Morphine leads to contraction of the ileal circular muscle via inhibition of the nitric pathway in mice
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Abstract

Morphine inhibits small intestinal transit in mice, although few μ-opioid receptors are present in the ileum. The present study focused on the action of morphine in the isolated mouse ileum to reveal the mechanism by which morphine inhibits mouse small intestinal transit. In the isolated circular muscle, morphine caused tonic contraction. This contraction was potently inhibited by naloxone and the μ-opioid receptor antagonist cyprodime. Moreover, the response was almost completely inhibited by tetrodotoxin and N⁵-glutamine-arginine, but only moderately inhibited by atropine and indomethacin. In the isolated longitudinal muscle, morphine caused no or only slight contractions. Furthermore, electrically induced contraction was dose-dependently depressed by morphine, an effect that was not reversed by naloxone. These findings indicate that 1) morphine-induced circular muscle contraction occurs in the mouse ileum, 2) the contraction occurs through μ-opioid receptors mainly by inhibiting the release of nitric oxide from nitrergic nerves, although cholinergic nerves are at least partly involved in this contractile mechanism, and 3) inhibition of descending relaxation of peristalsis by morphine may slow small intestinal transit.

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1. Introduction

Morphine, which is used for relief of pain associated with cancer, the postoperative setting, and visceral conditions, causes serious constipation. We previously investigated small intestinal transit in mice and reported that small intestinal transit was inhibited by morphine, and that the slowing of small intestinal movement was alleviated by Daikenchuto, a Japanese herbal medicine (Nakamura et al., 2002). Other studies have reported that subcutaneous morphine depresses small intestinal transit in mice and guinea pigs (Tan-No et al., 2003; Gallantine and Meert, 2005). The mechanism by which morphine inhibits small intestinal transit is suggested to be due to its contractile effects on the circular muscle of the ileum in guinea pigs (Lenard et al., 1999; Nakamura et al., 2002). Lenard et al. (1999) speculated that morphine-induced tonic contraction may occur via the inhibition of the release of nitric oxide (NO), as the contraction is abolished by tetrodotoxin and N⁵-glutamine-arginine (L-NNA).

Morphine binds to opioid receptors in the central and peripheral nervous systems and induces several side effects as well as antinociceptive effects. Receptor-binding assays revealed that morphine has about 20 to 90 times as high affinity to μ-opioid receptors as to δ- and κ-opioid receptors (Mignat et al., 1995; Frances et al., 1992).

In terms of tissue distribution of opioid receptors, μ-, δ-, and κ-opioid receptor immunoreactivities have been demonstrated in the rat (Gray et al., 2006) and guinea pig ileum (Sternini et al., 2004). Moreover, it was shown that guinea pig ileum had κ- and μ-opioid receptors, mouse vas deferens had δ-opioid receptors (Smith et al., 1988), and rat rectum had δ- and μ-opioid receptors (Shaw, 1979). Thus, guinea pig ileum is often used for the assessment of μ- and κ-opioid receptors, and morphine is found to produce an inhibition of electrically evoked contraction in the longitudinal muscle. On the other hand, it is reported that few μ-opioid receptors are present in the mouse ileum, and δ- and κ-opioid receptors are dominant, as electrically induced

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contractions are inhibited by δ- and κ-opioid receptor agonists, but not by morphine (Smith et al., 1988). It is not clear why morphine inhibits small intestinal transit in mice, as only a few μ-opioid receptors are distributed in the mouse ileum.

In the present study, we observed the contractile effects of morphine in the circular muscle of the mouse ileum. To our knowledge, these effects have never been reported.

2. Materials and methods

2.1. Animals

Male ddY-strain mice (5–7 weeks old; Japan SLC, Hamamatsu, Japan) were used. Animals were housed in a room at 24±1 °C and 60±5% relative humidity with light on from 7:00 to 19:00 and allowed free access to food and water. All animal experiments were carried out according to the “Principles of Laboratory Animal Care” (NIH publication number 85–23, revised 1985) and the Guidelines of the Animal Investigation Committee, Chiba University, Japan.

