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European Journal of Pharmacology 428 (2001) 127–136

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Antisense oligodeoxynucleotides to μ- and δ-opioid receptor mRNA block the enhanced effects of opioids during intestinal inflammation

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Received 12 April 2001; received in revised form 6 August 2001; accepted 7 August 2001

Abstract

Intestinal inflammation enhances the inhibitory effects of μ- and δ-opioids in the gut, possibly related to an increased receptor expression. We evaluated the effects of opioids after intraperitoneal administration of antisense oligodeoxynucleotides to μ- and/or δ-opioid receptor mRNA. Inflammation was induced in mice by intragastric administration of croton oil; gastrointestinal transit was assessed with charcoal and permeability with [^{51}Cr]etylenediaminetetraacetate ([^{51}Cr]EDTA). Baseline values were unaltered after antisense oligodeoxynucleotides. In controls, antisense oligodeoxynucleotides to μ-opioid receptor mRNA decreased the antitransit effects of morphine (27%) and [*N*-MePhe^{3},D-Pro^{4}]morphiceptin (PL017) (26%), and the reduction was significantly greater during inflammation (50% and 47%). A similar effect was observed on permeability (control: 41–21% decrease; inflamed: 66–45%). In both assays, antisense oligodeoxynucleotides to δ-opioid receptor mRNA also reduced the effects of [D-Pen^{2,5}]enkephalin (DPDPE) in a higher percentage during inflammation (43–32% controls, 60–49% inflamed). We show that antisense oligodeoxynucleotides to μ- and/or δ-opioid receptor mRNA are efficiently blocking the intestinal effects of opioids during inflammation, suggesting that an increased transcription of these receptors in the gut mediates the enhanced effects of opioids during inflammation.

Keywords: Antisense oligodeoxynucleotide; Gastrointestinal transit; Inflammation; Intestinal permeability; Intestine; Opioid receptor

1. Introduction

Cloning the different types of opioid receptors has provided new tools to study their synthesis and expression during different physio-pathological conditions (Evans et al., 1992; Thompson et al., 1993; Chen et al., 1993). The administration of antisense oligodeoxynucleotides designed to block the transcription of specific opioid receptor mRNA, selectively reduces the antinociceptive effects of μ- (Rossi et al., 1997), δ- (Sánchez-Blázquez et al., 1997) and κ-opioid receptor agonists (Pasternak et al., 1999). In the gut, the inhibitory effects of opioids on intestinal function are mediated by opioid receptors located in the central nervous system (brain and spinal cord) as well as by peripheral opioid receptors, on the intramural plexuses (Bagnol et al., 1997) and probably in epithelial cells (Lang et al., 1996; Nano et al., 2000). The relevance of the central versus peripheral opioid receptors in mediating the effects of opioids after systemic administration has not been established. Using the intracerebroventricular administration of antisense oligodeoxynucleotides to μ-opioid receptor mRNA, we have recently shown a reduction in the central effects of μ- but not δ-opioid receptor agonists on the inhibition of gastrointestinal transit and permeability (Pol et al., 1999). In addition, we have recently demonstrated that the potency of μ- and δ-opioid receptor agonists on the inhibition of gastrointestinal transit and permeability is significantly increased during intestinal inflammation, and that these effects are mediated by peripheral opioid receptors. The results of pharmacological experiments performed with β-funaltrexamine suggest that the number of functional μ-opioid receptors is increased during inflammation (Valle et al., 2001).

The aim of the present study was to investigate whether the intraperitoneal administration of antisense oligodeoxynucleotides to opioid receptor mRNA would alter the effects of systemically (subcutaneous) administered opioids. Several groups have demonstrated that antisense oligodeoxynucleotides administered by the intraperitoneal

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0014-2999/01/$ - see front matter
PII: S0014-2999(01)01281-X

route penetrate into most peripheral tissues (including the gut) but not into the brain (Karamysehv et al., 1993; Butler et al., 1997). Our working hypothesis was that, administered by this route, antisense oligodeoxynucleotides would block opioid receptor synthesis in the gut (with little or no effect in the central nervous system) and reduce the enhanced effects of opioids during intestinal inflammation. We used antisense oligodeoxynucleotides to μ- and δ-opioid receptor mRNA (alone and combined) and tested the effects of the mixed μ/δ-opioid receptor agonist morphine, which binds to central and peripheral opioid receptors, the peripheral μ-opioid receptor agonist *N*-[MePhe^3,D-Pro^4]morphiceptin (PL017), and the δ-opioid receptor agonist [D-Pen^{2,5}]enkephalin (DPDPE), which has a predominant peripheral action when administered subcutaneously. The selectivity of morphine for the δ-opioid receptor was also investigated using specific antisense oligodeoxynucleotides to μ- and δ-opioid receptor mRNA.

2. Methods

2.1. Animals

Experiments were performed with male Swiss CD-1 mice, weighing 20 to 25 g. Animals were housed under 12-h light and 12-h dark conditions in a room with controlled temperature (22 °C) and humidity (66%). Mice had free access to food and water and were allowed to become acclimated to their housing conditions for at least 1 week before the study. All experiments were conducted between 9:00 a.m. and 2:00 p.m. The study protocol was approved by the local Committee of Animal Use and Care of our Institution.

2.2. Intestinal inflammation induced by croton oil

In this study, we used a model of intestinal inflammation induced by the intragastric administration of two doses (0.05 ml) of croton oil 24 h apart; control animals received the same volume of intragastric saline. In both instances, animals were fasted for 18 h, except for free access to water, which was available for the duration of the study. In the present paper, animals without intestinal inflammation are referred to as controls throughout the study. Gastrointestinal transit and permeability studies were performed 4 days after the first dose of croton oil (Fig. 1). Morphological changes induced by croton oil were established by optical microscopy and included disruption of the mucosa with an increased number of macrophages (108%) in the mucosa and submucosa, and massive infiltration of lymphocytes in the submucosa (Puig and Pol, 1998).

