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Effects of μ-opioid receptor agonists on intestinal secretion and permeability during acute intestinal inflammation in mice ☆

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Abstract

We evaluated and compared the effects of μ-opioid receptor agonists on mucosal fluid transport and permeability, during acute intestinal inflammation. We hypothesized that inflammation would sensitize μ-opioid receptors in the submucosal plexus and/or enterocytes enhancing the effects of μ-opioid receptor agonists. Inflammation was induced by intragastric administration of croton oil, whereas controls received saline. Fluid transport was assessed by enteropooling, and intestinal permeability by blood-to-lumen passage of [^{51}Cr] etylenediaminetetraacetate ([^{51}Cr] EDTA). Intestinal inflammation induced a significant increase in enteropooling (1.9 times) and permeability (2.5 times). In saline- and croton oil-treated animals, μ-opioid receptor agonists produced dose-related inhibitions of enteropooling and intestinal permeability. During inflammation, the potency of morphine increased 4.8 and 3.7 times, inhibiting enteropooling and intestinal permeability, respectively; the potencies of fentanyl and PL017 similarly increased by approximately three (enteropooling) and two times (permeability) in croton oil animals. All effects were reversed by naloxone and naloxone methiodide. The results show that inflammation increases the inhibitory potency of μ-opioid receptor agonists on secretion and permeability, suggesting a sensitization of peripheral μ-opioid receptors.

Keywords: Enteropooling; Inflammation, peripheral; Intestine; Opioid; Opioid receptor; Permeability

1. Introduction

Opioids have long been used in the symptomatic treatment of diarrhoea, due to their inhibitory effects on intestinal transit and secretion. Many studies have characterised the effects of these compounds on gut motility; however, the antisecretory properties of opioids have been poorly characterised. Opioid receptors are present in the brain and spinal cord and also in the gastrointestinal tract, with particular abundance in the myenteric and submucosal plexuses (Bagnol et al., 1997), and in enterocytes of intestinal villi and crypts (Lang et al., 1996). The role of opioid receptors in water and electrolyte transport seems to be complex and not fully elucidated (De Luca and Coupar, 1996). Systemic opioid administration decreases intestinal motility and fluid and electrolyte secretion, binding to opioid receptors located at supraspinal, spinal and peripheral sites. Studies in mice, have confirmed the central effects of μ- and κ-opioid receptors increasing absorption of intestinal fluid and electrolytes (Jiang et al., 1990). Moreover, studies in vitro have demonstrated a proabsorptive role for μ- and δ-opioid receptor agonists in submucosal plexus (Sheldon et al., 1990) and epithelial enterocytes (Lang et al., 1996).

Intestinal injury induced by infection, chemicals, etc., can stimulate fluid and electrolyte secretion in excess of the mucosal absorptive capacity and generate net fluid accumulation into the intestinal lumen, that contributes to produce diarrhoea (Chang et al., 1983). Permeation across the intestinal epithelium occurs by two main routes: transcellular (across epithelial cells) and paracellular (between the cells), the latter occurring both, through tight junctions and the underlying intercellular space. At present, tight junctions are considered to be the most important barrier in the regulation of paracellular permeability (Bijlsma et al., 1997).

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The role of μ-opioid receptors on intestinal fluid transport and permeability during intestinal inflammation have not been investigated. Our laboratory has characterised and validated (Pol et al., 1995) a model of acute intestinal inflammation in mice, induced by the intragastric administration of croton oil (substantiated by electron microscopy). In this model, the potency of s.c. morphine inhibiting gastrointestinal transit (motility), was increased approximately three times (Pol and Puig, 1997), suggesting that the inflammatory stimuli ''sensitized'' μ-opioid receptors in the gut (plexus and/or enterocytes). We hypothesized that the opioid receptors involved in the inhibitory control of intestinal secretion would also be ''sensitized'' during acute inflammation; thus, in the present study, we have investigated the effects of μ-opioid receptor agonists on fluid transport (enteropooling) and permeability (blood-to-lumen transfer of [^{51}Cr] etylenediaminetetraacetate or [^{51}Cr] EDTA).

