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The effect of nociceptin, an endogenous ligand for the ORL_1 receptor, on rat colonic contraction and transit

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Abstract

To assess the role of ORL_1 (opioid receptor-like 1) receptor in the bowel movement, we investigated the effect of nociceptin on colonic contraction and transit in rats. Nociceptin (0.1–100 nM) concentration-dependently caused an immediate tonic contraction followed by rhythmic waves of contractions in the isolated colon. The response to nociceptin (10 nM) was not affected by the classical opioid receptor antagonists, naloxone, naltrindole and *nor*-binaltorphimine. Suppression of effect of inhibitory neurotransmitters using pituitary adenylate cyclase activating polypeptide(6–38) (PACAP-(6–38); 3 μM), vasoactive intestinal polypeptide(10–28) (VIP-(10–28); 3 μM) and N^{ω}-nitro-L-arginine methyl ester (L-NAME; 100 μM) did not influence the nociceptin-induced contractions. In anesthetized rats, intravenous administration of nociceptin (1 μg/kg) or morphine (1 mg/kg) caused phasic contractions in the proximal colon. Pretreatment with naloxone (300 μg/kg, i.v.) abolished the contractions induced by morphine, but not by nociceptin. The rate of large intestinal transit was dose-dependently accelerated by nociceptin (0.03–3 μg/kg, s.c.), but was retarded by morphine (1.7–5 mg/kg, s.c.). These results indicate that stimulation of ORL_1 receptor accelerates the colonic contraction and transit independently from opioid receptors.

Keywords: Nociceptin; Opioid; Colon; Motility; Contraction; Transit

1. Introduction

Nociceptin, also called orphanin FQ, is a novel 17-amino acid peptide, which is an endogenous ligand for the ORL_1 (opioid receptor-like 1) receptor (Meunier et al., 1995; Reinscheid et al., 1995). ORL_1 receptor transcripts have been reported to be widely distributed in peripheral organs including the gastrointestinal tract as well as the central nervous system (Wang et al., 1994). Stimulation of ORL_1 receptor inhibits adenylate cyclase activity like opioid receptors. However, there is some difference in the pharmacological effects between opioids and nociceptin; opioids and nociceptin cause analgesia and hyperalgesia, respectively (Meunier et al., 1995; Reinscheid et al., 1995). Pharmacological studies have revealed the peripheral effects of nociceptin including the inhibition of electrically induced contraction of vas deferens, stimulation of erection and inhibition of tachykinin release and tracheal contraction (Berzetei-Gurske et al., 1996; Calo et al., 1996; Giuliani and Maggi, 1996; Champion et al., 1997; Patel et al., 1997). Some of these effects are similar to those by opioids.

Bowel motility is controlled by a local network of intramural nerves and opioids are demonstrated to modulate the smooth muscle functions (Kromer, 1988). In the colon, opioid substances decrease the transit rate while increasing the smooth muscle tone through actions on opioid receptors at both peripheral and central nervous systems (Gillan and Pollock, 1980; Fioramonti et al., 1985; Grider and Makhlouf, 1987b; Shook et al., 1987; Broccardo and Improta, 1992). In contrast, there has been no report so far dealing with the contribution of the ORL_1 receptor in the control of colonic motility.

Elucidation of control mechanism of colonic motility will unveil the pathophysiology of constipation and diarrhea. In this study, to ascertain the role of ORL_1 receptors in the bowel movement, we investigated the effect of the endogenous ligand for the ORL_1 receptor, nociceptin, on colonic contraction and transit in vitro and in vivo.

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2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats weighing 180–370 g were housed in standard conditions (23 ± 2°C, 55 ± 5% relative humidity, 12 h light/12 h dark) and received food and water ad libitum. All experiments were carried out in accordance with the Declaration of Helsinki and/or with the guide for the care and use of laboratory animals approved by the Japanese Pharmacological Society.

2.2. Responses of isolated colon

Rats were stunned and killed by bleeding. The proximal colon was removed and emptied of the contents. A segment with longitudinal orientation (1.5 cm in length) was mounted vertically in a 15 ml organ bath and connected to a force-displacement transducer for monitoring the change of the isometric tension. The organ bath was filled with a Krebs–Henseleit solution (mM: NaCl 119, KCl 4.7, $MgSO_4$ 1.5, KH_2PO_4 1.2, $CaCl_2$ 2.5, $NaHCO_3$ 25 and glucose 11.1) kept at 32°C and bubbled with 95% O_2 and 5% CO_2. The preparation was equilibrated for 60–90 min under an initial tension of 1 g.

