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Further evidence for a peripheral component in the enhanced antinociceptive effect of systemic morphine in mononeuropathic rats: involvement of κ-, but not δ-opioid receptors

Gwénaëlle Catheline, Valérie Kayser *, Gisèle Guilbaud

Unité de Recherches de Physiopharmacologie du Système Nerveux, I.N.S.E.R.M. U 161, 2 rue d'Alésia, 75014 Paris, France

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Abstract

The contribution of a peripheral action of morphine in the augmented antinociceptive effect of this substance was re-evaluated in a well established rat model of peripheral unilateral mononeuropathy (chronic constriction of the common sciatic nerve), using a relatively low dose of systemic morphine (1 mg/kg i.v.) and local low doses of specific antagonists of κ- (nor-binaltorphimine) or δ- (naltrindole) opioid receptors. Vocalization thresholds to paw pressure were used as a nociceptive test. Escalating doses of nor-binaltorphimine (10–30 μg injected locally into the nerve injured paw) significantly and dose dependently reduced the effect of morphine on this paw but not on the contralateral paw, an effect which plateaued at 30 μg. By contrast, the local injection of naltrindole (30–40 μg into the nerve injured paw) had no effect on morphine analgesia. The doses of opioid receptor antagonists used, injected i.v., in the contralateral paw, or alone in the nerve injured paw had no significant effect. These results suggest that the peripheral effect of systemic morphine in this model of neuropathic pain could be mediated not only by μ- but also by κ-opioid receptors.

Keywords: Peripheral antinociception; Mononeuropathic rat; Paw pressure; Systemic morphine; κ-Opioid receptor antagonist; δ-Opioid receptor antagonist

1. Introduction

The treatment of painful syndromes following peripheral nervous system injury is a real therapeutic challenge (Ollat, 1991) and the use of opioids to control neuropathic pain remains highly controversial (Arner and Meyerson, 1988; Portenoy et al., 1990; Kupers et al., 1991; Rowbotham et al., 1991; Jadad et al., 1992). Despite this controversy, in a well established rat model of peripheral mononeuropathy produced by four loose ligatures around the common sciatic nerve (chronic constriction injury model) which has many of the features of a neuropathic pain state in humans (Bennett and Xie, 1988; Attal et al., 1990), we demonstrated the antinociceptive effect of morphine and various selective agonists at μ- ((D-Ala^2,*N*-Me-Phe^4,Gly^5-ol)-enkephalin (DAMGO)), δ- (tyr-D-Ser(*O*-*t*-butyl)-Gly-Phe-Leu-Thr (BUBU)) and κ- ((*trans*-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl) cyclohexyl] benzene-acetamide methanesulfonate) (U 69,593)) opioid receptors, on the vocalization threshold to paw pressure (Neil et al., 1990; Attal et al., 1991; Desmeules et al., 1993; Kayser et al., 1995), which seems to contradict the classical view that neuropathic pain is opioid resistant. These effects were observed 2 weeks after the sciatic injury, when the behavioural pain related disorders have reached a maximum (Bennett and Xie, 1988; Attal et al., 1990; Desmeules et al., 1995). Whatever the opioid used, the antinociceptive action was more marked on the paw on the nerve injured side than for the contralateral paw. One contributory mechanism for this enhanced effect might be a peripheral action of the opioid receptor agonists on the nerve injured paw. This hypothesis was tested and confirmed in a previous study, using systemic morphine (1 mg/kg i.v.) and low doses of local naloxone or its quaternary salt (naloxone methiodide), which has a low capacity to cross the blood-brain barrier suggesting the involvement of peripheral μ-type opioid receptors (Kayser et al., 1995).

The peripheral opioid antinociception, against noxious pressure at least, may be mediated through μ-, κ- and even δ-opioid receptors under inflammatory conditions (Barber and Gottlich, 1992; Stein, 1993; Kayser and Guilbaud, 1994). Following our investigation of opioids and

* Corresponding author. Tel.: (33-1) 4589-3662; Fax: (33-1) 4588-1304.

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neuropathic pain, we have investigated in the present study the antinociceptive activity of systemic morphine (1 mg/kg i.v.) after peripheral application of low doses of specific antagonists of κ- and δ-opioid receptors (nor-binaltorphimine and naltrindole respectively) by using the measure of the vocalization thresholds to paw pressure as a nociceptive test in the chronic constriction injury model. Preliminary data have been reported (Catheline et al., 1995).

2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Charles Rivers, France) 9–10 weeks old, weighing 175–200 g on arrival and 310–340 g at the moment of testing were used ($n = 76$). They were housed 4–5 per cage, habituated to the colony room for a week before the experiments and kept under a 12/12 h day/night cycle and a constant room temperature of 22°C. Food and water were available ad libitum. The Guidelines on Ethical Standards For Experimental Pain in Animals of the International Association for the Study of Pain (IASP, 1983) were followed. The number of animals used was kept to a minimum in each experimental group. In particular, the control group receiving intravenous (i.v.) morphine and intraplantar (i.pl.) saline was limited to 9 animals, since the effect of morphine was well reproducible and roughly comparable to that described in previous experiments (Neil et al., 1990; Attal et al., 1991; Kayser et al., 1995).

