ELSEVIER

European Journal of Pharmacology 339 (1997) 63–67

ejp

Short Communication

Effects of μ- and κ-opioid receptors on postoperative ileus in rats

Benedicte Y. De Winter [a], Guy E. Boeckxstaens [b], Joris G. De Man [a], Tom G. Moreels [a], Arnold G. Herman [a], Paul A. Pelckmans [a,*]

[a] *Division of Gastroenterology and Pharmacology, Faculty of Medicine, University of Antwerp, Universiteitsplein 1, 2610 Antwerp, Belgium*
[b] *Division of Gastroenterology and Hepatology, Academic Medical Centre, Meibergdreef 9, 1105 Amsterdam, The Netherlands*

Received 31 July 1997; revised 5 September 1997; accepted 12 September 1997

Abstract

In a rat model of postoperative ileus, induced by abdominal surgery, we investigated the effect of μ- and κ-opioid receptors. Different degrees of inhibition of the gastrointestinal transit, measured by the migration of Evans blue, were achieved by skin incision, laparotomy or laparotomy plus manipulation of the gut. Morphine (1 mg/kg), a preferential μ-opioid receptor agonist, significantly inhibited the transit after skin incision, while the transit after the laparotomy with or without manipulation was not significantly affected. Fedotozine (5 mg/kg), a peripheral κ-opioid receptor agonist, enhanced the transit after laparotomy plus manipulation, while naloxone (1 mg/kg), a non-specific opioid receptor antagonist, further inhibited the transit after laparotomy plus manipulation. Naloxone and fedotozine alone had no effect on the transit after skin incision or laparotomy without manipulation. However, naloxone prevented the effect of morphine on the transit after skin incision and of fedotozine on the laparotomy plus manipulation. These results support a role for peripheral κ-opioid receptors in the pathogenesis of postoperative ileus induced by abdominal surgery. © 1997 Elsevier Science B.V.

Keywords: Ileus; Gastrointestinal transit; Opioid; κ-Opioid receptor; Antinociception

1. Introduction

Postoperative ileus is a common complication after abdominal surgery involving the activation of an inhibitory nervous reflex pathway with the afferent limb consisting of capsaicin sensitive fibers and the efferent limb consisting of adrenergic inhibitory fibers (Dubois et al., 1973; Furness and Costa, 1974; Bueno et al., 1978; Holzer et al., 1986; Livingston and Passaro, 1990; Holzer et al., 1992). However, other mechanisms such as the activation of inhibitory non-adrenergic non-cholinergic (NANC) nerves may contribute as well (Abrahamsson et al., 1979; Glise and Abrahamsson, 1980). We previously showed the involvement of both adrenergic and nitrergic NANC nerves in the pathogenesis of postoperative ileus in the rat (De Winter et al., 1997). In this model, different degrees of inhibition of the gastrointestinal transit were achieved by skin incision, laparotomy and laparotomy with mechanical stimulation of the small intestine and caecum (De Winter et al., 1997).

Opioids are widely used after abdominal surgery for analgesia. However opioids are known to inhibit gastrointestinal propulsion and to prolong postoperative ileus in rats and humans (Jaffe and Martin, 1985; Yukioka et al., 1987; Brown et al., 1988; Livingston and Passaro, 1990; Benson et al., 1994; Ferraz et al., 1995). The most widely used opioid for analgesia is morphine, a preferential μ-opioid receptor agonist, shown to inhibit the gastrointestinal transit in rats via a central and a peripheral pathway (Weisbrodt et al., 1980; Galligan and Burks, 1983; Manara et al., 1986; Thörn et al., 1996). In contrast, fedotozine, a new peripheral κ-opioid receptor agonist, is able to reverse the inhibition of the gastrointestinal transit induced by peritoneal irritation in rats suggesting a potential role in the treatment of ileus (Rivière et al., 1993, 1994; Friese et al., 1997). The purpose of the present study therefore was to investigate the role of κ-opioid receptors in experimental ileus induced by abdominal surgery.

