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Pharmacology, Biochemistry and Behavior 77 (2004) 695–703
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

Nefopam potentiates morphine antinociception in allodynia and hyperalgesia in the rat

Philippe Girard*, Yannick Pansart, Jean-Marie Gillardin

Laboratoires Biocodex, Service de Pharmacologie, Zac de Mercières, 60200 Compiègne, France

Received 28 October 2003; received in revised form 15 January 2004; accepted 19 January 2004

Abstract

The objective of this study was to resolve discrepancies regarding the possible antinociceptive synergy between morphine and nefopam in animal models of pain. Firstly, we have examined the antinociceptive activity of nefopam, a nonopioid antinociceptive compound that inhibits monoamine reuptake, in pain models of allodynia and hyperalgesia induced by carrageenan injection, or skin and muscle incision of the rat hind paw. Single subcutaneous administration of nefopam at 30 mg/kg blocked carrageenan- and incision-induced thermal hyperalgesia, and weakly but significantly diminished carrageenan-induced tactile allodynia. A weaker dose of nefopam (10 mg/kg) only reduced carrageenan-induced tactile allodynia and incision-induced thermal hyperalgesia. Secondly, we assessed the usefulness of the coadministration of nefopam with morphine. Combination of a nonanalgesic dose of nefopam (10 mg/kg) with a nonanalgesic dose of morphine (0.3 or 1.0 mg/kg) completely inhibited carrageenan- or incision-induced thermal hyperalgesia, respectively. In carrageenan-induced tactile allodynia, coadministration of weak analgesic doses of nefopam (10 and 30 mg/kg) with a nonanalgesic dose (1 mg/kg) or moderately analgesic dose (3 mg/kg) of morphine significantly reduced or reversed allodynia, respectively. In conclusion, coadministration of nefopam with morphine enhances the analgesic potency of morphine, indicating a morphine sparing effect of nefopam.

Keywords: Nefopam; Morphine; Potentiation; Carrageenan; Incision; Thermal; Mechanical; Tactile; Hyperalgesia; Allodynia

1. Introduction

Injury and/or inflammation to peripheral tissues, after surgery or various damages, produces sensory changes, such as prolonged pain, increased sensitivity to painful stimuli (hyperalgesia) and/or nonpainful stimuli (allodynia) reviewed by Millan (1999).

Morphine is the main analgesic used in postoperative pain. Some patients are not well protected from pain, and after repeated administrations, side effects appear, like respiratory depression, and its efficacy decreases due to the development of tolerance (Cooper et al., 1997; Guignard et al., 2000). In order to improve analgesia associated with morphine, coadministration of another analgesic drug possessing a different mechanism of action is often attempted (Sutters et al., 1999; Suzuki et al., 1999; Mimoz et al., 2001; Reynolds et al., 2003).

Nefopam is a centrally acting antinociceptive compound (Piercey and Schroeder, 1981; Tresnak-Rustad and Wood, 1981) with both supraspinal and spinal sites of action (Berge et al., 1986; Fasmer et al., 1987). This analgesic drug (Conway and Mitchell, 1977; Heel et al., 1980), which is structurally unrelated to other analgesics, induces antinociception in animals (Conway and Mitchell, 1977; Bernatzky and Jurna, 1986; Girard et al., 2001; Buritova and Besson, 2002) and in humans (Beaver and Feise, 1977; Heel et al., 1980; Guirimand et al., 1999). Its main mechanism of action involves the inhibition of monoamine reuptake in the central nervous system in vitro (Tresnak-Rustad and Wood, 1981; Rosland and Hole, 1990) and in vivo (Vonvoigtlander et al., 1983; Hunskaar et al., 1987; Ohkubo et al., 1991; Fuller and Snoddy, 1993). Nefopam does not bind to opiate receptors (Heel et al., 1980) and its antinociceptive activity is not inhibited by the opioid antagonist naloxone in the hot plate test (Piercey and Schroeder, 1981).

In view of these characteristics, the study of the coadministration of nefopam with morphine has been investigated in some animal models (Conway and Mitchell, 1977; Kvam, 1979) and in a few clinical studies (McLintock et al.,

* Corresponding author. Tel.: +33-3-44-86-82-28; fax: +33-3-44-86-82-34.
E-mail address: p.girard@biocodex.fr (P. Girard).

