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Analgesic effects of loperamide in bone cancer pain in mice

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Abstract

The intratibial inoculation of NCTC 2472 cells induces an osteosarcoma in C3H/HeJ mice. These mice show thermal hyperalgesic responses which may be blocked by the local administration of opiates over the tibial tumoral mass (Menèndez L, Lastra A, Hidalgo A, Meana A, Garcia E, Baamonde A. Peripheral opioids act as analgesics in bone cancer pain in mice. NeuroReport 2003b;14:867–9). The aim of this report was to characterize the analgesic responses obtained by activating peripheral opioid receptors in bone cancer pain. Here, we initially describe that this osteosarcoma induces mechanical as well as thermal hyperalgesia. Loperamide, an opioid agonist unable to cross the blood–brain barrier, inhibits both thermal and mechanical hyperalgesia when s.c. injected, locally over the tibial tumoral mass (7.5–75 μg) or distantly, under the fur of the neck (4 mg/kg). These analgesic effects seem peripherally mediated since they are reverted by the administration of naloxone methiodide (10 mg/kg) and because the withdrawal latencies of the contralateral, non-affected, paws remain unaltered. Furthermore, only cyprodime (1 mg/kg) but not naltrindole (0.1 mg/kg) or nor-binaltorphimine (10 mg/kg) blocked these effects, showing the involvement of μ-opioid receptors in the peripheral analgesia induced by loperamide on thermal and mechanical hyperalgesia. The advantages of using peripheral acting opiates – devoid of central colateral effects – for the treatment of cancer related pain are suggested.

Keywords: Cancer pain; Osteosarcoma; Loperamide; Peripheral opioid receptors

1. Introduction

The management of pain induced by the presence of a neoplastic malignancy is a cause of concern since available therapies, mainly based on the use of opiate drugs, do not always succeed in relieving this complication (Cleeland et al., 1994). Since bone neoplastic states are the most common situation leading to cancer-related pain (Brescia et al., 1990), a considerable effort has been addressed towards the experimental study of bone cancer pain in the past few years. In this sense, the development of experimental models of cancer pain in mice and rats has considerably broadened the knowledge of the neurochemical and behavioral events underlying this type of pain.

Initially, Schwei et al. (1999) proposed a model based on the inoculation of osteolytic fibrosarcoma (NCTC 2472) cells into the femur of C3H/HeJ mice yielding a painful osteosarcoma. The description of pain reactivity induced by the presence of this osteosarcoma together with the immunohistochemical modifications of several spinal neurotransmitter systems involved in nociception demonstrated that cancer-induced pain is a unique entity different from other modalities of chronic pain, such as inflammatory or neuropathic ones (Honore et al., 2000; Luger et al., 2002). After the initial inoculation of NCTC 2472 into the femur (Schwei et al., 1999), these fibrosarcoma cells have been further inoculated into different bones such as the calcaneous (Wacnik et al., 2001), the humerus (Wacnik et al., 2003) and the tibia (Menéndez et al., 2003a). In all such cases, a primary bone tumor (osteosarcoma) is developed in mice. In addition, another experimental approach based on the intratibial inoculation of mammary adenocarcinoma

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metastasic cells (MRMT-1) in rats was described (Medhurst et al., 2002). The development of bone tumor after the inoculation of these MRMT-1 cells in rats induces mechanical hyperalgesia (Medhurst et al., 2002) as well as sensitization of spinal nociceptive neurons detected by electrophysiological studies (Urch et al., 2003; Donovan-Rodriguez et al., 2004).

The availability of these models has favoured the assessment of the analgesic efficacy rendered by the administration of different drugs such as opiates (Luger et al., 2002; Menéndez et al., 2003a,b), cannabinoids (Kehl et al., 2003), biphosphonates (Walker et al., 2002), COX-2 inhibitors (Sabino et al., 2002; Fox et al., 2004) and endothelin type A receptor antagonists (Peters et al., 2004).

