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Endogenous opioids are involved in morphine and dipyrone analgesic potentiation in the tail flick test in rats

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Abstract

The combined administration of low doses of opiates with non-steroidal anti-inflammatory drugs can produce additive or supra-additive analgesic effects while reducing unwanted side effects. We have recently reported that co-administration of morphine with dipyrone (metamizol) produces analgesic potentiation both in naïve and in morphine-tolerant rats. The purpose of this work was to determine the role of opioids on the acute potentiation observed between morphine and dipyrone i.v. in the rat tail flick test. To do this, two experiments were done. In the first one, naloxone was administered 10 min before morphine (3.1 mg/kg), dipyrone (600 mg/kg) or their combination at the same doses. Control animals received saline instead of naloxone. In the second experiment, naloxone (or saline) was given 2 min after reaching the maximal peak effect with each individual analgesic treatment. When naloxone was i.v. administered prior to analgesics, it completely blocked morphine effects, partially prevented morphine/dipyrone antinociception and delayed dipyrone-induced nociception. At 3.1 mg/kg, naloxone produced an increased nociception. When naloxone was given after analgesics, it dose-dependently blocked the effects of morphine alone and in combination with dipyrone but with different potency in each case. As to dipyrone, naloxone delayed the time to antinociceptive peak effect. Taken together, these results support the notion that endogenous opioids are involved in the analgesic potentiation observed with the combination of morphine plus dipyrone.

Keywords: Analgesia; Metamizol; Endogenous opioid; Potentiation; Non-steroidal anti-inflammatory drug

1. Introduction

Dipyrone, the pirazolone derivative also known as metamizol, is a non-steroideal anti-inflammatory drug widely used as analgesic in Europe and Latin America. As with other analgesics of this group, dipyrone and its active metabolites 4-methylaminoantipyrine and 4-aminoantypirine decrease prostaglandin synthesis (Weithmann and Alpermann, 1985), mainly through cyclooxygenase-2 activity inhibition (Campos et al., 1999). When injected directly into the periaqueductal grey matter, dipyrone produces antinociception in intact, but not in spinally transected rats (Carlsson et al., 1986). If dipyrone is injected into the nucleus raphe magnus, a rapid antinociceptive effect is also seen (Jones, 1996). This last effect seems to be due to an activation of the descending inhibitory pain control system (Tortorici and Vanegas, 1994; Vanegas et al., 1997; Hernández and Vanegas, 2001).

Two additional mechanisms of action have been proposed for dipyrone. One is the activation of the L-arginine/nitric oxide/cyclic GMP (cGMP)/K^+ channel pathway, and the other is an interaction with the glutamatergic system. In this respect, it has been shown that N^G-L-nitro-arginine methyl ester (L-NAME), a nitric oxide synthase inhibitor, blocks dipyrone's antinociceptive effect in the formalin test (Aguirre-Bañuelos and Granados-Soto, 1999), and in a model of inflammation induced by prostaglandin E_2 (Lorenzetti and Ferreira, 1996). ATP-sensitive K^+ channel blockers prevent dipyrone's actions in a model of nociception induced by prostaglandin E_2 injection (Alves and Duarte, 2002), and calcium dependent K^+ channel inhibitors block dipyrone antinociception in rats injected with formalin

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(Ortiz et al., 2003). As to the interaction with the glutamatergic system, there is evidence that dipyrone decreases glutamate binding in rat brains and diminishes the hyperalgesia produced by glutamate administration (Beirith et al., 1998). Besides, another glutamatergic-mediated nociceptive response, auto-tail biting in mice, is blocked by metabotropic glutamate receptor antagonists (Siebel et al., 2004).

