enhancements of responses to noxious and non-noxious stimuli. Neuroscience Letters, 181, 98-102.

Curtis, D.R. & Watkins, J.C. (1963). Acidic amino acids with strong excitatory actions on mammalian neurons. Journal of Physiology, London, 166, 1-14.

Daniel, H., Hemart, N., Jaillard, D. & Crepel, F. (1992). Coactivation of metabotropic glutamate receptors and of voltage-gated calcium channels induces long-term depression in cerebellar Purkinje cells *in vitro*. Brain Research, 90, 327-331.

Davar, G., Hama, A., Deykin, A., Vos, B. & Maciewicz, R. (1991). MK-801 blocks the development of thermal hyperalgesia in a rat model of experimental painful neuropathy. Brain Research, 553, 327-330.

Davies, S.N. & Lodge, D. (1987). Evidence for involvement of *N*-methylaspartate receptors in 'wind-up' of class 2 neurones in the dorsal horn of the rat. Brain Research,, 424, 402-406.

Davis, S. & Laroche, S. (1996). Activation of metabotropic glutamate receptors induce differential effects on synaptic transmission in the dentate gyrus and CA1 of the hippocampus in the anaesthetized rat. Neuropharmacology, 35, 337-346.

De Biasi, S. & Rustioni, A. (1988). Glutamate and substance P coexist in primary afferent terminals in the superficial laminae of spinal cord. Proceedings to the National Academy of Science, USA, 85, 7820-7824.

Desai, M.A., Smith, T.S. & Conn, P.J. (1992). Multiple metabotropic glutamate receptors regulate hippocampal function. Synapse, 12, 206-213.

Devor, M. & Wall, P.D. (1978). Reorganisation of spinal cord sensory map after peripheral nerve injury. Nature, 276, 75-76.

Diaz, A. & Dickenson, A.H. (1997). Blockade of spinal N- and P-type, but not L-type, calcium channels inhibits the excitability of rat dorsal horn neurones produced by subcutaneous formalin inflammation. Pain, 69, 93-100.

Dickenson, A.H. & Aydar, E. (1991). Antagonism at the glycine site on the NMDA receptor reduces spinal nociception in the rat. Neuroscience Letters, 121, 263-266.

Dickenson, A.H. & Sullivan, A.F. (1987a). Evidence for a role of the NMDA receptor in the frequency dependent potentiation of deep rat dorsal horn nociceptive neurones following C fibre stimulation. Neuropharmacology,. 26, 1235-1238.

Dickenson, A.H. & Sullivan, A.F. (1987b). Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurones. Neuroscience Letters, 83, 207-211.

Dickenson, A.H. & Sullivan, A.F. (1987c). Subcutaneous formalin-induced activity of dorsal horn neurones in the rat: differential response to an intrathecal opiate administered pre or post formalin. Pain, 30, 349-360.

Dickenson, A.H. & Sullivan, A.F. (1990). Differential effects of excitatory amino acid antagonists on dorsal horn nociceptive neurones in the rat. Brain Research, 506, 31-39.

Diemer, N.H., Jøorensen, M.B., Johansen, F.F., Sheardown, M. & Honoré, T. (1992). Protection against ischemic hippocampal CA1 damage in the rat with a new non-NMDA antagonist, NBQX. Acta Neurologica Scandinavia, 86, 45-49.

Doherty, A.J., Palmer, M.J., Henley, J.M., Collingridge, G.L. & Jane, D.E. (1997). (*RS*)-2-chloro-5-hydroxyphenylglycine (CHPG) activates $mGluR_5$, but not $mGluR_1$, receptors expressed in CHO cells and potentiates NMDA responses in the hippocampus. Neuropharmacology, 36, 265-267.

Donevan, S.D. & Rogawski, M.A. (1993). GYKI 52466, a 2,3,-benzodiazepine, is a highly selective, noncompetitive antagonist of AMPA/Kainate receptor responses. Neuron, 10, 51-59.

Dougherty, P.M., Palecek, J., Palecková, V., Sorkin, L.S. & Willis, W.D. (1992). The role of NMDA and non-NMDA excitatory amino acid receptors in the excitation of primate spinothalamic tract neurons by mechanical, chemical, thermal, and electrical stimuli. Journal of Neuroscience, 12, 3025-3041.

Dougherty, P.M. & Willis, W.D. (1991). Modification of the responses of primate spinothalamic neurons to mechanical stimulation by excitatory amino acids and an *N*-methyl-D-aspartate antagonist. Brain Research, 542, 15-22.

Dube, G.R. & Marshall, K.C. (1997). Modulation of excitatory synaptic transmission in locus coeruleus by multiple presynaptic metabotropic glutamate receptors. Neuroscience, 80, 511-521.

Dubuisson, D. & Dennis, S.G. (1977). The formalin test: a quantitative study of the analgesic effect of morphine, meperidine, and brain stem stimulation in rats and cats. Pain, 4, 161-174.

Dumuis, A., Pin J.-P., Oomagari, K., Sebben, M. & Bockaert, J. (1990). Arachidonic acid released from striatal neurons by joint stimulation of ionotropic and metabotropic quisqualate receptors. Nature, 347, 182-184.

Dumuis, A., Sebben, M., Fagni, L., Prézeau, L., Manzoni, O., Cragoe Jr., E.J. & Bockaert, J. (1993). Stimulation of arachidonic acid release by glutamate receptors depends on $Na^+/Ca^{2+}$ exchanger in neuronal cells. Molecular Pharmacology, 43, 976-981.

Duvoisin, R.M., Zhang, C. & Ramonell, K. (1995). A novel metabotropic glutamate receptor expressed in the retina and olfactory bulb. Journal of Neuroscience, 15, 3075-3083.

East, S.J., Hill, M.P. & Brotchie, J.M. (1995). Metabotropic glutamate receptor agonists inhibit endogenous glutamate release from rat striatal synaptosomes. European Journal of Pharmacology, 277, 117-121.

Eaton, S.A., Jane, D.E., Jones, L.St.J., Porter, R.H.P., Pook, P.C.-K., Sunter, D.C., Udvarhelyi, P.M., Roberts, P.J., Salt, T.E. & Watkins, J.C. (1993). Competitive antagonism at metabotropic glutamate receptors by (*S*)-4-carboxyphenylglycine and (*RS*)-α-methyl-4-carboxyphenylglycine. European Journal of Pharmacology, 244, 195-197.

Eisenberg, E., LaCross, S. & Strassman, A.M. (1995). The clinically tested *N*-methyl-D-aspartate receptor antagonist memantine blocks and reverses thermal hyperalgesia in a rat model of painful mononeuropathy. Neuroscience Letters, 187, 17-20.

Eisenberg, E., Vos, B.P. & Strassman, A.M. (1993). The NMDA antagonist memantine blocks pain behavior in a rat model of formalin-induced facial pain. Pain, 54, 301-307.

Elliott, K.J., Brodsky, M., Hynansky, A.D., Foley, K.M. & Inturrisi, C.E. (1995). Dextromethorphan suppresses both formalin-induced nociceptive behaviour and the formalin-induced increase in spinal cord c-*fos* mRNA. Pain, 61, 401-409.

Ferreira, S.H. & Lorenzetti, B.B. (1994). Glutamate spinal retrograde sensitization of primary sensory neurons associated with nociception. Neuropharmacology, 11, 1479-1485.

Fitzjohn, S.M., Irving, A.J., Palmer, M.J., Harvey, J., Lodge, D. & Collingridge, G.L. (1996). Activation of group I mGluRs potentiates NMDA responses in rat hippocampal slices. Neuroscience Letters, 203, 211-213.

Fletcher, E.J., Martin, D., Aram, J.A., Honoré, L. & Honoré, T. (1988). Quinoxalinediones selectively block quisqualate and kainate receptors and synaptic

events in rat neocortex and hippocampus and frog spinal cord *in vitro*. British Journal of Pharmacology, 95, 585-597.

Flor, P.J., Lindauer, K., Puttner, I., Rüegg, D., Lukic, S., Knöpfel, T. & Kuhn, R. (1995). Molecular cloning, functional expression and pharmacological characterization of the human metabotropic glutamate receptor type 2. European Journal of Neuroscience, 7, 622-629.

Foster, A.C. & Fagg, G.E. (1984). Acidic amino acid binding sites in mammalian neuronal membranes: their characteristics and relationship to synaptic receptors. Brain Research Review, 7, 103-164.

Forsythe, I.D. & Westbrook, G.L. (1988). Slow excitatory post-synaptic currents mediated by *N*-methyl-D-aspartate receptors on cultures of mouse hippocampal neurons. Journal of Physiology, 396, 515-534.

Fotuhi, M., Sharp, A.H., Glatt, C.E., Hwang, P.M., von Krosigk, M., Snyder, S.H. & Dawson, T.M. (1993). Differential localization of phosphoinositide-linked metabotropic glutamate receptor (mGluR1) and the inositol 1,4,5-trisphosphate receptor in rat brain. The Journal of Neuroscience, 13, 2001-2012.

Fukunaga, K., Soderling, T.R. & Miyamoto, E. (1992). Activation of $Ca^{2+}$/calmodulin-dependent protein kinase II and protein kinase C by glutamate in cultured rat hippocampal neurons. Journal of Biological Chemistry, 267, 22527-22533.

Fundytus, M.E., Fisher, K., Dray, A., Henry, J.L. & Coderre, T.J. (1998). *In vivo* antinocieptive activity of anti-rat $mGluR_1$ and $mGluR_5$ antibodies in rats. NeuroReport, 9, 731-735.

Furuyama, T., Kiyama, H., Sato, K., Park, T.H., Maeno, H., Takagi, H. & Tohyama, M. (1993). Region-specific expression of subunits of ionotropic glutamate receptors (AMPA-type, KA-type and NMDA receptors) in the rat spinal cord with special reference to nociception. Molecular Brain Research, 18, 141-151.

Gao, W-C. & Qiao, J-T. (1998). Nitric oxide contributes to both spinal nociceptive transmission and its descending inhibition in rats: an immunocytochemical study. Neuroscience Letters, 240, 143-146.

Geiger, J.R.P., Melcher, T., Koh, D.S., Sakmann, B., Seeburg, P.H., Jonas, P. & Monyer, H. (1995). Relative abundance of subunit mRNAs determining gating and $Ca^{2+}$ permeability of AMPA receptors in principal neurons and interneurons in rat CNS. Neuron, 15, 193-204.

Gerber, G., Kangrga, I., Ryu, P.D., Larew, J.S.A. & Randic, M. (1989). Multiple effects of phorbol esters in the rat spinal dorsal horn. Journal of Neuroscience, 9, 3606-3617.

Gerber, G. & Randic, M. (1989). Participation of excitatory amino acid receptors in the slow excitatory synaptic transmission in the rat spinal cord *in vitro*. Neuroscience Letters, 106, 220-228.

Gereau, R.W.,4th & Conn, P.J. (1995). Roles of specific metabotropic glutamate receptor subtypes in regulation of hippocampal CA1 pyramidal cell excitability. Journal of Neurophysiology, 74, 122-129.

Gilbert, M.E. & Mack, C.M. (1990). The NMDA antagonist, MK-801, suppresses long-term potentiation, kindling, and kindling-induced potentiation in the perforant path of the unanesthetized rat. Brain Research, 519, 89-96.

Glaum, S.R. & Miller, R.J. (1992). Metabotropic glutamate receptors mediate excitatory transmission in the nucleus of the solitary tract. Journal of Neuroscience, 12, 2251-2258.

Glaum, S.R. & Miller, R.J. (1993). Activation of metabotropic glutamate receptors produces reciprocal regulation of ionotropic glutamate and GABA responses in the nucleus of the tractus solitarius of the rat. Journal of Neuroscience, 13, 1636-1641.

Glaum, S.R. & Miller, R.J. (1995). Presynaptic metabotropic glutamate receptors modulate omega-conotoxin-GVIA-insensitive calcium channels in the rat medulla. Neuropharmacology, 34, 953-964.

Glaum, S.R., Slater, N.T., Rossi, D.J. & Miller, R.J. (1992). Role of metabotropic glutamate (ACPD) receptors at the parallel fiber-Purkinje cell synapse. Journal of Neurophysiology, 68, 1453-1462.

Glaum, S.R., Sunter, D.C., Udvarhelyi, P.M., Watkins, J.C. & Miller, R.J. (1993). The actions of phenylglycine derived metabotropic glutamate receptor antagonists on multiple (1*S*,3*R*)-ACPD responses in the rat nucleus of the tractus solitarius. Neuropharmacology, 32, 1419-1425.

Gomeza, J., Brabet, M.S., Parmentier, M.L., Restituito, S., Bockaert, J. & Pin, J.P. (1996). Coupling of metabotropic glutamate receptor 2 and 4 to G α 16, and chimeric G α q/I proteins: characterization of new antagonists. Molecular Pharmacology, 50, 923-930.

