Peripheral Effects of Opioids in a Model of Chronic Intestinal Inflammation in Mice1,2

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ABSTRACT

The study describes a model of chronic intestinal inflammation in mice. Inflammation was induced by the administration of one dose of croton oil (CO) (acute CO) or two doses (chronic CO) of intragastric CO, whereas controls received saline (SS); GI transit was measured with charcoal. Chronic CO induced intestinal inflammation substantiated by optical microscopy, weight loss (20%) and a 25% increase in GI transit. The ED50 values in SS animals were 1.67 ± 0.13 mg/kg for morphine and 0.038 ± 0.006 mg/kg for fentanyl; chronic CO significantly decreased the ED50 values to 0.16 ± 0.03 mg/kg (morphine) and 0.006 ± 0.0005 mg/kg (fentanyl). Thus the potency of morphine increased 10.4 times and that of fentanyl 6.3 times. The effects of enkephalin, but not those of U-50488H, were also significantly enhanced during chronic CO. The antitransit effects of p.o. loperamide increased 11.7 times during chronic CO. All effects were reversed by specific antagonists. The fraction of the active opioid receptor that mediates the antitransit effects of morphine was evaluated using β-funaltrexamine. In chronic CO, the doses of β-funaltrexamine needed to antagonize 1 mg/kg of morphine were significantly higher than in SS and acute CO, and the ED50/Kd ratio was 20 times lower. These results suggest an increase in the active concentration of μ-opioid receptors during chronic inflammation.

Studies performed in different models of articular inflammation have demonstrated an increased antinoceptive effect of opioids (Stein, 1995), mediated by peripheral OR located in the rat paw (Stein et al., 1989). Different mechanisms have been postulated to explain the enhanced effects of opioids during acute and chronic articular inflammation; a “sensitization” of peripheral receptors, induced by the local inflammatory response, could account for the increased potency during acute inflammation (Antonijevic et al., 1995). During chronic inflammation, an increase in the actual number of functionally active OR (Zhang et al., 1996), together with an enhanced axonal transport (Hassan et al., 1993), without changes in the level of mRNA that codifies μ-OR, has been documented (Schäfer et al., 1995).

In addition to their antinociceptive effects, opioids inhibit GIT in different animal models and in the human. Both the antitransit and the antinoceptive effects are mediated by μ-OR, although other OR subtypes are also involved. In a recent investigation, we have reported that acute intestinal inflammation increases the antitransit effects of morphine approximately 3-fold (Pol et al., 1994). On the basis of the results obtained in the chronic arthritic rat model, we hypothesized that chronic intestinal inflammation could further enhance the antitransit effects of opioids. In our former model, the intragastric administration of CO induced an acute inflammatory response of the small intestine within 3 h of its administration. Under these experimental conditions, a significant increase in the antitransit effects of μ and δ (but not κ) opioids (Pol et al., 1994) mediated by peripheral OR was demonstrated. The direct correlation between inflammation and enhanced effects of opioids was validated after treatment with castor oil, a cathartic agent that does not induce intestinal inflammation (Pol et al., 1996); treatment with castor oil did not alter the antitransit effects of opioids.

The aims of the present investigation were 1) to characterize and validate a model of chronic intestinal inflammation induced by the intragastric administration of CO, 2) to determine the potency of receptor-specific opioids and their reversibility by antagonists during chronic inflammation, 3) to establish the peripheral component of the response, and 4) to investigate the mechanisms that may mediate the enhanced response to opioids during chronic intestinal inflammation.

Materials and Methods

Animals. Male Swiss CD-1 mice (Charles River, S.A. Barcelona, Spain), weighing 20 to 25 g were housed five per cage in a room with

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2 Preliminary results were presented at the 5th European Society of Anaesthesiologists, Lausanne, Switzerland, May 1997.