The aims of our investigation were: (1) to evaluate and compare changes in intestinal fluid transport and permeability, during acute intestinal inflammation, (2) to determine the potency of μ-opioid receptor agonists on both fluid transport and permeability in the absence and presence of inflammation, (3) to assess the peripheral component of their effects during inflammation and (4) to establish the reversibility of the inhibitory effects by μ-opioid receptor antagonists.

2. Methods

2.1. Animals

Male Swiss CD-1 mice (Charles River Spain), weighing 25–30 g, were used in all experiments. Animals were housed under 12 h light/12 h dark conditions in a room with controlled temperature (22°C) and humidity (60%). Mice had free access to food and water, and were used after a minimum of 4 days of acclimatization to the housing conditions. All experiments were conducted between 0900 and 1700 h. The study protocol was approved by the local Committee of Animal Use and Care of our Institution.

2.2. Inflammation induced by croton oil

Before the experiments, animals were fasted for 18 h, except for free access to water, which was available for the duration of the study. Intestinal inflammation was induced by the p.o. administration of 0.05 ml of croton oil, while control animals received the same volume of p.o. saline (Pol et al., 1994). Enteropooling and intestinal permeability studies were performed 3.5 h after saline or croton oil. Morphological changes induced by croton oil have been previously reported by our group (Pol et al., 1995) and were established by electron microscopy. They included an increased number of clear vesicles in the cytoplasm of epithelial cells (mainly near the luminal pole), swollen mitochondria with disrupted cristae and enlarged spaces filled with fine granular material in the extravascular compartment of the villi, in mice treated with croton oil.

2.3. Enteropooling or intraluminal accumulation of fluid

Mucosal transport of fluid was determined using the enteropooling assay (Rivière et al., 1990), that evaluates the net accumulation of fluid in the lumen of the small intestine (Robert et al., 1976). After fasting (18 h) and p.o. administration of croton oil or saline, animals were sacrificed by cervical dislocation (Fig. 1); the small intestine was clamped at the pyloric valve and the ileo-caecal junction and carefully removed from the abdomen. The small intestine was weighed (W_1), emptied of fluid, reweighed (W_2) and the length (L) measured. The difference in weight divided by the length, shows the ''enteropooling'' in milligrams of fluid per centimeter of intestine.

$$\text{Enteropooling} = (W_1 - W_2)/L$$

The effects of morphine, fentanyl and PL017 on intraluminal accumulation of fluid were determined in both treatment groups (saline and croton oil). Morphine and PL017 were administered subcutaneously 30 min, and fentanyl 20 min before enteropooling. Control groups received vehicle (saline) injections. The μ-opioid receptor antagonists naloxone and naloxone methiodide were injected intraperitoneally 15 min before the μ-opioid receptor agonists.

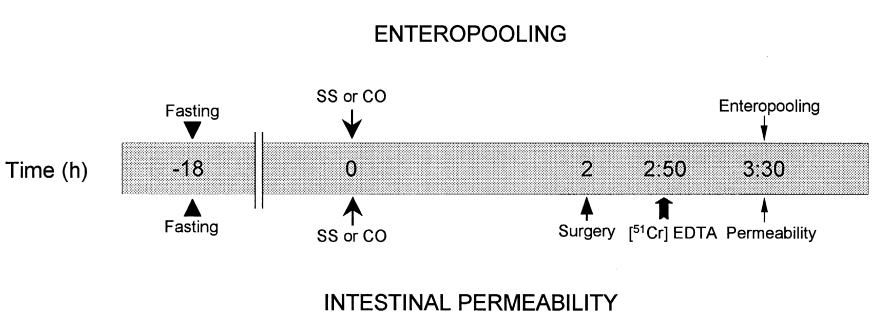

Fig. 1. Experimental design showing the time of surgery, the [^{51}Cr] EDTA administration and the enteropooling and intestinal permeability evaluation. Animals were fasted 18 h before saline or croton oil.