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doi:10.1016/j.pbb.2004.01.018

1988; Mimoz et al., 2001). However, these studies present discrepant results. For example, in the mouse radiant heat test, one study did not show enhancement of morphine antinociception by nefopam (Conway and Mitchell, 1977), but a later study showed that nefopam enhances morphine's analgesic effect (Kvam, 1979). Furthermore, two clinical studies indicated that nefopam has a morphine-sparing effect (McLintock et al., 1988; Mimoz et al., 2001). In order to address these discrepancies and directly evaluate the morphine-sparing characteristics of nefopam, the present study was undertaken using long-term inflammatory (carrageenan) and postoperative (incision) pain models (Hargreaves et al., 1988; Brennan et al., 1996; Zahn et al., 1997).

Intraplantar injection of carrageenan into one rat hind paw is a well-established method and possesses many similarities to clinical inflammatory diseases, like persistent strong hyperalgesia. This inflammatory agent produces a local inflammation, which can induce thermal hyperalgesia and tactile allodynia previously observed in animals (Hargreaves et al., 1988; Tabo et al., 1998; Xu et al., 2000; Yamamoto et al., 2001). In the postoperative pain model, an incision through the skin, fascia and muscle of the plantar aspect of the rat hind paw induces mechanical (Brennan et al., 1996; Zahn et al., 1997) and heat hyperalgesia (Field et al., 1997). These procedures mimic some painful conditions in chronic, injured patients after surgery and/or peripheral nerve lesions (Brennan et al., 1996).

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Janvier breeding) were housed in air-conditioned, temperature (22 ± 2 °C)- and hygrometry (50 ± 20%)-controlled rooms with a 12:12-h light/dark lighting schedule. Diet (UAR, France) and filtered tap water were available ad libitum. Experiments were run at least 4 days after the animals arrived in the laboratory. All the experiments were carried out in accordance with the recommendations of the International Association for the Study of Pain (IASP) Committee for Research and Ethical Issues Guidelines [*Pain 16* (1983) pp. 109–110].

2.2. Induction of carrageenan unilateral hindpaw inflammation

Male rats (220–250 g) were used in groups of 10. Peripheral inflammation was induced by intraplantar injection of 2% carrageenan solution (0.1 ml) into the middle of the plantar surface of the right hind paw, immediately after the control test (basal response), in nonanaesthetised rats. Tactile allodynia and thermal hyperalgesia were assessed 1, 2, 3, 4 and 5 h after carrageenan injection.

2.3. Induction of unilateral hindpaw incision

The method of Brennan et al. (1996) was used. Male rats (300–350 g) were used in groups of 10. The first day (Day 0), a control value of the withdrawal threshold or latency was measured before surgery. Then, animals were anaesthetised with pentobarbital (75 mg/kg ip was used in order to limit pain). The left hind paw was cleaned with Poliodine, and a 1-cm longitudinal incision was made through the skin and muscle of the plantar aspect. The skin was sutured and an intramuscular injection of penicillin (Extencillin) was given to avoid infection. The rat was placed in an individual cage with sterilised bedding. Mechanical and thermal hyperalgesia were assessed the second day (Day 1) at various times until 3 h after the treatments.

2.4. Hand-held electronic algometer

All rats were first tested before the injection of carrageenan or before incision to obtain basal responses. Animals were placed on an elevated plastic mesh grid (10 × 10 mm) which allowed access to the paws and they were left to habituate for a few minutes. The pressure algometer consisted of a hand-held force transducer made by Bioseb (France) which resembles the Somedic type 739 used by Moller et al. (1998).

For tactile allodynia threshold measures, a stainless steel spring ended by a hair, like a filament, connected to the transducer, was applied under the paw until a withdrawal or the bending of the filament. The maximum paw withdrawal threshold (PWT) reached with this spring was around 15–16 g, which is nearly the same PWT (15.1 g) reached using von Frey filaments with the up–down method for control animals (Chaplan et al., 1994; Yamamoto et al., 2001). Carrageenan was injected into the right hind paw, and 2.5 h after, the test drug or a combination of the drugs, was administered by subcutaneous route.

The algometer probe was applied manually with an increasing force until the rat withdrew his paw and allowed on-line display. Withdrawal threshold was determined in two sessions averaged and spaced of a few minutes.

2.5. Thermal hyperalgesia testing

The method of Hargreaves et al. (1988) was used. Male rats (300–350 g) were used in groups of 10. Rats were placed individually in a clear plastic chamber (Ugo Basile Plantar test apparatus) and left to acclimatise for 5 min before testing. Light from an 8-V, 50-W halogen bulb (64607 Osram) was delivered to the plantar surface of one of the rat's hind paws through the base of the plastic box. The beam was about 12 mm in diameter (as used by Tabo et al., 1998; Xu et al., 2000; Bianchi and Panerai, 1988). The time taken for the rat to withdraw its left hind paw was noted. Animals not responding were

removed after 30 s (cutoff time, similar to Taguchi et al., 1999). Two applications of the radiant heat stimulus were done, separated by 1- to 2-min intervals, and the mean of the two measures was taken. The intensity was selected in order to reach a basal withdrawal latency of 8–10 s, and to reduce the variability.

