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Reversal of tolerance to the antitransit effects of morphine during acute intestinal inflammation in mice

Olga Pol & [1]Margarita M. Puig

Anesthesiology Research Unit, IMIM, Department of Anesthesiology, Hospital Universitario del Mar, Barcelona, Spain

1 The aim of investigation was to establish and compare the reversibility of tolerance to the antitransit effects of morphine by three different procedures: (a) acute inflammation of the gut, (b) lorglumide a cholecystokinin$_A$ (CCK_A) receptor antagonist, or (c) MK-801, an N-methyl-D-aspartate (NMDA) receptor ion channel blocker. The type of interaction between morphine and lorglumide or MK-801 on the inhibition of gastrointestinal transit (GIT) in naive animals was also evaluated.

2 Male Swiss CD-1 mice were implanted with 75 mg of morphine base or placebo pellets. Gastrointestinal transit was assessed with a charcoal meal and results expressed as % inhibition of GIT. Inflammation was induced by the intragastric (p.o.) administration of croton oil (CO), while controls received castor oil (CA) or saline (SS). Morphine was administered by subcutaneous (s.c.) or intracerebroventricular (i.c.v.) injection, to naive and tolerant animals treated with CO, CA or SS. Dose-response curves for s.c. morphine were also perforrmed in naive and tolerant mice receiving 5.2 or 7.4 nmol (s.c.) lorglumide or MK-801, respectively.

3 The ED_{50} values for inhibition of GIT by s.c. morphine were: 45.9 ± 2.7 and 250.1 ± 3.1 nmol in naive and tolerant animals, respectively, demonstrating a five fold decrease in the potency of morphine. In naive animals, inflammation (CO) decreased the ED_{50} of morphine three times (14.4 ± 2.2 nmol). However, no tolerance to s.c. morphine (ED_{50} 16.4 ± 2.6 nmol) was manifested during intestinal inflammation. After i.c.v. administration, a similar degree of tolerance to morphine was observed (4.8 fold decrease in potency). Intestinal inflammation had no effect on the ED_{50} values of i.c.v. morphine in naive and tolerant animals, showing that reversal of tolerance is related to local mechanism/s. Mean values for intestinal pH were 6.9 ± 0.04 and 6.2 ± 0.04 in SS and CO treated mice, respectively. In addition, morphine was 74 times more potent by the i.c.v. than by the s.c. route (naive-SS).

4 Morphine and lorglumide interacted synergistically in naive animals; in addition, the administration of lorglumide reversed tolerance to s.c. morphine. No interaction (additivity) was observed in naive animals when morphine and MK-801 were administered in combination. However, the drug completely reversed tolerance to the antitransit effects of morphine.

5 The present investigation shows that acute inflammation of the gut reverses tolerance to the antitransit effects of s.c. morphine by a peripheral mechanism. Qualitatively similar results were obtained after the administration of lorglumide or MK-801. Our results suggest that a local decrease in pH could play an important role during inflammation, while antagonism of endogenous compensatory systems would explain the reversal of tolerance induced by lorglumide or MK-801.

Keywords: Morphine tolerance; opioids; gastrointestinal transit; intestinal inflammation; cholecystokinin-A receptor antagonist; N-methyl-D-aspartate receptor channel blocker

Introduction

Opioids are extensively used as analgesics in the treatment of pain of moderate to severe intensity. Chronic administration of morphine induces tolerance to the analgesic and other pharmacological effects, including constipation. However, the rate and magnitude of the development of tolerance to the different effects is not completely clear (Jaffe, 1990). Tolerance to opioids is manifested by a decrease in the intensity of the response to a constant dose (Foley, 1991) and a shift to the right of the dose-response curve to the agonists. Tolerance to μ opioid receptor agonists has been widely investigated, but the mechanisms involved are not well understood. At present, an increase in adenylate cyclase activity (Nestler, 1992), uncoupling of the μ opioid receptors to G-proteins (Tao *et al*., 1993), and/or sequestration of the μ opioid receptors in the presence of an unaltered μ opioid receptor gene expression (Buzas *et al*., 1996), are the most likely mechanisms involved.

The intestine has been widely used to evaluate the pharmacological effects of opioids and ileus/constipation constitute one of the major side effects after acute and chronic administration. The characteristics of the development of tolerance to the intestinal effects of opioids are not well characterized in animals or man. However, in experimental models of nociception, different groups of drugs have been shown to prevent/reverse tolerance to the antinociceptive effect of opioids; among them, calcium channel blockers (Dierssen *et al*., 1990) and cholecystokinin (CCK) or N-methyl-D-aspartate (NMDA) receptor antagonists (Trujillo & Akil, 1991; Xu *et al*., 1992) seem to be most effective. In addition, other investigators have shown a lack of tolerance to the antinociceptive effects of morphine in different models of chronic inflammation (Neil *et al*., 1990; Stein *et al*., 1996). However, the impact or influence of the above mentioned drugs and/or intestinal inflammation, on the reversal of tolerance to the antitransit effects of morphine have not been investigated.