2.3. Gastrointestinal transit

Gastrointestinal transit was measured according to the procedures used in our laboratory. After animals were fasted for 18 h, 0.25 ml of a suspension of 10% vegetable charcoal in 5% gum acacia (Sigma, St. Louis, MO) was administered intragastrically, and gastrointestinal transit was evaluated 20 min later (Pol et al., 1996). Animals were then killed by cervical dislocation, and the small intestine was separated from the omentum avoiding stretching. The length of intestine from the pyloric sphinc-

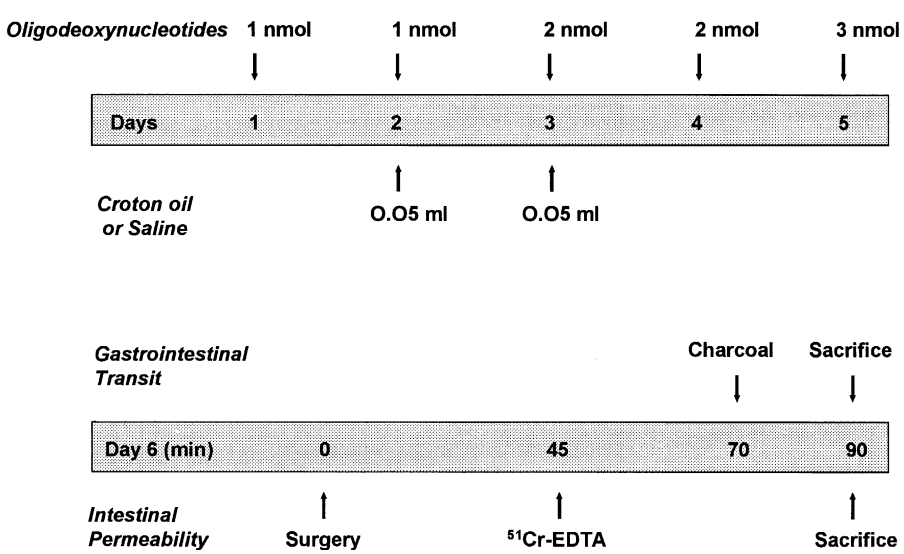

Fig. 1. Experimental design showing the protocol of administration of antisense oligodeoxynucleotides and croton oil (upper panel) and the time/s of evaluation (lower panel) of gastrointestinal transit (charcoal) and permeability ([^{51}Cr]EDTA).

ter to the ileocecal junction, and the distance travelled by the charcoal were measured. For each animal, gastrointestinal transit was calculated as the percentage (%) of distance travelled by the charcoal, relative to the total length of the small intestine (% of gastrointestinal transit).

2.4. Intestinal permeability

Intestinal permeability of the small intestine was assessed by measuring the blood-to-lumen transfer of [^{51}Cr]etylenediaminetetraacetate ([^{51}Cr]EDTA), according to the procedure used in our laboratory (Valle et al., 2000). After fasting for 18 h, mice were laparotomized under light halothane anaesthesia, and both renal pedicles were ligated to prevent excretion of the radioactive marker into the urine. Animals were allowed to recover for a period of 45 min; at this time 4 μCi of [^{51}Cr]EDTA was injected intravenously into a vein of the tail. Forty-five minutes later, animals were killed by cervical dislocation, the small intestine was removed, and the intestinal lumen was washed with 1 ml of saline (Fig. 1, lower panel). The [^{51}Cr]EDTA present in the fluid was measured with a gamma counter (LKB-Wallac, 1282 CompuGamma). Detected counts per minute are expressed as the percentage of the total counts administered. In these experiments, opioid receptor agonists were given subcutaneously, 15 min before the intravenous administration of [^{51}Cr]EDTA.

The experimental design for gastrointestinal transit and permeability evaluation is shown in Fig. 1.

2.5. Antisense oligodeoxynucleotides

The antisense oligodeoxynucleotides used in the study were: (a) antisense oligodeoxynucleotides to μ-opioid receptor mRNA 16-32 5′C*T*GATGTTCCCTGGG*C*C-3′, corresponding to nucleotides 16 to 32 of the murine μ-opioid receptor gene sequence (Sánchez-Blázquez et al., 1997; Pol et al., 1999); (b) antisense oligodeoxynucleotides to δ-opioid receptor mRNA 7–26 5′ G*C*ACGGGCAGAGGGCACC*A*G 3′, corresponding to nucleotides 7 to 26 of the murine δ-opioid receptor gene sequence (Lai et al., 1994); and (c) antisense oligodeoxynucleotides to random sequence-mRNA with the sequence 5′-G*C*CTTATTTACTACTTTC*G*C-3′ served as control (Gillardon et al., 1994; Sánchez-Blázquez et al., 1997). All antisense oligodeoxynucleotides were synthesised by GibcoBRL (Barcelona, Spain) and antisense oligodeoxynucleotide solutions were dissolved in sterile saline immediately before use.

In all experiments, antisense oligodeoxynucleotides were administered intraperitoneally according to the following schedule: 1 nmol on days 1 and 2; 2 nmol on days 3 and 4; and 3 nmol on day 5. On day 6, the inhibitory effects of opioids on gastrointestinal transit or permeability were evaluated (Fig. 1). To assess the specificity of the treatments with antisense oligodeoxynucleotides to μ- and δ-opioid receptor mRNA, we used mice that received intraperitoneal vehicle and mice injected with antisense oligodeoxynucleotides to random-sequence mRNA, using 6–8 animals per group.

2.6. Groups of experiments

The inhibitory effects of μ and δ opioids administered subcutaneously were evaluated in controls and mice with intestinal inflammation. All drugs were tested on gastrointestinal transit and permeability: (1) the effects of morphine were assessed in animals pretreated with antisense oligodeoxynucleotides to μ-opioid receptor mRNA and antisense oligodeoxynucleotides to δ-opioid receptor mRNA alone, as well as their combination antisense oligodeoxynucleotides to (μ + δ)-opioid receptor mRNA; (2) the effects of PL017 were established in animals pretreated with antisense oligodeoxynucleotides to μ-opioid receptor mRNA; and (3) DPDPE was tested in animals pretreated with antisense oligodeoxynucleotides to δ-opioid receptor mRNA.