2.6. Drugs

Nefopam hydrochloride (Biocodex Laboratories, France), morphine (supplied by Francopia, France) and carrageenan lambda (Sigma, France) were dissolved in physiologic saline solution (0.9% sodium chloride). Nefopam and morphine were administered subcutaneously. Control animals received physiological saline.

2.7. Statistical methods

Data are expressed as mean ± S.E.M. The statistical test used was repeated-measures analysis of variance (ANOVA) followed by post hoc comparisons based on Bonferroni or Dunnett methods to determine differences in significance between groups. A P value less than .05 was assumed as the significance level.

3. Results

3.1. Carrageenan-induced tactile allodynia

On the test day, all the animals exhibited baseline PWTs of the noninjected hind paw around 15–16 g. Carrageenan intraplantar injection significantly reduced the nociceptive PWT of the injected paw to around 6–9 g, but did not affect the PWT of the noninjected contralateral hind paw. This tactile allodynia was present 2 h after carrageenan injection and for at least 5 h.

The subcutaneous administration of nefopam during the inflammation period, 2.5 h after injection of the algogen, slightly but significantly reduced tactile allodynia 30 min after its administration (Fig. 1A). At this time, the PWT was reduced from 14.5 ± 0.4 g for the noninjected paw to 6.0 ± 0.4 g for the inflamed paw, and nefopam, 10 and 30 mg/kg, increased this paw inflamed threshold to 9.8 ± 0.9 and 8.6 ± 0.9 g, respectively. This antiallodynic effect was considered moderate because it did not restore the control threshold, and was absent at the other times tested.

In the same protocol, morphine showed a dose-related antiallodynic effect (Fig. 1B). At 1 mg/kg, morphine did not exhibit antinociceptive activity, but at 3 mg/kg, morphine significantly augmented the PWT at 30, 90 and 150 min after morphine injection, and restored it to around 50% of the control threshold. At a dose of 10 mg/kg, morphine completely blocked carrageenan-induced tactile allodynia during the experiment, and restored the control PWT to control values.

Coadministration of nefopam with a nonanalgesic dose of morphine (1 mg/kg), induced the appearance of a significant antiallodynic activity 30 min after administration and until 5 h after carrageenan injection (Fig. 1C): the PWT was reduced from 16.0 ± 0.4 g for the noninjected paw to 6.5 ± 0.4 g for the inflamed paw and morphine, at 1 mg/kg, did not modify this inflamed paw threshold (6.2 ± 0.7 g), 3 h after carrageenan injection. At the same time, coadministration of nefopam at 10 and 30 mg/kg with morphine significantly reduced this tactile allodynia, with an increase of the inflamed paw threshold to 9.5 ± 1.3 and 12.4 ± 1.1 g, respectively.

Simultaneous injection of nefopam with a mild analgesic dose of morphine (3 mg/kg) yielded a restoration of control PWT values until the end of the experiment (Fig. 1D). For example, morphine at 3 mg/kg produced a small but significant increase of the inflamed paw threshold (from 7.1 ± to 0.5 to 9.9 ± 1.0 g), 4 h after carrageenan injection. At the same time, coadministration of nefopam at 10 and 30 mg/kg with morphine restored the PWT values close to control (12.8 ± 0.9 and 13.8 ± 0.7 g, respectively).

Thus, coadministration of nefopam with morphine, at a nonanalgesic (1 mg/kg) or a mild analgesic (3 mg/kg) dose, yielded an increase in the nociceptive PWT or a tendency to restore the control withdrawal threshold, respectively, and these effects were close to those produced by single doses of 3 or 10 mg/kg of morphine, respectively.

Under the same conditions, single administration of nefopam or morphine, or combination of both drugs, did not significantly modify the PWT of the contralateral uninflamed hind paw.

3.2. Carrageenan-induced thermal hyperalgesia

On the test day, all the animals exhibited baseline paw withdrawal latencies (PWLs) of the noninjected paw around 11.5–17.5 s. Carrageenan intraplantar injection significantly reduced the nociceptive PWL of the injected paw to around 6–8 s, but did not affect the PWL of the noninjected contralateral hind paw. This heat hyperalgesia, which began to develop 2 h after carrageenan injection, was more pronounced at 3 h, and remained stable until 5 h.

