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Differences in the morphine-induced inhibition of small and large intestinal transit: Involvement of central and peripheral μ-opioid receptors in mice

Kenjiro Matsumoto [a,b,*], Hiroyuki Umemoto [a], Tomohisa Mori [c], Ryuya Akatsu [a], Shinichiro Saito [a], Kimihito Tashima [a], Masahiro Shibasaki [c], Shinichi Kato [b], Tsutomu Suzuki [c], Syunji Horie [a]

[a] Laboratory of Pharmacology, Faculty of Pharmaceutical Sciences, Josai International University, 1 Gumyo, Togane, Chiba 283-8555, Japan
[b] Division of Pathological Sciences, Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Ymashina, Kyoto 607-8414, Japan
[c] Department of Toxicology, Hoshi University School of Pharmacy and Pharmaceutical Sciences, 2-4-41Ebara, Shinagawa-ku, Tokyo 142-8501, Japan

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ABSTRACT

Constipation is the most common side effect of morphine. Morphine acts centrally and on peripheral sites within the enteric nervous system. There are a few comprehensive studies on morphine-induced constipation in the small and large intestine by the activation of central and peripheral μ-opioid receptors. We investigated the differences in the inhibition of the small and large intestinal transit in normal and morphine-tolerant mice. Morphine reduced the geometric center in the fluorescein isothiocyanate–dextran assay and prolonged the bead expulsion time in a dose-dependent manner. The inhibitory effects of morphine were blocked by μ-opioid antagonist β-funaltrexamine, but not by δ- and κ-opioid antagonists. The peripheral opioid receptor antagonist, naloxone methiodide, partially blocked morphine's effect in the small intestine and completely blocked its effect in the large intestine. The intracerebroventricular administration of naloxone significantly reversed the delay of small intestinal transit but did not affect morphine-induced inhibition of large intestinal transit. Naloxone methiodide completely reversed the inhibition of large intestinal transit in normal and morphine-tolerant mice. Naloxone methiodide partially reversed the morphine-induced inhibition of small intestinal transit in normal mice but completely reversed the effects of morphine in tolerant mice. Chronic treatment with morphine results in tolerance to its inhibitory effect on field-stimulated contraction in the isolated small intestine but not in the large intestine. These results suggest that peripheral and central opioid receptors are involved in morphine-induced constipation in the small and large intestine during the early stage of treatment, but the peripheral receptors mainly regulate constipation during long-term morphine treatment.

1. Introduction

Constipation is the major side effect of chronic opioid administration and relief from the adverse gastrointestinal effects markedly enhances patients' quality of life (McQuay, 1999; Portenoy, 1996). In the case of morphine, the dose required for its analgesic effect is much higher than that required for its gastrointestinal side effect, about fifty times higher in human and about four times in experimental animal (Mori et al., 2013; Matsumoto et al., 2006). Therefore, when morphine is used as an analgesic, constipation is a significant issue (Megens et al., 1998).

Opioid receptors are widely distributed throughout the central and peripheral nervous systems, and play a fundamental role in pain relief and development of adverse effects (Quock et al., 1999). It is widely accepted that opioids act directly on peripheral μ-opioid receptors in intestinal neurons, which decreases gastrointestinal motility, and consequently induces constipation. Central opioid receptors are also thought to be involved in morphine-induced constipation because central administration of morphine induces delayed gastrointestinal transit (Kuo et al., 2014; Mori et al., 2013; Pol et al., 1999). Opioid receptor antagonists that cross blood-brain barrier act central and peripheral opioid receptor and antagonize both opioid induced analgesic effect and constipation.

* Corresponding author at: Division of Pathological Sciences, Department of Pharmacology and Experimental Therapeutics, Kyoto Pharmaceutical University, Misasagi, Ymashina, Kyoto 607-8414, Japan.
E-mail address: kenjiro@mb.kyoto-phu.ac.jp (K. Matsumoto).

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Therefore, peripherally restricted opioid antagonists, block opioid receptor in intestine, are useful agents for treating opioid-induced constipation (Camillert et al., 2014; Holzer 2012; 2010).

The quality of life of patients who experience cancer-related pain is worsened by tolerance to opioid analgesics, which involves the loss of their analgesic potency. An increase in the required dose of opioids leads to an increase in possible side effects. Morphine induces tolerance to analgesia, while tolerance to constipation hardly develops. However, there are a few comprehensive studies on morphine-induced constipation in the small and large intestine by the activation of central and peripheral μ-opioid receptors. In the present study, we investigate the involvement of central and peripheral μ-opioid receptors in morphine-induced constipation by using the μ-opioid receptor antagonist naloxone methiodide, which does not cross the blood–brain barrier (Lewanowitsch and Irvine, 2002). Furthermore, we investigated the contribution of central and peripheral opioid receptors to morphine-induced constipation in chronic morphine-treated mice.

In the present study, the fluorescein isothiocyanate (FITC)–dextran test is used for investigating the small intestinal transit in mice. This method provides precise information about intestinal transit, especially small intestinal transit (Overhaus et al., 2004). As previously reported, the large intestine, especially the rectum, is the region most involved in opioid-induced constipation (Ono et al., 2014). Therefore, we compared localization of the μ-opioid receptor in small and large intestine using an immunohistochemical staining and investigated large intestinal transit (from transverse distal colon to rectum) using a bead expulsion assay. Furthermore, to clarify the involvement of peripheral opioid receptors in small and large intestine, we examined the inhibitory effect of morphine on field-stimulated contraction. We designed this study to investigate the involvement of the small and large intestines in morphine-induced inhibition of gastrointestinal transit by using the in vivo and in vitro methods.