Gordh, T., Karlsten, R. & Kristensen, J. (1995). Intervention with spinal NMDA, adenosine, and NO systems for pain modulation. Annals of Medicine, 27, 229-234.

Grandes, P., Mateos, J.M., Rüegg, D., Kuhn, R. & Knöpfel, T. (1994). Differential cellular localization of three splice variants of the mGluR1 metabotropic glutamate receptor in rat cerebellum. NeuroReport, 5, 2249-2252.

Greene, C.C., Schwindt, P.C. & Crill, W.E. (1992). Metabotropic receptor mediated afterdepolarization in neocortical neurons. European Journal of Pharmacology, 226, 279-280.

Greene, C.C., Schwindt, P.C. & Crill, W.E. (1994). Properties and ionic mechanisms of a metabotropic glutamate receptor-mediated slow afterdepolarization in neocortical neurons. Journal of Neurophysiology, 72, 693-704.

Guerineau, N.C., Gahwiler, B.H. & Gerber, U. (1994). Reduction of resting $K^+$ current by metabotropic glutamate and muscarinic receptors in rat CA3 cells: mediation by G-proteins. Journal of Physiology, 474, 27-33.

Guilbaud, G., Gautron, M., Jazat, F., Ratinahirana, H., Hassig, R. & Hauw, J.J. (1993). Time course of degeneration and regeneration of myelinated nerve fibres following chronic loose ligatures of the rat sciatic nerve: can nerve lesions be linked to the abnormal pain-related behaviours. Pain, 53, 147-158.

Guiramand, J., Vignes, M. & Recasens, M. (1991). A specific transduction mechanism for the glutamate action on phosphoinositide metabolism via the quisqualate metabotropic receptor in rat brain synaptoneurosomes: II. Calcium dependency, cadmium inhibition. Journal of Neurochemistry, 57, 1501-1509.

Haley, J.E., Sullivan, A.F. & Dickenson, A.H. (1990). Evidence for spinal *N*-methyl-D-aspartate receptor involvement in prolonged chemical nociception in the rat. Brain Research, 518, 218-226.

Harris, E.W., Ganong, A.H. & Cotman, C.W. (1984). Long-term potentiation in the hippocampus involves activation of *N*-methyl-D-aspartate receptors. Brain Research, 323, 132-137.

Hartrick, C.T., Wise, J.J. & Pattterson, J.S. (1997). Preemptive intrathecal ketamine delays mechanical hyperalgesia in the neuropathic rat. Anesthesia and Analgesia, 86, 557-560.

Harvey, J., Palmer, M.J., Irving, A.J., Clarke, V.R. & Collingridge, G.L. (1996). NMDA receptor dependence on mGlu-mediated depression of synaptic transmission of the CA1 region of the rat hippocampus. British Journal of Pharmacology, 119, 1239-1247.

Hayashi, Y., Momiyama, A., Takahashi, T., Ohishi, H., Ogawa-Meguro, R., Shigemoto, R., Mizuno, N. & Nakanishi, S. (1993). Role of a metabotropic glutamate receptor in synaptic modulation in the accessory olfactory bulb. Nature, 366, 687-690.

Hayashi, Y., Sekiyama, N., Nakanishi, S., Jane, D.E., Sunter, D.C., Birse, E.F., Udvarhelyi, P.M. & Watkins, J.C. (1994). Analysis of agonist and antagonist activities of phenylglycine derivatives for different cloned metabotropic glutamate receptor subtypes. Journal of Neuroscience, 14, 3370-3377.

Hayashi, Y., Tanabe, Y., Aramori, I., Masu, M., Shimamoto, K., Ohfune, Y. & Nakanishi, S. (1992). Agonist analysis of 2-(carboxycyclopropyl)glycine isomers for cloned metabotropic glutamate receptor subtypes expressed in Chinese hamster ovary cells. British Journal of Pharmacology, 107, 539-543.

Hayes, R.L., Mao, J., Price, D.D., Germano, A., D'Avella, D., Fiori, M. & Mayer, D.L. (1992). Pretreatment with gangliosides reduces abnormal nociceptive responses associated with a rodent mononeuropathy. Pain, 48, 391-396.

Herrero, I., Castro, E., Miras-Portugal, M.T. & Sánchez-Prieto, J. (1996). Two components of glutamate exocytosis differentially affected by presynaptic modulation. Journal of Neurochemistry, 67, 2346-2354.

Herrero, I., Miras-Portugal, M.T. & Sánchez-Prieto, J. (1992a). Positive feedback of glutamate exocytosis by metabotropic presynaptic receptor stimulation. Nature, 360, 163-166.

Herrero, I., Miras-Portugal, M.T. & Sánchez-Prieto, J. (1992b). Activation of protein kinase C by phorbol esters and arachidonic acid required for the optimal potentiation of glutamate exocytosis. Journal of Neurochemistry, 59, 1574-1577.

Herrero, I., Miras-Portugal, M. & Sánchez-Prieto, J. (1994). Rapid desensitization of the metabotropic glutamate receptor that facilitates glutamate release in rat cerebrocortical nerve terminals. European Journal of Neuroscience, 6, 115-120.

Herrero, I., Vázquez, E., Miras-Portugal, M.T. & Sánchez-Prieto, J. (1996). Decrease in $[Ca^{2+}]c$ but not in cAMP mediates L-AP4 inhibition of glutamate release: PKC-mediated suppression of this inhibitory pathway. European Journal of Neuroscience, 8, 700-709.

Hollmann, M. & Heinemann, S. (1994). Cloned glutamate receptors. Annual Review of Neuroscience, 17, 31-108.

Hollmann, M., O'Shea-Greenfield, A., Rogers, S.W. & Heinemann, S. (1989). Cloning by functional expression of a member of the glutamate receptor family. Nature, 342, 643-648.

Hölscher, C. (1997). Nitric oxide, the enigmatic neuronal messenger: its role in synaptic plasticity. Trends in Neuroscience, 20, 298-303.

Hölscher, C., Anwyl, R., & Rowan, M.J. (1997). Activation of group II metabotropic glutamate receptors blocks induction of long-term potentiation and depotentiation in area CA1 of the rat *in vivo*. European Journal of Pharmacology, 322, 155-163.

Houamed, K.M., Kuijper, J.L., Gilbert, T.L., Haldeman, B.A., O'Hara, P.J., Mulvihill, E.R., Almers, W. & Hagen, F.S. (1991). Cloning, expression, and gene structure of a G protein-coupled glutamate receptor from rat brain. Science, 252, 1318-1321.

Huang, L., Breakwell, N.A., Rowan, M.J. & Anwyl, R. (1997). PCC-IV inhibits the induction of long-term potentiation in the dentate gyrus *in vitro*. European Journal of Pharmacology, 332, 161-165.

Hume, R.I., Dingledine, R. & Heinemann, S.F. (1991). Identification of a site in glutamate receptor subunits that controls calcium permeability. Science, 253, 1028-1031.

Hunter, J.C. & Singh, L. (1994). Role of excitatory amino acid receptors in the mediation of the nociceptive response to formalin in the rat. Neuroscience Letters, 174, 217-221.

Hylden, J.L.K., Nahin, R.L., Traub, R.J. & Dubner, R. (1989). Expansion of receptive fields of spinal lamina I projection neurons in rats with unilateral adjuvant-induced inflammation: the contribution of dorsal horn mechanisms. Pain, 37, 229-243.

Igwe, O.J. & Ning, L. (1994). Regulation of the second-messenger systems in the rat spinal cord during prolonged peripheral inflammation. Pain, 58, 63-75.

Ikeda, K., Nagasawa, M., Mori, H., Arakis, K., Sakimura, K., Watanabe, M., Inoue, Y. & Mishina, M. (1992). Cloning and expression of the epsilon 4 subunit of the NMDA receptor channel. FEBS Letters, 313, 34-38.

Ikeda, S.R., Lovinger, D.M., McCool, B.A. & Lewis, D.L. (1995). Heterologous expression of metabotropic glutamate receptors in adult rat sympathetic neurons: subtype-specific coupling to ion channels. Neuron, 14, 1029-1038.

Irving, A.J., Schofield, J.G., Watkins, J.C., Sunter, D.C. & Collingridge, G.L. (1990). 1*S*,3*R*-ACPD stimulates and L-AP3 blocks $Ca^{2+}$ mobilization in rat cerebellar neurons. European Journal of Pharmacology, 186, 363-365.

Ishida, M., Saitoh, T., Shimamoto, K., Ohfune, Y. & Shinozaki, H. (1993). A novel metabotropic glutamate receptor agonist: marked depression of monosynaptic excitation in the newborn rat isolated spinal cord. British Journal of Pharmacology, 109, 1169-1177.

Ishii, T., Moriyoshi, K., Sugihara, H., Sakurada, K., Kadatoni, H., Yokoi, M., Akazawa, C., Shigemoto, R., Mizuno, N., Masu, M. & Nakanishi, S. (1993). Molecular characterization of the family of the *N*-methyl-D-aspartate receptor subunits. Journal of Biological Chemistry, 268, 2836-2843.

Ito, I., Kohda, A., Tanabe, S., Hirose, E., Hayashi, M., Mitsunaga, S. & Sugiyama, H. (1992). 3,5-Dihydroxyphenyl-glycine: a potent agonist of metabotropic glutamate receptors. NeuroReport, 3, 1013-1016.

Iversen, L., Mulvihill, E., Haldeman, B., Diemer, N.H., Kaiser, F., Sheardown, M. & Kristensen, P. (1994). Changes in metabotropic glutamate receptor mRNA levels following global ischemia increase of a putative presynaptic subtype (mGluR4) in highly vulnerable rat brain areas. Journal of Neurochemistry, 63, 625-633.

Izumi, Y., Clifford, D.B. & Zorumski, C.F. (1991). 2-Amino-3-phosphonopropionate blocks the induction and maintenance of long-term potentiation in rat hippocampal slices. Neuroscience Letters, 122, 187-190.

Jaffe, D.B. & Brown, T.H. (1994). Metabotropic glutamate receptor activation induces calcium waves within hippocampal dendrites. Journal of Neurophysiology, 72, 471-474.

Jahr, C.E. & Stevens, C.F. (1987). Glutamate activates multiple single channel conductances in hippocampal neurons. Nature, 325, 522-525.

Jane, D.E., Jones, P.L. St. J., Pook, P.C.-K., Salt, T.E., Sunter, D.C. & Watkins, J.C. (1993). Stereospecific antagonism by (+)-α-methyl-4-carboxyphenylglycine (MCPG) of (1*S*,3*R*)-ACPD-induced effects in neonatal rat motoneurones and rat thalamic neurones. Neuropharmacology, 32, 725-727.

Jane, D.E., Jones, P.L. St. J., Pook, P.C.-K., Tse, H.-W. & Watkins, J.C. (1994). Actions of two new antagonists showing selectivity for different sub-types of metabotropic glutamate receptor in the neonatal rat spinal cord. British Journal of Pharmacology, 112, 809-816.

Jane, D.E., Pittway, K., Sunter, D.C., Thomas, N.K. & Watkins, J.C. (1995). New phenylglycine derivatives with potent and selective antagonist activity at presynaptic glutamate receptors in neonatal spinal cord. Neuropharmacology, 34, 851-856.

Jane, D.E., Thomas, N.K., Tse, H.W. & Watkins, J.C. (1996). Potent antagonists at the L-AP4- and (1*S*,3*S*)-ACPD-sensitive presynaptic metabotropic glutamate receptors in the neonatal rat spinal cord. Neuropharmacology, 35, 1029-1035.

Johansen, P.A. & Robinson, M.B. (1995). Identification of 2-amino-2-methyl-4-phosphonobutanoic acid as an antagonist at the mGluR4a receptor. European Journal of Pharmacology, 290, R1-R3.

Johansen, T.H., Drejer, J., Watjen, F. & Nielsen, E.O. (1993). A novel non-NMDA receptor antagonist shows selective displacement of low-affinity [$^3$H]kainate binding. European Journal of Pharmacology, 246, 195-204.

Joly, C., Gomeza, J., Brabet, I., Curry, K., Bockaert, J. & Pin, J.P. (1995). Molecular, functional, and pharmacological characterization of the metabotropic glutamate receptor type 5 splice variants: comparison with mGluR1. Journal of Neuroscience, 15, 3970-3981.

Jonas, P. & Burnashev, N. (1995). Molecular mechanisms controlling calcium entry through AMPA-type glutamate receptor channels. Neuron, 15, 987-990.

Jones, M.W. & Headley, P.M. (1995). Interactions between metabotropic and ionotropic glutamate receptor agonists in the rat spinal cord *in vivo*. Neuropharmacology, 34, 1025-1031.