Regarding the doses of the opioids tested in the study, for morphine and PL017, we used the ED_{80} doses derived from dose–response relationships obtained in our laboratory. For DPDPE, the doses that produced a maximal inhibitory effect in the different experimental conditions were used (Table 1).

2.7. Drugs

We used morphine sulphate (Alcaiber, Madrid, Spain), PL017 ([*N*-MePhe^3, D-Pro^4]-morphiceptin) (Peninsula Laboratories, Belmont, CA, USA) and DPDPE ([D-Pen^{2,5}]-enkephalin) (Research Biochemicals, USA). Drugs were dissolved in sterile pyrogen-free 0.9% sodium chloride just before use and injected subcutaneously (10 ml/kg).

2.8. Data analysis

The inhibitory effects of the opioid receptor agonists are expressed as the percent inhibition of the gastrointestinal

Table 1
Individual doses (mg/kg) of each opioid used in the study

Assay	Transit		Permeability	
Treatment	Control	Inflammation	Control	Inflammation
Morphine	7	1	15	3
PL017	3	0.5	10	1.5
DPDPE	10	1	10	0.05

For morphine and PL017 the doses are the ED_{80} values derived from dose–response relationships in control conditions and during inflammation. Doses that produced a maximal inhibitory effect in the different experimental conditions were used for DPDPE (Puig and Pol, 1998; Valle et al., 2001).

transit or permeability in drug-treated animals (test) when compared with the mean gastrointestinal transit or permeability measured in the corresponding group of control mice ($n = 6–8$).

$$\%\,\text{inhibition} = [(\text{control} - \text{test})/(\text{control})] \times 100$$

Data are expressed as group means ± S.E. Statistical analysis for significant differences between two groups was assessed by Student's *t*-test. When multiple groups were compared, one- or two-way analysis of variance (ANOVA) was used, followed by a Student–Newman–Keuls test, whenever applicable. A value of $P < 0.05$ was considered significant.

3. Results

3.1. Effect of pretreatment with antisense oligodeoxynucleotides to μ, δ and (μ + δ)-opioid receptor mRNA on gastrointestinal transit and permeability, in the presence and absence of intestinal inflammation

Experiments were performed in order to determine the effects of the intraperitoneal administration of antisense oligodeoxynucleotides to μ- and δ-opioid receptor mRNA individually, and in combination, on basal gastrointestinal transit and permeability. Antisense oligodeoxynucleotides to random-sequence mRNA and vehicle-treated animals were also tested. The results show that the administration of antisense oligodeoxynucleotides did not alter basal gastrointestinal transit or permeability, either in controls or in mice with intestinal inflammation (Table 2). A two-way ANOVA (group, treatment) demonstrated a significant effect of the group with inflammation ($P < 0.0001$), but not of the treatment (antisense oligodeoxynucleotides) or their interaction. In these experiments, inflammation of the gut increased gastrointestinal transit and permeability 1.5 and 3 times, respectively, when compared to control values. Pretreatment with any of the antisense oligodeoxynu-

Table 2
Percent gastrointestinal transit and permeability under basal conditions in the different groups of the study

Assay	Transit (%)		Permeability (%)	
Treatment	Control	Inflammation	Control	Inflammation
Vehicle	50.5 ± 1.7	75.8 ± 2.3[a]	0.41 ± 0.05	1.22 ± 0.10[a]
μ-ODN	44.9 ± 3.1	68.1 ± 2.8[a]	0.37 ± 0.01	1.10 ± 0.06[a]
δ-ODN	46.1 ± 2.4	69.8 ± 2.5[a]	0.38 ± 0.02	1.09 ± 0.10[a]
(μ + δ)-ODN	44.8 ± 2.7	69.7 ± 2.5[a]	0.37 ± 0.04	1.10 ± 0.04[a]
random-ODN	48.4 ± 0.7	72.4 ± 3.1[a]	0.39 ± 0.02	1.18 ± 0.07[a]

Results are expressed as mean values ± S.E. for 6–8 animals per group. In all instances inflammation increases % transit and permeability. No significant differences are observed between the different treatments (vehicle, antisense oligodeoxynucleotides) in each group of study (control, inflammation) (Student–Newman–Keuls test). μ-ODN, antisense oligodeoxynucleotides to μ-opioid receptor mRNA; δ-ODN, antisense oligodeoxynucleotides to δ-opioid receptor mRNA; (μ + δ)-ODN, antisense oligodeoxynucleotides to (μ + δ)-opioid receptor mRNA and random-ODN, antisense oligodeoxynucleotides to random-sequence mRNA.

[a] Indicates a $P < 0.01$ when controls and animals with intestinal inflammation were compared (Student's *t*-test).