2.4. Intestinal permeability

Permeability of the small intestine was assessed by measuring the passage of [^{51}Cr] EDTA from blood to lumen using a technique adapted from Miller et al (1991). After fasting (18 h), animals received p.o. saline or croton oil, and 2 h later, the renal pedicles were ligated (Fig. 1). Mice were laparotomized under ether anaesthesia, and both renal pedicles were ligated to prevent rapid excretion of the radioactive marker into the urine. Animals were allowed to recover for a period of 50 min, and at this time, 4 μCi of [^{51}Cr] EDTA was injected intravenously into a vein of the tail. Forty minutes later, animals were sacrificed by cervical dislocation, the small intestine was removed and the intestinal lumen was washed with 1 ml of saline. The [^{51}Cr] EDTA present in the lumen was then measured with a gamma counter (LKB-WALLAC, 1282 Compugamma). Detected counts per minute (cpm) were expressed as the percentage of the initial administered dose. In these experiments, subcutaneous morphine and PL017 were given 15 min, and fentanyl 5 min before [^{51}Cr] EDTA. Control groups received vehicle injections. Naloxone and naloxone methiodide were injected intraperitoneally, 15 min before the μ-opioid receptor agonists.

2.5. Drugs

We used morphine hydrochloride (Alcaiber, Madrid, Spain), fentanyl (Syntex Latino, Madrid, Spain), PL017 [*N*-MePhe^3, D-Pro^4]morphiceptin (Peninsula Laboratories, Belmont, CA., USA), (−) naloxone hydrochloride (Sigma) and naloxone methiodide (Research Biochemicals, Wayuland, MA). Drugs were dissolved in sterile pyrogen-free 0.9% sodium chloride just before use and injected subcutaneously at the nape of the neck in a volume of 10 ml/kg.

2.6. Statistical analysis

The inhibitory effects of the opioid receptor agonists are expressed as the percentage of inhibition of enteropooling or intestinal permeability in a drug-treated animal (test) when compared with the mean enteropooling or intestinal permeability measured in the corresponding group of vehicle-treated mice.

$$\%\text{Inhibition} = [(\text{vehicle} - \text{test})/(\text{vehicle})] \times 100$$

Data are expressed as a group mean ± S.E. All statistical calculations were performed as described by Tallarida and Murray (1986). ED_{50} ± S.E. (dose that produced a 50% effect) values were determined by linear regression analysis of dose–response relations based on at least six animals per dose. Tests for parallelism and validity of the tests were estimated by parallel line assay. Statistical analyses for significant differences between two groups were obtained by Student's *t*-test; when multiple groups were compared, one-way analysis of variance (ANOVA), followed by Student–Newman–Keuls test, was used whenever applicable. A value of $P < 0.05$ was considered as significant.

3. Results

3.1. Effects of croton oil on enteropooling and intestinal permeability

Enteropooling and intestinal permeability were evaluated 180 and 210 min, respectively, following p.o. administration of saline or croton oil. Enteropooling values in saline- and croton oil-treated mice were 6.9 ± 0.5 and 12.3 ± 0.9 mg/cm, respectively (Fig. 2A), demonstrating a 1.9-fold increase ($P < 0.05$) after croton oil administration. Blood-to-lumen transfer of [^{51}Cr] EDTA was 0.41 ± 0.04% in controls, and 1.04 ± 0.05% during inflammation

