Available online at www.sciencedirect.com
ScienceDirect
Pharmacology, Biochemistry and Behavior 88 (2007) 122–129
PHARMACOLOGY BIOCHEMISTRY AND BEHAVIOR
www.elsevier.com/locate/pharmbiochembeh

# Activation of peripheral and spinal histamine $H_3$ receptors inhibits formalin-induced inflammation and nociception, respectively

Keri E. Cannon [a], Rob Leurs [b], Lindsay B. Hough [a,*]

[a] *Center for Neuropharmacology and Neuroscience, Albany Medical College MC-136, Albany, NY, USA*
[b] *Leiden/Amsterdam Center of Drug Research (LACDR), Division of Medicinal Chemistry, Department of Pharmacochemistry, Vrije Universiteit Amsterdam, De Boelelaan 1083, 1081 HV Amsterdam, The Netherlands*

Received 8 May 2007; received in revised form 6 July 2007; accepted 16 July 2007
Available online 25 July 2007

**Abstract**

Pharmacological activation of histamine $H_3$ receptors is known to reduce the release of inflammatory peptides, thereby reducing pain and inflammation, but the site(s) and mechanism(s) of these effects are currently unknown. The present study addressed these questions by examining the effects of the $H_3$ agonist immepip and the $H_3$ antagonist thioperamide on nociceptive behaviors and swelling produced during the rat formalin test. Systemic administration of immepip (5 and 30 mg/kg, s.c.) significantly attenuated formalin-induced flinching but not licking responses during both phases. This attenuation was reversed by either systemic (15 mg/kg, i.p.) or intrathecal (20 or 50 μg) administration of thioperamide. Furthermore, immepip (30 mg/kg, s.c.) significantly inhibited formalin-induced swelling, an action which was completely reversed by systemic (15 mg/kg, i.p.), but not intrathecal (50 μg) thioperamide. Also consistent with this pattern, intrathecal immepip (50 μg) reduced flinching responses, but had no effect on formalin-induced paw swelling. The present findings suggest that activation of $H_3$ receptors located on peripheral and spinal terminals of deep dermal fibers attenuates formalin-induced swelling and flinching, respectively. Pharmacological stimulation of $H_3$ receptors could be an important therapeutic approach for many disorders related to deep dermal or inflammatory pain.
© 2007 Elsevier Inc. All rights reserved.

*Keywords:* Histamine; $H_3$ receptor; Inflammation; Antinociception; Analgesia; Immepip; Thioperamide

## 1. Introduction

There is considerable interest in histamine $H_3$ receptors as emerging pharmaceutical targets (Esbenshade et al., 2006). Although $H_3$ receptor density is highest in the brain, *in situ* hybridization studies have also found these receptors to exist in dorsal root ganglia, spinal cord and selected peripheral tissues (Pollard et al., 1993; Heron et al., 2001; Pillot et al., 2002). In addition, activation of peripheral $H_3$ receptors inhibits peptide release, suggesting a sensory fiber localization (Ohkubo et al., 1995; Delaunois et al., 1995; Imamura et al., 1996; Nemmar et al., 1999). Immunochemical studies recently confirmed the existence of $H_3$ receptors on sensory neurons in the dorsal root ganglia, and on sensory fibers in the skin spinal cord (Cannon et al., 2007).

Since peptidergic fibers have been implicated in nociception (Morton and Hutchison, 1989; Millan, 1999; Furst, 1999), the inhibition of the activity of these fibers by $H_3$ receptor agonists might be expected to modulate pain transmission. In fact, oral administration of the $H_3$ agonist pro-drug BP 2-94 to mice reduced nociceptive responses in the phenylbenzoquinone writhing and formalin tests, but failed to reduce nociceptive hot plate responding (Rouleau et al., 1997). This work suggested that $H_3$ agonists may attenuate some types of nociceptive transmission, but the sites and mechanisms responsible for these effects remained unclear.

