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Peripheral and spinal mechanisms of antinociceptive action of lumiracoxib

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Abstract

The possible participation of the nitric oxide (NO)-cyclic GMP-K^+ channel pathway, serotonergic or opioidergic system on lumiracoxib-induced local or intrathecal antinociception was assessed in the formalin test. Local or intrathecal administration of lumiracoxib dose-dependently produced antinociception in the second phase of the test. Moreover, local or intrathecal pretreatment with N^G-L-nitro-arginine methyl ester (L-NAME, NO synthesis inhibitor), 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ, guanylyl cyclase inhibitor), glibenclamide (ATP-sensitive K^+ channel blocker), charybdotoxin and apamin (large- and small-conductance Ca^{2+}-activated-K^+ channel blockers, respectively) or margatoxin (voltage-dependent K^+ channel blocker), but not N^G-D-nitro-arginine methyl ester (D-NAME) or vehicle, significantly prevented lumiracoxib-induced antinociception. The intrathecal injection of methiothepin (serotonin receptor antagonist) reduced lumiracoxib-induced intrathecal antinociception. Local peripheral or intrathecal naloxone did not modify either local or intrathecal lumiracoxib-induced antinociception. Results suggest that lumiracoxib activates the NO-cyclic GMP-K^+ channels to produce local and intrathecal antinociception. Data also suggest that lumiracoxib activates the intrathecal serotonergic system, but not opioid receptors either at peripheral or spinal sites.
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Keywords: Lumiracoxib; K^+ channel; NO-cyclic GMP pathway; Charybdotoxin; Apamin; Margatoxin; Serotonergic system; Opioid receptor

1. Introduction

Cyclooxygenase-2 inhibitors represent a novel class of non-steroidal anti-inflammatory drugs (NSAIDs) with potent anti-inflammatory and anti-hyperalgesic activity in animal models of pain (Seibert et al., 1994) as well as in clinical trials (Kellstein et al., 2004; Atherton et al., 2004). Lumiracoxib is a novel selective inhibitor of cyclooxygenase-2 that shows potent anti-inflammatory activity in in vitro and ex vivo assays (Atherton et al., 2004). The analgesic effect of cyclooxygenase-2 inhibitors in inflammatory pain models is attributed to the inhibition of the synthesis of prostaglandins, which sensitize primary afferent neurons. The anti-hyperalgesic effect of lumiracoxib has been recently reported in a cancer pain model (Fox et al., 2004). However, since an antiallodynic effect was also observed (Fox et al., 2004), other mechanisms, different from prostaglandin synthesis inhibition, were suggested.

There is evidence that the antinociceptive effect of rofecoxib, another cyclooxygenase-2 selective inhibitor, involves the activation of the nitric oxide (NO)-cyclic GMP-K^+ channel pathway (Déciga-Campos and López-Muñoz, 2004) and serotonergic system (Sandrini et al., 2002). In addition, recent studies suggest that diclofenac-induced peripheral antinociception is produced through the activation of the NO-cyclic GMP pathway (Tonussi and Ferreira, 1994; Ortiz et al., 2003b; Alves et al., 2004). Like diclofenac, other NSAIDs stimulate the NO-cyclic GMP pathway at the periphery (Duarte et al., 1990, 1992; Ferreira et al., 1991; Granados-Soto et al., 1995; Islas-Cadena et al., 1999; Aguirre-Bañuelos and Granados-Soto,

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2000; Lázaro-Ibáñez et al., 2001; Ortiz et al., 2003b; Alves et al., 2004) and at the spinal cord (Lorenzetti and Ferreira, 1996). NO and cyclic GMP can activate several targets including different types of K^+ channels (Bolotina et al., 1994; Carrier et al., 1997; Levy and Strassman, 2004; Liu et al., 2004). Accordingly, Duarte and coworkers have recently reported that NO donors- and dibutyril cyclic GMP-induced peripheral antinociception is reversed by ATP-sensitive K^+ channel blockers (Soares et al., 2000; Soares and Duarte, 2001) thus establishing a link between the NO-cyclic GMP pathway, the opening of K^+ channels and antinociception.

After acute injury, repetitive C fiber input evokes a state of spinal facilitation. Among the receptors mediating this phenomenon are *N*-methyl-D-aspartate (NMDA) and substance P receptors. Activation of these receptors increases intracellular concentrations of Ca^{2+} which in turn activates NO synthase as well as cyclooxygenase-2 (Yaksh, 1999). NO has been postulated as a neurotransmitter at the spinal level conveying nociceptive information (Meller et al., 1992) and there is evidence that NO-induced nociception could be modulated by some NSAIDs (Björkman, 1995). On the other hand, several observations suggest that endogenous opioids may be involved in NSAIDs-induced antinociception. Accordingly, diclofenac significantly increased β-endorphins plasma levels in humans (Sacerdote et al., 1985), whereas that the diclofenac-induced antinociceptive in rats can be reversed by naloxone (Björkman, 1995). Activation of the descending serotonergic system has been proposed as an additional mechanism for some NSAIDs (Björkman, 1995; Pini et al., 1997). Data indicates that acetylsalicylic acid is able to increase the brain serotonin (5-HT) content in rats (Pini et al., 1997). In addition, diclofenac-induced antinociception can be reversed by chemical destruction of the raphe nucleus region or spinal and supraspinal administration of 5-HT receptor antagonists such as methiothepin and ritanserin (Björkman, 1995). Taken together these data suggest that some NSAIDs could activate a descending inhibitory serotonergic system which in turn would produce spinal antinociception.

On the basis of the above observations, this work was undertaken to determine whether the NO-cyclic GMP-K^+ channel pathway, and the opioidergic and serotonergic systems have any participation on the local and intrathecal antinociception induced by the cyclooxygenase-2 selective inhibitor lumiracoxib, a NSAID structurally related with diclofenac, in the 1% rat formalin test.

