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European Journal of Pharmacology 441 (2002) 177–183

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Metamizol potentiates morphine antinociception but not constipation after chronic treatment

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Received 31 January 2002; accepted 5 February 2002

Abstract

This work evaluates the antinociceptive and constipating effects of the combination of 3.2 mg/kg s.c. morphine with 177.8 mg/kg s.c. metamizol in acutely and chronically treated (once a day for 12 days) rats. On the 13th day, antinociceptive effects were assessed using a model of inflammatory nociception, pain-induced functional impairment model, and the charcoal meal test was used to evaluate the intestinal transit. Simultaneous administration of morphine with metamizol resulted in a markedly antinociceptive potentiation and an increasing of the duration of action after a single (298 ± 7 vs. 139 ± 36 units area (ua); $P < 0.001$) and repeated administration (280 ± 17 vs. 131 ± 22 ua; $P < 0.001$). Antinociceptive effect of morphine was reduced in chronically treated rats (39 ± 10 vs. 18 ± 5 au) while the combination-induced antinociception was remained similar as an acute treatment (298 ± 7 vs. 280 ± 17 au). Acute antinociceptive effects of the combination were partially prevented by 3.2 mg/kg naloxone s.c. ($P < 0.05$), suggesting the partial involvement of the opioidergic system in the synergism observed. In independent groups, morphine inhibited the intestinal transit in $48 \pm 4\%$ and $38 \pm 4\%$ after acute and chronic treatment, respectively, suggesting that tolerance did not develop to the constipating effects. The combination inhibited intestinal transit similar to that produced by morphine regardless of the time of treatment, suggesting that metamizol did not potentiate morphine-induced constipation. These findings show a significant interaction between morphine and metamizol in chronically treated rats, suggesting that this combination could be useful for the treatment of chronic pain.

Keywords: Morphine; Metamizol; Synergism; Antinociception; Constipation; Chronic treatment

1. Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDS) and opioids are widely used in clinics for pain treatment. In spite of their proved efficacy, both types of drugs produce unwanted side effects that limit their use for prolonged periods of time. Opioids are still the drugs of choice for the treatment of many moderate to severe pain syndromes (Reisine and Pasternak, 1996); however, they produce constipation sometimes even after a single administration (Kromer, 1988). Another common undesired side effect of opioids is the development of analgesic tolerance after prolonged treatment that can lead to dose escalation (Bhargava, 1994). On the other hand, NSAIDS are very effective for the treatment of moderate pain but their use is limited because of gastrointestinal irritation (Vane and Botting, 1996). Among NSAIDS, metamizol is a good alternative due to its high efficacy and good gastric tolerability (Rodríguez et al., 1994; Planas et al., 1998), and is widely used in Latin America, Germany and other European countries (Miralles et al., 1987).

A strategy to attenuate the unwanted side effects of high doses of analgesic drugs is to combine low doses of both opioids and NSAIDS. This approach not only reduces the risks associated with the use of high doses of individual compounds but can also result in an improved analgesic treatment (Grotto et al., 1965; Wang and Sandoval, 1971; Bentley and Head, 1987; Malmberg and Yaksh, 1993; López-Muñoz et al., 1993a,b, López-Muñoz, 1994; Sandrini et al., 1999). For example, preclinical studies have shown that metamizol increases morphine-induced analgesia when these drugs are co-administered (López-Muñoz, 1994; Taylor et al., 1998). Our group has further characterised the effects of this analgesic combination testing a wide range of doses of metamizol (56.2 to 1000 mg/kg s.c.) and morphine

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0014-2999/02/$ - see front matter
PII: S0014-2999(02)01344-4

(1 to 10 mg/kg s.c.) in a model of inflammatory nociception (pain-induced functional impairment model in the rat; López-Muñoz, 1994). Among the 24 combinations tested, 13 produced additive effects and 11 supra-additive effects as compared with the antinociception produced by the same doses of the individual drugs. The combination that resulted in maximal antinociceptive potentiation was that of 177.8 mg/kg metamizol with 3.2 mg/kg morphine.