Subsequently, the $H_3$ agonist immepip was shown to attenuate mechanical (tail pinch) responses in rats, but neither tail flick nor hot plate reflexes were affected (Cannon et al., 2003). Additional experiments using $H_3$ knockout mice, pharmacological antagonists, and systemic and intrathecal treatments in rats showed unequivocally that activation of spinal $H_3$ receptors attenuates nociceptive responses to low-intensity tail pinch stimulation (Cannon et al., 2003). More recently, a range of thermal and mechanical intensities were

* Corresponding author. Center for Neuropharmacology and Neuroscience, Albany Medical College, 47 New Scotland Ave., Albany, NY 12206, USA. Tel.: +1 518 262 5786.
*E-mail address:* houghl@mail.amc.edu (L.B. Hough).

0091-3057/$ - see front matter © 2007 Elsevier Inc. All rights reserved.
doi:10.1016/j.pbb.2007.07.014

employed to confirm that the acute antinociceptive profile of $H_3$ agonists is both modality – (*i.e.* mechanical *vs.* thermal) and intensity – (low *vs.* high mechanical) specific (Cannon et al., 2005). It was concluded that immepip probably inhibits low-intensity mechanical nociception by activation of $H_3$ receptors located on the spinal terminals of Aδ and possibly C high-threshold mechanoreceptors (Cannon et al., 2005). Such an antinociceptive profile is extremely unusual.

In contrast, much less is known about the modulation of inflammatory pain by $H_3$ agonists. Earlier studies revealed that the $H_3$ agonist pro-drug BP 2-94 attenuates swelling and nociceptive behaviors in some mouse models of inflammatory pain (Rouleau et al., 1997; Rouleau et al., 2000). The $H_3$ agonist R-α-methylhistamine was also recently shown to have systemic anti-inflammatory activity (Poveda et al., 2006). In addition, preliminary findings from our laboratory suggested that the $H_3$ agonist immepip attenuates formalin-induced flinching responses in rats (Cannon et al., 2005). However, the site(s) and mechanism (s) of the antinociceptive and anti-inflammatory actions of $H_3$ agonists following formalin remain unknown. These questions are addressed in the present study by evaluating pharmacological manipulations with the $H_3$ agonist immepip and the $H_3$ antagonist thioperamide on formalin-induced nociceptive behaviors and swelling in rats. Several potential sites of action for these drugs are considered, including spinal neurons, spinal terminals of nociceptive sensory fibers, peripheral terminals of these fibers within the skin, and sites within the brain.

## 2. Methods and materials

### 2.1. Animals

All experiments were approved by the Institutional Animal Care and Use Committee of Albany Medical College. Male