2.2. Drugs

Drugs used in this study were morphine hydrochloride (Takeda Chemical Ind., Osaka, Japan), tetrodotoxin (Wako Pure Chemicals Ind., Ltd., Osaka, Japan), and acetylcholine chloride, atropine sulfate, indomethacin, N^G-nitro-l-arginine, l-arginine hydrochloride, naloxone hydrochloride dihydrate, cyprodime hydrobromide, and Leu-enkephalin (Sigma Chemical Co., St. Louis, MO, USA). Indomethacin was dissolved in ethanol and diluted with Krebs–Henseleit solution. Cyprodime hydrobromide was dissolved in dimethylsulfoxide and diluted with distilled water. The other drugs were dissolved in distilled water. The vehicles containing ethanol or dimethylsulfoxide had no influence on morphine-induced responses.

2.3. Measurement of contractile force in the mouse ileum under basal conditions

Mice were killed by exsanguination and the ileum was excised. The distal end of 5 cm from the ileocecal junction was discarded and the ileum was placed in Krebs–Henseleit solution (mM): NaCl, 112.08; KCl, 5.90; MgCl_2, 1.18; CaCl_2, 1.97; NaH_2PO_4, 1.22; NaHCO_3, 25.00, and glucose, 11.49. The luminal contents were gently flushed with the nutrient solution. Then, the ileum was cut in segments of circular muscle (approximately 5 mm wide) and longitudinal muscle (approximately 8 mm long) and mounted in a 5-ml organ bath containing Krebs–Henseleit solution. The bath was maintained at 37 °C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The contractions were recorded by an isometric transducer (TB-611T, Nihon Kohden, Tokyo, Japan) connected to an amplifier (AD-632J, Nihon Kohden, Tokyo, Japan). After 60 min equilibration, acetylcholine (1×10^{-6} M) was added several times with an interval of 15 min to stabilize the reactivity of the preparation, and then morphine was added. In some experiments, the preparation was incubated with each antagonist for 3 min before morphine was tested. In the case of indomethacin and L-NNA treatments, the preparation was incubated for 20 and 5 min, respectively. Some data were normalized as a percentage of the corresponding control contraction, and others were expressed as tension (g) of responses.

2.4. Electrical stimulation of mouse ileum

Mouse ileum was excised in the same way as described above. Longitudinal muscle (approximately 15 mm long) and circular muscle (approximately 5 mm wide) was attached to a platinum needle-ring electrode and a parallel-type electrode, respectively, and mounted in a 5-ml organ bath containing Krebs–Henseleit solution. The bath was maintained at 37 °C and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The contractions were recorded by an isometric transducer (TB-611T, Nihon Kohden, Tokyo, Japan) connected to an amplifier (AP-621G, Nihon Kohden, Tokyo, Japan). After 60 min equilibration, the circular muscle (0.5 g tension) was transmurally stimulated with square pulses of 1.5 ms duration at a rate of 10 Hz and supramaximal voltage for 10 s every 2 min by a stimulator (SEN-2201, Nihon Kohden, Tokyo, Japan). The circular muscle (0.2 g tension) was stimulated under two different conditions. In the first condition, normal Krebs–Henseleit solution was used and the preparation was transmurally stimulated with square pulses of 1.5 ms duration at a rate of 10 Hz and supramaximal voltage for 30 s every 2.5 min. In the second condition, Ca^{2+} concentration in Krebs–Henseleit solution was halved (0.985 mM) and stimulus frequency was changed into 1 Hz. The height of the twitch responses evoked by transmural stimulation was measured before and after morphine challenge.

Fig. 1. Contractile effects of morphine (Mor) in circular muscle of the mouse ileum. (A) Typical recording and (B) the dose-dependent effect. Each value represents the mean±S.E.M. of 6 mice.
All data were expressed as a percentage of the twitch response before the morphine challenge.

2.5. Statistical analysis

All data are expressed as mean±S.E.M. Statistical analyses were performed with the Student’s t test or Aspin–Welch t test for comparison of two groups. *P<0.05 was considered statistically significant.