2. Materials and methods

2.1. Animals

Experiments were performed on adult female Wistar rats (body weight range, 180–220 g) of 6 to 7 weeks of age. The animals were obtained from our own breeding facilities and had free access to food and drinking water before experiments. All experiments followed the Guidelines on Ethical Standards for Investigation of Experimental Pain in Animals (Zimmermann, 1983). Additionally, the study was approved by the Institutional Animal Care and Use Committee (Centro de Investigación y de Estudios Avanzados, México, DF, México).

2.2. Intrathecal surgery

Chronic catheterization of the intrathecal subarachnoid space was performed as described by Yaksh and Rudy (1976). The rats were anesthetized with a ketamine–xylazine mixture (45–12 mg/kg, i.p.), placed in a stereotaxic head holder, and the atlanto-occipital membrane exposed. The latter membrane was pierced, and a polyethylene catheter (PE-10, 8.0 cm length) was inserted intrathecally and advanced caudally to the level of the thoracolumbar junction. The wound was then sutured and the animals were allowed to recover from surgery for at least 5 days before testing. Rats showing any signs of motor impairment were euthanized in a CO_2 chamber.

2.3. Measurement of antinociceptive activity

Antinociception was assessed using the formalin test (Dubuisson and Dennis, 1977). The rats were placed in open Plexiglas observation chambers for 30 min to allow them to acclimate to their surroundings; then they were removed for formalin administration. Fifty microliters of diluted formalin (1%) was injected s.c. into the dorsal surface of the right hind paw with a 30-gauge needle. The animals were returned to the chambers and nociceptive behavior was observed immediately after formalin injection. Mirrors were placed in each chamber to enable unhindered observation. Nociceptive behavior was quantified as the number of flinches of the injected paw during 1-min periods every 5 min, up to 60 min after injection (Wheeler-Aceto and Cowan, 1991). Flinching was readily discriminated and was characterized as rapid and brief withdrawal, or as flexing of the injected paw. Formalin-induced flinching behavior was biphasic (cf. Dubuisson and Dennis, 1977; Wheeler-Aceto and Cowan, 1991). The initial acute phase (0–10 min) was followed by a relatively short quiescent period, which was then followed by a prolonged persistent response (15–60 min). At the end of the experiment the rats were sacrificed in a CO_2 chamber.

2.4. Drugs

Lumiracoxib was a gift of Novartis Farmacéutica (Mexico City). N^G-L-nitro-arginine methyl ester (L-NAME), N^G-D-nitro-arginine methyl ester (D-NAME) and 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ) were purchased from Research Biochemical International (Natick, MA,

USA). Glibenclamide (glyburide), charybdotoxin, apamin, margatoxin, naloxone and methiothepin were purchased from Sigma (St. Louis, MO, USA). L-NAME, D-NAME, charybdotoxin, apamin, margatoxin, naloxone and methiothepin were dissolved in saline. Lumiracoxib, glibenclamide and ODQ were dissolved in dimethylsulfoxide (DMSO) 20% for local peripheral injection, whereas that these drugs were dissolved in 100% DMSO for intrathecal administration.

2.5. Examination of catheter position

At the end of the experiment the correct position of the catheter was assessed by the intrathecal administration of 2% lidocaine (10 μl) followed by saline (10 μl). Motor paralysis of the hind limb of the rat occurring within 15 min after anesthetic administration was considered as an indication of correct position of the catheter. In addition, 1% methylene blue (10 μl) was injected intrathecally. The spinal cord was harvested and cut through the L4–L5 intervertebral disk to look for the catheter tip under a dissecting microscope (4× magnification). Rats showing the catheter tip positioned at sites other than the dorsal spinal cord or dye staining of paravertebral musculature were not considered for data analysis.

2.6. Study design

For local peripheral drug administration, rats received a s.c. injection (50 μl) into the dorsal surface of the right hind paw of vehicle (20% DMSO) or increasing doses (30, 100, 300 μg in 50 μl 20% DMSO) of lumiracoxib 20 min before formalin injection into the ipsilateral paw. To determine whether lumiracoxib acted locally, it was administered to the left (contralateral) paw 20 min before formalin was injected into the right paw, and the corresponding effect on nociceptive behavior was assessed. To determine whether lumiracoxib-induced peripheral antinociception was mediated by either the NO-cyclic GMP pathway, K^+ channel or opioidergic system, effect of pretreatment (−10 min) with the appropriate vehicle (20% DMSO for ODQ and glibenclamide or saline for L-NAME, D-NAME, charybdotoxin, apamin, margatoxin and naloxone) or L-NAME (10–100 μg/paw), ODQ (12.5–50 μg/paw), D-NAME (100 μg/paw), glibenclamide (10–100 μg/paw), apamin (0.3–3 μg/paw), charybdotoxin (0.1–1 μg/paw), margatoxin (1–100 ng/paw) and naloxone (50 μg/paw) on the antinociceptive effect induced by local peripheral lumiracoxib (300 μg/paw) was assessed. Each rat received 3 injections and appropriate controls for multiple injections and vehicles were performed before starting the formal study. Doses and drug administration schedule of NO, cyclic GMP, K^+ channel inhibitors, opioid receptor antagonist and lumiracoxib for peripheral administration were selected based on previous reports (Ocaña et al., 1990; Galeotti et al., 1999; Rodrigues and Duarte, 2000; Soares et al., 2000; Soares and Duarte, 2001) and on pilot experiments in our laboratory.

