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BRAIN RESEARCH

Research Report

Orexin-2 receptors inhibit primary afferent fiber-evoked responses of ventral roots in the neonatal rat isolated spinal cord

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ARTICLE INFO

Article history:
Accepted 8 April 2008
Available online 29 May 2008

Keywords:
Orexin
Neonatal spinal cord
Slow ventral root potential
Wind-up
Rat

ABSTRACT

Orexin-A and orexin-B are hypothalamic peptides. Orexin-A binds equally to both orexin-1 and orexin-2 receptors but orexin-B has a preferential affinity for orexin-2 receptor. In the spinal dorsal horn, orexins have been shown to be concentrated in the superficial laminae. In the present study, the authors examined the effect of spinally applied orexin-A and orexin-B on the primary afferent fiber-evoked nociceptive reflex in the isolated spinal cord of the neonatal rat. In the isolated spinal cord preparation from 0–3day old rats, single-shock stimulation of a dorsal root (L3–L5) at a strength which can activate C-fibers induced a slow depolarizing response lasting about 30s (slow ventral root potential: slow VRP) in the ipsilateral ventral root of the same segment. Bath application of orexin-A and orexin-B inhibited the slow VRP in a concentration-dependent manner. Bath application of SB-334867, a selective orexin-1 receptor antagonist, had no effect on the depressant effect of orexin-A on slow VRP. Bath application of [Ala^{11},D-Leu^{15}]-orexin B, a selective orexin-2 receptor agonist, depressed the slow VRP. Both orexin-A and orexin-B depressed the level of temporal summation of synaptic activity evoked by 20 repetitive stimulations of the dorsal root. These data suggest that orexin-2 receptor, but not orexin-1 receptor, may play an inhibitory role in nociceptive transmission in the neonatal rat spinal cord.

1. Introduction

Orexin-A and orexin-B are hypothalamic peptides of 33 and 28 amino acids, respectively, and have a role in the regulation of feeding behavior (Sakurai et al., 1998), energy metabolism (Schwartz, 1998; Lubkin and Stricker-Krongrad, 1998), and the sleep–wake cycle (Hagan et al., 1999). Orexin-A and orexin-B act via G-protein coupled receptors named orexin-1 receptor and orexin-2 receptor (Sakurai et al., 1998). Orexin-A binds to both orexin-1 and orexin-2 receptors but orexin-B has a preferential affinity for orexin-2 receptor (Sakurai et al., 1998).

Intravenous injection of orexin-A has been reported to be analgesic in the adult rats (Bingham et al., 2001; Holland et al., 2006). It has been reported that, in the adult rats, intrathecally administered orexin-A, but not orexin-B, produced an anti-nociceptive effect in inflammatory pain models such as the formalin test (Yamamoto et al., 2002) and the carrageenan test (Yamamoto et al., 2003a), and in neuropathic pain models such as the partial sciatic nerve ligation model (Yamamoto et al., 2003b) and the diabetic neuropathy pain model (Kajiyama et al., 2005). These effects of orexin-A were reported to be completely antagonized by pretreatment with SB-334867,

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Abbreviations: ANOVA, one-way analysis of variance; CSF, cerebrospinal fluid; NMDA, *N*-methyl-D-aspartate; slow VRP, slow ventral root potential

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doi:10.1016/j.brainres.2008.04.016

Table 1 – Summary of the drug effects

Drug	Fast reflex response	Slow VRP
Orexin-A	–	↓
Orexin-B	–	↓
[Ala^{11},D-Leu^{15}]-orexin B	–	↓
SB-334867	–	–
Orexin-A + SB-334867	–	↓

– no effect, ↓ depressant effect.

a selective orexin-1 receptor antagonist (Yamamoto et al., 2002, 2003a; Kajiyama et al., 2005). On the other hand, in the infant rat (75–125g), orexin-B has been reported to excite certain superficial dorsal horn neurons, some of which exert inhibitory influences on other cells in the region (Grudt et al., 2002) and this indicated that activation of spinal orexin-2 receptor inhibits nociceptive transmission in the spinal cord. These data indicated that orexin-1 receptor, but not orexin-2 receptor, is involved in the nociceptive transmission in the adult rat spinal cord and that orexin-2 receptor plays an important role in nociceptive transmission in the infant rat spinal cord. Thus, in the neonatal rat spinal cord, the role of orexin-1 receptor and orexin-2 receptor in nociceptive transmission is unpredictable. In the present study, we examined the role of orexin-1 receptor and orexin-2 receptor in the primary afferent fiber-evoked nociceptive reflex of the isolated neonatal rat spinal cord. In the isolated spinal cord preparation, activation of primary afferent fibers by electrical stimulation at a strength which can activate C-fibers evokes a depolarization of a slow time course in ventral roots (hereafter referred to as the slow ventral root potential; slow VRP) and this slow VRP has been shown to be markedly depressed by tachykinin antagonists as well as an opioid agonist (Akagi et al., 1985; Yanagisawa et al., 1984).