A particular aspect related to opiates has been the demonstration that the local administration of loperamide or of low doses of morphine can inhibit the thermal hyperalgesia induced by intratibial NCTC fibrosarcoma cell inoculation, these effects being prevented by the administration of the peripheral opioid antagonist naloxone methiodide (Menéndez et al., 2003b). These results suggest the possibility that bone cancer-induced hyperalgesia could be alleviated by opiates without producing any effect on the central nervous system. Therefore, if analgesia could be attained by stimulating peripheral opioid receptors, many of the centrally mediated opiate side effects, such as sedation, respiratory depression or drug dependence, should be completely absent. The studies performed on painful inflammatory models have been the initial ones to demonstrate that analgesia could be achieved by the stimulation of peripheral opioid receptors, and constitute the experimental core advocating the use of peripheral acting opiate agonists for analgesic purposes (DeHaven-Hudkins et al., 1999; Stein et al., 2003). In fact, the up-regulation of opioid receptors in peripheral nociceptors has been demonstrated in these inflammatory models (Stein, 1991; Zollner et al., 2003). More recently, peripheral opioid analgesia has also been reported in experimental neuropathic pain (Truong et al., 2003).

In this report, we try to characterize the analgesic properties of loperamide, an opiate agonist which does not cross the blood–brain barrier (Wuster and Herz, 1978; Schinkel et al., 1996), on bone cancer-induced pain. Thus, based on the previous description of the inhibitory effects exerted by local loperamide on the thermal hyperalgesia induced by the intratibial inoculation of NCTC 2472 cells (Menéndez et al., 2003b), the particular objectives of the present study were (1) to elucidate whether this type of osteosarcoma could also induce mechanical hyperalgesia aiming to test if locally administered loperamide could inhibit not only thermal, but also mechanical bone cancer-induced hyperalgesia; (2) to assess if the systemic (not local) administration of loperamide could also induce antihyperalgesic responses through the activation of peripheral opioid receptors; and (3) to characterize the type of opioid receptor (μ-, δ-, or κ-opioid receptors) involved in the antihyperalgesic actions of loperamide on bone cancer-induced hyperalgesia.

2. Material and methods

2.1. Animals

Experiments were performed in 5- to 6-week old (28–30 g weight) C3H/HeJ mice (CRIFFA, Spain) maintained in the Animalario of the Universidad de Oviedo (Reg. 33044 13A) with water and food ad libitum. All the experimental procedures were approved by the Comité Ético de Experimentación Animal de la Universidad de Oviedo (Asturias, Spain).

2.2. Drugs

Loperamide hydrochloride (Sigma) was dissolved in 1% DMSO and administered either locally or systemically. The local administration of loperamide was made by the subcutaneous injection of 0.2 ml of loperamide over the tibial tumoral mass. For systemic administration of loperamide, a subcutaneous injection was made under the fur of the neck. Naloxone methiodide (Sigma) was solved in saline and injected subcutaneously into the neck 15 min before testing. Cyprodime hydrobromide (Sigma), naltrindole hydrochoride (Tocris) and nor-binaltorphimine dihydrochloride (Tocris) were solved in saline and injected subcutaneously into the neck 30 min before testing. Xylacine (Rompun®) and Ketamine (Imalgene®) were diluted in saline and i.p. injected. The systemic administration of drugs was done in a final volume of 10 ml/kg.

2.3. Cell culture and implantation

NCTC 2472 cells (American Type Culture Collection, ATCC) were cultured in NCTC 135 medium (Sigma) containing 10% horse sera (Sigma) and passaged weekly according to ATCC guidelines. For their administration, cells were detached by scraping and then centrifuged at 1400 rpm. The pellet was suspended in PBS (10^6 cells/ 200 μl) and then used for intratibial injections.

For implantation, animals were anesthetized with a mixture of xylazine (10 mg/kg) and ketamine (90 mg/kg) i.p. injected. The right knee of mice was bent and placed facing the experimenter and a minimal skin incision was made, exposing the tibial plateau. A 25-gauge needle was used to perforate the tibial plateau and, once removed, another needle (30 gauge) coupled to a Hamilton syringe filled with cell suspension was carefully introduced into the medullary cavity of the tibia. Finally, 10^5 NCTC 2472 cells suspended in 20 μl of PBS were slowly injected. Control groups were injected with 20 μl of PBS containing 10^5 NCTC 2472 cells killed by quickly freezing and thawing them twice without cryoprotection. The surgical procedure was completed with a stitch of the knee skin.