These studies clearly suggest that co-administration of metamizol and morphine has beneficial antinociceptive effects. However, the mechanisms of action involved in the antinociceptive potentiation induced by this combination are not clear and the involvement of the opioid system is controversial (Taylor et al., 1998; Aguirre-Bañuelos and Granados-Soto, 1999). Moreover, no studies have been made to determine the efficacy of this combination after repeated administrations and studies concerning the potential synergism of unwanted side effects are lacking. Recent evidence suggests that, under certain circumstances, the combination of opioids with NSAIDS can result not only in potentiation of analgesic effects but also in an enhancement of adverse effects (Montes et al., 2000). For this reason, it is important to study the synergistic interactions of both desirable and undesirable actions of drug combinations.

The purpose of this work was to evaluate the antinociceptive and constipating effects of the combination of morphine with metamizol after repeated administration using the combination previously described as optimal to produce the maximal antinociceptive potentiation in the pain-induced functional impairment model in the rat. A second objective was to evaluate the involvement of the opioid system in the acute antinociceptive effects induced by the same combination of maximal potentiation.

2. Materials and methods

2.1. Animals

Male Wistar rats [Crl:(WI)BR] (180–220 g), from our own breeding facilities, were used in this study. Animals were housed in an animal room at a constant temperature (22 ± 2 °C) with a 12:12-h light–dark cycle (lights on at 7:00 h), with free access to water and commercial food. Twelve hours before experiments, food was withheld but animals had free access to drinking water. All experiments were performed under approval of our Institutional Ethical Committee and followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). The number of experimental animals was kept to a minimum and they were used only once.

2.2. Drugs

Uric acid and naloxone hydrochloride were purchased from Sigma (St. Louis, MO, USA). Morphine hydrochloride was obtained from Merck (Darmstadt, Germany) via the Mexican Ministry of Health. Metamizol sodium was purchased from Hoechst (Mexico City, Mexico). Vegetable charcoal and arabic gum were purchased from a commercial supplier. Uric acid was suspended in mineral oil. Metamizol, morphine and naloxone were dissolved in saline solution and administered in a volume of 2 ml/kg. Vegetable charcoal and arabic gum were suspended in 0.2% carboxymethyl cellulose. The doses mentioned in the text refer to the salts of substances.

2.3. Measurement of antinociceptive activity

Antinociception was assessed using the pain-induced functional impairment model in the rat (PIFIR model, López-Muñoz et al., 1993b). The detailed methodology has been previously described. Briefly, under ether anaesthesia, rats were injected with 50 μl of uric acid (30%) into the right knee joint to induce nociception. Immediately afterwards, an electrode was attached to each hind-paw of the animals. Rats were allowed to recover from anaesthesia and then placed on a stainless-steel cylinder of 30 cm diameter. This cylinder was rotated at 4 rpm for periods of 2 min every 30 min in order to force animals to walk and the time of electrode contact on the cylinder was recorded with a computer. The time of contact of the injured hind limb reached a zero value 2 h and 30 min after uric acid injection. At this time, antinociceptive drugs were administered (see below) and the time of electrode contact was recorded during 2 min every 30 min, for four additional hours. Antinociception was evaluated as the recovery of the contact time of the injured limb. Rats were euthanized at the end of the experiment.

2.4. Measurement of gastrointestinal transit

Gastrointestinal transit was assessed using the charcoal meal test (Schulz et al., 1979; Manara et al., 1986). Rats were administered s.c. with antinociceptive drugs (see below) and 30 min later received 2 ml of a suspension of 5% vegetable charcoal with 5% arabic gum via an intragastric flexible tube. Animals were killed by cervical dislocation 30 min after oral meal administration. Immediately afterwards, the stomach and small intestine were removed to measure the length of the intestine (from the pyloric sphincter to the ileocoecal junction) and the distance travelled by the charcoal meal. The propulsive activity of the gut was determined by calculating the percentage of gastrointestinal transit, i.e., the distance travelled by charcoal meal divided by the total length of small intestine, multiplied by 100.

