Available online at www.sciencedirect.com
SCIENCE DIRECT®
European Journal of Pharmacology 491 (2004) 21–30
www.elsevier.com/locate/ejphar

Involvement of spinal lipoxygenase metabolites in hyperalgesia and opioid tolerance

Tuan Trang[a], Benjamin McNaull[a], Remi Quirion[b], Khem Jhamandas[a,*]

[a] Department of Pharmacology and Toxicology, Faculty of Health Sciences, Queen's University, Kingston, Ontario, Canada, K7L 3N6
[b] Douglas Hospital Research Centre and Department of Psychiatry, McGill University, Montreal, Quebec, Canada, H9H 1R3

Received 8 December 2003; received in revised form 2 March 2004; accepted 5 March 2004

Abstract

This study investigated role of spinal lipoxygenase metabolites in induction of hyperalgesia and development of opioid analgesic tolerance. In the rat, nociception was measured using formalin and tail-flick tests. Intrathecal administration of leukotriene receptor agonist (LTB_4) augmented the second phase of the formalin response and marginally increased sensitivity to acute thermal stimulation in the tail-flick test, responses suppressed by 6-(6-(3*R*-hydroxy-1*E*,5*Z*-undecadien-1-yl)-2-pyridinyl)-1,5*S*-hexanediol (U75302), a leukotriene BLT receptor antagonist. Treatment with 15-hydroxyperoxyeicosatetranoic acid (HPETE) increased phase II formalin activity, but had no effect on tail-flick responses. 12-HPETE failed to produce an effect in either nociceptive test. In the second part of this study, chronic spinal morphine for 5 days produced progressive decline in morphine antinociception and loss in analgesic potency. These effects were attenuated by co-administration of morphine with selective and nonselective lipoxygenase inhibitors. These results suggest involvement of lipoxygenase metabolites in both pain modulation and induction of opioid tolerance at the spinal level.

Keywords: Hyperalgesia; Lipoxygenase; Opioid tolerance; Spinal, rat

1. Introduction

Metabolism of arachidonic acid via activity of the cyclo-oxygenase and lipoxygenase pathways yields prostaglandins and several lipoxygenase metabolites, respectively. It is widely recognized that in states of peripheral inflammatory pain, prostaglandins generated locally act to sensitize peripheral nociceptors to noxious stimuli, resulting in the induction of hyperalgesia. There is evidence that at the peripheral level, lipoxygenase metabolites exert similar effects. Intraplantar injection of leukotriene receptor agonist (LTB_4) or 8(*R*),15(*S*)-dihydroxy-eicosa-5*cis*-9,11,13-*trans*-tetraenoic acid (8*R*,15*S*-diHETE), which are metabolites derived from the 5- and 15-lipoxygenase pathways, respectively, evokes a profound hyperalgesic response likely involving sensitization of C- and A-delta nociceptors (Rackham and Ford-Hutchinson, 1983; Levine et al., 1984, 1985; Martin et al., 1987, 1988). LTB_4 has been shown to produce both thermal and mechanical hyperalgesia (Bisgaard and Kristensen, 1985; Martin et al., 1987, 1988). Pretreatment of animals with a 5-lipoxygenase inhibitor or a leukotriene BLT receptor antagonist effectively blocks hyperalgesia and inflammatory response associated with peripheral injections of carrageenan (Tonussi and Ferreira, 1999; Jain et al., 2001).

At the level of the central nervous system, considerable evidence has shown that activity of prostaglandins also produces nociception. Activation of spinal prostaglandin receptors sensitizes primary afferent fibres and increases excitability of dorsal horn neurons (Malmberg and Yaksh, 1992; Vasko et al., 1994), an effect that underlies induction of thermal hyperalgesia (Taiwo and Levine, 1988; Uda et al., 1990) and tactile allodynia (Minami et al., 1994). Whether lipoxygenase metabolites play a similar role in spinal nociception is not known; however, indirect evidence favours this possibility. Ritchie et al. (2000) demonstrated that hyperalgesia induced by intrathecal injections of substance P or *N*-methyl-D-aspartate (NMDA) is suppressed by spinal administration of lipoxygenase inhibitors, suggesting lipoxygenase metabolites are involved in the expression of this nociceptive response. Additionally, lipoxygenase mRNA has been localized in

* Corresponding author. Tel.: +1-613-533-6119; fax: +1-613-533-6412.
E-mail address: jhamanda@post.queensu.ca (K. Jhamandas).

0014-2999/$ - see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.ejphar.2004.03.022

the spinal cord (Kawajiri et al., 2000); thus, lipoxygenase metabolites could be generated in this region in response to nociceptive stimuli. Recently, Gilbert et al. (2003) reported that induction of spinal cysteinyl leukotriene receptor (CysLT1R) in mice contributes to inflammation-associated neuropathic pain. To further elucidate the role of lipoxygenase metabolites in spinal nociception, the first part of this study examined whether direct spinal administration of metabolites yielded by the 5-, 12- and 15-lipoxygenase pathways induces pro-nociceptive responses in the formalin test, a model of persistent inflammatory pain, and in the tail-flick test, a model of acute thermal nociception.

The second part of this study investigated the role of lipoxygenase metabolites in the development of spinal morphine tolerance, a phenomenon attributed to the development of progressive latent hyperalgesia involving activity of spinal pro-nociceptive transmitters (L-glutamate, calcitonin gene-related peptide (CGRP), substance P) and prostaglandins. Blockade of glutamate, CGRP or substance P receptors effectively inhibits induction of tolerance to spinal morphine analgesia (Mao et al., 1994; Powell et al., 2000, 2003) and suppresses precipitated morphine withdrawal (Trang et al., 2002). Previous evidence suggests that pro-nociceptive actions of L-glutamate and substance P are mediated through the release of spinal prostaglandins (Marriott et al., 1991a,b; Malmberg and Yaksh, 1992; Hua et al., 1999). Our studies demonstrate that activity of sensory neurotransmitters contributing to the development of morphine tolerance–dependence is also expressed via spinal prostaglandins. Thus, spinal administration of cyclooxygenase inhibitors, including a cyclooxygenase-2 selective inhibitor, known to decrease prostaglandin synthesis, blocks the development of morphine tolerance (Powell et al., 2000; Wong et al., 2000) and attenuates morphine withdrawal (Dunbar et al., 2000; Trang et al., 2002). However, inhibition of cyclooxygenase activity diverts arachidonic acid into the lipoxygenase pathway yielding lipoxygenase metabolites (Kirchner et al., 1997; Gilroy et al., 1998), some of which modulate opioid analgesia (Vaughan et al., 1997; Gök et al., 1999; Christie et al., 2000). Recent evidence has shown significant induction in expression and activity of lipoxygenase following repeated morphine exposure (Patel et al., 2003). Activity of lipoxygenase has also been implicated in the genesis of opioid physical dependence. Capasso (1999) demonstrated that inhibition of lipoxygenase activity effectively suppresses acute opioid withdrawal in isolated guinea pig ileum. Using an in vivo rat model, we showed that spinal administration of lipoxygenase inhibitors significantly reduces both the behavioural and neurochemical manifestations of precipitated morphine withdrawal (Trang et al., 2003). Whether lipoxygenase activity also contributes to the development of opioid tolerance is not known. To determine this, the present study examined effects of selective and nonselective lipoxygenase inhibitors on the development of analgesic tolerance to chronic morphine in the spinal model.

2. Methods

2.1. Intrathecal catheterization and drug injection

All experiments were carried out in accordance with the guidelines of the Canadian Council on Animal Care using protocols approved by the University Animal Care Committee. Male Sprague–Dawley rats (200–250 g) (Charles River, Quebec) were housed in separate cages and maintained on a 12-h-light/12-h-dark cycle with access to food and water ad libitum. Animals were implanted with indwelling intrathecal catheters using the method previously described by Yaksh and Rudy (1976). Briefly, animals were placed in a stereotaxic frame and anesthetized using halothane (4%). A small puncture was made in the atlanto-occipital membrane and a polythene catheter (PE-10; 7.5 cm long) inserted caudally so that the tip rested on the lumbar enlargement of the spinal cord and the rostral end was exteriorized to facilitate drug administration. Surgical wounds were closed with sutures and animals allowed to recover for 1 week. Animals showing signs of motor dysfunction (forelimb or hindlimb paralysis) were excluded from experiments. Drugs were injected in a 10-μl volume (i.t.) followed by 10 μl of 0.9% saline to flush the catheter.

