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BRAIN RESEARCH

Research Report

Acute PAR2 activation reduces GABAergic inhibition in the spinal dorsal horn ☆

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ABSTRACT

We investigated the mechanism underlying inhibition of spinal dorsal horn GABAergic neurotransmission to elucidate the role of protease-activated receptor-2 (PAR2). Initially, we confirmed that PAR2 agonist SL-NH_2 applied intrathecally produced mechanical hyperalgesia. Then we performed patch-clamp experiments in substantia gelatinosa neurons of spinal cord slice, and found that spontaneous inhibitory post-synaptic currents (sIPSCs) were significantly decreased in both frequency and amplitude when neurons were incubated with PAR2 agonist SL-NH_2 for a brief time period (2 min). The GABA-mediated currents were significantly reduced, and there was no impact on glycine-mediated currents during this SL-NH_2 treatment. These results suggest that PAR2 activation enhanced the pain response, potentially via inhibition of dorsal horn GABAergic neurotransmission.

1. Introduction

Protease-activated receptors (PARs) are a novel family of G-protein-coupled receptors that are found in various tissues throughout the body. They have recently attracted the attention of neuroscientists owing to their profound effects in the central and peripheral nervous systems (Noorbakhsh et al., 2003). The activation of protease-activated receptor-2 (PAR2) potentially regulates cell membrane ion channel through a series of signaling molecules, thus enhancing the neuronal excitability and causing pain (Lu et al., 2010). Recently, numerous researches have explored the role of PAR2 in peripheral inflammatory pain. For example, intraplantar injection of PAR2 agonist in rats causes marked and sustained hyperalgesia through its interaction with TRPA1 channels (Dai et al., 2007), TRPV4 channels (Grant et al., 2007), and TRPV1 channels (Dai et al., 2004). However, few studies have focused on the possibility that PAR2 agonist acts directly within the spinal cord to produce pronociceptive effects.

Alier et al. (2008) first reported that intrathecally applied PAR2 agonist produces mechanical and thermal hyperalgesia. And moreover, it augments the thermal and mechanical hyperalgesia produced in an inflammatory pain model. Surprisingly, their electrophysiological studies failed to demonstrate an increase in excitatory transmission or neuronal excitability in spinal cord dorsal horn following application of

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PAR2 agonist, and they did not report an analysis regarding PAR2 agonist's effect on inhibitory neurotransmission. As modulation of the inhibitory tone at the dorsal horn level maintains an important role in processing nociceptive information relayed to supraspinal centers (Drew et al., 2004; Malan et al., 2002), it is possible that PAR2 agonist acts directly within the spinal cord through modulating the inhibitory neurotransmission. Hence, in this study we investigated the effect of PAR2 activation on spontaneous inhibitory post-synaptic currents (sIPSCs) recorded from lamina II neurons. If there is evidence supporting our hypothesis of direct activation, we aim to explore the effect on GABAergic and glycinergic neurotransmission further.

2. Results

In the behavioral study, we observed a significant decrease in the nociceptive threshold in rats after intrathecal injection of the PAR2 agonist SL-NH_2. Interestingly, this effect mostly disappeared when the PAR2 agonist SL-NH_2 and PAR2 antagonist FS-NH_2 were administered together (Fig. 1).

We next recorded sIPSCs in lamina II neurons by maintaining the holding potential at 0 mV. The PAR2 agonist SL-NH_2 was perfused at the concentration of 100 uM for 2 min. Most neurons (7–9 of 15) responded to SL-NH_2, and SL-NH_2 produced a significant decrease of sIPSCs in both frequency ($p<0.01$) and amplitude ($p<0.05$) (Figs. 2A–C). And this effect disappeared when PAR2 antagonist FS-NH_2 and SL-NH_2 were perfused together (Figs. 3A–C).

To further explore the GABAergic and glycinergic inhibitory synaptic transmission, we examined the effects of PAR2 agonist SL-NH_2 on GABA and glycine induced outward currents by maintaining the holding potential at 0 mV. The GABA-mediated current was significantly reduced by SL-NH_2 ($p<0.05$) (Figs. 4A–B), while PAR2 agonist has no effects on glycine-mediated current (Figs. 5A–B). Also, it appears that the suppression of GABA-mediated currents is greater than the reduction in sIPSCs amplitudes, and this may be due to GABA-mediated currents are recorded by application of exogenous GABA while the sIPSCs are recorded spontaneously.