Several reports have studied the role of opioids in dipyrone's effect in different models of nociception with variable results. According to Akman et al. (1996), i.c.v., i.t. and s.c. injections of dipyrone decrease the writhing reflex in mice. Since this effect can be antagonized by systemic administration of naloxone, these authors proposed that dipyrone releases endogenous opioid peptides. Tortorici and Vanegas (2000) found that rats repeatedly injected with dipyrone in the periaqueductal grey developed tolerance to dipyrone and cross-tolerance to morphine. Moreover, fully tolerant rats to dipyrone showed typical signs of opioid withdrawal when challenged with an i.p. naloxone dose, indicating that opioids are relevant to centrally-mediated dipyrone effects. In contrast with these results, Beirith et al. (1998) reported that a s.c. naloxone injection did not prevent the antinociception caused by i.p. dipyrone in the formalin test in mice, while Taylor et al. (1998) found that naloxone was unable to reverse the antinociceptive effect of dipyrone alone or in combination with morphine in the writhing test, suggesting that endogenous opioids do not play a role in this experimental preparation.

Co-administration of non-steroidal anti-inflammatory drugs with opioids can result in analgesic potentiation (López-Muñoz et al., 2004; Zelcer et al., 2005). In particular, dipyrone increases the antinociceptive effects of morphine in monoarthritic rats (López-Muñoz, 1994) without increasing adverse side effects (Hernández-Delgadillo et al., 2002). A similar effect was seen in mice using the writhing test (Taylor et al., 1998). In a previous study, our group reported that co-administration of sub-effective i.v. doses of morphine and dipyrone resulted in long-lasting potentiation in the tail flick test (Hernández-Delgadillo et al., 2003). This effect persisted in both morphine-tolerant and rats repeatedly treated with dipyrone when they were switched to the morphine–dipyrone combination (Hernández-Delgadillo and Cruz, 2004). Although these studies clearly suggest that co-administration of dipyrone and morphine produces supra-additive effects, the mechanism of action involved in such synergism is not clear and the involvement of the opioid system is controversial (Taylor et al., 1998; Aguirre-Bañuelos and Granados-Soto, 1999).

The purpose of this work was to determine the role of opioids on the acute potentiation observed between morphine and dipyrone (i.v.) in the tail flick test. We report here that doses of naloxone that completely antagonise morphine are only partially effective in delaying or decreasing morphine–dipyrone supra-additive effects. To almost abolish the potentiated response produced by this combination it is necessary to administer higher naloxone doses. It is concluded that endogenous opioids are released as a consequence of morphine–dipyrone i.v. administration, but other mechanisms seem to be involved in the delayed potentiation observed by the end of the experiment.

2. Materials and methods

2.1. Animals

Male Wistar rats (180 to 220 g) from our breeding facilities were housed in an animal room at 22±2 °C with a 12:12 h light–dark cycle (lights on at 7:00 h) and free access to food and water. Experiments were performed during the light phase of the cycle. Animals were handled twice a day for 2 days before the experiment in order to reduce stress. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983) and were approved by our local Committee on Ethics on Animal Experimentation.

2.2. Drugs

Morphine sulphate was obtained from Laboratorios Pisa, dipyrone sodium from Aventis (Mexico City, Mexico), and heparin sodium from Sigma Chemical Co. (St. Louis, MO, USA). All drugs were dissolved in sterile saline solution and administered intravenously in a final volume of 1 ml during 2 min using an infusion pump (KD Scientific, USA). After each drug injection, the catheter was flushed with heparin solution in a volume exceeding the estimated catheter dead space.

2.3. Surgical procedure

In rats anaesthetised with ether, a polyethylene catheter (PE50) flushed with heparin solution (500 units/ml) was inserted and fixed into the right jugular vein. The distal end of the catheter was guided subcutaneously to the top of the neck, where it was exteriorised and sealed with a metal plug. A 24-h period was allowed for recovery, after which a 24-gauge stainless steel needle attached to a 5-ml Becton Dickinson syringe was inserted into the outer tip of the jugular cannula for drug administration.

