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Dipyrone potentiates morphine-induced antinociception in dipyrone-treated and morphine-tolerant rats

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Abstract

Coadministration of morphine and dipyrone produces acute and chronic antinociceptive potentiation in drug-naïve rats. In this work, the effectiveness of the combination was determined in rats pretreated with morphine or dipyrone. Nine groups of male rats received (i.v.) 3.1 mg/kg morphine, 600 mg/kg dipyrone, or the morphine-dipyrone combination twice a day for five administrations (three groups per treatment). From the 6th to the 10th administration, one group out of each treatment continued without change, while the other two were switched to one of the other two possible treatments. In morphine-tolerant rats, morphine plus dipyrone produced a transient antinociceptive potentiation. In dipyrone-treated animals, this combination produced a long-lasting potentiation. In animals only treated with the combination, antinociception was clear since the beginning, although it decreased after the 6th injection. No cross-tolerance was seen between morphine and dipyrone. These data suggest that dipyrone potentiates morphine-induced antinociception in dipyrone-treated as well as in morphine-tolerant rats.

Keywords: Opioid; Metamizol; Antinociception; Potentiation; Tolerance; Cross-tolerance

1. Introduction

The development of analgesic tolerance after repeated morphine administration is a common undesired side effect that can limit clinical opioid use for prolonged periods of time (Bhargava, 1994). This phenomenon leads to a gradual drug dose escalation with a potentially associated increase of undesirable effects (Mercadante, 1999). A strategy to reduce the unwanted side effects of high doses of analgesics is to combine low doses of opioids with nonsteroidal antiinflammatory drugs (Ripamonti and Dickerson, 2001; MacPherson, 2002). This approach may also result in improved analgesic efficacy (Maves et al., 1994; Christie et al., 1999). The acute potentiation produced by coadministration of opioids and nonsteroidal antiinflammatory drugs is one of the best characterised effects of these combinations (Grotto et al., 1965; Bentley and Head, 1987; Malmberg and Yaksh, 1993; Picard et al., 1997; Lashbrook et al., 1999). Supra-additive effects have been observed with acute analgesic administration, but little is known about the efficacy of combining low doses of opioids and nonsteroidal antiinflammatory drugs for prolonged periods.

Among nonsteroidal antiinflamatory compounds, dipyrone (also known as metamizol) is widely used in many countries for pain management due to its high efficacy and good gastric tolerability (Patel et al., 1980; Rodriguez et al., 1994; Planas et al., 1998; Sanchez et al., 2002). Some preclinical studies have shown that dipyrone enhances morphine-induced antinociception when both drugs are acutely coadministered (Carlsson and Jurna, 1987; Lopez-Muñoz, 1994; Taylor et al., 1998; Aguirre-Bañuelos and Granados-Soto, 1999; Hernández-Delgadillo et al., 2002). Our group has recently reported that the morphine-dipyrone combination produces antinociceptive potentiation evaluated in the tail-flick test and delays tolerance

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development throughout repeated administrations (Hernández-Delgadillo et al., 2003). To our knowledge, no studies have addressed whether this synergism persists in morphine-tolerant or dipyrone-pretreated rats. The purpose of the present work was to study the antinociceptive efficacy of morphine plus dipyrone coadministration in rats previously treated repeatedly with each of the individual drugs in the tail-flick test.

2. Materials and methods

2.1. Animals

Male Wistar rats (180–220 g) were housed in an animal room under controlled temperature (22±2 °C) and a 12:12 h light–dark cycle (lights on at 7:00 h) with free access to drinking water and commercial food. In order to reduce stress, all rats were handled twice a day for 2 days before testing the drugs. Our local Committee on Ethics on Animal Experimentation approved all experimental procedures, which followed the regulations established in the Mexican official norm for the use and care of laboratory animals "NOM-062-ZOO-1999". All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983).

2.2. Drugs

Morphine sulphate was obtained from Laboratorios Pisa, dipyrone sodium purchased from Aventis (Mexico City, Mexico) and heparin sodium bought from Sigma (St. Louis, MO, USA). All drugs were dissolved in sterile saline solution and administered intravenously.

2.3. Surgical procedure and drug administration

In ether anaesthetised rats, 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. After surgery, rats were individually housed and allowed a 24-h recovery period. 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. Drugs were injected in a 1-ml volume 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.