Low-frequency repetitive stimulation of the dorsal root at high intensity evokes a progressive increase in the action potential discharge elicited in spinal neurons by each of the successive stimuli in a train. This phenomenon, termed wind-up, is a typical model of sensitization of spinal neurons in the dorsal horn and this wind-up phenomenon is thought to be one of central mechanisms for hyperalgesia in inflammatory pain or neuropathic pain (Thompson et al., 1994; Yamamoto and Yaksh, 1992a,b). It has been reported that the analgesic potency of orexin-A is higher in the inflammatory pain model than in the hot plate test (Yamamoto et al., 2002). This suggested that orexin-A preferentially suppresses the development of spinal sensitization and that orexin-A produces only a weak effect on the thermal nociceptive transmission in the spinal cord. In the present study, we also examined the effect of orexin-A and orexin-B on the development of the wind-up phenomenon in the neonatal rat spinal cord.

2. Results

The effects of drugs applied to the spinal cord are summarized in Table 1.

Bath application of either orexin-A (10–1000 nM, $n = 6$) or orexin-B (10–1000 nM, $n = 5$) to the spinal cord exerted a depressant effect on the dorsal root evoked slow VRP in a concentration-dependent manner [Figs. 1 and 2, $p < 0.05$ by one-way analysis of variance (ANOVA)]. On the other hand, orexin-A had no effect on the fast reflex responses at any concentration examined in this study (Fig. 1, $n = 6$, $p > 0.5$ by ANOVA). Orexin-B had also no effect on the fast reflex responses at any concentration examined in this study ($n = 5$, $p > 0.5$ by ANOVA).

Bath application of SB-334867, a selective orexin-1 receptor antagonist, to the spinal cord had no effect on the dorsal root evoked slow VRP at a concentration between 0.1 and 1000 nM ($n = 5$, $p > 0.6$ by ANOVA). Bath application of 1000 nM SB-334867 with 1000 nM orexin-A did not affect the depressant

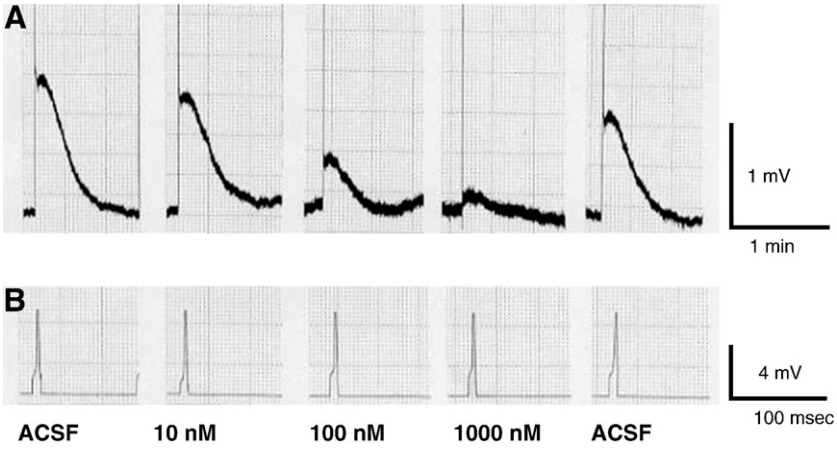

Fig. 1 – Effect of orexin-A on the primary afferent evoked ventral root potential in the neonatal rat spinal cord. A single-shock stimulus at a strength which can activate C-fibers (200 μs in duration and 20–30 V in amplitude) was given to the dorsal root (L3–L5) and the potential was recorded extracellularly from the ipsilateral ventral root. Orexin-A was applied to the spinal cord in a cumulative manner. (A) Sample records of slow ventral root potential (VRP) recorded by a pen recorder. (B) Sample records of the fast reflex response that were stored in a transient memory device and recorded by a pen recorder with a 500-fold expansion of the time base.