2.4. Tail flick test

We used a standardised tail flick apparatus (UGO BASILE, Italy) with the thermal stimulus intensity adjusted to a baseline tail flick latency of 6.0±0.5 s. Animals were screened for thermal nociception and those showing no flicking within 5.5 to 6.5 s were discarded (approximately 10–15% of the total). The cut-off time was set at 15 s to avoid tissue damage. The mean baseline latency, derived from two tests, was obtained before each drug injection for each rat. After drug administration, tail withdrawal latency was determined every 15 min during the first hour and every 30 min until completing 3 h. Rats were euthanised at the end of the experiments with carbon dioxide.

2.5. Study design

2.5.1. Naloxone administered prior to analgesics

A total of 96 animals were divided in 4 experimental series. The first series included three groups ($n=8$, each), each

receiving saline and, 10 min later, one of the following treatments: a) 600 mg/kg dipyrone, b) 3.1 mg/kg morphine; or c) 600 mg/kg dipyrone+3.1 mg/kg morphine. The other three series (3 groups each) were used to evaluate the effect of three naloxone doses (0.3, 1.0 and 3.1 mg/kg) administered 10 min before dipyrone, morphine or the morphine–dipyrone combination ($n=8$, each group).

2.5.2. Naloxone administered after analgesics

Six groups ($n=8$, each) were used for this study. Saline or naloxone (0.3 or 3.1 mg/kg) was administered 2 min after reaching the antinociceptive peak effect. This time varied depending on the analgesic, i.e., in rats treated with 3.1 mg/kg morphine or the combination of morphine plus dipyrone, naloxone was given at the 17th min, in dipyrone-treated rats, naloxone was administered at min 32.

2.6. Data analysis

All results were expressed as the mean±S.E.M of eight determinations. Antinociception was evaluated by: a) tail withdrawal latency and; b) the area under the curve for each time course. The area under the curve was calculated by the trapezoidal rule (Gibaldi, 1991). We used a non-paired Student's t test to test for significant differences between two independent groups; a one-way analysis of variance (ANOVA), followed by Dunnett's test, to compare drug effects in several experimental groups with respect to a control; and a two-way analysis of variance to compare the effect of treatment, time and interaction between two groups. All statistical procedures were performed with SigmaStat (version 2.03, Jandel).

3. Results

3.1. Naloxone administered prior to analgesics

Morphine (3.1 mg/kg) produced an antinociceptive effect that reached its maximum 15 min after administration. Naloxone completely prevented this effect at the three doses tested (Fig. 1A). For reasons of clarity, data corresponding to 1.0 mg/kg naloxone are not included in this figure since they overlap with those produced by 3.1 mg/kg. The main difference between these doses was that only 3.1 mg/kg naloxone produced a significant increase in nociception with respect to control animals (latencies to tail withdrawal: 4.9±0.4 s vs. 6.2±0.15 s, respectively; $P<0.05$, Student's t test; Fig. 1A).

Dipyrone produced a clear antinociception that reached its maximum 30 min after injection (Fig. 1B). The lowest naloxone dose (0.3 mg/kg) had no effect, but 1 and 3.1 mg/kg completely changed the antinociception time course. No peak was observed at 30 min, but there was a gradual increase in response that reached an apparent steady state 2 h after dipyrone administration. The difference between 1.0 and 3.1 mg/kg naloxone was that only the latter produced a significant increase in nociception with respect to the control group (latencies to tail withdrawal: 4.33±0.6 vs. 5.94±0.16, respectively; $P<0.05$; Student's t test; Fig. 1B).