We have previously shown that acute intestinal inflammation increases approximately three times the potency of s.c. morphine to inhibit gastrointestinal transit (GIT) (Pol *et al*., 1994) by a peripheral mechanism. In the present investigation, our working hypothesis was that local events occurring at the inflamed site such as 'sensitization' of μ opioid receptors, could restore the response to morphine in tolerant animals. Among the local events occurring during acute inflammation, a decrease in pH is usually present, a phenomena that has been shown to increase the efficacy of opioids (Selley *et al*., 1993).

[1]Author for correspondence at: Department of Anesthesiology, Hospital Universitario del Mar, Paseo Marítimo 22, 08003 Barcelona, Spain.

Thus in our study, we measured pH changes in the different experimental conditions. Since the effects of morphine on GIT are mediated by μ opioid receptors located in the brain and the gut, we assessed tolerance (at peripheral and central sites) after the s.c. and i.c.v. administration of morphine. In addition, we examined the reversibility of tolerance to the antitransit effects of morphine after the administration of lorglumide, a CCK-A antagonist (Van der Bent *et al*., 1994) or MK-801, an NMDA receptor ion channel blocker (Trujillo & Akil, 1991). The type of interaction between morphine and lorglumide or MK-801 on the inhibition of GIT was also evaluated. Preliminary results have been presented at the XXVI International Narcotics Research Conference (Scotland, U.K., July 9–13, 1995).

Methods

Animals

Experiments were performed on male Swiss CD-1 mice, weighing 20–25 g. Animals were housed under 12 hour light/12 hour dark conditions in a room with controlled temperature (22°C) and humidity (66%). Mice had free access to food and water and were used after a minimum of four days acclimatization to the housing conditions. All experiments were conducted between 9 h 00 min and 14 h 00 min. The International Association for the Study of Pain guidelines on ethical standards for investigations in animals were followed.

Gastrointestinal transit (GIT)

GIT was measured according to procedures used in our laboratory (Pol *et al*., 1995; Puig *et al*., 1996). Briefly, food was removed 18 h before the experiment but animals had free access to water. At this time, a charcoal meal (0.25 ml of 10% charcoal in 5% gum acacia) was administered intragastrically and GIT was evaluated 20 min later. Animals were killed and the small intestine separated from the omentum avoiding stretching. The length of intestine from the pyloric sphincter to the ileocecal junction and the distance travelled by the charcoal meal were measured. For each animal, GIT was calculated as the percentage (%) of distance travelled by the charcoal, relative to the total length of the small intestine (% of GIT). The inhibitory effects of drugs on GIT are expressed as a percentage of inhibition of the transit in a drug-treated animal (test GIT) when compared with the mean transit measured in a group of vehicle-treated mice ($n=20$). % inhibition = [(vehicle GIT – test GIT)/(vehicle GIT)] × 100.

Inflammation of the gut

Acute inflammation was induced by the intragastric administration of 0.05 ml of croton oil (CO) according to the method described previously (Pol *et al*., 1994; 1996b). Two other groups of animals receiving the same volume of saline (SS) or castor oil (CA) served as controls (Pol *et al*., 1996c). All groups of animals were weighed and placed into separate cages. After 3 h, the animals were again weighed and GIT was measured with a charcoal meal.

Histological examination

Animals were killed three hours after p.o. administration of CO, CA or SS, and the small intestine rapidly excised. Samples of the proximal jejunum were fixed with 2.5% glutaraldehyde in phosphate buffer (200–400 mOsm; pH: 7.2–7.4) for 24 h and processed either for light or electron microscopic examination. For light microscopic studies, the samples were embedded in paraffin and 5 μ thick longitudinal and radial sections were obtained with a sliding microtome; dewaxed sections were stained with haematoxylin and eosin. For electron microscopic studies, small blocks were washed in phosphate buffer and postfixed with 2% osmium tetroxide for 2 h; blocks were embedded in araldite and ultrathin sections were obtained with an ultramicrotome; the sections were stained with uranyl acetate and lead citrate. Five animals treated with CO, CA or SS were used for histological studies.

