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European Journal of Pharmacology 357 (1998) 171–178

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Peripheral component in the enhanced antinociceptive effect of systemic U-69,593, a κ-opioid receptor agonist in mononeuropathic rats

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Received 21 July 1998; revised 4 August 1998; accepted 11 August 1998

Abstract

The contribution of a peripheral action of the κ-opioid receptor agonist U-69,593 (*trans*-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl) cycloexil] benzene-acetamide methanesulfonate) in the augmented antinociceptive effect of this substance was investigated in a well-established rat model of peripheral unilateral neuropathy (chronic constriction of the common sciatic nerve). Relatively low dose of systemic U-69,593 (0.75 mg/kg intravenous (i.v.)) and intraplantar (i.pl.) low doses of specific antagonists of κ-(nor-binaltorphimine) or μ-(D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2: CTOP) opioid receptors were used. Vocalization thresholds to paw pressure were used as a nociceptive test. The i.pl. injection of nor-binaltorphimine (10–15 μg injected into the nerve-injured hind paw) had no effect on the antinociceptive effect of U-69,593. Higher doses (20–30 μg i.pl. nor-binaltorphimine) significantly reduced the effect of U-69,593 on this paw but not on the contralateral paw, an effect which plateaued at 30 μg. By contrast, the i.pl. injection of CTOP (1 μg into the nerve-injured paw) had no effect on U-69,593 antinociception, whereas it reduced the effect of systemic morphine in these animals. The doses of nor-binaltorphimine used, injected into the contralateral paw or i.v., failed to modify the antinociceptive effects of U-69,593 on either paw. These results provide evidence for a peripheral component in the enhanced antinociceptive effect of systemic U-69,593 in this model of neuropathic pain.

Keywords: Antinociception, peripheral; Mononeuropathic rat; Paw pressure; κ-Opioid receptor agonist; U-69,593

1. Introduction

Traditionally, opioid analgesia has been attributed to the activation of opioid receptors exclusively within the central nervous system. Over the past 15 years, however, a large number of studies have discovered and characterized peripheral opioid actions. Such effects occur primarily in inflamed but also in normal tissue (Kayser et al., 1990, Kayser and Guilbaud, 1994; Antonijevic et al., 1995; Kolesnikov et al., 1996), and have been found both in animal experiments and under clinical conditions in patients (Stein and Yassouridis, 1997). Opioid receptors have been demonstrated on peripheral terminals in thinly myelinated and unmyelinated sensory nerves in animals (Fields et al., 1980; Hassan et al., 1993; Coggeshall et al., 1997) and opioid receptor messenger ribonucleic acid (mRNA) has been detected in dorsal root ganglia, which contain the cell bodies of primary afferent neurons (Maekawa et al., 1994; Schäfer et al., 1995; Buzas and Cox, 1997). All three opioid receptor types (μ, δ, κ) can be functionally active in peripheral tissues (Barber and Gottschlich, 1992; Stein, 1993, Coggeshall et al., 1997).

Various animal models have been developed to investigate neuropathic pain. Of these, the well-established rat model of peripheral mononeuropathy produced by persistent moderate constriction of the common sciatic nerve (Bennett and Xie, 1988; Attal et al., 1990) has been studied extensively in our laboratory. We have shown that in this model, systemic morphine and selective agonists at μ-([D-Ala2,*N*-MePhe4,Gly5-ol]-enkephalin: DAMGO), δ-([D-Thr2,Leu5]-enkephalin-Thr: DTLET and [D-Cys(StBu)2,Leu5]-enkephalin-Thr(OtBu)]: BUBUC) and κ-(*trans*-3,4-dichloro-*N*-methyl-*N*-[7-(1-pyrrolidinyl) cycloexil] benzene-acetamide methanesulfonate: U-69,593) opioid receptors produce dose-dependent effects on the vocalization threshold to paw pressure test and have an enhanced effect on the nerve-injured side (Neil et al., 1990; Attal et al., 1991; Desmeules et al., 1993; Catheline et al., 1996a,b; Idänpään-Heikkilä et al., 1997). The contri-

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0014-2999/98/$19.00
PII: S0014-2999(98)00597-4

bution of a peripheral opioid receptor mechanism in the enhanced antinociceptive effect of systemic morphine has been demonstrated (Kayser et al., 1995), which could be mediated not only by μ- but also by κ-opioid receptors (Catheline et al., 1996a). Consistent with these findings, we also found a significant effect 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 204408) on the neuropathic rats, which was reversed by i.pl. nor-binaltorphimine (Keïta et al., 1995).