Jones, N.M., Lawrence, A.J. & Beart, P.M. (1998). *In vivo* microdialysis reveals facilitatory metabotropic glutamate receptors regulating excitatory amino acid release in rat nucleus tractus solitarius. Neurochemistry International, 32, 31-38.

Jones, P.G. & Roberts, P.J. (1993). Stimulation of phosphoinositide hydrolysis in the guinea pig cortex by isomers of 1-aminocyclo-pentane-1,3-dicarboxylate (ACPD). Molecular Neuropharmacology, 3, 17.

Jouvenceau, A., Dutar, P. & Billard, J.M. (1995). Presynaptic depression of inhibitory postsynaptic potentials by metabotropic glutamate receptors in rat hippocampal CA1 pyramidal cells. European Journal of Pharmacology, 281, 131-139.

Kajander, K.C. & Bennett, G.J. (1992). Onset of a painful peripheral neuropathy in rat: a partial and differential deafferentation and spontaneous discharge in Aβ and Aδ primary afferent neurons. Journal of Neurophysiology, 68, 734-744.

Kajander, K.C., Wakisaka, S. & Bennett, G.J. (1992). Spontaneous discharge originates in the dorsal root ganglion at the onset of a painful peripheral neuropathy in the rat. Neuroscience Letters, 138, 225-228.

Kamiya, H., Shinozaki, H. & Yamamoto, C. (1996). Activation of metabotropic glutamate receptor type 2/3 suppresses transmission at rat hippocampal mossy fibre synapses. Journal of Physiology, 493, 447-455.

Kangrga, I. & Randic, M. (1991). Outflow of endogenous aspartate and glutamate from the rat spinal dorsal horn *in vitro* by activation of low-and high-threshold primary afferent fibers. Modulation by μ-opioids. Brain Research, 553, 347-352.

Kawamata, M. & Omote, K. (1996). Involvement of increased excitatory amino acids and intracellular $Ca^{2+}$ concentrations in the spinal dorsal horn in an animal model of neuropathic pain. Pain, 68, 85-96.

Keinänen, K., Wisden, W., Sommer, B., Werner, P., Herb, A., Verdoorn, T.A., Sakmann, B. & Seeburg, P.H. (1990). A family of AMPA-selective glutamate receptors. Science, 249, 556-560.

Kelso, S.R., Nelson, T.E. & Leonard, J.P. (1992). Protein kinase C-mediated enhancement of NMDA currents by metabotropic glutamate receptors in xenopus oocytes. Journal of Physiology, 449, 705-718.

Kemp, J.A. & Leeson, P.D. (1993). The glycine site of the NMDA receptor: five years on. Trends in Pharmacological Science, 14, 20-25.

Kemp, M., Roberts, P., Pook, P., Jane, D., Jones, A., Jones, P., Sunter, D., Advarhelyi, P. & Watkins, J. (1994). Antagonism of presynaptically mediated depressant responses and cyclic AMP-coupled metabotropic glutamate receptors. European Journal of Pharmacology, 266, 187-192.

Kenshalo, D.R., Jr., Leonard, R.B., Chung, J.M. & Willis, W.D. (1982). Facilitation of the responses of primate spinothalamic cells to cold and mechanical stimuli by noxious heating of the skin. Pain, 12, 141-152.

Kim, Y.I., Na, H.S., Yoon, Y.W., Han, H.C., Ko, K.H. & Hong, S.K. (1997). NMDA receptors are important for both mechanical and thermal allodynia from peripheral nerve injury in rats. NeuroReport, 8, 2149-2153.

King, A.E. & Lopez-Garcia, J.A. (1993). Excitatory amino acid receptor-mediated neurotransmission from cutaneous afferents in rat dorsal horn *in vitro*. Journal of Physiology, 472, 443-457.

King, A.E., Thompson, S.W.N., Urban, L. & Woolf, C.J. (1988). An intracellular analysis of amino acid induced excitations of deep dorsal horn neurones in the rat spinal cord slice. Neuroscience Letters, 89, 286-292.

Kingston, A.E., Burnett, J.P., Mayne, N.G. & Lodge, D. (1995). Pharmacological Analysis of 4-carboxyphenylglycine derivatives: comparison of effects on mGluR1α and mGluR5a subtypes. Neuropharmacology, 34, 887-894.

Kinoshita, A., Ohishi, H., Neki, A., Nomura, S., Shigemoto, R., Takada, M., Nakanishi, S. & Mizuno, N. (1996). Presynaptic localization of a metabotropic glutamate receptor, mGluR8, in the rhinencephalic areas: a light and electron microscope study in the rat. Neuroscience Letters, 207, 61-64.

Kinoshita, A., Ohishi, H., Nomura, S., Shigemoto, R., Nakanishi, S. & Mizuno, N. (1996). Presynaptic localization of a metabotropic glutamate receptor, mGluR4a, in the cerebellar cortex: a light and electron microscope study in the rat. Neuroscience Letters, 207, 199-202.

Klein, J., Reymann, K.G. & Riedel, G. (1997). Activation of phospholipases C and D by the novel metabotropic glutamate receptor agonist tADA. Neuropharmacology, 36, 261-263.

Klimscha, W., Horváth, G., Szikszay, M., Dobos, I. & Benedek, G. (1998). Antinociceptive effect of the *S*(+)-enantiomer of ketamine on carrageenan hyperalgesia after intrathecal administration in rats. Anesthesia and Analgesia, 86, 561-565.

Koga, T., Sakai, N., Tanaka, C. & Saito, N. (1996) Presynaptic and $Ca^{2+}$-independent PKC subspecies modulates NMDA1 current. NeuroReport, 7, 477-480.

Koh, J.-Y., Palmer, E. & Cotman, C.W. (1991). Activation of the metabotropic glutamate receptor attenuates *N*-methyl-D-aspartate neurotoxicity in cortical cultures. Proceedings to the National Academy of Science, USA, 88, 9431-9435.

Köhler, M., Burnashev, N., Sakmann, B. & Seeburg, P.H. (1993). Determination of $Ca^{2+}$ permeability in both TM1 and TM2 of high affinity kainate receptor channels: diversity of RNA editing. Neuron, 10, 491-500.

Kolhekar, R. & Gebhart, G.F. (1994). NMDA and quisqualate modulation of visceral nociception in the rat. Brain Research, 651, 215-226.

Kolhekar, R., Meller, S.T. & Gebhart, G.F. (1993). Characterization of the role of spinal *N*-methyl-D-aspartate receptors in thermal nociception in the rat. Neuroscience, 57, 385-395.

Kozikowski, A.P., Tückmantel, W., Liao, Y., Manev, H., Ikonomovic, S. & Wroblewski, J.T. (1993). Synthesis and metabotropic receptor activity of the novel rigidified glutamate analogues (+)- and (-)-*trans*-azetidine-2,4-dicarboxylic acid and their *N*-methyl derivatives. Journal of Medicinal Chemistry, 36, 2706-2708.

Kristensen, J.D., Karlsten, R., Gordh, T. & Berge, O.-G. (1994) The NMDA antagonist 3-(2-carboxypiperazin-4-yl)propyl-1-phosphonic acid (CPP) has antinociceptive effect after intrathecal injection in the rat. Pain, 56, 59-67.

Krnjevic, K. & Phillis, J.W. (1963). Iontophoretic studies of neurones in the mammalian cerebral cortex. Journal of Physiology, London, 165, 274-305.

Kutsuwada, T., Kashiwabuchi, N., Mori, H., Sakimura, K., Kushiya, E., Araki, K., Meguro, H., Masaki, H., Kymanishi, T., Arakawa, M. & Mishina, M. (1992). Molecular diversity of the NMDA receptor channel. Nature, 358, 36-41.

Laird, J.M. & Bennett, G.J. (1993). An electrophysiological study of dorsal horn neurons in the spinal cord of rats with an experimental peripheral neuropathy. Journal of Neurophysiology, 69, 2072-2085.

Laird, J.M., Mason, G.S., Webb, J., Hill, R.G. & Hargreaves, R.J. (1996). Effects of a partial agonist and a full antagonist acting at the glycine site of the NMDA receptor on inflammation-induced mechanical hyperalgesia in rats. British Journal of Pharmacology, 117, 1487-1492.

Lam, H.H., Hanley, D.F., Trapp, B.D., Saito, S., Raja, S., Dawson, T.M. & Yamaguchi, H. (1996). Induction of spinal cord neuronal nitric oxide synthase (NOS) after formalin injection in the rat hind paw. Neuroscience Letters, 210, 201-204.

Lekan, H.A. & Carlton, S.M. (1995). Glutamatergic and GABAergic input to rat spinothalamic tract cells in the superficial dorsal horn. Journal of Comparative Neurology, 361, 417-428.

Lerma, J., Paternain, A.V., Naranjo, J.R. & Mellstrim, B. (1993). Functional kainate selective glutamate receptors in cultured hippocampal neurons. Proceedings to the National Academy of Science, USA, 90, 11688-11692.

Li, J.-L., Ohishi, H., Kaneko, T., Shigemoto, R., Neki, A., Nakanishi, S. & Muzuno, N. (1996). Immunohistochemical localization of a metabotropic glutamate receptor, mGluR7, in ganglion neurons of the rat; with special reference to the presence of glutamatergic ganglion neurons. Neuroscience Letters, 204, 9-12.

Li, H., Ohishi, H., Kinoshita, A., Shigemoto, R., Nomura, S. & Mizuno, N. (1997). Localization of a metabotropic glutamate receptor, mGluR7, in axon terminals of presumed nociceptive, primary afferent fibers in the superficial layers of the spinal dorsal horn: an electron microscope study in the rat. Neuroscience Letters, 223, 153-156.

Libri, V., Constanti, A., Zibetti, M. & Postlethwaite, M. (1997). Metabotropic glutamate receptor subtypes mediating slow inward tail current (IADP) induction and inhibition of synaptic transmission in olfactory cortical neurones. British Journal of Pharmacology, 120, 1083-1095.

Lin, Q., Peng, Y.B. & Willis, W.D. (1996). Possible role of protein kinase C in the sensitization of primate spinothalamic tract neurons. Journal of Neuroscience, 16, 3026-3034.

Littman, L., Chase, L.A., Renzi, M., Garlin, A.B., Koerner, J.F., Johnson, R.L. & Robinson, M.B. (1995). Effects of quisqualic acid analogs on metabotropic glutamate receptors coupled to phosphoinositide hydrolysis in rat hippocampus. Neuropharmacology, 34, 829-841.

Littman, L., Munir, M., Flagg, S.D. & Robinson, M.B. (1992). Multiple mechanisms for inhibition of excitatory amino acid receptors coupled to phosphoinositide hydrolysis. Journal of Neurochemistry, 59, 1893-1904.

Littman, L. & Robinson, M.B. (1994). The effects of L-glutamate and *trans*-(+-)-1-amino-1,3-cyclopentanedicarboxylate on phosphoinositide hydrolysis can be pharmacologically differentiated. Journal of Neurochemistry, 63, 1291-1302.

Llano, I., Dreessen, J., Kano, M. & Konnerth, A. (1991). Intradendritic release of calcium induced by glutamate in cerebellar Purkinje cells. Neuron, 7, 577-583.

Lodge, D. & Collingridge, G. (1991). The pharmacology of excitatory amino acids. In: D. Lodge & G.L. Collingridge (Eds.), Trends in Pharmacological Science, Special report: The pharmacology of excitatory amino acids (pp. 1-3). Cambridge: Elsevier.

Lodge, D. & Johnson, K.M. (1991). Noncompetitive excitatory amino acid receptor antagonists. In: D. Lodge & G.L. Collingridge (Eds.), Trends in Pharmacological Science, Special report: The pharmacology of excitatory amino acids (pp. 13-18). Cambridge:Elsevier.

Lombardi, G., Alesiani, M., Leonardi, P., Cherici, G., Pellicciari, R. & Moroni, F. (1993). Pharmacological characterization of the metabotropic glutamate receptor inhibiting D-[$^3$H]-aspartate output in rat striatum. British Journal of Pharmacology, 110, 1407-1412.

Lombardi, G., Leonardi, P. & Moroni, F. (1996). Metabotropic glutamate receptors, transmitter output and fatty acids: studies in rat brain slices. British Journal of Pharmacology, 117, 189-195.

Lombardi, G., Pellegrini-Giampietro, D.E., Leonard, P., Cherici, G., Pellicciari, R. & Moroni, F. (1994). The depolarization-induced outflow of D-[$^3$H]aspartate from rat brain slices is modulated by metabotropic glutamate receptors. Neurochemistry International, 24, 525-532.

