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The antinociceptive effect of leukotriene D_4 receptor antagonist, MK-571, in mice: possible involvement of opioidergic mechanism

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Abstract

The effect of a leukotriene D_4 receptor antagonist, (3-(3-(2-(7-chloro-2-quinolinyl)ethenyl)phenyl(3-dimethyl amino-3-oxo propyl)thio)methyl)thio) propanoic acid (L-660,711; MK-571), was investigated on nociceptive responses in mice using three different assays: acetic-acid-induced abdominal constrictions, formalin response and tail-flick test. MK-571 (8–32 mg/kg, i.v.) produced dose-dependent protection against acetic-acid-induced abdominal constriction (ED_{50} = 30 mg/kg). The compound (10–80 mg/kg, i.p.) was also effective, in a dose-dependent manner, on the second phase of the formalin response (ED_{50} = 26 mg/kg). However, it had no effect on the first phase of the formalin response and in the tail-flick test. Naloxone (1 mg/kg, i.v.), an opioid antagonist, almost completely blocked the antinociceptive effect of MK-571 in both acetic-acid-induced abdominal constriction and the second phase of the formalin test. These results provide evidence for an antinociceptive action of MK-571 at peripheral sites and suggest that opioid mechanisms are involved.

Keywords: Leukotriene; Opioid; Nociception; LTD_4 receptor antagonist; Naloxone

1. Introduction

Leukotrienes, which are 5-lipoxygenase products of arachidonic acid, are involved in the inflammatory process (Samuelsson, 1983). Leukotriene B_4, a potent chemotactic and chemocinetic agent for neutrophils (Ford-Hutchinson et al., 1980), causes hyperalgesia in several species including rats and humans (Levine et al., 1984; Bisgaard and Kristensen, 1985). It has also been shown that the leukotriene B_4 reduces the threshold of activation of C-nociceptors (Martin et al., 1987) and sensitizes intrapulpal A-delta fibers (Madison et al., 1992). Peptido-leukotrienes (leukotriene C_4, D_4, E_4), slow-reacting substances of anaphylaxis, are potent smooth-muscle-contracting agents. They mediate inflammatory reactions by producing changes in blood flow and by increasing vascular permeability (Dahlén et al., 1981; Ford-Hutchinson, 1985).

Pharmacological studies have focused on investigating the role of leukotriene B_4 in nociception rather than the role of peptido-leukotrienes. However, different reports exist regarding the type of leukotriene released in nociceptive responses. In one study, it was shown that zymosan injection into the peritoneum increased leukotriene B_4 release in mice (Berkenkopf and Weichman, 1988), whereas another study reported that there was a significant increase in leukotriene C_4 release while leukotriene B_4 production was unaffected by zymosan in mice (Doherty et al., 1985).

In spite of the numerous studies using 5-lipoxygenase inhibitors to elucidate the role of leukotrienes in nociception (Dallob et al., 1987; Amann et al.,1996), the number of studies with leukotriene receptor antagonists is limited. In our previous study, we showed that the leukotriene D_4 receptor antagonist, L-648,051 (4-[3-(4-acetyl-3-hydroxy-2-propylphenoxy)propylsulfonyl]-γ-oxo-benzenebutanoic acid), decreased the number of the acetic-acid-induced abdominal constrictions in mice and had an antinociceptive effect when combined with a subanalgesic dose of morphine in the hot-plate test (abstract by Gök et al., 1995).

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In the light of the foregoing, the aim of the present study was to investigate the antinociceptive effect of MK-571, a selective leukotriene D_4 receptor antagonist, on the response to chemical (acetic acid and formalin) and thermal (tail-flick) stimulation in mice. Furthermore, to investigate the possible involvement of the opioidergic system, naloxone — an opioid antagonist — was used to reverse the resultant antinociception.