The subcutaneous administration of nefopam during the inflammation period, 2.5 h after injection of the algogen, blocked thermal hyperalgesia at 30 mg/kg, but was inactive at 10 mg/kg (Fig. 2A). The highest dose of nefopam induced quick and strong antinociception 30 min after its administration, and until 5 h. For example, 3 h after its injection, carrageenan reduced the PWL from 12.6 ± 0.7 s for the noninjected paw to 6.6 ± 1.1 s for the inflamed paw. Nefopam at 30 mg/kg significantly increased this paw inflamed latency to 14.0 ± 1.3 s, and this antihyperalgic effect of nefopam allowed a restoration of the control latency.

In the same protocol, morphine showed a potent and dose-related antihyperalgic effect (Fig. 2B). At 0.3 mg/kg,

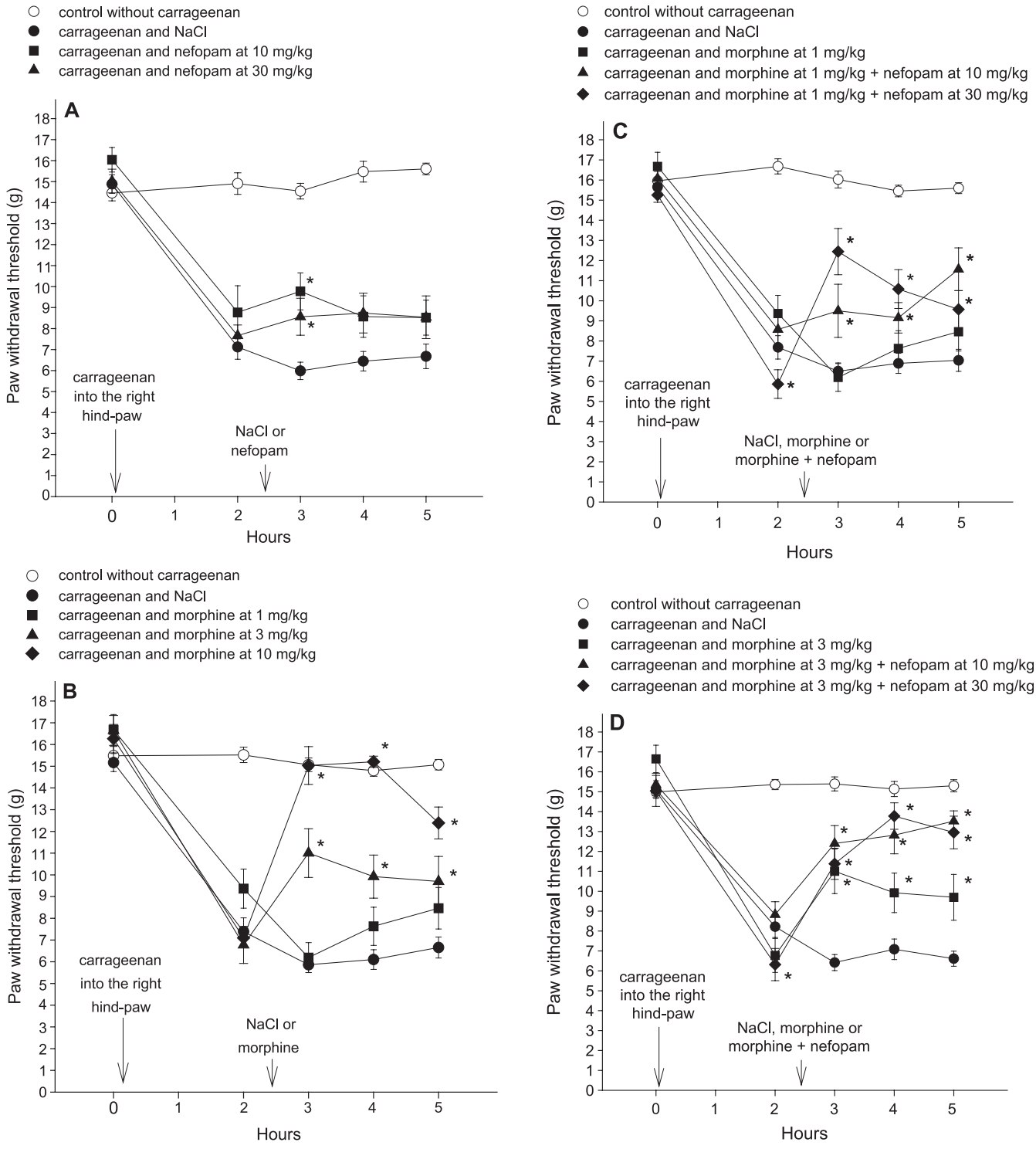

Fig. 1. Effects of subcutaneous nefopam (A), morphine (B) or both drugs' coadministration at various doses (C and D) on carrageenan-induced tactile allodynia in rats. PWTs were determined by a stainless steel filament placed on a spring to apply a pressure under the midplantar surface of one hind paw until the rat withdraws his paw. Baseline (Time 0) measurements were taken before animals received an intraplantar injection of carrageenan. Results are expressed as the mean of the PWT (g ± S.E.M.) of 10–30 animals per group. (* ANOVA statistical test with significance differences at the level of 5% for treated groups compared to the group receiving carrageenan and NaCl.)

