ELSEVIER

European Journal of Pharmacology 429 (2001) 139–145

ejp

www.elsevier.com/locate/ejphar

Peripheral effects of morphine in neuropathic rats: role of sympathetic postganglionic nerve fibers

Antti Pertovaara *, Hong Wei

Department of Physiology, Institute of Biomedicine, University of Turku, Kiinamyllynkatu 10, FIN-20520 Turku, Finland
Department of Physiology, Institute of Biomedicine, University of Helsinki, Helsinki, Finland

Accepted 27 July 2001

Abstract

We studied the contribution of peripheral opioid receptors to the morphine-induced antinociception in rats with a spinal nerve ligation-induced neuropathy. Intraplantar (i.pl.) injection of morphine produced a stronger suppression of nociceptive reflex responses of the neuropathic limb following ipsilateral, than contralateral, administration, whereas the morphine-induced effect on the control limb was independent of the injection side. Antinociception induced by systemically administered morphine was significantly attenuated by i.pl. injection of a peripherally acting opioid receptor antagonist in neuropathic but not in sham-operated rats. Following chemical sympathectomy with 6-hydroxydopamine, antinociception was achieved at a lower dose ipsilaterally, than contralaterally, following i.pl. administration of morphine, and the morphine-induced antinociception was attenuated by a peripherally acting opioid receptor antagonist. These results indicate that peripheral opioid receptors may contribute to the morphine-induced antinociception in the spinal nerve ligation-induced model of neuropathy. Sympathectomy of the neuropathic limb may underlie, at least partly, the increased peripheral efficacy of morphine in neuropathy.

Keywords: Antinociception; Morphine; Neuropathic pain; Sympathectomy

1. Introduction

A peripherally acting analgesic drug might be highly beneficial in treating pathophysiological pain, since it provides a possibility to selectively treat pain with minor central side-effects. There is abundant evidence indicating that peripheral mechanisms may significantly contribute to the morphine-induced antinociceptive effect under inflammatory conditions (Ferreira and Nakamura, 1979; Joris et al., 1987; Stein et al., 1988). Moreover, morphine has been shown to have peripheral antinociceptive actions in animals with the chronic constriction model of mononeuropathy (Kayser et al., 1995; Catheline et al., 1996). Some studies suggest that chromic gut-induced inflammation may actually induce the hypersensitivity observed in chronic constriction model of neuropathy (Maves et al., 1993). In addition, other recent studies have emphasized the role of inflammation in experimental models of neuropathy (Bennett, 1999). This raises the question whether the peripheral antinociceptive actions observed in mononeuropathic animals were due to inflammation or neuropathy. Therefore, we determined the peripheral action of morphine in another model of neuropathy that is caused by tightly ligating two spinal nerves (Kim and Chung, 1992). A sham operation of the spinal nerves does not produce neuropathic symptoms suggesting that nerve injury might have a more important role in this model of neuropathy. This hypothesis is supported by the histological findings that a visible inflammatory reaction of the peripheral nerves appears less marked in the spinal nerve ligation model (Röyttä et al., 1999) than in the chronic constriction injury model (Luukko et al., 1994).

The method for the induction of neuropathy, ligation of the peripheral or spinal nerve, leads to an accompanying destruction of somatic as well as sympathetic nerve fibers (Baron et al., 1988). The possible consequences of nerve ligation on peripheral morphine sensitivity might be as well to injury of somatic nerves, autonomic nerves, or both. In the present study, we attempted to determine the contribution of the injury of somatic vs. autonomic nerves to the possible peripheral action of morphine by comparing

* Corresponding author. Tel.: +358-2-333-7578; fax: +358-2-250-2610.

E-mail address: Antti.Pertovaara@utu.fi (A. Pertovaara).

0014-2999/01/$ - see front matter
PII: S0014-2999(01)01315-2

the effect of nerve ligation with that produced by a chemical sympathectomy.

2. Materials and methods

The experiments were performed with adult, male Hannover Wistar rats (weight 200–300 g; The Finnish Laboratory Animal Center, Kuopio, Finland). The experimental protocol adhered to the European Community guidelines for the use of experimental animals and it was accepted by the Institutional Animal Care Committee of the University of Helsinki and the Regional Government of Southern Finland. The animals were housed in polycarbonate cages with a deep layer of sawdust, one to three animals in each cage, in a thermostatically controlled room at 20 ± 2 °C. The room was artificially illuminated from 6 a.m. to 8 p.m. The rats received commercial pelleted rat–mouse feed and tap water ad libitum.