Lorenzini, P., Bisso, G.M., Fortuna, S. & Michalek, H. (1996). Differential responsiveness of metabotropic glutamate receptors coupled to phosphoinositide hydrolysis to agonists in various brain areas of the adult rat. Neurochemical Research, 21, 323-329.

Löscher, W. (1998). Pharmacology of glutamate receptor antagonists in the kindling model of epilepsy. Progress in Neurobiology, 54, 721-741.

Lovinger, D.M. (1991). *Trans*-1-aminocyclopentane-1,3-dicarboxylic acid (*t*-ACPD) decreases synaptic excitation in rat striatal slices through a presynaptic action. Neuroscience Letters, 129, 17-21.

Lovinger, D.M. & McCool, B.A. (1995). Metabotropic glutamate receptor-mediated presynaptic depression at corticostriatal synapses involves mGluR2 or 3. Journal of Neurophysiology, 73, 1076-1083.

Lowenstein, C.J., Dinerman, J.L. & Snyder, S.H. (1994). Nitric oxide: A physiologic messenger. Annals of Internal Medicine, 120, 227-237.

Lujan, R., Nusser, Z., Roberts, J.D., Shigemoto, R. & Somogyi, P. (1996). Perisynaptic location of metabotropic glutamate receptors mGluR1 and mGluR5 on dendrites and dendritic spines in the rat hippocampus. European Journal of Neuroscience, 8, 1488-1500.

Luthi, A., Gahwiler, B.H. & Gerber, U. (1997). 1*S*,3*R*-ACPD induces a region of negative slope conductance in the steady-state current-voltage relationship of hippocampal pyramidal cells. Journal of Neurophysiology, 77, 221-228.

Ma, Q.P. & Woolf, C.J. (1995). Noxious stimuli induce an *N*-methyl-D-aspartate receptor-dependent hypersensitivity of the flexion withdrawal reflex to touch: implications for the treatment of mechanical allodynia. Pain, 61, 383-390.

MacDermott, A.B., Mayer, M.L., Westbrook, G.L., Smith, S.J. & Barker, J.L. (1986) NMDA-receptor activation increases cytoplasmic calcium concentration in cultured spinal cord neurones. Nature, 321, 519-522.

MacDonald, J.F. & Wojtowicz, J.M. (1980) Two conductance mechanisms activated by applications of L-glutamic, L-aspartic, DL-homocysteic, *N*-methyl-D-aspartic, and KL-kainic acids to cultured mammalian central neurones. Canadian Journal of Physiological Pharmacology, 58, 1393-1397.

Macek, T.A., Winder, D.G., Gereau, R.W. 4th, Ladd, C.O. & Conn, P.J. (1996). Differential involvement of group II and group III mGluRs as autoreceptors at lateral and medial perforant path synapses. Journal of Neurophysiology, 76, 3798-3806.

Malmberg, A.B., Chen, C., Tonegawa, S. & Basbaum, A.I. (1997). Preserved acute pain and reduced neuropathic pain in mice lacking PKC gamma. Science, 278, 279-283.

Malmberg, A.B. & Yaksh, T.L. (1993). Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. Pain, 54, 291-300.

Malmberg, A.B. & Yaksh, T.L. (1994). Voltage-sensitive calcium channels in spinal nociceptive processing: blockage of N- and P-type channels inhibits formalin-induced nociception. Journal of Neuroscience, 14, 4882-4890.

Manahan-Vaughan, D., Reiser, M., Pin, J.P., Wilsch, V., Bockaert, J., Reymann, K.G. & Riedel, G. (1996). Physiological and pharmacological profile of *trans*-azetidine-2,4-dicarboxylic acid: metabotropic glutamate receptor agonism and effects on long-term potentiation. Neuroscience, 72, 999-1008.

Manahan-Vaughan, D. & Reymann, K.G. (1995). 1S,3R-ACPD dose-dependently induces a slow-onset potentiation in the rat hippocampal CA1 region *in vivo*. Neuropharmacology, 34, 1103-1105.

Manahan-Vaughan, D. & Reymann, K.G. (1996). Metabotropic glutamate receptor subtype agonists facilitate long-term potentiation within a distinct time window in the dentate gyrus *in vivo*. Neuroscience, 74, 723-731.

Manzoni, O.J., Castillo, P.E. & Nicoll, R.A. (1995). Pharmacology of metabotropic glutamate receptors at the mossy fiber synapses of the guinea pig hippocampus. Neuropharmacology, 34, 965-971.

Manzoni, O.J., Fagni, L., Pin, J.P., Rassendren, F., Poulet, F., Sladeczek, F. & Bockaert, J. (1990). *Trans*-1-amino-cyclopentyl-1,3-dicarboxylic stimulation quisqualate phosphoinositide-coupled receptors but not ionotropic glutamate receptors in striatal neurons and *Xenopus* oocytes. Molecular Pharmacology, 38, 1-6.

Manzoni, O.J., Finiels-Marlier, F., Sassetti, I., Bockaert, J., le Peuch, C. & Sladeczek, F.A. (1990). The glutamate receptor of the Qp-type activates protein kinase C and is regulated by protein kinase C. Neuroscience Letters, 109, 146-151.

Manzoni, O., Michel, J.-M. & Bockaert, J. (1997). Metabotropic glutamate receptors in the rat nucleus accumbens. European Journal of Neuroscience, 9, 1514-1523.

Manzoni, O.J., Poulat, F., Do, E., Sahuquet, A., Sassetti, I., Bockaert, J. & Sladeczek, F.A. (1991). Pharmacological characterization of the quisqualate receptor coupled to phospholipase C (Qp) in striatal neurons. European Journal of Pharmacology, 207, 231-241.

Mao, J., Mayer, D.J., Hayes, R.L. & Price, D.D. (1993). Spatial patterns of increased spinal cord membrane-bound protein kinase C and their relation to increase sin 14C-2-deoxyglucose metabolic activity in rats with painful peripheral mononeuropathy. Journal of Neurophysiology, 70, 470-481.

Mao, J., Price, D.D., Hayes, R.L. Lu, J. & Mayer, D.J. (1992). Differential role of NMDA and non-NMDA receptor activation in induction and maintenance of thermal

hyperalgesia in rats with painful peripheral mononeuropathy. Brain Research, 598, 271-278.

Mao, J., Price, D.D., Hayes, R.L., Lu, J., Mayer, D.J. & Frenk, H. (1993). Intrathecal treatment with dextrorphan or ketamine potently reduces pain-related behaviours in a rat model of peripheral mononeuropathy. Brain Research, 605, 164-168.

Mao, J., Price, D.D., Mayer, D.J. & Hayes, R.L. (1992). Pain-related increases in spinal cord membrane-bound protein kinase C following peripheral nerve injury. Brain Research, 588, 144-149.

Mao, J., Price, D.D., Mayer, D.J., Lu, J. & Hayes, R.L. (1992). Intrathecal MK-801 and local nerve anesthesia synergistically reduce nociceptive behaviours in rats with experimental peripheral mononeuropathy. Brain Research, 576, 254-262.

Mao, J., Price, D.D., Phillips, L.L., Lu, J. & Mayer, D.J. (1995). Increases in protein kinase C gamma immunoreactivity in the spinal cord dorsal horn of rats with painful mononeuropathy. Neuroscience Letters, 198, 75-78.

Mao, J., Price, D.D., Zhu, J., Lu, J. & Mayer, D.J. (1997). The inhibition of nitric oxide-activated poly (ADP-ribose) synthetase attenuates transsynaptic alteration of spinal cord dorsal horn neurons and neuropathic pain in the rat. Pain, 72, 355-366.

Martin, L.J., Blackstone, C.D., Huganir, R.L. & Price, D.L. (1992). Cellular localization of a metabotropic glutamate receptor in rat brain. Neuron, 9, 259-270.

Masu, M., Tanabe, Y., Tsuchida, K., Shigemoto, R. & Nakanishi, S. (1991). Sequence and expression of a metabotropic glutamate receptor. Nature, 349, 760-765.

Mayer, M. L. & Westbrook, G. L. (1987). Permeation and block of *N*-methyl-D-aspartic acid receptor channels by divalent cations in mouse cultured central neurones. Journal and Physiology, 394, 501-527.

Mayer, M. L., Westbrook, G. L. & Guthrie, P. B. (1984). Voltage-dependent block by $Mg^{2+}$ of NMDA responses in spinal cord neurones. Nature, 309, 261-263.

McCormick, D.A. & von Krosigk, M. (1992). Corticothalamic activation modulates thalamic firing through glutamate "metabotropic" receptors. Proceedings of the National Academy of Sciences, USA, 89, 2774-2778.

McGahon, B. & Lynch, M.A. (1994). A study of the synergism between metabotropic glutamate receptor activation and arachidonic acid in the rat hippocampus. NeuroReport, 5, 2353-2357.

McLennan, H., Huffman, R.D. & Marshall, K.C. (1968). Patterns of excitation of thalamic neurones by amino-acids and by acetylcholine. Nature, 219, 387-388.

Meguro, H., Mori, H., Araki, K., Kushiya, E., Kutsuwada, T., Yamazaki, M., Kumanishi, T., Arakawa, M., Sakimura, K. & Mishina, M. (1992). Functional characterization of a heteromeric NMDA receptor channel expressed from cloned cDNAs. Nature, 357, 70-74.

Meldrum, B. & Garthwaite, J. (1990). Excitatory amino acid neurotoxicity and neurodegenerative disease. Trends in Pharmacological Science, 11, 379-387.

Meller, S.T. (1994). Thermal and mechanical hyperalgesia: a distinct role for different excitatory amino acid receptors and signal transduction pathways? APS Journal, 3, 215-231.

Meller, S.T., Cummings, C.P., Traub, R.J. & Gebhart, G.F. (1994). The role of nitric oxide in the development and maintenance of the hyperalgesia produced by intraplantar injection of carrageenan in the rat. Neuroscience, 60, 367-374.

Meller, S.T., Dykstra, C.L. & Gebhart, G.F. (1993). Acute mechanical hyperalgesia is produced by coactivation of AMPA and metabotropic glutamate receptors. NeuroReport, 4, 879-882.

Meller, S.T., Dykstra, C.L. & Gebhart, G.F. (1996). Acute thermal hyperalgesia in the rat is produced by activation of *N*-methyl-D-aspartate receptors and protein kinase C and production of nitric oxide. Neuroscience, 71, 327-335.

Meller, S.T. & Gebhart, G.F. (1993). Nitric oxide (NO) and nociceptive processing in the spinal cord. Pain, 52, 127-136.

Meller, S.T. & Gebhart, G.F. (1994). Spinal mediators of hyperalgesia. Drugs, 47, 10-20.

Meller, S.T., Gebhart, G.F. & Maves, T.J. (1992). Neonatal capsaicin treatment prevents the development of the thermal hyperalgesia produced in a model of neuropathic pain in the rat. Pain, 51, 317-321.

Mendell, L.M. (1966). Physiological properties of unmyelinated fiber projections to the spinal cord. Experimental Neurology, 16, 316-332.

Menétrey, D. & Besson, J.M. (1982). Electrophysiological characteristics of dorsal horn cells in rats with cutaneous inflammation resulting from chronic arthritis. Pain, 13, 343-364.

Millan, M.J. & Sequin, L. (1994). Chemically-diverse ligands at the glycine B site coupled to *N*-methyl-D-aspartate (NMDA) receptors selectively block the late phase of formalin-induced pain in mice. Neuroscience Letters, 178, 139-143.

Miller, K.E., Clements, J.R., Larson, A.A. & Beitz, A.J. (1988). Organization of glutamate-like immunoreactivity in the rat superficial dorsal horn: light and electron microscopic observations. Synapse, 2, 28-36.

Mistry, R. & Challiss, R.A.J. (1996). Differences in agonist and antagonist activities for two indices of metabotropic glutamate receptor-stimulated phosphoinositide turnover. British Journal of Pharmacology, 117, 1735-1743.

Mistry, R., Prabhu, G., Godwin, M. & Challiss, R.A. (1996). Stimulatory effects of the putative metabotropic glutamate receptor antagonist L-AP3 on phosphoinositide turnover in neonatal rat cerebral cortex. British Journal of Pharmacology, 117, 1309-1317.

Mjellem-Joly, N., Lund, A., Berge, O.-G. & Hole, K. (1991). Potentiation of a behavioural response in mice by spinal coadministration of substance P and excitatory amino acid agonists. Neuroscience Letters, 133, 121-124.

Monaghan, D.T., Bridges, R.J. & Cotman, C.W. (1989). The excitatory amino acid receptors: their classes, pharmacology, and distinct properties in the function of the central nervous system. Annual Reviews of Pharmacology and Toxicology, 29, 365-402.