2.2. Behavioural assessment of nociception

2.2.1. Formalin test

The formalin test has been shown to be a reliable model of clinical pain associated with inflammation (Dubuisson and Dennis, 1977). One hour prior to testing, animals were placed in a Plexiglas chamber for habituation to the test environment. A mirror was situated behind the chamber to facilitate observation during testing. Agents tested (see below) were administered intrathecally immediately prior to formalin injection.

For formalin injection, animals were lightly anesthetized with halothane so that a transient loss of spontaneous movement was observed. Subsequently, 50 μl of 1.5% formalin in saline was injected subcutaneously into the right plantar surface of the hindpaw; control animals received an intraplantar injection of saline. Animals were returned to the observation chamber immediately following injection. Time zero of the study was defined as injection of formalin or saline. It is well established that the formalin-evoked nociceptive response is characterized by shaking, licking and elevation of the injected paw (Abbott et al., 1981; Sugimoto et al., 1986). These behavioural responses were measured with a hand-held stopwatch for 5-min periods from 0 to 60 min and converted into seconds (maximum score of 300 s per interval). Tjolsen et al. (1992) have previously shown that there are two distinct phases of the formalin-evoked

nociceptive response: an early phase (phase 1) lasting the first 5 min that is the result of direct chemical stimulation of nociceptors and a late phase (phase 2) caused by a latent inflammatory reaction lasting from 20 to 60 min. Scores for the late phase (phase 2) of the formalin test were averaged over the 20–60-min interval. Animals were used only for one measurement in each experiment.

2.2.2. Tail-flick test

The tail-flick test was used to evaluate response to an acute thermal nociceptive stimulus (D'Amour and Smith, 1941). In this test, radiant heat was applied to the dorsal surface of the tail using an analgesic meter (Owen et al., 1981), and the time latency for removal of the tail from the stimulus was recorded. In the hyperalgesia study, the heat source was adjusted to yield a baseline response of 5.5–6.5 s, which allowed for the detection of increased pain response. In the analgesic tolerance study, baseline response was adjusted to 2–3 s to allow measurement of morphine-induced antinociception. A cutoff of 10 s was used in both studies to prevent tissue injury.

2.2.3. Paw-pressure test

The paw-pressure test was employed in the tolerance study to assess response to a mechanical nociceptive stimulus (Loomis et al., 1987). Using an air-filled inverted syringe, pressure was applied to the dorsal surface of the hindpaw. Pressure was gradually increased until a paw-withdrawal response was observed and the value (mm Hg) recorded. A cutoff of 300 mm Hg was used to prevent tissue injury. Previous experiments have reported no interaction between responses in the tail-flick and paw-pressure tests (Loomis et al., 1985).

2.3. Experimental paradigm

2.3.1. Study 1: role of spinal lipoxygenase metabolites in nociception

The formalin test and the thermal tail-flick test were employed to evaluate the role of lipoxygenase metabolites in nociception. Animals were used for one study and evaluated using only one of these nociceptive tests. Immediately prior to testing, animals were given an intrathecal injection of either saline, nordihydroguaiaretic acid (NDGA, a nonselective lipoxygenase inhibitor), LTB_4 (a 5-lipoxygenase metabolite), 12-hydroxyperoxyeicosatetranoic acid (HPETE) (a 12-lipoxygenase metabolite), or 15-HPETE (a 15-lipoxygenase metabolite). 6-(6-(3*R*-hydroxy-1*E*,5*Z*-undecadien-1-yl)-2-pyridinyl)-1,5*S*-hexanediol (U75302), a leukotriene BLT receptor antagonist, was administered 10 min prior to LTB_4 injection.

Assessment of the formalin-evoked nociceptive response was made in blind fashion by two investigators for a total of 60 min according to the guidelines outlined above. The ability of acute intrathecal drug treatment to influence the formalin response was measured by their effect on the total time spent exhibiting formalin-evoked nociceptive behaviours.

In the thermal nociceptive tail-flick test (see above), animals were tested every 10 min for the first 60 min, and a final measurement was taken at the 90-min time point. The ability of drug treatment to influence the thermal nociceptive response was indicated by changes in the latency of response as compared to baseline.

2.4. Study 2: role of spinal lipoxygenase metabolites in the development of opioid tolerance

2.4.1. Induction of spinal morphine tolerance

To induce morphine tolerance, animals were administered morphine (15 μg, i.t.) once daily between 10:00 and 11:00 a.m. for 5 days, as described in an earlier study by Powell et al. (1999). Tail-flick and paw-pressure nociceptive tests (see above) were performed both before (baseline) and 30 min after drug injection (drug-induced response). The peak antinociceptive effect of morphine has been shown to occur 30 min after injection (Gouarderes et al., 2000). Following the treatment period on day 6, animals were given ascending doses of morphine every 30 min until a maximal level of antinociception was reached in both the tail-flick and paw-pressure tests. The results of this testing was used to generate cumulative morphine dose–response curves from which the morphine ED_{50} value, an indicator of morphine agonist potency, was derived. A progressive decline in the daily antinociceptive effect of morphine and an increase in the ED_{50} value of morphine indicate a state of tolerance.

2.4.2. Effect of intrathecal administration of lipoxygenase inhibitors on morphine tolerance

To examine the role of lipoxygenase metabolites in the development of spinal opioid tolerance, NDGA, a nonselective lipoxygenase inhibitor, baicalein, a 12-lipoxygenase inhibitor, or (2-[12-hydroxydodeca-5,10-diynyl]-3,5,6-trimethyl-*p*-benzoquinone) AA-861, a 5-lipoxygenase inhibitor, was co-administered with intrathecal morphine once daily for 5 days. Nociceptive testing was performed daily and cumulative dose–response curves generated on the sixth day as described above. The action of lipoxygenase inhibitors on the induction of tolerance was measured by examining their effect on the decline in magnitude of morphine antinociception over the 5-day administration period and on the morphine ED_{50} values assessed at the end of this period.

2.5. Drugs

Formalin (10%) (Sigma Diagnostics, USA) was diluted with saline to make a 1.5% formalin solution. LTB_4, 12-HPETE, 15-HPETE and U75302 were purchased from Caymen Chemicals (Canada) and stored in ethanol solution at −20 °C. For injection, ethanol was removed by evap-

oration to dryness under nitrogen gas and then dissolved in physiological saline (0.9%). Morphine sulphate (BDH Pharmaceuticals, Canada) was also dissolved in saline. NDGA (nordihydroguaiaretic acid), AA-861 (2-(12-hydroxydodeca-5,10-diynyl)-3,5,6-trimethyl-1,4-benzoquinone) and baicalein were dissolved in 5% cyclodextrin (Sigma-Aldrich, Canada).

2.6. Data analysis

All values in the figures and text are expressed as mean ± standard error of the mean (S.E.M.) of n observations. Statistical significance ($p < 0.05$) was determined by one-way analysis of variance (ANOVA) followed by appropriate post hoc tests (Newman–Keuls). Tail flick and paw-pressure values were converted to a maximum percent effect (MPE): MPE = 100 × [post-drug response − baseline response]/[cutoff value − baseline response]. The ED_{50} values were determined using a nonlinear regressional analysis (Prizm 2, GraphPad Software).