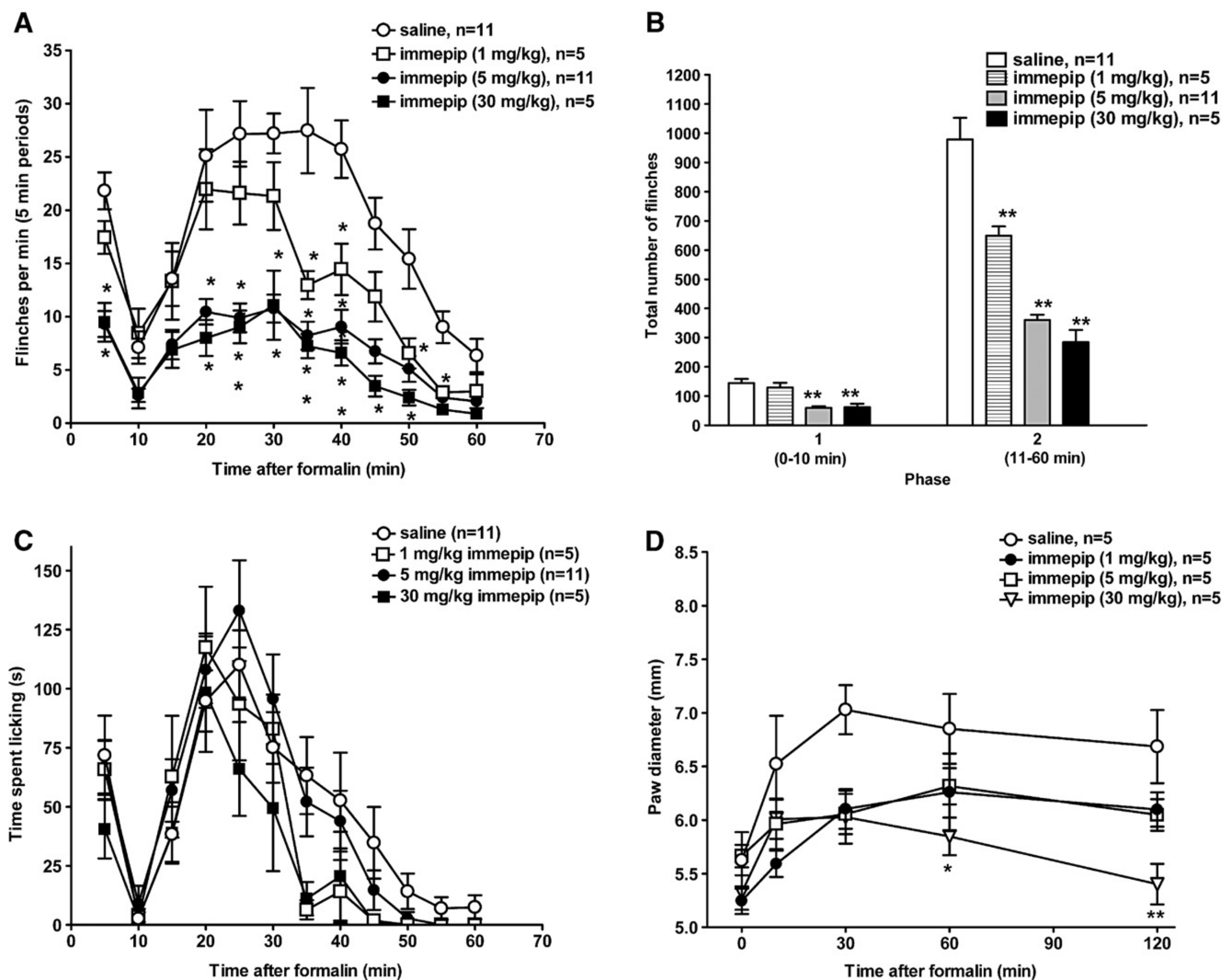


Fig. 1. Effects of the $H_3$ agonist immepip on formalin-induced flinching responses, licking responses, and paw swelling. Rats received immepip (1, 5, or 30 mg/kg, s.c.) or saline vehicle, followed 30 min later by intraplantar formalin. A: Number of flinches per min was recorded and grouped into 5 min periods (ordinate, mean ± S.E.M.) for the times after formalin shown (abscissa, min). B: The total number of flinches was summed for each phase (ordinate, mean ± S.E.M.). Phase 1 was established as 0–10 min, whereas Phase 2 was established as 11–60 min. C: Time spent licking (ordinate, s, mean ± S.E.M.) is shown in 5 min intervals (abscissa, min). D: Paw diameter (ordinate, mm, mean ± S.E.M.) was recorded at the times shown (abscissa, min) after formalin injection. Data are pooled from two separate experiments: a) saline and immepip (5 mg/kg) treatments ($n$=6) in which paw volume was not measured (data in Fig. 1A–1C), and b) all four treatment groups in which behavior and paw volume was measured (data in all 4 figures, $n$=5). *, ** $P$<0.05, $P$<0.01 *vs.* saline at same time interval, respectively.

Sprague–Dawley rats (300–350 g, Taconic Farms, Germantown, NY) housed in groups of two or three were maintained on a 12 h light/dark cycle (lights on from 7:00 to 19:00 h). Food and water were provided *ad libitum*.

### 2.2. Drugs

Immepip dihydrobromide (synthesized at Vrije Universiteit, Amsterdam, Netherlands, Vollinga et al., 1994) and thioperamide maleate (Tocris, Ellisville, MO) were dissolved in saline and neutralized with 1 N NaOH to pH 6.5. All doses of immepip and thioperamide are expressed as bases. Neutral buffered formalin (10%, Sigma) was diluted in saline to produce a 2.5% formalin solution with a pH of 7.0.