2.5. Study design

2.5.1. Protocol 1: acute administration of antinociceptive drugs

Eight independent groups of animals ($n = 6$, each) were s.c. injected with saline solution, once a day for 12 days. On

day 13th, they were tested either for nociception or for intestinal transit as previously described. In four of the groups, nociception was induced by uric acid administration and the antinociceptive effects of either 3.2 mg/kg morphine, 177.8 mg/kg metamizol or the combination of the same doses of morphine and metamizol were evaluated. A saline-treated group served as control. In the other four groups, the intestinal transit of the charcoal meal was measured after the administration of either saline (control group), morphine, metamizol or morphine plus metamizol, at the same doses used for antinociceptive studies.

2.5.2. Protocol 2: repeated administration of antinociceptive drugs

Six independent groups of rats were administered, in pairs, with 3.2 mg/kg morphine, 177.8 mg/kg metamizol or the combination of the same doses of morphine plus metamizol, once a day, for twelve days. On day 13th, three groups were injected with uric acid, and 2.5 h later with morphine, metamizol or the combination of morphine plus metamizol at the same doses used before, and evaluated for antinociceptive effects. The other three groups were injected with the individual agents or the combination of morphine plus metamizol, and then tested for intestinal transit.

2.5.3. Protocol 3: role of the opioidergic system on the synergy of the antinociceptive effects of the combination of morphine plus metamizol

Four additional groups were tested to determine whether naloxone was able to prevent the antinociceptive effect produced by the combination of morphine plus metamizol in acutely treated animals. All groups were injected with uric acid as previously described. After 2 h and 30 min, when the hind limb dysfunction was maximal, animals were injected with 3.2 mg/kg naloxone s.c., followed, 10 min later, by either saline, morphine, metamizol or the combination of morphine plus metamizol (at the same doses used before). As in previous experiments, the antinociceptive effects were evaluated for 4 h.

2.6. Data and statistical analysis

All results are presented as the mean ± S.E.M. of six animals per group. Data from antinociception studies are expressed as the percentage of functionality index (%FI), i.e., the contact time of the injured limb divided by the contact time of the control limb, and multiplied by 100. The area under the curve (AUC) for each time course of antinociceptive effects was calculated by the trapezoidal rule. Unpaired Student's *t*-test was used for comparisons between two means of independent groups. A two-way analysis of variance (time, treatment, interaction), followed by a Tukey test, was used to compare the effects of the combination of morphine plus metamizol with the sum of the effects of the individual agents at each time point. This statistical test was also used to compare the effects of the combination of morphine plus metamizol, with the individual effects of each analgesic drug in acutely and chronically treated animals. Differences were considered to reach statistical significance when $P < 0.05$.

3. Results

3.1. Antinociceptive effects of the combination of morphine plus metamizol

Intraarticular injection of uric acid induced a progressive dysfunction of the injured limb that reached its maximum at 2.5 h (data not shown). At that moment (zero time), the functionality index was zero and antinociceptive drugs were administered. Fig. 1 shows the time course of the effects of 3.2 mg/kg morphine, 177.8 mg/kg metamizol and the