2.2. Surgical procedure

The neuropathy was produced on the right hindpaw according to the method described by Bennett and Xie (1988) and Attal et al. (1990). Under sodium pentobarbital anaesthesia (Nembutal, 50 mg/kg i.p.), four ligatures (catgut chrome 5/0) were tied loosely, with approximately 1 mm spacing, around the common sciatic nerve. The animals developed pain related disorders including allodynia and hyperalgesia to mechanical and thermal stimulation, according to the IASP classification (Merskey, 1986). The abnormal sensitivity is maximal 2 weeks after the beginning of the constriction, with a recovery at 8–10 weeks (Attal et al., 1990; Desmeules et al., 1995).

2.3. Behavioural test

A pre-operative threshold (mean of two consecutive stable thresholds) was determined for both hindpaws of each rat. Then, 2 weeks after surgery, when the abnormal pain behaviour is at a stable maximum, a preliminary or control threshold (mean of two consecutive stable thresholds) was determined before injection of the drugs. Vocalization thresholds were then measured every 10 min (to avoid nociceptor 'fatigue'), until they returned to baseline.

Test sessions, beginning at 09.30 h, were carried out in a quiet room, away from the colony room. The experimenter was unaware of the drugs and doses used. The rats were randomly assigned to groups of 5 for a series of tests.

The vocalization thresholds expressed in grams were determined by a modification of the Randall and Selitto method (Kayser and Guilbaud, 1990). An increasing pressure was applied to the hindpaw (with a Basile analgesimeter, Apelex, tip diameter of the stylus applied to the dorsal paw in the sciatic nerve territory, between the third and the fourth metatarsus: 1 mm) until the rat squeaked. This response represents a more integrated nociceptive behaviour than the withdrawal of the paw and is especially sensitive to the analgesic compounds particulary in this pain model (Attal et al., 1991; Ardid and Guilbaud, 1992; Desmeules et al., 1993). The withdrawal reflex that usually occurs before vocalization was prevented by gently holding the rat's hindpaw in position under the pusher until vocalization.

2.4. Drugs and test dosages

The following drugs were used: morphine hydrochloride (Meram, Paris, France), nor-binaltorphimine dihydrochloride (RBI, Research Biochemicals, Natick, MA, USA), a specific κ-opioid receptor antagonist, naltrindole hydrochloride (RBI, Research Biochemicals, Natick, MA, USA), a specific δ-opioid receptor antagonist, saline (NaCl 0.9%).

Drugs were freshly prepared in sterile physiological saline (0.9% NaCl). Each animal was used only once and received a single dose of drug or saline. Morphine was administered intraveinously (i.v.) into the lateral tail vein. Intraplantar injections of nor-binaltorphimine or naltrindole were made into the paw in a volume of 0.2 ml. Control rats in each group received the same volume of saline (0.9% NaCl).

In each series of experiments, nor-binaltorphimine, naltrindole or saline was injected just after morphine. The injections were performed without anaesthesia: each rat was placed in a Plexiglas cylinder, with a small hole at the bottom, so that only the tail or the hindpaw was free for injection. Using this minimally stressful method, the injections were performed very rapidly. Rats were then placed in their cages for 10 min before the beginning of the experiments.

2.4.1. First experimental series

In a first series of experiments, 3 groups of mononeuropathic rats receiving i.v. morphine (1 mg/kg) were injected locally into the nerve injured paw with 10 ($n = 6$), 20 ($n = 9$) or 30 ($n = 6$) μg of nor-binaltorphimine. These doses were based on our previous studies in mononeuropathic rats (Keita et al., 1995).

Two other groups of neuropathic rats, receiving 1

mg/kg i.v. morphine, were injected i.v. ($n = 6$) or i.pl. into the contralateral paw ($n = 6$) with the top dose of 30 μg of nor-binaltorphimine. These experiments were conducted to assess whether the preventive effects of local nor-binaltorphimine were mediated through a central site of action. In addition, to assess the capacity of the κ-opioid receptor antagonist, given alone, to alter the nociceptive threshold in mononeuropathic rats, an additional group ($n = 6$) treated with i.v. saline instead of morphine, was injected i.pl. into the nerve injured paw with 30 μg of nor-binaltorphimine.