2.4. Evaluation of antinociceptive activity

A standardised tail flick apparatus, with a radiant heat source connected to an automatic timer (UGO BASILE, Italy) was used. In this model, antinociception is manifested as an increase in tail withdrawal latency (D'Amour and Smith, 1941; modified by Nance and Sawynok, 1987). The stimulus intensity was adjusted to a baseline tail flick latency of 6.0±0.5 s. Before the first drug injection, animals were screened for thermal nociception; those animals showing no flicking within 5.5–6.5 s (approximately 10–15% of the total) were excluded from the study. To avoid tissue damage, the cut-off time in the absence of response was set at 15 s. 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 during the subsequent 2 h. Rats were euthanised at the end of the experiments with carbon dioxide.

2.5. Study design

Nine groups (n=8, each) of animals were treated with either 3.1 mg/kg morphine, 600 mg/kg dipyrone, or the combination of the same doses of morphine plus dipyrone (3 groups per treatment) twice a day until completing five administrations (1st treatment phase). These doses were chosen based on dose–response curves previously done because they are close to their EC_{50} values (Hernández-Delgadillo et al., 2003). During the 2nd phase (from the 6th to the 10th administration), one group out of each treatment continued without change, while the other two groups were switched to one of the other two possible treatments.

2.6. Data and statistical analysis

All results are expressed as the mean±S.E.M. of eight determinations. Antinociception was evaluated by three different parameters: (a) an increase in tail withdrawal latency; (b) the percentage of maximum possible effect; and (c) the area under the curve for each time course. The percentage of maximum possible effect (%MPE) was calculated at the peak effect, using the formula $\%MPE = [(A-B)/(15-B)]\times 100$, where B and A were the tail flick latencies before and after drug administration, and 15 was the cut-off time value. The area under the curve was calculated by the trapezoidal rule (Gibaldi, 1991). A one-way analysis of variance for repeated measures, followed by Dunnett's test, was used to compare the drug effects after repeated administrations with respect to those observed with the first injection. A two-way analysis of variance (administration, treatment, and their interaction) followed by a Tukey test was applied to compare the observed effects of the combination of morphine plus dipyrone and the expected sum of individual effects at each administration. The expected values were calculated on the basis of addition of the effects of the individual component drugs (Seegers et al., 1981; Hernández-Delgadillo et al., 2002). The mean responses of two independent experimental groups were

compared using a Mann–Whitney test. The program used to perform statistical procedures was SigmaStat (version 2.03, Jandel Scientific).

3. Results

3.1. Antinociceptive effects of dipyrone and the morphine-dipyrone combination in morphine-tolerant rats

Complete tolerance to the antinociceptive effects of morphine developed after its 5th administration (Fig. 1). When morphine-tolerant animals were switched to dipyrone, this nonsteroidal antiinflammatory drug produced a similar antinociceptive effect to that seen when dipyrone was given to drug-naïve animals (compare the effects observed in Fig. 1 at the 6th administration with those seen in Fig. 2 at the 1st dose of dipyrone, area under the curve: 108±26 vs. 145.2±51.5; percentage of maximum possible effect: 26±6.4 vs. 36.1±7.2, n.s., Mann-Whitney test). When rats already tolerant to morphine received morphine plus dipyrone at the 6th administration, a clear and complete antinociceptive effect was observed. It is worth noting that the combined administration of these analgesics produced a significantly higher response than what could have been expected by the addition of their individual effects (dashed line). This treatment, however, lost efficacy when given repeatedly.

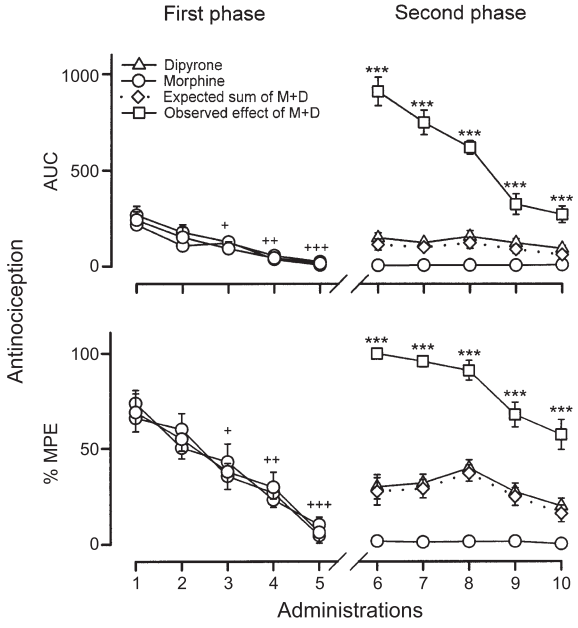

Fig. 1. Antinociceptive effects of repeated administration of 3.1 mg/kg morphine and the combination of the same dose of morphine plus 600 mg/kg dipyrone (M+D) in dipyrone-treated rats. Data are expressed as the area under the curve (AUC, upper panel) and as the percentage of maximum possible effect (%MPE, lower panel). Drug treatment was switched at the 6th administration. Each point represents the mean±S.E.M. of eight rats. Asterisks denote statistically significant differences from the expected sum of the effects of morphine plus dipyrone at each administration. *P<0.05; ***P<0.001; Tukey test.