Measurement of pH

Local changes in pH induced by inflammation were measured in the small intestine (25 cm down from the pyloric sphincter) of mice treated with CO, CA or SS (10 animals per group). Animals were killed three h after treatment and pH recorded with a micropH 2001 (Crison, Barcelona, Spain) and a glass electrode, calibrated just before the study with standard solutions (pH 4.0 and 7.0).

Induction of tolerance to morphine

Under light ether anaesthesia, mice were implanted with 75 mg morphine base or placebo pellets, at the nape of the neck. GIT was determined 72 h after pellet implantation. Dependence on morphine was demonstrated in SS, CA and CO treated animals after the s.c. administration of naloxone (68.7 nmol) which induced a withdrawal syndrome characterized by increased spontaneous activity, tremours, jumping, and in some animals, spontaneous vocalization (Brown *et al*., 1988).

Dose-response curves for morphine

Dose-response curves for morphine were obtained after s.c. or i.c.v. injection, in naive and tolerant animals treated with CO, CA or SS. Subcutaneous morphine was administered 30 min before and i.c.v. morphine immediately before the charcoal. In addition, dose-response curves for s.c. morphine were recorded in naive and tolerant mice (in the absence of inflammation, SS) that had received, 40 min before morphine, one of the following drugs: lorglumide (CCK_A antagonist), MK-801 (NMDA receptor ion channel blocker) or vehicle. Drugs were administered intraperitoneally (i.p.). In order to be able to compare the relative potency of morphine when administered by the s.c. and i.c.v. routes, all doses of morphine are expressed in nmol.

Drugs

The drugs used were: morphine sulphate (Alcaiber S.A., Madrid, Spain), lorglumide (ICN Ibérica, S.A., Barcelona, Spain), MK-801 ((+)-5-methyl-10,11-dihydro-5H-dibenzyl[a,d]cyclohepten-5,10-imine hydrogen maleate; dizocilpine, Research Biochemicals Incorporated, U.S.A.) and naloxone hydrochloride (Sigma Chemical Co., St Louis, Mo., U.S.A.). Drugs were dissolved in pyrogen-free 0.9% sodium chloride just before use and injected s.c. (at the nape of the neck) or i.p., in a final volume of 10 ml kg^{-1}. For the i.c.v. injections, drugs were delivered in a volume of 5 μl, by a Hamilton syringe (Microdispenser Socorex, PANREAC S.A.) fitted with a 26-gauge needle, according to the method of Haley & McCormick (1957). The site of injection was 2 mm caudal and 2 mm lateral to bregma, and 3 mm in depth from the skull surface.

Data analysis

The data are expressed as group means ± s.e. All statistical calculations were performed as described by Tallarida & Murray (1986). ED_{50} (dose which produced a 50% effect) values were determined by linear regression analysis of dose-response relations based on at least ten mice per dose. Tests for parallelism and validity of the tests were estimated by use of parallel line assays. Statistical analysis for significant differences between two groups were obtained by Student's t test; when multiple groups were compared, one or two way analysis of variance (ANOVA) followed by Student-Newman-Keuls test was used. A value of $P<0.05$ was considered significant.

Results

Effect of castor (CA) or croton oil (CO) on the gut

The intragastric administration of CA or CO to fasted mice induced (3 h after treatment): (a) weight loss of 9.8 and 10% in CA and CO treated mice, respectively, while SS animals lost 4% ($P<0.05$); (b) an increase in GIT of 12% (CA) and 20% (CO) that was not statistically significant between groups, and (c) electron microscopic evidence of inflammation in CO but not in CA animals. The main morphological changes included an increased number of clear vesicles in the cytoplasm of jejunal epithelial cells and enlarged spaces filled with fine granular material in the extravascular compartment. Since these findings have been published previously (Pol *et al*., 1994; 1996c), only five animals per group were used to corroborate the characteristics of the model. Thus, our results show that only animals treated with CO presented intestinal inflammation.

Dose-response curves for morphine during inflammation in naive and tolerant mice

Dose-response curves for s.c. morphine were recorded in naive and tolerant mice treated with CO (inflammation), CA or SS. Similar experiments were carried out after the i.c.v. administration of morphine. For each treatment and route of administration, linear parallel dose-response curves were obtained and their ED_{50} values calculated.

Table 1 shows the ED_{50} values of morphine after s.c. administration. In SS animals implanted with a morphine pellet, the dose-response curve for morphine was shifted to the right resulting in a 5.4 fold increase of the ED_{50}, thus demonstrating tolerance to morphine. In tolerant mice treated with CA (without inflammation) the potency of morphine decreased 4.6 times; no statistical differences were observed when SS and CA were compared. In the presence of inflammation (CO) the ED_{50} of morphine was the same in naive and morphine implanted (pellet) animals, showing that intestinal inflammation restores the potency of morphine in tolerant animals. The results also show that inflammation of the gut (CO) increases the potency of morphine 3.1 and 15.2 times in naive and tolerant animals respectively.