2.4.2. Second experimental series

In a second series of experiments, 2 groups of neuropathic rats, receiving 1 mg/kg i.v. morphine, were injected i.pl. into the nerve injured paw with 30 ($n = 10$) and 40 ($n = 6$) μg of naltrindole. These doses, in the same range, were determined on the basis of previous experiments, where nor-binaltorphimine and naltrindole injected at the dose of 1 mg/kg i.v. specifically reversed the effects of their agonists in mononeuropathic rats (Desmeules et al., 1993).

Two other groups, receiving 1 mg/kg i.v. morphine, were injected i.v. with 30 ($n = 3$) or 40 ($n = 6$) μg of naltrindole. Two additional groups of neuropathic rats treated with i.v. saline instead of morphine, were injected i.pl. with 30 ($n = 5$) or 40 ($n = 4$) μg of naltrindole.

2.5. Statistical analysis

Vocalization thresholds are reported in grams. Data are expressed as mean ± S.E.M. Values of the mean curves are expressed as percentages of the two control values measured just before drug administration. A paired Student's *t*-test was employed to compare the vocalization thresholds before and after the surgery for each paw. To evaluate the effects of co-injection of morphine and the different opioid receptor antagonists (naltrindole and nor-binaltorphimine) or saline, an analysis of variance (ANOVA) was performed taking as the variable the areas under the curves (AUC), expressed in g/min. The Fisher's post least significant difference (PLSD) test was used for multiple comparison between doses or saline. Results were regarded as being significant when P values were less than 0.05.

3. Results

Before nerve ligature, the vocalization threshold did not differ significantly between the hindpaws, being 337 ± 16 g and 322 ± 9 g. As reported earlier (Attal et al., 1990; Desmeules et al., 1993, 1995; Kayser et al., 1995), the mean threshold was markedly decreased for the nerve injured paw: 208 ± 5 g (62% of the pre-operative value), 2 weeks after the surgical procedure ($P < 0.0001$, paired *t*-test). This decreased threshold was also clearly lower than that of normal rats and it was considered to reflect mechanical allodynia-like behaviour (Merskey, 1986). By contrast, the mean vocalization threshold for the contralateral paw was 334 ± 30 g for the contralateral paw (104% of the pre-operative value), not significantly decreased, although it was diminished for some rats, as already described (Attal et al., 1991; Desmeules et al., 1995).

After the injection of morphine and/or the different opioid receptor antagonists, no significant behavioural changes were noted for either group of rats, other than an increase in the vocalization threshold.

3.1. Effect of an i.pl. injection of saline on the antinociceptive effect of morphine (Fig. 1, Table 1)

The effect of an i.pl. injection of saline (0.9% NaCl in a volume of 0.2 ml) into the nerve injured paw, on the antinociceptive effect of morphine administered i.v. at the dose of 1 mg/kg, was re-investigated in 9 mononeuropathic rats. In these animals, the antinociceptive effect of morphine was roughly comparable to that obtained 2 weeks after surgery in previous experiments (Neil et al., 1990; Attal et al., 1991; Kayser et al., 1995).

3.1.1. Nerve injured paw (Fig. 1A)

The antinociceptive effect of morphine lasted for 120 min with a maximum at 30 min. At this time point, the

Table 1
Maximal mean vocalization thresholds (g ± S.E.M.) for the nerve injured and the contralateral paw in mononeuropathic rats, before and after injection of systemic morphine (1 mg/kg i.v.) + saline, nor-binaltorphimine (nor-BNI) or naltrindole (NTI) into the nerve injured paw

Treatment (μg)			Nerve injured paw		Contralateral paw	
			Before injection	After injection	Before injection	After injection
Saline	i.pl.	($n = 9$)	204 ± 15	489 ± 42	318 ± 16	534 ± 22
nor-BNI	10	($n = 6$)	234 ± 5	444 ± 17	354 ± 10	576 ± 20
	20	($n = 9$)	231 ± 6	339 ± 18	342 ± 8	552 ± 18
	30 i.pl.	($n = 6$)	177 ± 13	375 ± 39	351 ± 18	531 ± 26
NTI	30	($n = 10$)	204 ± 7	453 ± 39	342 ± 14	552 ± 23
	40 i.pl.	($n = 6$)	222 ± 22	453 ± 63	345 ± 18	534 ± 33

The pre-operative threshold for all the rats was 337 ± 16 g and 322 ± 9 g for the 2 paws respectively.

