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Inhibition of osteosarcoma-induced thermal hyperalgesia in mice by the orally active dual enkephalinase inhibitor PL37. Potentiation by gabapentin

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ABSTRACT

We have previously shown that stimulation of peripheral opioid receptors by exogenous opiates counteracts the thermal hyperalgesia elicited by a tibial osteosarcoma due to intraosteal inoculation of NCTC 2472 cells to mice. Aiming to study whether pheripheral endogenous enkephalins could also counteract this painful symptom, we assayed in this model the effects of PL37, an orally active dual inhibitor of enkephalin inactivating enzymes. Oral administration of PL37 (25 mg/kg) completely supressed osteosarcoma-induced thermal hyperalgesia through the activation of μ-opioid receptors, since the administration of cyprodime (1 mg/kg) inhibited its antihyperalgesic effect. Neither naltrindole (0.1 mg/kg) nor nor-binaltorphimine (10 mg/kg) modified this PL37-induced antihyperalgesic effect. Moreover, the inhibition of the antihyperalgesic effect induced by PL37 after the administration of naloxone-methiodide (2 mg/kg), a non selective opioid antagonist that does not cross the blood-brain barrier, demonstrates the involvement of peripheral opioid receptors. In contrast, centrally mediated effects may be detected when assaying a higher dose of PL37 (50 mg/kg). Besides, the administration of gabapentin (6.25–25 mg/kg, i.p.) dose-dependently inhibited osteosarcoma-induced thermal hyperalgesia. Interestingly, the combined administration of subeffective doses of PL37 and gabapentin completely prevented this type of thermal hyperalgesia. An isobolographic analysis of this interaction demonstrated a synergistic interaction between both drugs.

1. Introduction

The endogenous opioid peptides met- and leu-enkephalin are rapidly inactivated at the synaptic cleft by two membrane-bound zinc metallopeptidases, called neutral endopeptidase and aminopeptidase N (Roques, 2000). The development of inhibitors of these two inactivating ecto-enzymes designated dual inhibitors has constituted the cornerstone pharmacological tool to study the effects induced by endogenous enkephalins (reviews in Roques et al., 1993; Noble and Roques, 2007; Jutkiewicz, 2007). Thus, these inhibitors were shown to strongly increase the extracellular levels of enkephalins in various brain structures (Daugé et al., 1996) and their specific action on the catabolism of enkephalins is supported by their loss of effect in preproenkephalin knock-out mice (Noble et al., 2008). The development of dual inhibitors has advanced from highly hydrophilic compounds, such as kelatorphan, that do not readily cross the blood-brain barrier, to molecules able to enter the brain, such as RB101 or RB120 and, more recently, PL37, that shows good oral bioavailability (Noble and Roques, 2007). In experimental inflammatory situations, antinociceptive effects have been described in arthritic rats (Perrot et al., 1993) and also in models of unilateral inflammatory pain caused by intraplantar injection of Freund's complete adjuvant (Maldonado et al., 1994) or carrageenan (Le Guen et al., 1999). Regarding neuropathic pain, dual inhibitors were reported to suppress mechanical hyperalgesia produced in rats by a constriction of the sciatic nerve (Lee et al., 1994) or mechanical allodynia detected in diabetic rats (Coudoré-Civiale et al., 2001). Although no previous study described the effects of enkephalin-degrading inhibitors in models of cancer-induced pain, several reports have described the analgesic effect of opiates on the nociceptive symptoms that appear in C3H/HeJ mice inoculated intraosteally with NCTC 2472 cells (Honoré et al., 2000; Wacnik et al., 2003; Menéndez et al., 2003a). In addition, it has been shown that opioid receptor agonists acting at the periphery can effectively block some nociceptive signs associated to the presence of malignant cells in the tibia, such as thermal or mechanical hyperalgesia (Menéndez et al., 2003a,b, 2005, 2007). On the contrary, other nociceptive behaviors, such as mechanical allodynia (Baamonde et al., 2006; El Mouedden and Meert, 2007) or spontaneous lifting behavior (El Mouedden and Meert, 2007), are insensitive to this pharmacological approach. In the present study, we