2.4. Unilateral hot plate (UHP) test

As previously described (Menéndez et al., 2002), mice were gently restrained and the plantar side of the tested paw was placed on the hot plate surface (53 ± 1 °C). The latency for paw withdrawal from the heated surface was manually recorded with a chronometer. The measurements of the withdrawal latencies of each hindpaw were made separately and alternately at 2-min intervals and the mean of two measures made in each hindpaw was considered. Those animals showing basal latencies equal to or higher than 20 s were discarded. A cut-off of 30 s was established in order to prevent tissue damage.

2.5. Paw pressure test

Mechanical hyperalgesia was assessed by a modification of the Randall-Selitto procedure inspired by the method described by Ferreira et al. (1988), where a constant pressure stimulus is applied. Mice were gently restrained and a pressure of 450 g was applied to their hindpaws with a Ugo Basile 7200 apparatus, until a struggle reaction appears. At this moment, the noxious stimulus was stopped and the latency in seconds was manually recorded with a chronometer. The measurements of the withdrawal latencies of each hindpaw were made separately and alternately at 2-min intervals and the mean of two measures made in each hindpaw was considered. A 60-s cut-off was established in order to prevent tissue damage.

2.6. Statistical analysis

The mean values and the corresponding standard errors were calculated for each behavioural assay. Statistical analysis was carried out using an initial two-way analysis of variance (ANOVA) when both treatment and time act as variables (the temporal course of mechanical hyperalgesia or the time course of the effects induced after the administration of loperamide) or a one-way ANOVA, which were followed by the Dunnett's t test in the dose–effect curves or by the Newman–Keuls when intergroup differences were calculated (the time course of the development of mechanical hyperalgesia or the effect induced by naloxone–methiodide). The comparisons between two groups only (the time courses of the effects induced by loperamide) were made by the Student's t test for unpaired data. Statistical significance was considered at $P<0.05$.

3. Results

3.1. Dose- and time-dependent effects of local loperamide (7.5–75 μg) on osteosarcoma-induced thermal hyperalgesia

At week 4 after intratibial NCTC cell inoculation, the measure of UHP latencies in both hindpaws revealed the instauration of thermal hyperalgesia in the paw affected by the osteosarcoma (Fig. 1A). Thirty minutes after the subcutaneous administration of loperamide (7.5–75 μg) over the tibial tumoral mass, the hyperalgesic responses were abolished in a dose-related way ($F_{4,51}=74.34$; $P<0.01$), without any modification on the latencies of the contralateral paws. The highest dose used (75 μg) did not only prevent the hyperalgesia, but also evoked an intense analgesic effect restricted to the injected paw (Fig. 1A). The time course of the effect induced after the administration of 75 μg of loperamide or solvent show a treatment- and time-dependent significant interaction ($F_{4,51}=27.05$; $P<0.01$). Loperamide produced analgesia at 0.5, 1 and 2 h after its injection (Fig. 1B), the peak effect being reached 30 min after its administration.

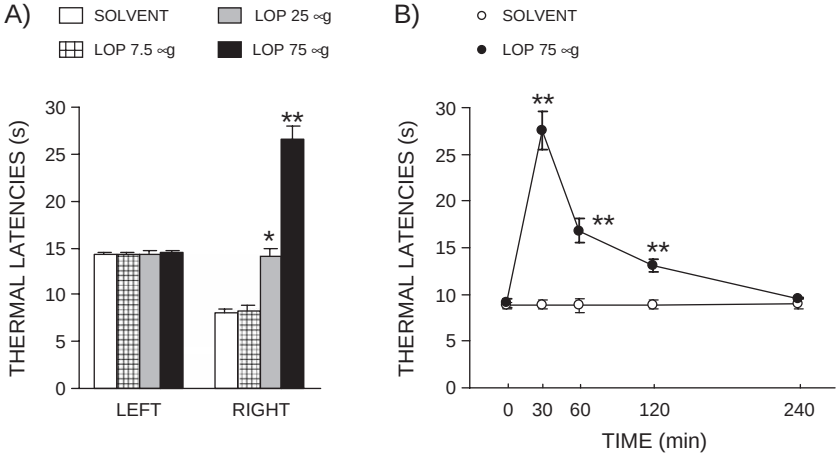

Fig. 1. Effects of local loperamide on the hindpaw thermal withdrawal latencies in CH3/HeJ mice inoculated 4 weeks before in their right tibiae with NCTC 2472 cells. Loperamide (LOP) or solvent (1% DMSO) were injected s.c. over the peritumoral region of the implanted limb. In panel (A), dose–effect curve of loperamide (7.5–75 μg, 30 min before testing) and in panel (B), time course of the local loperamide analgesic effects (75 μg). The means and their corresponding S.E. are represented. *$P<0.05$; **$P<0.01$, Dunnett's t test (A) or ** $P<0.01$, Student's t test (B), compared with solvent.