Following our investigation on peripherally mediated opioid analgesia, we have investigated in the present study the antinociceptive activity of systemic U-69,593 (0.75 mg/kg i.v.) after peripheral application of low doses of specific antagonists of μ- and κ-opioid receptors (D-Phe-Cys-Tyr-D-Trp-Orn-Thr-Pen-Thr-NH_2: CTOP and nor-binaltorphimine, respectively) by using the measure of the vocalization thresholds to paw pressure as a nociceptive test in the chronic constriction injury model. Even though the antinociceptive efficacy of κ-opioid receptor agonists has been studied to some extent under both normal and inflammatory conditions (Stein et al., 1988, 1989; Taiwo and Levine, 1991; Kolesnikov et al., 1996), the experimental evidence on neuropathic rats is still sparse.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley rats (Charles River, Saint-Aubin-lès-Elbeuf, France), $n = 63$, weighing 175 to 200 g on arrival, were used. The rats were housed five in a cage on a 12-h light/12-h dark cycle (lights on at 0700). The ambient temperature was kept at 22°C, and the rats had free access to standard laboratory food and tap water. The animals were allowed to habituate to the housing facilities for at least 1 week before the experiments were begun. The Guidelines of the Committee for Research and Ethical Issues of the 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.) U-69,593 and i.pl. (i.pl.) saline (0.9% NaCl in a volume of 0.2 ml injected subcutaneously in the rat plantar hind paw) was limited to six animals, since the effect of U-69,593 was reproducible and comparable to that described in previous experiments (Desmeules et al., 1993; Catheline et al., 1996b). For the same reason, we have not re-evaluated the capacity of the κ-opioid receptor antagonist, nor-binaltorphimine given alone, to alter the nociceptive threshold in mononeuropathic rats, since it has been done in previous experiments. We determined that, when injected i.pl. into the nerve-injured hind paw, the top dose of 30 μg of nor-binaltorphimine alone produced no effect on either the nerve-injured or contralateral hind paw (Keïta et al., 1995; Catheline et al., 1996a).

2.2. Surgery

The unilateral peripheral mononeuropathy was produced on the right hind paw according to the method described by Bennett and Xie (1988) and Attal et al. (1990). The animals were anesthetized with sodium pentobarbital (Nembutal, 50 mg/kg i.p.). The common sciatic nerve was exposed by blunt dissection at the level of the midthigh and four loose ligatures (5-0 chromic catgut, about 1-mm spacing) were placed around the nerve taking care not to interrupt the epineural circulation. To minimize the discomfort and possible painful mechanical stimulation, the rats were housed in large cages with saw dust bedding after the surgery. The neuropathic rats were able to eat and drink unaided.

2.3. Behavioral testing

Before surgery, a pre-operative threshold (mean of two consecutive stable thresholds) was determined for both hind paws. At 2 weeks after surgery, when the abnormal pain behavior is at a stable maximum (Bennett and Xie, 1988; Attal et al., 1990), a pre-injection threshold (mean of two consecutive stable thresholds) was determined before the injection of the drugs. Vocalization thresholds to paw pressure were then measured every 10 min, until they had returned to the level of the control values. The delay of 10 min was chosen to avoid tissue damage during the testing.

Test sessions, beginning at 0930, were carried out in a quiet room, away from the colony room. The animals were not acclimatized to the test situations beforehand. The experimenter was unaware of the drug combinations used. The rats were randomly assigned to groups of five for a series of tests.

The vocalization thresholds to paw pressure, 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 hind paw (with a Basile analgesymeter, 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 centrally integrated response is especially sensitive to analgesic compounds, particularly in this model of mononeuropathy (Attal et al., 1991; Desmeules et al., 1993; Kayser et al., 1995; Catheline et al., 1996a,b).

