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Original Article

Yokukansan (Kampo medicinal formula) prevents the development of morphine tolerance by inhibiting the secretion of orexin A

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ABSTRACT

Background: Yokukansan (YKS), a traditional herbal (Kampo) medicine consisting of seven herbs, is effective in the treatment of pain disorders, such as headache, postherpetic neuralgia, fibromyalgia, and trigeminal neuralgia, and we have previously shown it to be effective against morphine analgesic tolerance in rats. It has been reported that orexin receptor antagonists prevent the development of morphine tolerance and that YKS inhibits the secretion of orexin A in the hypothalamus. This study examined whether the inhibition of the secretion of orexin A by YKS is one mechanism underlying its effect against morphine analgesic tolerance.

Methods: Male Wistar rats were administered a subcutaneous injection of morphine hydrochloride (10 mg/kg/day) for 5 days. One group was preadministered YKS, starting 3 days before the morphine. The withdrawal latency following thermal stimulation was measured daily using a hot plate test. On day 5, the levels of orexin A in the plasma and the midbrain were measured, and the appearance of activated astrocytes in the midbrain was examined by immunofluorescence staining.

Results: The preadministration of YKS prevented the development of morphine tolerance. The repeated administration of morphine significantly increased the plasma and midbrain levels of orexin A and the activation of astrocytes. These increases were significantly inhibited by the preadministration of YKS.

Conclusion: These results suggest that the preadministration of YKS attenuated the development of antinociceptive morphine tolerance and that the inhibition of orexin A secretion may be one mechanism underlying this phenomenon.

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1. Introduction

Morphine is a powerful opioid analgesic widely used for relieving severe pain, such as the pain associated with cancer and surgery. However, its repeated administration may lead to dependence and the development of antinociceptive tolerance. Morphine tolerance is a complex physiological response involving glial cell activity,[1–3] neuroinflammation,[4–7] opioid receptor desensitization,[8] and the activation of spinal N-methyl-D-aspartate receptors.[9] In recent years, it has been reported that orexins are also involved in the development of antinociceptive tolerance.[10–12]

Orexins (also called hypocretins) are neuropeptides. Orexinergic neurons are mainly expressed in the lateral and perifornical areas of the posterior hypothalamus, with their axons widely distributed throughout the central nervous system, apart from in the cerebellum.[13] Orexins A and B are derived from the same precursor peptide, prepro-orexin,[14] and bind to the orphan G-protein coupled receptor orexin-1 receptor (OX1R), which is highly selective for orexin A, and to the orexin-2 receptor (OX2R), which is a nonselective receptor for both orexins A and B.[13] Orexins are involved in the modulation of many physiological processes, including sleep, arousal, feeding, and metabolism,[15] as well as in the autonomic regulation of the cardiovascular,[16] respiratory,[17] and neuroendocrine systems.[18] Many studies have indicated that orexin A also exhibits antinociceptive effects in the brain and spinal cord, based on different types of pain models with thermal, chemical,[19,20] and neuropathic nociception.[21,22] Conversely, orexin and OX1R may be involved in the development of tolerance to the analgesic effects of morphine. Studies have shown that pretreatment with an OX1R antagonist inhibited the development of morphine tolerance.[10–12]

Yokukansan (YKS) is a traditional herbal (Kampo) medicine that comprises seven herbs (Table 1). It is administered to patients who show symptoms such as emotional irritability, neurosis, and insomnia and to infants who suffer from night crying and convulsions.[23] Recently, it has been reported that YKS is also effective against pain disorders, including headache, postherpetic neuralgia, fibromyalgia, and trigeminal neuralgia.[24,25] Studies have demonstrated the antinociceptive effects of YKS in mice models with visceral pain[11] and rat models with chronic constriction injury.[26–28] We have previously reported that preadministration of YKS attenuated the development of antinociceptive morphine tolerance, and that the suppression of spinal glial cell activation may be one mechanism underlying this phenomenon.[29] We also showed that the administration of YKS reduced the secretion of orexin A in a rat stress model.[30]

In the present study, therefore, we investigated whether inhibition of the secretion of orexin A is involved in the effect of YKS against morphine analgesic tolerance.

Table 1 – The Component Galenicals of Yokukansan

Component galenicals of Yokukansan (YKS)	
Uncariae cum Uncis ramulus	3.0 g
Cnidii rhizoma	3.0 g
Bupleuri radix	2.0 g
Atratylodis Lanceae rhizoma	4.0 g
Poria	4.0 g
Angelicae radix	3.0 g
Glycyrrhizae radix	1.5 g

The weights indicate the relative amounts mixed.

2. Methods

2.1. Animals

Male Wistar rats (7–8 weeks old) were purchased from Nippon Bio-Supp. Center (Tokyo, Japan). During the experimental period, the animals were housed in standard plastic cages (W 24 × L 40 × H 20 cm) and were kept in our animal facility at 25 ± 2°C and 55% ± 5% humidity, with a light/dark cycle of 12 hours/12 hours. Food (CLEA Japan, CE-2, Tokyo, Japan), and water was provided *ad libitum*. The experiments were performed in accordance with the guidelines of the Committee of Animal Care and Welfare of Showa University. All experimental procedures were approved by the Committee of Animal Care and Welfare of Showa University (certificate number: 07062).

2.2. Administration of drugs

The dry powdered extracts of YKS (Lot No. 2110054010) used in the present study were supplied by Tsumura & Co. (Tokyo, Japan). The seven herbs comprising YKS (Table 1) were mixed and extracted with purified water at 95.1°C for 1 hour; the soluble extract was separated from the insoluble waste and concentrated by removing water under reduced pressure. The YKS was dissolved in distilled water and orally administered (1.0 g/kg/day). This dose was chosen on the basis of effective doses of YKS recommended in previous reports.[31] The rats which were not treated with YKS received water instead.

Morphine hydrochloride (T1-02591; Takeda Chemical Industries, Osaka, Japan) was dissolved in physiological saline and injected subcutaneously. The rats not treated with morphine were administered only saline.

2.3. Influence of morphine administration on orexin A secretion

The influence of a single administration of morphine on orexin A secretion was examined. Eighteen rats were randomly divided into three groups (each $n = 6$): Control, which were administered physiological saline; Mor (2 mg), subcutaneously administered with morphine at 2 mg/kg; and Mor (10 mg), subcutaneously administered with morphine at 10 mg/kg. At 30 minutes after dosing, the rats were anesthetized with intraperitoneally administered pentobarbital sodium (50 mg/kg) (Somnopentyl; Kyoritsu Seiyaku, Tokyo, Japan). Blood samples were taken from the inferior vena cava, and midbrain samples were then removed. To avoid the influence of daily fluctuations, all samples were obtained between 1:00 and 3:00 PM. The blood samples were centrifuged at 4°C and 3000 rpm for 10 minutes and the supernatants collected. The midbrain samples were mashed homogeneously with a