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aimed to explore whether one of the osteosarcoma-evoked nociceptive symptoms sensitive to the stimulation of peripheral opioid receptors (thermal hyperalgesia) may be inhibited by locally released endogenous enkephalins. To achieve this goal, we tested the antihyperalgesic efficacy of PL37, a dual inhibitor of enkephalin inactivating enzymes suitable for oral administration, in C3H/HeJ mice intratibially inoculated with NCTC 2472 cells.

Furthermore, we assessed the possibility of potentiating the PL37 effects by acute coadministration of gabapentin, an anticonvulsivant drug with analgesic properties whose ability to enhance the effects induced by opiates in different painful situations, including cancer pain (Caraceni et al., 1999, 2004) has been extensively documented.

Thus, we investigated the effect of PL37 on osteosarcoma-induced thermal hyperalgesia and the contribution of peripheral μ-, δ-, and κ-opioid receptors by using selective antagonists and naloxone-methiodide, a non specific compound acting almost exclusively at the periphery. Furthermore, we investigated the effect of combinations of gabapentin and PL37 on this osteosarcoma-induced thermal hyperalgesia.

2. Methods

2.1. Animals

The experiments were performed in 5–6 week old (26–33 g weight) C3H/HeJ mice bred in the Animalario de la Universidad de Oviedo (Reg. 33044 13A), housed six per cage and maintained on a 12-h dark–light cycle with free access to food and water. All the experimental procedures were approved by the Comité Ético de Experimentación Animal de la Universidad de Oviedo (Asturias, Spain). Each animal was used only once.

2.2. Drugs

PL37, 1-2-[(1-ethoxy-carbonyloxy-ethoxycarbonylmethyl)-carbamoyl]-3-phenyl-propyldisulfanylmethyl-3-methylsulfanyl-propyl-ammonium (Patent: EP 2006/067711) was dissolved in a mixture of ethanol and polyethylene glycol 400 in distilled water (1/4/5) and administered by the oral route. Its effects were measured at several times after administration for time course studies or 20 min after in the rest of experiments. Naloxone hydrochloride and naloxone-methiodide (Sigma) were dissolved in saline and administered 20 min before testing and cyprodime hydrobromide (Sigma), naltrindole hydrochloride (Tocris) and nor-binaltorphimine dihydrochloride (Tocris) were solved in saline and administered 30 min before testing. Gabapentin, dissolved in saline, was administered intraperitoneally at several times before testing for kinetic studies and 60 min before in the resting ones. Xylazine (Rompun®) and ketamine (Imalgene®) were diluted in distilled water.

2.3. Cell culture

NCTC 2472 cells (ATCC) were cultured in NCTC 135 medium (Sigma) with 10% horse serum (Sigma). For their administration, cells were detached by scraping and then centrifuged at 400 *g*. The pellet was suspended in phosphate buffer saline in a concentration of 10^5 cells in 5 μl. For cell implantation, animals were anesthetized with a mixture of xylazine (10 mg/kg) and ketamine (90 mg/kg). 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 syringue filled with 5 μl of cell suspension was carefully introduced into the medullary cavity of the tibia. Finally, the application of acrylic glue (Hystoacril®, Braun) and a stitch of the knee skin completed the surgical procedure. Control groups were injected with 5 μl of PBS containing 10^5 NCTC 2472 cells killed by quickly freezing and thawing them twice without cryoprotection. Mice were studied 4 weeks after the intratibial inoculation of tumoral cells.

2.4. Unilateral hot plate test

Mice were gently restrained and the plantar side of the tested paw was placed on the hot plate surface (51 ± 1 °C) (Menéndez et al., 2002). The latency for paw withdrawal from the heated surface was recorded. The measurements of the withdrawal latencies of each hindpaw were made separately at 2 min intervals and the mean of two measures was considered. A cut-off of 30 s was established in order to prevent tissue damage.