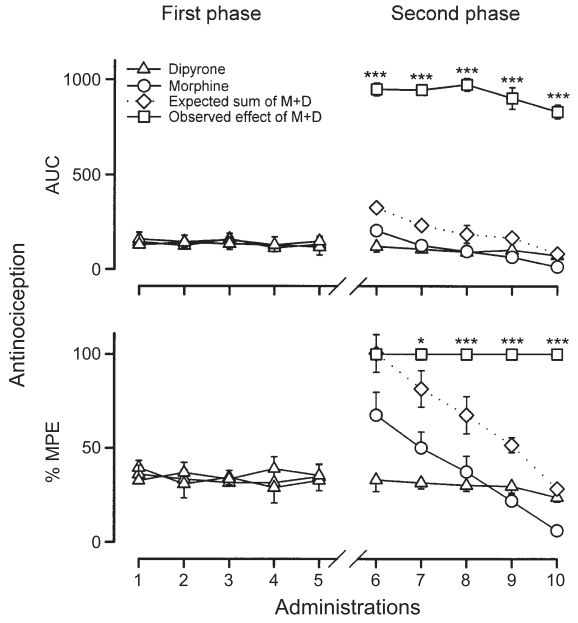

Fig. 2. Antinociceptive effects of repeated administration of 600 mg/kg dipyrone and the combination of 3.1 mg/kg morphine plus the same dose of dipyrone (M+D) in morphine-tolerant rats. Data are expressed as the area under the curve (AUC, upper panel) and as the percentage of maximum possible effect (%MPE, lower panel). Drug treatment was switched at the 6th administration. Each point represents the mean±S.E.M. of eight rats. Asterisks denote statistically significant differences between the observed effects of morphine plus dipyrone and the expected sum of the effects of this combination at each administration. ***P<0.001; Tukey test. Crosses indicate statistical significance with respect to the first morphine dose (^{+}P<0.05, ^{++}P<0.01, ^{+++}P<0.001; Dunnett's test).

3.2. Antinociceptive effects of morphine and the morphine-dipyrone combination in dipyrone-treated rats

Ten administrations of dipyrone produced relatively constant antinociception throughout the study (Fig. 2), i.e., no statistically significant differences were found among the responses observed at different administrations [$F(9,63)$= 1.1; P=0.37; analysis of variance for repeated measures]. Thus, by the time animals were changed to a different treatment, there was no evidence of tolerance development. When, at the sixth drug administration, rats were switched to morphine, an antinociception similar to that seen in non-treated animals was produced (compare the effects observed in Fig. 2 at the 6th administration of morphine with those seen in Fig. 1 at the 1st dose, area under the curve: 205±27.8 vs. 215.4±22.2; percentage of maximum possible effect: 67.4±12.3 vs. 73.7±7 n.s., Mann-Whitney test). With subsequent administrations, tolerance developed in a similar way to that in animals exclusively treated with morphine. Of interest is the finding that when dipyrone-treated animals were switched to the combined treatment of morphine plus dipyrone, a complete and relatively stable antinociception was observed.