2. Materials and methods

2.1. Animals and laboratory

Locally bred male albino mice (23–31 g) were used in this study. The mice were housed for 1 day in the test room before experimentation. Experiments were carried out in a temperature-controlled room at 23°C. To avoid seasonal and diurnal variations, all experiments were carried out in the same season (December–February) and the same time of the day (morning). The experimenter was blind to drug treatment in all experiments. Each animal was used only once. The antinociceptive tests were conducted following the ethical guidelines laid out by the Committee for Research and Animal Ethics of Ege University School of Medicine.

2.2. Acetic acid abdominal constriction test

The abdominal constriction (writhing) test described by Hayashi and Takemori (1971) was used. Acetic acid (0.6%) was injected into the peritoneum to produce typical abdominal constrictions (0.1 ml/10 g), which are characterized by stretching of the whole animal with concave arching of the back followed by extension of the hind limbs. Following the i.p. injection of acetic acid, animals were placed in individual transparent containers and the number of abdominal constrictions in a 30-min period were counted. MK-571 was administered intravenously in a volume of 0.1 ml/mouse (8–32 mg/kg) 5 min before the acetic acid injection.

2.3. Formalin test

The formalin test has been shown to be a model of clinical pain associated with inflammation (Hunskaar et al., 1985; Wheeler-Aceto et al., 1990). This test is characterized by two phases: the first (early) phase is a result of direct chemical stimulation of nociceptors, and the second (late) phase involves an inflammatory reaction (Shibata et al., 1989; Rosland et al., 1990). For this test, mice were acclimated to individual cylindrical Plexiglas observation chambers for at least 1 h prior to testing. According to the method of Dubuisson and Dennis (1977), formalin (5%) was administered subcutaneously (25 μl) into the right hindpaw of the mouse. The time either licking or biting of the injected paw was defined as the nociceptive response. The first 5 min of the formalin response was evaluated as the first phase and the response between 15 and 45 min was taken as the second phase. In pretreated groups, MK-571 (10–80 mg/kg, i.p.) was administered 15 min before the formalin injection.

2.4. Tail-flick assay

The nociceptive tail-flick reflex was assessed using a standard tail-flick apparatus (D'Amour and Smith, 1941) in which the radiant heat source was focused on the dorsal surface of the tail. The heat emitted from the apparatus was adjusted to maintain an intensity sufficient to elicit tail-flick latencies of approximately 2–4 s. A 10-s cut-off value was used to prevent tissue damage. Each animal was assessed for baseline tail-flick latency before saline or drug administration. The measure of latency was evaluated again 15, 30, 60 min after MK-571 (20, 40 and 80 mg /kg, i.p.) or saline administration. Each animal was used as its own control.

2.5. Rota-rod test

In order to assess possible nonspecific sedative and muscle-relaxant effects of MK-571, the mice were tested on the Rota-rod (Rosland et al., 1990). The Rota-rod apparatus consisted of a rod 3 cm in diameter, subdivided into five compartments by discs 24 cm in diameter. The mice were placed on the rotating bar (16 rpm) and the time the mice stayed on the rod was determined. The cut-off time used was 2 min.

2.6. Body temperature

Body temperature was measured by inserting a rectal thermometer probe 4–5 mm into the rectum and was expressed as temperature before and after administration of MK-571 or saline. The temperature was measured every 15 min for a 60-min period.

2.7. Drugs and injections

MK-571 (3-(3-(2-(7-chloro-2-quinolinyl)ethenyl) phenyl(3-dimethyl amino-3-oxo propyl)thio)methyl) thio)propanoic acid), a leukotriene D_4 receptor antagonist (Jones et al., 1989) and a generous gift of Merck Frosst (Quebec, Canada), was dissolved in sterile saline. Naloxone hydrochloride (Sigma) (1 mg/kg) was prepared in sterile saline and administered subcutaneously in a volume of 0.1 ml/12 g body weight 10 min before MK-571 injection in the acetic acid or formalin tests. The dose of naloxone was selected from the study of Quock et al. (1993). Control animals received appropriate volumes of

saline by different routes (i.v., i.p., s.c.). All drug solutions were prepared on the day of experiment.