### 2.3. Surgery

For rat intrathecal injections, the spinal subarachnoid space was chronically cannulated under general anesthesia as described previously (Yaksh and Rudy, 1976) with modifications (Hammond, 1988). The animals were then placed in separate cages and allowed to recover for a minimum of ten days.

### 2.4. Formalin test

For all experiments, animals were habituated to the formalin test environment by placing them in the test apparatus (Plexiglass chamber 15.25 in. × 15.8 in. × 9.5 in.) for 1 h prior to injection of formalin. Subjects were then given a subcutaneous (s.c.) injection of either immepip or saline. Thirty minutes later, the subjects were given an s.c. injection of 2.5% formalin (volume of 50 µl) into the plantar surface of the left hind paw. Some subjects received an intraperitoneal (i.p.) injection of thioperamide or saline 20 min prior to formalin. Other subjects received an intrathecal (i.t.) injection of immepip, thioperamide, or saline 10 min prior to formalin injection. The time intervals used for agonist and antagonist administration were adapted from Cannon et al. (2003).

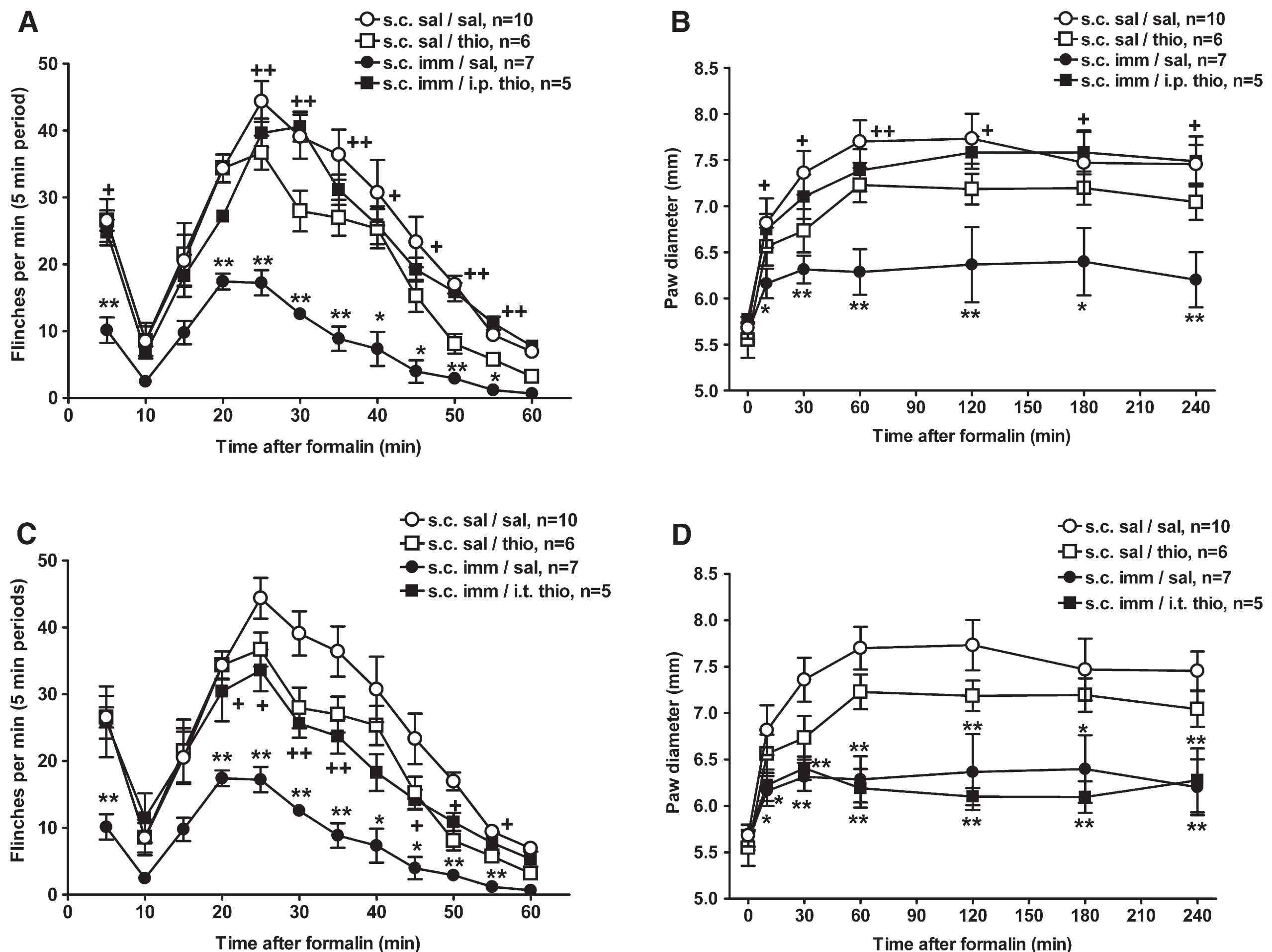


Fig. 2. Effects of the $H_3$ antagonist thioperamide on immepip-induced attenuation of flinching responses (A, C) and paw swelling (B, D) after formalin treatment. Rats received immepip (imm, 30 mg/kg, s.c.) or saline vehicle (sal, s.c.). A, B: Effects of systemic thioperamide were studied. Ten minutes after immepip, thioperamide (thio, 15 mg/kg, i.p.) or saline (sal) were injected, followed by intraplantar formalin 20 min later. C, D: Effects of intrathecal thioperamide were studied. Twenty minutes after immepip, intrathecal thioperamide (50 µg) or saline (sal) was given, followed by formalin 10 min later. Numbers of flinches per min, grouped in 5 min periods (A, C, ordinate, mean ± S.E.M.), and paw diameters (B, D, ordinate, mm, mean ± S.E.M.) were recorded at the times shown after formalin (abscissa, min). No significant differences were found in flinching responses or paw diameters between the following groups, thus permitting the groups to be pooled and identified as in parentheses: 1) s.c. saline/i.p. saline *vs.