2. Materials and methods

2.1. Operation protocol

All procedures received approval from the Commission for Medical Ethics from the University of Antwerp (U.I.A.). Male Wistar rats (150–225 g) were fasted for 48 h with

* Corresponding author. Tel.: +32-3-8213323; fax: +32-3-8254678; e-mail: pelckman@uia.ua.ac.be.

free access to water. The operation protocol was previously described in detail (De Winter et al., 1997). Briefly, the rats were divided in three groups in a randomised way and underwent an operation under ether anaesthesia. We previously demonstrated that ether anaesthesia had no effect on the gastrointestinal transit in our model (De Winter et al., 1997). The first group underwent an abdominal skin incision after shaving and disinfecting the abdomen. The second group underwent a laparotomy consisting of the incision of the abdominal skin, the abdominal muscle layers and the peritoneum. The third group underwent a laparotomy followed by the evisceration and manipulation of the small intestine and caecum during 5 min. After the operations the rats were allowed to recover for one hour. Then they received an intragastric injection of 0.1 ml Evans blue (50 mg in 1 ml 0.9% sodium chloride; Tanila et al., 1993) via a specially designed orogastric cannula introduced through the mouth. 20 min later the rats were killed and the intestinal transit was measured from the pylorus to the most distal point of migration of Evans blue and expressed in cm.

2.2. Experimental protocol

In a first series of experiments the effects of morphine (1 mg/kg), a preferential μ-opioid receptor agonist (Jaffe and Martin, 1985) and naloxone, a non-selective opioid receptor antagonist (Jaffe and Martin, 1985) were tested on the intestinal transit of Evans blue. The rats were randomly divided in four groups. The first group served as control group and received an intravenous (i.v.) injection of 0.9% sodium chloride in the tail vein. Then the rats underwent a skin incision, laparotomy or laparotomy plus manipulation. The second group received an i.v. injection of morphine (1 mg/kg), 1 min before the operations. The third group received an i.v. injection of naloxone (1 mg/kg) 1 min before the operations. The fourth group was injected i.v. with naloxone followed by morphine, 1 min before the operations.

In a second series of experiments we investigated the effect of fedotozine (5 mg/kg; Diop et al., 1994), a peripheral κ-opioid receptor agonist, on the intestinal transit of Evans blue after the three operations. The rats were randomly divided in three groups. The first group served as control group and received an i.v. injection of 0.9% sodium chloride. The second group was injected i.v. with fedotozine one minute before the operations. The third group received an i.v. injection of naloxone (1 mg/kg) followed by an i.v. injection of fedotozine one minute before the operations.

2.3. Drugs used

The following drugs were used: diethyl ether (Merck, Darmstadt, Germany), Evans blue (Sigma, St. Louis, USA), morphine hydrochloride (S.A. Belgopia, Louvain-la-Neuve, Belgium), naloxone hydrochloride dihydrate (Aldrich Chemical Company, Milwaukee, USA), sodium chloride 0.9% (Plurule®, Baxter, Lessines, Belgium). Fedotozine tartrate was a gift of Parke Davis (Zaventem, Belgium). Fedotozine, morphine and naloxone were dissolved in 0.9% sodium chloride.

2.4. Presentation of results and statistical analysis

The total length of the small intestine was not statistically different between the groups. Therefore, results are expressed as cm migration of Evans blue, the measurements were from the pylorus to the most distal point of migration of Evans blue. Group differences were assessed by simple factorial analysis of variance (ANOVA) and one way analysis of variance followed by the Bonferroni test for multiple comparisons. Values are shown as mean $\pm$ S.E.M. for n indicating the number of rats used. P-values of less than 0.05 were considered to be significant. All data were analysed with the SPSS for windows software (SPSS, Chicago, IL).

3. Results

3.1. Effect of morphine and naloxone on the intestinal transit

The total length of the small intestine was not statistically different between the different groups. In control rats, treated with 0.9% sodium-chloride, the transit after the skin incision was 62.2 ± 3.2 cm of 97.9 ± 2.2 cm ($n = 10$). The transit was not influenced by the skin incision as previously shown (De Winter et al., 1997). The laparotomy significantly delayed the intestinal transit to 35.8 ± 3.7 cm of 97.4 ± 1.8 cm ($n = 10$). This inhibition was even more pronounced when the laparotomy was associated with the manipulation of the small intestine and caecum: the transit was 19.3 ± 3.5 cm of 98.4 ± 1.9 cm ($n = 10$) (Fig. 1).