2.8. Statistical analysis

The data are expressed as means ± S.E.M. The results of acetic acid and formalin tests were analyzed by the Kruskal–Wallis analysis of variance test followed by Dunn's multiple comparison test or one-way analysis of variance followed by Newman–Keul's test, as appropriate. Statistical comparisons in terms over time within the groups in the tail-flick test and for body temperature measurements were analyzed by repeated measures analysis of variance followed by Tukey–Kramer multiple comparison test. If there was a significant effect of the test compound in a dose-dependent manner, the effective dose 50 (ED_{50}) with concomitant 95% confidence limits was determined according to the test of Litchfield and Wilcoxon (1949). The software of Pharma/PCS was used for this test.

3. Results

3.1. The effect of MK-571 on acetic-acid-induced abdominal constrictions

Fig. 1 shows the total number of abdominal constrictions in all groups for a 30-min period after acetic acid injection. Saline injection alone (control) into the peritoneum did not produce any abdominal constriction in mice ($n = 6$). In acetic-acid-injected control animals, abdominal constrictions were observed 1 min after the injection and peaked at 5–10 min ($n = 23$). Treatment with MK-571 reduced the number of abdominal constrictions in a dose-dependent manner. This decrease was significant at the doses of 16 and 32 mg/kg, i.v. ($P < 0.05$ and 0.001, respectively). The ED_{50} of MK-571 was found to be 30 mg/kg with concomitant 95% confidence limits of 19.2–47 mg/kg.

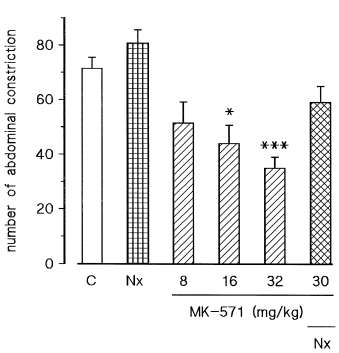

Fig. 1. The effect of MK-571 alone and in the presence of naloxone in the mouse acetic-acid-induced abdominal constriction test. Each bar represents the mean number of abdominal constrictions ± S.E.M. during 30 min after acetic acid injection. Open bar shows control (C) animals ($n = 23$); squared bar shows naloxone (Nx)-alone group (1 mg/kg, s.c.; $n = 9$); horizontal dashed bar indicates the increasing dose of MK-571: 8 mg/kg ($n = 8$), 16 mg/kg ($n = 10$), 32 mg/kg ($n = 13$); diagonal dashed bar shows naloxone (1 mg/kg, s.c.) + MK-571 ($ED_{50} = 30$ mg/kg, i.v.; $n = 11$). $^{*}P < 0.05$, $^{***}P < 0.001$, different from control.

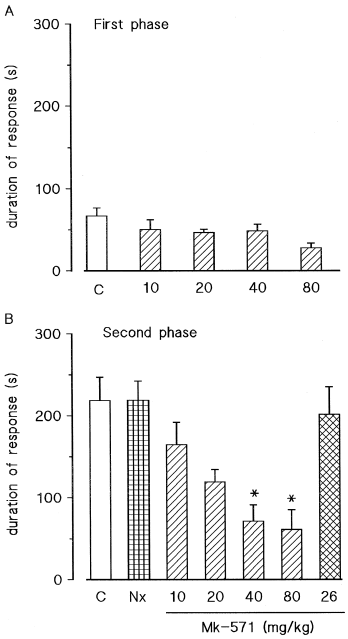

Fig. 2. The effect of MK-571 alone and in the presence of naloxone (1 mg/kg, s.c.) in the formalin test. Each bar represents the mean ± S.E.M of the licking or biting time (s) of the injected paw of mice during the first 5 min (A, first phase) and 15–45 min (B, second phase) after formalin injection. Open bar shows control (C) animals given saline i.p. ($n = 10$); squared bar shows naloxone (Nx)-alone group (1 mg/kg, s.c.; $n = 8$); horizontal dashed bar indicates animals pretreated with an increasing dose of MK-571 i.p.: 10 mg/kg ($n = 7$), 20 mg/kg ($n = 8$), 40 mg/kg ($n = 11$), 80 mg/kg ($n = 8$); diagonal dashed bar shows naloxone (1 mg/kg, s.c.) + MK-571 group ($ED_{50} = 26$ mg/kg, i,p.; $n = 12$). $^{*}P < 0.05$, different from control.