3. Results

3.1. Study 1: the role of spinal lipoxygenase metabolites in nociception

3.1.1. Effect of intraplantar formalin or saline injection in the rat hindpaw

As illustrated in Fig. 1A, intraplantar injection (50 μl) of 1.5% formalin produced a biphasic nociceptive response characterized by shaking, licking and elevation of the injected hindpaw. These behaviours were not seen in animals given intraplantar saline injection (50 μl). Specifically, formalin injection elicited an immediate nociceptive response that lasted for 5 min (phase I) in duration and

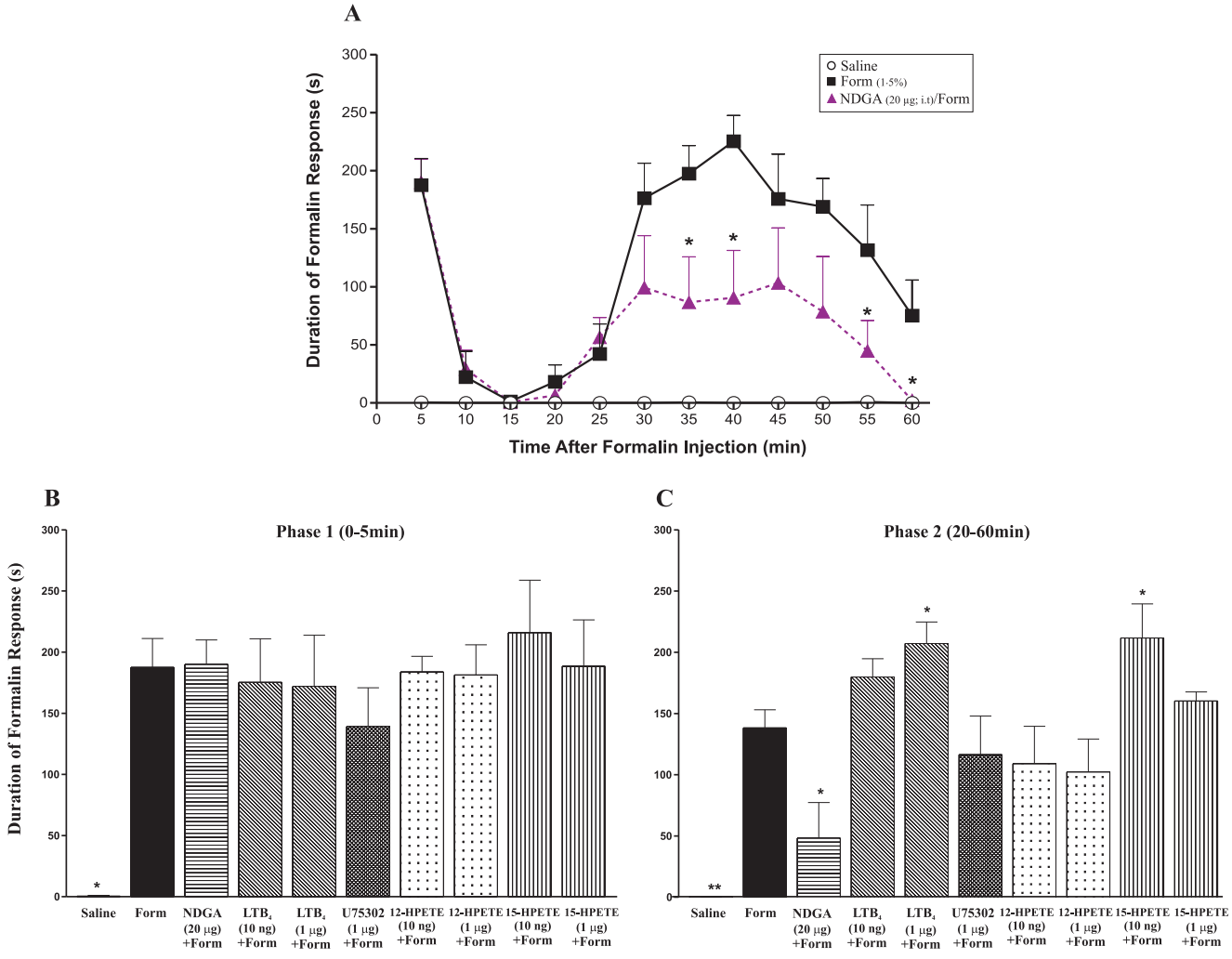

Fig. 1. Effect of intrathecal administration of lipoxygenase metabolites on the duration of formalin response. Agents were given immediately prior to intraplantar formalin (1.5%) injection and nociception scored for a 60-min period. (A) represents the time course of formalin response and effect of NDGA, a nonselective lipoxygenase inhibitor, (B) represents the duration of formalin response in phase 1 and (C) represents the average response in phase 2. The data are expressed as mean ± S.E.M., $n = 4–6$ animals per treatment group. Asterisks represent significant difference from formalin-only-injected animals; $^*p < 0.05$.

reflects direct chemical activation of peripheral nociceptors (Dickenson and Sullivan, 1987). The mean duration of the phase I formalin response was 187.5 ± 23.6 s ($n = 6$), a value significantly different from that in animals given corresponding intraplantar saline injection 0.5 ± 0.5 s ($n = 5$) ($p < 0.01$). This response was followed by a short quiescent period (5–20 min) during which animals exhibited minimal nociceptive behaviours (11.7 ± 11.3 s) (Fig. 1A). However, at 20 min post-formalin injection, there was a marked increase in nociceptive activity representative of phase II (20–60 min) of the formalin response. This late phase reflects a persistent inflammatory state (Malmberg and Yaksh, 1993; Yaksh and Malmberg, 1993). The average duration of the formalin-evoked nociceptive response in the second phase was 138.3 ± 14.3 s, which is significantly different from the saline-treated group (0.5 ± 0.1 s) ($p < 0.01$). Pretreatment with the nonselective lipoxygenase inhibitor, NDGA (20 μg, i.t.; $n = 5$), did not affect phase I responses but significantly attenuated phase II of the formalin response (Fig. 1A). Thus, lipoxygenase metabolites generated in the spinal cord likely contribute to the persistent nociceptive behaviours observed in the second phase of the formalin test.

3.1.2. Effect of lipoxygenase metabolites on the formalin response

To investigate whether lipoxygenase metabolites play a role in the formalin response, specific metabolites (LTB_4, 12-HPETE, 15-HPETE) were injected intrathecally. These lipoxygenase metabolites were chosen on the basis of results obtained in previous studies demonstrating their involvement in peripheral hyperalgesia in the rat model (Rackham and Ford-Hutchinson, 1983; Levine et al., 1984, 1986; Shin et al., 2002). Administration of LTB_4 or 15-HPETE had no effect on initial phase I response but significantly augmented second-phase formalin-evoked behaviours (Fig. 1B and C). Treatment with LTB_4 at a dose of 1 μg ($n = 5$) noticeably increased the duration of shaking, licking and elevation of the injected hindpaw in phase II (207.2 ± 17.5 s) ($p < 0.05$) as compared to the saline-treated control group. This response was effectively suppressed (116.4 ± 31.5 s) ($p < 0.05$) by pretreatment with a selective leukotriene BLT receptor antagonist, U75302 (1 μg; $n = 5$), suggesting that the hyperalgesic effects of LTB_4 is receptor mediated. Baseline formalin responses were not altered by acute intrathecal injection of U75302 (1 μg; $n = 5$) alone (not shown). Although the duration of the formalin response was increased by LTB_4 at a lower dose of 10 ng, this effect was not statistically significant. Administration of 15-HPETE at a dose of 10 ng ($n = 5$) also elevated phase II of the formalin-evoked response, but did not influence nociceptive activity in phase I (Fig. 1C). Interestingly, this effect of 15-HPETE was not seen at the higher dose of 1 μg ($n = 5$). Injection of 12-HPETE at a dose of 10 ng ($n = 4$) or at 1 μg ($n = 5$), failed to produce a noticeable effect on either the first or second phase of the formalin response. Collectively, these results indicate that 5- and 15-lipoxygenase-derived metabolites play a role in sensitization of nociceptive fibres associated with the formalin-evoked inflammatory response.