2.4. Drugs

The following drugs were used: morphine hydrochloride (Meram, Paris, France), U-69,593 a specific κ-opioid receptor agonist (Sigma, Saint-Quentin Fallavier, France),

nor-binaltorphimine dihydrochloride a specific κ-opioid receptor antagonist (Research Biochemicals, Natick, MA, USA), CTOP, a specific μ-opioid receptor antagonist (Neosystem Isochem, Strasbourg, France), and saline (0.9% NaCl).

Drugs were freshly prepared in sterile physiological saline. Morphine and U-69,593 were administered i.v. in a volume of 0.2 ml, into a lateral tail vein. Nor-binaltorphimine, CTOP or saline were injected subcutaneously in a volume of 0.2 ml, into the nerve-injured or contralateral plantar hind paw.

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 hind paw 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. The experimenter was unaware of the drug combinations injected. Each animal was used only once.

2.5. Experimental protocols

In each series of experiments, nor-binaltorphimine, CTOP or saline was injected just after U-69,593 or morphine.

In a first series of experiments, five groups of mononeuropathic rats received 0.75 mg/kg i.v. of U-69,593, then saline ($n = 6$, control group) or 10 μg, 15 μg, 20 μg or 30 μg of nor-binaltorphimine injected i.pl. into the nerve-injured paw ($n = 6$, $n = 6$, $n = 9$ and $n = 6$, respectively). These doses were based on our previous studies in mononeuropathic rats (Keïta et al., 1995; Catheline et al., 1996a,b).

Two other groups of neuropathic rats received 0.75 mg/kg i.v. of U-69,593, then 20 μg of nor-binaltorphimine injected i.v. or i.pl. into the contralateral paw ($n = 6$ in each group). These experiments were conducted to assess whether the antagonistic effects of i.pl. nor-binaltorphimine were mediated through a central site of action.

In a second series of experiments, two groups of neuropathic rats received 1 mg/kg i.v. of morphine, then saline (control group) or 1 μg of CTOP injected i.pl. into the nerve-injured paw ($n = 6$ in each group). Another group of rats received 0.75 mg/kg i.v. of U-69,593, then 1 μg of CTOP into the nerve-injured paw ($n = 6$). The 1 μg dose of CTOP was determined on the basis of previous experiments in an experimental model of inflammatory pain (Stein et al., 1989).

2.6. Statistics

Vocalization thresholds to paw pressure are given in grams. Data are expressed as means ± S.E.M. A paired student's *t*-test was used to compare the vocalization thresholds to paw pressure obtained before and after the surgery for each hind paw and to compare the drug effects with the control value. To evaluate the effects of co-injection of U-69,593 or morphine and the different opioid receptor antagonists (nor-binaltorphimine and CTOP) or saline, an analysis of variance (ANOVA) was performed taking as the variable the areas under the curves (AUCs) expressed in g/min. The observed significances were then confirmed with Scheffe's post-hoc test. The observed differences were regarded as significant when the P values were lower than 0.05.

3. Results

Before the nerve ligature, the mean vocalization thresholds to paw pressure (336 ± 10 g and 333 ± 7 g, $n = 63$) obtained from the left and right hind paws respectively, did not differ. In agreement with previous studies (Desmeules et al., 1993; Kayser et al., 1995; Catheline et al., 1996a,b; Idänpään-Heikkilä et al., 1997), at week 2 after the nerve ligature, the mean vocalization threshold to paw pressure of the nerve-injured hind paw was decreased to 237 ± 3 g (71 ± 3% of the pre-operative value) ($P < 0.001$, paired *t*-test). As described earlier, the mean vocalization threshold to paw pressure for the contralateral hind paw of 350 ± 2 g (104 ± 3% of the pre-operative value), was not decreased.

After the injection of morphine, U-69,593 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 thresholds to paw pressure.