Monyer, H., Burnashev, N., Laurie, D.J., Sakmann, B. & Seeburg, P.H. (1994). Development and regional expression in the rat brain and functional properties of four NMDA receptors. Neuron, 12, 529-540.

Monyer, H., Sprengel, R., Schoepfer, R., Herb, A., Higuchi, M., Lomeli, H., Burnashev, N., Sakmann, B. & Seeburg, P. H. (1992). Heteromeric NMDA receptors: molecular and functional distinction of subtypes. Science, 256, 1217-1221.

Morimoto, K., Katayama, K., Inoue, K. & Sato, K. (1991). Effects of competitive and noncompetitive NMDA receptor antagonists on kindling and LTP. Pharmacology Biochemistry & Behaviour, 40, 893-899.

Morisset, V. & Nagy, F. (1996). Modulation of regenerative membrane properties by stimulation of metabotropic glutamate receptors in rat deep dorsal horn neurons. Journal of Neurophysiology, 76, 2794-2798.

Moriyoshi, K., Masu, M., Ishii, T., Shigemoto, R., Mizuno, N. & Nakanishi, S. (1991). Molecular cloning and characterization of the rat NMDA receptor. Nature, 358, 36-41.

Moroni, F., Lombardi, G., Thomsen, C., Leonardi, P., Attucci, S., Peruginelli, F., Torregrossa, S.A., Pellegrini-Giampietro, D.E., Luneia, R. & Pellicciari, R. (1997). Pharmacological characterization of 1-aminoindan-1,5-dicarboxylic acid, a potent mGluR1 antagonist. Journal of Pharmacology and Experimental Therapeutics, 281, 721-729.

Morris, R.G.M., Anderson, E., Lynch, G.S. & Baudry, M. (1986). Selective impairment of learning and blockade of long-term potentiation by an *N*-methyl-D-aspartate receptor antagonist, AP5. Nature, 319, 774-776.

Mosconi, T. & Kruger, L. (1996). Fixed-diameter polyethylene cuffs applied to the rat sciatic nerve induce a painful neuropathy: ultrastructural morphometric analysis of axonal alterations. Pain, 64, 37-57.

Munro, F.E., Fleetwood-Walker, S.M. & Mitchell, R. (1994). Evidence for a role of protein kinase C in the sustained activation of rat dorsal horn neurons evoked by cutaneous mustard oil application. Neuroscience Letters, 170, 199-202.

Murphy, S.N. & Miller, R.J. (1988). A glutamate receptor regulates $Ca^{2+}$ mobilization in hippocampal neurons. Proceedings of the National Academy of Sciences, USA, 85, 8737-8741.

Murphy, S.N. & Miller, R.J. (1989). Two distinct quisqualate receptors regulate $Ca^{2+}$ homeostasis in hippocampal neurons in vitro. Molecular Pharmacology, 35, 671-680.

Murray, C.W., Cowan, A. & Larson, A.A. (1991). Neurokinin and NMDA antagonists (but not a kainic acid antagonist) are antinociceptive in the mouse formalin model. Pain, 44, 179-185.

Nakajima, Y., Iwakabe, H., Akazawa, C., Nawa, H., Shigemoto, R., Mizuno, N. & Nakanishi, S. (1993). Molecular characterization of a novel retinal metabotropic glutamate receptor mGluR6 with a high agonist selectivity for L-2-amino-4-phosphonobutyrate. Journal of Biological Chemistry, 268, 11868-11873.

Nakanishi, S. (1992). Molecular diversity of glutamate receptors and implications for brain function. Science, 258, 597-603.

Nakanishi, N., Schneider, N.A. & Axel, R. (1990). A family of glutamate receptor genes: evidence for the formation of heteromultimeric receptors with distinct channel properties. Neuron, 5, 569-581.

Näsström, J., Karlsson, U. & Post, C. (1992). Antinociceptive actions of different classes of excitatory amino acid receptor antagonists in mice. European Journal of Pharmacology, 212, 21-29.

Nebe, J., Vanegas, H., Neugebauer, V. & Schaible, H.G. (1997). Omega-agatoxin IVA, a P-type calcium channel antagonist, reduces nociceptive processing in spinal cord neurons with input from the inflamed but not from the normal knee joint--an

electrophysiological study in the rat in vivo. European Journal of Neuroscience, 9, 2193-2201.

Neil, K.E., Kendall, D.A. & Alexander, S.P. (1996). Coupling of metabotropic glutamate receptors to phosphoinositide mobilisation and inhibition of cyclic AMP generation in the guinea-pig cerebellum. British Journal of Pharmacology, 118, 311-316.

Neki, A., Ohishi, H., Kaneko, T., Shigemoto, R., Nakanishi, S. & Mizuno, N. (1996). Pre- and postsynaptic localization of a metabotropic glutamate receptor, mGluR2, in the rat brain: an immunohistochemical study with a monoclonal antibody. Neuroscience Letters, 202, 197-200.

Netzeband, J.G., Parsons, K.L., Sweeney, D.D. & Gruol, D.L. (1997). Metabotropic glutamate receptor agonists alter neuronal excitability and $Ca^{2+}$ levels via the phospholipase C transduction pathway in cultured Purkinje neurons. Journal of Neurophysiology, 78, 63-75.

Neugebauer, V., Kornhuber, J., Lücke, T. & Schaible, H.G. (1993). The clinically available NMDA receptor antagonist memantine is antinociceptive on rat spinal neurones. NeuroReport, 4, 1259-1262.

Neugebauer, V., Lücke, T. & Schaible, H.G. (1993). Differential effects of *N*-methyl-D-aspartate (NMDA) and non-NMDA receptor antagonists on the responses of rat spinal neurons with joint input. Neuroscience Letters, 155, 29-32.

Neugebauer, V., Lücke, T. & Schaible, H.G. (1994). Requirement of metabotropic glutamate receptors for the generation of inflammation-evoked hyperexcitability in rat spinal cord neurons. European Journal of Neuroscience, 6, 1179-1186.

Niedbala, B., Sanchez, A. & Feria, M. (1995). Nitric oxide mediates neuropathic pain behavior in peripherally denervated rats. Neuroscience Letters, 188, 57-60.

Nowak, L., Bregestovski, P., Ascher, P., Herbet, A. & Prochiantz, Z. (1984). Magnesium gates glutamate activated channels in mouse central neurones. Nature, 307, 462-465.

Nusser, Z., Mulvihill, E., Streit, P. & Somogyi, P. (1994). Subsynaptic segregation of metabotropic and ionotropic glutamate receptors as revealed by immunogold localization. Neuroscience, 61, 421-427.

O'Connor, J.J., Rowan, M.J. & Anwyl, R. (1994). Long-lasting enhancement of NMDA receptor-mediated synaptic transmission by metabotropic glutamate receptor activation. Nature, 367, 557-559.

O'Connor, J.J., Rowan, M.J. & Anwyl, R. (1995). Tetanically induced LTP involves a similar increase in the AMPA and NMDA receptor component of the excitatory postsynaptic current: investigations of the involvement of mGlu receptors. Journal of Neuroscience, 15, 2013-2020.

Ohfune, Y., Shimamoto, K., Ishida, M. & Shinozaki, H. (1993). Synthesis of L-2-(2,3-dicarboxycyclopropyl)glycine: novel conformationally restricted glutamate analog. Bioorganic Medical Chemistry Letters, 3, 15-18.

Ohishi, H., Akazawa, C., Shigemoto, R., Nakanishi, S. & Mizuno, N. (1995). Distribution of the mRNAs for L-2-amino-4-phosphonobutyrate-sensitive metabotropic glutamate receptors, mGluR4 and mGluR7, in the rat brain. Journal of Comparative Neurology, 360, 555-570.

Ohishi, H., Nomura, S., Ding, Y.Q., Shigemoto, R., Wada, E., Kinoshita, A., Li, J.L., Neki, A., Nakanishi, S. & Mizuno, N. (1995). Presynaptic localization of a

metabotropic glutamate receptor, mGluR7, in the primary afferent neurons: an immunohistochemical study in the rat. Neuroscience Letters, 202, 85-88.

Ohishi, H., Ogawa-Meguro, R., Shigemoto, R., Kaneko, T., Nakanishi, S. & Mizuno, N. (1994). Immunohistochemical localization of metabotropic glutamate receptors, mGluR2 and mGluR3, in rat cerebellar cortex. Neuron, 13, 55-66.

Ohishi, H., Shigemoto, R., Nakanishi, S. & Mizuno, N. (1993a). Distribution of the mRNA for a metabotropic glutamate receptor (mGluR3) in the rat brain: an in situ hybridization study. Journal of Comparative Neurology, 335, 252-266.

Ohishi, H., Shigemoto, R., Nakanishi, S. & Mizuno, N. (1993b). Distribution of the messenger RNA for a metabotropic glutamate receptor, mGlu receptor 2, in the central nervous system of the rat. Neuroscience, 53, 1009-1018.

Okada, D. (1992). Two pathways of cyclic GMP production through glutamate receptor-mediated nitric oxide synthesis. Journal of Neurochemistry, 59, 1203-1210.

Okamoto, N., Hori, S., Akazawa, C., Hayashi, Y., Shigemoto, R., Mizuno, N. & Nakanishi, S. (1994). Molecular characterization of a new metabotropic glutamate receptor mGluR7 coupled to inhibitory cyclic AMP signal transduction. Journal of Biological Chemistry, 269, 1231-1236.

Okano, K., Kuraishi, Y. & Satoh, M. (1995). Effects of intrathecally injected glutamate and substance P antagonists on repeated cold stress-induced hyperalgesia in rats. Biological & Pharmaceutical Bulletin, 18, 42-44.

O'Leary, D.M. & O'Connor, J.J. (1997). Potentiation of synaptic transmission in the rat dentate gyrus *in vitro* by (*S*)-3,5-dihydroxyphenylglycine ((*S*)-DHPG). Neuroscience Letters, 229, 29-32.

Ottersen, O.P. & Landsend, A.S. (1997). Organization of glutamate receptors at the synapse. European Journal of Neuroscience, 9, 2219-2224.

Palecek, J., Palecková, V., Dougherty, P.M., Carlton, S.M. & Willis, W.D. (1992). Responses of spinothalamic tract cells in mechanical and thermal stimulation of skin in rats with experimental peripheral neuropathy. Journal of Neurophysiology, 67, 1562-1573.

Palecek, J., Palecková, V., Dougherty, P.M. & Willis, W.D. (1994). The effect of *trans*-ACPD, a metabotropic excitatory amino acid receptor agonist, on the responses of primate spinothalamic tract neurons. Pain, 56, 261-269.

Patel, J., Moore, W.C., Thompson, C., Keith, R.A. & Salama, A.I. (1990). Characterization of the quisqualate receptor linked to phosphoinositide hydrolysis in neocortical cultures. Journal of Neurochemistry, 54, 1461-1466.

Paternain, A.V., Morales, M. & Lerma, J. (1995). Selective antagonism of AMPA receptors unmasks kainate receptor mediated responses in hippocampal neurons. Neuron, 14, 185-189.

Pellegrini-Giampietro, D.E., Gorter, J.A., Bennett, M.V.L. & Zukin, R.S. (1997). The GluR2 (GluR-B) hypothesis: $Ca^{2+}$-permeable AMPA receptors in neurological disorders. Trends in Neuroscience, 20, 464-470.

Pellegrini-Giampietro, D.E., Pulsinelli, W.A. & Zukin, R.S. (1994). NMDA and non-NMDA receptor gene expression following global brain ischemia in rats: effect of NMDA and non-NMDA receptor antagonists. Journal of Neurochemistry, 62, 1067-1073.

Pellegrini-Giampietro, D.E., Torregrossa, S.A. & Moroni, F. (1996). Pharmacological characterization of metabotropic glutamate receptors coupled to phospholipase D in the rat hippocampus. British Journal of Pharmacology, 118, 1035-1043.

Pellegrini-Giampietro, D.E., Zukin, R.S., Bennett., M.V.L., Cho, S. & Pulsinelli, W.A. (1992). Switch in glutamate receptor subunit gene expression in CA1 subfield of hippocampus following global ischemia in rats. Procedures to the National Academy of Science, USA, 89, 10499-10503.

Pellicciari, R., Luneia, R., Costantino, G., Marinozzi, M., Natalini, B., Jakobsen, P., Kanstrup, A., Lombardi, G., Moroni, F. & Thomsen, C. (1995). 1-Aminoindan-1,5-dicarboxylic acid: a novel antagonist at phospholipase C-linked metabotropic glutamate receptors. Journal of Medicinal Chemistry, 38, 3717-1719.

Petralia, R.S., Wang, Y.X., Niedzielski, A.S. & Wenthold, R.J. (1996). The metabotropic glutamate receptors, mGluR2 and mGluR3, show unique postsynaptic, presynaptic and glial localizations. Neuroscience, 71, 949-976.