AABBREVIATIONS: OR, opioid receptor; CO, croton oil; SS, saline; GIT, gastrointestinal transit; β-FNA, β-funaltrexamine; DPDPE, enkephalin, [D-Pen2,5]-U-50488H, trans-3,4-dichloro-N-methyl-N-(2-(1-pyrydylidnil) cyclohexyl)benzeneazetamine; MR-2266, (−)-a-5,9-diethyl-2’-hydroxy-2-(3-furylmethyl)-6,7-benzomorphan; ANOVA, analysis of variance.
Inflammatory diarrhea induced by CO. Two types of experiments were performed: acute and chronic treatment with CO. Acute inflammation was induced by a single administration of 0.05 ml of CO intragastrically; control animals received p.o. SS. Mice in the chronic treatment group received a second dose of CO or SS (0.05 ml) 24 h after the first one. In both instances, animals were fasted for 18 h before CO administration or testing (charcoal), except that they had free access to water. Chronic animals had access to food and water for a period of 6 h between the two doses of CO or between the second dose of CO and testing with charcoal. In all instances, weight loss, histological examination of the jejunum and GIT were determined at different time-points. Acute CO animals (one dose of CO) were tested 3, 6, 12, 24, 48 and 96 h after CO, and chronic CO animals (two doses of CO) 48, 72, 96, 120 and 144 h after the first dose of CO (fig. 1).

In order to determine changes in body weight and GIT, mice were weighed before treatment (CO or SS) and again at the different time-points indicated above, then they were sacrificed so that we could assess GIT or the presence of histological changes induced by CO.

Histological examination. After treatment with SS or CO, animals were sacrificed at different times according to the protocol, and the small intestine was rapidly excised. Samples of the proximal jejunum were fixed with 4% paraformaldehyde or 2.5% glutaraldehyde in phosphate buffer (200–400 mOsm; pH 7.2–7.4) for 24 h and then processed for either optical or electron microscopy examination, respectively. For optical studies, the samples were embedded in paraffin, and longitudinal and radial sections 5 μm thick were obtained with a sliding microtome; dewaxed sections were stained with hematoxylin and eosin. For electron microscopy studies, small blocks were washed in phosphate buffer and postfixed with 2% osmium tetroxide for 2 h; blocks were embedded in araldite, and sections were obtained with an ultramicrotome. The sections were stained with uranyl acetate and lead citrate. For each treatment (acute and chronic) and time-point, five animals receiving CO and five receiving SS were used for histological studies.

GIT. GIT in mice was measured according to procedures used in our laboratory (Pol et al., 1995; Puig et al., 1996). Animals were fasted 18 h before the experiments, except that they had free access to water. In the morning, a single dose (acute treatment) of CO or SS was administered, and GIT was measured 3, 6, 12, 24, 48 and 96 h later, with a charcoal meal. Animals in the chronic-treatment group received two doses of CO or SS, 24 h apart, and GIT was measured 48, 72, 96, 120 or 144 h after the first dose. The period of fasting did not significantly alter GIT in SS-treated animals (fig. 5).

Each mouse received intragastrically 0.25 ml of a suspension of 10% vegetable charcoal in 5% gum acacia (Sigma Chemical Co., St. Louis, MO) and was sacrificed 20 min afterwards; the stomach and small intestine were removed and the omentum separated, avoiding stretching. We measured and recorded both the length of the intestine from the pyloric sphincter to the ileocecal junction and the distance traveled by the charcoal front.

Opioid agonists (morphine, fentanyl, DPDPE and U-50488H) were administered s.c. at the nape of the neck, whereas the antagonists (naloxone, naltrexone, MR-2266 and β-FNA) were given i.p. In addition, the effects of p.o. loperamide, a peripherally acting mixed agonist, were also assessed. Subcutaneous drugs were given 30 min, and p.o. drugs 40 min, before the marker. The opioid antagonists naloxone, naltrexone and MR-2266 were given 15 to 20 min before the charcoal, whereas i.p. β-FNA was injected 150 min before testing. The effects of opioids were evaluated at the time of their peak effect, according to the route of administration.

Drugs. Drugs were obtained from the following sources: morphine hydrochloride (Alcaiber S.A., Madrid, Spain), fentanyl (Syntex Latino S.A., Madrid, Spain), loperamide hydrochloride, DPDPE, naltrexone and β-FNA hydrochloride (Research Biochemicals Inc., Wayuland, MA); naloxone hydrochloride (Sigma), and MR-2266 (a gift from Boehringer-Ingelheim, Mannheim, West Germany). All drugs were dissolved in pyrogen-free distilled water just before use and injected in a volume of 10 ml/kg. Animals in the control groups received vehicle (saline) injections.