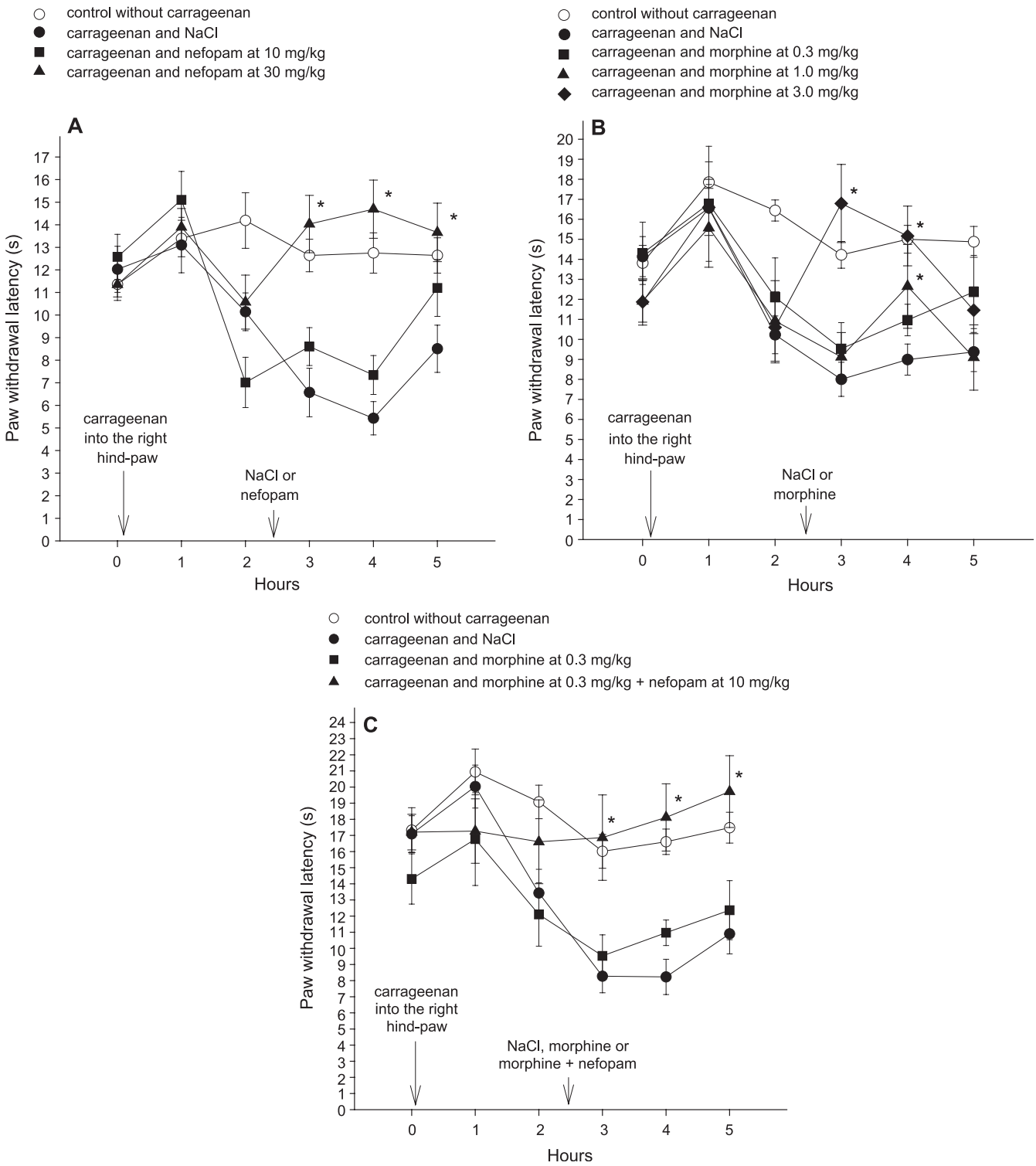

Fig. 2. Effects of subcutaneous nefopam (A), morphine (B) or both drugs' coadministration (C) on carrageenan-induced thermal hyperalgesia in rats. PWLs were determined by the rat plantar test. Baseline (Time 0) measurements were taken before animals received an intraplantar injection of carrageenan. Results are expressed as the mean of the PWL (s ± S.E.M.) of 10–20 animals per group. (* ANOVA statistical test with significance differences at the level of 5% for treated groups compared to the group receiving carrageenan and NaCl.)