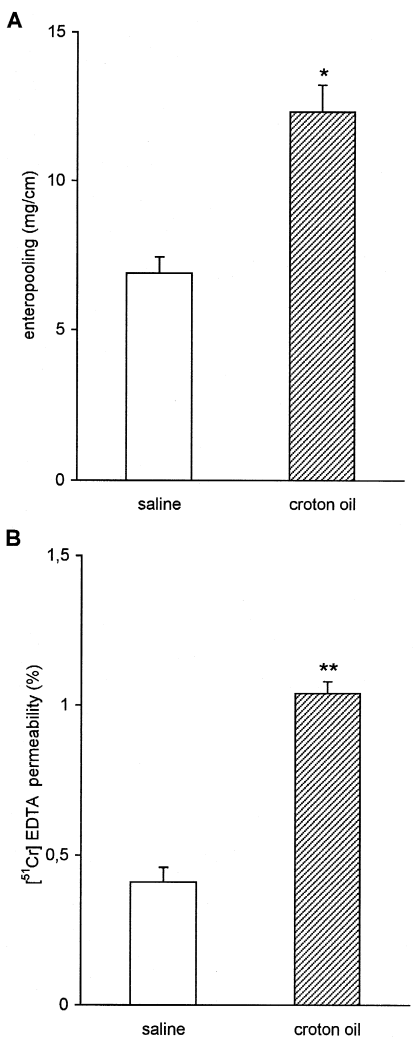

Fig. 2. Effects of p.o. administration of saline or croton oil on (A) enteropooling and (B) [^{51}Cr] EDTA permeability. Each column represents the mean value ± S.E. of at least eight animals. * $P < 0.01$ and ** $P < 0.001$, when compared to saline (Student's *t*-test).

(Fig. 2B), showing a 2.5-fold increase ($P < 0.01$) in intestinal permeability. The results demonstrated that acute inflammation (croton oil) significantly increases intestinal intraluminal fluid.

3.2. Inhibitory effects of morphine and fentanyl on enteropooling and intestinal permeability

The effects of morphine (mixed μ-opioid receptor agonist) and fentanyl (μ-opioid receptor agonist) on enteropooling and intestinal permeability were assessed in controls and during intestinal inflammation. Morphine produced a dose-related inhibition of enteropooling (Fig. 3A) and intestinal permeability (Fig. 3B) in saline- and croton oil-treated animals. In all instances, analysis of the dose–response curves showed coefficients of correlation close to 1, and no significant differences in the slopes of the curves. In animals treated with croton oil, the dose–response curves to morphine were significantly shifted to the left, demonstrating an increased response during inflammation. From the curves, ED_{50} values for morphine were obtained as a measure of potency (Table 1). The results show that during inflammation, the potency of morphine increased 4.8 and 3.7 times in the enteropooling and permeability tests, respectively.

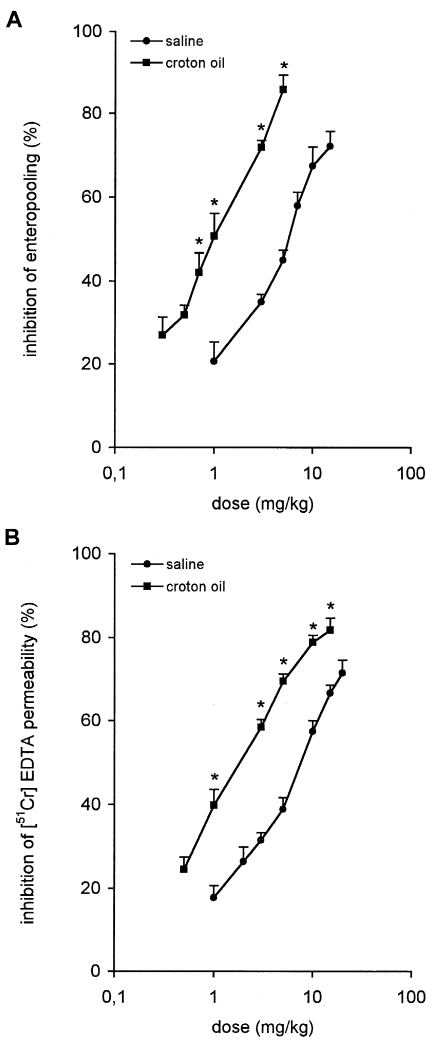

Fig. 3. Dose-related inhibition of (A) enteropooling and (B) [^{51}Cr] EDTA permeability by morphine in mice primed with saline or croton oil. Each point represents the mean ± S.E. of at least six animals. * $P < 0.01$, when compared to saline (Student's *t*-test).

