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Effects of intrathecal opioids combined with low-dose naloxone on motilin and its receptor in a rat model of postoperative pain

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ABSTRACT

Aims: To investigate the effects of intrathecal morphine and fentanyl combined with low-dose naloxone on the expression of motilin and its receptor in a rat model of postoperative pain.
Main methods: An intrathecal catheter was implanted, and saline, opioids (morphine and fentanyl) and naloxone were intrathecally administered 7 days later. An incisional pain model was established to induce pain behaviors in rats by unilateral plantar incision. Thermal hyperalgesia and mechanical allodynia were measured by using a radiant heat and electronic Von Frey filament, respectively. The expression of motilin in the hippocampus, stomach, duodenum, and plasma was determined by ELISA; and the expression of motilin receptor in the hippocampus was detected by Western blot assay.
Key findings: Motilin and its receptor were detected in the hippocampus. Acute incisional pain increased the motilin expression in the hippocampus and duodenum, while decreasing its expression in the gastric body and plasma. Postoperative analgesia with morphine + fentanyl upregulated the expression of motilin in the hippocampus; however, motilin was downregulated in peripheral sites. Naloxone at 1 ng/kg restored motilin to baseline levels. Acute pain, morphine + fentanyl, and naloxone all induced the expression of motilin receptor in the hippocampus.
Significance: Acute pain, postoperative analgesia with opioids, and naloxone significantly impacted the expression of hippocampal and peripheral motilin. Variation trends in all sites were not identical. Intrathecal injection of low-dose naloxone upregulated paw withdrawal thermal latency and enhanced the analgesic effects of opioids. The findings presented here provide a new basis for central and peripheral regulations in GI motility, clinical postoperative analgesia, and management of analgesic complications.

Introduction

Motilin, a brain-gut peptide hormone with a 22-amino-acid sequence, was first discovered by Brown et al., 1972; it is released periodically during the interdigestive state from the specialized neurons in the myenteric plexus and brain as well as the mucosa of the stomach and intestine (Beinfeld and Korchak, 1985; Brown et al., 1972; Itoh et al., 1978; Xu et al., 2005). Motilin acts as an endogenous ligand for motilin receptors (MTLRs), which are located primarily in the enteric nerves, smooth muscle, and gastric vagal nerve terminals of the gastrointestinal (GI) tract; MTLRs are abundant in the stomach and duodenum. Detection of motilin immunoreactivity and mRNA, and MTLRs in the mammalian central nervous system (CNS) structures such as cortex, cerebellum, hippocampus, amygdala, and hypothalamus has indicated the integrative role of motilin as a brain neurotransmitter and/or neuromodulator (Guan et al., 2003). Microinjection of motilin into the rat's ventral medial hypothalamus, lateral hypothalamus, hippocampus, and amygdala resulted in a significant enhancement of GI motility (Feng et al., 2007; Guan et al., 2003; Tang et al., 2000; Zhang et al., 2002), and such a stimulation may be attributed to the contraction of smooth muscle cells through MTLR (Boivin et al., 1990; Zhou and Wang, 1996). This is evident from the findings that erythromycin, a MTLR agonist, also stimulates the GI motility in diabetic rats (Jia et al., 2007). Motilin has also been reported to control GI motility through hippocampus, which has the highest MTLR density (Feng et al., 2007; Han et al., 2006; Lin and Dong, 2004).

Functional disorders of GI tract such as postoperative nausea and vomiting (PONV), functional gastroparesis, irritable bowel syndrome,

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constipation, diarrhea, and chronic abdominal pain, were reported to be closely related to endogenous motilin expression (Chapman et al., 2013; Jia et al., 2007; Sanger, 2012; Stanghellini et al., 2003). Incisional pain, postoperative opioid analgesia, and naloxone administration are quite common in patients who undergo surgeries. An accurate evaluation of endogenous motilin expression both in the peripheral sites and CNS during pathophysiological conditions would help in understanding, preventing, and treating GI disorders caused by these inevitable factors. The present study attempted to simulate the clinical procedure involving all mentioned factors and analyze their interactive effects on motilin expression in both peripheral and hippocampal regions. Specifically, the study aimed to investigate the effects of intrathecal morphine and fentanyl combined with low-dose naloxone on the expression of motilin and its receptor in a rat model of postoperative pain. The data described herein provide an understanding on the variation trend and potential relationship between motilin and hippocampus, and a basis for the potential mechanism of multilevel GI motility regulation.

Materials and methods

Animals

Adult Sprague–Dawley rats (6 to 8 weeks, 180 to 220 g) were obtained from Experimental Animal Center of Xuzhou Medical College (Xuzhou, Jiangsu Province, China) and housed under controlled relative humidity (20 to 30%), temperature (23 ± 2 °C), and 12/12 h light/dark cycle (light from 08:00 to 20:00 and dark at other times) with access to food and water ad libitum. Before experimental procedures, animals were allowed 7 days of acclimation, and efforts were made to limit any distress. Experimental protocols were approved by the Animal Care and Use Committee of Xuzhou Medical College, and the study was conducted per the Declaration of the National Institutes of Health Guide for Care and Use of Laboratory Animals (Publication No. 80-23, revised 1996).