2.5. Data analysis

The mean values and the corresponding standard errors were calculated for each assay. Intergroup comparisons were made by the Student's *t* test when only two groups were compared. The comparisons among several groups were done by using an initial one-way analysis of variance (ANOVA) followed by either the Dunnett's *t* test when groups received different doses of a drug or by the Newman–Keuls test when groups received different drug treatments. In all cases statistical significance was considered at $P<0.05$.

In order to evaluate the possible interaction between PL37 and gabapentin in the unilateral hot plate test, we performed an isobolographic analysis following the method described by Tallarida (2001, 2002) by using the computer program Pharm Tools Pro (version 1.27, The McCary Group Inc.). With this aim the dose–response curves of the thermal antihyperalgesic effects induced by both drugs on their own were constructed and the ED_{50} ± standard error (S.E.M.) was calculated. In order to calculate the ED_{50} values, we considered that each mouse showed an antihyperalgesic effect when its latency in the tumor-bearing paw surpassed the 50% of the maximal effect. The maximal antihyperalgesic effect (100%) was achieved when the latencies obtained in the injured paws reached the mean value obtained in the right paws of the control group (treated with killed cells). Isobolographic analysis assumes that the combination of drugs is made from equipotent doses of the individual drugs. Thus, a dose–response relationship was obtained by concurrent delivery of the two drugs in a constant dose ratio (fixed ratio) based on the ED_{50} values of each individual agent. To construct these curves, different groups of animals received one of the following doses of the combination: (PL37 ED_{50} + gabapentin ED_{50})/2; (PL37 ED_{50} + gabapentin ED_{50})/3; (PL37 ED_{50} + gabapentin ED_{50})/4 and (PL37 ED_{50} + gabapentin ED_{50})/8. From the resulting dose–response curve of the combination, the experimental ED_{50} was obtained. To determine if the interaction between the two drugs given in combination was synergistic, the theoretical additive ED_{50} was estimated from the dose–response curves of each drug administered individually, i.e., considering that the observed effect with the combination results from the sum of the individual effects of each component. This theoretical ED_{50} value is next compared with the experimental ED_{50} to determine if there is a statistically significant difference, as evaluated by using a Student's *t* test (Tallarida, 2001). The theoretical and experimental ED_{50} values of the studied combinations were also compared by calculating the interaction index (γ) as follows: γ = experimental ED_{50} value / theoretical ED_{50} value, where values lower than 1 indicate a synergistic interaction (Tallarida, 2002).

3. Results

3.1. Effects induced by PL37 on osteosarcoma-induced thermal hyperalgesia

Osteosarcoma-induced thermal hyperalgesia measured by the unilateral hot plate test was inhibited by the oral administration of

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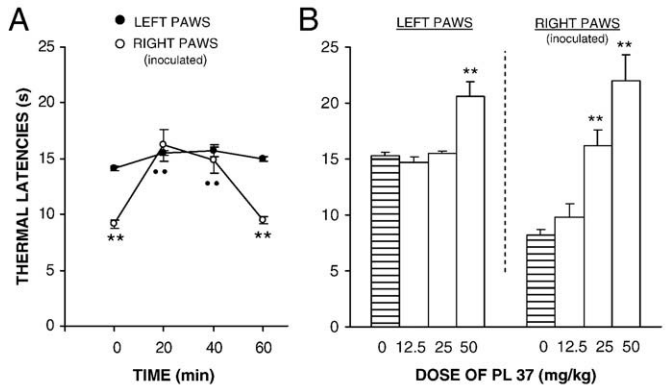

Fig. 1. A) Time course of the inhibition induced by PL37 (25 mg/kg; p.o.) on osteosarcoma-induced thermal hyperalgesia measured in the unilateral hot plate test in mice. Means and their corresponding S.E.M. are represented (n=6 per group). $^{**}P<0.01$, compared with the contralateral paw measured at the same time, Student's t test. ●●$P<0.01$, compared with the basal value (time 0) of the same paw, Dunnett's t test. B) Effect induced by different doses of PL37 (12.5–50 mg/kg; p.o.) on thermal latencies measured by the unilateral hot plate test in both paws of mice inoculated in their right paw with NCTC 2472 cells. Means and their corresponding S.E.M. are represented (n=5–6 per group). $^{**}P<0.01$, compared with solvent-treated paws, Dunnett's t test.