3.2. Dose- and time-dependent effects of local loperamide (7.5–75 μg) on osteosarcoma-induced mechanical hyperalgesia. Reversion by naloxone methiodide

Since no previous data related to the development of mechanical hyperalgesia have been reported after the implantation of NCTC 2472 cells intratibially, we assessed the paw pressure mechanical nociceptive thresholds before and at weeks 1, 2, 3 and 4 after NCTC 2472 cell intratibial inoculation. As shown in Fig. 2A, the intratibial implantation of the tumoral cells induced the onset of mechanical hyperalgesia in the inoculated paw 3 weeks after NCTC cell implantation. A significant interaction was observed between treatment (killed or live cells) and time ($F_{4,02}$= 11.9; $P<0.01$). When loperamide (7.5–75 μg) was locally administered at week 4, the hyperalgesia detected in the osteosarcoma-bearing paw was inhibited in a dose-related way ($F_{5,29}=35.96$; $P<0.01$), whilst the mechanical withdrawal latencies of the contralateral hindpaws remained unaffected (Fig. 2B). Also, the administration of 75 μg of loperamide induces an analgesic effect that was treatment- and time-dependent ($F_{4,7}=264.9$; $P<0.01$). Analgesia was significant 0.5, 1 and 2 h after its injection (Fig. 2C), the peak effect being reached 30 min after its administration.

The systemic administration of 10 mg/kg of naloxone–methiodide completely prevented the analgesic effect induced by the peritumoral administration of 75 μg of loperamide on osteosarcoma-induced mechanical hyperalgesia ($F_{4,82}=60.15$; $P<0.01$) (Fig. 2D), as occurred when thermal noxious stimuli were tested (Menéndez et al., 2003b).

3.3. Effects of the systemic (s.c.) administration of loperamide (4 mg/kg) on thermal and mechanical osteosarcoma-induced hyperalgesia

In order to study whether a systemic administration of loperamide distant from the tumoral region could also prevent osteosarcoma-induced hyperalgesia, the effects of 4 mg/kg of loperamide (which correspond to 120 μg per mouse), subcutaneously administered under the fur of the neck were tested. The administration of this dose of loperamide did not modify either the thermal (Fig. 3A) or the mechanical (Fig. 3C) withdrawal latencies in control mice implanted with killed NCTC cells. In contrast, when