Petralia, R.S., Wang, Y.X., Singh, S., Wu, C., Shi, L., Wei, J. & Wenthold, R.J. (1997). A monoclonal antibody shows discrete cellular and subcellular localizations of mGluR1 alpha metabotropic glutamate receptors. Journal of Chemical Neuroanatomy, 13, 77-93.

Phenna, S., Jane, S.D. & Chad, J.E. (1995). Increased perinuclear $Ca^{2+}$ activity evoked by metabotropic glutamate receptor activation in rat hippocampal neurones. Journal of Physiology, 486, 149-161.

Pickering, D.S., Thomsen, C., Suzdak, P.D., Fletcher, E.J., Robitaille, R., Salter, M.W., MacDonald, J.F., Huang, X.P. & Hampson, D.R. (1993). A comparison of two

alternatively spliced forms of a metabotropic glutamate receptor coupled to phosphoinositide turnover. Journal of Neurochemistry, 61, 85-92.

Pin, J.P., Joly, C, Heinemann, S.F. & Bockaert, J. (1994). Domains involved in the specificity of G protein activation in phospholipase C-coupled metabotropic glutamate receptors. EMBO Journal, 13, 342-348.

Pin, J.P., Waeber, C., Prezeau, L., Bockaert, J. & Heinemann, S.F. (1992). Alternative splicing generates metabotropic glutamate receptors inducing different patterns of calcium release in *Xenopus* oocytes. Proceedings of the National Academy of Sciences, USA, 89, 10331-10335.

Pisani, A., Calabresi, P., Centonze, D. & Bernardi, G. (1997a). Activation of group III metabotropic glutamate receptors depresses glutamatergic transmission at corticostriatal synapse. Neuropharmacology, 36, 845-851.

Pisani, A., Calabresi, P., Centonze, D. & Bernardi, G. (1997b). Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. British Journal of Pharmacology, 120, 1007-1014.

Pook, P.C.-K., Birse, E.F., Jane, D.E., Jones, A.W., Jones, P.L.St.J., Mewett, K.N., Sunter, D.C., Udvarhelyi, D.C., Wharton, B. & Watkins, J.C. (1993). Differential actions of the metabotropic glutamate receptor antagonists 4C-PG and αM4C-PG at L-AP4-like receptors in neonatal rat spinal cord. British Journal of Pharmacology, 108, 87P.

Pook, P.C.-K., Sunter, D.C., Udvarhelvi, P.M. & Watkins, J.C. (1992). Evidence for presynaptic depression of monosynaptic excitation in neonatal rat motoneurones by (1*S*,3*S*)- and (1*S*,3*R*)-ACPD. Experimental Physiology, 77, 529-532.

Price, D.D., Hayes, R.L., Ruda, M. & Dubner, R. (1978). Spatial and temporal transformation of input to spinothalamic tract neurons and their relation to somatic sensations. Journal of Neurophysiology, 41, 933-946.

Puig, S. & Sorkin, L.S. (1996). Formalin-evoked activity in identified primary afferent fibers - systemic lidocaine suppresses phase-2 activity. Pain, 64, 345-355.

Pulsinelli, W., Sarokin, A. & Buchan, A. (1993). Antagonism of the NMDA and non-NMDA receptors in global versus focal brain ischemia. Progress in Brain Research, 96, 125-135.

Qian, J., Brown, S.D. & Carlton, S.M. (1996). Systemic ketamine attenuates nociceptive behaviours in a rat model of peripheral neuropathy. Brain Research, 715, 51-62.

Rahman, S. & Neuman, R.S. (1996). Characterization of metabotropic glutamate receptor-mediated facilitation of *N*-methyl-D-aspartate depolarization of neocortical neurones. British Journal of Pharmacology, 117, 675-683.

Raigorodsky, G. & Urca, G. (1987). Intrathecal *N*-methyl-D-aspartate (NMDA) activates both nociceptive and antinociceptive systems. Brain Research, 422, 158-162.

Raigorodsky, G. & Urca, G. (1990). Spinal antinociceptive effects of excitatory amino acid antagonists: quisqualate modulates the action of *N*-methyl-D-aspartate. European Journal of Pharmacology, 182, 37-47.

Reichling, D.B. & MacDermott, A.B. (1993). Brief calcium transients evoked by glutamate receptor agonists in rat dorsal horn neurons: fast kinetics and mechanisms. Journal of Physiology, 469, 67-88.

Ren, K. & Dubner, R. (1993). NMDA receptor antagonists attenuate mechanical hyperalgesia in rats with unilateral inflammation of the hindpaw. Neuroscience Letters, 163, 22-26.

Ren, K., Hylden, J.L.K., Williams, G.M., Ruda, M.A. & Dubner, R. (1992). The effects of a non-competitive NMDA receptor antagonist, MK-801, on behavioral hyperalgesia and dorsal horn neuronal activity in rats with unilateral inflammation. Pain, 50, 331-344.

Ren, K., Williams, G.M., Hylden, J.L.K., Ruda, M.A. & Dubner, R. (1992). The intrathecal administration of excitatory amino acid receptor antagonists selectively attenuated carrageenan-induced behavioral hyperalgesia in rats. European Journal of Pharmacology, 219, 235-243.

Riedel, G., Casabona, G. & Reymann, K.G. (1995). Inhibition of long-term potentiation in the dentate gyrus of freely moving rats by the metabotropic glutamate receptor antagonist, MCPG. Journal of Neuroscience, 15, 87-98.

Riedel, G., Manahan-Vaughan, D., Kozikowski, A.P. & Reymann, K.G. (1995). Metabotropic glutamate receptor agonist *trans*-azetidine-2,4-dicarboxylic acid facilitates maintenance of LTP in the dentate gyrus *in vivo*. Neuropharmacology, 34, 1107-1109.

Riedel, G., Wetzel, W. & Reymann, K.G. (1994). (*R*,*S*)-α-methyl-4-carboxyphenylglycine ((+)-MCPG) blocks spatial learning in rats and long-term potentiation in the dentate gyrus *in vivo*. Neuroscience Letters, 167, 141-144.

Roche, A.K., Cook, M., Wilcox, G.L. & Kajander, K.C. (1996). A nitric oxide synthesis inhibitor (L-NAME) reduces licking behavior and Fos-labeling in the spinal cord of rats during formalin-induced inflammation. Pain, 66, 331-341.

Romano, C., Sesma M.A., McDonald, C.T., O'Malley, K. Van den Pol, A.N. & Olney, J.W. (1995). Distribution of metabotropic glutamate receptor mGluR5 immunoreactivity in rat brain. Journal of Comparative Neurology, 355, 455-469.

Rossi, P., D'Angelo, E. & Taglietti, V. (1996). Differential long-lasting potentiation of the NMDA and non-NMDA synaptic currents induced by metabotropic and NMDA receptor coactivation in cerebellar granule cells. European Journal of Neuroscience, 8, 1182-1189.

Russo, R.E., Nagy, F. & Hounsgaard, J. (1997). Modulation of plateau properties in dorsal horn neurones in a slice preparation of the turtle spinal cord. Journal of Physiology, 499, 459-474.

Sahara, Y. & Westbrook, G.L. (1993). Modulation of calcium currents by a metabotropic glutamate receptor involves fast and slow kinetic components in cultured hippocampal neurons. Journal of Neuroscience, 13, 3041-3050.

Sakurada, T., Sugiyama, A., Sakurada, C., Tan-No, K., Yonezawa, A., Sakurada, S. & Kisara, K. (1996). Effect of spinal nitric oxide inhibition on capsaicin-induced nociceptive response. Life Sciences, 59, 921-930.

Salt, T.E. & Eaton, S.A. (1995a). Modulation of sensory neurone excitatory and inhibitory responses in the ventrobasal thalamus by activation of metabotropic excitatory amino acid receptors. Neuropharmacology, 34, 1043-1051.

Salt, T.E. & Eaton, S.A. (1995b). Distinct presynaptic metabotropic receptors for L-AP4 and CCGI on GABAergic terminals: pharmacological evidence using novel α-methyl derivative mGluR antagonists, MAP4 and MCCG, in the rat thalamus *in vivo*. Neuroscience, 65, 5-13.

Saugstad, J.A., Kinzie, J.M., Mulvihill, E.R., Segerson, T.P. & Westbrook, G.L. (1994). Cloning and expression of a new member of the L-2-amino-4-phosphonbutyric acid-sensitive class of metabotropic glutamate receptors. Molecular Pharmacology, 45, 367-372.

Saugstad, J.A., Kinzie, J.M., Shinohara, M.M., Segerson, T.P. & Westbrook, G.L. (1997). Cloning and expression of rat metabotropic glutamate receptor 8 reveals a distinct pharmacological profile. Molecular Pharmacology, 51, 119-125.

Schoepp, D.D. (1994). Novel functions for subtypes of metabotropic glutamate receptors. Neurochemistry International, 24, 439-449.

Schoepp, D.D., Goldsworthy, J., Johnson, B.G., Salhoff, C.R. & Baker, S.R. (1994). 3,5-Dihydroxyphenylglycine is a highly selective agonist for phosphoinositide-linked metabotropic glutamate receptors in the rat hippocampus. Journal of Neurochemistry, 63, 769-772.

Schoepp, D.D., Johnson, B.G., Monn, J.A. (1992). Inhibition of cyclic AMP formation by a selective metabotropic glutamate receptor agonist. Journal of Neurochemistry, 58, 1184-1186.

Schoepp, D.D., Johnson, B.G., Salhoff, C.R., McDonald, J.W. & Johnston, M.V. (1991). *In vitro* and *in vivo* pharmacology of *trans-* and *cis*-(+-)-1-amino-1,3-cyclopentanedicarboxylic acid: dissociation of metabotropic and ionotropic excitatory amino acid receptor effects. Journal of Neurochemistry, 56, 1789-1796.

Schoepp, D.D., Johnson, B.G., Salhoff, C.R., Valli, M.J., Desai, M.A., Burnett, J.P., Mayne, N.G. & Monn, J.A. (1995). Selective inhibition of forskolin-stimulated cyclic AMP formation in rat hippocampus by a novel mGluR agonist, 2*R*,4*R*-aminopyrrolidine-2,4-dicarboxylate. Neuropharmacology, 34, 843-850.

Schoppa, N.E. & Westbrook, G.L. (1997). Modulation of mEPSCs in olfactory bulb mitral cells by metabotropic glutamate receptors. Journal of Neurophysiology, 78, 1468-1475.

Schouenbourg, J. & Dickenson, A.H. (1985). The effects of a distant noxious stimulation on A and C fibre evoked flexion reflexes and neuronal activity in the dorsal horn of the rat. Brain Research, 328, 23-32.

Schrader, L.A. & Tasker, J.G. (1997). Presynaptic modulation by metabotropic glutamate receptors of excitatory and inhibitory synaptic inputs to hypothalamic magnocellular neurons. Journal of Neurophysiology, 77, 527-536.

Scott, R.H., Sutton, K.G. & Dolphin, A.C. (1993). Interactions of polyamines with neuronal ion channels. Trends in Neuroscience, 16, 153-160.

Sekiyama, H., Hayashi, Y., Nakanishi, S., Jane, D.E., Tse, H.-W., Birse, E.F. & Watkins, J.C. (1996). Structure-activity relationships of new agonists and antagonists of different metabotropic glutamate receptor subtypes. British Journal of Pharmacology, 117, 1493-1503.

Sergueeva, O.A., Nikolai, B., Fedorov, B. & Reymann, K.G. (1993). An antagonist of glutamate metabotropic receptors, (*RS*)-α-methyl-4-carboxyphenylglycine, prevents the LTP-related increase in postsynaptic AMPA sensitivity in hippocampal slices. Neuropharmacology, 32, 933-935.

Sheardown, M.J., Neilsen, E.Ø., Hansen, A.J., Jacobsen, P. & Honoré, T. (1990). 2,3-Dihydroxy-6-nitro-7-sulfamoyl-benzo(F)-quinoxaline: a neuroprotectant for cerebral ischemia. Science, 247, 571-574.

Shen, K.-Z. & Johnson, S.W. (1997). A slow excitatory postsynaptic current mediated by G-protein-coupled metabotropic glutamate receptors in rat ventral tegmental dopamine neurons. European Journal of Neuroscience, 9, 48-54.

Sher, G.D. & Mitchell D. (1990). Intrathecal *N*-methyl-D-aspartate induces hyperexcitability in rat dorsal horn convergent neurones. Neuroscience Letters, 119, 199-202.