3.2. The effect of MK-571 on the formalin response

Fig. 2 shows the formalin response time for the first (0–5 min) and the second phases (15–45 min) in control and treated mice. Pretreatment with MK-571 (10, 20, 40 and 80 mg/kg, i.p.) 15 min before formalin injection did not produce any antinociceptive effect in the first phase while it caused a dose-dependent decrease in the reaction time of the second phase. The ED_{50} value of MK-571 was found to be 26 mg/kg with concomitant 95% confidence limits 14–47 mg/kg.

3.3. The effect of MK-571 on the tail-flick test

The results of tail-flick latencies are summarized in Table 1. In saline-treated or MK-571-treated mice, no differences were found between the pre- and post-injection tail-flick latencies ($P > 0.05$).

Table 1
The effect of MK-571 in the tail-flick test in mice
Post-drug values were determined 15, 30 and 60 min after MK-571 (i.p.) or saline injection. Each datum represents the mean ± S.E.M. of 14 animals in control group; 7, 13, 9 animals treated with MK-571 at the doses of 20, 40, 80 mg/kg, respectively.

Drugs	Dose	Latency (s)			
		Pre-drug	15th min	30th min	60th min
Control (saline)		3.8 ± 0.2	4.3 ± 0.2	4.1 ± 0.2	3.7 ± 0.3
MK-571 (mg/kg)	20	3.8 ± 0.3	3.9 ± 0.3	3.3 ± 0.1	3.6 ± 0.3
	40	4.0 ± 0.2	3.8 ± 0.1	3.7 ± 0.2	3.9 ± 0.1
	80	4.1 ± 0.1	4.2 ± 0.1	3.7 ± 0.2	3.8 ± 0.2

3.4. The effect of opioid antagonist on antinociceptive effect of MK-571

The selected dose of naloxone (1 mg/kg, s.c.) used in the present study did not produce, by itself, any significant effect on acetic-acid-induced abdominal constriction and the second phase of formalin response, as shown in Figs. 1 and 2B. When naloxone (1 mg/kg, sc.) was administered before injection of the ED_{50} dose of MK-571, it almost completely blocked the antinociceptive effect of the drug on acetic-acid-induced abdominal constriction and the second phase of the formalin response (Fig. 2B).

3.5. Rota-rod test

MK-571 did not affect the sensorimotor performance of animals when administered at the ED_{50} doses of 30 mg/kg, iv., and 26 mg/kg, i.p., for the acetic acid and formalin tests ($n = 10$), respectively. Sensorimotor performance was not affected by MK-571 even at the highest dose (80 mg/kg, i.p., $n = 7$) used in this study.

3.6. The effect of MK-571 on body temperature

In saline group, the difference between pre- and post-dose temperature was 0.2 ± 0.01°C (mean ± S.E.M.) 15 min after saline injection. MK-571, administered at doses of 20, 40 and 80 mg/kg, i.p., did not cause a significant reduction in temperature ($P > 0.05$, $n = 5–6$). The changes in temperature before and after injection of MK-571 at 20, 40 and 80 mg/kg, i.p., were -0.15 ± 0.2, -0.8 ± 0.4, 0.1 ± 0.3°C, respectively, 15 min following administration. Repeated measures of temperature for the 60-min period after MK-571 injection did not show any significant difference between pre- and post-dose values ($P > 0.05$).

4. Discussion

The findings of the present study showed that MK-571, a leukotriene D_4 receptor antagonist, produced dose-dependent antinociception against acetic-acid-induced constrictions and the second phase of the formalin response. This MK-571-induced antinociception was almost completely blocked by naloxone. MK-571 did not produce any significant effect in the tail-flick test. The doses of MK-571 needed to produce significant antinociception in the acetic acid and formalin tests were relatively high, suggesting that the effective dose of this compound may be changed due to the kind of stimulus, e.g., inflammatory states. The effectiveness of MK-571 does not seem to be related to nonspecific mechanisms and a hypothermic action since it did not produce any impairment in sensorimotor performance and in body temperature even at the highest dose used (80 mg/kg, i.p.).

