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Effects of methylnaltrexone on morphine-induced inhibition of contraction in isolated guinea-pig ileum and human intestine

Chun-Su Yuan *, Joseph F. Foss, Jonathan Moss

Committee on Clinical Pharmacology and the Department of Anesthesia and Critical Care, The University of Chicago, Chicago, IL 60637, USA

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Abstract

We investigated the effects of methylnaltrexone on morphine-induced inhibition of smooth muscle-strip contraction in isolated guinea-pig ileum and human small intestine. The longitudinal muscle-strip was immersed in a temperature-controlled (37°C) bath containing a physiological solution of 95% O_2 and 5% CO_2 with pH 7.4. Muscle contraction was elicited by transmural electrical stimulation with a pulse duration of 0.5 ms at frequencies of 1–50 Hz for 5–10 s at 1–3-min intervals. Muscle contraction was blocked by tetrodotoxin or atropine in both preparations. When methylnaltrexone was applied to the bath, the force produced by muscle contraction was enhanced up to approximately 30%. Stimulation-elicited muscle contraction was inhibited by morphine, which decreased the force of contraction $42 \pm 9.5\%$ (S.D.) in the human intestine preparation and $35 \pm 8.6\%$ in guinea-pig ileum at the inhibitory concentration 70% (IC_{70}). Methylnaltrexone effectively antagonized the effects of morphine-induced inhibition of muscle-strip contraction. In the guinea-pig ileum preparation, methylnaltrexone at 30, 100 and 300 nM blocked $25 \pm 10.5\%$, $74 \pm 7.2\%$ and $89 \pm 9.9\%$ of morphine-induced (300 nM) inhibition, respectively. In the human intestine preparation, methylnaltrexone at the same concentrations blocked $57 \pm 10.9\%$, $74 \pm 12.9\%$ and $92 \pm 7.2\%$ of morphine-induced (100 nM) inhibition, respectively. The relative ratio of methylnaltrexone to morphine was higher in human intestine (1:1) than in the guinea-pig ileum preparation (1:3). These data provide preliminary information for clinical studies to evaluate the efficacy of methylnaltrexone in preventing or reducing morphine-induced antimotility and antitransit actions.

Keywords: Morphine; Methylnaltrexone; Naloxone; Gut motility; Ileum, guinea-pig; Intestine, human, isolated

1. Introduction

Morphine sulfate and related opioids are widely used clinical analgesics. However, their administration is often accompanied by side effects including nausea, vomiting and constipation. These side effects are often severe enough to limit use of opiates even when medically indicated (Walsh, 1984; McCaffrey and Beebe, 1989; Glare and Lickiss, 1992). Morphine inhibits gastric emptying and propulsive motor activity of the intestine, thereby decreasing the rate of intestinal transit and producing constipation.

The etiology of opiate-induced antipropulsive response is somewhat controversial. Morphine can act within the central nervous system to alter autonomic outflow to the gut (Parolaro et al., 1977; Stewart et al., 1978; Galligan and Burks, 1983). However, opiates also can have a direct effect on the bowel (Daniel et al., 1959; Burks, 1973). The inhibition of gastrointestinal transit by morphine results primarily from direct action on opioid sites in the gut (Tavani et al., 1980; Manara et al., 1986). It seems likely, therefore, that opioid receptors at different anatomical sites affect gastrointestinal motility and propulsion. Understanding the pharmacological effects of opioids on gastrointestinal motility is further complicated by difference of effect among species, the region of the gastrointestinal tract examined, and the precise experimental conditions.

N-Methylnaltrexone bromide (methylnaltrexone), a novel quaternary ammonium antagonist of peripheral opioid receptors, has the broad therapeutic potential of a narcotic antagonist that does not cross the blood-

* Corresponding author. Department of Anesthesia and Critical Care, The University of Chicago Medical Center, 5841 S. Maryland Avenue, MC 4028, Chicago, IL 60637, USA. Tel. (312) 702-1916, fax (312) 702-3535, e-mail: cyuan@midway.uchicago.edu.

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brain barrier (Russel et al., 1982; Brown and Goldberg, 1985). It has the potential for decreasing the side effects of opioid pain medications that are mediated peripherally without affecting analgesia. Methylnaltrexone antagonized the emetic effects of morphine in animal models and in human subjects (Foss et al., 1993; Foss, unpublished data). The present study was designed to evaluate the efficacy of methylnaltrexone on morphine-induced inhibition of motility in isolated guinea-pig ileum and human small intestine.