For intrathecal administration, rats received an intrathecal injection of vehicle (100% DMSO, 10 μl) or increasing doses (30, 100, 300 μg in 10 μl DMSO) of lumiracoxib 20 min before formalin injection into the right paw, and nociceptive behavior was assessed. To determine whether lumiracoxib-induced intrathecal antinociception was mediated by either the NO-cyclic GMP pathway, K^+ channel, opioidergic or serotonergic system, effect of pretreatment (−10 min) with the appropriate vehicle (100% DMSO for glibenclamide and ODQ or saline for L-NAME, ODQ, D-NAME, charybdotoxin, apamin, margatoxin, naloxone and methiothepin) or L-NAME (12.5–50 μg/rat), D-NAME (50 μg/rat), ODQ (1–10 μg/rat), glibenclamide (25–75 μg/rat), apamin (0.1–1 ng/rat), charybdotoxin (0.1–1 ng/rat), margatoxin (0.1–1 ng/rat), naloxone (50 μg/rat) and methiothepin (0.3 μg/rat) on the antinociceptive effect induced by intrathecal lumiracoxib (300 μg, i.t.) was assessed. Each rat received 3 intrathecal injections and appropriate controls for multiple injections and vehicles were performed before starting the formal study. Doses and drug administration schedule of NO, cyclic GMP, K^+ channel inhibitors and opioid receptor antagonist and lumiracoxib for intrathecal administration were selected based on previous reports (Ocaña et al., 1990; Galeotti et al., 1999) and on pilot experiments in our laboratory. Rats in all groups were observed regarding behavioral or motor function changes induced by the intrathecal treatment. This was assessed, but not quantified, by testing the animals' ability to stand and walk in a normal posture, as proposed elsewhere (Chen and Pan, 2001).

2.7. Data analysis and statistics

All experimental results are given as the mean ± S.E.M. of the data obtained in 8 animals per group. Curves were constructed plotting the number of flinches as a function of time. The area under the number of flinches against time curves (AUC), an expression of the duration and intensity of the effect, was calculated by the trapezoidal rule. Reduction of number of flinches or AUC of the second phase is reported only, since we were not able to observe effect on phase 1. Analysis of variance (ANOVA), followed by Tukey's test was used to compare differences between treatments. Differences were considered to reach statistical significance when $P<0.05$.

3. Results

3.1. Antinociceptive effect of lumiracoxib

Subcutaneous formalin injection into the right hind paw produced a typical pattern of flinching behavior characterized by a biphasic time course (Fig. 1A and B, black squares). Phase 1 of the nociceptive response began immediately after formalin administration and then declined gradually in approximately 10 min. Phase 2 began about 15

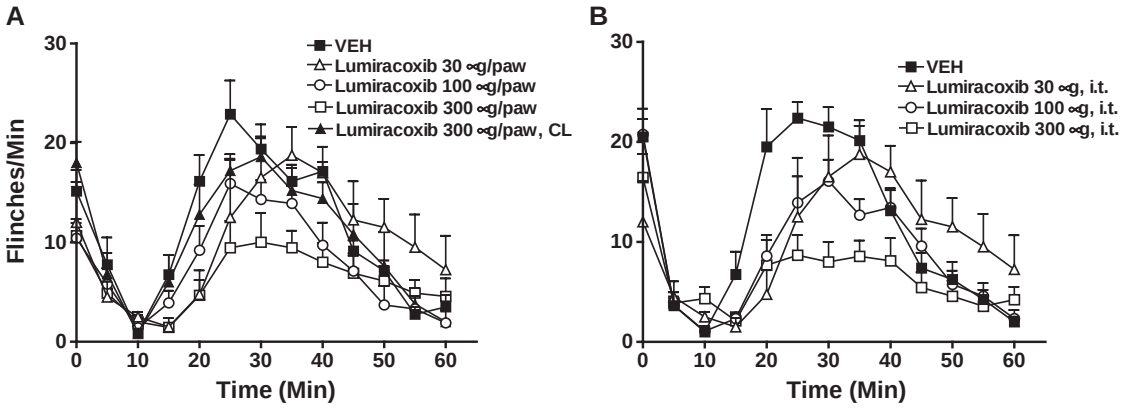

Fig. 1. Time course of the local peripheral (A) and intrathecal (B) antinociceptive effect of lumiracoxib in the formalin test. Data are presented as mean ($n=8$) ± S.E.M.

min after formalin administration and lasted about 1 h (cf. Dubuisson and Dennis, 1977). Catheter tip and dye staining were found around the dorsal spinal cord, suggesting that volume injected does not reach supraspinal structures. Ipsilateral local peripheral (Fig. 1A) and intrathecal (Fig. 1B) administration of lumiracoxib produced a dose-dependent reduction in the flinching behavior otherwise observed after formalin injection. In contrast, contralateral local peripheral injection of the NSAID (300 μg/paw) did not affect formalin-induced flinching behavior (Fig. 1A). Lumiracoxib significantly reduced the number of flinches during phase two ($P<0.05$), but not during phase one (Fig. 1A and B). After local peripheral or intrathecal administration of lumiracoxib, no changes in stand and walk in a normal posture was observed in either group, control or treated (data not shown).

3.2. Effect of L-NAME and ODQ on the local peripheral and intrathecal antinociceptive activity of lumiracoxib

Local peripheral pretreatment with the NO synthesis inhibitor L-NAME (100 μg/paw) or the NO-sensitive guanylyl cyclase inhibitor ODQ (50 μg/paw) did not produce any effect by themselves on formalin-induced flinching behavior. However, either L-NAME (10–100 μg/paw, Fig. 2A) or ODQ (12.5–50 μg/paw, Fig. 3A), but not D-NAME (100 μg/paw), reversed in dose-dependent manner the local peripheral antinociception induced by lumiracoxib ($P<0.05$). In a similar way, intrathecal administration of L-NAME (12.5–50 μg/rat, Fig. 2B) or ODQ (1–10 μg/rat, Fig. 3B), but not D-NAME (50 μg/rat), significantly diminished in a dose-dependent manner lumiracoxib-induced intrathecal antinociception ($P<0.05$). By them-

