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Research Report

Intrathecal midazolam regulates spinal AMPA receptor expression and function after nerve injury in rats

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ABSTRACT

Spinal γ-aminobutyric acid (GABA) and alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors have been implicated in the mechanisms of neuropathic pain after nerve injury; however, how these two receptors interact at the spinal level remains unclear. Here we show that intrathecal midazolam through activation of spinal GABAA receptors attenuated the expression and function of spinal AMPA receptors in rats following peripheral nerve injury. Thermal hyperalgesia and mechanical allodynia induced by chronic constriction nerve injury (CCI) in rats were attenuated by the short-acting benzodiazepine midazolam (20=10>5 μg>vehicle) administered intrathecally once daily for 7 postoperative days. CCI-induced upregulation of AMPA receptors within the spinal cord dorsal horn was also significantly reduced by the intrathecal midazolam (10, 20 μg) treatment. The inhibitory effects of midazolam (10, 20 μg) on neuropathic pain behaviors and AMPA receptor expression were prevented by co-administration of midazolam with the GABAA receptor antagonist bicuculline (3 μg), whereas intrathecal treatment with bicuculline (1 or 3 μg) alone in naive rats induced the upregulation of spinal AMPA receptor expression and nociceptive responses, indicating a tonic regulatory effect from endogenous GABAergic activity on the AMPA receptor expression and spinal nociceptive processing. These results indicate that modulation of spinal AMPA receptor expression and function by the GABAergic activity may serve as a mechanism contributory to the spinal nociceptive processing.

1. Introduction

Neuropathic pain after peripheral nerve injury is a chronic pain condition, which remains very difficult to treat. Spinal glutamatergic and γ-aminobutyric acid (GABA)ergic systems are among several proposed mechanisms of neuropathic pain and have been extensively investigated over the last two decades (Dubner, 1991; Dougherty and Willis, 1991; Yamamoto and Yaksh, 1992; Mao et al., 1995; Woolf and Mannion, 1999; Hammand, 2001). Evidence exists indicating that either activation of the glutamatergic system or a possible loss of GABAergic activity, or both, after peripheral nerve injury contributes to the development and maintenance of neuropathic pain behaviors in rats (Mao et al., 1995, 1997; Bridges et al., 2001; Cronin et al., 2004; Polgar et al., 2005; Scholz et al., 2005). These earlier studies suggest a possible interaction

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between these regulatory systems within the central nervous system.

Ionotropic glutamate receptors including alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptors are localized both presynaptically and postsynaptically within superficial laminae of spinal cord dorsal horn. AMPA receptors have been shown to play a significant role in the mechanisms of neuropathic pain and spinal nociceptive processing (Mao et al., 1992a,b; Leem et al., 1996; Kondo et al., 2002; Garry et al., 2003). The AMPA receptor consists of several subunits and previous studies have indicated that spinal AMPA receptor expression was altered after either peripheral nerve injury or inflammation, which contributed to the development of pathological pain conditions in rats (Harris et al., 1996; Alvarez et al., 2000).

On the other hand, it has been demonstrated that activation of spinal GABAergic system negatively regulated spinal nociceptive processing (Kontinen and Dickenson, 2000; Hammand, 2001; Nishiyama and Hanaoka, 2003), suggesting an important role for the GABAergic system in the balance between spinal excitatory and inhibitory elements after peripheral nerve injury. Benzodiazepines activate GABAA receptors, reduce excitatory transmitter release presynaptically as well as excitatory activity postsynaptically in spinal dorsal horn neurons (Haefely, 1988). Indeed, intrathecal midazolam (a benzodiazepine-GABAA receptor agonist) attenuated neuropathic pain behaviors (Hwang and Yaksh, 1997). Recently, presynaptic AMPA receptors on GABAergic terminals have been shown to have a bidirectional role in neuronal activity with the superficial spinal cord dorsal horn and contributed to the mechanisms of central sensitization and hyperalgesia (Lu et al., 2005). Moreover, the effect on GABA receptors may interact with that of AMPA receptor antagonists in rats (Nishiyama et al., 1999). Thus, it is possible that there might be interactions at the spinal level between the GABA and AMPA receptors and such interactions may have a functional role in neuropathic pain behaviors.