The abdominal constrictor response is considered to be a model of acute tissue-injury-induced pain and is suitable to study opioid analgesic mechanisms because of its sensitivity to the analgesic effect of morphine (Martin, 1983). Acetic acid, which is used to cause abdominal constriction in mice, is known to induce the rapid release of various mediators capable of increasing vascular permeability (Doherty et al., 1985), such as bradykinin, substance P, histamine, 5-hydroxytryptamine, acetylcholine, ATP, potassium and hydrogen ions, prostaglandins and leukotrienes (Lembeck, 1983). Leukotrienes, which are involved in vascular permeability and inflammation, have been widely investigated in various nociceptive tests in mice by several investigators (Doherty et al., 1985; Berkenkopf and Weichman, 1988). However, these studies focused on the role of leukotriene B_4 rather than peptido-leukotrienes, since leukotriene B_4 is a potent chemotactic factor for neutrophils and causes leukocyte-dependent hyperalgesia (Ford-Hutchinson et al., 1980; Levine et al., 1984). Peptido-leukotrienes also mediate inflammatory reactions by changing blood flow and increasing vascular permeability. It has previously been shown that i.p. injection of zymosan into mice led to increased leukotriene C_4 production, while it did not cause detectable production of leukotriene B_4 (Doherty et al., 1985). Thus, our results demonstrating the antinociceptive effect of the leukotriene D_4 receptor antagonist on acetic-acid-induced abdominal constrictions and suggesting an enhanced activity of leukotriene D_4 are in accordance with the results of these previous studies.

That an opioid antagonist blocked MK-571-induced antinociception suggests the involvement of the opioid system in pain transmission. A possible interaction between leukotrienes and the opioid system was supported by our previous study (Gök et al., 1995) showing that the leukotriene D_4 receptor antagonist, L-648,051, did not produce any antinociceptive effect when administered alone while it produced significant antinociception when combined with a subanalgesic dose of morphine in the hot plate test, which has been widely accepted as a sensitive assay for opioid agonists. In the current study, MK-571 also produced an antinociceptive effect in the inflammatory phase of the formalin response, assuming an enhanced

activity of peptido-leukotrienes on inflammatory pain. The blockage of this effect by naloxone is suggestive of opioid involvement. It is well-established that the μ-opioid system is supersensitive under inflammatory conditions in peripheral (Stein et al., 1988, 1989) and spinal (Hylden et al., 1991) sites. This increased activity of μ-opioid agonists has been attributed to a pharmacological action at receptors in inflamed tissue (Stein et al., 1988, 1989). The μ-opioid agonist, morphine, was also found to inhibit polymorphonuclear neutrophil aggregation induced by various agents including thromboxane B_2 and leukotriene B_4 (Pasotti et al., 1993). Thus, the antinociceptive effect of MK-571 observed in the current study could be explained by both a possible direct opioid-agonist-like action of the drug at opioid receptors and an indirect action through augmentation of endogenous opioid tone. So far, there is no clear evidence showing that leukotriene receptor antagonists bind to opioid receptors at peripheral or central sites. Recently, a relationship between leukotrienes and opioid system was reported in which opioids play a regulatory role on leukotriene generation from murine spleen cells, with morphine inhibiting and, conversely, dynorphin stimulating the generation of leukotriene C_4 (Wang et al., 1995). Changing vascular permeability and ion conductance by analgesic drugs is an important mechanism to produce their antinociceptive action. Thus, it is also plausible to speculate that MK-571 may display its effect through a possible interaction with the ion conductance in the receptor region. In fact, the vascular and nonvascular constrictor effects of peptido-leukotrienes were reversed by cromakalim, a K^+-ATP channel activator, which caused hyperpolarization (McLeod and Piper, 1992). Welch and Dunlow (1993) suggested that the interaction between K^+ channel openers and opioids on antinociception was due to the sharing of a common second messenger system such as Ca^{2+} by these drugs, rather than to a direct interaction at a similar receptor. Moreover, patch clamp electrophysiological studies and ion flux studies showed that naloxone exerts complex actions on K^+ channels and binds to an area near the charybdotoxin/tetraethylammonium binding locus of K^+ channels in T-cell (Millar et al., 1997).