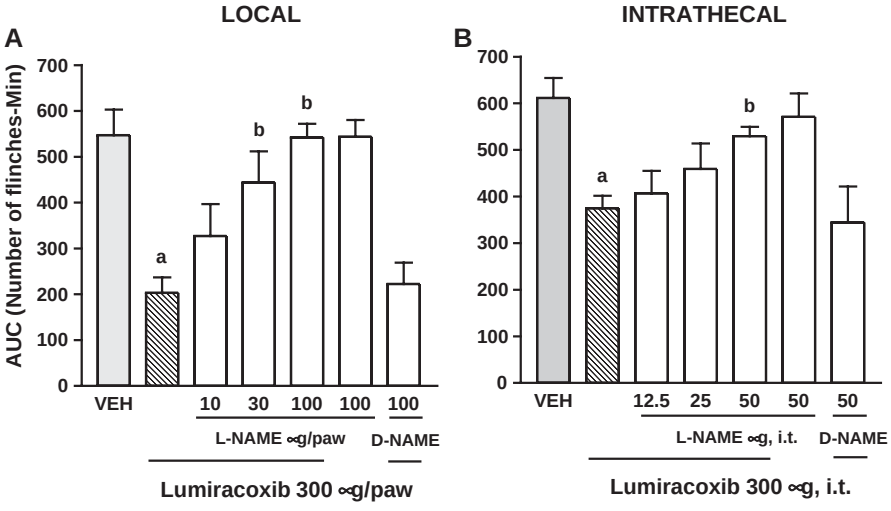

Fig. 2. Effect of N^G-L-nitro-arginine methyl ester (L-NAME) on the local peripheral (A) and intrathecal (B) antinociception produced by lumiracoxib. Rats received either local peripheral or intrathecal lumiracoxib (−20 min), either local peripheral or intrathecal N^G-L-nitro-arginine methyl ester and N^G-D-nitro-arginine methyl ester (D-NAME) pretreatment (−10 min) and an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC) of the second phase of the formalin test. Bars are the means ± S.E.M. of the data obtained in 8 animals. [a]Significantly different from the vehicle (VEH) group ($P<0.05$) and [b]significantly different from the lumiracoxib group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

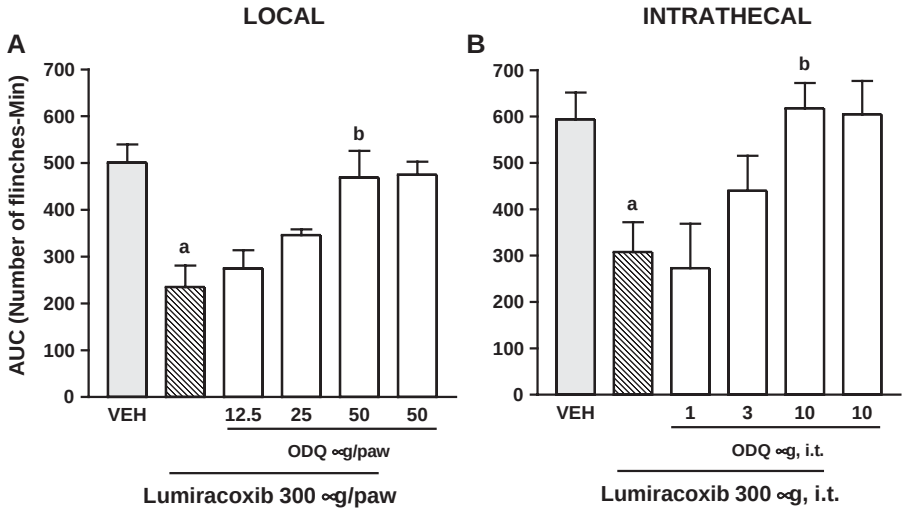

Fig. 3. Effect of 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one (ODQ) on the local peripheral (A) and intrathecal (B) antinociception produced by lumiracoxib. Rats received either local peripheral or intrathecal lumiracoxib (−20 min), either local peripheral or intrathecal 1*H*-(1,2,4)-oxadiazolo(4,2-*a*)quinoxalin-1-one pretreatment (−10 min) and an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC) of the second phase of the formalin test. Bars are the means ± S.E.M. of the data obtained in 8 animals. [a]Significantly different from the vehicle (VEH) group ($P<0.05$) and [b]significantly different from the lumiracoxib group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

selves, L-NAME (50 μg/rat, i.t.) or ODQ (10 μg/rat, i.t.) did not affect formalin-induced nociceptive behavior.

3.3. Effect of glibenclamide, charybdotoxin, apamin and margatoxin on the local peripheral and intrathecal antinociceptive activity of lumiracoxib

Local peripheral pretreatment with the ATP-sensitive K^+ channel blocker glibenclamide (10–100 μg/paw, Fig. 4A), small-conductance Ca^{2+}-activated K^+ channel blocker apamin (0.3–3 μg/paw, Fig. 5A), large-conductance Ca^{2+}-activated K^+ channel blocker charybdotoxin (0.1–1 μg/paw, Fig. 6A) and voltage-dependent K^+ channel blocker margatoxin (1–100 ng/paw, Fig. 7A) dose-dependently prevented the antinociception produced by local peripheral lumiracoxib ($P<0.05$). Given alone, peripheral administration of K^+ channel inhibitors did not modify formalin-induced nociceptive behavior. A similar pattern was observed at the spinal cord, as intrathecal administration of glibenclamide (25–75 μg/rat, Fig. 4B), apamin (0.1–1 ng/rat, Fig. 5B), charybdotoxin (0.1–1 ng/rat, Fig. 6B) and margatoxin (0.1–1 ng/rat, Fig. 7B) dose-dependently prevented the antinociception produced by intrathecal lumiracoxib ($P<0.05$). At the tested doses, the K^+ channel inhibitors did not modify formalin-induced nociceptive behavior.