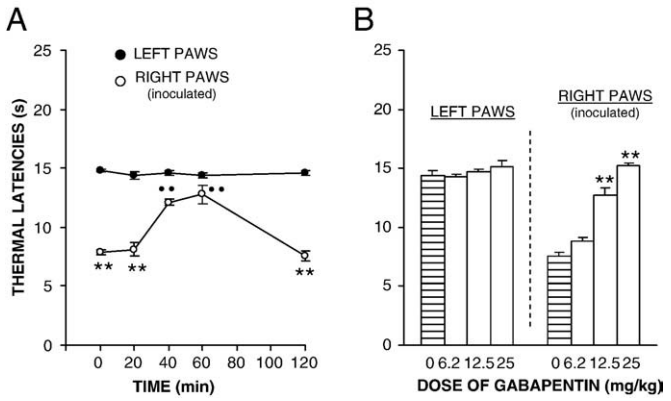

Fig. 3. A) Time course of the effect of gabapentin (25 mg/kg; i.p.) on osteosarcoma-induced thermal hyperalgesia measured in the unilateral hot plate test in mice. Means and their corresponding S.E.M. are represented. $^{**}P<0.01$, compared with the contralateral paw measured at the same time, Student's t test. ●●$P<0.01$, compared with the basal value (time 0) of the same paw, Dunnett's t test. B) Effect induced by different doses of gabapentin (6.25–25 mg/kg; i.p) on thermal latencies measured in the unilateral hot plate test in both paws of mice inoculated in their right paw with NCTC 2472 cells. Means and their corresponding S.E.M. are represented (n=6–7 per group). $^{**}P<0.01$, compared with solvent-treated paws, Dunnett's t test.

PL37 (25 mg/kg). This inhibitory effect peaked 20–40 min after PL37 administration, disappearing at time 60 (Fig. 1A). A dose–response curve was constructed 20 min after its administration, showing that the administration of 25 mg/kg of PL37 exclusively increases the latencies measured in the inoculated paws, previously shortened by the development of the osteosarcoma, whereas the administration of 50 mg/kg produced a prolongation of the latencies in both affected and unaffected paws (Fig. 1B).

3.2. Effects induced by opioid receptor antagonists on the inhibition of osteosarcoma-induced thermal hyperalgesia by PL37

The antihyperalgesic effect induced by 25 mg/kg of PL37 (p.o., 20 min before testing) in the unilateral hot plate test was completely inhibited by the administration of naloxone-methiodide (2 mg/kg, i.p., 20 min before testing, Fig. 2A). In contrast, although the bilateral analgesic effect induced by 50 mg/kg of PL37 was completely reverted by naloxone hydrochloride (2 mg/kg, i.p., data not shown), they were only partially reduced in both paws after the administration of 2 mg/kg of naloxone-methiodide (Fig. 2B).

The administration of the selective μ-opioid receptor antagonist cyprodime (1 mg/kg; s.c., 30 min before testing) completely prevented the antihyperalgesic effect induced by 25 mg/kg of PL37, while the δ-opioid receptor antagonist naltrindole (0.1 mg/kg; s.c., 30 min before testing) or the κ-opioid receptor antagonist nor-binaltorphimine (10 mg/kg; s.c., 30 min before testing) did not modify the effect induced by PL37 (Fig. 2C). The administration of opioid receptor antagonists alone did not modify thermal withdrawal latencies (data not shown).