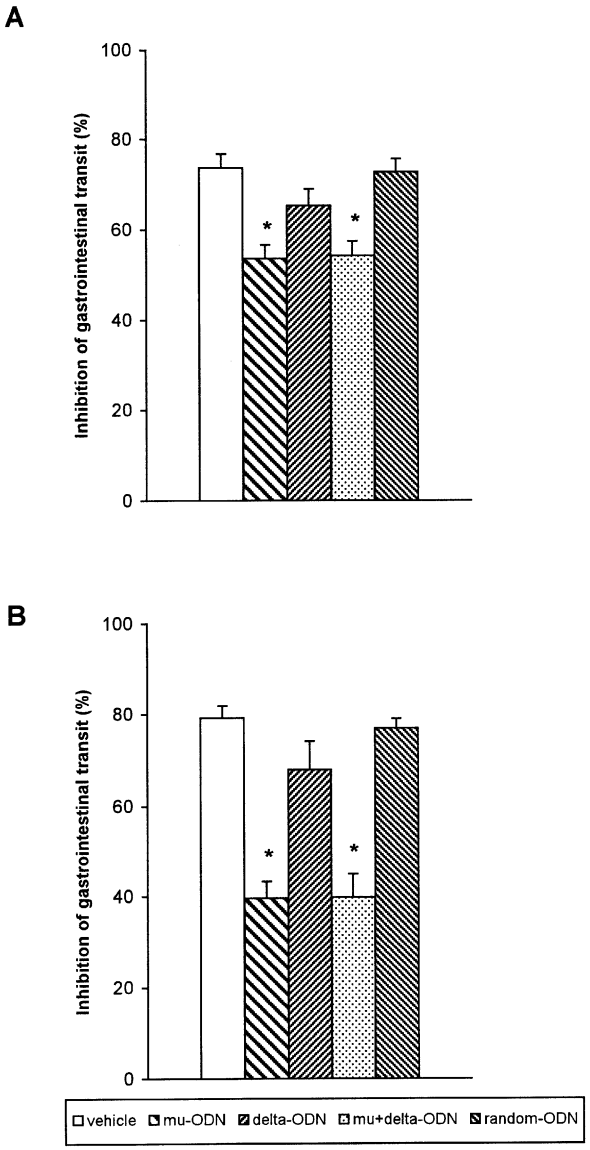

Fig. 2. Percent inhibition of gastrointestinal transit induced by the subcutaneous administration of morphine in controls (7 mg/kg, panel A) and in animals with intestinal inflammation (1 mg/kg, panel B). Animals were treated with vehicle, antisense oligodeoxynucleotides to μ-, δ-, (μ + δ)-opioid receptor or random mRNA, according to the shade-codes included in the lower part of the figure. Each column represents the mean ± S.E. for 6–8 animals. In each panel (A, B) the * indicates significant differences between animals treated with antisense oligodeoxynucleotides to μ- or (μ + δ)-opioid receptor mRNA and animals treated with vehicle, antisense oligodeoxynucleotides to δ-opioid receptor or random-sequence mRNA ($P < 0.05$; Student–Newman–Keuls test). mu-ODN, antisense oligodeoxynucleotides to μ-opioid receptor mRNA; delta-ODN, antisense oligodeoxynucleotides to δ-opioid receptor mRNA; mu + delta-ODN, antisense oligodeoxynucleotides to (μ + δ)-opioid receptor mRNA and random-ODN antisense, oligodeoxynucleotides to random-sequence mRNA.

cleotides (or combination) decreased transit and permeability by approximately 8–12%.

3.2. Effects of morphine in animals pretreated with antisense oligodeoxynucleotides to μ- or δ-opioid receptor mRNA administered alone or in combination

The effects of morphine on transit and permeability were evaluated in controls and in mice with intestinal inflammation, in animals pretreated with antisense oligodeoxynucleotides to μ-, δ-, (μ + δ)-opioid receptor mRNA or antisense oligodeoxynucleotides to random-sequence mRNA. We first present the results for gastrointestinal transit. In control animals, the administration of morphine (7 mg/kg) to animals receiving intraperitoneal saline induced a 73.8 ± 3.0% inhibition of transit. In animals pretreated with antisense oligodeoxynucleotides to μ- or (μ + δ)-opioid receptor mRNA (Fig. 2A), the effect of morphine was significantly decreased to 53.5 ± 3.1% and 54.2 ± 3.2%, respectively ($P < 0.05$), that is by approximately 27% (Table 3). The effect of morphine was unaltered in animals receiving antisense oligodeoxynucleotides to δ-opioid receptor or random-sequence mRNA. During inflammation, the antitransit effects of morphine are enhanced, and thus we tested a dose (1 mg/kg) that induced the same inhibition as that induced by 7 mg/kg of morphine in control animals. Under these experimental conditions, morphine induced a 79.2 ± 2.6% inhibition of transit, both in vehicle and antisense oligodeoxynucleotides to random-sequence mRNA-pretreated animals; however, the inhibitory effect of morphine (1 mg/kg) was significantly decreased to 39.6 ± 3.7 and 39.8 ± 5.1% in animals pretreated with antisense oligodeoxynucleotides to μ- or (μ + δ)-opioid receptor mRNA, respectively (Fig. 2B). The results were analysed by a two-way ANOVA (group and treatment), which demonstrated a significant effect of the treatment with antisense oligodeoxynucleotides ($P < 0.0001$) and of the interaction ($P < 0.008$). In each group (control and inflammation), the effect of the treatment was related to the administration of antisense oligodeoxynucleotides to μ- or (μ + δ)-opioid receptor mRNA ($P < 0.05$, one way-ANOVA). The significant effect of the interaction was related to a greater antagonism of the effects of morphine during inflammation in animals pretreated with active antisense oligodeoxynucleotides. Table 3 shows percent changes in the effects of morphine induced by the antisense oligodeoxynucleotides in controls (27% decrease) and in mice with intestinal inflammation (50% decrease). The results indicate that the effects of equipotent (antitransit) doses of morphine under control conditions, are blocked to a greater extent by antisense oligodeoxynucleotides in the presence of inflammation.

When intestinal permeability was evaluated in control animals, the administration of morphine (15 mg/kg) induced a 68.7 ± 2.5% inhibition. In control animals, pretreatment with antisense oligodeoxynucleotides to μ- or (μ + δ)-opioid receptor mRNA (Fig. 3A) significantly decreased the inhibitory effects of morphine to 40.2 ± 4.3% and 41.1 ± 4.3%, respectively; however no significant effects were observed after treatment with antisense oligodeoxynucleotides to δ-opioid receptor or random-sequence mRNA. During inflammation (Fig. 3B), morphine (3 mg/kg) produced a 77.1 ± 2.9% inhibition of permeability, an effect which was significantly decreased to 26.2 ± 3.6% and 26.4 ± 4.1% in animals pretreated with antisense oligodeoxynucleotides to μ- or (μ + δ)-opioid receptor mRNA, respectively; no significant effects were observed in animals receiving antisense oligodeoxynucleotides to δ-opioid receptor or random-sequence mRNA. Two-way ANOVA (group and treatment) showed a significant effect of the treatment (oligodeoxynucleotides, $P < 0.0001$) and of the interaction ($P < 0.005$). In each group (control and inflammation), the effect of the treatment was related to the administration of antisense oligodeoxynucleotides to μ- or (μ + δ)-opioid receptor mRNA ($P < 0.05$, one-way ANOVA), and the interaction was related to the greater antagonism of the effects of morphine during inflammation (66% versus 41%, $P < 0.04$, Table 3).