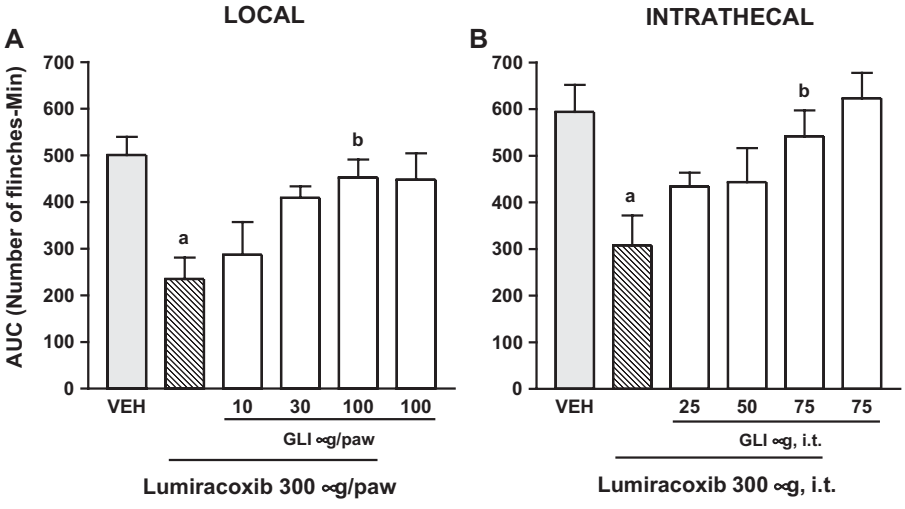

Fig. 4. Effect of glibenclamide (GLI) on the local peripheral (A) and intrathecal (B) antinociception produced by lumiracoxib. Rats received either local peripheral or intrathecal lumiracoxib (−20 min), either local peripheral or intrathecal glibenclamide pretreatment (−10 min) and an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC) of the second phase of the formalin test. Bars are the means ± S.E.M. of the data obtained in 8 animals. [a] Significantly different from the vehicle (VEH) group ($P<0.05$) and [b] significantly different from the lumiracoxib group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

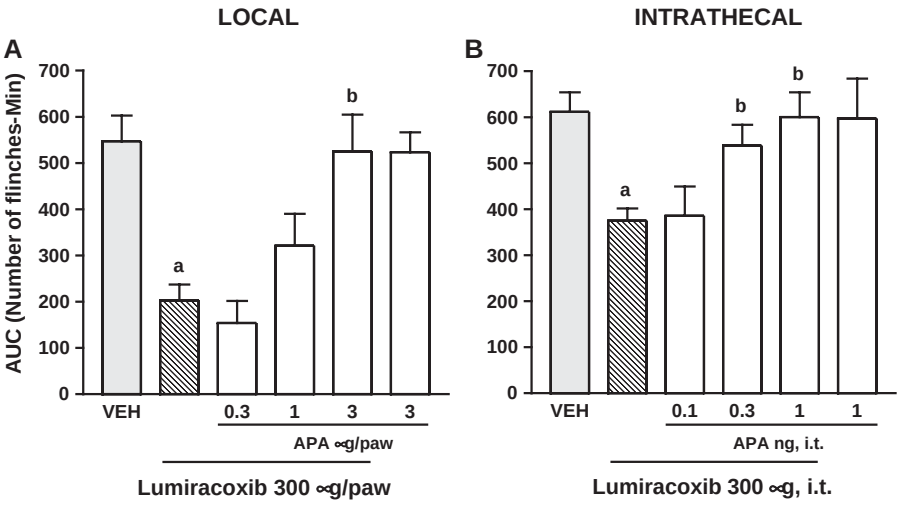

Fig. 5. Effect of apamin (APA) on the local peripheral (A) and intrathecal (B) antinociception produced by lumiracoxib. Rats received either local peripheral or intrathecal lumiracoxib (−20 min), either local peripheral or intrathecal apamin pretreatment (−10 min) and an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC) of the second phase of the formalin test. Bars are the means ± S.E.M. of the data obtained in 8 animals. [a]Significantly different from the vehicle (VEH) group ($P<0.05$) and [b]significantly different from the lumiracoxib group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

3.4. Effect of naloxone and methiothepin on the local peripheral and intrathecal antinociceptive activity of lumiracoxib

Local peripheral or intrathecal administration of the opioid receptor antagonist naloxone did not produce any effect on formalin-induced flinching behavior as compared to saline. Moreover, local peripheral (50 μg/paw) or intrathecal (50 μg/rat) naloxone was not able to reduce either local peripheral (Fig. 8A) or intrathecal (Fig. 8B) antinociception induced by lumiracoxib. On the contrary, the intrathecal administration of the 5-HT receptor antagonist methiothepin (0.3 μg/rat), but not vehicle, significantly reduced lumiracoxib-induced intrathecal antinociception (Fig. 9).

4. Discussion

4.1. Local peripheral and intrathecal antinociceptive effect of lumiracoxib

In the present study we were able to observe local peripheral and intrathecal antinociception with lumiracoxib. The local antinociceptive effect of lumiracoxib was not due to a systemic action since the administration of the drug in the

