Can Novel AMPA and NMDA Receptor Antagonists Induce Analgesia?

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Abstract: The glutamate receptors in the nervous system are related to nociceptive response. These receptors include the AMPA (α-amino-3-hydroxy-5-methyl-4-isoxazolepropionate) receptor and the NMDA (N-methyl-D-aspartate) receptor. The purpose of this study was to investigate whether novel antagonists of these glutamate receptors could inhibit the nociceptive response in the spinal cord of male Wistar rats. Rats intrathecally (i.t.) received 0.1 to 10 pmol of Ly-293558 (a novel AMPA antagonist) and 10 to 1000 pmol of Ly-233053 (a novel NMDA antagonist) dissolved in 50 μl of physiological saline. A 50-μl volume of 2.0% formalin solution was injected as a noxious stimulus into the hindpaw 15 min after the i.t. injections. We measured the total time the animal spent licking the hindpaw in the first 5 min (early phase) and from 10 to 30 min (late phase) after formalin injection. The licking time during the early phase was significantly and dose-dependently decreased with intrathecal administrations of both Ly-293558 and Ly-233053 (p<0.05). However, Ly-293558 induced this effect at much lower concentrations. During the late phase, only the highest dose of each antagonist significantly shortened licking time. Our results indicate that these two novel AMPA and NMDA receptor antagonists when intrathecally administrated could induce antinociceptive effects during both the acute phase (peripheral sensitization) and late phase (central sensitization) of formalin-induced nociceptive stimulation without producing motor dysfunction.

Key words: analgesia, excitatory amino acid receptor, NMDA receptor, AMPA receptor, NMDA antagonist: Ly 233053, AMPA antagonist: Ly 293558

Introduction

Increased pain in response to noxious stimulation following peripheral tissue injury depends on an increase in the sensitivity of primary afferent nociceptors at the site of injury.

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(peripheral sensitization), and on an increase in the excitability of neurons in the CNS (central sensitization). Antagonists of Excitatory Amino Acid (EAA) receptors, such as the AMPA and NMDA receptors, have been shown to possess antinociceptive actions\textsuperscript{5}). For example, intrathecal injection of CNQX, an NMDA receptor antagonist, in rats attenuates nociceptive response to tissue injury during the early phase (peripheral sensitization) and particularly during the late phase (central sensitization) of the formalin test\textsuperscript{6}).

An important quality of an analgesic intended for spinal administration is the ability to produce antinociception without concomitant impairment of motor function. The purpose of this study was to investigate whether two novel glutamate receptor antagonists, Ly 293558, an AMPA antagonist and Ly 233053, a competitive NMDA antagonist, could inhibit the nociceptive response in the spinal cord of male Wistar rats without causing motor dysfunction. We used the formalin test as a behavioral model of injury-induced peripheral and central sensitization, and measured the total time the animal spent licking the hindpaw in the first 5 min (early phase) and from 10 to 30 min (late phase) after formalin injection.

**Materials**

After the protocol was reviewed and approved by the Institutional Animal Care and Use Committee, we used male Wistar rats (Harlan Wistar, Inc., Indianapolis, IN, U.S.A.) weighing 200–230 g. The animals were housed at 22.0 ± 0.5°C in a humidity controlled room under a 12-hr light/dark cycle (lights on at 6:00 am). The animals were allowed food and water ad libitum.

Under light ether anesthesia, a caudal cutaneous vertical incision (1 cm in length) was made to facilitate lumbar puncture. We waited at least 1 hour, and then intrathecal injections of saline as a control, Ly293558 or Ly233053 in fully awake rats (n=5 for each injection), were performed with a 27 gauge needle between the L5 and L6 vertebrae. Appropriate doses of these antagonists were determined in a preliminary study. A single dose of each of the antagonists was selected in order to produce mild antinociceptive effects that would not produce behavioral responses\textsuperscript{5}). The concentrations of Ly293558 (the novel AMPA antagonist, 0.1 pmol to 10 pmol) and Ly233053 (the novel NMDA antagonist, 10 pmol to 1000 pmol) used did not produce motor dysfunction or behavioral responses. All agents were dissolved in 50 μl of saline and given intrathecally 15 min prior to formalin injection.

For nociceptive testing, rats were given a standard subcutaneous injection of 50 μl of 2.0% formalin into the plantar surface of one hindpaw. Rats were then placed in a 30 cm × 30 cm × 30 cm Plexiglas box. We measured the total time the animal spent licking the hindpaw in the first 5 min (early phase) and from 10 to 30 min (late phase) after formalin injection.

We observed animals for 2 hours. The following morning we examined animals for evidence of motor dysfunction such as gait disturbance. Then rats were sacrificed by intracardiac injection of KCl 2 mEq in 1 ml under ether anesthesia. The data were analyzed using one-way ANOVA. We considered differences statistically significant when the p value was less than 0.05.
Results

Controlled total licking time with saline injection was 104±5 sec (early phase) and 151±35 sec (late phase), respectively (mean±SE).

Fig. 1 shows the effects of Ly 293558 (AMPA antagonist) and Ly 233053 (NMDA antagonist) in the early phase. Each doses of AMPA antagonist (0.1–10 pmol) and NMDA antagonist (10–1000 pmol) significantly shortened licking time, and this inhibition was dose-dependent.

Fig. 2 shows the effects of Ly 293558 and Ly 233053 in the late phase. Only the highest doses of AMPA antagonist (10 pmol) and NMDA antagonist (1000 pmol) significantly shortened licking time.

Discussion

The present study demonstrated that formalin-induced nociceptive behaviors are reduced by pretreatment with the novel EAA antagonists Ly 293558 (AMPA antagonist) and Ly 233053 (NMDA antagonist).