2. Materials and methods

With approval from the Institutional Animal Care and Use Committee of the University of Chicago, young adult male guinea-pigs of the Hartley strain, weighing between 250 and 450 g, were killed by CO_2 narcosis. The abdomen was opened by a midline incision and the terminal portion of the ileum was removed and placed in oxygenated Krebs solution, containing (in mM) NaCl 119.0, KCl 4.7, $NaHCO_3$ 25.0, KH_2PO_4 1.2, $CaCl_2$ 2.5, $MgCl_2$ 1.5 and dextrose 11.0. The last 10 cm of the terminal ileum was discarded because excitatory α-adrenoceptors are located near the ileocecal junction (Munro, 1953).

With approval from the Institutional Review Board of the University of Chicago, small samples of the proximal jejunum or distal ileum of eight patients, ages 31–78 years, were isolated during the normal course of gastric bypass or ileal loop surgery before pathological procedures. Immediately after removal, the intestinal segments were placed in a nutrient solution consisting of (in mM) NaCl 119.7, KCl 4.7, $NaHCO_3$ 23.0, KH_2PO_4 1.2, $CaCl_2$ 1.8, $MgCl_2$ 1.2 and dextrose 7.9 (Hayashi et al., 1986).

Longitudinal smooth muscle-strips, approximately 8–10 mm long and 2 mm wide, were dissected out from guinea-pig ileum and human small intestine. These strips consisted of the longitudinal muscle fibers, the circular muscle fibers (most of them in cross-section). The preparations were immersed in a temperature-controlled (37°C) bath and continuously bubbled with a mixture of 95% O_2 and 5% CO_2. The pH of the solution was maintained at 7.4.

A muscle-strip was tied to a Grass FT-03 mechanical transducer (Quincy, MA) with the hook anchoring the upper end of the strip. A preload of 0.5–1.0 g was applied to the muscle-strip. The transducer was connected to a Gould 3000 series polygraph recorder via a Gould universal amplifier (Cleveland, OH), and the force of isometric contractions of the longitudinal muscle-strip was measured.

Then the muscle-strip was placed between a pair of platinum electrodes. The distance between the electrodes was wide enough to enable undisturbed muscle contractions yet narrow enough to enable electrical stimulation of intramural nerve terminals. Before the preparation was electrically stimulated (Grass stimulator, model S8800), a stimulus isolation unit (SIU 5B, Grass Instrument) was coupled between the stimulator and the electrodes. At 1–3-min intervals, a train of square pulses of 0.5 ms was delivered at frequencies of 1–50 Hz for 5–10 s. In some experiments, single shock stimulation with variable frequencies was also used. In both isolated guinea-pig ileum and human small intestine preparations, longitudinal muscle-strip contraction elicited by electrical stimulation was blocked by tetrodotoxin (0.3 μM; Sigma, St. Louis, MO) or atropine (1.0 μM; Sigma, St. Louis, MO), suggesting the neurogenic nature of this contraction.

Other drugs used were as follows: morphine sulfate (Wyeth-Eikinsins, Radnor, PA), naloxone (Sigma, St. Louis, MO) and methylnaltrexone (synthesized by Mallinckrodt Specialty Chemicals, St. Louis, MO).

Smooth muscle-strip contraction was analyzed as the isometric force (amplitude) produced. Due to the variation in size of the muscle-strips tested, the absolute force of contraction elicited by electrical stimulation varied from tissue to tissue. Therefore, the value of the force produced by stimulation before any drug addition was normalized to 100% and considered control. After application of drug(s), the force produced by muscle-strip contraction was measured and reported as a percentage change from the force observed at control level. Data were analyzed using Student's t-test and Mann-Whitney U-test with $P < 0.05$ considered statistically significant.

3. Results

In the temperature-controlled bath, some guinea-pig ileum muscle-strips contracted spontaneously. The rate and the force of spontaneous contraction changed unpredictably over time, but contraction elicited by electrical stimulation was stable for a prolonged observation period.