Data analysis. GIT was calculated as the percentage of the distance traveled by the charcoal relative to the total length of the small intestine (% GIT). The inhibitory effects of opioids on GIT are expressed as percent inhibition of transit in opioid-treated animals (test GIT) compared with the mean transit obtained in a group of vehicle-treated mice (n = 20).

\[ \text{% inhibition} = \left[ \left( \text{vehicle GIT} - \text{test GIT} \right) / \text{vehicle GIT} \right] \times 100 \]

Data are expressed as group mean ± S.E. All statistical calculations were performed as described by Tallarida and Murray (1986). ED\textsubscript{50} ± S.E. values were determined by linear regression analysis of dose-response relations based on at least 10 mice per dose. Tests for parallelism and validity of the tests were estimated by using the parallel-line assay. In the present investigation, ED\textsubscript{50} is defined as the dose that produces a 50% effect based on the E\textsubscript{max} estimated from the double reciprocal plot. Statistical analysis for significant differences among two groups was obtained by Student’s t test. When multiple groups were compared, one-way analysis of variance (ANOVA) was used, followed by a Student-Newman-Keuls test whenever applicable. A value of P < .05 was considered significant. All data-points shown are mean values, and in the figures, vertical bars represent the S.E.

Results

Effects of the administration of CO on total body weight. Acute and chronic administration of CO produced a significant decrease in body weight, accompanied by loose, watery stools. Control animals receiving p.o. SS, lost 4% and 6% of body weight during acute and chronic treatment, respectively, and no diarrhea was observed (fig. 2). Maximal weight loss in animals treated with acute CO was 10% (6 h); during chronic CO, maximal weight loss was 20% (96 h); differences between groups were statistically significative (P < .01). The results show that when compared with the SS groups, acute CO significantly decreased body weight at 3, 6, 12 and 24 h (P < .01), whereas the same effect occurred after chronic CO at 48, 72, 96 and 120 h (P < .01).

Morphological changes induced by the administration of CO. All preparations were obtained from the proximal jejunum of SS and CO mice. In acute CO animals, light-microscopical examination did not reveal histological changes between the two groups.
differences between SS and CO at any time tested. Using electron microscopy, however, we observed morphological changes demonstrating the presence of inflammation 3 h and 6 h after CO administration. Thus an increased number of clear and dark vesicles in the cytoplasm of epithelial cells, mainly near the luminal pole, and swollen mitochondria with disrupted cristae were present only in CO-6h treated animals (fig. 3). No abnormalities were seen in the blood vessel walls, but enlarged spaces filled with fine granular material occurred in the extravascular compartment of the villi in mice treated with CO-6h. These findings are analogous to those previously reported by our laboratory (Pol et al., 1994).

Histological preparations from animals treated with chronic CO (at 48, 96 and 120 h) were examined under light and electron microscopy. In these animals, light microscopy revealed a clear disruption of the mucosa and an infiltration of lymphocytes in the submucosa, whereas controls (SS) did not show morphological changes. Maximal inflammatory response was observed 96 h after treatment (fig. 4).

Effects of CO on GIT. GIT was assessed in acute and chronic mice treated with p.o. SS or CO. The results show that acute CO significantly increased GIT at 3, 6 and 12 h (P < .01) and did not induce any significant change thereafter (fig. 5). In chronic CO animals, a significant increase was observed at 48, 72, 96, 120 h (P < .001), when compared with the respective controls (chronic SS). When acute and chronic treatments (SS and CO) were compared, transit in the SS groups was unaltered, whereas GIT during acute and chronic CO was similarly increased.

Effects of morphine on GIT during acute and chronic intestinal inflammation. The effects of morphine on GIT were evaluated at different time-points after CO
(acute and chronic) administration. Saline-gavaged mice served as control. In acute mice, morphine produced a dose-related inhibition of GIT in all experimental conditions. Dose-response curves to morphine performed in SS-treated animals at 3, 6, 12 and 24 h were superimposed, and no significant differences were observed between their ED$_{50}$ values ($P > .05$, ANOVA). In acute CO, the dose-response curves to morphine were shifted to the left at 3, 6 and 12 h; however, the curve at 24 h after CO was superimposed to the control (SS). All dose-response curves were parallel, and no significant differences were observed between the slopes. The ED$_{50}$ value for morphine in SS animals at 6 h was $1.68 \pm 0.10$ mg/kg (table 1); ED$_{50}$ values significantly decreased to $0.60 \pm 0.04$ mg/kg, $0.55 \pm 0.02$ mg/kg and $1.09 \pm 0.08$ mg/kg for evaluation times 3, 6 and 12 h, respectively ($P < .05$). Thus the increase in the potency of morphine induced by acute CO was more prominent 6 h after administration. Dose-response curves to morphine in chronic SS performed at the different time-points (48, 72, 96, 120 and 144 h), were also superimposed. During chronic CO the curves were shifted to the left, and their ED$_{50}$ values are shown in table 1. Thus the ED$_{50}$ for chronic SS at 96 h was $1.67 \pm 0.13$ mg/kg; chronic CO significantly decreased the ED$_{50}$ values of morphine at 48 ($0.83 \pm 0.07$ mg/kg), 72 ($0.48 \pm 0.06$ mg/kg), 96 ($0.16 \pm 0.03$ mg/kg), and 120 ($0.3 \pm 0.04$ mg/kg) h ($P < .01$ when compared with SS). Table 1 shows that the most pronounced increases in the potency of morphine were observed 6 h (acute) and 96 h (chronic) after CO administration. The table also shows that acute CO increased the potency of morphine 3.0 times, whereas during chronic CO it was increased 10.4 times. On the basis of these results, the subsequent evaluation of the effects of opioids was carried out at 6 h and 96 h for acute and chronic treatments, respectively.