Study reagents

MTLR 11-A rabbit anti-human MTLR immunoglobulin G (IgG) and secondary chicken anti-rabbit IgG-fluorescein (FITC) were purchased from Alpha Diagnostic Intel Inc. (San Antonio, USA) and Santa Cruz Biotechnology (Dallas, USA), respectively. Motilin enzyme-linked immunosorbent assay (ELISA) kit was purchased from Gene Biotech Company (Shang Hai Green, Shang Hai, China); drug substances, including morphine, fentanyl, and naloxone were purchased from Northeast Pharmaceutical Group (Shen Yang, China), Yichang Humanwell Pharmaceutical Company (Hu Bei China), and China National Medicine Pharmaceutical Company (Shang Hai, China), respectively. Doses of each drug were determined based on preliminary experiments.

Intrathecal catheterization

A lumbar intrathecal catheter was placed using a modified version of a reported technique for subarachnoid drug administration (Storkson et al., 1996). In brief, rats were anesthetized with 4% chloral hydrate (400 mg/kg, intraperitoneal injection); and their lumbar skin was shaved, cleaned, and incised. The intervertebral space between L5 and L6 was punctured with a hypodermic needle, and a PE10 tubing (OD 0.55 mm, ID 0.30 mm; AniLab Software and Instruments Company, China) was inserted. The distal end was secured and tunneled to the cervical region. The catheter was flushed with saline and sealed. Experiments were started 7 days after the placement of intrathecal catheter. At experiment end, the localization of the catheter was verified by injecting 10 μL of 2% lidocaine: only rats with a brief bilateral hind limb paresis after the injection were used for further experiments.

Incisional pain model

Incisional pain model was performed as described previously (Brennan et al., 1996). After rats were anesthetized by sevoflurane, a 1 cm longitudinal incision was made through the skin and fascia of the plantar aspect of the right hind paw; the underlying plantar muscle was elevated and incised longitudinally, leaving the muscle origin and insertion intact. The skin was apposed with 2 mattress sutures of 5–0 nylon. After surgery, the animals were allowed to recover in their cages.

Eighty-nine rats were intrathecally catheterized, of which 9 died after operation. In addition, 8 animals with a paralysis of the unilateral hind limbs after intrathecal injection of lidocaine were excluded from the experiment. The remaining 72 catheterized rats were randomly divided into 6 groups (n = 12): NS group (normal saline), P group (incisional pain), MFP group (morphine + fentanyl + incisional pain), MFPN1 group (morphine + fentanyl + 0.2 ng/kg naloxone + incisional pain), MFPN2 group (morphine + fentanyl + 1 ng/kg naloxone + incisional pain), and MFPN3 group (morphine + fentanyl + 5 ng/kg naloxone + incisional pain). Incisional pain was introduced for all rats, except NS animals. Rats in MFP, MFPN1, MFPN2, and MFPN3 groups were intrathecally injected with morphine (5 μg/kg) and fentanyl (0.25 μg/kg) 20 min before modeling. Intrathecal injection of naloxone was administered at 0.2, 1, and 5 ng/kg to MFPN1, MFPN2, and MFPN3 rats, respectively. Rats in NS and P groups were injected NS. All drugs were diluted to a 50 μL volume before injection.

At the following time points, paw withdrawal mechanical threshold (PWMT) and paw withdrawal thermal latency (PWTL) were assessed: 24 h before intrathecal catheterization (T0); 24 h before modeling (T1); and 1 h (T2), 3 h (T3), 6 h (T4), 24 h (T5), 48 h (T6), and 72 h (T7) after modeling.

Measurement of mechanical allodynia

Mechanical allodynia was assessed using electronic Von Frey filaments (IITC Life Science Inc., Victory Blvd, Woodland Hills, CA). Animals were placed in individual plastic boxes (20 × 25 × 15 cm) on a metal mesh floor and allowed to acclimate for 1 h. The filament was presented perpendicularly to the plantar surface with sufficient force to cause brisk withdrawal or paw flinching, which was considered as PMWT.

Measurement of thermal hyperalgesia

Thermal hyperalgesia was measured using PWTL as described by Hargreaves et al. (1988). In brief, rats were placed in clear plastic chambers (7 × 9 × 11 cm) and allowed to acclimatize to the environment for 1 h before testing. The radiant heat was directed to the plantar surface of each hind paw that was flushed against the glass or solution injection site through the glass plate. The nociceptive endpoints in the radiant heat test were the characteristic lifting or licking of the hind paw, and the time to the endpoint was considered the PWTL. The radiant heat intensity was adjusted to obtain basal PWTL of 12 to 15 s. A default cutoff of 20 s was used to prevent tissue damage. Thermal stimuli were delivered thrice to each hind paw at an interval of 5 min.

ELISA

Motilin levels in the plasma, stomach, duodenum, and hippocampus were determined using rabbit-specific ELISA kits per manufacturer's instructions. Six rats of each group were sacrificed 6 h after operation, and the plasma, stomach, duodenum, and hippocampus samples were quickly prepared for the assessment of motilin concentrations.