The antitransit effects of morphine in naive and tolerant animals were also assessed after i.c.v. administration. These experiments were performed in SS and CO treated animals, since CA did not induce significant differences from the control (SS) when morphine was given s.c. Parallel dose-response curves for i.c.v. morphine were obtained in naive and tolerant animals treated with SS or CO (Figure 1). In SS animals (no inflammation), tolerance was manifested by a shift to the right of the dose-response curve for morphine. In addition, the presence of intestinal inflammation (CO) did not alter the dose-response curves for i.c.v. morphine in naive or tolerant animals. From the data, ED_{50} values were obtained in the four study groups (Table 2). The results show that in the absence of inflammation (SS), the potency of morphine in tolerant mice was 4.8 lower than in naive mice; the same decrease was observed during inflammation. In addition, CO treatment did not alter the ED_{50} of morphine in naive or tolerant mice. The results show that morphine is 74 times more potent when administered by the i.c.v. than by the s.c. route in naive SS animals.

Mean values of intestinal pH ($n=5$ for each group) measured in tolerant SS, CA and CO treated animals were 6.9 ± 0.04 (SS), 6.85 ± 0.05 (CA), and 6.2 ± 0.04 (CO). The differences were statistically significant between the CO and the CA or SS groups ($P<0.01$; Student-Newman-Keuls test).

Effects of lorglumide and MK-801 on the inhibition of GIT induced by morphine in naive mice

Dose-response curves for s.c. morphine were recorded in naive animals receiving 5.2 nmol of lorglumide or 7.4 nmol of MK-801 (Figure 2). The administration of 5.2 nmol lorglumide alone induced a $2.1\pm0.2\%$ inhibition of GIT. Pretreatment with this dose of lorglumide induced a shift to the left of the dose-response curve for morphine with ED_{50} values of 46.2 ± 3.7 and 20.5 ± 2.2 nmol for morphine alone and morphine + lorglumide, respectively (Table 3). The interaction be-

Table 1 ED_{50} values for effect of subcutaneous morphine (nmol), on the % inhibition of GIT in naive and tolerant mice, treated with saline (SS), croton (CO) or castor oil (CA)

Treatment (p.o)	*Naive*	*Tolerant*	*Ratio (tolerant/naive)*
SS	45.9 ± 2.7^{a}	250.1 ± 3.1^{c}	5.4
CO	14.4 ± 2.2^{b}	16.4 ± 2.6^{b}	1.1
CA	40.2 ± 3.2^{a}	186.0 ± 3.0^{c}	4.6

Values shown are mean $ED_{50}\pm$s.e. (in nmol). For naive and tolerant mice treated with SS, CO or CA, identical letters (aa, bb, or cc) indicate that there are no significant differences between groups, while different letters (ab, ac or bc) show significant differences ($P<0.05$; Student-Newman-Keuls test).