The effects of test drugs on the time course of contractile responses were recorded on an ink-pen-writing recorder (Multicorder MC6625; Graphtec, Japan). The data were simultaneously stored in a computer analyzing system (MP100WS; Biopac Systems, Japan) for calculating the motor index of the contractile response. The motor index was calculated by measuring the area under the contractile wave for each 2 min period.

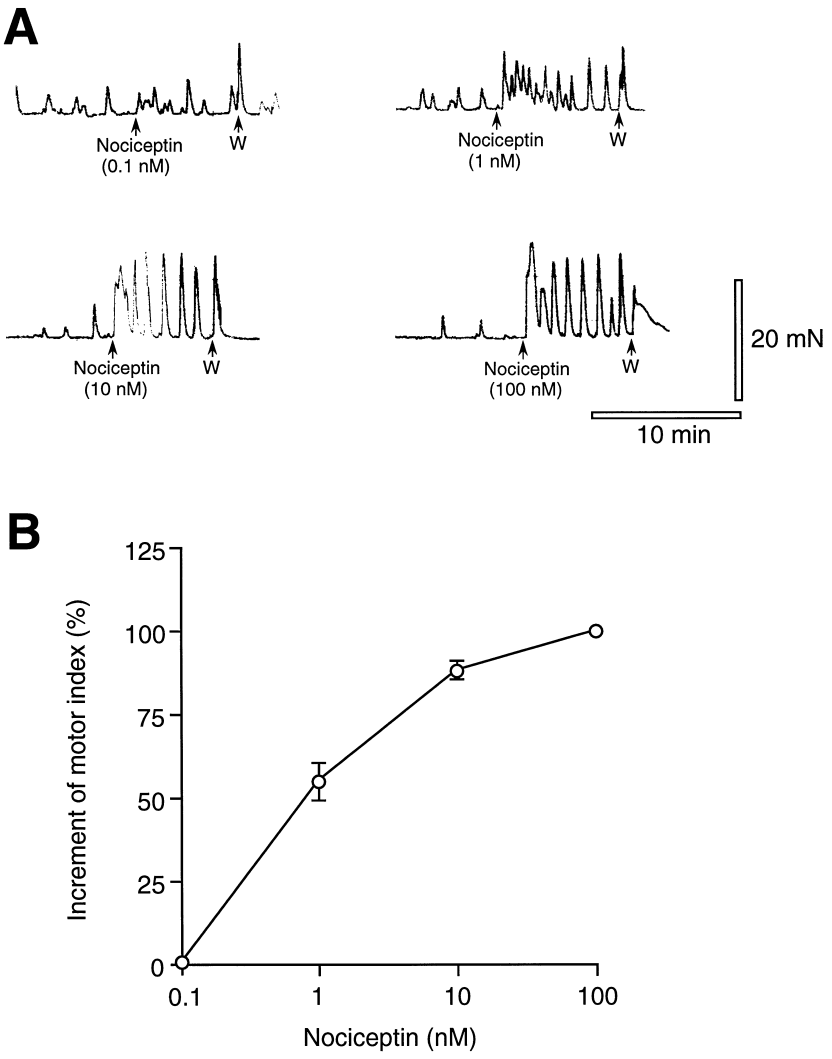

Fig. 1. Effect of nociceptin on motility of isolated rat proximal colon. (A) Typical tracings showing nociceptin (0.1–100 nM)-induced contractions. (B) Concentration–response curve of nociceptin. Motor index is expressed as a percentage of the increment induced by 100 nM nociceptin. Data represent the mean ± S.E.M. of six experiments.

2.3. Response of colon in anesthetized rats

Colonic motility studies in anesthetized rats were performed according to the procedures described previously (Nagasaki et al., 1989). In brief, rats were fasted for 20 h and anesthetized with urethane (1.2 g/kg s.c.). After midline laparotomy, a strain gauge force transducer (F-081S, Star Medical, Japan) was sutured on the serosal surface of the proximal colon to record the circular muscle contraction. For intravenous administration of drugs, the right femoral vein was cannulated. After the contractile response to bethanechol (30 μg/kg i.v.) became stable, the test drugs were administered intravenously.