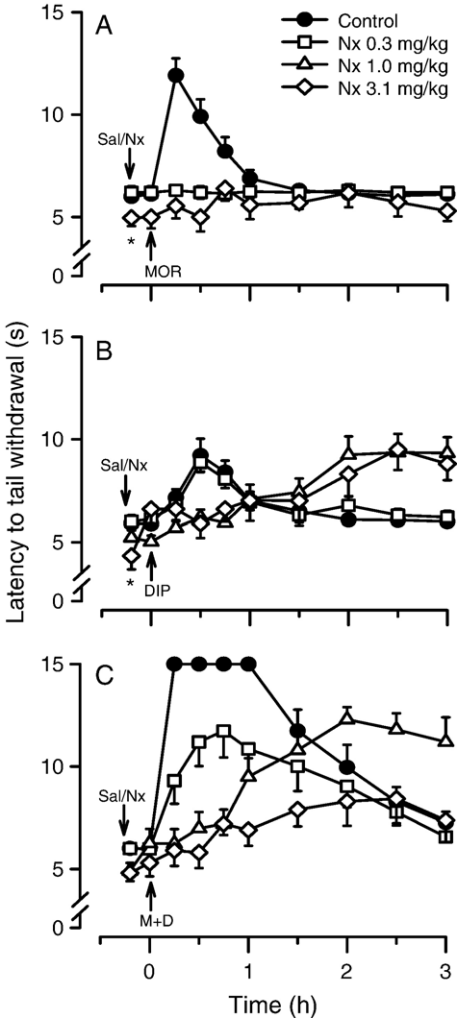

Fig. 1. Time course of the antinociceptive effect of 3.1 mg/kg morphine (MOR, panel A), 600 mg/kg dipyrone (DIP, panel B), or the combined administration of morphine and dipyrone at the same doses (M+D; panel C). Prior to analgesics, animals received saline (Sal) or naloxone (Nx). Each point represents the mean±S.E.M. of 8 data points. *$P<0.05$; Student's t test.

The combined administration of morphine and dipyrone resulted in supra-additive antinociceptive effects reaching the cut-off value (15 s) in the first 15 min and remaining at this maximum throughout the first hour of recording (Fig. 1C). Naloxone dose-dependently decreased the effectiveness of this combination and delayed the time at which maximum antinociception was achieved (Fig. 1C). It is worth noting that the doses of naloxone that were able to completely block morphine effects (panel A) could only partially prevent the antinociception produced by morphine plus dipyrone. Moreover, in clear contrast with that observed with dipyrone, in this experiment there was a clear difference between 1 and 3.1 mg/kg naloxone (panel C). Finally, the time courses corresponding to animals pretreated with 3.1 mg/kg naloxone were very similar whether they received dipyrone alone or in combination with morphine.

To rule out the possibility that the delayed antinociceptive peak response observed in rats pretreated with 3.1 mg/kg naloxone before the analgesic combination was due to the short

naloxone's half-life, an additional experiment was done using 3.1 mg/kg naltrexone, a more potent opioid antagonist with a longer half-life (Martin, 1977). No differences were found between the two groups [F (9,1)=0.005; P=0.944; two-way ANOVA; (data not shown)].

Fig. 2 shows a complementary analysis using the area under the curve data for each antinociception time course. This analysis confirms that naloxone completely blocked morphine-induced antinociception even at the lowest dose and had a pronociceptive effect at 3.1 mg/kg (negative area). As to dipyrone, naloxone was unable to block its antinociceptive effect. Paradoxically, the delay in dipyrone's time to peak produced by high naloxone doses resulted in an increased area. On the other hand, naloxone antagonised the antinociception produced by the analgesic combination of morphine and dipyrone, but with lower efficacy than against morphine. It is interesting to note that there was a significant difference between the effect of dipyrone alone versus dipyrone plus morphine in animals pretreated with enough naloxone to completely block that amount of morphine (5th vs. 10th bar; $P<0.05$; Student's t test).

3.2. Naloxone administered after analgesics

The second part of this work was designed to test if naloxone was able to counteract the supra-additive effects of morphine and dipyrone combination. When naloxone was administered immediately after morphine's peak effect, a rapid fall in antinociception was seen (Fig. 3A). The highest naloxone dose not only completely blocked morphine-induced antinociception, but also produced a very short-lasting hyperalgesia (3.9±0.5 s vs. 6.14±0.15; naloxone vs. control, respectively; $P<0.05$; Student's t test), manifested not only by a transient decrease in tail flick latency, but also by wet dog shakes, vocalizations and restlessness.