3.1. Effect of an i.pl. injection of saline on the antinociceptive effect of U-69,593 (Fig. 1)

In this group ($n = 6$), the pre-injection values were 354 ± 5 g and 225 ± 9 g for the contralateral and nerve-injured hind paws, respectively. In the nerve-injured hind paw, the effect of the 0.75 mg/kg dose of U-69,593 lasted for 100 min and peaked between 10 and 30 min. The mean vocalization threshold to paw pressure at 30 min was 564 ± 18 g (250 ± 4% of the pre-injection value) ($P < 0.001$, paired *t*-test). In the contralateral hind paw, the effect of the 0.75 mg/kg dose of U-69,593 lasted for 80 min and peaked between 10 and 40 min. The mean vocalization threshold to paw pressure at 40 min was 585 ± 15 g (165 ± 2%) ($P < 0.001$, paired *t*-test). Therefore, although the pre-injection value from the nerve-injured hind paw was lower than that of the contralateral hind paw, the animals were able to sustain equal pressures from both hind paws after the injection of U-69,593. Further, the overall effect of U-69,593 was enhanced in the nerve-injured hind paw compared with the contralateral hind paw ($P < 0.01$, paired *t*-test for the AUCs).

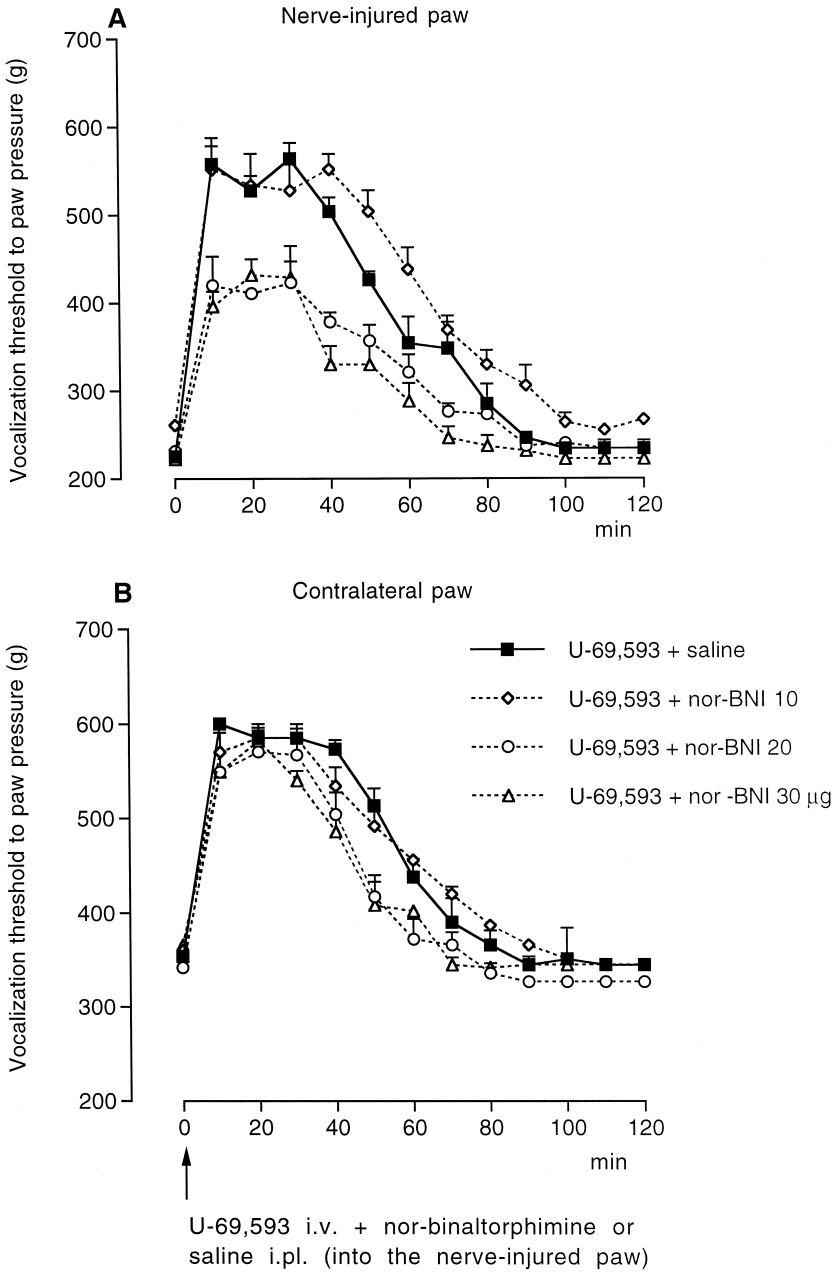

Fig. 1. Mean curves illustrating the ability of i.pl. nor-binaltorphimine (10, 20 and 30 μg, $n = 6$, 9 and 6, respectively) or saline ($n = 6$) to prevent the antinociceptive effect of systemic U-69,593 (0.75 mg/kg), as measured by the vocalization threshold to paw pressure. (A) nerve injured, (B) contralateral hind paw. Each value (in grams) is expressed as mean ± S.E.M. nor-BNI = nor-binaltorphimine.