Table 3
Percent decrease in the effects of the opioid receptor agonists (morphine, PL017 and DPDPE) on the inhibition of transit and permeability in controls and in mice with intestinal inflammation

Treatment	Opioid receptor agonist	Transit		Permeability	
		Control	Inflammation	Control	Inflammation
μ-ODN	Morphine	27.5 ± 4.2	50.0 ± 4.7[a]	41.4 ± 5.6	66.0 ± 5.8[a]
(μ + δ)-ODN	Morphine	26.5 ± 4.3	49.7 ± 5.5[a]	40.2 ± 5.2	65.7 ± 5.3[a]
μ-ODN	PL017	26.0 ± 3.1	47.0 ± 3.5[a]	20.6 ± 5.1	45.0 ± 5.5[a]
δ-ODN	DPDPE	42.8 ± 3.4	60.0 ± 5.3[a]	32.4 ± 4.9	49.4 ± 3.8[a]

Results are expressed as mean values ± S.E. for 6–8 animals per group. The doses of each opioid used in these experiments are shown in Table 1. μ-ODN, antisense oligodeoxynucleotides to μ-opioid receptor mRNA; (μ + δ)-ODN, antisense oligodeoxynucleotides to (μ + δ)-opioid receptor mRNA and δ-ODN, antisense oligodeoxynucleotides to δ-opioid receptor mRNA.

[a] For each opioid receptor agonist and assay (gastrointestinal transit or intestinal permeability), this indicates a $P < 0.05$, when comparing controls and animals with inflammation (Student's *t*-test).

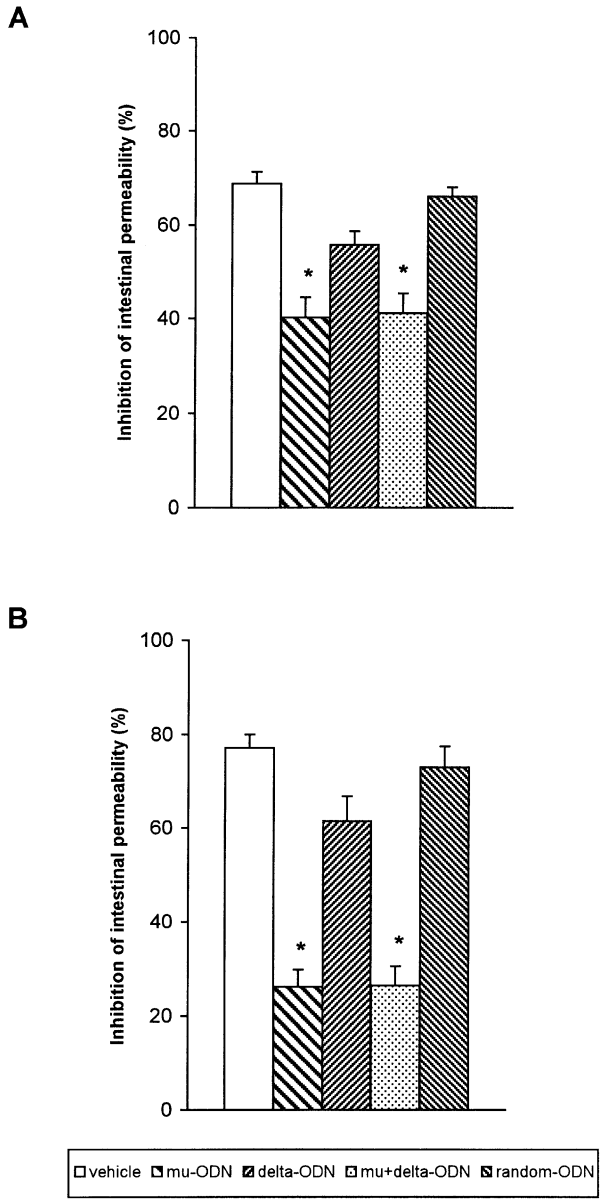

Fig. 3. Percent inhibition of permeability induced by morphine after treatment with vehicle, antisense oligodeoxynucleotides to μ-, δ- or (μ + δ)-opioid receptor or random-sequence mRNA. Panel A shows the effects of morphine (15 mg/kg) in control animals and panel B (3 mg/kg) in animals with intestinal inflammation. Each column represents the mean ± S.E. for 6–8 animals. In each panel (A, B) the * indicates significant differences between animals treated with antisense oligodeoxynucleotides to μ- or (μ + δ)-opioid receptor mRNA and animals treated with vehicle, antisense oligodeoxynucleotides to δ-opioid receptor or random-sequence mRNA ($P < 0.05$; Student–Newman–Keuls test). mu-ODN, antisense oligodeoxynucleotides to μ-opioid receptor mRNA; delta-ODN, antisense oligodeoxynucleotides to δ-opioid receptor mRNA; mu + delta-ODN, antisense oligodeoxynucleotides to (μ + δ)-opioid receptor mRNA and random-ODN, antisense oligodeoxynucleotides to random-sequence mRNA.