3. Results

3.1. Contractile effects of morphine in the circular muscle of the mouse ileum

In the circular muscle of the mouse ileum, morphine produced slow, long-lasting contractions (Fig. 1A). When morphine (1×10⁻⁹–1×10⁻⁴ M) was added cumulatively, contractile effects developed dose-dependently (n=6, Fig. 1B). In contrast, acetylcholine (1×10⁻⁶ M) caused relaxation in 16 of 21 preparations and contraction (0.010±0.002 g) in 5 of 21 preparations.

Further experiments were done using several pharmacological tools to elucidate the morphine-induced contraction. Atropine, indomethacin, and opioid antagonists had no effect on the basal tone, but tetrodotoxin (1×10⁻⁶ M, n=6) caused a tonic contraction (0.041±0.022 g) (Fig. 2A). L-NNA (1×10⁻⁴ M, n=4) also caused a contraction (0.041±0.015 g) similar to tetrodotoxin (Fig. 2B) and this contraction was abolished by pre-incubation with L-arginine (L-Arg; 2×10⁻³ M) (Fig. 2C). In the presence of atropine (3×10⁻⁷ M, n=9) and indomethacin (2×10⁻⁵ M, n=8), the morphine-induced (1×10⁻⁶ M) contraction was 68±12% and 58±21% of the control response, respectively.

(Fig. 3). Administration of tetrodotoxin led to an increase in baseline contractions, but morphine had no further contractile effects. After an increase in baseline contractions by L-NNA, morphine caused only small contraction (88±12% inhibited). Moreover, the morphine-induced contraction was 78±16% inhibited by naloxone (3×10⁻⁷ M, n=6) and 73±8% inhibited by the μ-opioid receptor antagonist cyprodime (3×10⁻⁶ M, n=5).

3.2. Effects of morphine on longitudinal muscle of the mouse ileum

In the longitudinal muscle preparation of the mouse ileum, acetylcholine was a full agonist of the contractile response.
Morphine (1 × 10⁻⁹–1 × 10⁻⁴ M) caused contraction in 21 of 47 preparations; more than half of the preparations did not react to morphine. In addition, even if a contraction developed in the longitudinal muscle, its amplitude was less than 10% of the 1 × 10⁻⁶ M acetylcholine-induced contraction.

3.3. Inhibitory effects of morphine on electrically induced contraction in longitudinal muscle of the mouse ileum

In a preliminary experiment, it was found that square pulses of 1.5 ms duration at a rate of 10 Hz lead to maximal twitch contractions, so this stimulating condition was used in subsequent experiments. Morphine (1 × 10⁻⁶–1 × 10⁻⁴ M, n = 5) inhibited the electrically induced twitch contraction in a dose-dependent manner; the maximal inhibition rate was 43 ± 9% (Fig. 4). Inhibition by morphine was not reversed by naloxone or cyprodime (1 × 10⁻⁶–1 × 10⁻⁵ M). On the other hand, inhibition by a δ-opioid receptor agonist Leu-enkephalin was partially reversed by naloxone (data not shown). The electrically induced twitch contraction was abolished by atropine (1 × 10⁻⁶ M) or tetrodotoxin (1 × 10⁻⁶ M).

3.4. Effects of morphine on electrically induced response in circular muscle of the mouse ileum

In circular muscle of the ileum, relaxation was induced by electrical stimulation with 1.5 ms duration at a rate of 10 Hz when normal Krebs–Henseleit solution was used (data not shown). This relaxation was abolished by tetrodotoxin. Addition of L-NNA to the preparation caused a definite increase in the tone, but abolished the subsequent stimulation-induced relaxation. After morphine was added, the electrically stimulated relaxation was not affected. On the other hand, when the preparation was stimulated with 1 Hz in the modified Krebs–Henseleit solution, relaxation was also induced and 1 × 10⁻⁴ M morphine partially inhibited the relaxation in the presence of 1 × 10⁻⁶ M atropine (Fig. 5).