2.1. Behavioral assessment of nociceptive responses

Before actual testing, the animals were habituated to the experimental conditions by allowing them to spend 1–2 h daily in the laboratory during 3 days.

For assessment of tactile allodynia, the hindlimb withdrawal threshold evoked by stimulation of the hindpaw with monofilaments (von Frey hairs) was determined while the rat was standing on a metal grid. The plantar skin of the hindpaw was stimulated with a series of calibrated monofilaments (forces ranging from 0.1 to 46.6 g; Stoelting, Wood Dale, IL). The monofilaments were applied in an ascending series until the rat withdrew its hindlimb. The lowest force producing a withdrawal response was considered the threshold. The threshold for each animal is the average of three separate measurements.

For assessment of mechanical hyperalgesia, the hindlimb withdrawal threshold evoked by noxious mechanical stimulation (paw pressure test) was determined with a Basile Analgesy meter (Ugo Basile, Varese, Italy). With this device, a mechanical force increasing at a rate of 32 g/s was applied to the hindpaw until the rat withdrew its hindlimb. The withdrawal threshold of each animal was the average of three separate measurements performed and the minimum interval between measurements was 1 min. The cut-off force was 500 g.

For assessment of thermonociception, the heat-induced tail-flick response latency was determined using a radiant heat device (Socrel DS-20, Ugo Basile, Varese, Italy). This device focuses a heat beam on the tail and automatically records the latency to tail removal. The average of three consecutive measurements performed was recorded. The minimum interval between consecutive measurements was 1 min. The cut-off latency in the tail-flick test was 9 s. The tail skin temperature was determined prior to heat stimulation with a contact thermode (Olli Temperature Meter 535, Kone, Helsinki, Finland).

2.2. Techniques for producing neuropathy or chemical sympathectomy

The unilateral ligation of two spinal nerves (L_5 and L_6) was performed under pentobarbitone anesthesia (50 mg/kg i.p.) as described in detail earlier (Kim and Chung, 1992; Röyttä et al., 1999). Briefly, left paraspinal muscles were separated from the spinous processes at the L_4–S_1 levels. The L_6 transverse process was partly removed to identify visually the L_4–L_6 spinal nerves. The left L_5 and L_6 spinal nerves were isolated and tightly ligated with 6-0 silk thread. Following ligation, the wound was sutured and the rats were allowed to recover. Animals that showed a major motor impairment were excluded from this study and they were sacrificed. Of the operated rats with a spinal nerve ligation, only those with unilateral allodynia to mechanical stimulation with monofilaments (hindlimb withdrawal thresholds in the operated side < 7 g) were selected for further studies. Additionally, sham-operated rats were used as controls. In sham operation, the procedure was identical, except that spinal nerves were not ligated.

To produce chemical sympathectomy, a group of animals were pretreated with 6-hydroxydopamine (50 mg/kg i.p. for 2 days followed by 100 mg/kg i.p. for 3 days; Zhou et al., 1998). In a parallel study, we report that an identical pretreatment with 6-hydroxydopamine effectively destroyed the postganglionic sympathetic nerve fibers as verified by histological techniques (Kalmari et al., 2001).

2.3. Course of the study

The experiment started by ligation of spinal nerves, sham operation or pretreatment with 6-hydroxydopamine. The baseline responses were determined about 1 week after the operation or after the end of the 6-hydroxydopamine pretreatment. When studying the effect of i.pl. administration of morphine or saline in neuropathic and sham-operated animals, the behavioral pain tests and tail skin temperature measurements were performed prior to and 10 min after their microinjection into the paw. Morphine was injected i.pl. in a cumulative fashion (100 μg/20 μl followed about 15 min later by an additional 100 μg/20 μl). In addition, saline was injected twice (20 μl) followed by another 20 μl 15 min later. In neuropathic animals, the effects by injecting morphine into the neuropathic and contralateral limb were tested in separate sessions at an interval of at least 3 days. When studying the reversal of morphine-induced antinociception by a peripherally acting opioid receptor antagonist, morphine was injected systemically (s.c.) at a dose of 2.5 mg/kg in neuropathic or sham-operated animals. About 35 min later, naloxone methiodide (10 μg/20 μl) was injected into the neuropathic or sham-operated hind paw. Pain behavior and

tail skin temperature were measured prior to morphine injection, 30 min after morphine injection, and 10 min following injection of naloxone methiodide. The effect of naloxone methiodide alone (10 μg/20 μl i.pl.) was studied in a separate session both in neuropathic and sham-operated animals. The effect of chemical sympathectomy on morphine-induced antinociception was studied in three separate sessions. In one session, morphine was administered i.pl. into one of the hindpaws at cumulative doses of 100 and 200 μg and the testing of the animals was performed as above. In another session, morphine was administered s.c. at a dose of 2.5 mg/kg and this was followed by i.pl. administration of naloxone methiodide (10 μg). Also in this group, the pain testing was performed as in the corresponding group without chemical sympathectomy. In the third group, naloxone methiodide (10 μg) was injected alone i.pl.