Effects of receptor-specific opioids and loperamide during acute and chronic inflammation. We performed a series of experiments to establish the predominant type of receptor involved in the enhanced response to morphine during acute and chronic CO. We evaluated the antitransit effects of fentanyl (a $\mu$-agonist), DPDPE (a $\delta$-agonist) and U-50488H (a $\kappa$-agonist). Dose-response curves to fentanyl were performed in acute and chronic mice treated with SS or CO. Because the curves to fentanyl in SS animals were superimposed at 6 h and 96 h, in figure 6 we have used as control the curve obtained at 96 h (ED$_{50} = 0.038 \pm 0.006$ mg/kg). In CO animals, the dose-response curves to fentanyl were parallel and shifted to the left, and the corresponding

![Optical microscopical examination of intestinal preparations obtained from the proximal jejunum 96 h after chronic p.o. administration of SS (panel A) or CO (panel B). A disruption of the mucosa and an infiltration of lymphocytes in the submucosa (SM) are seen in CO-treated animals. V, villus; C, crypt; MP, muscularis propria. Hematoxylin and eosin staining. Magnification: panel A, $\times$160; panel B, $\times$400.](image)

![Percent GIT in animals treated with acute and chronic SS or CO. Upper (acute) and lower (chronic) panels show the effects of treatment (SS, CO) at different times. Results are expressed as mean values, and vertical bars indicate the S.E.; $N = 10$ animals per group. The * indicates $P < .001$ when compared with SS (Student’s $t$ test).](image)

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED$_{50}$ Value</th>
<th>Treatment</th>
<th>ED$_{50}$ Value</th>
</tr>
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<tbody>
<tr>
<td>SS (6 h)</td>
<td>$1.68 \pm 0.10$</td>
<td>SS (96 h)</td>
<td>$1.67 \pm 0.13$</td>
</tr>
<tr>
<td>CO (3 h)</td>
<td>$0.60 \pm 0.04**$</td>
<td>CO (48 h)</td>
<td>$0.83 \pm 0.07**$</td>
</tr>
<tr>
<td>CO (6 h)</td>
<td>$0.55 \pm 0.02**$</td>
<td>CO (72 h)</td>
<td>$0.48 \pm 0.06**$</td>
</tr>
<tr>
<td>CO (12 h)</td>
<td>$1.09 \pm 0.08*$</td>
<td>CO (96 h)</td>
<td>$0.16 \pm 0.03**$</td>
</tr>
<tr>
<td>CO (24 h)</td>
<td>$1.58 \pm 0.09$</td>
<td>CO (120 h)</td>
<td>$0.30 \pm 0.04**$</td>
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<tr>
<td>Ratio SS/CO (6 h)</td>
<td>3.0</td>
<td>Ratio SS/CO (96 h)</td>
<td>10.4</td>
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</table>

* $P < .05$ and ** $P < .01$ when compared with SS animals.
ED$_{50}$ values were 0.018 ± 0.004 mg/kg and 0.006 ± 0.0005 mg/kg for acute and chronic CO, respectively. The results show that acute CO enhanced the effect of fentanyl 2.1 times, whereas chronic CO further enhanced its effect up to 6.3 times.