Application of morphine to the bath inhibited stimulation-elicited muscle contraction. The frequency of stimulation applied to the muscle-strip influenced the extent of morphine-induced inhibition. Contraction stimulated by 5 Hz was effectively inhibited after morphine application, but a frequency of 10 Hz was adopted in the experiments because at this frequency, morphine-induced inhibition of contraction had much less variation (Fig. 1).

When methylnaltrexone alone was applied to the bath, the force produced by muscle contraction was enhanced in a concentration-related manner. At concentrations of 3, 30 and 300 nM, methylnaltrexone increased the force of contraction $9 \pm 3.1\%$ ($n = 6$),

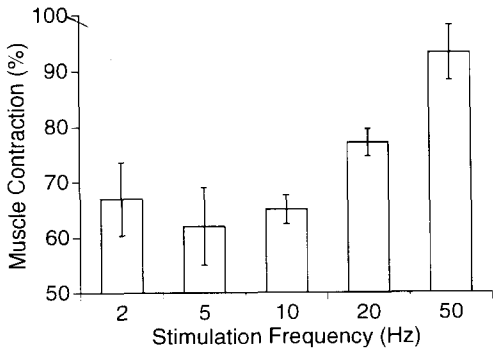

Fig. 1. Effects on the amplitude of guinea-pig ileum muscle-strip contraction elicited by transmural electrical stimulation at increasing frequencies in the presence of morphine (300 nM). Before application of morphine, muscle contraction was first elicited at each frequency and normalized to 100% (control).

$18 \pm 5.3\%$ ($n = 7$) and $27 \pm 8.2\%$ ($n = 9$) respectively, compared to control level.

Muscle contraction was inhibited by morphine application in a concentration-dependent fashion (Fig. 2). We selected 300 nM as the inhibitory concentration 70% (IC_{70}), where morphine decreased the force of contraction $35 \pm 8.6\%$ (S.D.). This concentration of morphine was used to evaluate the antagonist effects of methylnaltrexone and to compare them with those of naloxone, a commonly used opioid antagonist.

When methylnaltrexone, 30, 100 and 300 nM, was added to the bath before morphine application, morphine-induced inhibition of contraction elicited by electrical stimulation was blocked $25 \pm 10.5\%$, $74 \pm 7.2\%$ and $89 \pm 9.9\%$, respectively (Fig. 3A). To compare the effects of naloxone on inhibition with those of methylnaltrexone, naloxone was applied to the preparation. At 3, 10 and 30 nM naloxone blocked $28 \pm 10.6\%$, $63 \pm 9.5\%$ and $83 \pm 9.8\%$ of morphine-induced (300 nM) inhibition, respectively (Fig. 3A). Compared to methylnaltrexone, naloxone had similar effects on morphine-induced inhibition at approximately a 10-fold lower concentration.

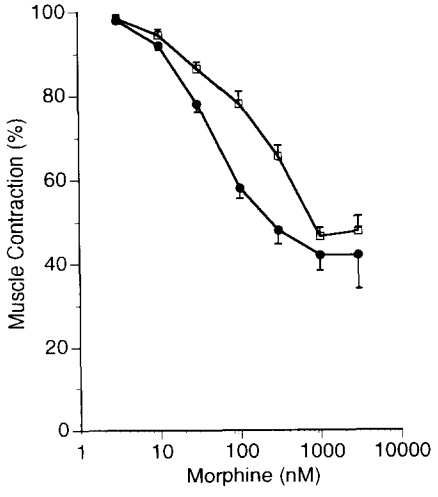

Fig. 2. Concentration-dependent response of morphine-induced inhibition on stimulation-elicited muscle contraction of isolated guinea-pig ileum preparation (□; IC_{70}, 300 nM) and human small intestine preparation (●; IC_{70}, 100 nM). The control activity level of muscle-strip contraction is normalized to 100%. During different levels of morphine application, percentage change in the muscle-strip contraction decreases as a function of the morphine concentration in these two preparations.