2.4. Drugs

Morphine hydrochloride was obtained from Orion Pharma, Espoo, Helsinki. Naloxone methiodide, an opioid antagonist that only poorly penetrates the blood–brain barrier, and 6-hydroxydopamine bromide were obtained from Research Biochemicals International, MA, U.S.A. Physiological saline was used for control injections. Intraplantar (i.pl.) injections were performed at a volume of 20 μl using a 50-μl Hamilton syringe connected via a polyethylene tubing to a 27-gauge hypodermic needle. During injections, one of the experimenters held the animal and the other performed the injection.

2.5. Statistics

For the assessment of monofilament-induced hindlimb withdrawal thresholds, nonparametric methods (Friedman or Kruskal–Wallis test followed by Dunn's test) were used. Other results were analyzed using one- or two-way analysis of variance followed by Tukey's test. $P < 0.05$ was considered to represent a significant difference.

3. Results

3.1. Baseline responses in different experimental groups

In a test of tactile allodynia (the monofilament test), the median baseline hindlimb withdrawal threshold ipsilateral to the spinal nerve ligation was 2.1 g (range 1.4–3.6 g), whereas in the contralateral (unoperated) hindlimb of the same animals the median threshold was 18.1 g (range 12.5–20.1 g). The corresponding threshold was 20.5 g (range 12.5–38 g) in the sham-operated group and 21.5 g (range 7.4–46.5 g) in the 6-hydroxydopamine-pretreated group. The monofilament-induced hindlimb withdrawal thresholds were significantly different between the different experimental conditions (KW = 15.7, $P < 0.002$). The threshold ipsilateral to the spinal nerve ligation was significantly lower than in any other experimental condition ($P < 0.05$, Dunn's test), whereas there was no significant difference in the threshold between other conditions.

In a test of mechanical nociception and hyperalgesia (the paw pressure test), the mean hindlimb withdrawal threshold of spinal nerve-ligated animals in baseline conditions was 77.2 ± 4.8 g ($\pm$S.E.M., $n = 7$) ipsilateral to the ligation and 132 ± 7 g contralateral to the ligation. The corresponding threshold was 139.2 ± 4 g ($n = 6$) in sham-operated animals and 128 ± 6 g ($n = 5$) in 6-hydroxydopamine-pretreated animals. The paw pressure thresholds were significantly different between the experimental conditions [$F(3,24) = 26.86$, $P < 0.0001$]. The threshold was significantly lower ipsilateral to the nerve ligation than in any other condition ($P < 0.001$, Tukey's test).

In a test of thermal nociception, the tail-flick test, the mean radiant heat-induced tail-flick latency was 3.2 ± 0.15 s in spinal nerve-ligated animals, 3.4 ± 0.22 s in sham-operated animals and 2.5 ± 0.3 s in 6-hydroxydopamine-pretreated animals. There was a significant difference in baseline tail-flick latencies between the groups [$F(2,17) = 4.28$, $P < 0.05$]. According to post hoc testing of tail-flick data, the only difference between the groups was between sham-operated and 6-hydroxydopamine-pretreated animals ($P < 0.05$, Tukey's test). The mean skin temperature of the tail in baseline conditions was 29.1 ± 0.3 °C in the nerve ligation group, 29.6 ± 0.2 °C in the sham-operated group and 27.1 ± 0.7 °C in the 6-hydroxydopamine-pretreated group. The differences in baseline temperatures between the groups were significant [$F(2,17) = 9.5$, $P < 0.005$]. The 6-hydroxydopamine-pretreated group had a lower tail skin temperature than other groups ($P < 0.01$, Tukey's test).

3.2. Intraplantar injection of morphine

The effects of drug or vehicle administrations on the monofilament-induced withdrawal threshold were determined only in the neuropathic hind limb. The high monofilament-induced thresholds in other conditions prevented a reliable dissociation of a "true" reflex response from an artifactual paw lifting by the test stimulus. Injection of 20 μl of physiological saline twice into the paw of the neuropathic hind limb had no significant effect (Fr = 5.18) on the monofilament-induced hind limb withdrawal threshold (Fig. 1A). Cumulative injection of morphine i.pl. produced a dose-related elevation of the withdrawal threshold of the neuropathic hind limb following administration into the neuropathic paw (Fr = 13.56, $P < 0.002$) but not following administration into the contralateral paw (Fr = 5.2; Fig. 1A).