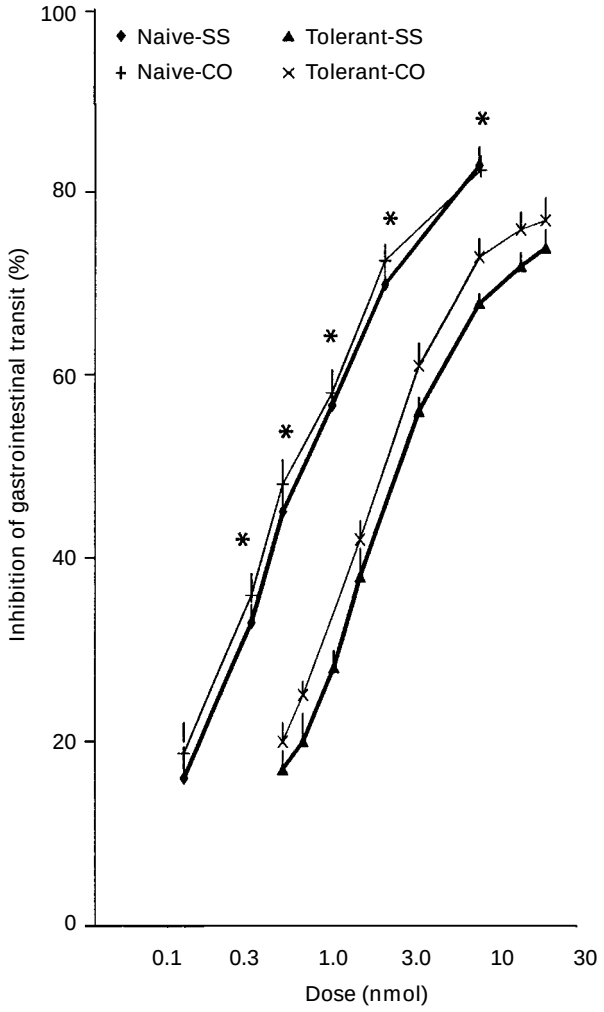

Figure 1 Inhibition of GIT induced by i.c.v. morphine in naive and tolerant animals treated with SS or CO. Gastrointestinal transit was evaluated 20 min after administration of charcoal. Each point represents the mean of 10 or more mice; vertical lines show s.e. $*P<0.05$, when naive and tolerant animals were compared (Student-Newman-Keuls test).

tween morphine and lorglumide appears to be synergistic based on the following evidence: (a) when the dose response-curves for morphine and morphine + lorglumide were analysed by two way ANOVA, significant effects of the treatment (morphine, morphine + lorglumide), the dose and their interaction ($P<0.02$; $n=10$ for each point) were obtained. Further comparison with one-way ANOVA showed that the effect of treatment was related to lorglumide, since the combination significantly increased the antitransit effects of morphine ($P<0.001$; $n=10$ for each dose). (b) The non-parallel displacement to the left of the dose-response curve of the combination, although lorglumide alone did not show a significant effect. (c) The sum of the effects produced by each drug alone was at each dose of morphine tested, significantly lower than the observed effects of the combination.

MK-801 alone at a dose of 7.4 nmol, produced a 1.4 ± 0.05% inhibition of GIT. In the presence of MK-801, the dose-response curve for morphine was slightly (but not significantly) shifted to the left (Figure 2). ED_{50} values for morphine alone and combined with MK-801 were 46.2 ± 3.7 and 36.9 ± 1.8 nmol, respectively. When data were analysed by two-way ANOVA, a significant effect of the dose ($P<0.001$; $n=10$ for each point), but not of the drugs or their interaction (drugs × doses) was observed. In addition, when the effect of MK-801 individually was added to the effect produced by each dose of morphine alone, the expected effects were not different from the observed effects, demonstrating additivity.

The results show that both the NMDA and CCK_A receptor antagonists have a modest inhibitory effect on GIT. However, only the latter enhanced the antitransit effects of morphine.

Table 2 ED_{50} values for effect of intracerebroventricular morphine (nmol), on the % inhibition of GIT in naive and tolerant mice, treated with saline (SS) or croton oil (CO)

Treatment (p.o.)	*Naive*	*Tolerant*	*Ratio (tolerant/naive)*
SS	0.62 ± 0.03^a	3.0 ± 0.10^b	4.8
CO	0.49 ± 0.02^a	2.4 ± 0.07^b	4.9
Ratio SS/CO	1.2	1.2	

Values shown are mean $ED_{50}\pm$s.e. (nmol). For naive and tolerant mice treated with SS or CO, identical letters (aa or bb) indicate that there are no significant differences between groups, while different letters (ab) show significant differences ($P<0.05$; Student-Newman-Keuls test).

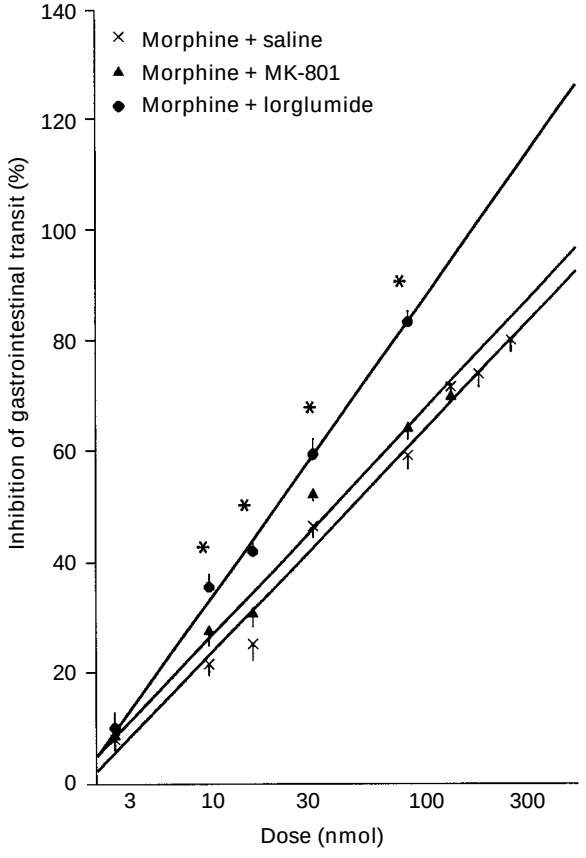

Figure 2 Inhibition of GIT induced by s.c. morphine in naive animals receiving (i.p.) SS, lorglumide or MK-801. Gastrointestinal transit was evaluated 20 min after administration of charcoal. Each point represents the mean of 10 or more mice; vertical lines show s.e. $^*P<0.05$, when morphine + lorglumide was compared to morphine alone (Student-Newman-Keuls test).