The transit after the skin incision was significantly different from the transit after the laparotomy with or without manipulation in the different treatment groups. There was also a statistical significant difference between the transit after the laparotomy and the transit after the laparotomy plus manipulation in the different treatment groups.

Morphine (1 mg/kg) significantly inhibited the transit after the skin incision from 62.2 ± 3.2 cm in control rats to 44.5 ± 2.2 cm ($n = 10$) in morphine treated rats (Fig. 1). Although morphine tended to decrease the transit after the laparotomy with or without manipulation compared to control rats, no statistical significance was reached. The transit after the laparotomy was 30.3 ± 2.9 cm ($n = 9$) and 14.8 ± 2.4 cm ($n = 10$) after the laparotomy plus manipulation (Fig. 1).

Naloxone alone (1 mg/kg) had no effect on the transit after the skin incision or the laparotomy as compared to

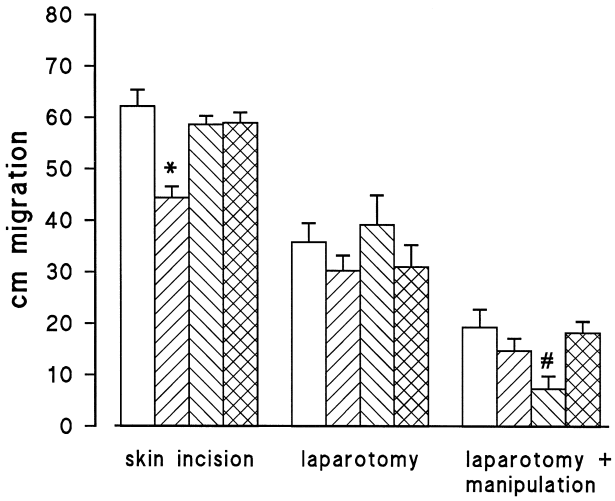

Fig. 1. Effect of skin incision, laparotomy or laparotomy with evisceration and manipulation of the small intestine and caecum on the intestinal transit in control rats (open bars, $n = 10$) and in rats treated with morphine 1 mg/kg (right-rising hatched bars, $n \geq 9$) or with naloxone 1 mg/kg (left-rising hatched bars, $n \geq 9$) or with naloxone plus morphine (cross-hatched bars, $n \geq 9$). Results are expressed as cm migration of Evans blue and shown as mean ± S.E.M. $^{*}P \leq 0.05$, significantly different from the transit in rats that underwent a skin incision; $^{\#}P \leq 0.05$ significantly different from the transit in control rats or rats treated with naloxone plus morphine after the laparotomy plus manipulation; one way analysis of variance followed by the Bonferroni test.

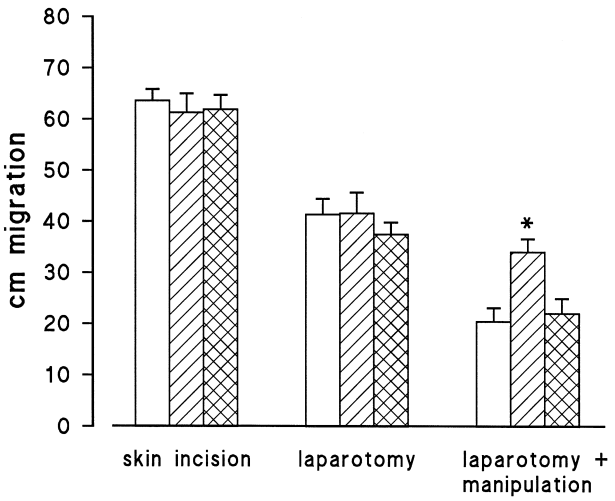

Fig. 2. Effect of skin incision, laparotomy or laparotomy with evisceration and manipulation of the small intestine and caecum on the intestinal transit in control rats (open bars, $n = 9$) and in rats treated with fedotozine 5 mg/kg (right-rising hatched bars, $n \geq 9$) or with naloxone 1 mg/kg plus fedotozine (cross-hatched bars, $n \geq 9$). Results are expressed as cm migration of Evans blue and shown as mean ± S.E.M. $^{*}P \leq 0.05$, significantly different from the transit in rats that underwent a laparotomy plus manipulation; one way analysis of variance followed by the Bonferroni test.

control rats ($n \geq 9$) (Fig. 1). However the transit after the laparotomy plus manipulation was significantly inhibited by naloxone from 19.3 ± 3.5 cm in control rats to 7.4 ± 2.5 cm ($n = 10$) in naloxone treated rats (Fig. 1).