When compared with the effect of saline, i.pl. administration of morphine produced a significant prolongation of the radiant heat-induced tail-flick latency [$F(2,48) = 10.45$,

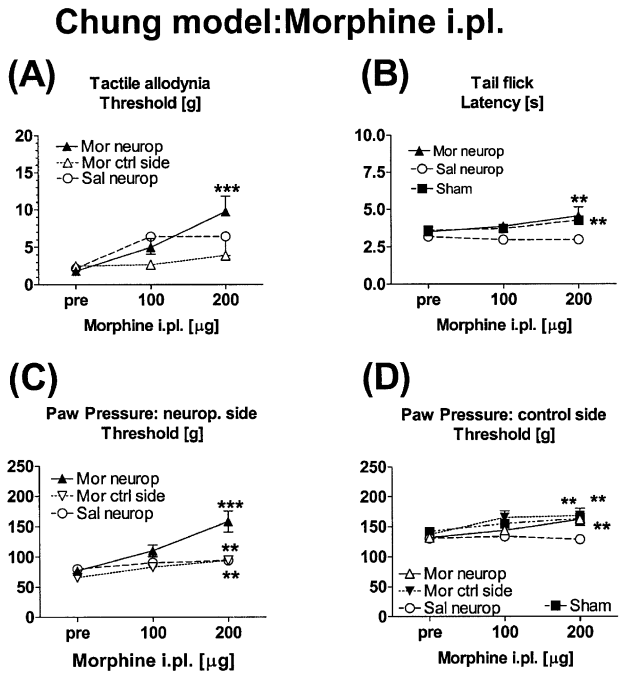

Fig. 1. Effect of a cumulative i.pl. administration of morphine (Mor; 100–200 μg) in neuropathic or sham-operated animals. The behavioral effects were determined 10 min following each i.pl. injection. (A) Tactile antiallodynic effects on the neuropathic limb according to the monofilament test. (B) Thermal antinociceptive effects according to the tail-flick test. (C) Mechanical antihyperalgesic effects on the neuropathic limb according to the paw pressure test. (D) Mechanical antinociceptive effects on control or sham-operated limbs according to the paw pressure test. In each graph, the mean value + S.E.M. is shown ($n = 5–7$). Mor neurop = morphine administered into the neuropathic paw; Mor ctrl side = morphine administered contralateral to the neuropathic paw; Sal neurop = saline administered into the neuropathic paw; Sham = morphine administered into the sham-operated paw of a nonneuropathic animal. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ [Dunn's test in (A), Tukey's test in (B) and (C); reference: the corresponding value prior to i.pl. injection = pre].

$P < 0.001$]. The morphine-induced prolongation of the tail-flick response was observed following a cumulative dose of 200 μg of morphine, independent of the side of administration (Fig. 1B). I.pl. administration of morphine did not produce any significant change in the tail skin temperature, independent of the side of morphine administration (not shown).

In the paw pressure test, the threshold of the neuropathic limb was significantly elevated by i.pl. administration of morphine [$F(2,45) = 16.15$, $P < 0.0001$]. The morphine-induced increase of the paw pressure threshold of the neuropathic limb was significantly higher following ipsilateral than contralateral injection [$F(1,30) = 15.69$, $P < 0.0005$], and the injection side-dependence of the threshold elevation was significant only at the higher dose of morphine [$F(2,30) = 3.34$, $P < 0.05$; Fig. 1C]. The paw pressure threshold of the unoperated control limb of neuropathic animals was also significantly elevated by i.pl. administration of morphine [$F(2,45) = 5.47$, $P < 0.01$], but the morphine-induced threshold elevation of the non-neuropathic limb was not higher following ipsilateral, than contralateral, injection [$F(1,30) = 2.17$; Fig. 1D]. I.pl. administration of morphine into the hind paw of sham-operated animals produced a significant elevation of the paw pressure threshold ipsilateral to morphine administration [$F(2,17) = 12.09$, $P < 0.005$; Fig. 1D]. The morphine-induced elevation of the paw pressure threshold in the sham-operated animals was not significantly different from that in the unoperated hind limb of the neuropathic animals [$F(2,45) = 1.45$].