Effects of lorglumide and MK-801 on the inhibition of GIT induced by morphine in tolerant mice

The effects of 5.2 nmol of lorglumide or 7.4 nnol of MK-801 on the dose-response curves for morphine were also evaluated in tolerant mice; in these animals, the inhibitory effect of each drug alone was unaltered. The resulting dose-response curves were superimposed and shifted to the left when compared to the curve with morphine alone (Figure 3). Similar results were obtained in tolerant animals treated with CO, indicating that lorglumide, MK-801 and intestinal inflammation can reverse tolerance to the antitransit effects of morphine.

In tolerant animals, the effects of treatment (morphine, morphine + lorglumide, morphine + MK-801 or morphine-CO) and dose were analysed by 2-way ANOVA. Each factor (treatment and doses) as well as their interaction had a significant effect ($P<0.001$; $n=10$ for each point). Comparison with one-way ANOVA showed that the effect of treatment was related to lorglumide, MK-801 or CO, since their combination with morphine significantly increased the inhibition of GIT when compared to morphine alone ($P<0.001$; $n=10$ for each point).

Table 3 shows the ED_{50} values for morphine in naive and tolerant animals. Our results demonstrate that tolerance to morphine is reversed by the administration of lorglumide or MK-801 or by acute inflammation. In naive animals the interaction of morphine + lorglumide was synergistic and had an ED_{50} of 20.5 ± 2.2; in tolerant animals, the ED_{50} was significantly increased to 36.5 ± 1.1 nmol demonstrating the reversal of tolerance and the absence of an interaction between morphine and lorglumide (additivity). The ED_{50}s of animals treated with MK-801 or CO remained unaltered in tolerant animals. Results were compared by one-way ANOVA.

Table 3 ED_{50} (nmol) of morphine + SS, morphine + lorglumide, morphine + MK-801 and morphine during inflammation (morphine + CO), on % inhibition of GIT in naive and tolerant mice

Drugs	*Naive*	*Tolerant*
Morphine + SS	46.2 ± 3.7^a	245.4 ± 1.8^c
Morphine + lorglumide	20.5 ± 2.2^b	36.5 ± 1.1^a
Morphine + MK-801	36.9 ± 1.8^a	48.4 ± 1.1^a
Morphine + CO	15.6 ± 2.2^b	16.4 ± 2.6^b

Values shown are mean $ED_{50}\pm$s.e. (nmol). For naive and tolerant mice treated with morphine + SS, morphine + lorglumide, morphine + MK-801 or morphine + CO identical letters (aa or bb) indicate that there are no significant differences between groups, while different letters (ab, ac or bc) show significant differences ($P<0.05$; Students-Newman-Keuls test).