Shigemoto, R., Kinoshita, A., Wada, E., Nomura, S., Ohishi, H., Takada, M., Flor, P.J., Neki, A., Abe, T., Nakanishi, S. & Mizuno, N. (1997). Differential presynaptic localization of metabotropic glutamate receptor subtypes in the rat hippocampus. Journal of Neuroscience, 17, 7503-7522.

Shigemoto, R., Kulik, A., Roberts, J.D., Ohishi, H., Nusser, Z., Kaneko, T. & Somogyi, P. (1996). Target-cell-specific concentration of a metabotropic glutamate receptor in the presynaptic active zone. Nature, 381, 523-525.

Shigemoto, R., Nakanishi, S. & Mizuno, N. (1992). Distribution of the mRNA for a metabotropic glutamate receptor (mGluR1) in the central nervous system: an in situ hybridization study in adult and developing rat. Journal of Comparative Neurology, 322, 121-135.

Shigemoto, R., Nomura, S., Ohishi, H., Sugihara, H., Nakanishi S. & Mizuno, N. (1993). Immunohistochemical localization of a metabotropic glutamate receptor, mGluR5, in the rat brain. Neuroscience Letters, 163, 53-57.

Shir, Y. & Seltzer, Z. (1990). A-fibers mediate mechanical hyperesthesia and allodynia and C-fibers mediate thermal hyperalgesia in a new model of causalgiform pain disorders in rats. Neuroscience Letters, 115, 62-67.

Skilling, S.R., Smullin, D.H., Beitz, A.J. & Larson, A.A. (1988). Extracellular amino acid concentrations in the dorsal spinal cord of freely moving rats following veratridine and nociceptive stimulation. Journal of Neurochemistry, 51, 127-132.

Skilling, S.R., Sun, X., Kurtz, H.J. & Larson, A.A. (1992). Selective potentiation of NMDA-induced activity and release of EAAs by dynorphin: possible roles in paralysis and neurotoxicity. Brain Research, 575, 272-278.

Sladeczek, F., Manzoni, O., Fagni, L., Dumuis, A., Pin, J.P., Sebben, M. & Bockaert, J. (1992). The metabotropic glutamate receptor (MGR): pharmacology and subcellular location. Journal of Physiology, Paris, 86, 47-55.

Sluka, K.A., Rees, H., Chen, P.S., Tsuruoka, M. & Willis, W.D. (1997). Capsaicin-induced sensitization of primate spinothalamic tract cells is prevented by a protein kinase C inhibitor. Brain Research, 772, 82-86.

Sluka, K.A. & Westlund, K.N. (1992). An experimental arthritis in rats: dorsal horn aspartate and glutamate increases. Neuroscience Letters, 145, 141-144.

Sluka, K.A. & Willis, W.D. (1997). The effects of G-protein and protein kinase inhibitors on the behaviorai responses of rats to intradermal injection of capsaicin. Pain, 71, 165-178.

Sommer, B., Keinänen, K., Verdoorn, T.A., Wisden, W., Burnashev, N., Herb, A., Köhler, M., Takagi, T., Sakmann, B. & Seeburg, P.H. (1990). Flip and flop: a cell-specific functional switch in glutamate-operated channels of the CNS. Science, 249, 1580-1585.

Sotgiu, M.L., Biella, G. & Riva, L. (1994). A study of early ongoing activity in dorsal horn units following sciatic nerve constriction. NeuroReport, 5, 2609-2612.

Sotgiu, M.L., Biella, G. & Riva, L. (1995). Poststimulus afterdischarges of spinal WDR and NS units in rats with chronic nerve constriction. NeuroReport, 6, 1021-1024.

Spanswick, D., Pickering, A.E., Gibson, I.C. & Logan, S.D. (1995). Excitation of sympathetic preganglionic neurons via metabotropic excitatory amino acid receptors. Neuroscience, 68, 1247-1261.

Stanfa, L.C., Misra, C. & Dickenson, A.H. (1996). Amplification of spinal nociceptive transmission depends on the generation of nitric oxide in normal and carrageenan rats. Brain Research, 737, 92-98.

Steel, J.H., Terenghi, G., Chung, J.M., Na, H.S., Carlton, S.M. & Polak, J.M. (1994). Increased nitric oxide synthase immunoreactivity in rat dorsal root ganglia in a neuropathic pain model. Neuroscience Letters, 169, 81-84.

Stefani, A., Pisani, A., Mercuri, N.B., Bernardi, G. & Calabrasi, P. (1994). Activation of metabotropic glutamate receptors inhibits calcium currents and GABA-mediated synaptic potentials in striatal neurons. Journal of Neuroscience, 14, 6734-6743.

Stefani, A., Spadoni, F. & Bernardi, G. (1996). L-AP4 inhibits high voltage-activated Ca2+ currents in pyramidal cortical neurones. NeuroReport, 7, 421-424.

Stefani, A., Spadoni, F. & Bernardi, G. (1998). Group III metabotropic glutamate receptor agonists modulate high voltage-activated Ca2+ currents in pyramidal neurons of the adult rat. Experimental Brain Research, 119, 237-244.

Stratton, K.R., Worley, P.F. & Baraban, J.M. (1989). Excitation of hippocampal neurons by stimulation of glutamate Qp receptors. European Journal of Pharmacology, 173, 235-237.

Study, R.E. & Kral, M.G. (1996). Spontaneous action potential activity in isolated dorsal root ganglion neurons from rats with a painful neuropathy. Pain, 65, 235-242.

Sucher, N.J., Awobuluyi, M., Choi, Y.-B. & Lipton, S.A. (1996). NMDA receptors: from genes to channels. Trends in Pharmacological Science, 17, 348-355.

Sun, X. & Larson, A.A. (1991). Behavioral sensitization to kainic acid and quisqualic acid in mice: comparison to NMDA and substance P responses. Journal of Neuroscience, 11, 3111-3123.

Suzdak, P.D., Sheardown, M.J. & Honoré, T. (1993). Characterization of the metabotropic glutamate receptor in mouse cerebellar granule cells: lack of effect of

2,3-dihydroxy-6-nitro-7-sulpharmoylbenzo(F)-quinoxalline (NBQX). European Journal of Pharmacology, 245, 215-220.

Swartz, K.J. & Bean, B.P. (1992). Inhibition of calcium channels in rat CA3 pyramidal neurons by a metabotropic glutamate receptor. Journal of Neuroscience, 12, 4358-4371.

Takaishi, K., Eisele, J.H., Jr. & Carstens, E. (1996). Behavioral and electrophysiological assessment of hyperalgesia and changes in dorsal horn responses following partial sciatic nerve ligation in rats. Pain, 66, 297-306.

Takahashi, T., Forsythe, I.D., Tsujimoto, T., Barnes-Davies, M. & Onodera, K. (1996). Presynaptic calcium current modulation by a metabotropic glutamate receptor. Science, 274, 594-597.

Tal, M. & Bennett, G.J. (1993). Dextrorphan relieves neuropathic heat-evoked hyperalgesia in the rat. Neuroscience Letters, 151, 107-110.

Tal, M., & Bennett, G.J. (1994). Neuropathic pain sensations are differentially sensitive to dextrorphan. NeuroReport, 5, 1438-1440.

Tal, M. & Eliav, E. (1996). Abnormal discharge originates at the site of nerve injury in experimental constriction neuropathy (CCI) in the rat. Pain, 64, 511-518.

Tanabe, Y., Masu, M., Ishii, T., Shigemoto, R. & Nakanishi, S. (1992). A family of metabotropic glutamate receptors. Neuron, 8, 169-179.

Tanabe, Y., Nomura, A., Masu, M., Shigemoto, R., Mizuno, N. & Nakanishi, S. (1993). Signal transduction, pharmacological properties, and expression patterns of two rat metabotropic glutamate receptors, mGluR3 and mGluR4. Journal of Neuroscience, 13, 1372-1378.

Tang, C.-M., Dichter, M. & Morad, M. (1989). Quisqualate activates a rapidly inactivating high conductance ionic channel in hippocampal neurons. Science, 243, 1474-1477.

Testa, C.M., Friberg, I.K., Weiss, S.W. & Standaert, D.G. (1998). Immunohistochemical localization of metabotropic glutamate receptors mGluR1a and mGluR2/3 in the rat basal ganglia. The Journal of Comparative Neurology, 390, 5-19.

Testa, C.M., Standaert, D.G., Young, A.B. & Penney, J.B. Jr. (1994). Metabotropic glutamate receptor mRNA expression in the basal ganglia of the rat. Journal of Neuroscience, 14, 3005-3018.

Thomas, N.K., Jane, D.E., Tse, H.W. & Watkins, J.C. (1996). α-Methyl derivatives of serine-O-phosphate as novel, selective competitive metabotropic glutamate receptor antagonists. Neuropharmacology, 35, 637-642.

Thompson, G.A., Jones, P.L. St. J. & Kilpatrick, I.C. (1995). The actions of a range of excitatory amino acids at (1*S*,3*R*)-1-aminocyclopentane-1,3-dicarboxylic acid-depolarizing receptors on neonatal rat motoneurones. Neuropharmacology, 34, 857-863.

Thomsen, C., Boel, E. & Suzdak, P.D. (1994). Actions of phenylglycine analogs at subtypes of the metabotropic glutamate receptor family. European Journal of Pharmacology, 267, 77-84.

Thomsen, C., Kristensen, P., Mulvihill, E., Haldeman, B. & Suzdak, P.D. (1992). L-2-amino-4-phosphonobutyrate (L-AP4) is an agonist at the type IV metabotropic

glutamate receptor which is negatively coupled to adenylate cyclase. European Journal of Pharmacology, 227, 361-362.

Thomsen, C., Mulvihill, E.R., Haldeman, B., Pickering, D.S., Hampson, D.R. & Suzdak, P.D. (1993). A pharmacological characterization of the mGluR1α subtype of the metabotropic glutamate receptor expressed in a cloned baby hamster kidney cell line. Brain Research, 619, 22-28.

Thomsen, C., Pekhletski, R., Haldeman, B., Gilbert, T.A., O'Hara, P. & Hampson, D.R. (1997). Cloning and characterization of a metabotropic glutamate receptor, mGluR4b. Neuropharmacology, 36, 21-30.

Thomsen, C. & Suzdak, P.D. (1993a). 4-carboxy-3-hydroxyphenyglycine, an antagonist at type I metabotropic glutamate receptor. European Journal of Pharmacology, 245, 299-301.

Thomsen, C. & Suzdak, P.D. (1993b). Serine-O-phosphate has affinity for type IV, but not type I, metabotropic glutamate receptor. NeuroReport, 4, 1099-1101.

Tingley, W.G., Roche, K.W., Thompson, A.K. & Huganir, R.L. (1993). Regulation of NMDA receptor phosphorylation by alternative splicing of the C-terminal domain. Nature, 364, 70-73.

Tizzano, J.P., Griffey, K.I. & Schoepp, D.D. (1995). Induction or protection of limbic seizures in mice by mGluR subtype selective agonists. Neuropharmacology, 34, 1063-1067.

Tölle, T.R., Berthele, A., Zieglgänsberger, W., Seeburg, P.H. & Wisden, W. (1993). The differential expression of 16 NMDA and non-NMDA receptor subunits in the rat spinal cord and in periaqueductal gray. Journal of Neuroscience, 13, 5009-5028.

Toms, N.J., Jane, D.E., Tse, H.W. & Roberts, P.J. (1995). Characterization of metabotropic glutamate receptor-stimulated phosphoinositide hydrolysis in rat cultured cerebellar granule cells. British Journal of Pharmacology, 116, 2824-2827.

Tracey, D.M., De Biasi, S., Phend, K. & Rustioni, A. (1991). Aspartate like immunoreactivity in primary afferent neurons. Neuroscience, 40, 673-686.

Trombley, P.Q. & Westbrook, G.L. (1992). L-AP4 inhibits calcium currents and synaptic transmission via a G-protein-coupled glutamate receptor. Journal of Neuroscience, 12, 2043-2050.

Trussell, L.O. & Fischbach, G.D. (1989). Glutamate receptor desensitization and its role in synaptic transmission. Neuron, 3, 209-218.

Trussell, L.O., Thio, L.L., Zorumski, C.F. & Fischbach, G.D. (1988). Rapid desensitization of glutamate receptors in vertebrate central neurons. Proceedings to the National Academy of Science, USA, 85, 4562-4566.

Tyler, E.C. & Lovinger, D.M. (1995). Metabotropic glutamate receptor modification of synaptic transmission in corticostriatal co-cultures: role of calcium influx. Neuropharmacology, 34, 939-952.

Ugolini, A., Corsi, M. & Bordi, F. (1997). Potentiation of NMDA and AMPA responses by group I mGluR in spinal cord motoneurons. Neuropharmacology, 36, 1047-1055.