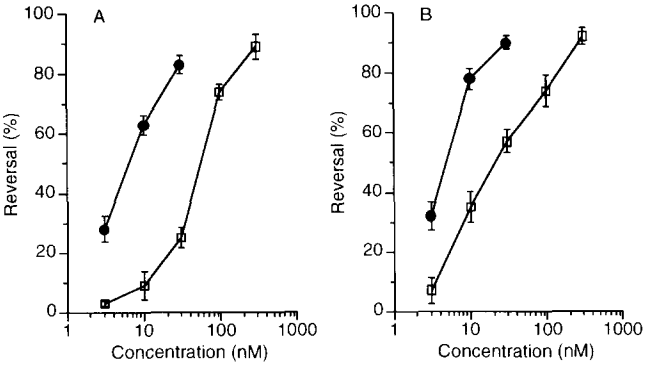

Fig. 3. Concentration-related reversal effects of methylnaltrexone (□) and naloxone (●) on morphine-induced inhibition of muscle-strip contraction. (A) Reversal effects on morphine-induced (300 nM) inhibition in isolated guinea-pig ileum preparation. EC_{70}, methylnaltrexone, 100 nM. (B) Reversal effects on morphine-induced (100 nM) inhibition in isolated human small intestine preparation. EC_{70}, methylnaltrexone, 100 nM. The control activity level of muscle-strip contraction is normalized to 100%. Effects of morphine at IC_{70} are treated as baseline (0%). In both preparations, naloxone has similar but more potent reversal effects compared to those effects of methylnaltrexone.

Most of the isolated human small intestine preparations exhibited spontaneous contraction. Electrical stimulation at 10 Hz abolished the spontaneous activity and elicited a stable muscle-strip contraction. The effects of morphine on contraction were observed after stimulation at 10 Hz, and the results were compared with those from the guinea-pig ileum preparations.

The addition of methylnaltrexone to the bath increased the force produced by the contraction of human intestine muscle-strips. At 30 nM concentration, methylnaltrexone increased the muscle contraction force $29 \pm 6.2\%$ ($n = 9$). This increase was significantly different from the effect on guinea-pig ileum preparations (18% increase at 30 nM, $P < 0.01$). This result suggests a stronger endogenous opioid action in regulation of human gastrointestinal motility. Application of naloxone alone to the bath produced a comparable increase in contractions in both guinea-pig and human muscle-strips.

In both the isolated proximal jejunum and distal ileum preparations of human intestine, morphine application inhibited muscle-strip contraction concentration dependently (Fig. 2). At 100 nM (IC_{70}), morphine decreased the force of contraction $42 \pm 9.5\%$.

It has been reported that the opioid mechanism operates with increasing functional significance from proximal to distal small intestine in the guinea-pig (Kromer et al., 1981). In our isolated human intestine preparation, at 100 nM concentration, morphine decreased the force of proximal jejunum-strip contraction $39 \pm 9.5\%$ ($n = 9$) and decreased the force of distal ileum-strip contraction $44 \pm 9.3\%$ ($n = 6$). Due to small sample size, patient age difference, and variable exposure to opioid medication during the surgery, a comparison of morphine effects on isolated jejunum and ileum-strip preparations could not be made in this study.

When methylnaltrexone, 30, 100 and 300 nM, was added to the bath before morphine application in isolated human intestine preparations, morphine-induced inhibition of contraction elicited by electrical stimulation was blocked $57 \pm 10.9\%$, $74 \pm 12.9\%$ and $92 \pm 7.2\%$, respectively (Fig. 3B). Compared to methylnaltrexone, naloxone had effects on morphine-induced inhibition similar to those observed in guinea-pig ileum preparations. At 3, 10 and 30 nM, naloxone blocked $32 \pm 10.8\%$, 78 ± 9.5 and $90 \pm 5.5\%$ of morphine-induced (100 nM) inhibition, respectively (Fig. 3B).

4. Discussion

The effect of opiates on gastrointestinal motility and transit is appreciated as a clinical phenomenon. In the rat, the ratio between subcutaneous analgesic and constipating doses of morphine is very close to four (Green, 1959). In orally treated mice, approximately 20 times less morphine is needed to antagonize castor oil diarrhea than to produce analgesia (Niemegeers et al., 1976). Clinically, it is desirable to maintain the analgesic effect of morphine while reducing such peripheral side effects as constipation, which may be mediated by receptors outside, anatomically or functionally, the blood-brain barrier.