In SS animals, we could not establish dose-response relationships after the s.c. administration of DPDPE; maximal inhibition using a dose range of 0.001 to 10 mg/kg (fig. 7) was approximately 25%. In acute CO and chronic CO animals, parallel dose-response curves were obtained that showed calculated $E_{\text{max}}$ values of 42.4 ± 1.8 and 48.1 ± 1.2, respectively (P < .05); the ED$_{50}$ values were 0.0082 ± 0.001 and 0.0041 ± 0.0009 mg/kg for acute and chronic CO, respectively (P < .05), which demonstrates that chronic CO increases the potency of DPDPE approximately 2 times when compared with acute-CO. The responses induced by each individual dose of DPDPE in SS, acute CO and chronic CO were compared using Student's $t$ test or one-way ANOVA. The results show that acute CO and chronic CO significantly increase the inhibitory effects of DPDPE at all doses tested (P < .05). In SS animals, low doses of DPDPE (0.005 and 0.01 mg/kg) had no effect or a negligible effect, whereas the same doses induced a significant inhibition of GIT during acute and chronic CO. These animals, the lowest dose of DPDPE that produced a measurable effect was 0.01 mg/kg (1.9 ± 1.3%); the same dose induced a 24.3 ± 2.7% and a 34.3 ± 1.9% inhibition during acute and chronic inflammation. Thus the results demonstrate that in the presence of inflammation, doses of DPDPE that were inactive in control animals produce a significant inhibition of GIT.

In control conditions (SS), the $\kappa$-agonist U-50488H administered in a dose range of 1 to 30 mg/kg induced a maximal inhibitory effect of 20%. Acute and chronic CO produced a modest increase in the inhibition of GIT (5%–8%), but the differences from SS were not statistically significative. The present experiments show that acute and chronic inflammation enhance the antitransit effects of $\mu$ and $\delta$ (but not $\kappa$) opioids.

The peripheral component of the antitransit effect of opioids during inflammation was evaluated using loperamide, a mixed opioid that, when given p.o., does not induce CNS effects (Hurwitz et al., 1994). Loperamide produced a dose-related inhibition of the GIT (fig. 8) in acute and chronic animals (SS and CO). Because the curves obtained in acute and chronic SS were superimposed, in figure 8 we have represented the results obtained at 96 h. Acute CO and chronic CO shifted the dose-response curves to the left in a parallel manner, and the resulting ED$_{50}$ values were 27.0 ± 0.2 mg/kg, 8.56 ± 0.4 mg/kg and 2.3 ± 0.08 mg/kg for the SS, acute CO and chronic CO groups, respectively. Thus the potency of p.o. loperamide increased approximately 3.1 and 11.7 times when compared with controls (table 2).

**Antagonism of the antitransit effects of opioids during acute and chronic CO.** In order to determine whether the enhanced effects of opioids during acute and chronic CO are mediated by OR, we used receptor-specific antagonists. In all experimental conditions, the effects on the ED$_{50}$ values of morphine, fentanyl and loperamide were completely antagonized by naloxone at doses of 0.1 (morphine and fentanyl) and 1 mg/kg (loperamide). Similarly, the antitransit effects of DPDPE (1 mg/kg) and U-50488H (5 mg/kg) were blocked by the specific antagonists naltrindole (1 mg/kg) and...
MR-2266 (3 mg/kg) in all treatment groups (SS and CO). The doses of the antagonists were selected on the basis of previous studies reporting selective blockade of the different types of OR (Magnam et al., 1982; Portoghese et al., 1988). The reversibility of the antitransit effects of opioids by the antagonists demonstrates that the enhanced effects observed during acute and chronic inflammation are mediated by interaction with OR.

Reversibility of the antitransit effects of morphine by β-FNA. In order to investigate the mechanism(s) of the enhanced effects of morphine, we used β-FNA, a competitive, nonreversible μ-opioid antagonist (Chen et al., 1995). These experiments were performed in order to estimate the fraction of the total number of OR that mediate the enhanced response of opioids during acute and chronic intestinal inflammation. We performed two types of experiments. In the first group, the inhibitory effect of a fixed dose of morphine (1 mg/kg) was tested in the presence of increasing amounts of i.p. β-FNA (5–100 μg). β-FNA was administered 150 min before the agonist; this time was selected to guarantee a full effect of the antagonist and at the same time avoid the initial agonist effect of β-FNA (Ward et al., 1982). In all experimental conditions, β-FNA antagonized the effects of morphine in a dose-dependent manner (fig. 9). However, significantly higher doses of β-FNA were needed to antagonize the antitransit effects of morphine in chronic CO (P < .01), whereas SS and acute CO behaved in a similar manner. On the basis of the different doses of β-FNA required to antagonized a fixed dose of morphine, it could be postulated that a similar population of OR mediates the response in SS and acute CO, whereas a “recruitment” of OR occurs in chronic CO.