5. Conclusion

The present results provide new information showing the antinociceptive effect of the leukotriene D_4 receptor antagonist, MK-571, at peripheral sites. Moreover, the blockage of this antinociception by naloxone suggests a possible interaction between peptido-leukotrienes and the opioid system in nociceptive responses. Further studies will clarify the mechanism(s) of leukotrienes in opioid-mediated processes in pain transmission.

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References

Amann, R., Schuligoi, R., Lanz, I., Peskar, B.A., 1996. Effect of a 5-lipoxygenase inhibitor on nerve growth factor-induced thermal hyperalgesia in the rat. Eur. J. Pharmacol. 306, 89–91.

Berkenkopf, J.W., Weichman, B.M., 1988. Product of prostacyclin in mice following intraperitoneal injection of acetic acid, phenylbenzoquinone and zymosan: its role in the writhing response. Prostaglandins 36, 693–709.

Bisgaard, H., Kristensen, J.K., 1985. Leukotriene B produces hyperalgesia in humans. Prostaglandins 30, 791–797.

Dahlén, S.E., Bjork, J., Hedqvist, P., Arfors, K.E., Hammarstrom, S., Lindgren, J.A., Samuelsson, B., 1981. Leukotrienes promote plasma leakage and leukocyte adhesion in postcapillary vessels: in vivo effects with relevance to the acute inflammatory response. Proc. Natl. Acad. Sci. U.S.A. 78, 3887–3891.

Dallob, A., Guindon, Y., Goldenberg, M.M., 1987. Pharmacological evidence for a role of lipoxygenase products in platelet-activating factor (PAF)-induced hyperalgesia. Biochem. Pharmacol. 36, 3201–3204.

D'Amour, F.E., Smith, D.L., 1941. A method for determining the loss of pain sensation. J. Pharmacol. Exp. Ther. 72, 74–79.

Doherty, N.S., Poubelle, P., Borgeat, P., Beaver, T.H., Westrich, G.L., Schrader, N.L., 1985. Intraperitoneal injection of zymosan in mice induces pain, inflammation and the synthesis of peptido-leukotrienes and prostaglandin E_2. Prostaglandins 30, 769–789.

Dubuisson, D., Dennis, S.G., 1977. The formalin test: a quantitative study of the analgesic effects of morphine, meperidine, and brainstem stimulation in rats and cats. Pain 4, 161–174.

Ford-Hutchinson, A.W., 1985. Leukotrienes: their formation and role as inflammatory mediators. Fed. Proc. 44, 25–29.

Ford-Hutchinson, A.W., Bray, M.A., Doig, M.V., Shipley, M.E., Smith, M.J.H., 1980. Leukotriene B, a potent chemokinetic and aggregating substance released from polymorphonuclear leukocytes. Nature 286, 264–265.

Gök, S., Gacar, N., Erciyes, N., Kalyoncu, N.I., 1995. The antinociceptive effect of a leukotriene receptor antagonist and a dual inhibitor of arachidonic acid by two tests in mice. Pharmacol. Res. 31, 118, (Suppl.).

Hayashi, G., Takemori, A.E., 1971. The type of analgesic–receptor interaction involved in certain analgesic assays. Eur. J. Pharmacol. 16, 63–66.

Hunskaar, S., Fasmer, O.B., Hole, K., 1985. Formalin test in mice, a useful technique for evaluating mild analgesics. J. Neurosci. Methods 14, 69–76.

Hylden, J.L.K., Thomas, D.A., Iadorola, M.J., Nahin, R.L., Dubner, R., 1991. Spinal opioid analgesic effects are enhanced in a model of unilateral inflammation/hyperalgesia: possible involvement of noradrenergic mechanisms. Eur. J. Pharmacol. 194, 135–143.