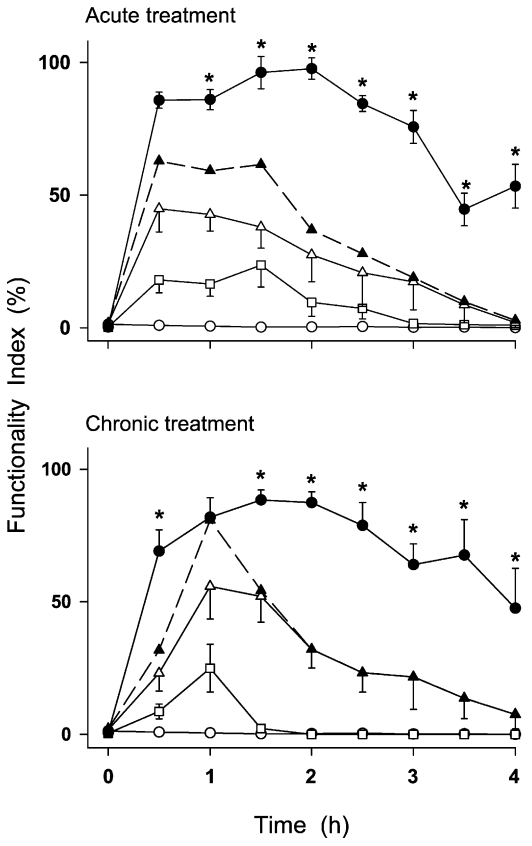

Fig. 1. Time courses of the acute and chronic antinociceptive effects of 3.2 mg/kg morphine (open squares), 177.8 mg/kg metamizol (open triangles) and the combination of the same doses of morphine and metamizol (filled circles), measured as functionality index recovery in rats submitted to pain-induced functionality impairment by intraarticular injection of uric acid. Dashed line represents the expected theoretical sum of the effects produced by the individual analgesic drugs. Control group was saline-treated (open circles). Data are expressed as means ± S.E.M. for six animals. * Significantly different from the addition of individual agents values in each time point ($P < 0.01$), as determined by two-way ANOVA followed by a Tukey test.

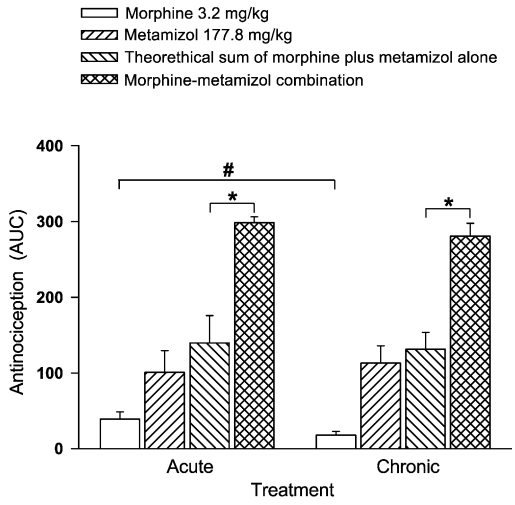

Fig. 2. Acute and chronic antinociceptive effects of 3.2 mg/kg morphine, 177.8 mg/kg metamizol and the combination of morphine plus metamizol. Data are expressed as the area under the curve (AUC) of the time course curves. Bars are the means ± S.E.M. for six animals. * Significantly different from the sum of the individual agents in each treatment ($P<0.001$) and #significantly different from the morphine-induced acute antinociceptive effect ($P<0.05$), as determined by two-way ANOVA followed by Tukey test.

morphine–metamizol combination after a single administration (upper panel) or after repeated administrations (lower panel). The curve corresponding to the expected sum of the antinociception produced by each individual analgesic is also included. This curve resulted from the addition of the individual effect produced by each analgesic drug in each time point. As shown in acutely treated animals, both morphine and metamizol produced a partial recovery of the functionality index that achieved its peak within 30 min and, afterwards, returned gradually to pre-drug levels. The maximal recovery observed after metamizol and morphine was 50% and 25% FI, respectively. When the same doses of the individual compounds were co-administered, a greater antinociceptive effect (90% FI) was obtained. This effect also reached its maximum 30 min after drugs injection, but remained relatively stable for the first 3 h and slowly decreased after this time without reaching control values. The comparison in each time point of the effect produced by the combination of morphine plus metamizol with the expected sum of the antinociception produced by each individual analgesic revealed that the experimentally observed antinociception was higher ($P<0.01$) than the expected theoretical sum of the antinociceptive effect of the analgesic drugs administered separately.

A very similar pattern of effects was seen in chronically treated animals. In this case, the combination of morphine plus metamizol also produced a higher response than the expected for the simple sum of the individual effects of each drug. The only difference was that the time to achieve the maximal response was longer (60 min) than that seen in acutely treated rats. As expected, saline-treated animals did not show any recovery of the functionality index along the 4 h of experimental recording.