Utilizing a rat model of chronic constriction nerve injury (CCI) (Bennett and Xie, 1988), we examined whether midazolam (a clinically available short-acting benzodiazepine and GABA analogue) given intrathecally would modulate the expression of spinal AMPA receptors and neuropathic pain behaviors in CCI rats through activation of spinal GABAA receptors.

2. Results

2.1. *Effects of midazolam on neuropathic pain behaviors: reduction by bicuculline*

The effects of midazolam on neuropathic pain behaviors were examined in seven groups of rats including (1) CCI plus vehicle, (2–4) CCI plus 5, 10 or 20 μg midazolam, (5) CCI plus 10 μg midazolam and 3 μg bicuculline, (6) sham plus 10 μg midazolam, and (7) sham plus vehicle. Each agent was given once daily (intrathecally) for 7 consecutive postoperative days, beginning immediately after operation. Midazolam (20=10 μg>5 μg>vehicle) reduced thermal hyperalgesia and mechanical allodynia in the hindpaw ipsilateral to CCI, as compared with vehicle-treated CCI rats on all of postoperative days (Fig. 1, $p<0.05$; $n=6$–7). In contrast, midazolam (10 or 20 μg) did not change baseline thermal and mechanical nociceptive responses in sham rats, nor did midazolam (5–20 μg) change thermal and mechanical nociceptive responses in the hindpaw contralateral to CCI (Fig. 1, $p>0.05$; $n=5$–7).

The effects of midazolam on neuropathic pain behaviors were primarily mediated through the GABAA receptor, because co-administration of midazolam (10 μg) with the GABAA receptor antagonist bicuculline (3 μg), given once daily for postoperative days 1–7, reversed the attenuation of thermal hyperalgesia and mechanical allodynia by midazolam (Fig. 1, $p<0.05$, $n=5$–7). The bicuculline dose was referenced from previous studies (Hwang and Yaksh, 1997; Kaneko and Hammond, 1997; Malan et al., 2002) and a lower dose of bicuculline (0.3 μg) was ineffective in our pilot experiment. The combined treatment of midazolam (10 μg) and bicuculline (3 μg) did not alter the thermal and mechanical nociceptive responses in the hindpaw contralateral to CCI (Fig. 1, $p>0.05$; $n=5$–7). These results indicate that intrathecal midazolam attenuated neuropathic pain behaviors in CCI rats, which was mainly mediated through the GABAA receptor within the spinal cord dorsal horn.

2.2. *Effects of midazolam on spinal AMPA receptor expression: reduction by bicuculline*

The effects of midazolam on the expression of spinal AMPA receptors after CCI were examined using Western blot. CCI but not sham operation induced an upregulation of AMPA receptors (GluR1, GluR2, GluR3) within the spinal cord dorsal horn ipsilateral to CCI on postoperative days 4 and 8 (not day 1) (Fig. 2, $p<0.05$, $n=4$–6), while there were no significant changes in the AMPA receptor expression within the contralateral spinal cord dorsal horn (data not shown). Intrathecal treatment with midazolam (10=20 μg, but not 5 μg, once daily for 7 postoperative days) reduced the upregulation of AMPA receptors in CCI rats as compare with CCI rats treated with a vehicle (Fig. 3, $p<0.05$, $n=4$–6). Similar to its effects on neuropathic pain behaviors, the GABAA receptor antagonist bicuculline reduced the effect of midazolam on spinal AMPA receptor expression when bicuculline (3 μg) was co-administered intrathecally with midazolam (10 μg) once daily for postoperative days 1–7 (Fig. 4, $p<0.05$, $n=5$–6). These results indicate that the upregulation of spinal AMPA receptor after CCI was mediated at least in part through the GABAA receptor within the spinal cord dorsal horn.

2.3. *Effects of bicuculline on nociceptive behaviors and spinal AMPA receptor expression in naïve rats*

In order to examine whether inhibition of GABAA receptor activity within the spinal cord would alter thermal and mechanical nociceptive responses as well as spinal AMPA receptor expression in naïve rats, four groups of rats were used including naïve rats treated with 0.3, 1 or 3 μg bicuculline. Each dose was given once daily to naïve rats for 7 consecutive days. Bicuculline (3 μg but not 0.3 μg or 1 μg) induced nociceptive responses to thermal and mechanical stimulation in naïve rats (Fig. 5, $p<0.05$, $n=5$), similar to that observed after CCI.