3.2. Effect of the i.pl. injection of nor-binaltorphimine (10–30 μg into the nerve-injured paw) on the effect of U-69,593 (Fig. 1)

The pre-injection values of these different groups ($n =$ 25 rats) were 358 ± 3 g and 247 ± 7 g for the contralateral and nerve-injured hind paws, respectively. In the nerve-injured paw, nor-binaltorphimine reduced the antinociceptive effect of U-69,593, in comparison with the control group of rats receiving i.pl. saline, with increasing doses (ANOVA, $F_{4,29} = 23.866$, $P < 0.001$).

With 10 μg of nor-binaltorphimine ($n = 6$), the maximal effect was observed 40 min after the injection. At this time, the mean vocalization threshold to paw pressure was 562 ± 16 g (215 ± 9% of the pre-injection value) ($P <$ 0.01, paired *t*-test). For 15 μg ($n = 6$, not shown) the peak value at 40 min after the injection was 570 ± 12 g (230 ± 6% of the pre-injection value) ($P < 0.01$, paired *t*-test). However, the effects of these two lower doses were not different from saline (Scheffe's post-hoc test for the AUCs).

With the dose of 20 μg ($n = 9$), the maximal effect reached at 30 min was 423 ± 24 g (183 ± 5% of the

pre-injection value) ($P < 0.01$, paired *t*-test), reduced by 67% compared with the effect of i.pl. saline. For the highest dose of 30 μg of nor-binaltorphimine ($n = 6$), the maximal effect at 30 min was 429 ± 36 g ($182 \pm 6\%$ of the pre-injection value) ($P < 0.01$, paired *t*-test), reduced by 66%. Further, the respective AUCs were reduced by 43% and 53%, in comparison with the control group of rats receiving i.pl. saline. These effects were significantly different from those obtained in this control group ($P < 0.05$, Scheffe's post-hoc test for the AUCs).

No changes in the vocalization thresholds to paw pressure after the co-injection of i.v. U-69,593 and i.pl. nor-binaltorphimine were observed in these animals for the contralateral paw, in comparison with the control group of rats receiving i.pl. saline (ANOVA, $F_{4,29} = 3.379$). The mean peak values were 585 ± 10 g ($162 \pm 5\%$ of the pre-injection value) ($P < 0.001$, paired *t*-test), 579 ± 6 g ($158 \pm 7\%$) ($P < 0.001$, paired *t*-test), 570 ± 15 g ($166 \pm 6\%$) ($P < 0.001$, paired *t*-test) and 585 ± 10 g ($159 \pm 4\%$) ($P < 0.001$, paired *t*-test), respectively for 10, 15, 20 and 30 μg, thus comparable whatever the i.pl. doses of nor-binaltorphimine (Fig. 1).

3.3. Effect of the i.v. injection of nor-binaltorphimine (20 μg) on the effect of U-69,593

In this group ($n = 6$), the pre-injection values were 354 ± 20 g and 237 ± 10 g for the contralateral and nerve-injured hind paws, respectively. No changes in the vocalization thresholds to paw pressure after the co-injection of i.v. U-69,593 and nor-binaltorphimine were observed in these animals, in comparison with the control group of rats receiving i.pl. saline (ANOVA, $F_{2,16} = 0.747$ for the nerve-injured hind paw and $F_{2,15} = 0.512$ for the contralateral hind paw). Maximal mean vocalization thresholds to paw pressure at 40 min after the injection were 564 ± 18 g ($237 \pm 2\%$ of the pre-injection value) ($P < 0.001$, paired *t*-test) for the nerve-injured hind paw and 591 ± 6 g ($167 \pm 6\%$) ($P < 0.001$, paired *t*-test) for the contralateral hind paw.