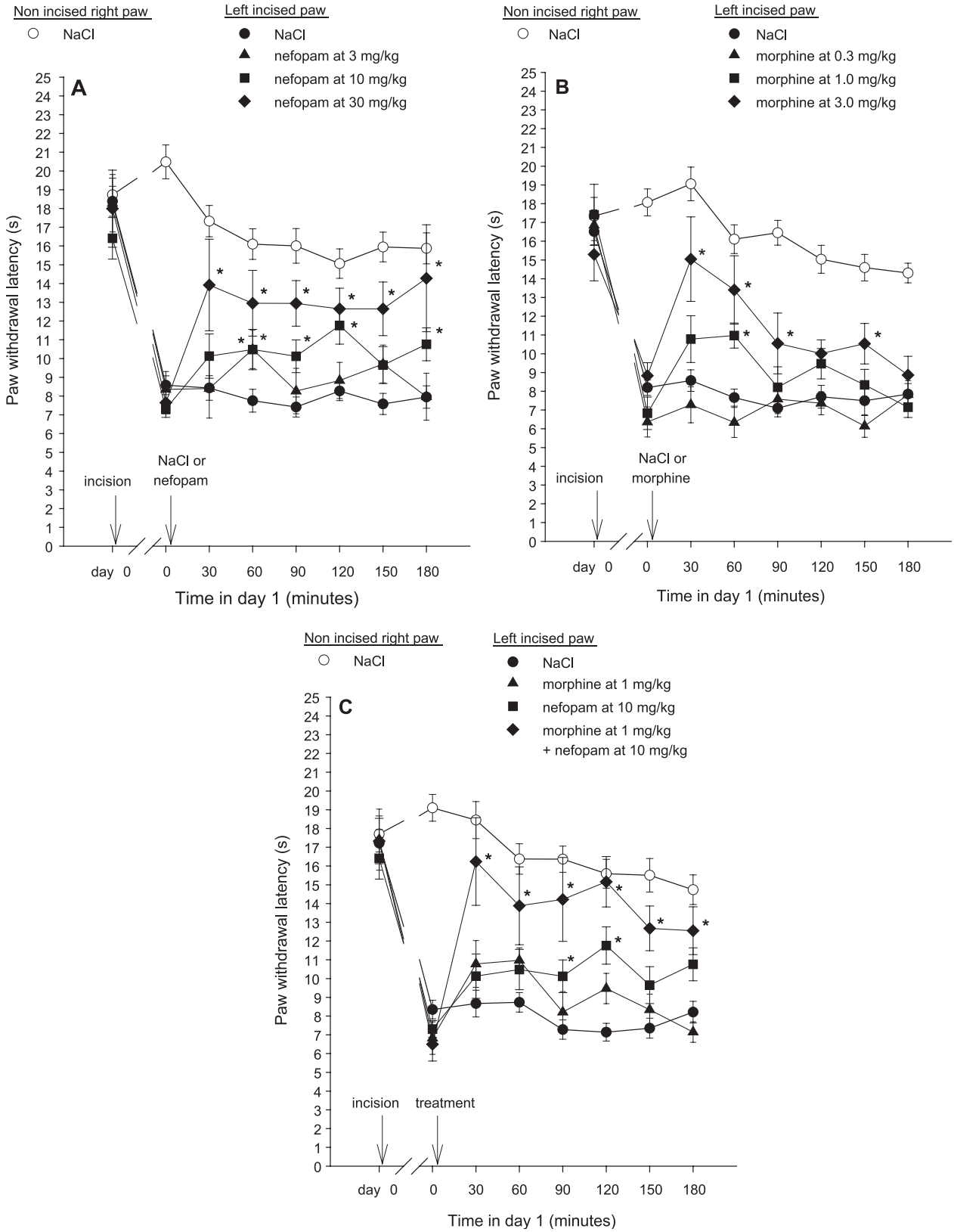

Fig. 3. Effects of subcutaneous nefopam (A), morphine (B) or both drugs' coadministration (C) on thermal hyperalgesia induced by incision of the plantar skin and muscle of the rat left hind paw. PWLs were determined by the rat plantar test. Baseline (Day 0) measurements were taken before surgery and PWL were reassessed the day after (Day 1). Results are expressed as the mean of the PWL (s ± S.E.M.) of 5–25 animals per group. (* ANOVA statistical test with significance differences at the level of 5% for treated groups compared to the group receiving NaCl and regarding the left incised paw.)

morphine did not possess any antinociceptive activity, but at 1 mg/kg, morphine significantly augmented the PWL at 90 min after morphine injection, and restored around 50% of the control latency. A dose of 3 mg/kg of morphine completely blocked carrageenan-induced heat hyperalgesia at 30 and 90 min, and restored the control PWL.

