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News

The analgesic effect of orexin-A in a murine model of chemotherapy-induced neuropathic pain

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ABSTRACT

Orexins are neuropeptides that are localized to neurons in the lateral and dorsal hypothalamus but its receptors are distributed to many different regions of the central nervous system. Orexins are implicated in a variety of physiological functions including sleep regulation, energy homeostats, and stress reactions. Furthermore, orexins administered exogenously have been shown to have analgesic effects in animal models. A type of intractable pain in patients is pain due to chemotherapy-induced peripheral neuropathy (CIPN). Several chemotherapeutic agents used for the treatment of malignant diseases induce dose-limiting neuropathic pain that compromises patients' quality of life. Here, we examined the analgesic effect of orexin-A in a murine model of CIPN, and compared it with the effect of duloxetine, the only drug recommended for the treatment of CIPN pain in patients. CIPN was induced in male BALB/c mice by repeated intraperitoneal injection of oxaliplatin, a platinum chemotherapeutic agent used for the treatment of advanced colorectal cancer. Neuropathic mechanical allodynia was assessed by the von Frey test, and the effect on acute thermal pain was assessed by the tail flick test. Intracerebroventricularly administered orexin-A dose-dependently attenuated oxaliplatin-induced mechanical allodynia and increased tail flick latencies. Oxaliplatin-induced mechanical allodynia was completely reversed by orexin-A at a low dose that did not increase tail flick latency. Duloxetine only partially reversed mechanical allodynia and had no effect on tail flick latency. The analgesic effect of orexin-A on oxaliplatin-induced mechanical allodynia was completely antagonized by prior intraperitoneal injection of SB-408124 (orexin type-1 receptor antagonist), but not by prior intraperitoneal injection of TCS-OX2-29 (orexin type-2 receptor antagonist). Our findings suggest that orexin-A is more potent than duloxetine in relieving pain CIPN pain and its analgesic effect is mediated by orexin type-1 receptors. Orexin type-1 receptor agonists may have potential therapeutic roles in the treatment of CIPN pain in patients.

1. Introduction

Orexins/hypocretins are endogenous neuropeptides that are produced from the precursor prepro-orexin by neurons that exist only in the lateral and dorsal hypothalamus. Orexin-producing neurons innervate many different regions of the brain (de Lecea et al., 1998; Sakurai et al., 1998). Two subtypes of orexin receptors, termed orexin type-1 (OX1) and orexin type-2 (OX2), have been identified. Orexin-A is a non-selective neuropeptide that has equal affinity for OX1 and OX2 receptors, while orexin-B is a selective neuropeptide that has 10-fold higher affinity for OX2 than OX1 receptors (Sakurai et al., 1998). Orexins have been implicated in numerous physiological functions, such as feeding (Yamada et al., 2000), energy homeostasis (Chemelli et al., 1999), sleep regulation (Kukkonen, 2012), cardiovascular regulation (Samson et al., 1999), and neuroendocrine function (Kuru et al., 2000). Orexin-producing neurons send projections to several regions of the central nervous system that are involved in analgesia, such as the midbrain periaqueductal gray, raphe nucleus and locus coeruleus nucleus (Peyron et al., 1998). These regions are known to play major roles in descending pain inhibition (Ossipov et al., 2010). Orexin-producing neurons also send projections directly to the spinal dorsal horn (van den Pol, 1999). In animal pain models, intracerebroventricular and spinal administrations of orexin-A have been shown to have antinociceptive effects suggesting a role of the orexin system in the modulation of nociceptive transmission (Bingham et al., 2001; Yamamoto et al., 2003; Cheng et al., 2003; Mobarakeh et al., 2005). In addition, we have shown that orexin neurons are activated under stress and persistent pain and that orexin is involved in stress-induced analgesia (Watanabe et al., 2005). Thus, drugs that activate orexin neurons or

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orexin receptors may have the potential to be a novel pharmacological approach for the treatment of pain.

There are still many types of pain that cannot be effectively treated even with the recent advancement of basic and clinical research on pain. Some types of neuropathic pain in particular are especially difficult to treat. A type of refractory neuropathic pain that seriously affect cancer patients is pain due to chemotherapy-induced peripheral neuropathy (CIPN). Several chemotherapeutic agents used for the treatment of malignant diseases induce peripheral neuropathic pain that compromises patients' quality of life and lead to changes in cancer treatment to non-neurotoxic agents with obvious negative implications for disease outcomes. Pregabalin, an analgesic which is effective in many types of neuropathic pain and is considered a standard treatment for neuropathic pain, is not effective in pain due to CIPN. Although many types of known analgesics are administered to patients in attempt to relieve CIPN pain, only duloxetine, a serotonin-norepinephrine reuptake inhibitor, has been shown to be effective in a prospective randomized study (Lavoie Smith et al., 2013). Thus duloxetine is the only drug that is recommended in the guidelines of the management of CIPN published by the American Society of Clinical Oncology (Hershman et al., 2014). However, even the effect of duloxetine is limited and it only produces a mild effect in these patients. Thus, there is an urgent need to develop a new analgesic for the management of CIPN in order to treat these cancer patients adequately and improve their quality of life during and after chemotherapy.

Recently we have developed a murine model of oxaliplatin-induced neuropathic pain which closely mimics patients' conditions and may be useful to investigate drugs for the treatment of CIPN pain (Toyama et al., 2014). In the present study, to explore the potential of orexin for the treatment of CIPN pain, we examined the analgesic effect of orexin-A in our model and compared it with the effect of duloxetine.