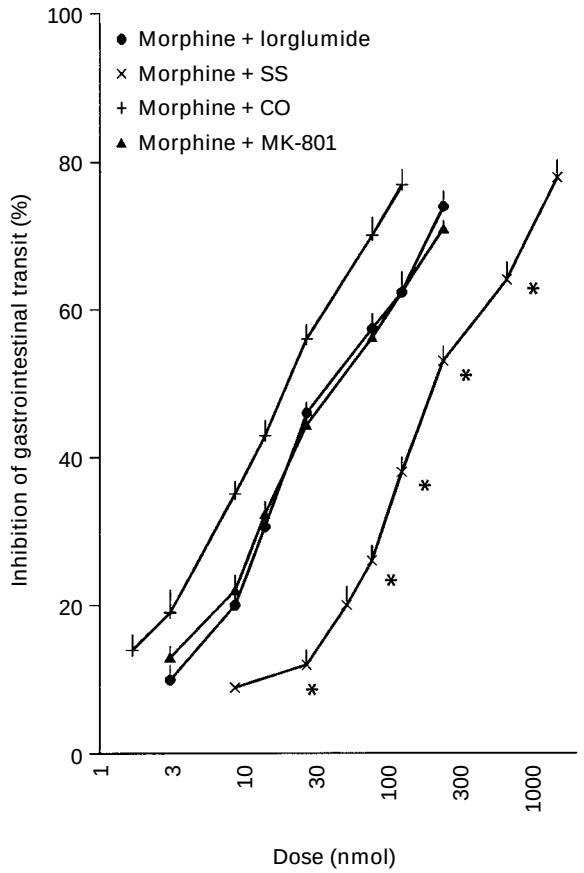

Figure 3 Inhibition of GIT induced by s.c. morphine in tolerant animals treated with SS, lorglumide, MK-801, or CO. Gastrointestinal transit was evaluated 20 min after administration of charcoal. Each point represents the mean of 10 or more mice; vertical lines show s.e. $*P<0.001$, when morphine alone (control group, SS) was compared to the three other groups (Student-Newman-Keuls test).

Discussion

The present investigation shows for the first time, that intestinal inflammation reverses tolerance to the antitransit effect of peripheral morphine. A qualitatively similar effect was observed after the administration of lorglumide or MK-801, although the underlying mechanisms are likely to be different.

We have recently characterized and validated (Pol *et al.*, 1994; 1995; 1996a) a model of acute intestinal inflammation in mice, induced by the intragastric administration of CO. Acute inflammation (substantiated by electron microscopy) increased the potency of s.c. morphine approximately three times. In the present study we have also used CA, an agent that induces hypersecretory diarrhoea without inflammation (Pol *et al.*, 1996c). When the antitransit effects of morphine were compared in the groups treated with CO or CA, the potency of morphine increased only in the CO group, demonstrating that inflammation is required in order to enhance the antitransit effects of morphine. Since the effects of s.c. morphine in SS and CA treated animals were indistinguishable, all subsequent experiments were performed in SS treated animals as control.

The relative potency of morphine when administered by central (i.c.v.) and systemic (s.c.) routes was also assessed. In these experiments, we converted all units ($\mu g\ \mu l^{-1}$ and $mg\ kg^{-1}$) to nmol, in order to be able to compare the ED_{50} values for morphine; the results show that i.c.v. morphine is 74 times more potent in inhibiting GIT than s.c. morphine. This finding probably involves different factors such as receptor density, local morphine concentration and kinetics, and has been previously described in the literature (Shook *et al.*, 1987).

The development of tolerance to the gastrointestinal effects of opioids has been shown to follow a different pattern than other pharmacological effects. In our model, tolerance was manifested by a decrease of approximately five times in the potency of morphine, supporting the results obtained by other investigators in small rodents (Wong *et al.*, 1980; Brown *et al.*, 1988). Our results show a similar degree or level of tolerance to morphine after s.c. and i.c.v. administration (ratios tolerant/naive were 5.4 and 4.8 for the s.c. and i.c.v. routes, respectively), demonstrating that brain and gut μ opioid receptors mediating the antitransit effects of morphine behave in a similar manner when chronically exposed to opioids.

The potency of morphine administered by the s.c. and i.c.v. routes was evaluated in the presence of intestinal inflammation (CO). As previously found, the potency of s.c. morphine increased three times during inflammation. However, after i.c.v. administration, the potency of morphine was analogous in SS and CO mice, demonstrating that the enhanced effects of morphine during inflammation are mediated by intestinal μ opioid receptors. As mentioned above, tolerance was substantiated by a five fold decrease in the potency of s.c. or i.c.v. morphine. Inflammation of the gut did not alter the response to i.c.v. morphine in tolerant animals. However, after s.c. administration no tolerance (as defined by a shift to the right of the dose-response curve) to morphine was observed. Since the decrease in the potency of i.c.v. morphine was comparable in SS and CO animals, we postulate that local events induced by the inflammatory process could be responsible for the reversal of tolerance to morphine.

Among other events, peripheral inflammation induces local acidosis; in our model we were able to document a significnat decrease in pH of 0.7 in CO-treated mice. In preparations of isolated neuronal membranes, acidosis increased the efficacy of opioid agonists to inhibit adenylate cyclase (Selley *et al.*, 1993), probably by enhancing the interaction of the μ opioid receptors with guanine-nucleotide-binding proteins (G-proteins). Since chronic exposure to opioids has been shown to uncouple the μ opioid receptors from G-proteins (Tao *et al.*, 1993), local acidosis could restore the potency of morphine in tolerant animals by this mechanism. In addition, tolerance induces sequestration of μ opioid receptors (Zadina *et al.*, 1995) and it is possible that tissue acidosis would disrupt the perineurum and facilitate the access of morphine to 'sequestered' μ opioid receptors in the gut (Antonijevic *et al.*, 1995). Thus, the enhanced effects of opioids during intestinal inflammation could be partially explained on the basis of a local decrease in pH. Accepting that chronic exposure to opioids induces changes in the configuration of the μ opioid receptors or the receptor-G protein complex, we hypothesize that local acidosis and/or other events occurring during intestinal inflammation could be responsible for the reversal of tolerance to morphine.