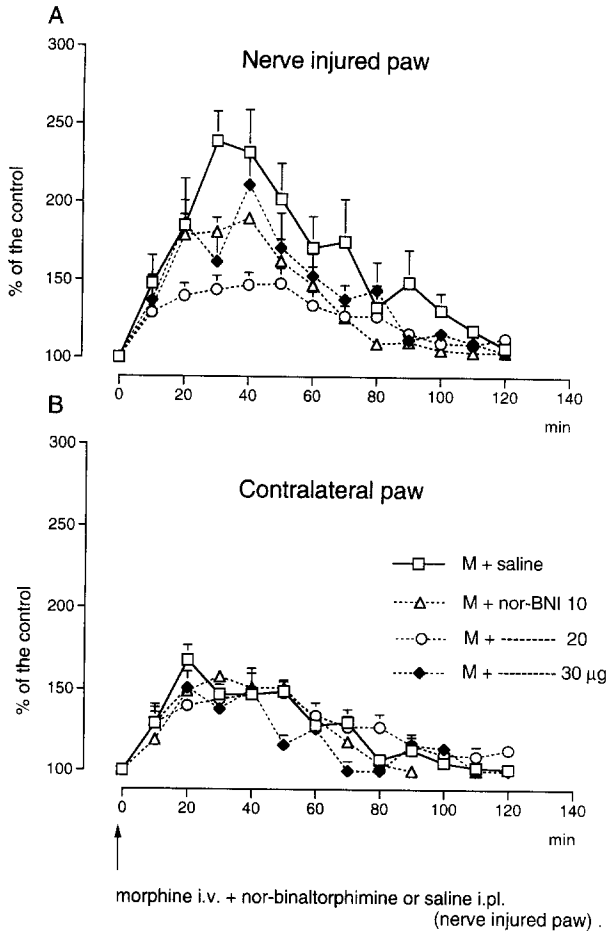

Fig. 1. Mean curves illustrating the ability of local nor-binaltorphimine (10, 20 and 30 μg) or saline (n = 6, 9, 6 and 9, respectively) to prevent the antinociceptive effect of systemic morphine (1 mg/kg), as measured by the vocalization threshold to paw pressure. (A) nerve injured, (B) contralateral hindpaw. Each value is expressed as a percentage of the mean control values ± S.E.M. M = morphine, nor-BNI = nor-binaltorphimine.

mean vocalization threshold was 489 ± 42 g (240 ± 20% of the pre-injection value).

3.1.2. Contralateral paw (Fig. 1B)

For this paw, the overall duration of the effect was shorter (80 min), with a maximum at 20 min; at this time point, the mean vocalization threshold was 534 ± 22 g (168 ± 7% of the pre-injection value). This effect was significantly less potent than on the nerve injured paw as indicated by the comparison of the AUCs ($P = 0.025$, unpaired t-test for the AUCs), confirming previous data (Neil et al., 1990; Attal et al., 1991; Kayser et al., 1995). Therefore, although the control threshold from the nerve injured paw was much lower than that of the contralateral paw, the animals were able to sustain roughly equal pressures from both hindpaws after the injection of morphine (Table 1).

3.2. Effect of an i.v. or an i.pl. injection of the κ-opioid receptor antagonist nor-binaltorphimine on the antinociceptive effect of morphine

3.2.1. Effect of the i.pl. injection of nor-binaltorphimine alone (30 µg into the nerve injured paw)

The effect of an i.pl. injection of nor-binaltorphimine (30 μg in a volume of 0.2 ml) into the nerve injured paw was determined in a group of mononeuropathic rats, which had received an acute i.v. injection of saline instead of morphine ($n = 7$). In these animals, the i.pl. injection of nor-binaltorphimine induced no significant effect on the nerve injured and the contralateral paw (maximal mean vocalization thresholds were 222 ± 26 g (121 ± 13% of the control value) and 303 ± 33 g (94 ± 11%) respectively).

3.2.2. Effect of the i.pl. injection of nor-binaltorphimine (10–30 µg into the nerve injured paw) on the effect of morphine (Figs. 1 and 2, Table 1)

The effect of nor-binaltorphimine (10, 20 or 30 μg, in a volume of 0.2 ml) injected into the nerve injured paw, was investigated in 3 groups of mononeuropathic rats receiving an i.v. injection of morphine ($n = 6$, $n = 9$ and $n = 6$ respectively).

3.2.2.1. Nerve injured paw (Fig. 1A, Fig. 2). In these animals, the overall antinociceptive effect of morphine on the nerve injured paw was significantly and dose-dependently reduced in comparison with the control group of rats receiving i.pl. saline ($P = 0.0029$, ANOVA, based on the AUCs). For rats receiving 10 μg of nor-binaltorphimine, the maximal vocalization threshold was 190 ± 7% of