2. Materials and methods

Experiments were approved by the Institutional Animal Use Committee of Teikyo University (Tokyo, Japan), and conducted in accordance with the National Institutes of Health guidelines and the International Association for the Study of Pain Committee for Research and Ethical Issues guidelines for animal research (Zimmermann, 1983).

2.1. Animals

Male BALB/c mice, 9 weeks old at the time of first drug administration, were used in the experiments. Male mice were chosen over female mice to avoid effects of hormonal changes of the menstrual cycle. All mice were housed on a 12:12 h dark–light cycle with food and water ad libitum.

2.2. Compounds

Oxaliplatin was obtained from Yakult Co., Ltd. (Tokyo, Japan) and was dissolved in distillated water. Orexin-A (Peptide Institute, Osaka, Japan) and duloxetine hydrochloride (Wako Pure Chemical Industries, Ltd., Osaka, Japan) were dissolved in saline. SB-408124, OX1 receptor antagonists (Tocris Bioscience, Bristol, U.K.), was dissolved in 1% (2-hydroxypropyl)-β-cyclodextrin (Sigma Chemical Co., St. Louis, MO, USA) and 10% DMSO in distilled water, and TCS-OX2-29, OX2 receptor antagonists (Tocris Bioscience, Bristol, UK), was dissolved in saline.

2.3. Oxaliplatin-induced neuropathic pain model

Oxaliplatin-induced neuropathic pain was induced in mice as reported previously (Toyama et al., 2014). Briefly, oxaliplatin (10 mg/kg) was injected intraperitoneally, once per week for 3 weeks (days 1, 8, and 15). Oxaliplatin was administered in a volume of 0.1 ml/10 g mouse weight. The repeated administrations of oxaliplatin induced mechanical allodynia, which could be assessed by decrease in mechanical pain threshold.

2.4. Pain tests

Mechanical threshold to induce paw withdrawal responses (paw withdrawal threshold) was assessed by stimulation of the plantar surface of the hind paw with von Frey hairs (Semmes-Weinstein Monofilaments; Stoelting Co., Wood Dale, IL). Mice were placed in a clear plastic chamber with a wire mesh floor, which provided full access to the planter surface of the hind paws. Mice were allowed to habituate for at least 15 min before testing. The paws were touched with one of a series of nine von Frey filaments with logarithmically incremental stiffness (0.023–3.630 g) starting with the filament of 0.407 g. The 50% mechanical withdrawal thresholds were determined using the up–down method (Chaplan et al., 1994).

In addition, effects of orexin-A on acute thermal pain were assessed by the tail flick test. Radiant heat was applied to the tail at 3 cm from the tip using a tail flick apparatus (IITC, Woodland Hills, Calif., USA). The latency of the mouse to flick the tail (tail flick latency) was measured. The intensity of the radiant heat was adjusted so that the latencies in the naïve mice would fall between 2.5 and 3.5 s. To avoid tissue damage, the heat stimulus was discontinued after 10 s (cut-off latency).

To assess the development of mechanical allodynia, paw withdrawal thresholds (mechanical thresholds) were measured prior to oxaliplatin administrations (baseline) and 7 days after the last oxaliplatin administration on the day of drug test (see below) prior to the administration of the test drug. Paw withdrawal thresholds were also measured after the administration of the test drug during the drug test. Tail flick latencies were also measured immediately after the von Frey test in the same mice.

2.5. Drug tests

2.5.1. Analgesic effects of orexin-A and duloxetine

Drug tests examining the analgesic effects of orexin-A and duloxetine were performed 7 days after the last injection of oxaliplatin by an experimenter blinded to the administered drugs.

Orexin-A (0.1, 0.3, or 1.0 nmol/3 μl) or vehicle was injected intracerebroventricularly (i.c.v.) to oxaliplatin-induced neuropathic mice and age-matched naïve mice using previously described methods (Pedigo et al., 1975; Navani and Yoburn, 2013). In brief, mice were briefly anesthetized with sevoflurane (<2 min) and a small incision was made on the head to expose the bregma. Injection was made 2 mm lateral to the bregma perpendicular to the surface of the skull into the right lateral ventricle via a 30 G needle. A polyethylene tubing was slipped over the needle to control the depth of the injection to 3 mm from the surface of the skull. Mice were subjected to the von Frey test and the tail flick test prior to and 30 min after the administration of orexin. In other oxaliplatin-induced neuropathic mice, duloxetine (10 or 30 mg/kg) or vehicle (saline) was injected intraperitoneally. Duloxetine was administered in a volume of 0.1 ml/10 g mouse weight. These mice were subjected to von Frey test and tail flick test prior to and 60 min after the administration of duloxetine.

2.5.2. Effects of OX1 and OX2 receptor antagonists on the analgesic effect of orexin-A

Other oxaliplatin-induced neuropathic mice were pre-treated with an intraperitoneal administration of SB-408124 (30 mg/kg), an OX1 receptor antagonist, TCS-OX2-29 (30 mg/kg), an OX2 receptor antagonist or vehicle. SB-408124 and TCS-OX2-29 were administered in a volume of 0.1 ml/10 g mouse weight. Ten minutes later, orexin-A (0.3 nmol/3 μl) was administered i.c.v. Mice were subjected to von Frey test and the tail flick test 30 min after the administration of orexin-A.

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