Our data showed that at 300 nM concentration (IC_{70}) in guinea-pig ileum and at 100 nM concentration (IC_{70}) in human small intestine, morphine decreased contractile activity 35% and 42%, respectively, compared to control levels. Methylnaltrexone effectively antagonized the effects of morphine in both these in vitro preparations. However, the relative ratio of methylnaltrexone to morphine in the human intestine was higher (100:100 or 1:1) than in the guinea-pig ileum preparation (100:300 or 1:3). This result suggests that a relatively higher concentration of methylnaltrexone may be required in humans than in guinea-pigs to block morphine-induced gastrointestinal effects. However, these differences in antagonism may reflect the conditions under which the human tissue was harvested. The isolated small intestine samples were obtained from patients who had received various amounts of opioids during the course of surgery. Results from the intestinal samples of one patient who did not receive opioids during the surgery were similar to results from samples of patients who had been treated with opioids. In addition, our study examined only specimens from young adult guinea-pigs and adult humans. Both age and species variability in autonomic function may exist.

Opioid peptides and their receptors are widely distributed in the central nervous system and throughout the gastrointestinal tract (Manara and Bianchetti, 1985). This distribution implies that the endogenous opiate system may participate in the regulation of gut function, including motility. Hughes et al. (1977) demonstrated biosynthesis of enkephalins within the guinea-pig myenteric plexus. The same group also found electrically evoked acetylcholine release from tissue was enhanced by naloxone, a widely used opioid antagonist. Kromer et al. (1981) observed that upon application of naloxone, contraction of segments of intestine was enhanced (for review, Kromer, 1989).

Results from our study demonstrate that methylnaltrexone enhances stimulation-elicited muscle-strip contraction in both guinea-pig ileum and human small intestine, suggesting an inhibitory modulation by endogenous opioids in these two species. The enhancement of muscle contraction in isolated human intestine is significantly greater than that in guinea-pig ileum tissue, indicating that endogenous opioid action in the regulation of human gastrointestinal motility may be stronger. It seems likely that methylnaltrexone has potential therapeutic significance if the endogenous opioid system plays a role in chronic idiopathic constipation.

The isolated guinea-pig ileum preparation is a commonly used model for studying opioid effects on intestinal motility. Different electrical stimulation paradigms have been applied to the preparation. For example, Paton used single shock or repetitive stimulation to produce partially or completely fused tetanus for a period of a few seconds to a minute. Morphine depression of the tension of stimulated guinea-pig ileum has been demonstrated (Paton, 1957). In one report, single shock at a frequency of six per minute was used (Kosterlitz and Watt, 1968). Recently, a train of pulses with variable frequencies was applied to both isolated guinea-pig ileum and human intestine preparations (Hayashi et al., 1986; Nomura and Hayashi, 1992). It has been shown that the extent of the inhibition by morphine varies, the inhibition being greater at low rather than high frequency stimulation (Paton, 1957; Szerb, 1982; Nomura and Hayashi, 1992). In our study, a train of pulses at a frequency of 10 Hz was used. Muscle contraction thus elicited was effectively

inhibited by morphine application in our experimental condition. Decrease in contractile activity in isolated guinea-pig ileum and human small intestine preparations was limited to effects on longitudinal muscle and may correlate with morphine effects on gastrointestinal transit. Other factors associated with gastrointestinal transit, such as circular muscle activation, were not examined in our preparation.

As a quaternary compound with limited central nervous system activity, methylnaltrexone will potentially allow for the reversal of opiate effects at peripheral sites such as the gut with no risk for reversal of analgesia. Selective antagonism of opiate side effects by tertiary compounds such as naloxone or naltrexone has been attempted, but was limited by the propensity for these compounds to reverse analgesia or to induce opiate withdrawal (Gowan et al., 1988; Jaffe and Martin, 1990; Culpepper-Morgan et al., 1992). The current study has additional value in showing the ability of methylnaltrexone to act directly on the bowel, which suggests that the drug may be efficacious when administered orally. Clinical observations of the side effects of epidurally administered opiates in humans revealed that constipation is rare, while pruritus and urinary retention are common (Cohn et al., 1986). Thus it seems likely that the effect of opiates on intestinal motility and transit in humans is not mediated by spinal opioid receptors.

In summary, our study demonstrates that methylnaltrexone reverses the inhibition of contraction in isolated guinea-pig ileum and human small intestine treated with morphine. Data from this investigation provide preliminary information for clinical studies to evaluate the efficacy of methylnaltrexone in preventing or reducing morphine-induced antimotility and antitransit actions.

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