3.1.3. Effect of lipoxygenase metabolites on acute thermal nociception

The effect of intrathecal lipoxygenase metabolite administration in the thermal nociceptive tail-flick test is represented in Fig. 2A and B. In saline-treated control animals, average baseline response to acute thermal stimulation over the 90-min testing period was 6.0 ± 0.3 s ($n = 5$). As illustrated in Fig. 2A, administration of LTB_4 produced a dose-dependent decrease in the latency of tail-flick response. At the lowest dose tested, 0.1 ng of LTB_4 did not alter tail-withdrawal response (6.3 ± 0.3 s) ($n = 8$) (not shown). A higher dose of 10 ng ($n = 8$) produced a transient reduction in the latency of response (5.0 ± 0.2 s) at 20 min following LTB_4 administration; however, this effect was not statistically significant. A more pronounced reduction in the latency of tail-flick response was observed after injection of LTB_4 at 1 μg ($n = 9$), with a maximal effect (4.3 ± 0.3 s)

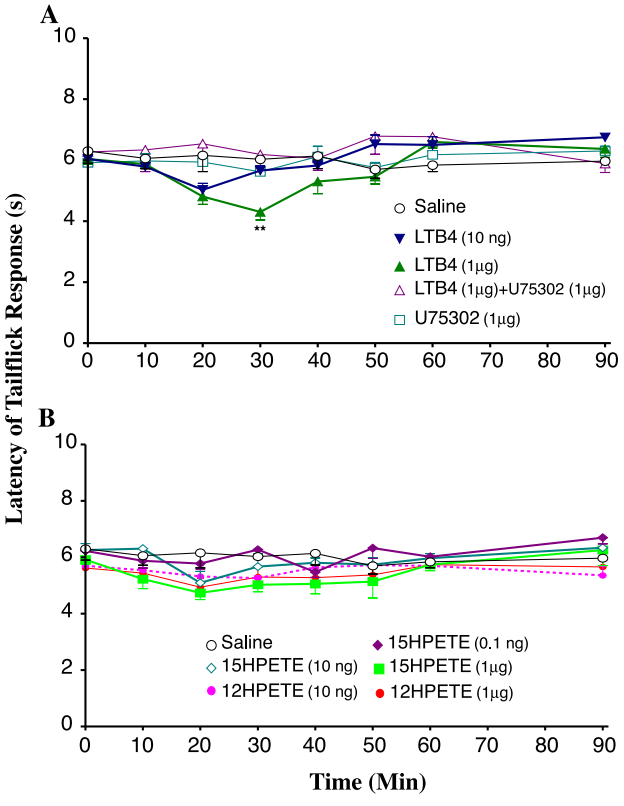

Fig. 2. Time course of latency of tail-flick response following intrathecal administration of various lipoxygenase metabolites. Nociceptive testing was performed at 10-min periods for a total of 90 min. (A) represents the effect of LTB_4, a 5-lipoxygenase metabolite, and U75302, a leukotriene BLT receptor antagonist, (B) illustrates the effect of 12-HPETE and 15-HPETE, which are 12- and 15-lipoxygenase metabolites, respectively, on tail-withdrawal response from an acute noxious thermal stimulus. The data are expressed as mean ± S.E.M., $n = 5–9$ animals per treatment group. Asterisks represent significant difference from saline-treated animals; $^*p < 0.05$, $^{**}p < 0.01$.

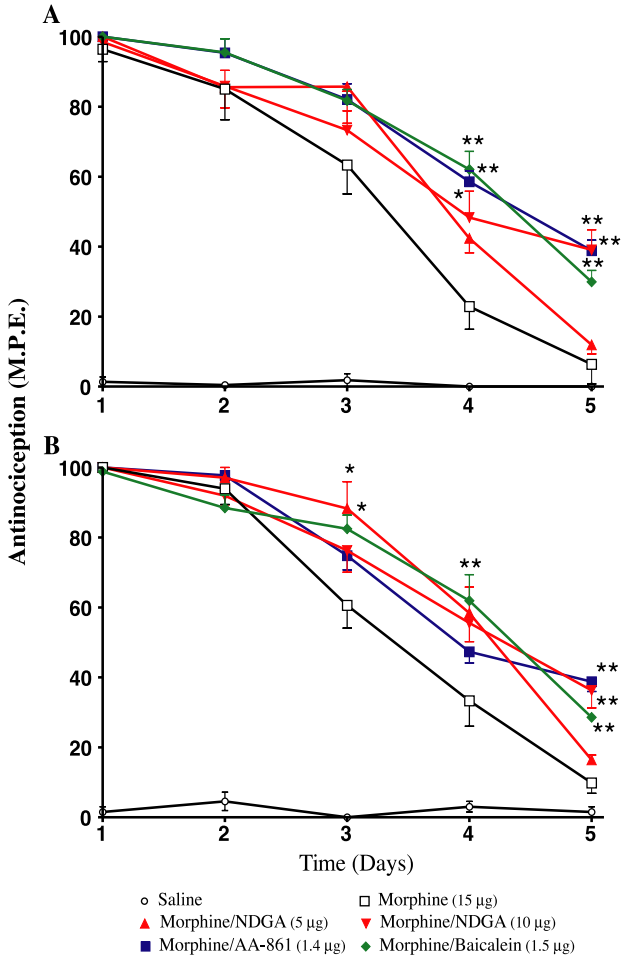

Fig. 3. Time course of the antinociceptive effect of daily intrathecal morphine (15 μg) alone and in combination with a nonselective lipoxygenase inhibitor (NDGA), a 5-lipoxygenase inhibitor (AA-861), and a 12-lipoxygenase inhibitor (baicalein) in the (A) tail-flick and (B) paw-pressure tests. Nociceptive testing was performed 30 min following each injection. The data are expressed as mean ± S.E.M., $n = 4–8$ animals per treatment group. Asterisks represent significant difference from morphine-only-treated animals; $*p < 0.05$, $**p < 0.01$.

($p < 0.01$) occurring at the 30-min interval and returning to baseline after 60 min. Injection of U75302 (1 μg, i.t.), a leukotriene BLT receptor antagonist, had no effect on baseline response when given alone; however, pretreatment of this antagonist abolished LTB_4-induced hyperalgesia, suggesting that the increased response to thermal stimulation elicited by LTB_4 is receptor mediated (Fig. 2). Administration of 15-HPETE (1 μg; $n = 7$) produced a slight but statistically insignificant reduction in tail-withdrawal response (Fig. 2B). Indeed, animals given 15-HPETE at a dose of 0.1 ng ($n = 6$) or 10 ng ($n = 7$), exhibited similar tail-flick latencies to saline-treated controls. Similarly, intrathecal administration of 12-HPETE at 10 ng ($n = 5$) and 1 μg ($n = 5$) failed to produce an effect on acute thermal response. Thus, these findings suggest that spinal lipoxygenase metabolites play a minimal role in acute thermal nociception in the rat.