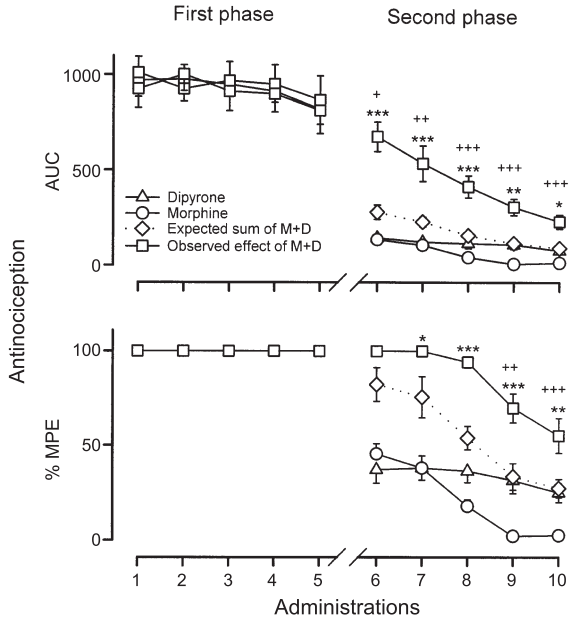

Fig. 3. Antinociceptive effects of repeated administration of 600 mg/kg dipyrone and 3.1 mg/kg morphine in rats pretreated with the combination of the same doses of morphine and dipyrone (M+D), expressed as the area under the curve (AUC, upper panel) and as the percentage of maximum possible effect (%MPE, lower panel). Drug treatment was switched at the 6th administration to morphine or dipyrone. Each point represents the mean±S.E.M. of eight rats. Asterisks denote statistically significant differences between the observed effects of morphine plus dipyrone and the expected sum of the effects of this combination at each administration. *P<0.05, **P<0.01, ***P<0.001; Tukey test. Crosses indicate statistical significance with respect to the first administration of the morphine-dipyrone combination. ^{+}P<0.05, ^{++}P<0.01, ^{+++}P<0.001; Dunnett's test.

3.3. Antinociceptive effects of morphine and dipyrone in rats pretreated with the morphine-dipyrone combination

Fig. 3 shows that in animals treated with the morphine-dipyrone combination, there was a significantly higher antinociception than that observed with the individual compounds in previous experiments. This effect decreased after the 6th injection of the combined analgesic treatment. In rats treated five times with morphine plus dipyrone and then switched to morphine alone, a lower antinociceptive action was seen and tolerance developed by the end of the treatment. When animals were switched to dipyrone, a mild but constant antinociception was seen.

4. Discussion

4.1. Potentiation of antinociception

It is well documented that combinations of analgesics that act at different receptors and/or on different transmission systems involved in pain modulation may reduce the effective dose of the single components (Bentley and Head, 1987; Cichewicz, 2004) and enhance pain relief (Picard et al., 1997; Curatolo and Sveticic, 2002). In a previous study, we analysed the efficacy of ten morphine plus dipyrone administrations to counteract thermal nociception in drug-naïve animals and found a marked response potentiation after acute and repeated administration (Hernández-Delgadillo et al., 2003). Since in clinic it is common to combine analgesics in patients that have already been treated with different drugs for pain management, we considered it of interest to extend these studies to rats pre-treated with morphine or dipyrone. In both cases, dipyrone and morphine coadministration resulted in antinociceptive potentiation. Important differences were found in the duration and magnitude of the effects.

In the present study, tolerance to the morphine-dipyrone combination was evident only from the 6th administration onwards in drug-naïve animals. In dipyrone-pretreated rats, tolerance to the combination was not observed, but it must be taken into account that only five administrations were tested. Most probably this phenomenon would have become evident with more prolonged treatments. Finally, in morphine-tolerant rats, the addition of dipyrone to the analgesic treatment enhanced antinociception to a higher degree than what could have been expected from simple additive effects. However, this potentiation significantly decreased throughout repeated administrations, suggesting a rapid development of tolerance to the analgesic combination.

Additive and supra-additive antinociceptive effects of an acute morphine plus dipyrone combination have been previously observed in different experimental models. Lopez-Muñoz (1994) showed that systemic administration of these drugs in combination was more effective to reverse nociception in monoarthritic rats than each of them alone. Carlsson and Jurna (1987) reported that an i.t. morphine injection potentiated the antinociceptive effects of dipyrone administered i.p. or into the periaqueductal grey of rats tested in the tail-flick model. According to Taylor et al. (1998), simultaneous i.p. administration of subeffective morphine and dipyrone doses resulted in antinociception potentiation in the writhing test. Moreover, Aguirre-Bañuelos and Granados-Soto (1999) showed that when these analgesics were coadministered at the same formalin injection site in rats, they produced significantly higher antinociception than that seen with either drug in the formalin test. Taken together, this evidence shows that antinociceptive potentiation between morphine and dipyrone occurs for a wide variety of noxious stimuli. From these data it also appears that potentiation involves both peripheral and central structures; however, the exact mechanism by which this effect occurs is still unknown.

In previous studies, we observed that dipyrone potentiated morphine antinociception after chronic treatment in rats injected with uric acid into the right knee joint as well as in rats evaluated in the tail flick test throughout repeated administrations of the morphine-dipyrone combination (Hernández-Delgadillo et al., 2002, 2003). In both cases,

the analgesics were administered systemically. It would be interesting to establish if the long-lasting potentiation observed under those conditions could be replicated using other routes of administration. Further experiments are warranted to address this issue.