3.3. Attempted reversal of morphine-induced effects by a peripherally acting naloxone

The tactile antiallodynic effect induced by systemic administration of morphine (2.5 mg/kg s.c.) was reversed by administration of naloxone methiodide (10 μg) into the neuropathic paw (Fig. 2A). This dose of naloxone methio-

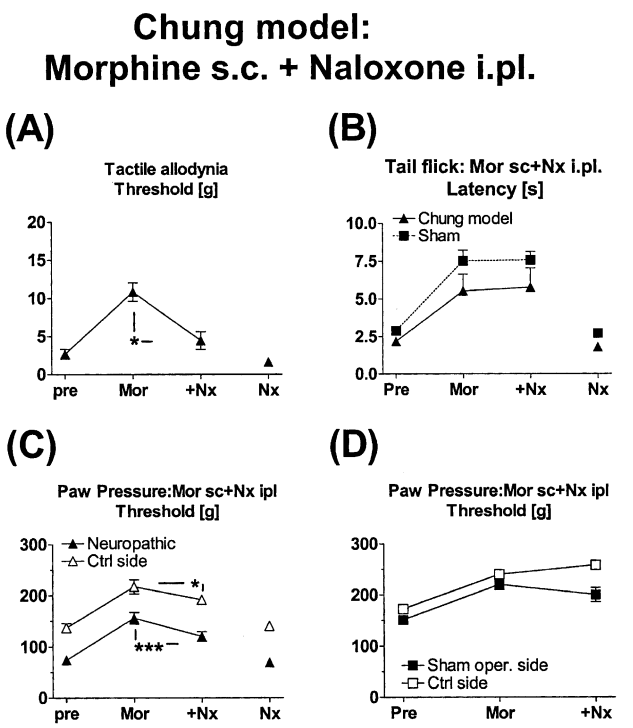

Fig. 2. Attempted reversal of morphine-induced effects by a peripherally acting opioid receptor antagonist, naloxone methiodide, in neuropathic or sham-operated animals. Morphine was administered s.c. at a dose of 2.5 mg/kg and naloxone methiodide i.pl. at a dose of 10 μg into the neuropathic or sham-operated hind paw. (A) Reversal of tactile antiallodynia in neuropathic limb. (B) Attempted reversal of thermal antinociception in the tail. (C) Reversal of mechanical antihyperalgesia or antinociception in neuropathic animals. (D) Attempted reversal of mechanical antinociception in sham-operated animals. Pre = prior to morphine injection; Mor = 30 min following s.c. morphine injection; Mor + Nx = 10 min following i.pl. naloxone methiodide injection and about 40 min following s.c. morphine injection; Nx = 10 min following i.pl. injection of naloxone methiodide alone in a separate session. $^{*}P < 0.05$, $^{***}P < 0.005$ [Dunn's test in (A), Tukey's test in other graphs]. The symbols represent mean values and the error bars S.E.M. ($n = 5–7$).

dide alone had no significant effect on tactile allodynia. Prolongation of the tail-flick latency induced by morphine (2.5 mg/kg s.c.) was not reduced by i.pl. administration of naloxone methiodide (10 μg; Fig. 2B). The elevation of paw pressure thresholds induced by morphine (2.5 mg/kg s.c.) in neuropathic animals was bilaterally attenuated by administration of naloxone methiodide (10 μg) into the neuropathic paw (Fig. 2C). In sham-operated animals, the effect of i.pl. administration of naloxone methiodide on morphine-induced elevation of the paw pressure thresholds was short of statistical significance (Tukey's test; Fig. 2D).