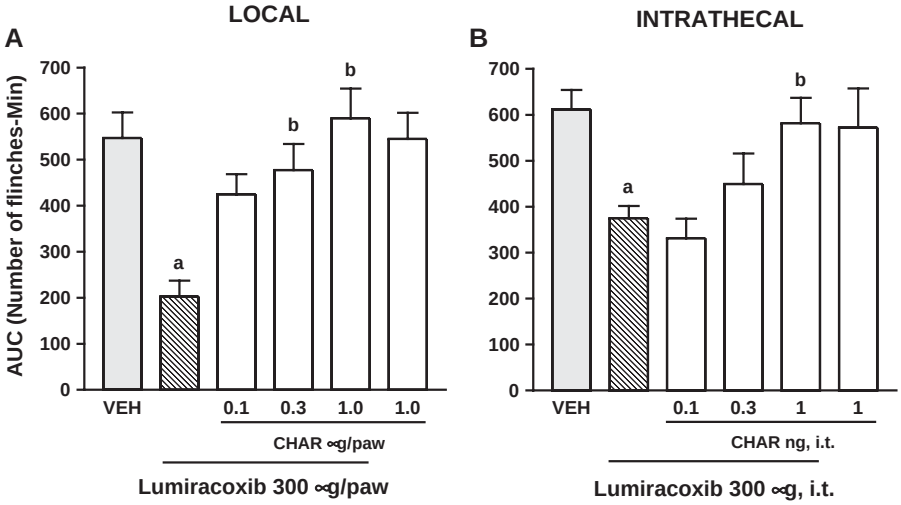

Fig. 6. Effect of charybdotoxin (CHAR) on the local peripheral (A) and intrathecal (B) antinociception produced by lumiracoxib. Rats received either local peripheral or intrathecal lumiracoxib (−20 min), either local peripheral or intrathecal charybdotoxin pretreatment (−10 min) and an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC) of the second phase of the formalin test. Bars are the means ± S.E.M. of the data obtained in 8 animals. [a]Significantly different from the vehicle (VEH) group ($P<0.05$) and [b] significantly different from the lumiracoxib group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

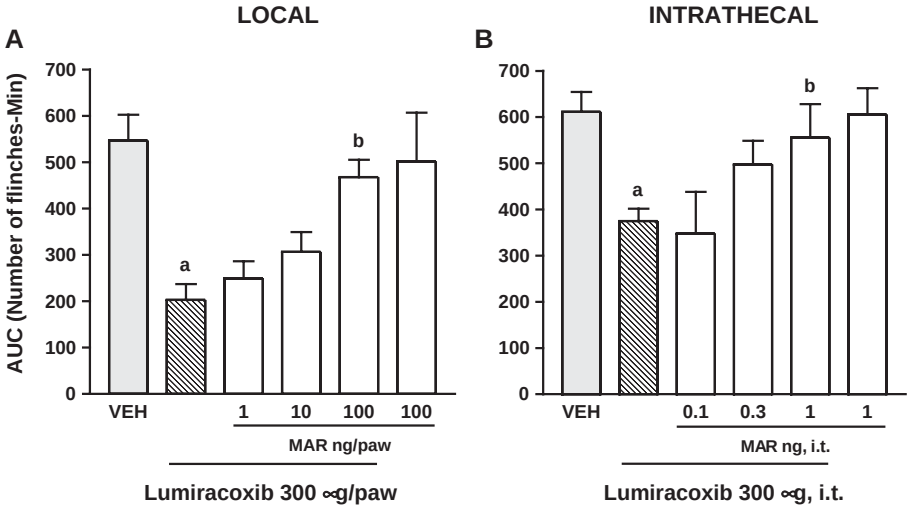

Fig. 7. Effect of margatoxin (MAR) on the local peripheral (A) and intrathecal (B) antinociception produced by lumiracoxib. Rats received either local peripheral or intrathecal lumiracoxib (−20 min), either local peripheral or intrathecal margatoxin pretreatment (−10 min) and an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC) of the second phase of the formalin test. Bars are the means ± S.E.M. of the data obtained in 8 animals. [a]Significantly different from the vehicle (VEH) group ($P<0.05$) and [b]significantly different from the lumiracoxib group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

contralateral paw was ineffective. Since lumiracoxib preferentially inhibits cyclooxygenase-2 (Atherton et al., 2004), our results suggest a relevant participation of local peripheral and spinal cyclooxygenase-2 in the nociceptive process induced by formalin. Even though, the participation of spinal cyclooxygenase-2 in the formalin test is contradictory, our results agree with previous observations about the intrathecal efficacy of cyclooxygenase-2 inhibitors (NS 398 and celecoxib) on formalin-induced nociception (Yamamoto and Nozaki-Taguchi, 1996, 2002), suggesting a relevant role for cyclooxygenase-2 at the spinal cord in this test. Previously it has been reported that the selective cyclooxygenase-2 inhibitor celecoxib injected directly into the paw failed to produce any significant antinociception after either 1% or 5% formalin. Based in the fact that time frame in the formalin test is not enough to allow the expression of cyclooxygenase-2 at the site of injury and the results with celecoxib, we proposed that local peripheral cyclooxygenase-2 may play a limited role in the formalin test (Torres-López et al., 2002). Therefore, the antinociceptive effect of peripheral lumiracoxib in

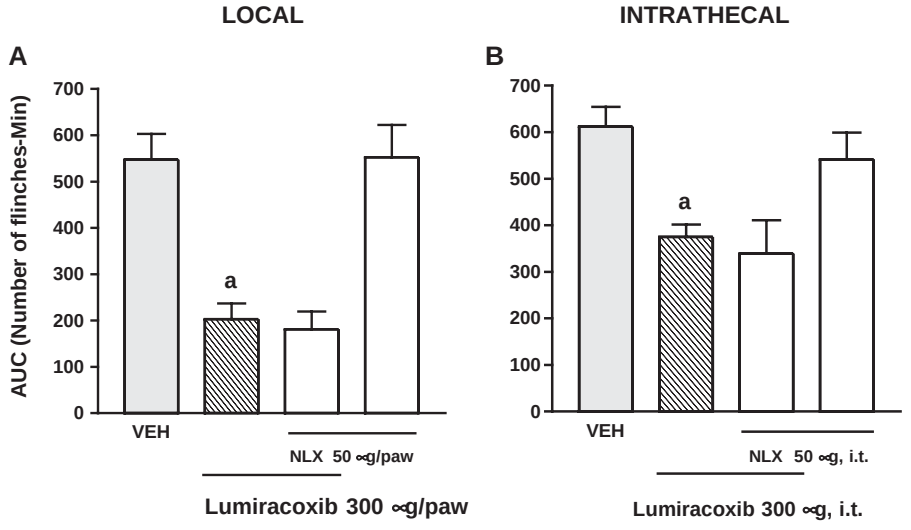

Fig. 8. Effect of naloxone (NLX) on the local peripheral (A) and intrathecal (B) antinociception produced by lumiracoxib. Rats received either local peripheral or intrathecal lumiracoxib (−20 min), either local peripheral or intrathecal naloxone pretreatment (−10 min) and an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC) of the second phase of the formalin test. Bars are the means ± S.E.M. of the data obtained in 8 animals. [a]Significantly different from the vehicle (VEH) group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