3.2. Study 2: role of spinal lipoxygenase metabolites in the development of opioid tolerance

Fig. 3A and B illustrates the effects of lipoxygenase inhibitors on the antinociceptive action of chronic intrathecal morphine in the tail-flick and paw-pressure tests. In saline-treated ($n = 4$) control animals, the baseline latency of response in the tail-flick test was 1.4 ± 1.4 s, and the threshold pressure to induce a paw-withdrawal response was 95 ± 8.5 mm Hg. These values were not significantly affected by repeated 5-day saline injection. Administration of morphine (15 μg) ($n = 5$) to naïve animals on day 1 produced maximal antinociception in both tests. However, repeated daily administration of this dose resulted in a progressive decline of morphine antinociception to baseline levels by day 5, reflecting development of tolerance to the analgesic actions of morphine (Fig. 3A and B). Co-administration of nonselective and selective lipoxygenase inhibitors attenuated this decline in morphine effect. NDGA, a nonselective lipoxygenase inhibitor, attenuated the decline

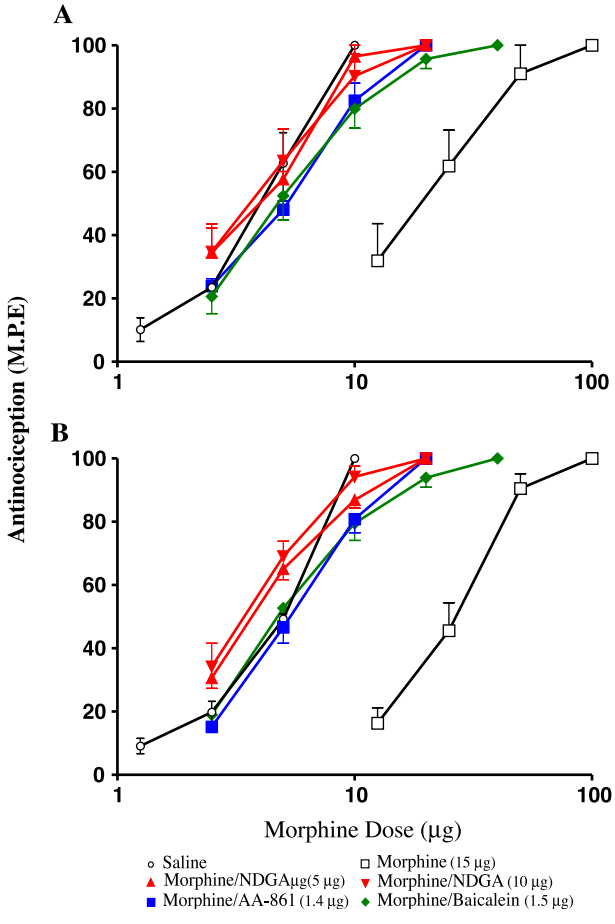

Fig. 4. Dose–response curves of the analgesic effects of acute intrathecal morphine following 5-day chronic treatment in (A) tail-flick and (B) paw-pressure tests. On day 6, animals were administered ascending doses of morphine every 30 min until a maximal level of antinociception was achieved in both nociceptive tests. The data are expressed as mean± S.E.M., $n = 4–8$ animals per treatment group.

in morphine antinociception. In the animals given 10 μg of NDGA ($n=6$) in combination with morphine, antinociception on days 4–5 ($p<0.05$) in both nociceptive tests was significantly greater than that in the morphine-only-treated group. Treatment with a lower dose of NDGA (5 μg) ($n=6$) slightly attenuated the decline in morphine antinociception; however, this effect was only statistically significant in the paw-pressure test on day 3 ($p<0.05$). Co-treatment of morphine with AA-861 (1.4 μg) ($n=8$), a selective 5-lipoxygenase inhibitor, also prevented the loss of morphine antinociception with significant effect on days 4–5 ($p<0.01$) in the tail-flick test and on day 5 ($p<0.01$) in the paw-pressure test. A similar response was observed in animals given baicalein (1.5 μg) ($n=8$), a selective 12-lipoxygenase inhibitor, with significance achieved on days 4–5 ($p<0.01$) in the tail-flick test and on days 3–5 ($p<0.01$) in the paw-pressure test. When administered at the dose tested without morphine, all three inhibitors failed to produce antinociception in either test (not shown).

Following the 5-day chronic treatment period, cumulative dose–response curves for the acute effects of morphine were generated on day 6 (Fig. 4A and B). Animals were given ascending doses of morphine at 30-min periods until a maximal level of antinociception was reached in both the tail-flick and paw-pressure tests. ED_{50} values for morphine were derived from the constructed dose–response curves (Table 1). As illustrated in Fig. 4A and B, morphine administration in saline control (morphine-naïve) animals produced a dose-dependent analgesic effect in both the tail-flick and paw-pressure tests (Table 1). Animals treated with 5-day intrathecal morphine (15 μg) required higher doses before maximal antinociception was achieved in these tests. This is reflected by a significant rightward shift in the cumulative dose–response curve and approximately a five-fold increase in ED_{50} values compared to saline controls, an effect indicating substantial loss in morphine analgesic potency (tolerance). Co-administration of a lipoxygenase inhibitor with morphine during tolerance induction prevented the rightward shift in the cumulative dose–response curve (Fig. 4A and B) and completely blocked the increase in ED_{50} values (Table 1). Indeed, ED_{50} values obtained in groups given morphine/NDGA (5 μg), morphine/NDGA (10 μg), morphine/AA-861 (1.4 μg) and morphine/baicalein (1.5 μg), were comparable to saline control values (Table 1). Administration of the lipoxygenase inhibitors alone for 5 days did not significantly alter the morphine ED_{50} values from that obtained with chronic saline treatment.

Table 1
Effect of lipoxygenase inhibitors on the development of spinal morphine tolerance

Chronic treatment	Tail flick	Paw pressure
	ED_{50} (μg) (mean ± S.E.M.)	ED_{50} (μg) (mean ± S.E.M.)
Saline	3.8 ± 0.5	4.5 ± 0.2
Morphine (15 μg)	18.9 ± 6.0[a]	23.5 ± 3.5[b]
Morphine/NDGA (5 μg)	2.9 ± 0.6	2.6 ± 0.4
Morphine/NDGA (10 μg)	2.8 ± 0.3	3.0 ± 0.1
NDGA (10 μg)	3.1 ± 0.6	3.9 ± 0.3
Morphine/AA-861 (1.4 μg)	4.3 ± 0.4	4.8 ± 0.4
AA-861 (1.4 μg)	4.1 ± 0.3	3.9 ± 0.7
Morphine/baicalein (1.5 μg)	4.6 ± 0.8	4.5 ± 0.5
Baicalein (1.5 μg)	3.8 ± 0.4	3.9 ± 0.5

Following the end of the 5-day chronic intrathecal treatment period, cumulative dose–response curves to acute morphine were generated on day 6. Animals were given ascending doses of morphine every 30 min until a maximal level of antinociception was reached in both the tail-flick and paw-pressure tests. ED_{50} values for morphine were derived from the constructed dose–response curves. [a]$p<0.05$ and [b]$p<0.01$ represent significant difference from saline-treated control group.

4. Discussion

The present study sought to investigate the role of spinal lipoxygenase metabolites in the modulation of nociception and in the development of opioid analgesic tolerance. The three metabolites, LTB_4, 12-HPETE and 15-HPETE, which originate from the 5-, 12- and 15-lipoxygenase pathways, respectively, were selected as test agents since they have previously been implicated in peripheral nociception (Levine et al., 1984, 1986; Martin et al., 1987, 1988; Shin et al., 2002). Injection of LTB_4 or 15-HPETE directly into the intrathecal space significantly enhanced nociceptive activity in the second phase of the formalin-evoked response. Administration of LTB_4, however, produced a marginal reduction in thermal latency in the tail-flick test, an effect not observed following treatment with 12- and 15-HPETE. In the second part of this study, co-administration of selective (AA-861, baicalein) and nonselective (NDGA) lipoxygenase inhibitors with intrathecal morphine for 5 days significantly attenuated the decline in antinociception and effectively blocked the loss of agonist potency associated with the development of tolerance. The results of this study provide evidence for a spinal action of lipoxygenase metabolites in the modulation of pain and in the induction of opioid tolerance.

Two distinct measures of nociception were used to assess the effects of spinal lipoxygenase metabolite administration: the formalin test, chosen because it is a reliable model of persistent inflammatory pain (Dubuisson and Dennis, 1977); the tail-flick test, chosen because it provides a model of acute response to noxious thermal input (D'Amour and Smith, 1941). The first phase of the formalin response results from direct activation of peripheral nociceptors mediated by spinal release of pro-nociceptive transmitters (L-glutamate, CGRP, substance P) (Smullin et al., 1990; Zhang et al., 1994), whereas second-phase activity represents sensitization of spinal neurons to the actions of these transmitters (Porro and Cavazzuti, 1993; Dickenson and Sullivan, 1987). The ability of NDGA, a nonselective lipoxygenase inhibitor, to significantly attenuate the second phase suggests that activity of endogenous spinal lipoxyge-

nase-derived metabolites contribute to the sensitization response. This is consistent with observations that spinal injection of LTB_4 or 15-HPETE augmented only the second phase of the formalin-induced response. It is also in accord with a previous study showing intraperitoneal administration of an LTD_4 receptor antagonist in mice effectively inhibits this phase (Gök et al., 1999).