Coadministration of a nonanalgesic dose of nefopam (10 mg/kg), with a nonanalgesic dose of morphine (0.3 mg/kg), induced the appearance of a significant antihyperalgic effect 30 min after administration and until 5 h after carrageenan injection (Fig. 2C). For example, 3 h after its administration, carrageenan reduced the PWL from 16.0 ± 1.0 s for the noninjected paw to 8.3 ± 1.0 s for the inflamed paw. At the same time, morphine at 0.3 mg/kg did not modify this inflamed paw threshold (9.5 ± 1.3 s), but coadministration of nefopam with morphine significantly increased the inflamed paw latency to 16.9 ± 2.6 s, a value equal to the control withdrawal latency. This effect was comparable to that associated with a single dose of 3 mg/kg of morphine.

Under the same conditions, single administration of nefopam or morphine, or combination of both drugs did not significantly modify the PWL of the contralateral uninflamed hind paw.

3.3. Incision-induced thermal hyperalgesia

An incision of the rat plantaris muscle led to an induction of thermal hyperalgesia measured by the rat plantar test, the day after the surgery. Baseline PWL induced by the radiant heat stimulus applied under each hind paw, was around 15–19 s before incision for both hind paws. PWL of the nonincised right hind paw remained stable inside this interval the next day during the 3 h of the experiment, and no statistical significance differences were observed, despite a slight decrease of the PWL. Incision of the left hind paw provoked a high and significant decrease of the PWL around 7–9 s, which remained stable for 3 h. Incisional surgery did not affect PWL to radiant heat stimuli of the contralateral hind paw.

The single-dose administration of nefopam by the subcutaneous route dose-dependently inhibited thermal hyperalgesia (Fig. 3A). At 3 mg/kg, nefopam showed a slight and brief reduction of nociception 60 min after its administration. At 10 mg/kg, nefopam significantly reduced the thermal hyperalgesia from 60 to 120 min after its injection. The higher dose of nefopam (30 mg/kg) completely blocked thermal hyperalgesia until 3 h after its administration, and the PWL became not significantly different from the PWL of the nonincised right hind paw. For example, 30 min after vehicle administration, the PWL was reduced from 17.3 ± 0.8 s for the nonincised paw to 8.4 ± 0.7 s for the incised paw. Nefopam at 3, 10 and 30 mg/kg diminished dose-dependently the thermal hyperalgesia, with PWL of 8.4 ± 1.6, 10.1 ± 1.2 and 13.9 ± 2.4 s, respectively.

Morphine single-dose administration by subcutaneous route also dose-dependently inhibited thermal hyperalgesia (Fig. 3B). At 0.3 mg/kg, morphine did not show any antinociceptive effect, but at 1.0 mg/kg, it significantly reduced the thermal hyperalgesia 60 min after its injection. A higher dose of morphine (3 mg/kg) inhibited thermal hyperalgesia, but the PWL stayed significantly different from the PWL of the nonincised right hind paw.

Coadministration of a low antinociceptive dose of nefopam (10 mg/kg) with a nonanalgesic dose of morphine (1 mg/kg) induced the appearance of a potent analgesic effect (Fig. 3C), which totally blocked thermal hyperalgesia for at least 3 h after their administration. The PWL became not significantly different from the PWL of the nonincised right hind paw. For example, 30 min after vehicle administration, the PWL was reduced from 18.4 ± 1.0 s for the nonincised paw to 8.7 ± 0.7 s for the incised paw. At the same time, morphine at 1 mg/kg did not modify the PWL (10.8 ± 1.2), but coadministration of nefopam with morphine significantly increased the PWL to 16.2 ± 2.3 s. This dose combination gave a larger antinociceptive effect than a single dose of 3 mg/kg of morphine.

4. Discussion

Clinically, in postoperative pain, morphine produces a consistent analgesic effect, but residual pain persists in some patients. Moreover, it can induce undesirable effects, ranging from constipation and respiratory depression to tolerance and physical dependence with chronic use. Moreover, clinical studies (Sjogren et al., 1993; Guignard et al., 2000) and animal experiments (Christensen and Kayser, 2000; Rivat et al., 2002) report that opiates may also elicit delayed and long-lasting hyperalgesia. An interesting hypothesis is to overcome these problems with the coadministration of another nonopioid analgesic compound as done in this study with nefopam.