Table 1
ED_{50} values (mg/kg) of morphine on enteropooling and intestinal permeability in control mice (saline) and during intestinal inflammation (croton oil)
Results are expressed as mean values ± S.E. of six or more animals per dose. For each assay, the ED_{50} in croton oil-treated mice was significantly different when compared to the ED_{50} in saline-treated animals. For each assay, different letters (a, b) indicate significant differences between treatment groups ($P < 0.05$, Student's *t*-test).

Assay	Group	ED_{50} ± S.E.	Slope	Ratio (saline/croton oil)
Enteropooling	Saline	5.0 ± 0.025^a	46.8 ± 4.0	4.8
	Croton oil	1.03 ± 0.024^b	46.9 ± 3.2	
Permeability	Saline	7.1 ± 0.021^a	47.6 ± 2.7	3.7
	Croton oil	1.87 ± 0.019^b	41.9 ± 1.6	

Fentanyl also induced dose-related inhibitions of enteropooling and intestinal permeability in saline- and croton-oil treated animals. The dose–response curves showed coefficients of correlation not significantly different from 1, and no differences in the slopes of the lines (Table 2). The potency of fentanyl, was increased significantly in animals treated with croton oil, as demonstrated by a shift to the left of the dose–response curve. When comparing the ED_{50} values, it could be demonstrated that fentanyl was 3.0 and 2.0 times more potent in croton oil than in saline-treated animals, respectively, decreasing enteropooling and intestinal permeability. These groups of experi-

Table 2
ED_{50} values (mg/kg) of fentanyl on enteropooling and intestinal permeability in control mice (saline) and during intestinal inflammation (croton oil)
Results are expressed as mean values ± S.E. of six or more animals per dose. For each assay, the ED_{50} in croton oil-treated mice was significantly different when compared to the ED_{50} in saline-treated animals. For each assay, different letters (a, b) indicate significant differences between treatment groups ($P < 0.05$, Student's *t*-test).

Assay	Group	ED_{50} ± S.E.	Slope	Ratio (saline/croton oil)
Enteropooling	Saline	0.039 ± 0.005^a	52.8 ± 1.0	3.0
	Croton oil	0.013 ± 0.004^b	59.2 ± 4.9	
Permeability	Saline	0.050 ± 0.030^a	53.7 ± 5.0	2.0
	Croton oil	0.025 ± 0.015^b	60.1 ± 3.5	

ments clearly demonstrate that intestinal inflammation significantly increases the potency of μ-opioid receptor agonists inhibiting intraluminal fluid accumulation.

3.3. Inhibitory effects of PL017 on enteropooling and intestinal permeability

These experiments were performed in order to evaluate the peripheral (intestinal) component of the effects of μ-opioid receptor agonists on fluid transport and permeability. PL017 is a μ-opioid receptor agonist that does not cross the blood-brain barrier (Chang et al., 1983), and when given systemically, produces its effects by binding to intestinal opioid receptors. Fig. 4 shows the dose–response relationships for PL017 on enteropooling (Fig. 4A) and intestinal permeability (Fig. 4B) measured in control animals and during inflammation. Inflammation shifted the dose–response curve to the left in a parallel manner and the potency of PL017, inhibiting enteropooling, increased approximately 3.6 times when compared to controls (Table 3). The inhibitory effects of PL017 on intestinal permeability increased 2.2 times, during inflammation. These results show that the enhanced effects of μ-opioid receptor agonists during inflammation are mediated by peripheral opioid receptors.

Table 3
ED_{50} values (mg/kg) of PL017 on enteropooling and intestinal permeability in control mice (saline) and during intestinal inflammation (croton oil)
Results are expressed as mean values ± S.E. of six or more animals per dose. For each assay, the ED_{50} in croton oil-treated mice was significantly different when compared to the ED_{50} in saline-treated animals. For each assay, different letters (a, b) indicate significant differences between treatment groups ($P < 0.05$, Student's *t*-test).