3.4. Effect of the i.pl. injection of nor-binaltorphimine (20 μg into the contralateral paw) on the effect of U-69,593

The pre-injection values of this group ($n = 6$) were 360 ± 4 g and 237 ± 7 g for the contralateral and nerve-injured hind paws, respectively. In these animals, the overall effect of U-69,593 on both hind paws was comparable to the control group receiving i.pl. saline (ANOVA, $F_{2,16} = 0.747$ for the nerve-injured hind paw and $F_{2,15} = 0.512$ for the contralateral hind paw). Maximal mean vocalization thresholds to paw pressure at 40 min were 567 ± 14 g ($239 \pm 4\%$ of the pre-injection value) ($P < 0.001$, paired *t*-test) for the nerve-injured hind paw and 597 ± 25 g ($166 \pm 5\%$) ($P < 0.001$, paired *t*-test) for the contralateral hind paw.

3.5. Effect of an i.pl. injection of a μ-opioid receptor antagonist, CTOP, on the antinociceptive effect of U-69,593 (Fig. 2)

In the control group of rats receiving 1 mg/kg i.v. of morphine and saline i.pl. into the nerve-injured hind paw ($n = 6$), the pre-injection values were 354 ± 3 g and 243 ± 15 g for the contralateral and nerve-injured hind paws, respectively. In the nerve-injured hind paw, the effect of morphine lasted for 80 min and peaked at 30 min. At this time, the mean vocalization threshold to paw pressure was 600 ± 10 g ($247 \pm 4\%$ of the pre-injection value) ($P < 0.001$, paired *t*-test). Similarly, in the contralateral hind paw, the effect of morphine lasted for 60 min and peaked at 30 min. At this time, the mean vocalization threshold to paw pressure was 453 ± 24 g ($128 \pm 8\%$) ($P < 0.01$, paired *t*-test).

In the rats receiving 1 mg/kg i.v. of morphine and 1 μg of CTOP i.pl. into the nerve-injured hind paw ($n = 6$), the pre-injection values were 351 ± 12 g and 246 ± 6 g for the contralateral and nerve-injured hind paws, respectively. In these rats, the effect of morphine on the nerve-injured hind paw was reduced in comparison with the control saline group (ANOVA, $F_{1,9} = 34.016$, $P < 0.001$, based on the AUCs). Maximal mean vocalization threshold to paw pressure at 30 min after the injection was 432 ± 9 g ($175 \pm 2\%$ of the pre-injection value) ($P < 0.001$, paired *t*-test), reduced by 72% compared with the effect of i.pl. saline. No changes in the vocalization thresholds to paw pressure were observed in these rats for the contralateral hind paw, in comparison with the control group of rats

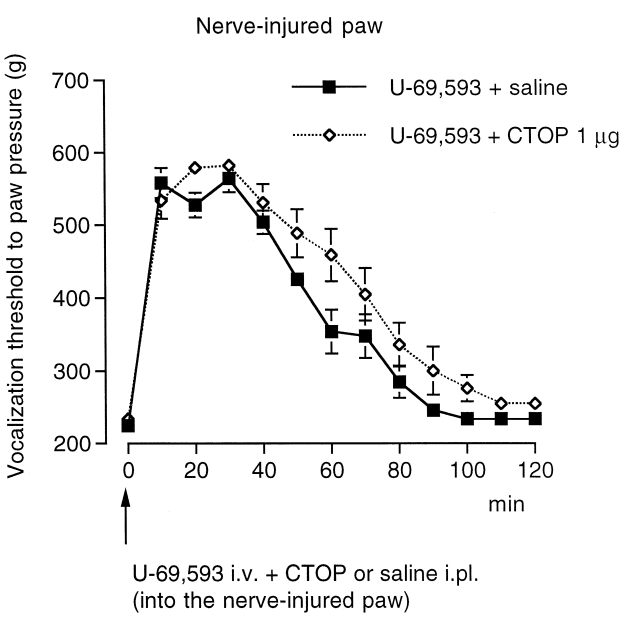

Fig. 2. Lack of change in the antinociceptive effects of systemic U-69,593 in rats receiving CTOP into the nerve-injured paw. The mean curves obtained from the nerve-injured paw after i.v. injection of 0.75 mg/kg of U-69,593 and saline ($n = 6$) or CTOP 1 μg ($n = 6$) into the nerve-injured paw. Each value (in grams) is expressed as mean ± S.E.M.

receiving i.pl. saline (ANOVA, $F_{1,8} = 0.338$). Maximal mean vocalization threshold to paw pressure at 30 min after the injection was 408 ± 24 g (118 ± 4% of the pre-injection value) ($P < 0.05$, paired *t*-test).