3.3. Effects of PL017 in animals pretreated with antisense oligodeoxynucleotides to μ-opioid receptor mRNA

In these experiments, we evaluated the effects of PL017 (a peripheral μ-opioid receptor agonist) on gastrointestinal transit and permeability in controls and in mice with intestinal inflammation after pretreatment with antisense oligodeoxynucleotides to μ-opioid receptor mRNA. In controls, PL017 (3 mg/kg) induced a 71.5 ± 2.1% inhibition of gastrointestinal transit, and pretreatment with antisense oligodeoxynucleotides to μ-opioid receptor mRNA (Fig. 4A) significantly decreased the effect to 52.9 ± 1.5%; no significant effect was observed in animals receiving antisense oligodeoxynucleotides to random-sequence mRNA. In mice with intestinal inflammation, PL017 (0.5 mg/kg) produced a 71.1 ± 2.6% inhibition of transit, which was significantly decreased to 37.7 ± 2.5 in animals pretreated with antisense oligodeoxynucleotides to μ-opioid receptor mRNA; no decrease was observed in animals receiving antisense oligodeoxynucleotides to random-sequence mRNA. Thus, pretreatment with antisense oligodeoxynucleotides to μ-opioid receptor mRNA reduced by 26% and 47% the effects of PL017 in controls and in mice with intestinal inflammation, respectively ($P < 0.001$, Table 3). The results were analysed by a two-way ANOVA and revealed a significant effect of the group (inflammation; $P < 0.03$), the treatment with antisense oligodeoxynucleotides to μ-opioid receptor mRNA ($P < 0.001$) and their interaction ($P < 0.02$).

Experiments performed on intestinal permeability showed that, in control animals, the administration of PL017 (10 mg/kg) induced a 72.6 ± 3.1% inhibition of intestinal permeability. Pretreatment with antisense oligodeoxynucleotides to μ-opioid receptor mRNA (Fig. 4B) significantly decreased the effect to 57.6 ± 4.4%, while no effect was observed after administration of antisense oligodeoxynucleotides to random-sequence mRNA. During inflammation, PL017 (1.5 mg/kg) produced a 71.8 ± 2.2% inhibition of permeability, an effect that was significantly decreased to 39.5 ± 4.7 in animals receiving antisense oligodeoxynucleotides to μ-opioid receptor mRNA. Thus, pretreatment with antisense oligodeoxynucleotides to μ-opioid receptor mRNA induced 20.6% and 45.0% decreases in controls and animals with inflammation, respectively ($P < 0.03$, Table 3). Two-way ANOVA showed a significant effect of the group ($P < 0.05$), the treatment ($P < 0.001$) and their interaction ($P < 0.05$). In each group (control and inflammation), the effect of the treatment was related to the administration of antisense oligodeoxynucleotides to μ-opioid receptor mRNA ($P < 0.05$, one-way ANOVA), and the effect of the interaction was associated with a greater antagonism of the effects of PL017 during inflammation in animals pretreated with antisense oligodeoxynucleotides to μ-opioid receptor mRNA.

3.4. Effects of DPDPE in animals pretreated with antisense oligodeoxynucleotides to δ-opioid receptor mRNA

The inhibitory effects of DPDPE on transit and permeability were also evaluated in animals treated with vehicle,

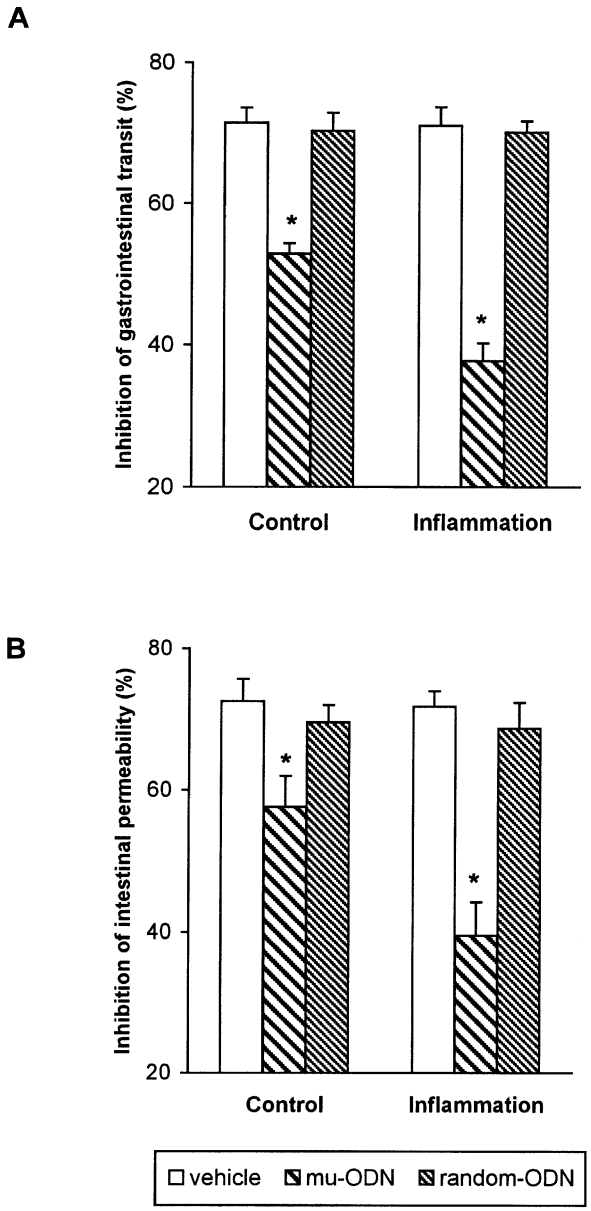

Fig. 4. Percent inhibition of gastrointestinal transit (panel A) and permeability (panel B) induced by the subcutaneous administration of PL017, in control (transit, 3 mg/kg and permeability, 10 mg/kg) and in animals with intestinal inflammation (transit, 0.5 mg/kg and permeability, 1.5 mg/kg). Animals were pretreated intraperitoneally with vehicle, antisense oligodeoxynucleotides to μ-opioid receptor or random-sequence mRNA according to the code-shade included in the figure. Each column represents the mean ± S.E. for 6–8 animals. In each panel (A, B) and group (control or inflammation) the * indicates significant differences between animals treated with antisense oligodeoxynucleotides to μ-opioid receptor mRNA and animals treated with vehicle or random-sequence mRNA ($P < 0.05$; Student–Newman–Keuls test). mu-ODN, antisense oligodeoxynucleotides to μ-opioid receptor mRNA and random-ODN, antisense oligodeoxynucleotides to random-sequence mRNA.