3.3. Effects of gabapentin on osteosarcoma-induced thermal hyperalgesia

No modification of withdrawal latencies was detected 20 min after the i.p. administration of 25 mg/kg of gabapentin to osteosarcoma-bearing mice. In contrast, a clear antihyperalgesic effect was detected 40 and 60 min after the injection of this dose (Fig. 3A).

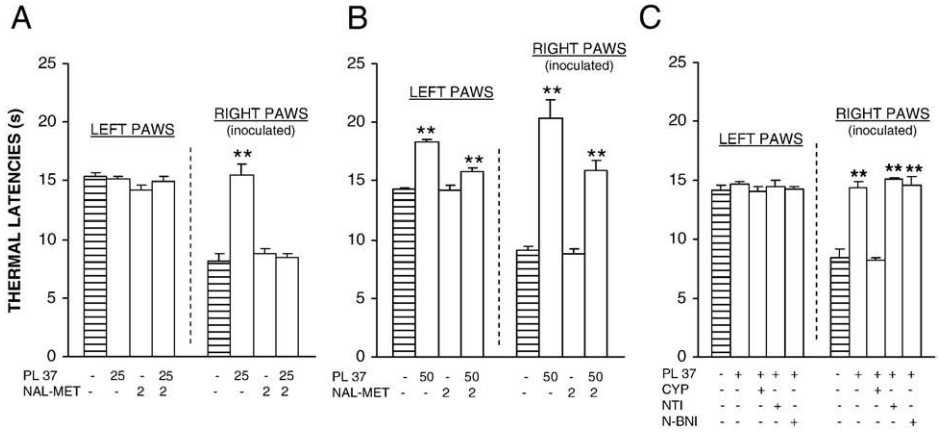

Fig. 2. A) Inhibition induced by 2 mg/kg of naloxone-methiodide (NAL-MET) of the antihyperalgesic effect evoked by 25 mg/kg of PL37 on osteosarcoma-induced thermal hyperalgesia measured in the unilateral hot plate test in mice. B) Partial inhibition induced by 2 mg/kg of naloxone-methiodide (NAL-MET) on the analgesic effect evoked by 50 mg/kg of PL37 in osteosarcoma-bearing mice. C) Effects induced by the administration of the selective μ- δ- and κ-opioid receptor antagonists cyprodime (CYP; 1 mg/kg), naltrindole (NTI; 0.1 mg/kg) and nor-binaltorphimine (n-BNI; 10 mg/kg) respectively, on the antihyperalgesic effect induced by 25 mg/kg of PL37 on osteosarcoma-induced thermal hyperalgesia. In all cases, means and their corresponding S.E.M. are represented (n=6–8 per group). $^{**}P<0.01$, compared with latencies obtained in the paws of solvent-treated mice, Newman–Keuls test.

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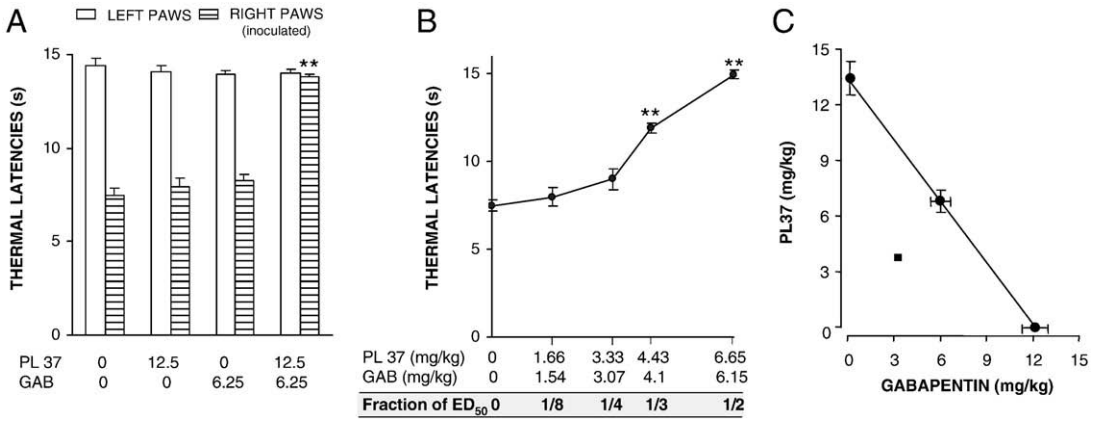