Jones, T.R., Zamboni, R., Belley, M., Champion, E., Charette, L., Ford-Hutchinson, A.W., Frenette, R., Gauthier, J.-Y., Leger, S., Masson, P., Mcfarlane, C.S., Piechuta, H., Rokach, J., Williams, H., Young, R.N., 1989. Pharmacology of L-660,711 (MK-571): a novel potent and selective leukotriene D_4 receptor antagonist. Can. J. Physiol. Pharmacol. 67, 17–28.

Lembeck, F., 1983. Mediators of vasodilation in the skin. Br. J. Dermatol. 25, 1, Suppl.

Levine, J.D., Lau, W., Kwiat, G., Goetzl, E.J., 1984. Leukotriene B_4 produces hyperalgesia that is dependent on polymorphonuclear leukocytes. Science 225, 743–745.

Litchfield, J.T., Wilcoxon, F., 1949. A simplified method of evaluating dose–effect experiments. J. Pharmacol. Exp. Ther. 96, 99–108.

Madison, S., Whitsel, E.A., Suarez-Roca, H., Maixner, W., 1992. Sensitizing effects of leukotriene B_4 on intradental primary afferents. Pain 49, 99–104.

Martin, W.R., 1983. Pharmacology of opioids. Pharmacol. Rev. 35, 283–323.

Martin, H., Basbaum, A., Kwiat, G., Goetzl, E., Levine, J., 1987. Leukotriene and prostaglandins sensitization of cutaneous high-threshold C- and A delta mechanonociceptors in hairy skin of rat hind limbs. Neuroscience 22, 651–659.

McLeod, J.D., Piper, P.J., 1992. Effect of K^+ channel-modulating drugs on the vasocontrictor responses of leukotrienes C_4, D_4 and angiotensin II in the guinea-pig isolated perfused heart. Br. J. Pharmacol. 105, 739–743.

Millar, D.B., Mazorow, D.L., Hough, C., 1997. The K^+ channel blocker, tetraethylammonium, displaces beta endorphine and naloxone from T-cell binding sites. J. Neuroimmunol. 78, 8–18.

Pasotti, D., Mazzone, A., Lecchini, S., Frigo, G.M., Ricevuti, G., 1993. The effect of opioid peptides on peripheral blood granulocytes. Riv. Eur. Sci. Med. Pharmacol. 15, 71–81.

Quock, R.M., Curtis, B.A., Reynolds, B.J., Mueller, J.L., 1993. Dose-dependent antagonism and potentiation of nitrous oxide antinociception by naloxone in mice. J. Pharmacol. Exp. Ther. 267, 117–122.

Rosland, J.H., Tjølsen, A., Mæhle, B., Hole, K., 1990. The formalin test in mice: effect of formalin concentration. Pain 42, 235–242.

Samuelsson, B., 1983. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 220, 568–575.

Shibata, M., Ohkubo, T., Takahashi, H., Kudo, T., Inoki, R., 1989. Modified formalin test: characteristic biphasic pain response. Pain 38, 347–352.

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

Stein, C., Millan, M.J., Shippenberg, T.S., Peter, K., Herz, A., 1989. Peripheral opioid receptors mediating antinociception in inflammation. Evidence for involvement of mu, delta and kappa receptors. J. Pharmacol. Exp. Ther. 248, 1269–1275.

Wang, H., Lin, J., Liu, J., 1995. Regulation of anti-SRBC antibody production by opioids and their mechanisms. Chin. Med. Sci. J. 10, 125–130.

Welch, S.P., Dunlow, L.D., 1993. Antinociceptive activity of intrathechally administered potassium channel openers and opioid agonists: a common mechanism of action? J. Pharmacol. Exp. Ther. 267, 390–399.

Wheeler-Aceto, H., Porrecca, F., Cowan, A., 1990. The rat paw formalin test: comparison of noxious agents. Pain 40, 229–238.