How LTB_4 or 15-HPETE augment formalin-induced nociception is not entirely clear. However, the ability of U75302, a selective leukotriene BLT receptor antagonist, to suppress LTB_4-induced hyperalgesia indicates that this response is receptor mediated. According to electrophysiological studies, LTB_4 sensitizes A- and C-fibres to thermal and mechanical stimuli in the rat paw (Martin et al., 1987, 1988). Increase in formalin response following intrathecal LTB_4 injection could be related to a direct or indirect sensitization of these spinal nociceptive fibres. Since LTB_4 is a potent chemotactic factor involved in recruitment of inflammatory cells, an indirect mechanism involving inflammatory mediators likely underlies this response (Ford-Hutchinson et al., 1980; Levine et al., 1984; Bisgaard and Kristensen, 1985). Indeed, LTB_4 has been shown to activate ploymorphonuclear leukocytes to induce release of 8R,15S-diHETE, a metabolite of the 15-lipoxygenase pathway that itself contributes to peripheral nociception (Levine et al., 1984, 1986). 15-HPETE is readily converted into 15-HETE, lipoxins and di-HETEs (Serhan et al., 1984a,b); thus, hyperalgesia elicited by spinal injection of this precursor could also be related to increased levels of 8*R*,15*S*-diHETE. The fact that LTB_4 and 15-HPETE produced hyperalgesia in the inflammatory formalin test while producing minimal effect in the acute thermal tail-flick test further supports involvement of inflammatory mechanisms in this response. Gilbert et al. (2003) have recently reported that intrathecal injection of a cysteinyl leukotriene receptor antagonist suppresses inflammation-associated neuropathic pain, but does not affect acute thermal nociceptive response in the tail-flick or hotplate test.

In a recent study, Hwang et al. (2000) reported that LTB_4, 5-HETE, 15-HPETE, 15-HETE and 12-HPETE directly activate the capsaicin-sensitive vanilloid receptor (VR1) in sensory dorsal root ganglion neurons. Among these metabolites, 12-HPETE was found to be the most potent vanilloid VR1 receptor agonist because of its structural similarity to capsaicin (Hwang et al., 2000). At the spinal level, vanilloid VR1 receptor expression has been identified in superficial dorsal horn primary afferent terminals co-expressed with pro-nociceptive transmitters, substance P and CGRP (Skofitsch and Jacobowitz, 1985; Tominaga et al., 1998; Valtschanoff et al., 2001). Activation of the vanilloid VR1 receptor by thermal or chemical stimulation causes profound hyperalgesia related to significant spinal release of these nociceptive transmitters (Jhamandas et al., 1984; Mantyh et al., 1997). Considering the role of VR1 in spinal nociception, it is conceivable that hyperaglesia resulting from spinal injection of lipoxygenase metabolites is partially related to activation of this receptor. However, the fact that spinal injection of 12-HPETE failed to affect nociceptive responses in either the formalin or tail-flick test suggests that the vanilloid system likely plays a lesser role in LTB_4 and 15-HPETE-induced hyperalgesia and that other spinal mechanisms may be involved in this response.

Recent evidence suggests that induction of a latent hyperalgesia contribute to the loss of analgesic potency that characterizes development of opioid tolerance at the spinal level (Mao et al., 1998; Crain and Shen, 2001; Shen and Crain, 2001). Since spinal injections of lipoxygenase metabolites in the present study induced sensitization to nociceptive input, we examined whether they contribute to the loss of morphine action following chronic drug exposure. If spinal lipoxygenase activity plays a role in the genesis of opioid tolerance, then intrathecal administration of inhibitors of this activity would be expected to prevent the decline in morphine antinociception. Indeed, daily intrathecal co-administration of NDGA (nonselective lipoxygenase inhibitor), AA-861 (5-lipoxygenase inhibitor), or baicalein (12-lipoxygenase inhibitor) with morphine attenuated the progressive loss of morphine effect and blocked the increase in morphine ED_{50} that is reflective of tolerance. Patel et al. (2003) recently reported a significant induction in the expression and activity of 12-lipoxygenase following chronic morphine exposure. Thus, it is possible that repeated morphine treatment leads to increased lipoxygenase expression at the spinal level, and inhibition of activity at different loci in the enzyme cascade decreases lipoxygenase metabolite synthesis and attenuates analgesic tolerance. Considering that intrathecal administration of lipoxygenase inhibitors alone did not elicit antinociception, the observed attenuation of tolerance is unlikely to result from an additivity of their effect with that of morphine. The effects of the different lipoxygenase inhibitors are attributable to lipoxygenase inhibition. NDGA is three times more potent at inhibiting lipoxygenase activity than cyclooxygenase (Hope et al., 1983; Salari et al., 1984), and both AA-861 and baicalein are highly selective for 5- and 12-lipoxygenase, respectively, with minimal effects on cyclooxygenase activity (Sekiya and Okuda, 1982; Yoshimoto et al., 1982; Ashida et al., 1983; Dailey and Imming, 1999). We recently reported that intrathecal administration of NDGA, baicalein, or AA-861 at doses used in this study markedly suppress the behavioural and neurochemical indices of antagonist-precipitated morphine withdrawal (Trang et al., 2003). Thus, these lipoxygenase inhibitors effectively prevent induction of opioid tolerance as well as physical dependence at the spinal level. The effects on tolerance reported here are strikingly similar to those seen previously following spinal intervention with nonselective and cyclooxygenase$_2$-selective inhibitors (Powell et al., 1999; Wong et al., 2000). This suggests metabolites derived from both pathways of arachidonic acid metabolism likely contribute to the development of spinal opioid tolerance and physical dependence. These findings also raise the interesting question as to whether

lipoxygenase and cyclooxygenase products activate a common cellular pathway, a possibility that remains to be examined in future experiments.

5. Conclusion

In conclusion, the results of this study demonstrate that spinal lipoxygenase activity contributes to both the induction of hyperalgesia and the development of spinal opioid tolerance. Pharmacological treatments aimed at inhibiting this activity or at blocking receptors activated by metabolites generated by the lipoxygenase pathway could prove beneficial in both the management of pain and the maintenance of opioid analgesic efficacy.

Acknowledgements

This work was supported by a research grant and a doctoral research award from the Canadian Institutes of Health and Research. We would like to thank Maaja Sutak for technical assistance in the project.

References

Abbott, F.V., Franklin, K.B., Ludwick, R.J., Melzack, R., 1981. Apparent lack of tolerance in the formalin test suggests different mechanisms for morphine analgesia in different types of pain. Pharmacol. Biochem. Behav. 15, 637–640.

Ashida, Y., Saijo, T., Kuriki, H., Makino, H., Terao, S., Maki, Y., 1983. Pharmacological profile of AA-861, a 5-lipoxygenase inhibitor. Prostaglandins 26, 955–972.

Bisgaard, H., Kristensen, J.K., 1985. Leukotriene B4 produces hyperalgesia in humans. Prostaglandins 30, 791–797.

Capasso, A., 1999. Further studies on the involvement of the arachidonic acid cascade in the acute dependence produced by mu, kappa and delta opioid agonists in isolated tissues. Neuropharmacology 38, 871–877.

Christie, M.J., Connor, M., Vaughan, C.W., Ingram, S.L., Bagley, E.E., 2000. Cellular actions of opioids and other analgesics: implications for synergism in pain relief. Clin. Exp. Pharmacol. Physiol. 27, 520–523.

Crain, S.M., Shen, K.F., 2001. Acute thermal hyperalgesia elicited by low-dose morphine in normal mice is blocked by ultra-low-dose naltrexone, unmasking potent opioid analgesia. Brain Res. 888, 75–82.