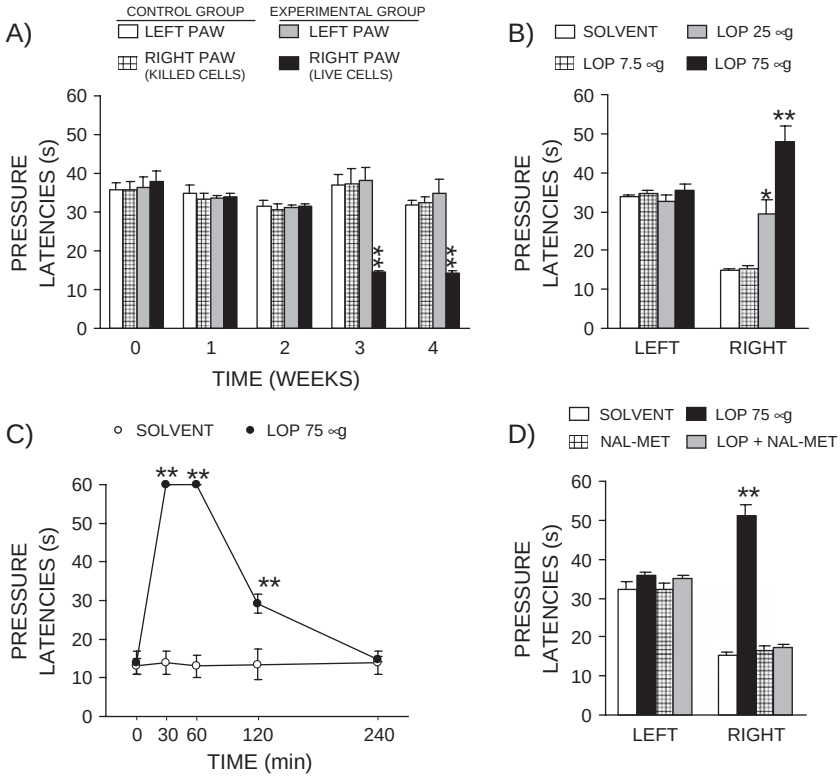

Fig. 2. Effects of local loperamide on the hindpaw mechanical withdrawal latencies in CH3/HeJ mice inoculated in their right tibiae with NCTC 2472 cells. In panel (A), time course of the withdrawal latencies obtained in mice inoculated with either live (experimental group) or killed (control group) cells. In panels (B), (C) and (D), loperamide (LOP) or solvent (1% DMSO) were injected s.c. over the peritumoral region of the implanted limb in mice injected 4 weeks before with NCTC 2472 cells. In panel (B), dose–effect curve of loperamide (7.5–75 μg, 30 min before); in panel (C), time course of the local loperamide analgesic effects (75 μg); in panel (D), effects of loperamide (75 μg, 30 min before) in the presence of naloxone methiodide (10 mg/kg; i.p, 15 min before) (NAL-MET). The means and their corresponding S.E. are represented. $^{**}P<0.01$, Newman–Keuls test, compared with control group (A) or solvent (D). $^{*}P<0.05$; $^{**}P<0.01$, Dunnett's t test (B) or $^{**}P<0.01$, Student's t test (C) compared with solvent.

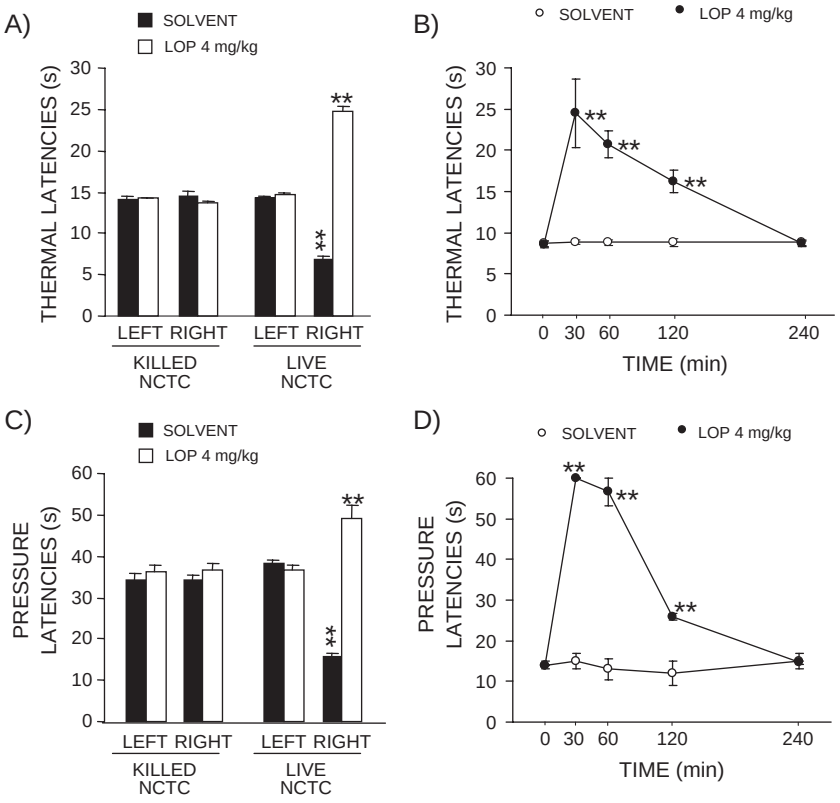

Fig. 3. Effects of systemic loperamide on the hindpaw thermal (A, B) and mechanical withdrawal latencies (C, D) in CH3/HeJ mice inoculated 4 weeks before in their right tibiae with NCTC 2472 cells. Loperamide (4 mg/kg, LOP) or solvent (1% DMSO) were injected subcutaneously under the fur of the neck and tested 30 min after, in mice implanted with live or killed NCTC 2472 cells (A, C) or at different times in mice implanted with live NCTC 2472 cells (B, D). The means and their corresponding S.E. are represented. $^{**}P<0.01$, Newman–Keuls test (A, C), $^{**}P<0.01$, Student's t test (B, D), compared with solvent.