Fig. 2 shows the AUC of the time course of the antinociceptive effects of morphine, metamizol and the combination of morphine plus metamizol in acutely and chronically treated rats. The AUC of the time course corresponding to the theoretical sum of the individual effects of morphine and metamizol is also included. As shown, the effect of the combination of morphine plus metamizol was significantly greater ($P<0.001$) than those predicted by the addition of the effects produced by each compound when were administered alone. These supra-additive effects of the analgesic combination were seen regardless of whether the animals received a single injection or repeated administrations of morphine plus metamizol. The comparison between acutely and chronically treated animals revealed that the schedule of morphine administration used in our experiments led to the development of tolerance to the antinociceptive effects of morphine ($P<0.05$), but not to those of metamizol or the morphine–metamizol combination.

3.2. Effect of naloxone on acute antinociceptive effect of the morphine–metamizol combination

Fig. 3 shows the antinociceptive effects (expressed as AUC) of 3.2 mg/kg morphine, 177.8 mg/kg metamizol and the combination of morphine plus metamizol in the presence and in the absence of the opioid antagonist, naloxone. Systemic administration of 3.2 mg/kg naloxone had no effect per se in rats treated with uric acid. As expected, pretreatment with naloxone prevented morphine-induced antinociception ($P<0.01$) while the metamizol-induced antinociception was unaltered. When the combination of

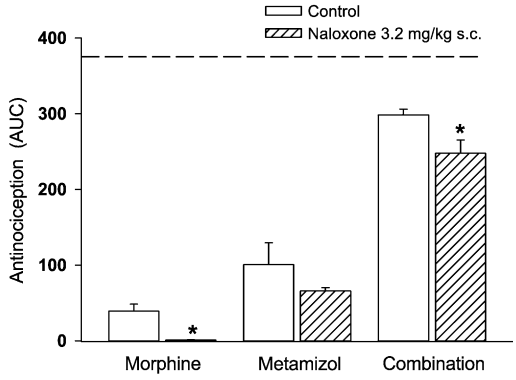

Fig. 3. Effect of naloxone on the acute antinociceptive effect produced by 3.2 mg/kg morphine, 177.8 mg/kg metamizol and the combination of morphine plus metamizol. Rats were pretreated with saline or naloxone. Data are expressed as the area under the curve (AUC) of the time course curves. Dashed line near the top represents the maximum AUC value (375 area units) that can be attained under these experimental conditions. Bars are the means ± S.E.M. for six animals. * Significantly different from the corresponding saline-pretreated group ($P<0.05$), as determined by Student's *t*-test for unpaired data.

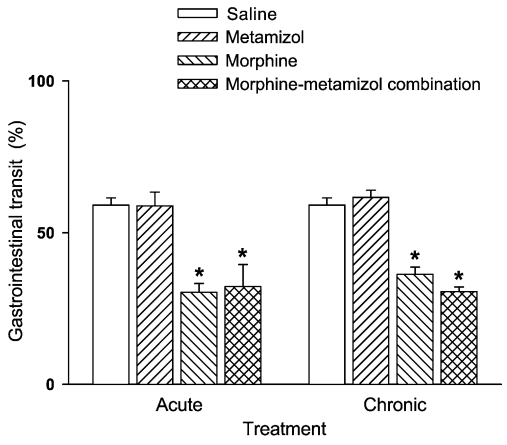

Fig. 4. Acute and chronic effects of 3.2 mg/kg morphine, 177.8 mg/kg metamizol and the combination of morphine plus metamizol on the gastrointestinal transit of charcoal meal. Data are expressed as the percentage of the gastrointestinal transit. Bars are the means ± S.E.M. for six animals. * Significantly different from saline-treated group in each treatment ($P < 0.05$), as determined by two-way ANOVA followed by a Tukey test.

morphine plus metamizol was given to animals previously treated with naloxone, the antinociceptive effect produced by this combination significantly reduced ($P < 0.05$) with respect to that observed in control rats pretreated with saline.