Dailey, L.A., Imming, P., 1999. 12-Lipoxygenase: classification, possible therapeutic benefits from inhibition, and inhibitors. Curr. Med. Chem. 6, 389–398.

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

Dickenson, A.H., Sullivan, A.F., 1987. Peripheral origins and central modulation of subcutaneous formalin-induced activity of rat dorsal horn neurones. Neurosci. Lett. 83, 207–211.

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

Dunbar, S.A., Karamov, I.G., Buerkle, H., 2000. The effect of spinal ibuprofen on opioid withdrawal in the rat. Anesth. Analg. 91, 417–422.

Ford-Hutchinson, A.W., Bray, M.A., Smith, M.J., 1980. Lipoxygenase products and the polymorphonuclear leucocyte. Agents Actions 10, 548–550.

Gilbert, A., Le Gouill, C., Thompson, C., Pasternak, G., Stankova, J., Rola-Pleszczynski, M., 2003. Expression and modulation of the cysteinyl leukotriene receptor 1 (cyslt1r) in the central nervous system. Soc. Neurosci. Abstr., 695.11.

Gilroy, D.W., Tomlinson, A., Willoughby, D.A., 1998. Differential effects of inhibitors of cyclooxygenase (cyclooxygenase 1 and cyclooxygenase 2) in acute inflammation. Eur. J. Pharmacol. 355, 211–217.

Gök, S., Onal, A., Cinar, M.G., Evinc, A., 1999. The antinociceptive effect of leukotriene D(4) receptor antagonist, MK-571, in mice: possible involvement of opioidergic mechanism. Eur. J. Pharmacol. 386, 195–200.

Gouarderes, C., Sutak, M., Zajac, J.M., Jhamandas, K., 2000. Role of adenosine in the spinal antinociceptive and morphine modulatory actions of neuropeptide FF analogs. Eur. J. Pharmacol. 406, 391–401.

Hope, W.C., Welton, A.F., Fiedler-Nagy, C., Batula-Bernardo, C., Coffey, J.W., 1983. In vitro inhibition of the biosynthesis of slow reacting substance of anaphylaxis (SRS-A) and lipoxygenase activity by quercetin. Biochem. Pharmacol. 32, 367–371.

Hua, X.Y., Chen, P., Marsala, M., Yaksh, T.L., 1999. Intrathecal substance P-induced thermal hyperalgesia and spinal release of prostaglandin E2 and amino acids. Neuroscience 89, 525–534.

Hwang, S.W., Cho, H., Kwak, J., Lee, S.Y., Kang, C.J., Jung, J., Cho, S., Min, K.H., Suh, Y.G., Kim, D., Oh, U., 2000. Direct activation of capsaicin receptors by products of lipoxygenases: endogenous capsaicin-like substances. Proc. Natl. Acad. Sci. U. S. A. 97, 6155–6160.

Jain, N.K., Kulkarni, S.K., Singh, A., 2001. Role of cysteinyl leukotrienes in nociceptive and inflammatory conditions in experimental animals. Eur. J. Pharmacol. 423 (1), 85–92.

Jhamandas, K., Yaksh, T.L., Harty, G., Szolcsanyi, J., Go, V.L., 1984. Action of intrathecal capsaicin and its structural analogues on the content and release of spinal substance P: selectivity of action and relationship to analgesia. Brain Res. 306, 215–225.

Kawajiri, H., Zhuang, D., Qiao, N., Yoshimoto, T., Yamamoto, M., Iseki, S., Hamaguchi, K., 2000. Expression of arachidonate 12-lipoxygenase in rat tissues: a possible role in glucagon secretion. J. Histochem. Cytochem. 48, 1411–1419.

Kirchner, T., Aparicio, B., Argentieri, D.C., Lau, C.Y., Ritchie, D.M., 1997. Effects of tepoxalin, a dual inhibitor of cyclooxygenase/5-lipoxygenase, on events associated with NSAID-induced gastrointestinal inflammation. Prostaglandins Leukot. Essent. Fat. Acids 56, 417–423.

Levine, J.D., Lau, W., Kwiat, G., Goetzl, E.J., 1984. Leukotriene B4 produces hyperalgesia that is dependent on polymorphonuclear leukocytes. Science 225, 743–745.

Levine, J.D., Gooding, J., Donatoni, P., Borden, L., Goetzl, E.J., 1985. The role of the polymorphonuclear leukocyte in hyperalgesia. J. Neurosci. 5, 3025–3029.

Levine, J.D., Lam, D., Taiwo, Y.O., Donatoni, P., Goetzl, E.J., 1986. Hyperalgesic properties of 15-lipoxygenase products of arachidonic acid. Proc. Natl. Acad. Sci. U. S. A. 83, 5331–5334.

Loomis, C.W., Cervenko, F.W., Jhamandas, K., Sutak, M., Milne, B., 1985. Analgesia and autonomic function following intrathecal administration of morphine and norepinephrine to the rat. Can. J. Physiol. Pharmacol. 63, 656–662.

Loomis, C.W., Milne, B., Cervenko, F.W., 1987. Determination of cross tolerance in rat spinal cord using intrathecal infusion via sequential mini-osmotic pumps. Pharmacol. Biochem. Behav. 26, 131–139.

Malmberg, A.B., Yaksh, T.L., 1992. Hyperalgesia mediated by spinal glutamate or substance P receptor blocked by spinal cyclooxygenase inhibition. Science 257, 1276–1279.

Malmberg, A.B., Yaksh, T.L., 1993. Spinal nitric oxide synthesis inhibition blocks NMDA-induced thermal hyperalgesia and produces antinociception in the formalin test in rats. Pain 54, 291–300.

Mantyh, P.W., Rogers, S.D., Honore, P., Allen, B.J., Ghilardi, J.R., Li, J., Daughters, R.S., Lappi, D.A., Wiley, R.G., Simone, D.A., 1997. Inhibition of hyperalgesia by ablation of lamina I spinal neurons expressing the substance P receptor. Science 278, 275–279.

Mao, J., Price, D.D., Mayer, D.J., 1994. Thermal hyperalgesia in asso-

ciation with the development of morphine tolerance in rats: roles of excitatory amino acid receptors and protein kinase C. J. Neurosci. 14, 2301–2312.

Mao, J., Price, D.D., Lu, J., Mayer, D.J., 1998. Antinociceptive tolerance to the mu-opioid agonist DAMGO is dose-dependently reduced by MK-801 in rats. Neurosci. Lett. 250, 193–196.

Marriott, D., Wilkin, G.P., Coote, P.R., Wood, J.N., 1991a. Eicosanoid synthesis by spinal cord astrocytes is evoked by substance P; possible implications for nociception and pain. Adv. Prostaglandin Thromboxane Leukotriene Res. 21B, 739–741.

Marriott, D.R., Wilkin, G.P., Wood, J.N., 1991b. Substance P-induced release of prostaglandins from astrocytes: regional specialisation and correlation with phosphoinositol metabolism. J. Neurochem. 56, 259–265.

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

Martin, H.A., Basbaum, A.I., Goetzl, E.J., Levine, J.D., 1988. Leukotriene B4 decreases the mechanical and thermal thresholds of C-fiber nociceptors in the hairy skin of the rat. J. Neurophysiol. 60, 438–445.

Minami, T., Uda, R., Horiguchi, S., Ito, S., Hyodo, M., Hayaishi, O., 1994. Allodynia evoked by intrathecal administration of prostaglandin E2 to conscious mice. Pain 57, 217–223.

Owen, J.A., Milne, B., Jhamandas, K., Nakatsu, K., 1981. Assembly of an inexpensive tail flick analgesia meter. J. Pharmacol. Methods 6, 33–37.