Consecutive injection of naloxone and morphine reversed the inhibitory effect of morphine on the transit after the skin incision and had no effect on the transit after the laparotomy (Fig. 1). The combination of naloxone and morphine also reversed the inhibitory effect of naloxone on the transit after the laparotomy plus manipulation (Fig. 1).

3.2. Effect of fedotozine on the intestinal transit

The total length of the small intestine was not statistically different between the different groups. In control rats treated with 0.9% sodium chloride, the transit after the skin incision was 63.6 ± 2.2 cm of 100.2 ± 2.3 cm ($n = 9$). The transit after the laparotomy was significantly delayed to 41.4 ± 3.1 cm of 98.2 ± 2.2 cm ($n = 9$). After the laparotomy plus manipulation, the transit was 20.4 ± 2.7 cm of 97.1 ± 1.8 cm ($n = 9$) (Fig. 2).

Fedotozine (5 mg/kg) had no effect on the transit after the skin incision or the laparotomy as compared to control rats ($n \geq 9$) (Fig. 2). However, fedotozine significantly enhanced the transit after the laparotomy plus manipulation from 20.4 ± 2.7 cm in control rats to 34.1 ± 2.6 cm in fedotozine treated rats ($n = 9$) (Fig. 2).

Naloxone plus fedotozine had no effect on the transit after the skin incision or the laparotomy ($n \geq 9$) (Fig. 2). However, consecutive injection of naloxone and fedotozine reversed the effect of fedotozine alone on the transit after the laparotomy plus manipulation; the transit was 22.0 ± 2.9 cm ($n = 9$) (Fig. 2).

The difference between the transit after the laparotomy and the transit after the laparotomy plus manipulation was significant in the control rats and in the rats treated with naloxone plus fedotozine. However, after treatment of the rats with fedotozine alone, the transit after the laparotomy plus manipulation was no longer significantly different from the transit after the laparotomy alone.

4. Discussion

Postoperative ileus is a common complication after abdominal surgery of which the pathogenesis is still debated. In an experimental model of postoperative ileus in the rat, we previously demonstrated that skin incision had no effect on the gastrointestinal transit of Evans blue. However, laparotomy inhibited the gastrointestinal transit significantly, an effect mediated by adrenergic nerves. Mechanical stimulation of the gut resulted in a further reduction of the gastrointestinal transit mediated by nitrergic nerves (De Winter et al., 1997). In the present study, we provide evidence that peripheral κ-opioid receptors may be involved in the inhibitory reflex triggered by mechanical stimulation of the gut.

Opioids like morphine are widely used to control postoperative pain despite their depressant activity on the

gastrointestinal motility (Jaffe and Martin, 1985; Yukioka et al., 1987; Livingston and Passaro, 1990; Benson et al., 1994; Ferraz et al., 1995). In the present study however, we demonstrate that the opioid fedotozine, a peripheral κ-opioid receptor agonist, reversed the additional inhibition induced by mechanical stimulation of the gut. This effect of fedotozine was specific as it was reversed by naloxone. The concentration of naloxone we used was a non-receptor specific dose, antagonising both μ- and κ-opioid receptors (Sengupta et al., 1996). Furthermore, fedotozine had no effect on the transit after skin incision and no effect on the delay in transit evoked by laparotomy showing that it was rather specific to mechanical stimulation. These results suggest that activation of peripheral κ-opioid receptors decreases the inhibitory reflex triggered by mechanical stimulation. Blockade of these receptors by naloxone indeed resulted in a further decrease in transit confirming this hypothesis. Endogenous opioids might attenuate the inhibition of the gastrointestinal transit induced by the manipulation, by activating the peripheral κ-opioid receptors. This positive effect of the peripheral κ-opioid receptors on the gastrointestinal transit is enhanced by exogenously administered κ-opioid receptor agonists like fedotozine.