3.3. Effect of the morphine–metamizol combination on intestinal transit

Fig. 4 shows the percentage of the intestinal transit of charcoal meal after acute and chronic treatment with 177.8 mg/kg metamizol, 3.2 mg/kg morphine and the morphine–metamizol combination. In saline-treated animals, the charcoal meal travelled approximately 60% of the total length of small intestine, in 30 min. Metamizol was not effective in altering the intestinal transit, while morphine inhibited gastrointestinal propulsion in 48 ± 4% and 38 ± 4% after acute and chronic treatment, respectively. The combination of morphine plus metamizol produced a similar inhibition of gastrointestinal transit as individual morphine, in both acute and chronic treatment ($P < 0.05$). The time of intestinal transit evaluation (30 min) was selected on the basis of preliminary data in which we determined that there was no significant difference between the results obtained when the intestinal transit was evaluated at 30 or 60 min after charcoal meal administration.

4. Discussion

The search of potentially useful treatments for pain alleviation has led to the development of preclinical models of nociception for the study of new experimental drugs and to optimise the use of analgesic drugs used in clinical practice. Among them, the PIFIR model allows the evaluation of the time course of antinociceptive effects in the same animal, and is particularly useful for the characterisation of drug interactions (López-Muñoz et al., 1993a,b). Using this model, in a previous study, we found that the combined administration of single doses of morphine and metamizol resulted in different degrees of antinociceptive potentiation (López-Muñoz, 1994). The purpose of the present work was to further characterise the morphine–metamizol combination that proved to be optimal to produce a maximal antinociceptive potentiation, using two different schedules of administration.

Some reports have described that metamizol produces an acute enhancement of morphine-induced antinociception when both drugs are administered simultaneously (López-Muñoz, 1994; Taylor et al., 1998). Our results are consistent with these findings because metamizol markedly potentiated morphine-induced antinociception and increased the duration of the antinociceptive effects not only after a single administration but also after repeated treatment. To our knowledge, this is the first report of chronic potentiation of morphine-induced antinociception by metamizol.

The mechanisms underlying the synergism of the acute antinociceptive effects of morphine and metamizol are not clear; in particular, the role of the opioidergic system is controversial. For example, the antinociceptive potentiation produced by the co-administration of morphine with metamizol has been reported to be insensitive to the opioid antagonist naloxone at doses up to 1 mg/kg i.p. in a model of visceral pain (Taylor et al., 1998). On the other hand, Aguirre-Bañuelos and Granados-Soto (1999) found that the administration of naloxone (0.1 mg/kg i.p.) partially decreased the antinociceptive potentiation produced by the combination of morphine plus metamizol locally administered in the same site of formalin injection. Our results show that naloxone pretreatment partially reduced the antinociceptive effect of the morphine–metamizol combination tested in acutely treated rats. Naloxone was not tested in chronically treated animals because it could have precipitated a withdrawal syndrome that would interfere with the interpretation of the results. Based on these data, it can be suggested that the opioidergic system is partially involved in the antinociceptive potentiation produced by the combination of morphine plus metamizol tested under our experimental conditions. However, the involvement of other systems cannot be discarded and further experiments are required to characterise the pharmacological basis of this potentiation. Of interest is the finding of Aguirre-Bañuelos and Granados-Soto (1999), who have suggested that the peripheral activation of the arginine–nitric oxide–cGMP pathway could play a role in the antinociceptive synergism of morphine with metamizol. According to the available evidence, the mechanisms involved in the synergism between morphine and metamizol could depend on the model of nociception, the dose and the route of administration of antinociceptive drugs.