2. Materials and methods

2.1. Experimental animals

Male ddY-strain mice (Japan SLC, Hamamatsu, Japan) weighing 25–32 g were used. The ddY strain is outbred and has been maintained as a closed colony. Mice of this strain show good reproductive performance and superior growth. We used ddY mice in this study because we have consecutive data in opioid-induced antinociceptive effect and constipation. Animals were housed in a temperature-controlled room at 24 °C with lights on from 07:00 to 19:00 and had free access to food and water. All experiments were performed in compliance with the "Guiding Principles for the Care and Use of Laboratory Animals" approved by the Japanese Pharmacological Society and the guidelines approved by the Ethical Committee on Animal Care and Animal Experimentation of Josai International University (#12). The number of animals used was kept to a minimum for meaningful interpretation of the data, and animal discomfort was kept to a minimum.

2.2. Drugs

The drugs used in this study were morphine hydrochloride (Takeda Chemical Ind., Osaka, Japan), naloxone hydrochloride (MP Biomedicals, Irvine, CA), naltrindole hydrochloride, nor-binaltorphimine dihydrochloride, naloxone methiodide (Sigma-Aldrich, St. Louis, MO, USA), and β-funaltrexamine hydrochloride (Tocris-Cookson, Bristol, UK). All drugs were dissolved in saline and were administered subcutaneously (s.c.) using a volume of 0.1 mL/10 g of mouse body weight. The opiate antagonists naloxone methiodide (3 mg/kg), naloxone (2 mg/kg), naltrindole hydrochloride (3 mg/kg), nor-binaltorphimine dihydrochloride (20 mg/kg), and β-funaltrexamine hydrochloride (40 mg/kg) were administered 15 min, 30 min, 30 min, 24 h, and 24 h before morphine injection, respectively. We previously reported antinociceptive effect and delay of gastrointestinal transit of novel μ-opioid, μ/κ-opioid, and μ/δ-opioid agonist compared with morphine in ddY mice (Matsumoto et al., 2014b, 2008, 2006). In these studies, we used naloxone methiodide, naloxone, naltrindole hydrochloride, nor-binaltorphimine dihydrochloride, and β-funaltrexamine hydrochloride in an amount sufficient to substantially inhibit corresponding opioid receptors.

2.3. Immunohistochemistry

Segments of the mouse rectum, distal colon, transverse colon, and ileum were removed, fixed by immersion in fresh 4% paraformaldehyde in 0.1 M phosphate buffer for 2 h at 4 °C, and then washed thrice with phosphate-buffered saline. The tissues were cryoprotected overnight in 0.1 M phosphate buffer containing 20% sucrose, frozen in Optimal Cutting Temperature compound (Sakura Finetek, Toyko, Japan) mounting medium, and then sectioned on a cryostat (Leica Instruments, Nussloch, Germany) at a thickness of 30 μm. The sections were thaw-mounted onto Superfrost Plus slides (Matsunami Glass Ind. Ltd., Osaka, Japan).

The immunohistochemical procedures were performed as described by Matsumoto et al. (2014a). Prior to staining, the slide-mounted sections were successively incubated in 10% normal donkey serum containing 0.2% Triton X-100 and 0.1% sodium azide in phosphate-buffered saline for 1 h, and were then washed thrice with phosphate-buffered saline for 10 min each. Subsequently, sections were incubated in rabbit anti-μ-opioid receptor (extracellular) antibody (1:100, Alomone Labs, Jerusalem, Israel), guinea-pig anti-neuronal nitric oxide synthase (nNOS, 1:4000, Euro Diagnostica, Malmö, Sweden), goat anti-choline acetyltransferase (CHAT, 1:1000, Millipore, Temecula, CA, USA), and goat anti-5-hydroxytryptamine (5-HT, 1:2000, ImmunoStar, New Richmond, WI, USA) for 40 h at room temperature. After washing in phosphate-buffered saline, the sections were incubated with donkey anti-rabbit FITC (1:400; Jackson ImmunoResearch Laboratories, West Grove, PA, USA) for 4 h at room temperature.

Double labeling of μ-opioid receptors with specific neurochemical markers was used to identify the neurotransmitters that are co-expressed by neurons bearing μ-opioid receptors. μ-Opioid receptor staining was carried out first using the FITC (described above) as the label, followed by guinea-pig anti-neuronal nitric oxide synthase (nNOS; 1:4000, Euro Diagonistica, Malmö, Sweden), goat anti-choline acetyltransferase (CHAT; Millipore, Billerica, Massachusetts, USA), or goat-serotonin (5-HT; 1:2000, ImmunoStar, Wisconsin, USA) antibody staining using the indirect labeling procedure.

2.4. Bead expulsion test

Mice were fasted, with water available ad libitum, for 18 h before the experiments. Fifteen minutes after the administration of morphine (0.3–10 mg/kg s.c.) or saline, a glass bead (approximately 3 mm in diameter) was inserted into the distal colon to a depth of 2 cm from the anus with a silicone tube (approximately 2 mm in diameter). The time required to expel the bead was measured up to 120 min.

2.5. FITC–dextran method

Mice were fasted, with water available ad libitum, for 18 h before the experiments. Animals were anesthetized and