4. Discussion

Morphine has been reported to cause contraction in circular muscle of the guinea pig ileum (Lenard et al., 1999; Nakamura et al., 2002). The present study is the first report showing that morphine also causes contraction of circular muscle in the mouse ileum. Although it is known that only a few μ-opioid receptors exist in the mouse ileum (Smith et al., 1988), μ-opioid receptors are predominantly responsible for the contractile effects of morphine, as the contraction was inhibited by not only naloxone but also by the μ-opioid receptor antagonist cyprodime.

The basal tone of tension in smooth muscle is determined by the balance between excitatory and inhibitory nerves. In the circular muscle preparation, tetrodotoxin caused contraction, which suggests that inhibitory nerves may dominantly control the resting tone in this preparation. Moreover, it was confirmed that NO was one of the inhibitory neurotransmitters, as L-NNA caused contraction.

The mechanism of morphine-induced contraction was further studied using several pharmacological tools. The contraction by morphine was completely inhibited by tetrodotoxin, suggesting that the contraction may be involved in the release of excitatory or inhibitory neurotransmitters. As contraction by morphine was strongly inhibited by L-NNA, it is suggested that morphine may act by inhibiting the release of NO from the inhibitory nerve. Furthermore, cholinergic nerves are at least partly involved in the mechanisms of contractile effects of morphine, as the contraction was inhibited by atropine. Additionally, prostanoid pathways are possibly involved in this mechanism, because morphine-induced contraction was apt to be inhibited by indomethacin.

We also observed the action of morphine on longitudinal muscle. More than half of the preparations did not react to morphine. In the preparations in which morphine led to a contraction, the amplitude of the contraction was small.

It is known that electrically induced contraction in longitudinal muscle of the guinea pig ileum is inhibited by morphine. In the mouse ileum, however, the electrically induced contraction was inhibited by δ- and κ-opioid receptor agonists but not by morphine (Smith et al., 1988). In our experiments, twitch contraction in the mouse ileum was inhibited by 1 × 10⁻⁴ M morphine, but this effect was not reversed by naloxone and cyprodime. On the other hand, inhibition of twitch contraction by the δ-opioid receptor agonist Leu-enkephalin was partially reversed by naloxone. Thus, the inhibitory effects of morphine are suggested not to be due to opioid-related actions. Their inhibitory mechanisms remain to be revealed and further studies are needed.

When circular muscle of the ileum was electrically stimulated, relaxation was induced. Because relaxation was abolished by L-NNA, it is suggested that relaxation was induced by NO. After morphine was added, the stimulation-induced relaxation was not affected.

Shimo and Ishii (1978) and Hayashi et al. (1981) reported that the inhibitory effects of morphine on electrically stimulated reaction were enhanced when stimulus frequency became lower and when Ca²⁺ concentration in the nutrient solution became lower, respectively. Thus, in this study, Ca²⁺ concentration in the Krebs–Henseleit solution was halved (0.985 mM) and stimulus frequency was reduced to 1 Hz. After administration of 1 × 10⁻⁴ M morphine, the stimulation-induced relaxation was partially inhibited in several preparations. This result suggests that morphine partially inhibits the release of NO. This speculation is consistent with the mechanism of morphine-induced contraction.
Additionally, intestinal peristalsis is probably involved in the appearance of constipation that occurs with morphine treatment. Fujita et al. (2004) and Grider (2003) have reported that descending relaxation of peristalsis in the circular muscle, which occurs at the anal side of the tract, is induced by NO in the mouse ileum and colon. It was also reported that the descending relaxation was inhibited by Met-enkephalin (Ivancheva and Radomirov, 2002) and that NO production during descending relaxation was decreased by Met-enkephalin (Grider, 1994). It is possible that morphine similarly inhibits the descending relaxation of peristalsis.

In conclusion, it is suggested that the inhibitory effects of morphine on small intestinal transit in mice are associated with contractile effects on circular muscle in the ileum through μ-opioid receptors, resulting in inhibition of descending relaxation of peristalsis. These effects are considered to be one of the causes of constipation.

References