The role of the endogenous opioidergic system in the antinociceptive effects of metamizol is also controversial. According to some authors, a non-opioid mechanism is responsible of the antinociceptive effects produced by metamizol because naloxone is not able to reduce metamizol antinociception (Taylor et al., 1998; Beirith et al., 1998). In contrast, other studies suggest that endogenous opioidergic system does play a role in metamizol antinociceptive effects because naloxone reverses the acute antinociceptive effects produced by metamizol (Akman et al., 1996; Tortorici et al., 1996). Moreover, a recent report has shown that repeated metamizol microinjections into the periaqueductal grey matter induce antinociceptive tolerance and display signs of opioid withdrawal upon systemic administration of naloxone in rats, suggesting that metamizol activates endogenous opioid system (Tortorici and Vanegas, 2000). Our results do not support the involvement of an opioidergic mechanism in the metamizol-induced antinociception, because naloxone was unable to block the acute antinociceptive effects produced by metamizol and the antinociceptive efficacy of metamizol was not decreased when this NSAID was tested in chronically treated rats. The differences observed in these reports could reflect differences in the model of nociception used, the dose and the route of administration of metamizol.

The development of tolerance to the antinociceptive effects of morphine after repeated administration is an unwanted side effect that limits its chronic use (Bhargava, 1994). In the present study, tolerance to the antinociceptive effects of morphine (3.2 mg/kg), but not to the morphine–metamizol combination, developed after repeated administration. Interestingly, there is a clinical report indicating that the administration of metamizol prior to an abdominal surgery reduced postoperative morphine requirements and avoided the opioid dose escalation (Rockemann et al., 1996).

The characterisation of analgesic combinations should include not only the desirable but also the unwanted side effects. Constipation is a common undesired side effect that can appear even after the first morphine administration (Dhasmana et al., 1987; Kromer, 1988) and cannot be easily dissociated from analgesic effects (Porreca et al., 1984; Parolaro et al., 1986, 1988). Due to this fact, one of the objectives of this work was to study the effect of the morphine–metamizol combination on the charcoal meal test, after single and repeated administrations. Our data show that the combination of morphine with metamizol produced an inhibition of gastrointestinal transit similar to that produced by morphine regardless of the time of treatment, suggesting that metamizol did not potentiate morphine-induced constipation.

In contrast with the well-described development of tolerance to the analgesic effects of opioids after repeated administration, the development of tolerance to gastrointestinal transit inhibition is still the matter of investigation. Several authors have shown that tolerance develops to morphine-induced constipation in mice implanted with morphine pellets (Petersen and Fujimoto, 1983; Weisbrodt et al., 1977), in rats chronically implanted with intrathecal catheters (Dhasmana et al., 1987) and in rats treated with increasing doses of morphine twice a day for several days (Gmerek et al., 1985). In contrast, Dhasmana et al. (1987) showed that tolerance did not develop to the constipating effects of sufentanil and alfentanil in rats chronically implanted with intrathecal catheters. Our data show that not only 3.2 mg/kg morphine but also the morphine–metamizol combination given chronically (once a day per 12 days) to rats did not result in the development of tolerance to the constipating effects. This could be due to the relatively low dose of morphine used in our experiments and the low frequency of administration, factors that, along with the time of opioid exposure, are recognised to influence the development of tolerance (Dhasmana et al., 1987; Gmerek et al., 1985).

In summary, our results show that: (a) metamizol markedly potentiate morphine-induced antinociception in acutely and chronically treated rats in a model of inflammatory nociception; (b) the acute antinociceptive potentiation produced by the morphine plus metamizol combination partially involves the opioidergic system; (c) morphine-induced constipation is not potentiated by metamizol; and (d) the repeated administration of morphine or the morphine–metamizol combination does not result in the development of tolerance to the constipating effects. Present results show a useful potentiation of the antinociceptive effects of morphine with metamizol without potentiation of constipation in chronically treated animals, suggesting that the morphine–metamizol combination could be potentially useful for the treatment of chronic pain.

Acknowledgements

The authors wish to thank Mr. A. Huerta, Mr. L. Oliva and Mr. I. Méndez for technical assistance. Hernández-Delgadillo G.P., Ventura R. and Diaz I. are CONACYT fellows.

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