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# Analgesic effects of mGlu1 and mGlu5 receptor antagonists in the rat formalin test

N. Sevostianova, W. Danysz*

*Merz Pharmaceuticals GmbH, Eckenheimer Landstrasse 100, 60318 Frankfurt/Main, Germany*

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**Abstract**

mGlu1 and mGlu5 receptors have been implicated in pain associated with inflammation. In the present study, the formalin test was used to measure sustained pain with components of tissue injury. The aims of the present study were to assess: (i) the role of mGlu1 and mGlu5 receptors in inflammatory pain using selective antagonist EMQMCM, 1.25–5 mg/kg, as the mGlu1 receptor antagonist, and MPEP or MTEP, 2.5–10 mg/kg, as mGlu5 receptor antagonist; (ii) the possible interaction between mGlu1 and mGlu5 receptor antagonists and morphine; and (iii) whether tolerance develops to the analgesic effects of these antagonists after prolonged treatment. EMQMCM, MTEP and MPEP significantly reduced the manifestation of both phases of formalin response. However, all these mGlu receptor antagonists did not affect the withdrawal latencies in a model of acute pain (Hargreaves test), which has a different underlying mechanism. In the present study, the suppressive effect on formalin-induced pain behaviour was much stronger when mGlu1 and mGlu5 receptor antagonists were co-injected compared to administration of a single antagonist, but this effect was not seen when mGlu receptor antagonist was co-administered with morphine. This is in contrast to the pronounced inhibitory effects after co-treatment with morphine and the uncompetitive NMDA receptor antagonist memantine. The present study also provides the first direct in vivo evidence that prolonged administration of MTEP (5 mg/kg) over 7 days leads to the development of tolerance to its antinociceptive effects. Such tolerance was not observed when EMQMCM (5 mg/kg) was administered in the same manner. In conclusion, these results provide additional arguments for the role of group I mGlu receptors in pain with inflammatory conditions.
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*Keywords:* Pain; mGlu receptors; Formalin test; mGlu1; mGlu5; Morphine; Tolerance

## 1. Introduction

The great body of evidence over the last several decades indicates that the excitatory amino acid glutamate plays a pivotal role in nociceptive processing. Glutamate acts at several types of receptors, including ionotropic (cation-specific ion channels divided into three groups: *N*-methyl-D-aspartate (NMDA), α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate (AMPA) and kainate receptors) and metabotropic receptors (coupled to G-proteins that modulate the intracellular second messengers). The vast number of studies in animals and in humans demonstrated the ability of NMDA receptor antagonists to attenuate central sensitization and hyperalgesia (Price et al., 1994; Mao, 1999). In addition to ionotropic receptors, the involvement of metabotropic glutamate receptors (mGlu) in pain has been shown in behavioural studies.

Metabotropic glutamate receptors are divided into three groups with growing evidence that group I mGlu receptors are implicated in nociceptive transmission and central sensitization. Group I mGlu receptors, comprising mGlu1 and mGlu5 receptors, are coupled to phospholipase C, and their activation leads to inositol 1,4,5-trisphosphate (IP3) production with subsequent release of $Ca^{2+}$ from intracellular stores (Crawford et al., 2000). Intrathecal administration of group I mGlu receptor agonists induces spontaneous nociceptive behaviours and allodynia (Fisher and Coderre, 1998; Dolan

* Corresponding author. Tel.: +49 69 150 3564; fax: +49 69 596 2150.
*E-mail address:* wojciech.danysz@merz.de (W. Danysz).

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and Nolan, 2000; Lorrain et al., 2002), whereas mGlu receptor antagonists produce antihyperalgesic effects in animal models of persistent pain (Fisher and Coderre, 1996; Walker et al., 2001a). Several studies showed that group I mGlu receptors have a modulatory role upon NMDA-induced responses via PKC-dependent mechanisms (Pisani et al., 1997; Pisani et al., 2001). Moreover, the abundant expression of mGlu1 and mGlu5 receptors on peripheral sensory neurons and superficially in dorsal horn of the spinal cord, where primary afferent fibres terminate, indicate the involvement of group I mGlu receptors in processes of nociceptive transmission and plasticity (Valerio et al., 1997; Neugebauer, 2001). It has recently been suggested that mGlu receptors contribute to pain states associated with inflammation. Results from electrophysiological and behavioural studies in rats are consistent with the involvement of group I mGlu receptors in the spinal processing of sustained nociceptive input with an inflammatory component evoked by formalin (Fisher and Coderre, 1996) and carrageenan or mustard oil applications (Young et al., 1997). However, although blockade of group I mGlu receptors reverses thermal hyperalgesia in models with inflammatory or nerve injuries, it does not attenuate mechanical hyperalgesia, which also constitutes the neuropathic pain (Walker et al., 2001a; Hudson et al., 2002). In the present study, the formalin test was used to measure sustained pain with tissue injury components. Formalin-induced behaviour is characterised by two phases, where the first phase reflects the acute pain state and the second phase is attributed to spinal cord hyperexcitability and is referred to as the ''tonic'' pain phase (Coderre and Yashpal, 1994; McCall et al., 1996; Martindale et al., 2001).