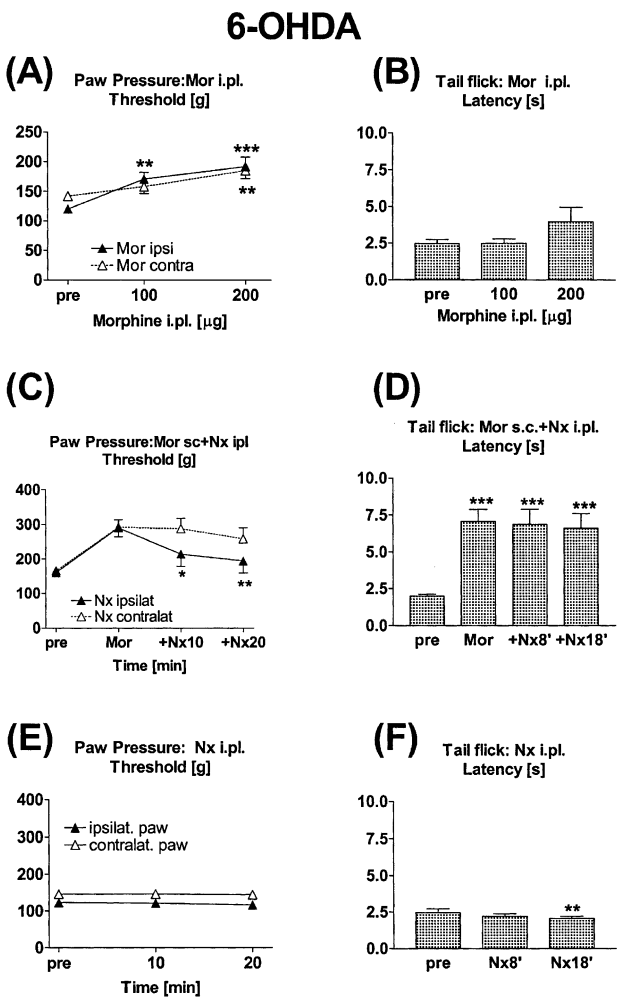

Fig. 3. Behavioral assessment of peripheral morphine actions in chemically sympathectomized rats. (A) Mechanical antinociceptive effect of morphine administered in a cumulative fashion i.pl. ipsi- or contralaterally to the test hindpaw. (B) Thermal antinociceptive effect of morphine on the tail following i.pl. administration. (C) Locally restricted reversal of morphine-induced mechanical antinociception by naloxone methiodide (Nx; 10 μg) administered i.pl. ipsi- or contralaterally to the test hind paw. (D) Attempted reversal of morphine-induced thermal antinociception in the tail by i.pl. administration of naloxone methiodide. (E) Paw pressure thresholds following i.pl. administration of naloxone methiodide alone (10 μg) ipsi- or contralateral to the test hind paw. (F) Tail-flick latency following i.pl. administration of naloxone methiodide alone. In (C) and (D), Mor = following morphine 2.5 mg/kg s.c.; +Nx8–20 = 8–20 min following i.pl. injection of naloxone methiodide and 38–50 min following s.c. injection of morphine. In (E) and (F), the numbers below the *x*-axis indicate time following i.pl. injection of naloxone methiodide. In all graphs, pre = prior to drug injections. The mean values + S.E.M. are shown ($n = 5–6$). $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.005$ (Tukey's test; reference: the corresponding prevalue).

3.4. Morphine-induced effects in sympathectomized animals

In 6-hydroxydopamine-pretreated animals, morphine administered i.pl. (cumulative doses of 100 and 200 μg) produced a highly significant elevation of the paw pressure threshold [$F(2,24) = 13.21$, $P = 0.0001$]. According to two-way analysis of variance, this threshold elevation was not significantly higher ipsilateral, than contralateral, to the morphine injection [$F(1,24) = 0.01$], independent of the morphine dose [$F(2,24) = 1.39$]. However, according to post hoc testing (Tukey's test), a lower dose of morphine was needed to produce a significant elevation of the paw pressure threshold following ipsilateral, than contralateral, injection (Fig. 3A). I.pl. administration of morphine (100–200 μg) did not produce any significant prolongation of the tail-flick latency [$F(2,14) = 2.23$; Fig. 3B].

The elevation of paw pressure thresholds induced by systemically administered morphine (2.5 mg/kg s.c.) in 6-hydroxydopamine-pretreated animals was reversed by i.pl. administration of naloxone methiodide (10 μg) into the ipsilateral, but not contralateral, hind paw (Fig. 3C). The prolongation of the tail-flick latency induced by systemically administered morphine in 6-hydroxydopamine-pretreated animals was not attenuated by i.pl. administration of naloxone methiodide (10 μg; Fig. 3D). I.pl. administration of naloxone methiodide alone in 6-hydroxydopamine-pretreated animals had no significant influence on paw pressure thresholds (Fig. 3E). However, tail-flick latencies were significantly reduced following naloxone methiodide alone [$F(2,17) = 8.33$, $P < 0.01$; Fig. 3F] and this was not accompanied by a change in the tail skin temperature [$F(2,17) = 0.208$].

4. Discussion

According to the present results, mechanical hypersensitivity in animals with a spinal nerve ligation-induced neuropathy is more effectively suppressed following peripheral administration of morphine into the neuropathic than the contralateral hind paw. Moreover, administration of a peripherally acting opioid receptor antagonist, naloxone methiodide, into the neuropathic hind paw reversed the antiallodynic and antihyperalgesic effect induced by systemically administered morphine in the neuropathic limb. However, the same dose (10 μg) of peripherally acting naloxone in the neuropathic hind paw did not reverse the morphine-induced prolongation of the tail-flick response, nor did peripherally acting naloxone (10 μg) have a

significant effect on antinociception induced by systemically administered morphine in sham-operated animals. These findings indicate that peripheral mechanisms contribute to the morphine-induced suppression of pain responses in animals with a spinal nerve ligation-induced neuropathy. This finding is in line with previous results indicating that morphine has peripheral actions in the chronic sciatic nerve constriction-induced model of neuropathy (Kayser et al., 1995). It should be noted, however, that following central, particularly supraspinal, administration in animals with a spinal nerve ligation-induced neuropathy, morphine produces antiallodynic and antinociceptive effects at doses that are an order of magnitude lower than following i.pl. administration (Wei et al., 1998).