In the second group of experiments, we used the ratio between the ED₅₀ of morphine and the dissociation constant (Kₐ), as an estimate of the number of spare OR in the different experimental conditions. Dose-response curves to morphine in the presence of a fixed dose of β-FNA (20 μg) were obtained in SS, acute CO and chronic CO. The curves were parallel, and the calculated E₅₀ values were not significantly different among themselves (range 81.3%–82.7%). From these curves, equiactive doses of morphine in the presence and absence of β-FNA were determined, and the Kₐ was calculated for each group (Furchgott and Bursztyn, 1967; Tallarida and Murray, 1986). Kₐ values were 23.9 ± 2.4 (SS), 11.0 ± 1.9 (acute CO) and 52.4 ± 3.6 mg/kg (chronic CO); the ED₅₀/Kₐ ratios were similar in SS (0.070) and acute CO (0.050), whereas a significant decrease (21 times) was observed in chronic CO (0.0032).

**Fig. 8.** Antitransit effects of p.o. loperamide, in control animals (SS) and during acute (CO-6h) and chronic (CO-96h) CO treatment. Results obtained with SS-6h were analogous to those observed in SS-96h. Each point represent the mean ± S.E. of 10 or more animals. The * indicates P < .05 when comparing acute (CO-6h) and chronic CO (CO-96h) to SS (Student-Newman-Keuls test).

**TABLE 2**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>ED₅₀ value (mg/kg)</th>
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<tbody>
<tr>
<td>Saline (SS-96h)</td>
<td>27.0 ± 0.2</td>
</tr>
<tr>
<td>Acute (CO-6h)</td>
<td>8.56 ± 0.4*</td>
</tr>
<tr>
<td>Chronic (CO-96h)</td>
<td>2.3 ± 0.08**</td>
</tr>
<tr>
<td>Ratio SS/acute CO</td>
<td>3.1</td>
</tr>
<tr>
<td>Ratio SS/chronic CO</td>
<td>11.7</td>
</tr>
</tbody>
</table>

* P < .05 and ** P < .01 when compared with saline.

**Fig. 9.** Antagonism of the antitransit effects of 1 mg/kg of morphine by increasing doses of i.p. β-FNA, in animals treated with saline, acute CO and chronic CO. All points in the SS and CO-6h curves are significantly different from the corresponding points in the CO-96h curve. Each point represents the mean value obtained from 10 or more mice, and vertical bars indicate the S.E. The * indicates P < .01 (Student-Newman-Keuls test).
**Discussion**

In the present investigation, we describe a model of intestinal inflammation in mice induced by the intragastric administration of two consecutive doses of CO given 24 h apart, measuring the effects 96 h after the first dose. A similar model of acute inflammation induced by a single dose of CO has been previously described and validated by our group (Pol et al., 1994). The aim of the present investigation was to assess possible differences in the effects of opioids during acute and chronic inflammation and to investigate the mechanism(s) of the enhanced effects.

In order to measure GIT, we used a charcoal meal. This method essentially measures motor activity of the GI tract and reflects a combination of gastric emptying and intestinal transit; thus our results do not establish a distinctive site (gastric or intestinal) of action of opioids, but a combination of both. Because results for the potency of opioids inhibiting GIT have been proved to be similar whether charcoal or radioactive markers are used (Shook et al., 1987, 1989), we performed our experiments with charcoal in order to avoid environmental contamination.

In the chronic model, we could demonstrate a characteristic inflammatory response of the small intestine, evidenced by light microscopy; the infiltration of lymphocytes and the disruption of the mucosae revealed the presence of chronic inflammation (Palmer et al., 1995). In our study, a precise quantification of the intensity of the inflammatory response was not attempted. When acute CO and chronic CO treatments were compared, significant morphological differences were observed under light microscopy; in addition, animals treated with chronic CO had a more pronounced weight loss than those with acute CO. However, the increase in GIT was similar in both groups, probably because limitations of the model do not permit an accurate evaluation of GIT when approximately an 80% inhibition is reached.