The purpose of the present study was to assess the role of mGlu1 and mGlu5 receptors in nociception evoked by injection of formalin using selective antagonists. Up to now, the majority of previous behavioural studies have been performed using non-selective mGlu1 receptor ligands, which have poor bioavailability and penetration to the brain (Spooren et al., 2003). Due to the lack of selective ligands, much less is known about the role of mGlu1 than mGlu5 receptors in nociception. EMQMCM (JNJ16567083, 3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfone) is of special interest, as it is one of the first selective noncompetitive and potent mGlu1 receptor antagonist that penetrates the blood–brain barrier. In the present study, the mGlu1 receptor antagonist EMQMCM (Lesage et al., 2002) and two mGlu5 receptor antagonists, MPEP (2-methyl-6-(phenylethynyl)pyridine (Varney et al., 1999)) and MTEP (3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine (Busse et al., 2004)), were tested either alone or in combination. Additionally, the Hargreaves test was employed to assess whether antagonists of group I mGlu receptors reduce acute pain with a different underlying mechanism than in the first phase of the formalin response. A considerable number of studies have demonstrated the enhancement of analgesic action after application of opiates in combination with NMDA receptor antagonists. Since group I mGlu receptors are also involved in synaptic plasticity and in synaptic pathways of pain transmission, one can assume that co-administration of group I mGlu receptor antagonists with an opioid receptor agonist will result in enhancement of analgesic action. In the present study, therefore, the possible interaction between mGlu1 and mGlu5 receptor antagonists and morphine was verified in the formalin test. In addition, since the treatment of chronic pain requires long-term administration of drugs, another aim was to investigate whether tolerance develops to the analgesic effects of EMQMCM and MTEP after prolonged treatment.

## 2. Materials and methods

### 2.1. Subjects

Adult experimentally naive male Sprague-Dawley rats (200–300 g; Janvier, France) were housed in groups of four with food and water available *ad libitum* and alternating 12 h/12 h day–night cycle (lights on at 07:00) for at least 6 days before the experiments were started. Colony room temperature and humidity were maintained at $20 \pm 1$ °C and $60 \pm 3\%$, respectively. All experiments were conducted during the light period of a day–night cycle. The study was approved by the Ethical Committee, Regierungspraesidium Darmstadt, Hessen and performed in accordance with the recommendations and policies of the U.S. National Institutes of Health Guidelines for the Use of Animals. Each animal was used only once.

### 2.2. Drugs

Two percent formaldehyde was made from 1 part of formalin (~36.6%; formalin, Fluka, Taufkirchen, Germany) and 17.3 parts of saline. Morphine sulphate (opioid receptor agonist; Sigma, Deisenhofen, Germany, 3 mg/kg) and memantine (HCl, 1-amino-3,5-dimethyladamantane, uncompetitive NMDA receptor antagonist, Merz Pharmaceuticals, Frankfurt/Main, Germany, 5 mg/kg) were dissolved in physiological saline. EMQMCM (JNJ16567083, (3-ethyl-2-methyl-quinolin-6-yl)-(4-methoxy-cyclohexyl)-methanone methanesulfone, 1.25–5 mg/kg), MPEP (2-methyl-6-(phenylethynyl)pyridine, 2.5–10 mg/kg) and MTEP ((3-[(2-methyl-1,3-thiazol-4-yl)ethynyl]-pyridine, 2.5–10 mg/kg) were synthesised by Merz Pharmaceuticals and dissolved in 10% water solution of Tween 80. Morphine was administered s.c.; glutamate receptor antagonists were injected i.p. The injection volume of morphine and memantine was 1 ml/kg, all antagonists of mGlu receptors were administered in a volume of 2 ml/kg.

For interaction studies, mode and time of administration were the same as for single injection experiments, using appropriate vehicles, i.e. each animal had two injections (see Fig. 2–4 legends for description). In experiments aimed to investigate tolerance, either EMQMCM or MTEP (5 mg/kg each) was given once daily for 7 days, and 24 h after the last injection the challenging dose of either EMQMCM or MTEP (5 mg/kg each) was administered 30 min before formalin (see Fig. 5 legend for description).

### 2.3. Formalin test

Rats were placed individually in an open Plexiglas chamber (bowl-like cage $40 \times 35$ cm) with a mirror angled at 45° positioned behind to allow an unobstructed view of the paws by the observer. The animals were habituated to the observation bowl for 30 min prior to the experimental sessions. Formalin (50 μl) was injected s.c. into the plantar surface of the rat hind paw using a 27-gauge needle. After injection, rats were immediately returned to the observation bowl and the formalin-induced behaviours were recorded for a period of 60 min. All tested substances were injected 30 min before the injection of formalin. The duration of licking and biting of the injected paw was scored using a custom-made software program and quantified every 6 min for the 60-min observation period. The 6-min interval was chosen based on an earlier report on the time course of the first (0–6 min) and second (12–60 min) phases of the formalin-induced facial grooming (Eisenberg et al., 1996).

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