Nefopam have been shown to possess potent analgesic properties mediated by mechanisms of action different from those of morphine. Nefopam's antinociceptive effect involves the inhibition of monoamine reuptake in the central nervous system (Tresnak-Rustad and Wood, 1981; Rosland and Hole, 1990; Vonvoigtlander et al., 1983; Hunskaar et al., 1987; Fuller and Snoddy, 1993). Moreover, it does not bind to opiate receptors (Heel et al., 1980) and does not cause respiratory depression (Heel et al., 1980; McLintock et al., 1988). Finally, there appears to be no cross-tolerance between nefopam and morphine (Conway and Mitchell, 1977).

In the literature, few animal studies have investigated the effects of the coadministration of nefopam with morphine. In the radiant heat-induced tail flick reflex in mice, subcutaneous pretreatment of a nonanalgesic dose of nefopam (18 mg/kg), with subcutaneous morphine at 5 mg/kg, did not enhance the antinociceptive effect of morphine (Conway

and Mitchell, 1977). In a later study using the same test, a nonanalgesic oral dose of nefopam (20 mg/kg) showed a significant enhancement of analgesia with intraperitoneal morphine at 10 mg/kg (Kvam, 1979). In two clinical studies, concerning upper abdominal surgery (McLintock et al., 1988) and hepatic resection (Mimoz et al., 2001), nefopam has been shown to spare morphine consumption. To address this discrepancy, the present study evaluated the coadministration of nefopam with morphine in comparison to single administration of a morphine injection in long-term inflammatory and postoperative pain models, which resemble painful conditions observed after surgery and/or peripheral nerve lesions.

In the inflammatory pain model, carrageenan has been shown to induce tactile allodynia (Xu et al., 2000; Yamamoto et al., 2001) and thermal hyperalgesia for many hours (Hargreaves et al., 1988; Tabo et al., 1998). Subcutaneous administration of nefopam at 30 mg/kg blocked the development and the maintenance of heat hyperalgesia and significantly reduced tactile allodynia.

In the postoperative pain model, incision of the skin and muscle hind paw produced immediate and long-lasting heat and mechanical hyperalgesia of the injured hind paw, without affecting nociceptive thresholds of the contralateral hind paw (Brennan et al., 1996; Zahn et al., 1997; Field et al., 1997; Wang et al., 2000). Subcutaneous administration of nefopam inhibited thermal hyperalgesia in a dose-dependent fashion with a total blockade at 30 mg/kg.

In both inflammatory and postoperative pain models, morphine displayed potent and dose-dependent antinociceptive activity, as previously shown in the same dose range (Zahn et al., 1997; Field et al., 1997; Wang et al., 2000; Joris et al., 1990; Hylden et al., 1991).

In carrageenan-induced tactile allodynia, coadministration of weak analgesic doses of nefopam with nonanalgesic or moderately analgesic doses of morphine significantly reduced or totally blocked allodynia, respectively. Moreover, coadministration of nonanalgesic dose of nefopam with a nonanalgesic dose of morphine completely inhibited carrageenan- or incision-induced thermal hyperalgesia, respectively.

This study demonstrated that combination of two drugs with different mechanisms of action, an opioid agonist (morphine) and a monoamine reuptake inhibitor (nefopam), induces antinociceptive synergy in inflammatory and postoperative pain models. Enhancement of morphine antinociception by other monoamine reuptake inhibitors has been shown with clomipramine, imipramine, amitriptyline and fluoxetine in mice (Sierralta et al., 1995), rats (Taiwo et al., 1985; Ventafridda et al., 1990; Eisenach and Gebhart, 1995; Nayebi et al., 2001) or monkeys (Gatch et al., 1998). By inhibiting monoamine reuptake, these drugs, as well as nefopam, can induce an increase of noradrenaline, serotonin and/or dopamine levels in the central nervous system. Because these mediators have been shown to modulate pain transmission on their own (Dennis et al., 1980; Millan, 1999; Bardin et al., 2000; Telner et al., 1979; Lin et al., 1981), the enhancement of morphine-associated antinociception can thus be rationalized.

The morphine-sparing effect of nefopam should reduce morphine consumption and possibly alleviate the development of tolerance and addiction. Future studies will address the possible contribution of morphine metabolites to the morphine-enhancing effects of nefopam.

Finally, the combination of nefopam and morphine might offer the possibility of increasing the percentage of patients who receive good postoperative analgesia without increased risk of opioid-related side effects (McLintock et al., 1988).

Acknowledgements

We thank Prof. R.H. Levy (University of Washington, Seattle, WA, USA) for his constructive interest in our work and for his help with the manuscript.

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