Values are mean ± SE (n=5)

![Graph showing licking time in early phase after treatment with AMPA and NMDA antagonists.]

Values are mean ± SE (n=5)

![Graph showing licking time in late phase after treatment with Ly 293558 and Ly 233053.]

Fig. 1. The early phase of hindpaw licking response after treatment with Ly 293558 (AMPA antagonist) and Ly 233053 (NMDA antagonist).

Fig. 2. The late phase of hindpaw licking response after Ly 293558 and Ly 233053 treatment. Values are expressed as mean±SE (n=5). Open bar indicates saline treatment. Closed bars indicate Ly 293558. Line bars indicate Ly 233053. *indicate p<0.05 vs hindpaw licking response after Ly 293558 and Ly 233053 treatment.
The early phase of the formalin test may be due to immediate and direct effects on sensory response (peripheral sensitization) and the late phase due to inflammatory response at injection site (central sensitization). Neural activity generated during the early phase of the formalin response can induce changes in CNS function (i.e., central sensitization), which in turn influence processing during the late phase. Woolf and Thompson showed that a brief nociceptive input causes prolonged increases in central excitation as gauged by rat hind limb flexion reflex activity. Endogenous release of EAAs may lead to the development of neuroplasticity in spinal nociceptive neurons following an intense nociceptive stimulus, and lead to the development of persistent nociception, as occurs following formalin injection. EAA treatment does not simply produce a static hyperalgesic effect that adds to formalin nociception throughout testing, but rather it shifts the late phase of the formalin test to an earlier time point.

Receptor-binding studies in the rat spinal cord have shown that NMDA and AMPA receptors are concentrated in the substantia gelatinosa of the dorsal horn. Current research suggests that, within the spinal dorsal horn, the NMDA receptor plays a role in neuronal plasticity, such as wind-up, central sensitization and hyperalgesia. On the other hand, the AMPA receptors mediate fast excitatory transmission involving both innocuous and acute nociceptive input.

It has been proposed that long-term potentiation (LTP; a kind of neuroplasticity that changes synaptic function), is due both to an increase in neurotransmitter release and to an enhanced postsynaptic AMPA/kainate response.

Haley, Sullivan and Dickenson demonstrated in mice that pretreatment with intrathecal AP-5, a competitive NMDA antagonist, or intravenous MK-801, a non-competitive NMDA antagonist, produced a marked inhibition of dorsal horn activity in the late phase after subcutaneous formalin injection, but only induced small nonsignificant effects on activity during the early phase. However, the behavioral response to formalin stimulation in rats showed an antinociceptive effect of the non-competitive NMDA antagonist, MK-801, on both the first and the second phase of the formalin test, although it was more potent against the second phase response. In mice, several AMPA antagonists, such as CNQX, DNQX and competitive NMDA antagonists, such as AP5, AP7 and CPP, reduced the early phase formalin response in behavioral tests (the late phase was not tested).

Our present results show that Ly 2935558, an AMPA-kainate antagonist (0.1–10 pmol), and Ly 233053, an NMDA antagonist (10–1000 pmol), significantly and dose-dependently reduced the duration of licking time in the early phase. Whereas in the late phase, only the highest doses of AMPA antagonist (10 pmol) and NMDA antagonist (1000 pmol) significantly reduced the licking time.

Why do these antagonists have more of an effect in the early phase that the late phase? These results may be due to the pharmacological characteristics of these agents, such as a rapid onset and a short duration of action. It has been shown that Ly 233053, a potent and selective antagonist of NMDA receptors, possesses a rapid onset, a relatively short duration and neuroprotective effect in the treatment of acute conditions such as cerebral ischemia.

No motor dysfunction was observed at the dosages we used. Paresis of the hind limb had developed at 100-fold higher concentrations of these agents in a preliminary study. Schoepp et al. suggested that the effective analgesic dose of Ly 233053 may not produce psychomimetic effects (catalepsy) in humans. Sang et al. showed that Ly 293558 has
analgesic efficacy at doses that do not cause troubling side effects in humans\(^{18}\).

In lamina II and the superficial part of lamina III in the rat spinal cord, dense areas of neurons relating to nociception are present. The expression of GluR2 is especially high in this region, suggesting that such high expression is a characteristic of the AMPA type receptor involved in nociception. It has been shown that Ly 293558 inhibits \(^{3}\text{H}\)-AMPA ligand binding and AMPA-induced depolarization\(^{11}\).

A recent study clearly showed that AMPA-KA receptors play a role in pain transmission in humans\(^{18}\). Intravenously administered Ly 293558 significantly decreased both spontaneous pain and the spread of mechanical hyperalgesia evoked by intradermal capsicin, but did not significantly reduce brief suprathreshold pain sensations in normal skin. Based on the differential analgesic response between two human experimental models of pain, it was suggested that AMPA-KA receptors primarily contribute to the processes leading to sensitization of central neurons (phase II).

The observation that Ly 293558 reduces pain and allodynia in the capsaicin model has implications for therapy. Mechanical allodynia and hyperalgesia are often seen in patients with acute pain after surgery and chronic pain after nerve or soft tissue injuries, and quantitative sensory testing in many such patients suggests central neural sensitization. N-methyl-D-aspartate receptor blockade has reduced pain in some of these patients but has been accompanied by side effects that usually prevent complete relief\(^{18}\).

In summary, intrathecal administration of the novel AMPA and NMDA receptor antagonists had antinociceptive effects during both the acute and late phase of formalin-induced nociceptive stimulation without causing motor dysfunction.

The authors would like to thank Mrs. Margaret Niedenthal, Eli-Lilly Co. (Indianapolis, IN, U.S.A.) who kindly provided us with Ly 293558 and Ly 233053.

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[Received February 17, 2000: Accepted May 18, 2000]