Our results are in line with previous results demonstrating that peripheral κ-opioid receptor agonists could reverse ileus induced by peritoneal irritation (Rivière et al., 1993, 1994; Friese et al., 1997). Fedotozine was also able to reverse the inhibition of the migrating myoelectric complex induced by laparotomy and caecum palpation (Rivière et al., 1993). The exact mechanism by which peripheral κ-opioid receptor agonists modulate the transit after induction of ileus is not clear. Possibly, they increase the threshold to activate the inhibitory reflex pathway. Sengupta et al. (1996) demonstrated that fedotozine attenuated the firing of primary afferent neurones in the rat after noxious colorectal distension. The antinociceptive activity of fedotozine was demonstrated in a rat model of duodenal pain and colon irritation (Diop et al., 1994; Langlois et al., 1994). Also according to Stein (1993), the most likely mechanism of antinociception of the opioids is the activation of opioid receptors located on primary afferent neurones. Therefore we hypothesise that during manipulation of the gut primary afferent fibers in the bowel wall are activated and in turn stimulate nitrergic neurones resulting in an inhibition of the transit. Endogenous opioids or peripheral κ-opioid receptor agonists can reduce the activation of these afferent fibers resulting in a less profound reduction of the transit.

In the present study, morphine significantly delayed the transit after the skin incision, while the transit after the laparotomy with or without manipulation was not significantly altered. The effect of morphine was reversed by naloxone. Interestingly, morphine and fedotozine had a differential effect on the transit after the skin incision and after the laparotomy plus manipulation indicating a different mode of action. This different mode of action most likely results from the activation of different opioid receptors. Morphine is acting preferentially on μ-opioid receptors (Jaffe and Martin, 1985) while fedotozine binds to peripheral κ-opioid receptors (Rivière et al., 1993). These results suggest that only peripheral κ-opioid receptor agonists, and not μ-opioid receptor agonists, can decrease the inhibition of the transit induced by mechanical stimulation of the gut. They also accentuate the specificity of fedotozine as a κ-opioid receptor agonist.

In conclusion, we confirmed that morphine has an inhibitory effect on the gastrointestinal transit and that this effect is reversed by naloxone. We demonstrated that the transit after the laparotomy plus manipulation was enhanced by fedotozine while it was further inhibited by naloxone. These results support a role for peripheral κ-opioid receptors in the pathogenesis of postoperative ileus induced by abdominal surgery. Therefore, peripheral κ-opioid receptor agonists may be of benefit in the treatment of postoperative ileus.

Acknowledgements

The authors wish to thank Mrs. L. Van de Noort for typing the manuscript. Benedicte De Winter is a research assistant of the Fund for Scientific Research, Flanders Belgium (FWO). This work was supported by the FWO-grant No. G.0220.96.

References

Abrahamsson, H., Glise, H., Glise, K., 1979. Reflex suppression of gastric motility during laparotomy and gastroduodenal nociceptive stimulation. Scand. J. Gastroenterol. 14, 101–106.

Benson, M.J., Roberts, J.P., Wingate, D.L., Rogers, J., Deeks, J.J., Castillo, F.D., Williams, N.S., 1994. Small bowel motility following major intra-abdominal surgery: The effects of opiates and rectal cisapride. Gastroenterology 106, 924–936.

Brown, N.J., Coupar, I.M., Rumsey, R.D.E., 1988. The effect of acute and chronic administration of morphine and morphine withdrawal on intestinal transit time in the rat. J. Pharm. Pharmacol. 40, 844–848.

Bueno, L., Ferre, J.P., Ruckebusch, Y., 1978. Effects of anesthesia and surgical procedures on intestinal myoelectric activity in rats. Am. J. Dig. Dis. 23, 690–695.

De Winter, B.Y., Boeckxstaens, G.E., De Man, J.G., Moreels, T.G., Herman, A.G., Pelckmans, P.A., 1997. Effect of adrenergic and nitrergic blockade on experimental ileus in rats. Br. J. Pharmacol. 120, 464–468.

Diop, L., Rivière, P.J.M., Pascaud, X., Junien, J.-L., 1994. Peripheral κ-opioid receptors mediate the antinociceptive effect of fetodozine on the duodenal pain reflex in rat. Eur. J. Pharmacol. 271, 65–71.

Dubois, A., Weise, V.K., Kopin, I.J., 1973. Postoperative ileus in the rat: Physiopathology, etiology and treatment. Ann. Surg. 178, 781–786.