* s.c. saline/i.t. saline (s.c. sal/sal, open circles), 2) s.c. saline/i.p. thioperamide *vs.* s.c. saline/i.t. thioperamide (s.c. sal/thio, open squares), and 3) s.c. immepip/i.p. saline *vs.* s.c. immepip/i.t. saline (s.c. imm/sal, closed circles). *,** $P<0.05$, $P<0.01$ *vs.* sal/sal at same time interval, respectively. $^{+,++}P<0.05$, $P<0.01$ *vs.* imm/sal at same time interval, respectively.

During each experiment, the number of flinches (*i.e.* flinching or shaking of the injected paw) was counted every min for 60 min and results were expressed as the average number of flinches per min (in 5 min periods). In addition, the total number of flinches was summed for each phase. Phase 1 was defined as the period of time beginning immediately after formalin injection and lasting 10 min. Phase 2 was defined as the time beginning 11 min post-formalin injection and ending 60 min. Digital calipers were used to measure the vertical thickness of the injected paw at the metatarsal level in all subjects (except as noted in Fig. 1). Baseline paw measurements were taken immediately prior to s.c. injections of immepip or saline (−30 min) and again just before formalin treatment (0 min). Paw measurements were repeated at the times indicated after formalin.

### 2.5. Statistics

Results are expressed as average number of flinches per min (grouped in 5 min periods), total number of flinches per phase, time spent licking (s), and paw swelling (mm), mean ± S.E.M. Two types of statistical analyses were performed: 1) repeated measures analysis of variance (ANOVA) and planned comparisons for analysis of the number of flinches per min or paw swelling measurements, and 2) Student's *t*-test for analysis of the total number of flinches per phase (Statistica, CSS, Inc., Tulsa, OK).