2.4. Large intestinal transit

Transit rates of large intestines were determined according to the procedures described previously (Nijs et al.,

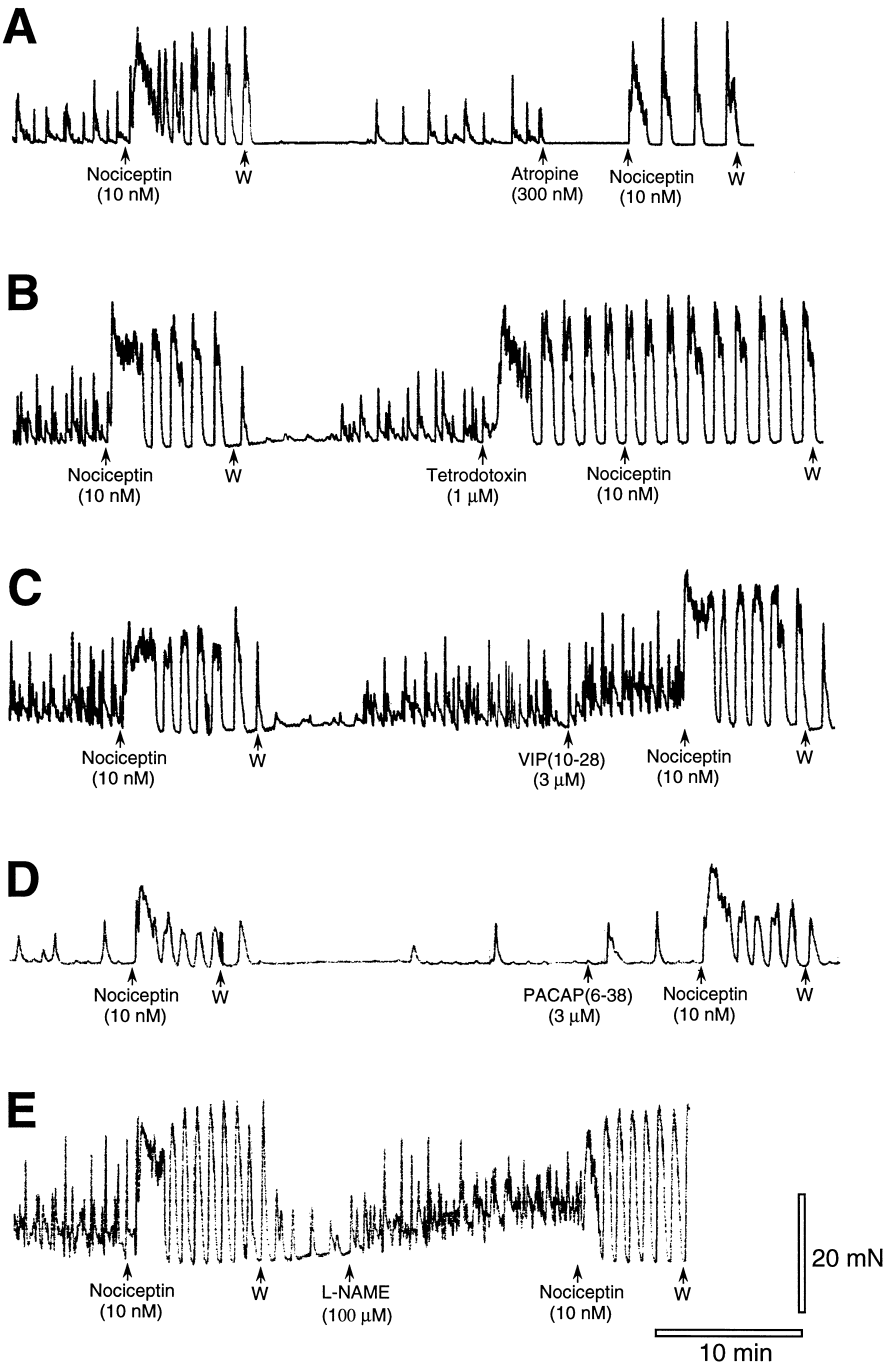

Fig. 2. Typical tracings showing the effects of atropine (A; 300 nM), tetrodotoxin (B; 1 μM), VIP(10–28) (C; 3 μM), PACAP(6–38) (D; 3 μM) and L-NAME (E; 100 μM) on nociceptin-induced contractions of isolated rat proximal colon.