Naloxone, at 0.3 mg/kg, did not change dipyrone effects when administered 2 min after its peak antinociceptive effect, but at 3.1 mg/kg, it produced a fall in antinociception that was slower and less pronounced than that seen in morphine-treated animals. Thereafter, a gradual recovery of antinociception was observed (Fig. 3B).

When naloxone was given to rats treated with morphine and dipyrone, a dose-dependent antagonism was seen. With 1 mg/kg naloxone, the tail flick latency returned to basal values 2.5 h after administration, while with 3.1 mg/kg naloxone, it took only 60 min (Fig. 3C).

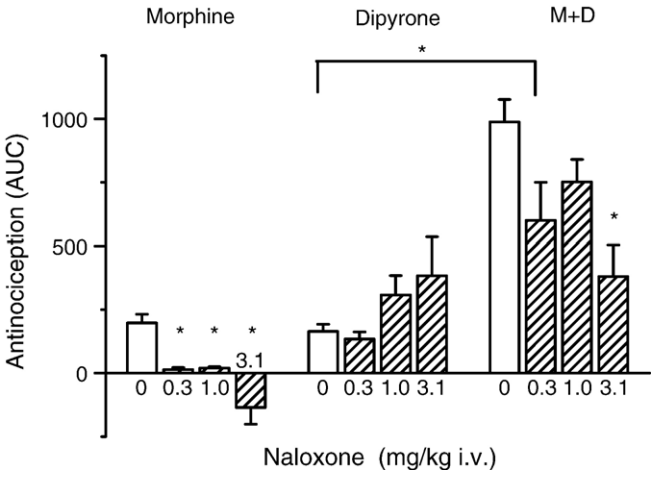

Fig. 2. Antinociception produced by 3.1 mg/kg morphine, 600 mg/kg dipyrone, or morphine plus dipyrone (M+D, same doses) evaluated as the area under the curve (AUC) of each time course. Each bar represents the mean±S.E.M. of 8 data points. Asterisks indicate that the value differs significantly from the respective control (*$P<0.05$; Dunnett's test) or, when in bracket, that the two values are significantly different (*$P<0.05$; Student's t test).

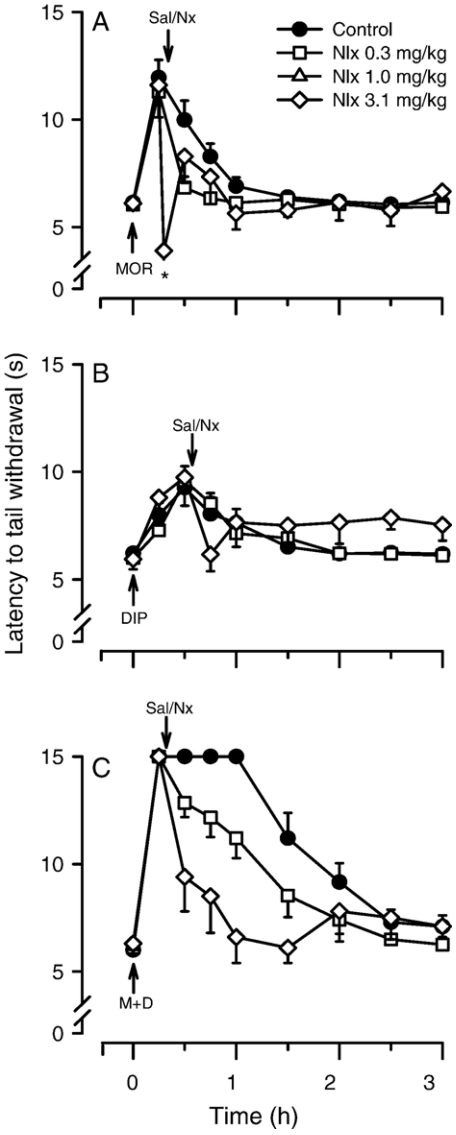

Fig. 3. Antinociception time course corresponding to 3.1 mg/kg morphine (MOR, panel A), 600 mg/kg dipyrone (DIP, panel B), and morphine plus dipyrone at the same doses (M+D; panel C). Naloxone (Nx) or saline (Sal) was given 2 min after the peak effect. Each point represents the mean±S.E.M. of 8 data points.