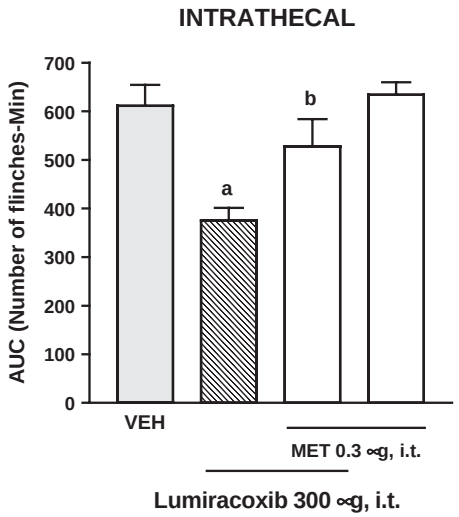

Fig. 9. Effect of intrathecal methiothepin (MET) on the intrathecal antinociception produced by lumiracoxib. Rats received intrathecal lumiracoxib (−20 min), intrathecal methiothepin pretreatment (−10 min) and an injection of 1% formalin (50 μl) at time zero. Data are expressed as the area under the number of flinches against time curve (AUC) of the second phase of the formalin test. Bars are the means ± S.E.M. of the data obtained in 8 animals. [a]Significantly different from the vehicle (VEH) group ($P<0.05$) and [b]significantly different from the lumiracoxib group ($P<0.05$), as determined by analysis of variance followed by the Tukey's test.

the current study was unexpected. Our data, however, agree with previous studies of our group showing that local peripheral injection of meloxicam and nimesulide, considered as preferential cyclooxygenase-2 inhibitors (Jackson and Hawkey, 2000), produced a significant antinociception in this model. This effect was attributed to the peripheral activation of the NO-cyclic GMP pathway (Islas-Cadena et al., 1999; Aguirre-Bañuelos and Granados-Soto, 2000) as could be the case for lumiracoxib (see below).

4.2. Effect of L-NAME and ODQ on the local peripheral and intrathecal antinociceptive activity of lumiracoxib

The local peripheral or intrathecal antinociceptive effect of lumiracoxib was blocked in a dose-dependent manner by the NO synthesis inhibitor L-NAME and NO-sensitive soluble guanylyl cyclase inhibitor ODQ (Moro et al., 1996), but not by saline or the inactive isomer of L-NAME, D-NAME. These results suggest that the NO-cyclic GMP pathway is involved in lumiracoxib-induced local peripheral and intrathecal antinociception. The data confirm the participation of the peripheral NO-cyclic GMP pathway in the antinociception produced by several NSAIDs (Duarte et al., 1990, 1992; Tonussi and Ferreira, 1994; Granados-Soto et al., 1995; Lorenzetti and Ferreira, 1996; Islas-Cadena et al., 1999; Aguirre-Bañuelos and Granados-Soto, 2000; Lázaro-Ibáñez et al., 2001; Ortiz et al., 2003a,b; Alves et al., 2004) and particularly cyclooxygenase-2 selective inhibitors as rofecoxib (Déciga-Campos and López-Muñoz, 2004). However, so far there are no reports about the effect of the intrathecal administration of NO synthesis and guanylyl cyclase inhibitors on the intrathecal activity of NSAIDs. In the current study, the intrathecal administration of L-NAME and ODQ did not modify formalin-induced flinching behavior, but significantly diminished the antinociceptive effect of intrathecal lumiracoxib. These data suggest that the NO-cyclic GMP pathway is involved in lumiracoxib-induced intrathecal antinociception. Accordingly, it has been reported that NO can either decrease or increase the mechanical responsiveness of nociceptors and its action might depend on the baseline level of neuronal excitability (Levy and Strassman, 2004). In addition, an analogue of cyclic GMP produced inhibition of spontaneous activity and mechanical responses of nociceptive afferents (Levy and Strassman, 2004; Liu et al., 2004). Contrariwise, the systemic administration of *S*-(+)-ketoprofen was unaffected by intrathecal L-NAME (Díaz-Reval et al., 2004). Moreover, the intrathecal antinociceptive effect of diclofenac or ibuprofen in glutamate-induced hyperalgesia was reversed by intrathecal L-arginine, but not by D-arginine (Björkman, 1995), thus suggesting that these NSAIDs could act via the functional inhibition of the pronociceptive actions of NO. In line with this observation, but contrary to our results, intrathecal administration of L-NAME produced antinociception in the formalin test (Malmberg and Yaksh, 1993). Differences observed between these reports and our results could be due to the type and intensity of the noxious stimuli, rat strain and particularly to the dose or concentration reached at the active site (Granados-Soto, 2003). In this sense, evidence suggests that low doses are associated with antinociception, whereas that medium or high doses of NO or cyclic GMP produce nociception (Prado et al., 2002; Tegeder et al., 2002).