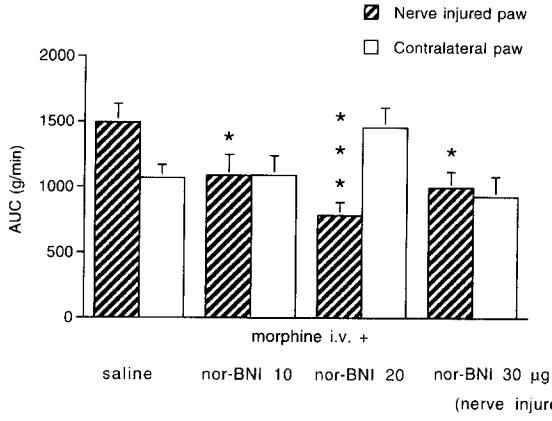

Fig. 2. Mean areas under the curves (g/min ± S.E.M.) for the preventive effect of nor-binaltorphimine (10, 20 and 30 μg i.pl. into the nerve injured paw) or saline on the antinociceptive action of 1 mg/kg i.v. morphine on the vocalization threshold to paw pressure: * $P < 0.05$, *** $P < 0.001$ (ANOVA, Fisher's PLSD) comparison with saline for the nerve injured and contralateral paws, respectively. nor-BNI = nor-binaltorphimine.

the control value, 40 min after the injection (444 ± 17 g). With the dose of 20 μg, the maximal effect, reached at 50 min was 147 ± 8% of the control (339 ± 18 g). The highest dose of nor-binaltorphimine (30 μg) had a maximal effect at 40 min, 212 ± 15% of the control value (375 ± 39 g). The multiple comparison of the AUCs between the effect of co-injected nor-binaltorphimine or saline revealed that the effect of the antagonist was significantly different from that of saline for each injected dose (10 μg $P = 0.041$, 20 μg $P = 0.0003$, 30 μg $P = 0.014$, Fisher's PLSD test for the AUCs) (Fig. 1A and Fig. 2). Nevertheless, the effect of the lowest dose of 10 μg and the highest dose of 30 μg were comparable ($P = 0.66$, Fisher's PLSD test for the AUCs) (Fig. 1A and Fig. 2).

3.2.2.2. Contralateral paw (Fig. 1B, Fig. 2). No significant changes in the vocalization thresholds after the co-injection of morphine and nor-binaltorphimine were observed for this paw, compared with the effect of i.pl. saline ($P = 0.062$, ANOVA, based on the AUCs) (Fig. 1B and Fig. 2). The mean peak values were 163 ± 10% of the control value (576 ± 20 g), 161 ± 5% (552 ± 18 g), and 151 ± 11% (531 ± 26 g) respectively for 10, 20 and 30 μg, thus roughly comparable whatever the i.pl. doses of nor-binaltorphimine.

The AUCs corresponding to the nerve injured paw receiving 20 μg i.pl. nor-binaltorphimine and the contralateral paw in the control group of rats receiving i.pl. saline were comparable ($P = 0.063$, unpaired t-test for the AUCs), indicating that the increased antinociceptive effect of morphine on the nerve injured paw was abolished (Fig. 2).

3.2.3. Effect of the i.v. injection of nor-binaltorphimine (30 μg) on the effect of morphine (Fig. 3)

Six neuropathic rats received the top dose of nor-binaltorphimine (30 μg in a volume of 0.2 ml) i.v. and 1 mg/kg i.v. morphine. No changes in the vocalization thresholds after the co-injection of i.v. morphine and nor-binaltorphimine were observed in these animals, in comparison with the control group of rats receiving i.pl. saline. Maximal vocalization thresholds were 198 ± 26% of the control at 30 min (354 ± 42 g) for the nerve injured paw (Fig. 3) and 132 ± 13% (447 ± 45 g) for the contralateral paw (not shown). These effects were not significantly different from those obtained in the control saline group ($P = 0.37$ for the nerve injured paw and $P = 0.13$ for the contralateral paw, Fisher's PLSD test for the AUCs).

3.2.4. Effect of the i.pl. injection of nor-binaltorphimine (30 μg into the contralateral paw) on the effect of morphine (Fig. 3)

The effect of a local injection of the top dose of nor-binaltorphimine into the contralateral paw was determined in a group of 6 mononeuropathic rats receiving i.v. morphine. In these animals, the overall effect of morphine

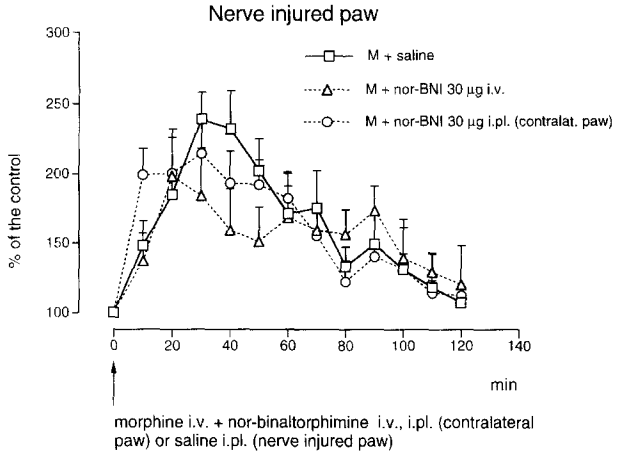

Fig. 3. Lack of change in the antinociceptive effects of systemic morphine in rats receiving nor-binaltorphimine i.v. or in the contralateral paw. The mean curves obtained from the nerve injured paw after injection of 1 mg/kg i.v. morphine and saline in the nerve injured paw, or nor-binaltorphimine 30 μg i.v. and in the contralateral paw (n = 9, 6 and 6, respectively). Each value is expressed as a percentage of the mean control values ± S.E.M. M = morphine, nor-BNI = nor-binaltorphimine.