4. Discussion

Analgesic potentiation has been reported for the combined administration of morphine and dipyrone in different models of antinociception, including arthritic inflammation (López-

Muñoz, 1994; Hernández-Delgadillo et al., 2002), the formalin test (Aguirre-Bañuelos and Granados-Soto, 1999); the tail flick test (Carlsson and Jurna, 1987; Hernández-Delgadillo et al., 2003), and the writhing reflex test (Taylor et al., 1998; Miranda et al., 2005). Some attempts have been made to determine the mechanisms underlying this potentiation, leading to different results for each particular model. Aguirre-Bañuelos and Granados-Soto (1999) observed that the local supra-additive antinociception obtained with morphine and dipyrone in the formalin test was partially prevented by the opioid antagonist naloxone and dose-dependently blocked by the nitric oxide synthase inhibitor L-NAME. Based on this, they concluded that an opioid mechanism and the nitric oxide-cyclic GMP pathway were involved. By contrast, Taylor et al. (1998), using a visceral model of pain, were unable to reverse morphine and dipyrone potentiation with naloxone and concluded that opioids were not involved in it.

In this work we have used two approaches to determine if endogenous opioids play a role in the antinociceptive potentiation observed when morphine is given in combination with dipyrone. The experimental arguments supporting that endogenous opioids are released as a result of this particular analgesic treatment are the following: a) A dose of naloxone that is completely effective to block morphine does not block the effects of morphine plus dipyrone, while a higher naloxone dose does. This suggests that there are more opioids to antagonise in the latter than in the former situation; b) A high dose of naloxone (3.1 mg/kg) modifies dipyrone effects when it is administered before and after dipyrone; c) There is a clear dose–response relationship for naloxone's ability to prevent and to reverse the antinociceptive potentiation produced by morphine and dipyrone co-administration.

These results are in line with previous reports indicating that dipyrone, whether administered in the periaqueductal grey (Akman et al., 1996; Tortorici et al., 1996) or systemically (Vazquez et al., 2005), activates endogenous opioidergic circuits along the descending pain control system (Vasquez and Vanegas, 2000; Hernández and Vanegas, 2001). The release of endogenous opioids by dipyrone could enhance exogenous opiate effects, explaining the need for a higher amount of naloxone to counteract the antinociception produced by morphine plus dipyrone.

The delayed antinociception observed by the end of the experiment in naloxone-treated animals still remains to be explained. The first idea was that there was a competition between naloxone, morphine and the continuously released endogenous opioids for the receptors that was tilted in favour of agonists when naloxone concentration decayed as a function of its short elimination half-life. However, since naltrexone, an opioid antagonist with a longer half-life than naloxone did not prevent the delayed antinociception, it is reasonable to suppose that other substances are mediating this effect.

It is well-known that different mechanisms are involved in the antinociceptive action of dipyrone depending on the model used to induce nociception and on the route of drug administration (Siebel et al., 2004). At least three mechanisms in addition to endogenous opioid release have been proposed. One is the well described cyclooxygenase inhibition (Weithmann and Alpermann, 1985; Campos et al., 1999), which is common to other non-steroidal anti-inflammatory drugs; another possibility is a delayed activation of the L-arginine/nitric oxide/cGMP/K^+ channel pathway (Lorenzetti and Ferreira, 1996; Aguirre-Bañuelos and Granados-Soto, 1999; Alves and Duarte, 2002). Finally, dipyrone could interact with the glutamatergic system (Beirith et al., 1998; Siebel et al., 2004). At this point our results strongly suggest that endogenous opioid release plays an important role in morphine–dipyrone supra-additive antinociception, but other mechanisms seem to be involved, especially by the end of the antinociception time course. Their complete characterisation remains to be elucidated.

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