Patel, H.H., Fryer, R.M., Gross, E.R., Bundey, R.A., Hsu, A.K., Isbell, M., Eusebi, L.O., Jensen, R.V., Gullans, S.R., Insel, P.A., Nithipatikom, K., Gross, G.J., 2003. 12-Lipoxygenase in opioid-induced delayed cardioprotection: gene array, mass spectrometric, and pharmacological analyses. Circ. Res. 92, 676–682.

Porro, C.A., Cavazzuti, M., 1993. Spatial and temporal aspects of spinal cord and brainstem activation in the formalin pain model. Prog. Neurobiol. 41, 565–607.

Powell, K.J., Hosokawa, A., Bell, A., Sutak, M., Milne, B., Quirion, R., Jhamandas, K., 1999. Comparative effects of cyclo-oxygenase and nitric oxide synthase inhibition on the development and reversal of spinal opioid tolerance. Br. J. Pharmacol. 127, 631–644.

Powell, K.J., Ma, W., Sutak, M., Doods, H., Quirion, R., Jhamandas, K., 2000. Blockade and reversal of spinal morphine tolerance by peptide and non-peptide calcitonin gene-related peptide receptor antagonists. Br. J. Pharmacol. 131, 875–884.

Powell, K.J., Quirion, R., Jhamandas, K., 2003. Inhibition of neurokinin-1-substance P receptor and prostanoid activity prevents and reverses the development of morphine tolerance in vivo and the morphine-induced increase in CGRP expression in cultured dorsal root ganglion neurons. Eur. J. Neurosci. 18, 1572–1583.

Rackham, A., Ford-Hutchinson, A.W., 1983. Inflammation and pain sensitivity: effects of leukotrienes D4, B4 and prostaglandin E1 in the rat paw. Prostaglandins 25, 193–203.

Ritchie, J., Pitcher, G.M., Sharif, R.N., 2000. Lipoxygenase inhibition depresses hyperalgesia induced by intrathecal administration of substance P, NMDA and AMPA in the rat. Soc. Neurosci. Abstr. 26, 1958.

Salari, H., Braquet, P., Borgeat, P., 1984. Comparative effects of indomethacin, acetylenic acids, 15-HETE, nordihydroguaiaretic acid and BW755C on the metabolism of arachidonic acid in human leukocytes and platelets. Prostaglandins Leukot. Med. 13, 53–60.

Sekiya, K., Okuda, H., 1982. Selective inhibition of platelet lipoxygenase by baicalein. Biochem. Biophys. Res. Commun. 105, 1090–1095.

Serhan, C.N., Hamberg, M., Samuelsson, B., 1984a. Lipoxins: novel series of biologically active compounds formed from arachidonic acid in human leukocytes. Proc. Natl. Acad. Sci. U. S. A. 1984, 5335–5339.

Serhan, C.N., Hamberg, M., Samuelsson, B., 1984b. Trihydroxytetraenes: a novel series of compounds formed from arachidonic acid in human leukocytes. Biochem. Biophys. Res. Commun. 118 (3), 943–949.

Shen, K.F., Crain, S.M., 2001. Cholera toxin-B subunit blocks excitatory opioid receptor-mediated hyperalgesic effects in mice, thereby unmasking potent opioid analgesia and attenuating opioid tolerance/dependence. Brain Res. 919, 20–30.

Shin, J., Cho, H., Hwang, S.W., Jung, J., Shin, C.Y., Lee, S.Y., Kim, S.H., Lee, M.G., Choi, Y.H., Kim, J., Haber, N.A., Reichling, D.B., Khasar, S., Levine, J.D., Oh, U., 2002. Bradykinin-12-lipoxygenase-VR1 signaling pathway for inflammatory hyperalgesia. Proc. Natl. Acad. Sci. U. S. A. 99, 10150–10155.

Skofitsch, G., Jacobowitz, D.M., 1985. Calcitonin gene-related peptide coexists with substance P in capsaicin sensitive neurons and sensory ganglia of the rat. Peptides 6, 747–754.

Smullin, D.H., Skilling, S.R., Larson, A.A., 1990. Interactions between substance P, calcitonin gene-related peptide, taurine and excitatory amino acids in the spinal cord. Pain 42, 93–101.

Sugimoto, M., Kuraishi, Y., Satoh, M., Takagi, H., 1986. Involvement of medullary opioid-peptidergic and spinal noradrenergic systems in the regulation of formalin-induced persistent pain. Neuropharmacology 25, 481–485.

Taiwo, Y.O., Levine, J.D., 1988. Characterization of the arachidonic acid metabolites mediating bradykinin and noradrenaline hyperalgesia. Brain Res. 458, 402–406.

Tjolsen, A., Berge, O.G., Hunskaar, S., Rosland, J.H., Hole, K., 1992. The formalin test: an evaluation of the method. Pain 51, 5–17.

Tominaga, M., Caterina, M.J., Malmberg, A.B., Rosen, T.A., Gilbert, H., Skinner, K., Raumann, B.E., Basbaum, A.I., Julius, D., 1998. The cloned capsaicin receptor integrates multiple pain-producing stimuli. Neuron 21, 531–543.

Tonussi, C.R., Ferreira, S.H., 1999. Tumour necrosis factor-alpha mediates carrageenin-induced knee-joint incapacitation and also triggers overt nociception in previously inflamed rat knee-joints. Pain 82, 81–87.

Trang, T., Sutak, M., Quirion, R., Jhamandas, K., 2002. The role of spinal neuropeptides and prostaglandins in opioid physical dependence. Br. J. Pharmacol. 136, 37–48.

Trang, T., Sutak, M., Quirion, R., Jhamandas, K., 2003. Spinal administration of lipoxygenase inhibitors suppresses behavioural and neurochemical manifestations of nalipoxygenaseone-precipitated opioid withdrawal. Br. J. Pharmacol. 140, 295–304.

Uda, R., Horiguchi, S., Ito, S., Hyodo, M., Hayaishi, O., 1990. Nociceptive effects induced by intrathecal administration of prostaglandin D2, E2, or F2 alpha to conscious mice. Brain Res. 510, 26–32.

Valtschanoff, J.G., Rustioni, A., Guo, A., Hwang, S.J., 2001. Vanilloid receptor VR1 is both presynaptic and postsynaptic in the superficial laminae of the rat dorsal horn. J. Comp Neurol. 436, 225–235.

Vasko, M.R., Campbell, W.B., Waite, K.J., 1994. Prostaglandin E2 enhances bradykinin-stimulated release of neuropeptides from rat sensory neurons in culture. J. Neurosci. 14, 4987–4997.

Vaughan, C.W., Ingram, S.L., Connor, M.A., Christie, M.J., 1997. How opioids inhibit GABA-mediated neurotransmission. Nature 390, 611–614.

Wong, C.S., Hsu, M.M., Chou, R., Chou, Y.Y., Tung, C.S., 2000. Intrathecal cyclooxygenase inhibitor administration attenuates morphine antinociceptive tolerance in rats. Br. J. Anaesth. 85, 747–751.

Yaksh, T.L., Rudy, T.A., 1976. Analgesia mediated by a direct spinal action of narcotics. Science 192, 1357–1358.

Yaksh, T.L., Malmberg, A.B., 1993. Spinal actions of NSAIDS in blocking spinally mediated hyperalgesia: the role of cyclooxygenase products. Agents Actions Suppl. 41, 89–100.

Yoshimoto, T., Yokoyama, C., Ochi, K., Yamamoto, S., Maki, Y., Ashida, Y., Terao, S., Shiraishi, M., 1982. 2,3,5-Trimethyl-6-(12-hydroxy-5,10-dodecadiynyl)-1,4-benzoquinone (AA-861), a selective inhibitor of the 5-lipoxygenase reaction and the biosynthesis of slow-reacting substance of anaphylaxis. Biochim. Biophys. Acta 713 (2), 470–473.

Zhang, R.X., Mi, Z.P., Qiao, J.T., 1994. Changes of spinal substance P, calcitonin gene-related peptide, somatostatin, Met-enkephalin and neurotensin in rats in response to formalin-induced pain. Regul. Pept. 51, 25–32.