4.1. Role of neuropathy vs. inflammation

There is abundant evidence indicating that peripheral actions have an important contribution to the morphine-induced antinociception under inflammatory conditions (Machelska et al., 1999). The chromic ligatures used for chronic constriction of the sciatic nerve induce a marked inflammatory reaction that has been considered to have a role in hypersensitivity induced by this model of neuropathy (Maves et al., 1993). Together, these earlier findings raised the question whether the contribution of peripheral mechanisms to the morphine-induced antinociception described previously in the chronic nerve constriction model of neuropathy (Kayser et al., 1995) is due to inflammation or neuropathy. Spinal nerve ligation-induced model of neuropathy is expected to produce considerably milder inflammatory reaction, particularly in the distal parts of the limb, and this expectation is supported by some histological observations (Luukko et al., 1994; Röyttä et al., 1999). However, since morphine appeared to have significant peripheral actions also in the spinal nerve ligation-induced model of neuropathy but not in sham-operated animals, it seems that nerve injury might lead to an enhanced contribution of peripheral mechanisms to morphine-induced antinociception. Earlier findings indicate that the increased peripheral action of morphine in neuropathy could be mediated both by μ- and κ-opioid receptors (Catheline et al., 1996).

4.2. Role of postganglionic sympathetic nerve fibers

Ligation of a spinal nerve as well as a peripheral nerve destroys not only somatic but also sympathetic nerve fibers (Baron et al., 1988). This raised the question whether sympathetic deafferentation might contribute to enhanced peripheral action of morphine following nerve injury. To address this question, peripheral actions of morphine were studied in chemically sympathectomized animals. The pretreatment with 6-hydroxydopamine is known to produce a marked destruction of postganglionic sympathetic nerve fibers (Zhou et al., 1998) as also histologically verified in our parallel study (Kalmari et al., 2001). According to the present results, sympathectomy had no influence on baseline pain responses. Following sympathectomy, a lower dose of morphine was needed to produce antinociception ipsi-than contralaterally to the i.pl. administration. Importantly, the antinociceptive effect induced by systemically administered morphine in sympathectomized animals was reversed ipsi-but not contralaterally to the i.pl. injection of peripherally acting naloxone. Significant attenuation of morphine-induced antinociception by peripherally acting naloxone was not observed in sham-operated animals with an intact sympathetic nervous system. These findings indicate that sympathectomy may cause enhanced contribution of peripheral mechanisms to morphine-induced antinociception. Moreover, these findings raise the possibility that the enhanced contribution of peripheral opioid receptors observed in neuropathic animals is actually caused by a nerve injury-related sympathectomy and not by injury of the somatic nerves. In a previous study, sympathectomy alone did not significantly enhance the antinociceptive effect of morphine administered i.pl. when compared with the effect of morphine in control animals (Zhou et al., 1998). This previous finding is not contradictory to the present results, since also in the present study the evidence for a local effect obtained using i.pl. injection of morphine was only marginally significant. In the present study the peripheral action of morphine was more clearly demonstrated using i.pl. injection of a peripherally acting naloxone to produce a local reversal of the morphine-induced antinociception.

4.3. Location of morphine action in the periphery

Sympathectomy does not attenuate the peripheral action of morphine in inflammed animals, whereas selective destruction of nociceptive C-fibers does (Zhou et al., 1998). This previous finding indicates that opioid receptors involved in peripheral antinociceptive actions are likely to be located on nociceptive primary afferent fibers and not on sympathetic nerve fibers. This concept is, at least partly, in line with the present results, since morphine had peripheral actions in sympathectomized animals of the present study. Peripherally acting naloxone not only produced a locally restricted reversal of morphine-induced mechanical antinociception or antihyperalgesia as indicated by the paw pressure test but it also produced a reversal of the morphine-induced tactile antiallodynia as indicated by the monofilament test. Since the threshold response in the paw pressure test is likely to be mediated by nociceptive fibers, the former finding fits the concept that the peripheral action of morphine was on nociceptive primary afferent fibers. However, the threshold of the neuropathic limb in the monofilament test is very low and it is likely to be based on activation of mechanoreceptive afferent fibers in rats as well as in humans (Torebjörk et al., 1992). Thus, the peripheral action of morphine on tactile allodynia may

be indirect, i.e., allodynia may be dependent on afferent barrage in nociceptive primary afferent fibers, morphine reduces this nociceptive afferent barrage due to a peripheral action, and this leads to antiallodynia. Alternatively, neuropathy produces in mechanoreceptive afferent fibers a phenotypic switch after which morphine is able to reduce their responses due to a peripheral mechanism.