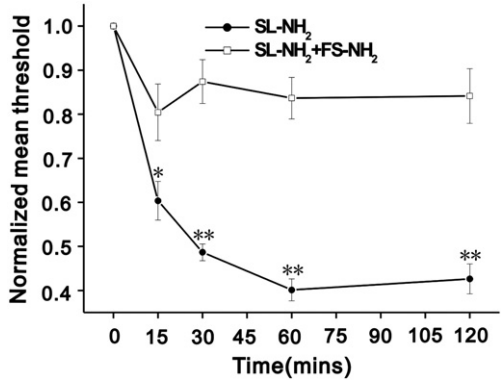

Fig. 1 – Nociceptive threshold measured in rat hindpaws before (time 0) and after intrethecal injection of SL-NH_2 (10 μg SL-NH_2 in 30 μl normal saline) or SL-NH_2 plus FS-NH_2 (10 μg SL-NH_2 and 10 μg FS-NH_2 in 30 μl normal saline). Mechanical withdrawal threshold was expressed as percentage of pre-treatment baseline value. Data are expressed as mean ± SEM. $^{*}p<0.05$, $^{**}p<0.01$ compared with the corresponding group at the same time point. SL-NH_2, SLIGRL-NH_2, PAR2 agonist peptide; FS-NH_2, FSLLRY-NH_2, PAR2 antagonist peptide.

3. Discussion

Previous research has demonstrated that intrathecal injection of PAR2 agonist induces and augments mechanical allodynia and thermal hyperalgesia in an inflammatory pain model (Alier et al., 2008). In this study, we further demonstrated that intrathecal administration of a certain concentration of PAR2 agonist can also induce mechanical hyperalgesia in normal rats, indicating that PAR2 receptors are not only involved in the process of maintaining chronic pathological pain but also play a role in the process of inducing pain. Moreover, our study has demonstrated that direct activation of PAR2 in the spinal cord had a decreased effect on spontaneous inhibitory neurotransmission and GABA-mediated outward currents in lamina II neurons, suggesting that PAR2 activation in the spinal cord potentially augments the transfer of nociceptive information by affecting the GABAergic rather than the glycinergic inhibitory tone of spinal dorsal horn neurons.

Although PAR2 is known to be expressed on neurons and astrocytes in rodent and human central nervous systems (Noorbakhsh et al., 2006), its presence in spinal cord is still controversial. In Alier et al.'s study, their immunohistochemical results suggested that PAR2 is absent from the spinal cord. This observation seems to both contradict data from our study and other functional studies that suggested that PAR2 resides on the central terminals of primary afferent nerves (Alier et al., 2008). By using the intrathecal loop dialysis system, intrathecal delivery of the PAR2-derived peptide SLIGRL stimulated PGE2 release and produced hyperalgesia, suggesting that activation of spinal PAR2 results in the stimulation of the spinal cyclooxygenase cascade and a prostaglandin dependent thermal hyperalgesia (Koetzner et al., 2004). Still, under the condition of paclitaxel-induced neuropathic pain, intrathecal delivery of PAR2 antagonist reversed paclitaxel-induced mechanical allodynia and heat hyperalgesia through suppression of paclitaxel-induced PKA and PKC activation in the spinal cord (Chen et al., 2011). All these evidences with our results suggested that PAR2 may play an important role during the process of pain at the spinal cord level.

PAR2 is involved in a variety of pathological pain conditions. It can be peripherally activated by a variety of highly expressed endogenous proteases such as trypsin and mast cell tryptase, producing a pain response (Hoogerwerf et al., 2004; Zhang et al., 2011). For PAR2 in the central nervous system, there are trypsin homologs that had been identified in the brain or spinal cord, which are all capable of regulated proteolytic signaling through activating PAR2 (Blaber et al., 2002; Chen et al., 1995; Sawada et al., 2000), however their roles in pain states need further study. There are many evidences for mast cell tryptase to activate PAR2 in inflammatory and neuropathic pain states in central nervous system. Under