In the animals ($n = 6$) receiving 0.75 mg/kg i.v of U-69,593 and 1 μg of i.pl. CTOP into the nerve-injured hind paw, the pre-injection values were 342 ± 4 g and 234 ± 6 g for the contralateral and nerve-injured hind paws, respectively. In this group, the effect of U-69,593 on both hindpaws was comparable that in the control group of rats receiving U-69,593 and i.pl. saline (ANOVA, $F_{1,11} = 2.408$ for the nerve-injured hind paw and $F_{1,6} = 0.024$ for the contralateral hind paw). Maximal mean vocalization thresholds to paw pressure at 40 min after the injection were 582 ± 12 g (248 ± 2% of the pre-injection value) ($P < 0.001$, paired *t*-test) for the nerve-injured hind paw (Fig. 2) and 570 ± 8 g (166 ± 2%) ($P < 0.001$, paired *t*-test) for the contralateral hind paw.

4. Discussion

Two weeks after the surgery, the rats with the chronic constriction injury of the sciatic nerve exhibited abnormal pain sensitivity, with decreased thresholds to mechanical stimulation, as shown previously (Attal et al., 1990; Desmeules et al., 1993; Kayser et al., 1995; Catheline et al., 1996a,b; Idänpään-Heikkilä et al., 1997). In the control group of rats receiving i.pl. saline, the κ-opioid receptor agonist U-69,593 increased the vocalization thresholds to paw pressure confirming the efficacy of relatively low i.v. doses of U-69,593 in this rat model of peripheral mononeuropathy (Desmeules et al., 1993; Catheline et al., 1996a). The antinociceptive effects of U-69,593, with a dose as low as 0.75 mg/kg i.v. were comparable in magnitude to those of systemic morphine (1 mg/kg i.v.) in the same behavioral test. In further accordance with our previous data, the effect of U-69,593 was enhanced in the nerve-injured hind paw compared with the contralateral hind paw. Even though the maximal effect of U-69,593 was equal on both hind paws, the statistical analysis revealed a significant difference between the overall effects (AUCs) of U-69,593 on the two paws. This was caused by both the decreased base-line thresholds of the nerve-injured hind paw and the increased duration of the effect of U-69,593 on this paw. No visible motor impairment was demonstrated, contrasting with the effect of higher doses of i.v. U-69,593 in this rat model of mononeuropathy (Desmeules et al., 1993).

We have previously shown that the antinociceptive effect of U-69,593 was reversed by a single dose (1 or 2 mg/kg i.v.) of the κ-selective opioid receptor antagonist nor-binaltorphimine in neuropathic rats (Catheline et al., 1996a). In agreement with these findings, in the present study, the effect of systemic U-69,593 was reduced on the nerve-injured hind paw, when i.pl. low doses (20–30 μg) of nor-binaltorphimine were injected into this hind paw. It is important to note that nor-binaltorphimine (20 μg into the nerve-injured hind paw) failed to alter the effect of U-69,593 on the contralateral hind paw. Since the same dose of nor-binaltorphimine was ineffective when injected i.v., the present results strongly support the involvement of peripheral κ-opioid receptors in the enhanced antinociceptive effect of systemic U-69,593 in the chronic constriction injury model. In fact, the i.pl. administration of CTOP, a selective μ-opioid receptor antagonist, was ineffective in reducing the effect of systemic U-69,593 in neuropathic rats, whereas it effectively reduced the effect of systemic morphine in these animals. This finding indicates, that the effect of systemic U-69,593 in neuropathic rats is unambiguously related to the activation of κ-opioid receptors. The peripheral opioid receptor system would interact with central systems (Kolesnikov et al., 1996), giving it great importance in the antinociceptive effect of systemic U-69,593. It might be argued that the antagonistic effect of i.pl. nor-binaltorphimine 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. However, we noted that the i.pl. injection of saline, did not influence U-69,593 analgesia in these animals. Further, CTOP, or the lowest doses of nor-binaltorphimine (10–15 μg) did not modify U-69,593 antinociception. In addition, a local anaesthetic effect of the i.pl. drug was excluded, since we determined in previous experiments that, the i.pl. injection of nor-binaltorphimine alone (30 μg) produced no effect on either the nerve-injured or contralateral hind paw (Keïta et al., 1995; Catheline et al., 1996a).