4.4. Conclusions

The present results indicate that morphine may have peripheral actions in animals with a spinal nerve ligation-induced neuropathy. Enhanced peripheral action of morphine in neuropathic animals might be due to an accompanying sympathectomy induced by nerve injury, since following sympathectomy alone morphine had significant peripheral actions. It remains to be studied whether it is possible to produce a significant attenuation of pain in neuropathic patients by peripherally acting opioid receptor agonists, without central side-effects.

Acknowledgements

We wish to thank Dr. E. Mecke for his help in the experiments. This study was supported by the Sigrid Jusélius Foundation, Helsinki, and the Academy of Finland.

References

Baron, R., Jänig, W., Kollmann, W., 1988. Sympathetic and afferent somata projecting in hindlimb nerves and the anatomical organization of the lumbar sympathetic nervous system of the rat. J. Comp. Neurol. 275, 460–468.

Bennett, G.J., 1999. Does a neuroimmune interaction contribute to the genesis of painful peripheral neuropathies? Proc. Natl. Acad. Sci. U. S. A. 96, 7737–7738.

Catheline, G., Kayser, V., Guilbaud, G., 1996. 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. Eur. J. Pharmacol. 315, 135–143.

Ferreira, S.H., Nakamura, M., 1979. Prostaglandin hyperalgesia: the peripheral analgesic activity of morphine, meperidine, and brain stem stimulation in rats and cats. Prostaglandins 18, 191–200.

Joris, J.L., Dubner, R., Hargreaves, K.M., 1987. Opioid analgesia at peripheral sites: a target for opioids released during stress and inflammation? Anesth. Analg. 66, 1277–1281.

Kalmari, J., Niissalo, S., Konttinen, Y.T., Pertovaara, A., 2001. Modulation of visceral nociceptive responses of rat spinal dorsal horn neurons by sympathectomy. NeuroReport 12, 797–801.

Kayser, V., Lee, S.H., Guilbaud, G., 1995. Evidence for a peripheral component in the enhanced antinociceptive effect of a low dose of systemic morphine in rats with peripheral mononeuropathy. Neuroscience 64, 537–545.

Kim, S.H., Chung, J.M., 1992. An experimental model for peripheral neuropathy produced by segmental spinal nerve ligation in the rat. Pain 50, 355–363.

Luukko, M., Konttinen, Y., Kemppinen, P., Pertovaara, A., 1994. Influence of various experimental parameters on the incidence of thermal and mechanical hyperalgesia induced by a constriction injury of the sciatic nerve in lightly anesthetized rats. Exp. Neurol. 128, 143–154.

Machelska, H., Binder, W., Stein, C., 1999. Opioid receptors in the periphery. Prog. Pain Res. Manage. 14, 45–58.

Maves, T.J., Pechman, P.S., Gebhart, G.F., Meller, S.T., 1993. Possible chemical contribution from chromic gut sutures produces disorders of pain sensation like those seen in man. Pain 54, 57–69.

Röyttä, M., Wei, H., Pertovaara, A., 1999. Spinal nerve ligation-induced neuropathy in the rat: sensory disorders and correlation between histology of the peripheral nerves. Pain 80, 161–170.

Stein, C., Millan, M.J., Yassouridis, A., Herz, A., 1988. Antinociceptive effects of μ- and κ-agonists in inflammation are enhanced due to a peripheral opioid receptor-specific mechanism. Eur. J. Pharmacol. 155, 255–264.

Torebjörk, H.E., Lundberg, L.E., LaMotte, R.H., 1992. Central changes in processing of mechanoreceptive input in capsaicin-induced secondary hyperalgesia in humans. J. Physiol. (London) 448, 765–780.

Wei, H., Panula, P., Pertovaara, A., 1998. A differential modulation of allodynia, hyperalgesia and nociception by neuropeptide FF in the periaqueductal gray of neuropathic rats. Neuroscience 86, 311–319.

Zhou, L., Zhang, Q., Stein, C., Schäfer, M., 1998. Contribution of opioid receptors on primary afferent versus sympathetic neurons to peripheral opioid analgesia. J. Pharmacol. Exp. Ther. 286, 1000–1006.