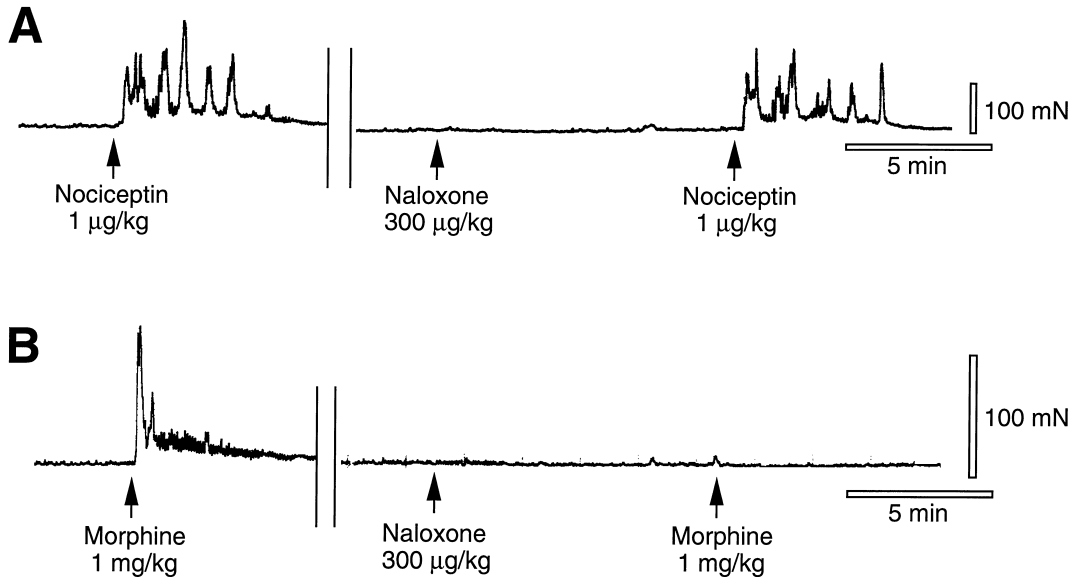

Fig. 3. Typical tracings showing the effect of naloxone (300 μg/kg i.v.) on colonic contractions induced by nociceptin (A; 1 μg/kg i.v.) and morphine (B; 1 mg/kg i.v.). Break in tracings represents a 30-min time period.

1993) with a slight modification. Rats were anesthetized with ether. After midline laparotomy, Evans' blue (0.5%) suspended in 1.5% carboxymethylcellulose sodium was injected into the proximal colon at a volume of 1 ml/rat, and then the abdomen was closed. One or four hours later, the animals were killed by inhalation of excess ether, and the large intestine was removed immediately. The length which the marker dye traveled was measured in the large intestine. The drugs were administered by a subcutaneous injection immediately before the marker dye injection.

2.5. Drugs

Nociceptin and pituitary adenylate cyclase activating polypeptide(6–38) (PACAP-(6–38)) were purchased from the Peptide Institute (Osaka, Japan). Vasoactive intestinal