on both hindpaws was comparable to the control saline group ($P = 0.86$ for the nerve injured paw and $P = 0.34$ for the contralateral paw, Fisher's PLSD test for the AUCs). The maximal vocalization thresholds were 214 ± 34% of the control value (456 ± 83 g) for the nerve injured paw (Fig. 3), and 158 ± 12% (447 ± 45 g) for the contralateral paw (not shown).

3.3. Effect of an i.v. or an i.pl. injection of a δ-opioid receptor antagonist, naltrindole, on the antinociceptive effect of morphine

3.3.1. Effect of the i.pl. injection of naltrindole alone (30 or 40 μg into the nerve injured paw)

The effect of an i.pl. injection of naltrindole (30 or 40 μg in a volume of 0.2 ml) into the nerve injured paw was determined in 2 groups of neuropathic rats receiving an i.v. injection of saline instead of morphine ($n = 5$ and $n = 4$ respectively). In these animals, no significant modifications of the threshold were observed. The maximal vocalization thresholds for 30 and 40 μg of naltrindole were for the nerve injured paw 95 ± 6% of the pre-injection value (213 ± 10 g) and 115 ± 7% (285 ± 30 g), and for the contralateral paw 91 ± 10% of the pre-injection value (330 ± 18 g) and 115 ± 15% (414 ± 57 g).

3.3.2. Effect of the i.pl injection of naltrindole (30 μg and 40 μg into the nerve injured paw) on the effect of morphine (Fig. 4, Table 1)

The effect of naltrindole (30 and 40 μg in a volume of 0.2 ml) injected into the nerve injured paw was tested in 2 groups of mononeuropathic rats ($n = 10$ and $n = 6$) receiving an i.v. injection of 1 mg/kg of morphine. In the 2 groups, the overall effect of morphine was not significantly

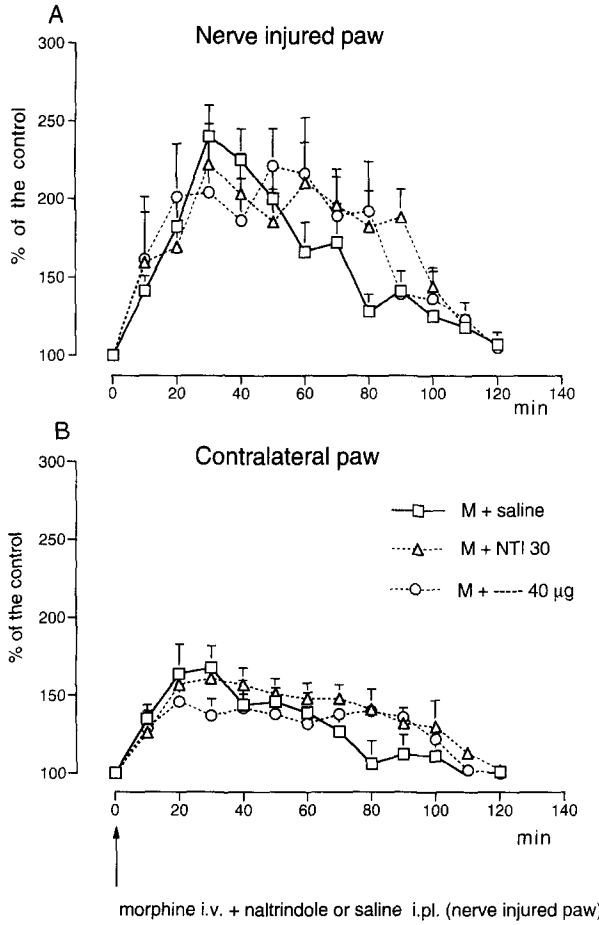

Fig. 4. Mean curves illustrating the inability of local naltrindole (30 and 40 μg) or saline (n = 10, 6 and 9, respectively) to prevent the antinociceptive effect of systemic morphine (1 mg/kg), as measured by the vocalization threshold to paw pressure. (A) nerve injured, (B) contralateral hindpaw. Each value is expressed as a percentage of the mean control value ± S.E.M. M = morphine, NTI = naltrindole.

different from the control saline group ($P = 0.617$ for the nerve injured paw and $P = 0.11$ for the contralateral paw, ANOVA, based on the AUCs) (Fig. 4A and B). Maximal vocalization thresholds for 30 and 40 μg respectively were 222 ± 12% (453 ± 39 g) and 204 ± 13% (453 ± 63 g) for the nerve injured paw, and 161 ± 9% (537 ± 30 g) and 155 ± 11% (534 ± 33 g) for the contralateral paw.