Assay	Group	ED_{50} ± S.E.	Slope	Ratio (saline/croton oil)
Enteropooling	Saline	1.16 ± 0.04[a]	29.7 ± 2.1	3.6
	Croton oil	0.32 ± 0.02[b]	28.8 ± 1.0	
Permeability	Saline	2.02 ± 0.03[a]	36.9 ± 2.3	2.2
	Croton oil	0.91 ± 0.03[b]	37.0 ± 2.2	

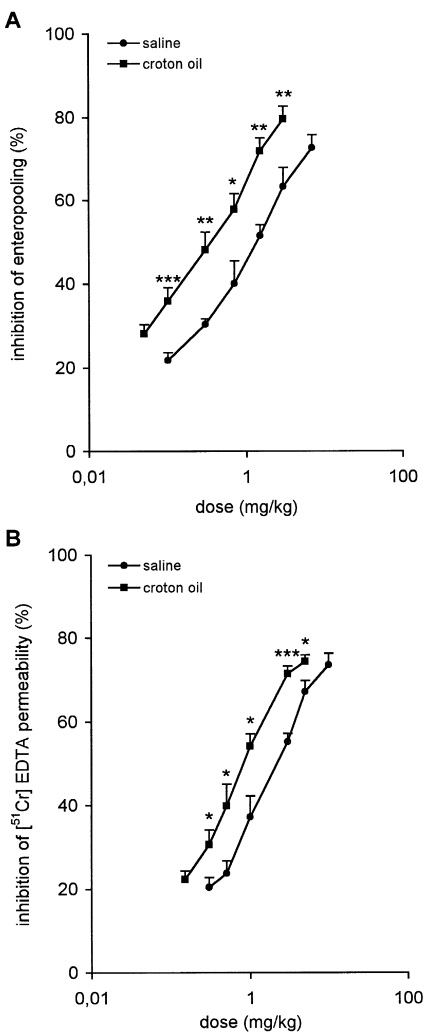

Fig. 4. Dose-related inhibition of (A) enteropooling and (B) [^{51}Cr] EDTA permeability by PL017 in mice primed with saline or croton oil. Each point represents the mean ± S.E. of at least six animals. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, when compared to saline (Student's *t*-test).

3.4. Antagonism of the inhibitory effects of morphine, fentanyl and PL017 by μ-opioid receptor antagonists

In order to evaluate the specificity of the responses observed during inflammation, the effects of μ-opioid receptor agonists were assessed after the administration of naloxone (0.1 mg/kg) or naloxone methiodide (0.3 mg/kg, a peripherally acting μ-opioid receptor antagonist), injected 15 min before the μ-opioid receptor agonists. These experiments were performed in saline- and croton oil-treated animals, testing the effects of the ED_{50} values of morphine, fentanyl, and PL017 on enteropooling and intestinal permeability. The results show that during inflammation, the effects of all μ-opioid receptor agonists on enteropooling (Fig. 5A) and permeability (Fig. 5B) were completely antagonized by naloxone and naloxone methiodide; similarly, the effects were completely antagonized in non-inflamed mice (saline-treated). For the sake of clarity in Fig. 5, we show enteropooling and permeability in animals treated with croton oil (as represented in Fig. 2), and the effect of each μ-opioid receptor agonist individually plus s.c. vehicle, naloxone or naloxone methiodide. The results demonstrate the opioid nature of the enhanced inhibitory effects of morphine, fentanyl and PL017, during intestinal inflammation. In addition, the results show that at the ED_{50} values, the effects can be completely antago-