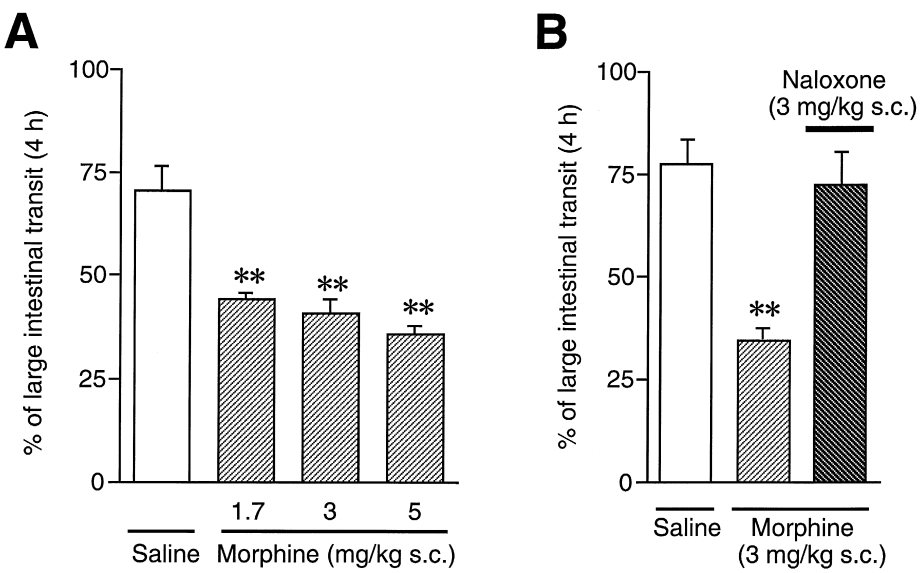

Fig. 4. Effect of morphine on large intestinal transit in rats. Drugs were subcutaneously administered immediately after instillation of marker dye into the proximal colon. Four hours later, the large intestine was removed. (A) Dose–response relationship for the effect of morphine. (B) Effect of naloxone (3 mg/kg) on morphine (3 mg/kg)-induced delay of large intestinal transit. Data represent the mean ± S.E.M. of 5–8 rats. $**P < 0.01$ compared with saline group.

polypeptide(10–28) (VIP-(10–28)) was purchased from Peninsula Lab., (CA, USA). *Nor*-binaltorphimine dihydrochloride (*nor*-BNI) was purchased from Research Biochemicals International (Natick, MA, USA). All other chemicals used were of reagent grade.

2.6. Statistics

Data were analyzed by one-way analysis of variance (ANOVA) followed by the Dunnet's multiple comparison test. Probabilities less than 5% ($P < 0.05$) were considered significant.

3. Results

3.1. Responses of isolated colon

Nociceptin caused an immediate tonic contraction followed by rhythmic waves of contractions which were quite stable in the rat isolated colon (Fig. 1A and Fig. 2). The motor index of nociceptin-induced contractions was concentration-dependent in the range between 0.1 and 100 nM (Fig. 1B). The responses to nociceptin (10 nM) were not affected by the μ, δ and κ opioid receptor antagonists, naloxone (100 nM), naltrindole (100 nM) and *nor*-BNI (100 nM) (data not shown). The contractions were decreased in frequency but unaffected in amplitude by atropine (300 nM) (Fig. 2A).

Tetrodotoxin induced phasic contractions similar to nociceptin. In the presence of tetrodotoxin, nociceptin did not evoke any responses (Fig. 2B). VIP(10-28) (3 μM), a VIP antagonist, and N^{ω}-nitro-L-arginine methyl ester (L-NAME; 100 μM), a NO-synthetase inhibitor, increased the basal tone of the colonic segment, suggesting that there is an inhibitory influence on basal tension mediated by the VIP and nitrergic nerves (Fig. 2C,E). On the other hand, PACAP-(6–38) (3 μM), a PACAP antagonist, did not influence the basal tone (Fig. 2D). Nociceptin-induced contractions were unaffected by the presence of PACAP-(6–38), VIP-(10–28) and L-NAME (Fig. 2C,D,E).

3.2. Response of colon in anesthetized rats

Intravenous administration of morphine (1 mg/kg) induced a phasic contraction accompanied by an increase in the basal tone of the proximal colon in anesthetized rats. The contractions induced by morphine were almost completely abolished by naloxone at a dose of 300 μg/kg (Fig. 3B). Nociceptin also caused a phasic contraction of the proximal colon at a dose of 1 μg/kg. In contrast to morphine, the effect of nociceptin was not affected by naloxone (Fig. 3A).