3.3.3. Effect of the i.v. injection of naltrindole (30 μg and 40 μg) on the effect of morphine

Two groups of rats ($n = 3$ and $n = 6$ respectively) received 30 and 40 μg of i.v. naltrindole (in a volume of 0.2 ml) and i.v. morphine (1 mg/kg). No significant change in the effect of morphine was elicited, with the maximal vocalization thresholds being for 30 μg and 40 μg respectively 226 ± 24% (495 ± 54 g) and 177 ± 9% (414 ± 21 g) for the nerve injured paw, and 157 ± 5% (576 ± 18 g) and 157 ± 3% (555 ± 13 g) for the contralateral paw.

4. Discussion

In the present study, using the measure of the vocalization threshold to mechanical paw pressure, based on a localised noxious stimulation which activates relatively well defined supraspinal structures, and is highly sensitive to detecting antinociceptive activity of low doses of various opioids (Kayser and Guilbaud, 1990), we clearly confirmed (Kayser et al., 1995) the participation of a peripheral opioid receptor mechanism in the enhanced antinociceptive effect of morphine, administered systemically at a relatively low dose, in mononeuropathic rats, a model of non-inflammatory pain. To differentiate between peripheral and central actions of systemic morphine, reversibility by the local versus systemic application of equivalent doses of standard opioid receptor antagonists (e.g. the κ-opioid receptor antagonist nor-binaltorphimine and the δ-opioid receptor antagonist naltrindole) was examined.

The findings strongly support the occurrence of κ-, but not δ-opioid receptor-specific effects at peripheral sites which, in addition to the involvement of peripheral μ-opioid receptors (Kayser et al., 1995), could account for the observed increased antinociceptive potency of systemic morphine against paw pressure in this rat model of neuropathic pain (Neil et al., 1990; Attal et al., 1991; Desmeules et al., 1993; Kayser et al., 1995). These results will be discussed in turn.

4.1. Preventive effect of the κ-opioid receptor antagonist nor-binaltorphimine

In mononeuropathic rats, the enhanced efficacy of morphine against pressure exerted on the nerve injured paw was significantly and dose-dependently prevented by the κ-opioid receptor antagonist nor-binaltorphimine injected directly into the nerve injured paw at doses of 10–30 μg. The same concentrations of nor-binaltorphimine were ineffective when injected i.v. The antagonistic effect of local nor-binaltorphimine was obtained with a dose as low as 10 μg and was significantly increased when 20 μg was used. For this dose, the increased antinociceptive potency of morphine on the nerve injured paw was almost abolished. A higher dose (30 μg nor-binaltorphimine) did not have a greater effect. In sharp contrast, the top dose of 30 μg injected into the contralateral paw was unable to reverse the effect of morphine in the mononeuropathic rats. It should be noted that the local injection of nor-binaltorphimine alone (30 μg into the nerve injured paw of rats which had received an acute injection of saline instead of morphine) induced no significant effect. It seems unlikely that the antagonistic effect of the local antagonist could be due to mechanical damage due to the introduction of the needle into the nerve injured paw and/or to ischaemic lesions due to the volume injected, since intraplantar injection of saline did not influence morphine

analgesia in these animals, as reported previously (Kayser et al., 1995).

There is evidence (Spanagel et al., 1994) that, when a single dose of nor-binaltorphimine is employed, a selective and persistent antagonism of κ-opioid receptor-mediated analgesia is achieved. The present results thus strongly support the involvement of peripheral κ-opioid receptors in the enhanced antinociceptive effect of the preferential μ-opioid receptor agonist morphine in the chronic constriction injury model. These findings are in agreement with our recent studies showing that the impressive antinociceptive effect produced by the κ-opioid receptor agonist, U69,593 (0.75 mg/kg i.v.) in this model was also significantly and dose dependently reduced by the local injection of nor-binaltorphimine in the nerve injured paw (10–30 μg) (Catheline et al., 1996). In addition, the local administration of the peripherally selective κ-opioid receptor agonist, (*R,S*)-*N*-[2-(*N*-methyl-3,4-dichloro-phenylacetamido-)-2-(3-carboxyphenyl)-ethyl]pyrrolidine hydrochloride (ICI 20448) (20–50 μg) had a significant antinociceptive effect on the nerve injured paw; this effect was reversed by local nor-binaltorphimine (20–30 μg) (Keita et al., 1995). These results are reminiscent of previous studies that examined the effects of local vs. systemic application of κ-opioid receptor agonists on the paw pressure test in rats with peripheral inflammation (Stein et al., 1988, 1989; Taiwo and Levine, 1991). Since prodynorphin- and proenkephalin-derived peptides have been detected in sensory ganglia (Botticelli et al., 1981; Przewlocki et al., 1988) and in peripheral terminals of sensory nerves (Weihe et al., 1985; Hassan et al., 1993), it is possible that the peripheral action of the κ-opioid receptor antagonist nor-binaltorphimine could be related to peripheral alterations in endogenous opioid substances, such as dynorphin (an opioid peptide considered to be the endogenous ligand of κ-opioid receptors), and in peripheral κ-opioid binding sites. It has previously been shown that κ-opioid receptor binding in the spinal cord is decreased 10 days after sciatic nerve ligation (Stevens et al., 1991; Besse et al., 1992), suggesting an increase in κ-synaptic activity. In addition, a marked and sustained up-regulation of dynorphin has been observed in biochemical studies of dorsal horn neurones on the nerve injury side (Bennett et al., 1989; Kajander et al., 1990; Draisci et al., 1991).