antisense oligodeoxynucleotides to random-sequence or δ-opioid receptor mRNA (Fig. 5). The antisense oligodeoxynucleotides to δ-opioid receptor mRNA decreased the effects of DPDPE on gastrointestinal transit by 42.8% and 60% in controls (at a dose of 10 mg/kg) and in mice with intestinal inflammation (1 mg/kg), respectively ($P < 0.03$, Table 3). Similar effects were observed on permeability,

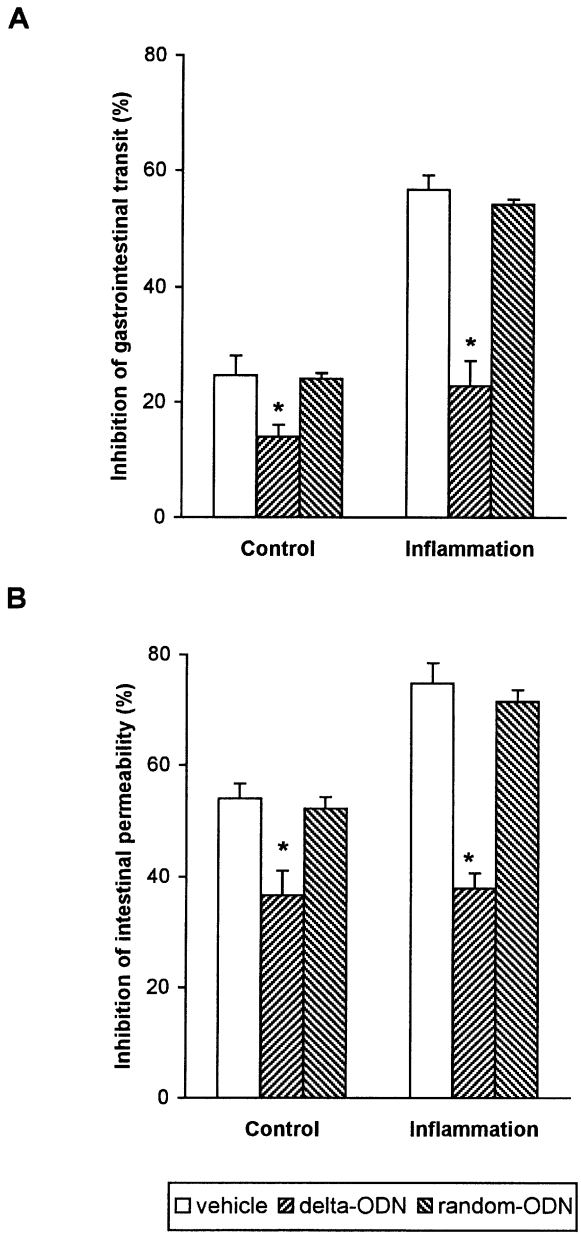

Fig. 5. Percent inhibition of gastrointestinal transit (A) and permeability (B) induced by the subcutaneous administration of DPDPE in controls (10 mg/kg in each test) and in animals with intestinal inflammation (transit, 1 mg/kg and permeability, 0.05 mg/kg). Animals were pretreated intraperitoneally with vehicle, antisense oligodeoxynucleotides to δ-opioid receptor or random-sequence mRNA. Each column represents the mean ± S.E. for 6–8 animals. In each panel (A, B) and group (control or inflammation) the * indicates significant differences between animals treated with antisense oligodeoxynucleotides to δ-opioid receptor mRNA and animals treated with vehicle or random-sequence mRNA ($P < 0.05$; Student–Newman–Keuls test). delta-ODN, antisense oligodeoxynucleotides to δ-opioid receptor mRNA and random-ODN, antisense oligodeoxynucleotides to random sequence mRNA.

where pretreatment with antisense oligodeoxynucleotides to δ-opioid receptor mRNA induced decreases of 32.4% (control, dose of 10 mg/kg) and 49.4% (inflammation, 0.05 mg/kg) ($P < 0.04$, Table 3). A two-way ANOVA revealed a significant effect of the group ($P < 0.0001$), the treatment ($P < 0.001$) and their interaction ($P < 0.05$) both on gastrointestinal transit (Fig. 5A) and on permeability (Fig. 5B). In all instances, the effect of treatment (antisense oligodeoxynucleotides) was related to the administration of antisense oligodeoxynucleotides to δ-opioid receptor mRNA (Student–Newman–Keuls test; $P < 0.05$) and that of the interaction (group by treatment) was related to a greater blockade of the effects of DPDPE during inflammation.

In Table 3, we summarise the percent decrease in the effects of the opioid receptor agonists induced by antisense oligodeoxynucleotides under all experimental conditions used in the study. The table shows that blockade of the synthesis of μ- or δ-opioid receptors decreases the effect of the opioid receptor agonists in control animals by approximately 20–43%, while during inflammation the administration of the same doses of antisense oligodeoxynucleotides reduces the effect of the opioid receptor agonists by 45–66%.

4. Discussion

Recent experiments from our laboratory have shown that during intestinal inflammation, the inhibitory effects of μ- and δ-opioids on gastrointestinal transit and permeability are increased approximately 10 times (Puig and Pol, 1998; Valle et al., 2001). In the present investigation we tested the hypothesis that the enhanced effects observed during inflammation could be related to an increase in the local synthesis of μ- and δ-opioid receptors. Using the intraperitoneal administration of antisense oligodeoxynucleotides to μ- and δ-opioid receptor mRNA as tools to block the expression of these receptors, we were able to demonstrate that the effects of μ- and δ-opioid receptor agonists are decreased to a greater extent during inflammation than under control conditions. We postulate that the inflammatory process could activate the transcription/translation of the mRNA that codes for μ- and δ-opioid receptors, and that antisense oligodeoxynucleotides would then be more effective in blocking the response to exogenously administered opioids. Thus, our results suggest a de novo synthesis of μ- and δ-opioid receptors during inflammation.