In the present study, we created dose-response curves to morphine at different times after CO, in order to establish approximately an 80% inhibition is reached. A similar model of acute inflammation induced by a single dose of CO has been previously described and validated by our group (Pol et al., 1994). The aim of the present investigation was to assess possible differences in the effects of opioids during acute and chronic inflammation and to investigate the mechanism(s) of the enhanced effects.

In all experimental conditions, the antitransit effects of systemic morphine are mediated by interaction with OR located at central and peripheral sites (Shook et al., 1987). We have investigated the peripheral component using p.o. loperamide, an opioid that has a potent anti-diarrheal effect mediated by intestinal μ/δ OR (Hurwitz et al., 1994). Our results show a similar increase in the potency of loperamide and morphine during acute and chronic inflammation, which suggests that the enhanced effects of μ/δ opioids are mediated by peripheral OR. We cannot completely exclude the possibility that loperamide binds to the charcoal and decreases its potency. However, in the study we used inactivated charcoal that should not bind or inactivate other compounds. In addition, loperamide was administered 40 min before the marker, so the possibility of loperamide and charcoal mixing (binding) in the gut is small.

Previous experiments carried out in our laboratory show that 40 min after the administration of a p.o. marker, the distance traveled is about 80% of the small intestine, and the marker is mostly present in the large intestine (Pol et al., 1994). Analogous results were obtained during acute and chronic inflammation.

Similarly, the possibility that CO altered the intestinal absorption of loperamide cannot be excluded. If absorption into the blood was increased, loperamide would still have a peripheral effect, because it does not easily cross the blood-brain barrier; in this case the effects of loperamide could be slightly decreased because of a "dilutional" effect in plasma. A decreased absorption could be expected during inflammatory diarrhea, because the increase in GIT reduces the time of contact of the drug with the intestinal wall (Awouters et al., 1993). In addition, intestinal absorption of loperamide in control conditions is less than 5%, so a further reduction would have negligible effects on the present results.

In all experimental conditions, the antitransit effects of morphine and loperamide were antagonized by naloxone, and the effects of δ and κ opioid agonists were blocked by specific antagonists. These results demonstrate that the enhanced effects of opioids during inflammation are mediated by binding to specific OR.

When analyzing the ED₅₀ values of morphine, we observed that its potency increased 3.0 and 10.4 times during acute CO and chronic CO, respectively; thus acute and chronic inflammation distinctly increased the antitransit effects of morphine which suggests different mechanisms in each experimental condition. On the basis of the results obtained in the arthritic rat model (Schäfer et al., 1995), we used β-FNA to assess the mechanism(s) involved in the enhanced response to morphine during inflammation. β-FNA is a competitive, nonreversible μ opioid antagonist that binds covalently to μ OR (Jiang et al., 1990; Mjanger and Yaksh, 1991). In one group of experiments, we used a fixed dose of morphine in the SS animals produced a significant inhibition during inflammation, which suggests the presence of "silent" δ OR in our experimental model. These receptors would be activated during inflammation and would thus explain the manifestation of an effect by otherwise inactive doses. The fact that the ED₅₀ and Eₘₐₓ values of DPDPDE are significantly different in acute and chronic CO suggests changes in the number of active receptors and/or the efficacy of the opioid.

In our model, the antitransit effects of κ agonist were modest, were not dose-related and were unaltered in the presence of acute or chronic inflammation.

The antitransit effects of systemic morphine are mediated by interaction with OR located at central and peripheral sites (Shook et al., 1987). We have investigated the peripheral component using p.o. loperamide, an opioid that has a potent anti-diarrheal effect mediated by intestinal μ/δ OR (Hurwitz et al., 1994). Our results show a similar increase in the potency of loperamide and morphine during acute and chronic inflammation, which suggests that the enhanced effects of μ/δ opioids are mediated by peripheral OR. We cannot completely exclude the possibility that loperamide binds to the charcoal and decreases its potency. However, in the study we used inactivated charcoal that should not bind or inactivate other compounds. In addition, loperamide was administered 40 min before the marker, so the possibility of loperamide and charcoal mixing (binding) in the gut is small.

Previous experiments carried out in our laboratory show that 40 min after the administration of a p.o. marker, the distance traveled is about 80% of the small intestine, and the marker is mostly present in the large intestine (Pol et al., 1994). Analogous results were obtained during acute and chronic inflammation.