4.2. Lack of effect of the δ-opioid receptor antagonist, naltrindole

Surprisingly, the clear action of the κ-opioid receptor antagonist on the antinociceptive effect of morphine contrasts the absence of an obvious preventive effect by the δ-opioid receptor antagonist naltrindole. In fact, the potent antinociceptive effect produced by i.v. morphine on the nerve injured paw remained roughly unmodified in rats receiving escalating doses of the δ-opioid receptor antagonist naltrindole (30–40 μg into the nerve injured paw): the mean curves obtained with the i.pl. antagonist were almost the same as in neuropathic rats receiving i.pl. saline or systemic naltrindole (30–40 μg i.v.). Based on this, we considered it unjustified to test the effect of naltrindole injected into the contralateral paw on the effect of morphine. It should be noted that an increased dose of naltrindole (50 μg into the nerve injured paw) in an additional group of rats ($n = 4$) antagonised the effect of i.v. morphine on both hindpaws (maximal mean vocalization thresholds were decreased by 36% on the nerve injured paw and 30% on the contralateral paw in comparison with the group of rats receiving i.pl. saline). This effect, observed also with low i.pl. doses of naloxone and methylnaloxone in mononeuropathic rats (although slight and not dose-related) (Kayser et al., 1995), suggests either a diffusion of naltrindole into the systemic circulation or a loss of specificity of the antagonist. These negative results strongly suggest that the participation of peripheral δ-opioid receptors in the enhanced antinociceptive effect of morphine is not major, at least in these pathologic conditions. These results are in contradiction with previous studies in which it has been shown that under inflammatory conditions δ-opioid receptors mediate the peripheral opioid antinociception against noxious pressure. Even though we have no clear explanation for this discrepancy, it should be pointed out that, when examined, δ-opioid receptor agonists were always less potent than μ ligands at peripheral sites (Fereirra et al., 1982; Stein et al., 1989; Levine and Taiwo, 1989; Antonijevic et al., 1995). In addition, these findings are consistent with other data obtained from the systemic application of δ-opioid receptor agonists, in which, although marked, the effects of δ-opioid receptor agonists in normal rats and in models of inflammatory and neuropathic pain were lower than those observed with the μ- or the κ-opioid receptor agonists and rapidly plateaued (Neil et al., 1986; Desmeules et al., 1993; Lee et al., 1994; Kayser et al., 1995). To clarify this issue, experiments are in progress in which the effects of systemic application of δ-opioid receptor agonists and local low doses of naltrindole in mononeuropathic rats are examined.

In conclusion, although depending on the particular circumstances, all three receptor types can be present and functionally active in peripheral tissue (Stein, 1993; Antonijevic et al., 1995), it appears that the peripheral action of systemic morphine in mononeuropathic rats is not only mediated via peripheral μ-opioid receptors, as demonstrated using naloxone and naloxone methiodide (Kayser et al., 1995), but also via peripheral κ-opioid receptors (Keita et al., 1995) and not by δ-opioid receptors. This observation might be linked to the enhanced axonal transport of μ- and κ-opioid receptors in sensory neurons observed under inflammatory conditions such as the i.pl. injection of Freund's adjuvant or interleukin-1β in rats (Jeanjean et al., 1994). As previously discussed (Kayser et al., 1995), although inflammatory conditions are not obvious 2 weeks after surgery in the chronic constriction injury model

(Attal et al., 1990, Maves et al., 1993; Clatworthy et al., 1995), the peripheral antinociceptive effect observed in the present pain model suggests mechanisms resembling those proposed for inflammatory models (Stein, 1993; Antonijevic et al., 1995). This may be linked to the presence of macrophages around the injured nerve fibres (Bonetti et al., 1993; Sommer et al., 1993; Lotan et al., 1994), the role of which is to promote the regeneration process in the peripheral nervous system (Stoll and Hartung, 1992), and the activation of opioid receptors located on peripheral sensory neurons (Stein, 1993; Antonijevic et al., 1995).

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