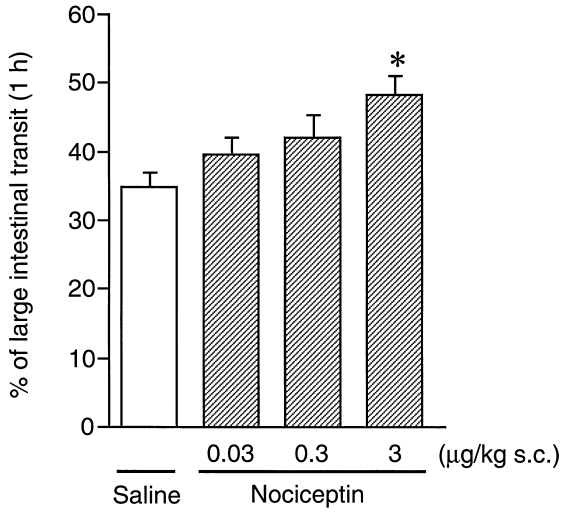

Fig. 5. Effect of nociceptin on large intestinal transit in rats. Drugs were subcutaneously administered immediately after instillation of 0.5% Evans' blue into the proximal colon. One hour later, the large intestine was removed. Data represent the mean ± S.E.M. of 14 rats. * $P < 0.05$ compared with saline group.

3.3. Large intestinal transit

The rate of large intestinal transit was dose-dependently retarded by subcutaneous administration of morphine (1.7–5 mg/kg) (Fig. 4A), and the retardation was completely blocked by naloxone (Fig. 4B). In contrast to morphine, nociceptin (s.c.) caused a dose-dependent acceleration of the rate of large intestinal transit (Fig. 5).

4. Discussion

In the present study, the concentration of nociceptin required to induce the contraction of isolated colonic smooth muscles was in good agreement with the previously reported affinity for the ORL_1 receptor (Meunier et al., 1995; Reinscheid et al., 1995). The contractions induced by nociceptin were unchanged in the presence of classical (μ, δ and κ) opioid receptor antagonists. In addition, ORL_1 transcripts have been reported to be present in the gastrointestinal tract (Wang et al., 1994). Therefore, it is suggested that nociceptin-induced contraction of isolated colonic smooth muscle is mediated by the ORL_1 receptor.

The time course of isolated colonic contractions induced by nociceptin was similar to that induced by other opioid substances (Gillan and Pollock, 1980; Grider and Makhlouf, 1987b; Laniyonu et al., 1989). Nociceptin-induced contractions were also mimicked and masked by tetrodotoxin. Intestinal smooth muscles are under the control of an inhibitory transmitter and removal of the inhibition by tetrodotoxin or opioid substances unveils the inherent rhythmic myogenic activity (Wood, 1972; Bortoff and Muller, 1975; Gillan and Pollock, 1980; Grider and

Makhlouf, 1987b). Classical opioid peptides also induce phasic contractions of isolated colonic smooth muscles by suppressing the VIP release (Grider and Makhlouf, 1987a,b). Nociceptin might exert its effect through suppression of inhibitory neurotransmitter release, like classical opioid peptides.

VIP, PACAP and nitric oxide (NO) are the major inhibitory neurotransmitters in the gastrointestinal tract (Grider et al., 1985; Schwörer et al., 1993; Matini et al., 1995). To elucidate the contribution of these inhibitory neurotransmitters, we examined the effect of VIP-(10–28), PACAP-(6–38) and L-NAME on nociceptin-induced contractions. However, prevention of effects of these inhibitory neurotransmitters neither induced phasic contractions nor affected the nociceptin-induced contraction. These results suggest that the modulation of VIP, PACAP and NOS-positive inhibitory neurons dose not mediate the nociceptin-induced contractions in rats.

The rate of large intestinal transit was dose-dependently retarded by morphine. The effect of morphine was completely blocked by naloxone treatment as shown in several species (Kaufman et al., 1988). Other classical opioid substances have also been reported to decrease the rate of large intestinal transit (Raffa et al., 1987; Shook et al., 1987, 1989). In contrast to these opioid substances, nociceptin increased the rate of large intestinal transit. Acceleration of large intestinal movement together with induction of hyperalgesia, suggest an important role of nociceptin in the gastrointestinal disease states such as the irritable bowel syndrome with pain.

Systemic administration of nociceptin immediately stimulated the colonic motility and transit. The rapid onset of contractile response suggests that this novel peptide exerts its effect directly at the level of the peripheral target portion although the role of a centrally mediated mechanism cannot be ruled out.

In the present study, nociceptin stimulated colonic contraction, and moreover accelerated the rate of colonic transit. Together with the demonstration of presence of ORL_1 receptors in gastrointestinal tracts, the results support the hypothesis that nociceptin plays a physiological role in the colonic motility. Nociceptin seems to modulate the colonic transit independently from the classical opioid peptides.

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