4.3. Effect of glibenclamide, charybdotoxin, apamin and margatoxin on the local peripheral and intrathecal antinociceptive activity of lumiracoxib

The results reported here suggest that modulation of K^+ channels at the peripheral and spinal level may represent an important step in the mechanism of antinociception induced by lumiracoxib. Either local peripheral or intrathecal administration of glibenclamide (ATP-sensitive K^+ channel blocker; Davies et al., 1991; Edwards and Weston, 1993), apamin and charybdotoxin (small- and large-conductance Ca^{2+}-activated K^+ channel blockers, respectively; Romey et al., 1984; Stretton et al., 1992), and margatoxin (voltage-gated K^+ channel blocker; Garcia-Calvo et al., 1993) significantly reduced the antinociceptive action of lumiracoxib, suggesting that lumiracoxib activates these channels at peripheral and intrathecal sites. This observation confirms previous reports about the participation of K^+ channels on NSAIDs-induced peripheral antinociception (Lázaro-Ibáñez et al., 2001; Granados-Soto et al., 2002; Alves and Duarte, 2002; Ortiz et al., 2002, 2003a,b; Alves et al., 2004). The fact that several NSAIDs, particularly molecules structurally related with lumiracoxib, such as fenamates, behave as K^+ channel openers in in vitro studies (Farrugia et al., 1993; Li

et al., 1999; Wu et al., 2001) is in line with this suggestion. However, to the best of our knowledge this is the first report about the possible participation of K^+ channels in NSAIDs-induced antinociception at the spinal cord. These data are according with the presence of several types of K^+ channels in spinal cord dorsal horn neurons (Yamashita et al., 1994; Safronov, 1999) and with the intrathecal antinociceptive effect of K^+ channel openers (Yamazumi et al., 2001).

It is known that besides its effects on large-conductance Ca^{2+}-activated K^+ channel, charybdotoxin is also able to inhibit intermediate-conductance Ca^{2+}-activated and voltage-gated K^+ channels (Kv), in particular Kv1.3 (Ouadid-Ahidouch et al., 1999). Therefore, blockade of lumiracoxib-induced antinociception by apamin and charybdotoxin suggest that this NSAID may be producing its antinociceptive effect through activation of small-, intermediate- and large-conductance Ca^{2+}-activated K^+ channel as well as voltage-gated K^+ channels (Kv1.3). The fact that the selective inhibitor of voltage-gated K^+ channels Kv1.3 margatoxin, at concentrations able to inhibit Kv1.3 (Garcia-Calvo et al., 1993), reduced the effect produced by lumiracoxib is in line with this suggestion. Taken together, results suggest that the local peripheral and intrathecal antinociceptive effect of lumiracoxib may result from activation of ATP-sensitive and small-, intermediate- and large-conductance Ca^{2+}-activated as well as voltage-gated (Kv1.3) K^+ channels.

In contrast with mechanical tests (Rodrigues and Duarte, 2000; Soares and Duarte, 2001; Sachs et al., 2004) in which the stimulation of the NO-cyclic GMP pathway seems to lead to the opening of ATP-sensitive K^+ channels via protein kinase G, our results suggest that, in the formalin test, other K^+ channels (see above) may be involved. This could be due to the differences in the pain test. 5-HT and histamine are the main mediators to produce overt behaviors in the formalin test (Parada et al., 2001); while in the carrageenan test there is no overt behavior, but sensitization, and the stimulation of the NO-cyclic GMP pathway seems to counteract ongoing nociceptor sensitization. Taken together data suggests that antinociceptive effect of lumiracoxib seems to reflect a blockade of the activation of the nociceptors via a NO-cyclic GMP-K^+ channel pathway.

4.4. Effect of naloxone and methiothepin on the local peripheral and intrathecal antinociceptive activity of lumiracoxib

Peripheral and spinal opioid mechanisms were analyzed by testing the effects of naloxone on lumiracoxib-induced antinociception. In the present experiments, local peripheral or intrathecal administration of naloxone was not able to modify the antinociceptive activity of local peripheral or intrathecal lumiracoxib, thus precluding the involvement of peripheral or spinal opioid mechanisms in lumiracoxib effect. The lack of effect could not be attributed to the doses of naloxone used as this dose has shown to be high enough to block morphine-induced antinociception in several pain models (Solomon and Gebhart, 1988; Alves et al., 2004).

Intrathecal administration of the high-affinity 5-HT_1/5-HT_2/5-HT_7 receptor antagonist methiothepin (Hoyer et al., 1994), but not vehicle, significantly reduced lumiracoxib-induced intrathecal antinociception. This data agree with previous observations suggesting that inhibition or destruction of the central serotonergic system reduces the antinociceptive effect of either diclofenac, acetaminophen (Björkman, 1995; Pini et al., 1996; Courade et al., 2001) or morphine (Yaksh, 1979). The mechanism by which lumiracoxib or other NSAIDs interact with the spinal serotonergic system remains to be elucidated, but it could involve 5-HT release (McCormack, 1994). In this sense, there are reports that acetaminophen and rofecoxib are able to increase brain content of 5-HT (Pini et al., 1996, 1997; Sandrini et al., 2002). Upon release, 5-HT could target specific 5-HT receptors in the spinal cord. Since methiothepin is a high-affinity 5-HT_1/5-HT_2/5-HT_7 receptor antagonist, our data suggest that these three receptors could be involved in lumiracoxib-induced intrathecal antinociception in the formalin test. More specifically, the candidate spinal receptor could be either the 5-HT_1 or 5-HT_2, which activation is linked to spinal antinociception (Oyama et al., 1996; Courade et al., 2001), but not the 5-HT_7 receptor as its activation is associated with pronociception (Millan, 2002). However, on the basis of these experiments, the possible participation of other types of 5-HT receptors cannot be ruled out.

In summary, data suggest that lumiracoxib-induced local peripheral and intrathecal antinociception result from activation of the NO-cyclic GMP-K^+ channel pathway, but not activation of opioidergic mechanisms. In addition, our data suggest that the intrathecal antinociceptive activity of lumiracoxib may result from activation of the descending inhibitory serotonergic system, besides the reduction of cyclooxygenase-2-produced prostaglandins.

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