Ferraz, A.A.B., Cowles, V.E., Condon, R.E., Carilli, S., Ezberci, F., Frantzides, C.T., Schulte, W.J., 1995. Nonopioid analgesics shorten the duration of postoperative ileus. Am. Surg. 61, 1079–1083.

Friese, N., Chevalier, E., Angel, F., Pascaud, X., Junien, J.-L., Dahl, S.G., Rivière, P.J.M., 1997. Reversal by κ-agonists of peritoneal irritation-induced ileus and visceral pain in rats. Life Sci. 60, 625–634.

Furness, J.B., Costa, M., 1974. Adynamic ileus, its pathogenesis and treatment. Med. Biol. 52, 82–89.

Galligan, J.J., Burks, T.F., 1983. Centrally mediated inhibition of small intestinal transit and motility by morphine in the rat. J. Pharmacol. Exp. Ther. 226, 356–361.

Glise, H., Abrahamsson, H., 1980. Reflex vagal inhibition of gastric motility by intestinal nociceptive stimulation in the cat. Scand. J. Gastroenterol. 15, 769–774.

Holzer, P., Lippe, I.T., Holzer-Petsche, U., 1986. Inhibition of gastrointestinal transit due to surgical trauma or peritoneal irritation is reduced in capsaicin-treated rats. Gastroenterology 91, 360–363.

Holzer, P., Lippe, I.T., Amann, R., 1992. Participation of capsaicin-sensitive afferent neurons in gastric motor inhibition caused by laparotomy and intraperitoneal acid. Neuroscience 48, 715–722.

Jaffe, J.H., Martin, W.R., 1985. Opioid analgesics and antagonists. In: Goodman Gilman, A., Goodman, L.S., Rall, T.W., Murad, F. (Eds.), Goodman and Gilman's. The Pharmacological Basis of Therapeutics. MacMillan Publishing Company, New York, pp. 491–531.

Langlois, A., Diop, L., Rivière, P.J.M., Pascaud, X., Junien, J.-L., 1994. Effect of fedotozine on the cardiovascular pain reflex induced by distension of the irritated colon in the anesthetized rat. Eur. J. Pharmacol. 271, 245–251.

Livingston, E.H., Passaro, E.P., 1990. Postoperative ileus. Dig. Dis. Sci. 35, 121–132.

Manara, L., Bianchi, G., Ferretti, P., Tavani, A., 1986. Inhibition of gastrointestinal transit by morphine in rats results primarily from direct drug action on gut opioid sites. J. Pharmacol. Exp. Ther. 237, 945–949.

Rivière, P.J.M., Pascaud, X., Chevalier, E., Le Gallou, B., Junien, J.-L., 1993. Fedotozine reverses ileus induced by surgery or peritonitis: action at peripheral κ-opioid receptors. Gastroenterology 104, 724–731.

Rivière, P.J.M., Pascaud, X., Chevalier, E., Junien, J.-L., 1994. Fedotozine reversal of peritoneal-irritation-induced ileus in rats: Possible peripheral action on sensory afferents. J. Pharmacol. Exp. Ther. 270, 846–850.

Sengupta, J.N., Su, X., Gebhart, G.F., 1996. κ, but not μ or δ, opioids attenuate responses to distention of afferent fibers innervating the rat colon. Gastroenterology 111, 968–980.

Stein, C., 1993. Peripheral mechanisms of opioid analgesia. Anesth. Analg. 76, 182–191.

Tanila, H., Kauppila, T., Taira, T., 1993. Inhibition of intestinal motility and reversal of postlaparotomy ileus by selective α_2-adrenergic drugs in the rat. Gastroenterology 104, 819–824.

Thörn, S.-E., Wattwil, M., Lindberg, G., Säwe, J., 1996. Systemic and central effects of morphine on gastroduodenal motility. Acta Anaesthesiol. Scand. 40, 177–186.

Weisbrodt, N.W., Sussman, S.E., Stewart, J.J., Burks, T.F., 1980. Effect of morphine sulfate on intestinal transit and myoelectric activity of the small intestine of the rat. J. Pharmacol. Exp. Ther. 214, 333–338.

Yukioka, H., Bogod, D.G., Rosen, M., 1987. Recovery of bowel motility after surgery. Br. J. Anaesth. 59, 581–584.