4.2. Potentiation versus reversal of tolerance

Repeated morphine administration can induce the development of tolerance to its analgesic effects (Twycross, 1997; Gutstein and Akil, 2001; Martin and Eisenach, 2001). There is evidence that a wide variety of agents can reverse this tolerance. Among these, the best studied drugs are cyclooxygenase inhibitors (Powell et al., 1999), competitive and noncompetitive *N*-methyl-D-aspartic acid receptor antagonists (Tiseo and Inturrisi, 1993; Tiseo et al., 1994; Elliot et al., 1994; Kolesnikov and Pasternak, 1999; Popik et al., 2000), antagonists acting at the glycine site of the *N*-methyl-D-aspartic acid receptor (Kolesnikov et al., 1994; Christensen et al., 2000), and drugs that inhibit the nitric oxide synthase (Kolesnikov et al., 1993; Powell et al., 1999). Adding these agents to morphine-tolerant animals produced a progressive and slow restoration of antinociception over time. This gradual recovery can be seen even when morphine is still in the experimental preparation (Kolesnikov et al., 1993; Powell et al., 1999; Popik et al., 2000).

Dipyrone and its active metabolites 4-methylaminoantipyrine and 4-aminoantypirine inhibit prostaglandin synthesis (Weithmann and Alpermann, 1985). This effect is mediated through cyclooxygenase-2 activity inhibition (Campos et al., 1999). Although there are no available data to directly support that dipyrone acts as a *N*-methyl-D-aspartic acid receptor antagonist, this possibility cannot be discarded because according to Beirith et al. (1998), dipyrone produces a slight inhibition (37%) of tritiated glutamate binding in cerebral cortical membranes of mice and rats. Based on these actions it is reasonable to suppose that dipyrone would be able to reverse morphine-induced tolerance. Contrary to this idea is the here reported pattern opposed to the well-described gradual reversal of tolerance; i.e., there was an immediate antinociception recovery with the first administration of the morphine-dipyrone combination in morphine-tolerant animals, decreasing rather than increasing with subsequent administrations. These results suggest that the antinociception recovery observed in the present experiments may not represent the reversal of tolerance, but may instead reflect analgesic potentiation.

Interestingly, in contrast with the transient increase in nociception observed in morphine-tolerant rats, there was a sustained potentiation when the morphine-dipyrone combination was administered to dipyrone-pretreated rats, thus suggesting that it is better to add morphine to dipyrone than to add dipyrone to morphine when changing from a single to a combined analgesic treatment.

4.3. Lack of cross tolerance between morphine and dipyrone

The experimental design presented in this paper allows comparisons of dipyrone efficacy in morphine-treated rats and vice versa. Dipyrone was equieffective in drug-naïve rats and in completely morphine-tolerant animals. Similarly, morphine produced the same antinociception in drug-free and in dipyrone-treated rats, indicating no cross tolerance between these substances. These results contrast with those reported by Tortorici and Vanegas (2000) in which morphine lacked an effect when given to rats rendered tolerant to dipyrone by repeated injections into the periaqueductal grey. In addition to the well-known peripheral effects of nonsteroidal anti-inflammatory drugs, dipyrone produces antinociception by acting upon central nervous system structures (Carlsson and Jurna, 1987; Akman et al., 1996; Jones, 1996; Tortorici and Vanegas, 2000). These central effects can be blocked or reduced by naloxone suggesting that endogenous opioid systems are involved (Tortorici et al., 1996). This allows the possibility of cross tolerance between morphine and dipyrone. The lack of cross tolerance in the present work could be due to our use of relatively short-term analgesic treatments and/or the systemic route of administration used for the compounds. Until very recently it was generally believed that nonsteroidal antiinflammatory drugs do not produce tolerance to their analgesic effects (Sunshine and Olson, 1994; Roberts and Morrow, 2001). In agreement with the work by Tortorici and Vanegas (2000), in a recent study we found that 600 mg/kg dipyrone, i.v., twice a day, for 11 days resulted in complete tolerance to its antinociceptive effects (Hernández-Delgadillo et al., 2003). Further studies are needed to determine if morphine cross tolerance could arise in animals rendered completely tolerant to dipyrone.

4.4. Concluding remarks

A limiting factor in the clinical use of opioid analgesic drugs is analgesic tolerance development with repeated administration. Considering the importance of opioids in the management of pain, information that provides strategies to attenuate, delay or reverse tolerance associated with their use can be valuable. Our data suggest that the dipyrone and morphine combination may be an effective therapeutic strategy to manage pain even when tolerance to morphine exists. Additionally, our results also suggest that the effectiveness of this combination lasts longer when used after dipyrone than after morphine pretreatment.

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