Fig. 4. A) Lack of effect of the administration of a subeffective dose of PL37 (12.5 mg/kg; p.o.; 20 min before testing) or gabapentin (6.25 mg/kg; i.p.; 60 min before testing) on the osteosarcoma-induced thermal hyperalgesia and antihyperalgesic effect observed when both drugs are administered in combination measured 20 min after the administration of PL37 and 60 min after gabapentin. Means and their corresponding S.E.M. are represented ($n=5$ per group). $^{**}P<0.01$, compared with the corresponding solvent-treated paws at every time, Newman–Keuls test. B) Antihyperalgesic effect induced in the right paws of osteosarcoma-bearing mice by the combined administration of PL37 (p.o.; 20 min before testing) and gabapentin (i.p.; 60 min before testing) at fixed doses that represent the 1/8, 1/4, 1/3 and 1/2 of their ED_{50} values. Means and their corresponding S.E.M. are represented ($n=6$ per group). $^{**}P<0.01$, compared with solvent-treated mice, Dunnett's t test. C) Isobologram showing the interaction between PL37 (p.o., 20 min before testing) and gabapentin (i.p., 60 min before testing) in the unilateral hot plate test. Horizontal and vertical bars indicate S.E.M. The oblique line between the x and y axes is the theoretical additive line. The point in the middle of this line is the theoretical additive point calculated from the individual drug ED_{50} values. The point below the line is the experimental ED_{50} value obtained with the combination.

Next, the antihyperalgesic effects induced by different doses of gabapentin were measured 60 min after i.p. injection. The dose of 6.25 mg/kg of gabapentin was subeffective, whereas the administration of 12.5 mg/kg produced a significant antihyperalgesic effect and a greater dose (25 mg/kg) completely suppressed the hyperalgesic effect induced by the osteosarcoma (Fig. 3B). None of these doses of gabapentin modified the withdrawal latencies of contralateral paws.

3.4. Potentiation of the antihyperalgesic effect induced by PL37 in osteosarcoma-bearing mice by the coadministration of gabapentin

According to the results shown above, the acute oral administration of PL37 (12.5 mg/kg, 20 min before testing) or of gabapentin (6.25 mg/kg; i.p., 60 min before testing) do not produce any antihyperalgesic effect when administered alone. In contrast, when mice received 12.5 mg/kg of PL37 (20 min before testing) plus 6.25 mg/kg of gabapentin (60 min before testing) osteosarcoma-induced thermal hyperalgesia was completely inhibited, being withdrawal latencies measured in osteosarcoma-bearing limbs undistinguishable from those obtained in the unaffected ones (Fig. 4A).

An isobolographic analysis was performed by studying the effects of the combination of different doses of both drugs. Initially, the ED_{50} value obtained for PL37 when administered 20 min before was 13.4 mg/kg p.o and that for gabapentin when administered 60 min before, 12.2 mg/kg i.p. (Table 1). Thus, the proportion of each drug in the total dose administered was 0.53 for PL37 and 0.47 for gabapentin.

Table 1
Effect of the administration of PL37 and gabapentin alone and in combination in the unilateral hot plate test

	ED_{50} (mg/kg)
PL37	13.4±0.9
Gabapentin	12.2±0.85
Theoretical combination	12.8±0.61
Experimental combination	7.03±1.1[a]
Interaction index	0.55±0.03

(ED_{50} values, in mg/kg).
ED_{50}: Effective dose resulting in a 50% of the antihyperalgesic effect. Data are the mean ± S.E of the estimate.
[a] Significantly different from the theoretical combination data ($P<0.05$), by the Student's t test.