Similarly, the possibility that CO altered the intestinal absorption of loperamide cannot be excluded. If absorption into the blood was increased, loperamide would still have a peripheral effect, because it does not easily cross the blood-brain barrier; in this case the effects of loperamide could be slightly decreased because of a “dilutional” effect in plasma. A decreased absorption could be expected during inflammatory diarrhea, because the increase in GIT reduces the time of contact of the drug with the intestinal wall (Awouters et al., 1993). In addition, intestinal absorption of loperamide in control conditions is less than 5%, so a further reduction would have negligible effects on the present results.

In all experimental conditions, the antitransit effects of morphine and loperamide were antagonized by naloxone, and the effects of δ and κ opioid agonists were blocked by specific antagonists. These results demonstrate that the enhanced effects of opioids during inflammation are mediated by binding to specific OR.

When analyzing the ED₅₀ values of morphine, we observed that its potency increased 3.0 and 10.4 times during acute CO and chronic CO, respectively; thus acute and chronic inflammation distinctly increased the antitransit effects of morphine which suggests different mechanisms in each experimental condition. On the basis of the results obtained in the arthritic rat model (Schäfer et al., 1995), we used β-FNA to assess the mechanism(s) involved in the enhanced response to morphine during inflammation. β-FNA is a competitive, nonreversible μ opioid antagonist that binds covalently to μ OR (Jiang et al., 1990; Mjanger and Yaksh, 1991). In one group of experiments, we used a fixed dose of morphine in the
presence of increasing doses of β-FNA; in this situation, the observed response reflects the fraction of OR not blocked by the antagonist. The experiments were performed in control animals and during acute and chronic inflammation. The effects of morphine in SS and acute CO were similarly reversed by β-FNA (fig. 9), which suggests that acute inflammation does not significantly alter the fraction of active μ OR. However, significantly higher doses of β-FNA were required to antagonize morphine during chronic inflammation, and an increase in the concentration of active μ OR induced by chronic inflammation could be postulated. In another group of experiments, we evaluated possible changes in “spare” receptors during acute and chronic CO, using the ratio ED50/KA. The ED50 is the dose of agonist that produces 50% of a maximal effect, whereas the KA is the dose of agonist needed to occupy 50% of the total number of receptors; the disparity between the ED50 and KA values indicates that only a fraction of the receptors present are needed to produce a certain level of response (ED50, for example). Thus a ratio ED50/KA smaller than 1 indicates the presence of spare receptors for a specific drug and biological preparation. In the present investigation, ED50/KA ratios in control (7 x 10−2) and acute-CO (5 x 10−2) animals were comparable and smaller than 1, which shows that acute inflammation does not significantly alter the number of spare receptors. In chronic CO animals, the ED50/KA ratio (3.2 x 10−3) decreased approximately 20 times, which suggests that in addition to spare receptors, other mechanisms are implicated in the enhanced response of morphine during chronic inflammation.

Our results suggest that the 3-fold increase in the effect of morphine observed during acute CO cannot be explained by the activation of “spare” receptors. The increase could be explained on the basis of a sensitization of OR induced by the local inflammatory process (low pH, enhanced coupling of opioids to their receptors, disruption of the perineurium, etc.). In a previous study we have shown that acute inflammation induces a significant decrease in pH (Pol and Puig, 1997) that could enhance receptor coupling to guanine-nucleotide-binding proteins (G proteins). These results are supported by the findings of Selley et al. (1993) in isolated membrane preparations, which demonstrated that acidosis increases the efficacy of opioid agonists in the inhibition of adenyl cyclase. The further increase in potency observed during chronic inflammation seems to be related to different mechanism(s). The results with β-FNA suggest an increase in the actual number of active OR that could occur as the result of increased synthesis of the receptor protein (increase in μ-mRNA) or in response to post-transcriptional changes in the absence of increased levels of mRNA. This possibility is supported by the demonstration, during chronic arthritis, of an increase in axonal transport and in the number of peripheral OR, without changes in the actual level of mRNA that codes for μ OR (Hassan et al., 1993). The molecular mechanisms involved in the enhanced effects of opioids during inflammation are under investigation in our laboratory.

In summary, our results show that chronic treatment with CO induces intestinal inflammation as evidenced by optical microscopy, weight loss and increased GIT. In this model, the antitrusalt effects of μ and δ (but not κ) opioids were significantly increased, and the enhanced effects were reversed by specific antagonists. The antitrusalt effects of p.o. loperamide, a peripherally acting opioid without central effects, were similarly increased during chronic inflammation. Experiments performed with β-FNA suggest that the number of active OR is increased during chronic inflammation of the gut.

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References