In addition to the events occurring at the receptor-complex level, other endogenous systems that apparently function as compensatory mechanisms could be involved in the development of tolerance to opioids (Han, 1995); among them, CCK and excitatory amino acids (EAAs), both implicated in primary sensory transmission, are likely to have a prominent role. In the present experiments, we have used two receptor-specific antagonists, lorglumide and MK-801, in order to block these endogenous compensatory mechanisms. The antagonists were selected because there is strong evidence in the literature for their effectiveness and selectivity for CCK_A and NMDA receptors, respectively (Makovec *et al.*, 1987; Woodruff *et al.*, 1987). The doses of the antagonists were used on the basis of previous data that evaluated their effects on intestinal motility (D'Amato *et al.*, 1991; Campbell *et al.*, 1991) or nociception (Kellstein & Mayer, 1991; Bilsky *et al.*, 1996). In addition, we performed pilot experiments with different doses of lorglumide (0.52, 5.2 and 52 nmol); a maximal increase in the antitransit effect of a fixed dose of morphine was obtained with 5.2 nmol of lorglumide, and thus, this dose was used in the study. Since MK-801 (at doses of 0.74, 7.4 and 74 nmol) did not induce changes in the effect of morphine, we employed 7.4 nmol, a dose that has been demonstrated to block NMDA receptors in diffferent experimental conditions.

In tolerant rats, an enhanced expression of the gene encoding CCK has been observed (Zhou *et al*., 1992) and CCK antagonists have been shown to prevent the development of tolerance to the antinociceptive effects of morphine (Xu *et al*., 1992). In the gut, the CCK family of peptides have an important role in the neural control of intestinal motility (Meyer *et al*., 1989). We have used lorglumide, a CCK_A receptor antagonist, in order to evaluate the reversal of tolerance to the antitransit effects of morphine. Our results show that lorglumide by itself has a negligible antitransit effect, but that at the dose-ratios used, interacts synergistically with morphine; interestingly, synergism is no longer present in tolerant animals, a phenomenon that has been previously observed when clonidine and morphine are combined (Roerig, 1995). The administration of a fixed dose of lorglumide reversed the tolerance to morphine, supporting a negative feedback control mechanism between opioids and CCK in the gut. The interaction between lorglumide and morphine on the inhibition of GIT in naive and tolerant animals could be of future importance if combinations of CCK antagonists and opioids are used in the treatment of pain.

Excitatory amino acid (EAA) antagonists such as MK-801 have been shown to produce analgesia (Dunbar & Yaksh, 1996), enhance the effects of morphine (Advokat & Rhein, 1995) and block or attenuate tolerance to opioids (Trujillo & Akil, 1994). Some of these results have been difficult to replicate and controversy still exists regarding the interaction of opioids and EAA antagonists on nociception. In the gut, *in situ* hybridization studies have identified myenteric neurones which express mRNA for the NMDA receptor (Broussard *et al*., 1994), suggesting that glutamate could have a neurotransmitter role in the enteric nervous system. However, our results show that at the dose used in the study, MK-801 alone did not alter GIT in control (SS) naive animals. When MK-801 was administered in combination with morphine, no interaction could be observed (additivity) but the drug completely reversed tolerance to morphine. Thus, although CCK_A and NMDA antagonists reversed morphine tolerance in our experimental conditions, the type of interaction between morphine and the antagonists lorglumide and MK-801 was different.

In the present investigation we showed that acute inflammation of the gut reverses tolerance to the antitransit effects of morphine by a peripheral mechanism. The observed effect was qualitatively similar to the reversal of tolerance observed after the administration of either a CCK_A antagonist or NMDA receptor ion channel blocker, but their mechanism/s appear to be distinct. We hypothesize that the decrease in local pH plays an important role during acute inflammation, while antagonisms of endogenous compensatory systems would explain the reversal of tolerance induced by lorglumide or MK-801. In addition, intestinal inflammation and acidosis could also alter the local regulation/expression of other 'compensatory systems' including CCK and glutamate.

Undoubtedly, the problem of tolerance to opioids during acute and chronic inflammation involves other systems and complex cellular events that cannot be explained by a modest change in pH. However, tissue acidosis could play a role in the reversal of tolerance to the antitransit effects of morphine during acute inflammation of the gut.

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