Vaccarino, F.M., Liljequist, S. & Tallman, J.F. (1991). Modulation of protein kinase C translocation by excitatory and inhibitory amino acids in primary cultures of neurons. Journal of Neurochemistry, 57, 391-396.

Valerio, A., Paterlini, M., Boifava, M., Memo, M. & Spano, P. (1997). Metabotropic glutamate receptor mRNA expression in rat spinal cord. NeuroReport, 8, 2695-2699.

Valerio, A., Rizzonelli, P., Paterlini, M., Moretto, G., Knöpfel, T., Kuhn, R., Memo, M. & Spano, P. (1997). mGluR5 metabotropic glutamate receptor distribution in rat and human spinal cord: a developmental study. Neuroscience Research, 28, 49-57.

Van den Pol, A.N. (1994). Metabotropic glutamate receptor mGluR1 distribution and ultrastructural localization in hypothalamus. Journal of Comparative Neurology, 349, 615-632.

Vázquez, E., Budd, D.C., Herrero, I., Nicholls, D.G. & Sánchez-Prieto, J. (1995). Co-existence and interaction between facilitatory and inhibitory metabotropic glutamate receptors and the inhibitory adenosine $A_1$ receptor in cerebrocortical nerve terminals. Neuropharmacology, 34, 919-927.

Vázquez, E., Herrero, I., Miras-Portugal, M.T. & Sánchez-Prieto, J. (1994). Role of arachidonic acid in the facilitation of glutamate release from rat cerebrocortical synaptosomes independent of metabotropic glutamate receptor responses. Neuroscience Letters, 174, 9-13.

Vázquez, E., Herrero, I., Miras-Portugal, M. & Sánchez-Prieto, J. (1995). Developmental change from inhibition to facilitation in the presynaptic control of glutamate exocytosis by metabotropic glutamate receptors. Neuroscience, 68, 117-124.

Vázquez, E. & Sanchez-Prieto, J. (1997). Presynaptic modulation of glutamate release targets different calcium channels in rat cerebrocortical nerve terminals. European Journal of Neuroscience, 9, 2009-2018.

Vidnyánszky, Z., Hamori, J., Negyessy, L., Rüegg, D., Knöpfel, T., Kuhn, R. & Gorcs, T.J. (1994). Cellular and subcellular localization of the mGluR5a metabotropic glutamate receptor in rat spinal cord. NeuroReport, 6, 209-213.

Vignes, M., Clarke, V.R., Davies, C.H., Chambers, A., Jane, D.E., Watkins, J.C. & Collingridge, G.L. (1995). Pharmacological evidence for an involvement of group II and group III mGluRs in the presynaptic regulation of excitatory synaptic responses in the CA1 region of rat hippocampal slices. Neuropharmacology, 34, 973-982.

Vizzard, M.A., Erdman, S.L. & de Groat, W.C. (1995). Increased expression of neuronal nitric oxide synthase (NOS) in visceral neurons after nerve injury. Journal of Neuroscience, 15, 4033-4045.

Wanaka, A., Shiotani, Y., Kiyama, H., Matsuyama, T., Kamada, T., Shiosaka, S. & Tohyama, M. (1987). Glutamate-like immunoreactive structures in primary sensory neurons in the rat detected by a specific antiserum against glutamate. Brain Research, 65, 691-694.

Watanabe, M., Inoue, Y., Sakimura, K. & Mishina, M. (1992). Developmental changes in distribution of NMDA receptor channel subunit mRNAs. NeuroReport, 3, 1138-1140.

Watanabe, M., Mishina, M. & Inoue, Y. (1994). Distinct spatiotemporal distributions of the *N*-methyl-D-aspartate receptor channel subunit mRNAs in the mouse cervical cord. Journal of Comparative Neurology, 345, 314-319.

Watkins, J.C. & Collingridge, G. (1994). Phenylglycine derivatives as antagonists of metabotropic glutamate receptors. Trends in Pharmacological Sciences, 15, 333-342.

Watkins, J.C. & Evans, R.H. (1981). Excitatory amino acid transmitters. Annual Review of Pharmacological Toxicology, 21, 165-204.

Watkins, J.C., Krogsgaard-Larsen, P. & Honoré, T. (1991). Structure-activity relationships in the development of excitatory amino acid receptor agonists and competitive antagonists. In: D. Lodge & G.L. Collingridge (Eds.), Trends in Pharmacological Science, Special report: The pharmacology of excitatory amino acids (pp. 4-12). Cambridge:Elsevier.

Weiss, S., Ellis, J., Hendley, D.D. & Lenox, R.H. (1989). Translocation and activation of protein kinase C in striatal neurons in primary culture: relationship to phorbol dibutyrate actions on the inositol phosphate generating system and neurotransmitter release. Journal of Neurochemistry, 52, 530-536.

Wermuth, C.G., Mann, A., Schoenfelder, A., Wright, R.A., Johnson, B.G., Burnett, J.P., Mayne, N.G. & Schoepp, D.D. (1996). (2*S*,4*S*)-2-amino-4-(4,4-diphenylbut-1-yl) - pentante -1,5-dioic acid: a potent and selective antagonist for metabotropic glutamate receptors negatively linked to adenylate cyclase. Journal of Medicinal Chemistry, 39, 814-816.

Westlund, K.N., McNeil, D.L. & Coggeshall, R.E. (1989). Glutamate immunoreactivity in rat dorsal root axons. Neuroscience Letters, 96, 13-17.

Westlund, K.N., McNeil, D.L., Patterson, J.T. & Coggeshall, R.E. (1989). Aspartate immunoreactive axons in normal rat L4 dorsal roots. Brain Research, 489, 347-351.

Winder, D.G. & Conn, P.J. (1992). Activation of metabotropic glutamate receptors in the hippocampus increases cyclic AMP accumulation. Journal of Neurochemistry, 59, 375-378.

Wisden, W. & Seeburg, P.H. (1993). A complex mosaic of high-affinity kainate receptors in rat brain. Journal of Neuroscience, 13, 3582-3598.

Woolf, C.J. (1983). Evidence for a central component of post-injury pain hypersensitivity. Nature, 306, 686-689.

Woolf, C.J. & Swett, J.E. (1984). The cutaneous contribution to the hamstring flexor reflex in the rat: an electrophysiological and anatomical study. Brain Research, 303, 299-312.

Woolf, C.J. & Thompson, S.W.N. (1991). The induction and maintenance of central sensitization is dependent on *N*-methyl-D-aspartic acid receptor activation; implications for the treatment of post-injury hypersensitivity states. Pain, 44, 293-299.

Wu, J., Lin, Q., Lu, Y., Willis, W.D. & Westlund, K.N. (1998). Changes in nitric oxide synthase isoforms in the spinal cord of rat following induction of chronic arthritis. Experimental Brain Research, 118, 457-465.

Yamada, K. & Nabeshima, T. (1997). Two pathways of nitric oxide production through glutamate receptors in the rat cerebellum in vivo. Neuroscience Research, 28, 93-102.

Yamamoto, T., Shimoyana, M. & Mizuguchi, T. (1993a). The effects of morphine, MK-801, an NMDA antagonist, and CP-96,345, an NK1 antagonist, on the hyperesthesia evoked by carrageenan injection in the rat paw. Anesthesiology, 78, 124-133.

Yamamoto, T., Shimoyama, N. & Mizuguchi, T. (1993b). Nitric oxide synthase inhibitor blocks spinal sensitization induced by formalin injection into the rat paw. Anesthesia and Analgesia, 77, 886-890.

Yamamoto, T. & Yaksh, T.L. (1992a). Comparison of the antinociceptive effects of pre- and posttreatment with intrathecal morphine and MK801, an NMDA antagonist, on the formalin test in the rat. Anesthesiology, 77, 757-763.

Yamamoto, T. & Yaksh, T.L. (1992b). Spinal pharmacology of thermal hyperesthesia induced by constriction injury of sciatic nerve. Excitatory amino acid antagonists, Pain, 49, 121-128.

Yashpal, K., Fisher, K., Chabot, J-G. & Coderre, T.J. (1998). Differential effects of NMDA and group I mGluR antagonists on both nociception and spinal cord protein kinase C translocation in the formalin test and a model of neuropathic pain in rats. Society for Neuroscience Abstracts, (*in press*).

Yashpal, K., Pitcher, G.M., Parent, A., Quirion, R. & Coderre, T.J. (1995). Noxious thermal and chemical stimulation induce increases in $^{3}$H-phorbol 12,13-dibutyrate binding in spinal cord dorsal horn as well as persistent pain and hyperalgesia, which is reduced by inhibition of protein kinase C. Journal of Neuroscience, 15, 3263-3272.

Yezierski, R.P. & Park, S.-H. (1993). The mechanosensitivity of spinal sensory neurons following intraspinal injections of quisqualic acid in the rat. Neuroscience Letters, 157, 115-119.

Yonehara, N., Takemura, M., Yoshimura, M., Iwase, K., Seo, H.G., Taniguchi, N. & Shigenage. Y. (1997). Nitric oxide in the rat spinal cord in Freund's adjuvant-induced hyperalgesia. Japanese Journal of Pharmacology, 75, 327-335.

Yoon, Y.W., Sung, B. & Chung, J.M. (1998). Nitric oxide mediates behavioral signs of neuropathic pain in an experimental rat model. NeuroReport, 9, 367-372.

Young, M.R., Fleetwood-Walker, S.M., Dickinson, T., Blackburn-Munro, G., Sparrow, H., Birch, P.J. & Bountra, C. ( 1997). Behavioural and electrophysiological evidence supporting a role for group I metabotropic glutamate receptors in the mediation of nociceptive inputs to the rat spinal cord. Brain Research, 777, 161-169.

Young, M.R., Fleetwood-Walker, S.M., Mitchell, R. & Dickenson, T. (1995). The involvement of metabotropic glutamate receptors and their intracellular signalling pathways in sustained nociceptive transmission in rat dorsal horn neurons. Neuropharmacology, 34, 1033-1041.

Young, M.R., Fleetwood-Walker, S.M., Mitchell, R. & Munro, F.E. (1994). Evidence for a role of metabotropic glutamate receptors in sustained nociceptive inputs to rat dorsal horn neurons. Neuropharmacology, 33, 141-144.

Zheng, F. & Gallagher, J.P. (1992). Metabotropic glutamate receptor agonists potentiate a slow afterdepolarization in CNS neurons. NeuroReport, 3, 622-624.

Zheng, F., Gallagher, J.P. & Connor, J.A. (1996). Activation of a metabotropic excitatory amino acid receptor potentiates spike-driven calcium increases in neurons of the dorsolateral septum. Journal of Neuroscience, 16, 6079-6088.

Zheng, F., Hasuo, H. & Gallagher, J.P. (1995). 1*S*,3*R*-ACPD-preferring inward current in rat dorsolateral septal neurons is mediated by a novel excitatory amino acid receptor. Neuropharmacology, 34, 905-917.

Zieglgänsberger, W. & Herz, A. (1971). Changes of cutaneous receptive fields of spino-cervical-tract neurones and other dorsal horn neurones by microelectrophoretically administered amino acids. Experimental Brain Research, 13, 111-126.

Zochodne, D.W., Murray, M., Nag, S. & Riopelle, R.J. (1994). A segmental chronic pain syndrome in rats associated with intrathecal infusion of NMDA: evidence for selective action in the dorsal horn. Canadian Journal of Neurological Sciences, 21, 24-28.

## Appendix A: Pain Terms

| | |
|---|---|
| Allodynia | Pain due to a stimulus which does not normally provoke pain. |
| Hyperalgesia | An increased response to a stimulus which is normally painful. |
| Neuropathic Pain | Pain initiated or caused by a primary lesion or dysfunction in the nervous system. |

## Appendix B: Contributions to Co-authored Manuscripts

Dr. Terence Coderre is a co-author on all five manuscripts. As my thesis advisor, he played a critical role in all aspects of the studies that are included in my Ph.D. dissertation. Together, Dr. Coderre and I planned/designed all experiments. With Dr. Coderre's advice and feedback, I developed all of the testing protocols (with exception to the mechanical allodynia testing protocol), and carried out all other aspects of each experiment. In addition, Dr. Coderre gave me advice with regard to data presentation for each manuscript, and once I had written each manuscript he thoroughly reviewed and commented on each of them before they were submitted for publication.

Drs. Catherine Cahill and Marian Fundytus, who are postdoctoral fellows in Dr. Coderre's laboratory, are co-authors on the fourth manuscript. I wanted to complete the experiments for this manuscript in a short period of time, which meant that surgeries, injections schedules and testing sessions involved large groups of animals at one time. Therefore, both postdoctoral fellows kindly helped me on the surgery days, and recorded the rats' nociceptive scores for me while I carried out the behavioural testing. Also, on several occasions, Dr. Fundytus helped by injecting some of the animals in the larger groups.

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