administered to osteosarcoma-bearing animals, this dose of loperamide completely suppressed both thermal and mechanical hyperalgesia, and in fact yielded higher latency values than those observed in control mice. The contralateral withdrawal latencies remained unaltered. The time courses of the effects observed when 4 mg/kg of loperamide

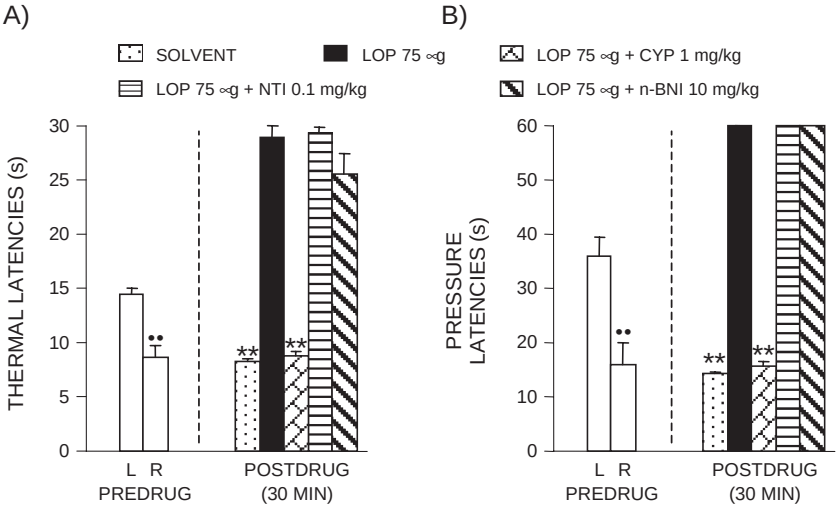

Fig. 4. Effects of the systemic (s.c.) administration of selective opioid antagonists on the analgesic effect induced by the local injection of loperamide on thermal (A) or mechanical (B) withdrawal latencies measured in animals intratibially implanted with NCTC 2472 cells 4 weeks before. Basal (predrug) measures of the implanted (right, R) and unimplanted (left, L) limbs are represented in the left hand side of both graphs. Loperamide (75 μg, 30 min before) (LOP) or solvent (1% DMSO) were injected s.c. over the peritumoral region of the implanted limb and tested alone or in the presence of cyprodime (CYP, 1 mg/kg), naltrindole (NTI, 0.1 mg/kg) or nor-binaltorphimine (n-BNI, 10 mg/kg,) s.c. injected 30 min before. The means and their corresponding S.E. are represented. $^{\bullet\bullet}P<0.01$, Student's t test when comparing paw latencies of right (implanted) and left (unimplanted) limbs, $^{**}P<0.01$, Newman–Keuls test, compared with LOP-treated mice.

or solvent were systemically administered show a treatment- and time-dependent significant interaction for either thermal ($F_{4,51}=14.58$; $P<0.01$) or mechanical hyperalgesia ($F_{4,51}=152.9$; $P<0.01$). In both cases, the analgesic effects lasted for, at least, 2 h (Fig. 3B and D).

3.4. Effects of systemic cyprodime (1 mg/kg), naltrindole (0.1 mg/kg) and nor-binaltorphimine (10 mg/kg) on the analgesic effect of local loperamide (75 μg) on thermal and mechanical osteosarcoma-induced hyperalgesia

Loperamide (75 μg) was locally administered together with a subcutaneous (under the fur of the neck) administration of either solvent, cyprodime (μ-opioid receptor selective antagonist), naltrindole (δ-opioid receptor selective antagonist) or nor-binaltorphimine (κ-opioid receptor selective antagonist) and their effects on thermal and mechanical osteosarcoma-induced hyperalgesia were tested 30 min later. As shown in Fig. 4A, the thermal latencies were strongly increased in the group treated with loperamide alone, this effect being completely prevented by the coadministration of 1 mg/kg of the μ-opioid receptor antagonist cyprodime, and completely unaffected by the coadministration of either naltrindole (0.1 mg/kg) or nor-binaltorphimine (10 mg/kg) ($F_{4,31}=78.47$; $P<0.01$). A similar result was obtained when mechanical hyperalgesia was assessed, the analgesic effect of loperamide only being reversed by cyprodime ($F_{4,67}=3386.8$; $P<0.01$) (Fig. 4B). Cyprodime, naltrindole or nor-binaltorphimine had no effect by themselves on the withdrawal latencies (data not shown).