For performing the experimental ED_{50}, the analgesic effect of four doses of both drugs corresponding to 1/2, 1/3, 1/4 and 1/8 of their ED_{50} administered at the corresponding times at which their analgesic effects peaked (20 min for PL37 and 60 min for gabapentin) was assayed. As shown in Fig. 4B, a dose–response curve was constructed by using these fixed combinations of ED_{50} fractions of both drugs, and from the data herein obtained, the experimental ED_{50} value of the combination was calculated. This experimental ED_{50} value was significantly lower than the theoretical ED_{50} (Fig. 4C) obtained if a purely additive interaction would occur, demonstrating that the antihyperalgesic effect produced by these drugs in combination is not additive. A further analysis shows that the interaction index is lower than 1, a value that indicates a synergistic interaction in which a twofold increase of the potency of the combination occurs (Table 1) (Tallarida, 2002).

4. Discussion

The intraosteal inoculation of NCTC 2472 fibrosarcoma-derived cells to C3H/HeJ mice provokes the growth of an osteolytic sarcoma that produces several nociceptive symptoms in the affected limb (Schwei et al., 1999; Honoré et al., 2000; Wacnik et al., 2003; Menéndez et al., 2003a). The aim of the present experiments was to investigate whether one of these nociceptive symptoms, thermal hyperalgesia, may be inhibited by oral administration of PL37, a dual inhibitor of the two enkephalin inactivating enzymes, neutral endopeptidase and aminopeptidase N. Furthermore, we have explored the possibility that the effect induced by PL37 may be potentiated by the simultaneous administration of gabapentin.

The oral administration of PL37 dose-dependently inhibited the thermal hyperalgesic responses detected 4 weeks after the intratibial inoculation of NCTC 2472 cells, demonstrating the ability of endogenous enkephalins protected from their catabolism to counteract this hyperalgesic response. The effect produced by 25 mg/kg of PL37 probably occurs by acting on peripheral opioid receptors since it is restricted to the affected paw and blocked by naloxone-methiodide, at a dose at which this drug does not produce central effects (Fürst et al., 2005). The possibility of a peripheral action of endogenous enkephalins when low doses of PL37 are used is consistent with the previous description of pre-proenkephalin A gene expression in dorsal root ganglia of rats and its subsequent transport to nociceptor

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synapses (Antunes et al., 2001) and with the reported peripheral inhibition of osteosarcoma-induced thermal hyperalgesia by loperamide or low doses of local morphine (Menéndez et al., 2003b). In addition, an increased release of enkephalins from leucocytes bearing neutral endopeptidase and aminopeptidase N (Salzet et al., 2000) recruited at the level of injured tissues was previously reported (Rittner et al., 2006).

Used at a higher oral dose (50 mg/kg), PL37 evoked a greater analgesic effect probably by acting both at peripheral and central nervous system levels, as suggested from the bilateral increase in withdrawal latencies and the partial inhibition produced by naloxone-methiodide. This incomplete reversion may be understood considering that this antagonist probably inhibits the peripheral component of the analgesic effect without affecting the central one.

Since our studies were focused on the peripheral effects induced by PL37, the dose used in further experiments was 25 mg/kg. The peripheral antihyperalgesic effect induced by this dose seems exclusively mediated through μ-, but not δ- or κ-, opioid receptors activation since the administration of cyprodime, a selective antagonist of μ-opioid receptors inhibited the responses whereas neither naltrindole (a δ-opioid receptor antagonist) nor nor-binaltorphimine (a κ-opioid receptor antagonist) modified them. Previous studies have shown that μ- or δ-opioid receptors can be involved in the analgesic effects induced by inhibitors of enkephalins catabolism. For instance, δ-opioid receptors are responsible of the inhibition induced by the local administration of the dual inhibitor kelatorphan on nociceptive neurons of rat dorsal horn (Dickenson et al., 1986). Related to the involvement of μ-opioid receptors, it has been described that the analgesic effect of RB101 in healthy mice is unaffected by the selective δ-opioid receptor antagonist naltrindole (Noble et al., 1992) and that the antinociceptive effect of this drug on rat paws inflamed by carrageenan is completely blocked by the selective μ-opioid receptor antagonist beta-flunaltrexamine (Le Guen et al., 1999). The fact that in our experiments peripheral μ-opioid receptors are involved in the effect of PL37 seems consistent with the previous observation that selective μ-opioid receptor agonists produce greater peripheral analgesic effects than δ-opioid receptors agonists in this model of tumoral thermal hyperalgesia (Baamonde et al., 2005).