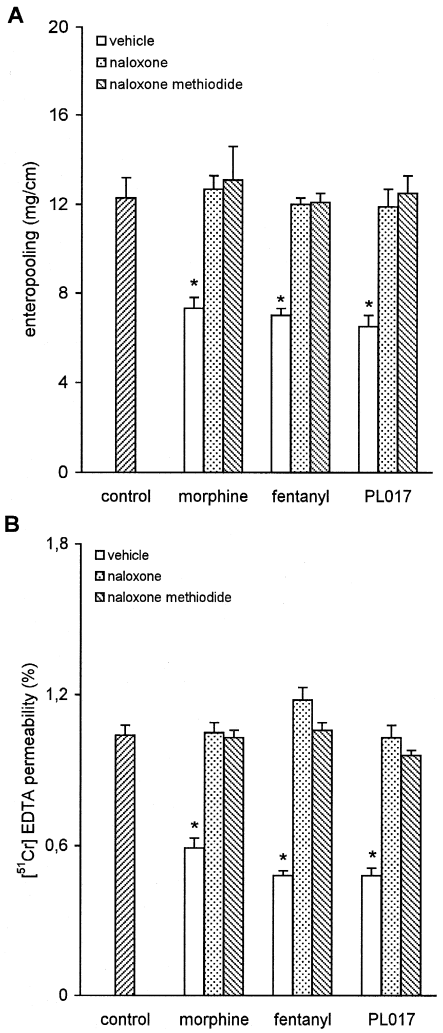

Fig. 5. Effect of vehicle, naloxone and naloxone methiodide on the inhibitory action of the ED_{50} doses to morphine, fentanyl and PL017 on (A) enteropooling and (B) [^{51}Cr] EDTA permeability, in animals treated with croton oil. Each column represents the mean ± S.E. of at least six animals. $^{*}P < 0.001$ (Student–Newman–Keuls test).

nized by a peripherally acting μ-opioid receptor antagonist.

4. Discussion

The aim of study was to determine and compare the inhibitory effects of μ-opioid receptor agonists on fluid transport and intestinal permeability in the absence (saline) and presence of intestinal inflammation (croton oil). In order to determine net mucosal water transport (pass of fluid into the lumen by paracellular and transcellular routes), we used the enteropooling assay that reflects both paracellular and transcellular routes and has been validated by different groups of investigators (Rivière et al., 1990; Farmer and Burks, 1991). Intestinal permeability was assessed by the blood-to-lumen transfer of [^{51}Cr] EDTA, an inert radiolabelled probe that crosses the epithelium by the paracellular pathway (Nylander et al., 1991; Bjarnason et al., 1995). This assay (but not enteropooling) requires renal pedicle ligation of all experimental animals (control and inflamed), and thus, the possible influence of a high blood urea nitrogen values on the enhanced effects of opioids during inflammation, would be negligible.

Our results show that acute intestinal inflammation induced by croton oil, produced a significant increase in fluid secretion (1.8 times) and intestinal permeability (2.5 times). The observed differences between the methods could be related to the relative lack of precision of the enteropooling technique, since the results are based on the weight of the gut before and after eliminating the intraluminal fluid. However, the transfer of [^{51}Cr] EDTA from blood to lumen accurately measures the passage of water and electrolytes through tight junctions.

Intestinal μ-opioid receptors have been reported to be present in the myenteric and submucosal plexuses (Bagnol et al., 1997), and in lower densities in epithelial cells of intestinal villi and crypts (Lang et al., 1996). Neuronal and extra-neuronal opioid receptors seem to play a role in the physiological inhibitory control of intestinal secretion. At present, there are no reports demonstrating the presence of opioid receptors in intestinal tight junctions that regulate water and electrolyte transport. However, the intragastric administration of loperamide reduces the blood-to-lumen leakage of [^{14}C]-erythrytol in ligated loops of rat colon (Farack and Loeschke, 1984). In our experimental conditions, μ-opioid receptor agonists (morphine, fentanyl and PL017) produced a dose-related inhibition of intestinal permeability in saline- and croton oil-treated animals. Since [^{51}Cr] EDTA is thought to pass to the intestinal lumen through paracellular pathways, a direct effect of μ-opioids decreasing the aperture of the tight junctions could be postulated. However, a neuronal (intrinsic innervation) versus a non-neuronal (tight junctions in the epithelial cells) site of action of μ-opioids cannot be demonstrated on the basis of the present experiments.