The intraperitoneal administration of antisense oligodeoxynucleotides did not significantly alter gastrointestinal transit or permeability under control conditions or in animals with intestinal inflammation. Similarly, the administration of the same antisense oligodeoxynucleotides to μ-opioid receptor mRNA by the intracerebroventricular route (at the same doses and treatment schedule) did not alter gastrointestinal transit or permeability in control animals (Pol et al., 1999). The present results suggest that, under baseline conditions, μ- or δ-opioid receptors seem to play a minor role in the physiological control of intestinal function. Gastrointestinal transit has been shown to be decreased by approximately 30% in μ-opioid receptor knockout mice when compared to that of heterozygous and wild-type animals (Roy et al., 1998); the dissimilarity with our results could be related to the presence of residual functional opioid receptors that remain active after treatment with antisense oligodeoxynucleotides (Sánchez-Blázquez et al., 1997).

Inflammation increased gastrointestinal transit and permeability approximately 1.5 and 3 times in control animals (Table 2), and this increase was unaltered in animals treated with antisense oligodeoxynucleotides to μ-, δ- or (μ + δ)-opioid receptor mRNA. In our model of inflammation, we observed a severe disruption of the inner layers of the gut (epithelium, mucosa and submucosa), whereas the nervous plexuses and muscular layers were unaffected. Thus the morphological changes seen in our model, could explain why permeability was enhanced during inflammation.

Croton oil is an irritant agent that induces inflammation in different tissues, such as the cornea (Villena et al., 1999), skin (Blazso et al., 1999), mucous membranes (Colorado et al., 1991) and small intestine (Puig and Pol, 1998). There are no references in the literature regarding possible toxic effects of croton oil in the liver or that it induces or impairs hepatic metabolism. Thus, possible systemic effects of croton oil after intragastric administration cannot be excluded at present. In our study, chronic croton oil administration decreased the ED_{50} of the opioid receptor agonists, whereas the amount of antisense oligodeoxynucleotides required to block their effects was significantly increased. This suggests that the metabolism of the opioid receptor agonists was not increased by croton oil, although, specific induction of the metabolism of the antisense oligodeoxynucleotides cannot be excluded.

The administration of antisense oligodeoxynucleotides to μ-opioid receptor mRNA to control animals significantly reduced the effects of morphine by 27.5–41.4% (on transit and permeability) and the effects were further decreased during inflammation (50–66%). Thus the increased potency of morphine observed during inflammation could be related to an enhanced μ-opioid receptor gene expression in the gut. Experiments carried out with μ-opioid receptor knockout mice show that the antinociceptive effects of morphine (Loh et al., 1998), its place-preference activity, physical dependence (Matthes et al., 1998) and immunosuppresion (Gavériaux-Ruff et al., 1998) are abolished, supporting a preferential binding of morphine to μ-opioid receptors. The possible contribution of δ-opioid receptors to the inhibitory effects of morphine in the gut was assessed in animals treated with antisense oligodeoxynucleotides to δ-opioid receptor mRNA. Under

these conditions, the effects of morphine were unaltered, thus suggesting that δ-opioid receptors are not actually involved. Moreover, the concurrent administration of antisense oligodeoxynucleotides to (μ + δ)-opioid receptor mRNA did not alter the inhibitory effects of morphine when compared to the effect of treatment with antisense oligodeoxynucleotides to μ-opioid receptor mRNA alone, which further supports the notion that in the gut, morphine binds predominantly to μ-opioid receptors. Additional evidence supporting a predominant effect of morphine on μ-opioid receptors is provided by the experiments of Matthes et al. (1998), who have shown that blockade of δ-opioid receptors by naltrindole does not alter the antinociceptive effects of morphine. To validate this observation in the gut, we evaluated the antitransit effects of morphine (7 mg/kg) in control animals after the administration of naltrindole (3 mg/kg, intraperitoneal). We observed a minor decrease in the antitransit effect of morphine that was not statistically significant (morphine + vehicle: 73.8 ± 3.0% inhibition, versus morphine + naltrindole 65.6 ± 2.6% inhibition; $P > 0.05$), a finding which supports the results of Matthes et al., (1998).

Previous studies have demonstrated that the effects of systemic PL017 and DPDPE on intestinal function are mediated by peripheral (intestinal) opioid receptors (Shook et al., 1987). In the present study, we show that the intraperitoneal administration of antisense oligodeoxynucleotides reduces the effects of peripheral opioid receptor agonists both in controls and in mice with intestinal inflammation, thus demonstrating that, when administered by this route, antisense oligodeoxynucleotides are able to block the expression of μ- and δ-opioid receptors in the gut. The present results support the peripheral site of action of these opioid receptor agonists, and suggest that there is an increased expression of both μ- and δ-opioid receptors during inflammation.

In conclusion, our study shows that the intraperitoneal administration of antisense oligodeoxynucleotides to μ- or δ-opioid receptor mRNA, blocks the effects of μ- and δ-opioid receptor agonists to a greater extent in the presence of inflammation. The results suggest that an increased transcription of these receptors in the gut mediates the enhanced effects of opioids during inflammation. Moreover, the present experiments show that the peripheral (intraperitoneal) administration of specific antisense oligodeoxynucleotides to μ- or δ-opioid receptor mRNA is effective in blocking μ- and δ-opioid receptor synthesis in the gut. Blockade of the expression of μ- or δ-opioid receptors did not significantly alter gastrointestinal transit or permeability, thus suggesting a poor physiological inhibitory control by the endogenous opioid system. Our results also show that, both under control conditions and during inflammation, morphine induces inhibition of gastrointestinal transit and permeability by binding mainly to μ-opioid receptors, with a small or negligible contribution of δ-opioid receptors.

Acknowledgements

The authors thank Mr. Sergi Leánez for his excellent technical assistance.

This work was partially supported by grants from CYCYT, PM98-0155 and FIS, 00/0658, Madrid; Fundació La Marató de TV3, 2032/97 and Generalitat de Catalunya 1999SGR00244, Barcelona, Spain.

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