4. Discussion

As stated in the introductory section, the starting point of these experiments is a previous report describing that the local administration of morphine or loperamide can prevent the thermal hyperalgesia produced by the development of a murine osteosarcoma (Menéndez et al., 2003b). The present data offer additional information about the effective range of doses of locally applied loperamide able to produce this thermal antihyperalgesic effect and show that this opioid agonist can prevent the mechanical hyperalgesia induced by the osteosarcoma. This inhibition of mechanical sensitization occurs in the same range of doses as those necessary to inhibit thermal hyperalgesia, and is also reverted by the administration of the peripheral antagonist naloxone methiodide at a dose high enough to block μ-, δ- and κ-opioid receptors, but devoid of central effects (Lewanowitsch and Irvine, 2002). Furthermore, these antihyperalgesic effects can be achieved after the systemic administration of the drug, evoking the activation of μ-, but not δ- or κ-opioid receptors, which is responsible for the analgesic effects of loperamide on bone cancer-induced pain.

Initially, the present results show that the intratibial implantation of NCTC 2472 cells induces mechanical hyperalgesia, measured by a paw-pressure test, 3 weeks after their inoculation. This is in accordance with the mechanical hyperalgesia observed 3 weeks after the inoculation of NCTC 2472 cells into the femur, as assessed by the direct palpation of the region affected by the tumor (Schwei et al., 1999). Although a previous electrophysiological study describes that the implantation of NCTC 2472 cells into and around the calcaneous elicits the sensitization of nociceptors to thermal, but not mechanical, stimuli (Cain et al., 2001), it may be considered that the putative sensitization of spinal neurons due to the tumor-evoked spontaneous activity of nociceptors could be responsible for the establishment of mechanical hyperalgesia.

In any case, we observed that, when fibrosarcoma tumoral cells are implanted inside the medullary cavity of the tibia, both mechanical and thermal hyperalgesia develop although the former appears 1 week before. Several facts could help to explain the delay between the instauration of mechanical and thermal hyperalgesia. For instance, since the peripheral nociceptors involved in the transmission of noxious heat or pressure are different (Julius and Basbaum, 2001; Viana et al., 2002; Woolf et al., 2004), it seems likely that their sensitization could be differently modulated. Furthermore, the mechanisms leading to the instauration of thermal and mechanical hyperalgesia in the spinal cord depend on different mediators (Meller and Gebhart, 1994) that could come into play separately. When these facts are borne in mind, it can be understood that the time needed for the development of both types of hyperalgesia may not be necessarily the same. In fact, it has even been described that a unique pathological process may lead to the production of only one type of hyperalgesia, but not to the other (Hou et al., 2003).

The local administration of loperamide over the tibial tumoral mass antagonized in a dose-related way the decrease of both thermal and mechanical latencies measured in the paws of the tumor-bearing limbs. The effective doses of loperamide, the time required to obtain the peak effect and the duration of such were rather similar in inhibiting either mechanical or thermal hyperalgesia. In both cases, loperamide exhibited a considerable analgesic efficacy so that its administration not only restored the normal thermal and mechanical latencies, but even evoked an increase of the withdrawal latencies above the normal ones for at least 1 h. Since the withdrawal thresholds of the contralateral paws remained unaltered and the peripherally acting opioid receptor antagonist, naloxone methiodide, inhibits the analgesia induced by loperamide on thermal (Menéndez et al., 2003b) and mechanical hyperalgesia, these analgesic effects seem to be peripherally mediated.