During inflammation, the main permeation pathway across the epithelium is paracellular (Madara and Stafford, 1989), and the subepithelial pressure induced by the inflammatory process could lead to electrolyte and fluid secretion within the lumen by this route (Goerg et al., 1983; Farack and Loeschke, 1984). In addition, inflammatory mediators decrease tight junction resistance, also increasing intestinal permeability (Gardiner et al., 1995; Wyatt et al., 1997). However, the effects of opioids on fluid secretion have not been carefully evaluated. In our experiments, morphine inhibited enteropooling and intestinal permeability in a dose-related manner both in controls and during inflammation. The increased potency observed during inflammation (4.8 and 3.7 times, on enteropooling and permeability, respectively) could be explained by a sensitization of the opioid receptors induced by the local inflammatory process. We have previously reported a sig-

nificant decrease in pH during inflammation (Pol and Puig, 1997) that could enhance receptor coupling to guanine-nucleotide-binding proteins (Selley et al., 1993), increasing the efficacy of opioid receptor agonists. However, a possible accumulation of the substances (opioids) in the inflamed tissue cannot be excluded. Because morphine is a well-known mixed μ/δ-opioid receptor agonist, we also studied the effects of fentanyl and PL017 (selective μ-opioid receptor agonists) during inflammation; these drugs had similar, but more modest effects both on enteropooling (3–3.6 times increasing potency) and permeability (2–2.2 times). Thus, our experiments show an increase in potency of μ/δ-opioid receptor agonists induced by the administration of croton oil. The expression of μ- and δ-opioid receptors in neuronal and epithelial cells during inflammation is being investigated in our laboratory using immunohistochemical methods.

The inhibitory effects of morphine on the gut after systemic administration are mediated by interaction with opioid receptors, located at central (spinal/supraspinal) and peripheral (gut) sites. It has been postulated that by this route, low doses (ED_{50} or below) of opioids predominantly bind to peripheral opioid receptors (Pol et al., 1996), while higher doses interact with central and peripheral opioid receptors. We have investigated the role of peripheral μ-opioid receptors in the regulation of intestinal fluid transport and permeability, using PL017, a selective μ-opioid receptor agonist with limited access to the central nervous system. The experiments were carried out in saline- and croton oil-treated animals, and the effects were compared to those of fentanyl, a selective μ-opioid receptor agonist that acts at central and peripheral sites. In both assays (enteropooling and permeability), fentanyl and PL017 showed comparable enhanced effects during inflammation, suggesting that in these experimental conditions, the drugs induce their inhibitory effects binding to peripheral (intestinal) opioid receptors. The peripheral component of the μ-opioid receptor agonists was further evaluated by the administration of naloxone methiodide, which completely reversed the effects of the ED_{50} values of all μ-opioid receptor agonists. These results show that during inflammation, the enhanced effects of μ-opioid receptor agonists are mediated by peripheral (intestinal) opioid receptors.

In our experiments, the increase in the potencies of μ-opioid receptor agonists were more prominent in the enteropooling than in the permeability assay. This finding could be related to the fact that enteropooling (although not quite precise), allegedly measures paracellular and transcellular fluid passage, and opioid receptors located in neuronal (plexus) and non-neuronal (crypts) sites, could be involved in the effects of μ-opioid receptor agonists. In all experimental conditions, the inhibitory effects of the μ-opioid receptor agonists were antagonized by naloxone, demonstrating that the enhanced effects of μ-opioid receptor agonists during inflammation are mediated by opioid receptors. We have previously reported that inflammation enhances the potency of morphine inhibiting gastrointestinal transit approximately 3 times (Pol et al., 1994). Interestingly, the present experiments show that inflammation increases the effects of morphine on enteropooling by 4.8 times, suggesting that both neuronal and extraneuronal opioid receptors could be sensitized during acute intestinal inflammation.

In summary, our results show that acute inflammation induces a significant increase in intestinal secretion and permeability. In both assays, the potency of μ-opioid receptor agonists was significantly increased during inflammation and the enhanced effects were completely reversed by naloxone. The effects of a peripherally acting opioid (PL017) were similar to those of drugs that cross the blood-brain barrier (fentanyl, morphine), demonstrating that peripheral (intestinal) opioid receptors mediate the enhanced effects of opioids during inflammation. The dose-related effects of opioids on intestinal secretion and permeability suggest that opioid receptors actively participate in the passage of water and electrolytes through tight junctions, and that inflammation induces a sensitization of μ-opioid receptors that mediate these effects.

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