In previous studies, it has been described that the administration of gabapentin to mice intrafemorally inoculated with NCTC 2472 cells may inhibit different behavioral parameters of nociception, such as limb use during ambulation or palpation-evoked flinching and guarding (Peters et al., 2005). The analgesic efficacy of gabapentin has been also demonstrated in other cancer-induced pain models (Kuraishi et al., 2003; Donovan-Rodriguez et al., 2005) and only one study failed to observe such analgesic effect (El Mouedden and Meert, 2007). Our data indicate that the acute administration of gabapentin dose-dependently inhibit osteosarcoma-induced thermal hyperalgesia. The effective doses of gabapentin (12.5–25 mg/kg, i.p.) were lower than those used in the mentioned previous study performed with NCTC 2472 cells (100 mg/kg, oral) (Peters et al., 2005), a fact probably due both to the different testing parameter measured and to the different routes of administration used.

Besides, the fact that the antinociceptive effects induced by gabapentin are preferentially detected at the injured level, could reflect a possible effect of the drug at the peripheral level. In this sense, it has been shown that, apart from its central analgesic action (Field et al., 1997), gabapentin can induce peripheral antinociceptive responses (Carlton and Zhou, 1998; Todorovic et al., 2003). Indeed an increased expression of mRNA levels of the calcium channel $\alpha_2\delta$-1 subunit in peripheral nociceptive fibres during the development of the same type of tumor has been described (Khasabova et al., 2007). Also, it has been reported that gabapentin can produce analgesia through the peripheral release of NO (Ortiz et al., 2006), a mechanism able to relieve osteosarcoma-induced thermal hyperalgesia (Menéndez et al., 2007). Even if the restricted effect to the injured paw suggests that gabapentin could act peripherally, a central effect cannot be discarded since the drug was systemically administered.

The results obtained by the coadministration of PL37 and gabapentin indicate that the analgesic effects induced by both drugs are strongly potentiated in such a way that doses of both drugs ineffective if administered alone completely abolish osteosarcoma-induced hyperalgesia when associated. In fact, data obtained by the isolobolographic analysis show that the experimental ED_{50} value obtained is significantly lower than the calculated theoretical ED_{50} value indicating a synergistic interaction (Tallarida, 2002). This result fits well with the potentiation between opiates and gabapentin described in different situations of chronic pain, including cancer processes (Caraceni et al., 1999, 2004) and constitutes the first study describing that a synergistic interaction between gabapentin and opiates may be obtained by using an inhibitor of enkephalin catabolism.

Overall, we show here that the oral administration of low doses of PL37, an inhibitor of enkephalin-degrading enzymes, may inhibit osteosarcoma-induced thermal hyperalgesia acting peripherally. Furthermore, this peripheral thermal antihyperalgesic effect induced by PL37 may be potentiated by the simultaneous administration of gabapentin, in such a way that doses of both drugs that are separately inactive become active when simultaneously administered. These results reinforce the interest for the development of dual inhibitors of enkephalin inactivating peptidases as new analgesics devoid of the drawbacks of morphine and surrogates mainly due to the ubiquitous stimulation of the widely distributed opioid receptors by exogenous opioid receptor agonists.

Acknowledgments

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