In the nor-binaltorphimine-treated rats, the mean curves obtained over the dose range of 20 to 30 μg of nor-binaltorphimine were comparable; the effect of U-69,593 on the nerve-injured paw was reduced by 43% and 53%, respectively, in comparison with the saline-treated group. This suggests that the peripheral antinociceptive effect of U-69,593 was completely antagonized. Thus, to test higher doses of nor-binaltorphimine appeared unjustified. These results are in line with our previous studies (Keïta et al., 1995) demonstrating an effect of ICI 204448, a κ-opioid receptor agonist that minimally crosses the blood–brain barrier in mononeuropathic rats, which rapidly plateaued, and thus probably resulted from a saturation of peripheral opioid receptors.

In the present study, no reduction of the antinociceptive action of U-69,593 in the vocalization threshold to paw pressure test, could be detected when nor-binaltorphimine was injected into the contralateral, uninjured hind paw. This finding suggests that either κ-opioid receptors in peripheral tissue would not be functionally active, or that a different dose of i.pl. nor-binaltorphimine is required to influence the effect of U-69,593 on the contralateral hind paw of the neuropathic rats. These data may appear to be

in contradiction to those of Kolesnikov et al. (1996), who demonstrated that the κ-opioid receptor agonist U-50,488H injected into the tail induced significant and nor-binaltorphimine-reversible antinociceptive effects in mice. However, the experimental procedure in this previous study was different: they used a different test (tail-flick test) in mice, and a different κ-opioid receptor agonist, U-50,488H.

As previously discussed (Kayser et al., 1995; Catheline et al., 1996b), the peripheral antinociceptive effect of systemic U-69,593 against mechanical stimuli of the nerve-injured hind paw, suggests mechanisms resembling those proposed for inflammatory pain models (see Stein and Yassouridis, 1997). Such mechanisms may be linked to a disruption of the perineurial barrier and subsequent facilitated access to opioid receptors on peripheral nerves (Antonijevic et al., 1995) and/or to an enhanced peripherally directed axonal transport of opioid receptors in sensory neurons, leading to an increase in their number on peripheral nerve terminals (Hassan et al., 1993; Jeanjean et al., 1994; Schäfer et al., 1995; Li et al., 1996). Even though inflammatory conditions are not obvious at 2 weeks after surgery (Attal et al., 1990; Maves et al., 1993; Clatworthy et al., 1995), the immune system is intimately involved in the mediation of the nervous system response to damage (Sommer et al., 1998; Wagner et al., 1998). Cytokines are present in normal peripheral nerve and are upregulated following nerve injury (Bourde et al., 1996; Reichert et al., 1996; Wagner and Myers, 1996). Upon nerve-injury, non-resident macrophages which are a primary source of cytokines, are recruited to the site of injury (Bonetti et al., 1993; Sommer et al., 1993; Lotan et al., 1994; Wagner et al., 1998). Macrophages participate importantly in the degeneration and regeneration processes following injury and in the development and resolution of neuropathic pain (Brown et al., 1991; Stoll and Hartung, 1992; Perry et al., 1993; Dahlin et al., 1996; Wagner et al., 1998). Accordingly, the peripheral antinociceptive action of systemic U-69,593 resulting in an enhanced effect on the nerve-injured hind paw, found in the present study, could be linked to macrophages recruitment around the injured nerve fibres and to a possible up-regulation of κ-opioid receptors in this rat model of peripheral neuropathic injury.

In conclusion, the present findings on the mononeuropathic rats, suggest again that peripheral opioid analgesic systems, especially κ-opioid systems, may prove to be a valuable pharmacological target in the design of novel analgesics.

Acknowledgements

This study was partly supported by the Association pour la Recherche sur le Cancer.

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