Previous reports describe that the presence of this type of neoplastic process in bone can lead to important neurochemical changes at the spinal level, such as the dynorphin or Fos expression or the hypertrophia of spinal glial cells (Schwei et al., 1999; Honore et al., 2000). Furthermore, in a similar model of bone cancer in rats, the sensitization of

spinal nociceptive neurons has been reported (Urch et al., 2003; Donovan-Rodriguez et al., 2004). In this context, the present data seem to indicate that, apart from the possible inhibition of these spinal alterations, the peripheral inhibition of nociceptor excitability may also constitute an effective strategy to alleviate some hyperalgesic responses due to the presence of tumoral cells in bone.

It should be remarked that loperamide shows similar efficacy and potency to inhibit both thermal and mechanical hyperalgesia since, as stated above, both types of hyperalgesia may depend on different mechanisms, a particular analgesic drug can show a preferent ability to inhibit one of these processes. For example, the peripheral administration of indomethacin selectively inhibits the development of thermal – but not mechanical – zymosan-induced hyperalgesia (Turnbach and Randich, 2001), whereas the blockade of endothelin type B receptors selectively prevents mechanical inflammatory hyperalgesia, without affecting thermal sensitization (Baamonde et al., 2004). Thus, our results seem to indicate that the stimulation of peripheral opioid receptors could lead to a broad analgesic effect on bone cancer-induced pain.

We further explored the ability of loperamide to prevent both types of tumoral hyperalgesia, when systemically administered. This point could be particularly interesting if the affected bone were located at a site difficult to reach for local injections (i.e., a vertebral body) or when the osteoclastic process simultaneously appears at several different locations. In our model, a distant s.c. administration of loperamide under the fur of the neck was able to completely prevent either thermal or mechanical hyperalgesia induced by the osteosarcoma in the hindpaw. Once again, the fact that the analgesic effect was restricted to the affected limb strongly suggests that this analgesic effect should be peripherally mediated and indicates that the biochemical modifications by which loperamide can induce its analgesic effects are probably localised in the body region affected by the osteosarcoma. In any case, this result demonstrates that, in order prevent the osteosarcoma-induced hyperalgesia, the local administration of loperamide is not necessary, but local concentrations able to produce the same effect can be achieved after the systemic administration of the drug.

Finally, loperamide shows the highest affinity for μ-opioid receptors, being its K_D at δ- and κ-opioid receptors 10 and 300 times higher than that at μ-opioid receptors, respectively (DeHaven-Hudkins et al., 1999). Therefore, a functional "in vivo" binding to δ- and κ-opioid receptors cannot be discarded. In order to elucidate the type of opioid receptor involved in the peripheral analgesic effects of loperamide on bone cancer-induced pain, this agonist was coadministered with selective μ-, δ- and κ-opioid receptor type antagonists (cyprodime, naltrindole and nor-binaltorphimine, respectively). The doses of these antagonists were selected from previous papers (Mendes et al., 2000; Baamonde et al., 1991; McLaughlin et al., 2003) searching the selective blockade of μ-, δ- and κ-opioid receptors, respectively. Since the selective μ-opioid receptor antagonist, cyprodime, but not the δ-selective antagonist, naltrindole, nor the κ-selective antagonist, nor-binaltorphimine, was able to antagonize the analgesic effect of loperamide on both thermal and mechanical osteosarcoma-induced hyperalgesia, it can be concluded that these analgesic effects are selectively mediated through μ-opioid receptors. Furthermore, this result also strongly suggests that μ-opioid receptors are overexpressed at the peripheral level in response to the presence of the osteosarcoma. In any case, since – as shown above – loperamide exerted its analgesic effects through the activation of μ-opioid receptors exclusively, a putative overexpression of δ- and κ-opioid receptors should not be ruled out. Indeed, in inflammatory diseases both the peripheral expression of μ-, δ- and κ-opioid receptors (Stein et al., 1989) and the ability of their respective agonists to induce analgesia (Ji et al., 1995) have been described.

Overall, the present results demonstrate that loperamide can prevent thermal and mechanical osteosarcoma-induced hyperalgesia, by acting through a peripheral population of μ-opioid receptors probably up-regulated during the development of this pathology. Since a similar efficacy can be attained after either local or systemic administration of the drug, our results support that the systemic administration of peripherally acting μ-opioid receptor agonists, such as loperamide, could be a suitable approach for the management of some types of bone cancer-induced pain.

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