## 3. Results

### 3.1. Effects of immepip on formalin-induced flinching

Dose-dependent inhibition of flinching responses was observed over a range of immepip doses (Fig. 1A; repeated measures ANOVA, drug by time interaction, $P<0.01$). The lowest dose of immepip (1 mg/kg, s.c.) produced flinching responses similar to the control responses during Phase 1 and the first half of Phase 2. However, during the second half of Phase 2, this low dose of immepip significantly reduced flinching responses (Fig. 1A). This reduction was also seen in the total number of flinches produced during Phase 2 (Fig. 1B). Larger doses of immepip (5 and 30 mg/kg) significantly attenuated formalin-induced flinching responses during Phase 1 and Phase 2 (Fig. 1A and B).

### 3.2. Effects of immepip on formalin-induced licking

In contrast to the effects on flinching, none of the three doses of immepip had a significant effect on formalin-induced licking responses (Fig. 1C). Because there appeared to be a trend toward reductions in licking after the highest dose of immepip, licking times were also summed for each animal for phase 1 and phase 2 (data not shown). Total licking times for either phase were not statistically different across the treatment groups by one-way ANOVA. Furthermore, separate *t*-test comparisons of either phase 1 or phase 2 total licking times from saline-treated *vs.* immepip (30 mg/kg)-treated subjects found no differences.

### 3.3. Effects of immepip on formalin-induced paw swelling

Injection of formalin into the hind paw resulted in swelling as early as 10 min after injection (Fig. 1D). This swelling reached a plateau by 30 min and was maintained for up to 240 min (Figs. 1D, 2B, D). Non-significant reductions in average paw diameters were achieved by the lower doses of immepip, whereas the largest dose (30 mg/kg) produced highly significant reductions in swelling (Fig. 1D repeated measures ANOVA, $P<0.01$). A repeat of this experiment in the antagonist studies (Fig. 2) confirmed a large, long-lasting inhibition of swelling (Fig. 2B, D).

### 3.4. Effects of thioperamide on immepip-induced inhibition of flinching

In order to determine if the actions of immepip are mediated by $H_3$ receptors, the effects of the selective $H_3$ antagonist thioperamide were examined in conjunction with immepip treatment on both formalin-induced flinching and swelling (Fig. 2). Repeated measures ANOVAs of flinching data from combinations of saline, immepip, and thioperamide (i.p. and i.t.) treatments showed significant main effects of drug ($P<0.01$), time ($P<0.01$), and significant drug by time interaction terms

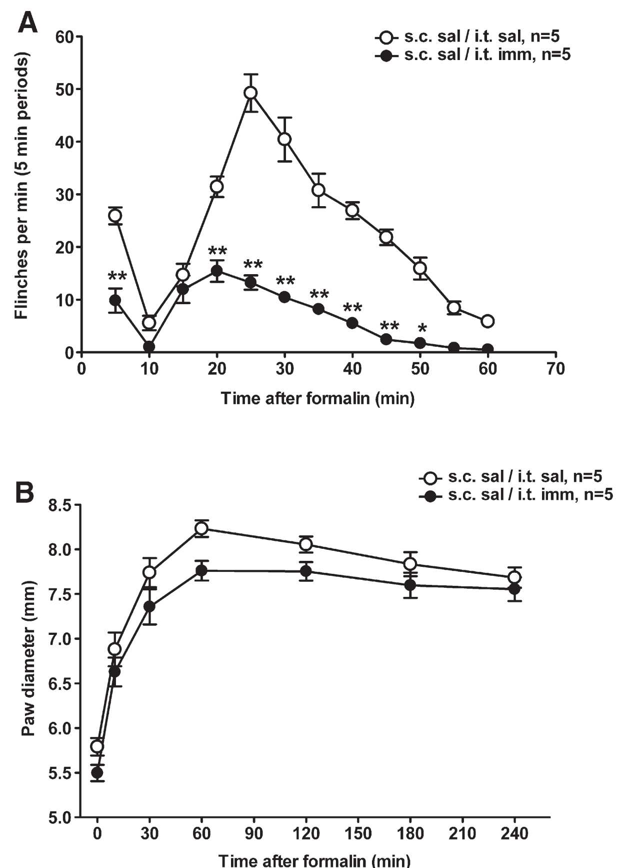


Fig. 3. Effects of intrathecal immepip on formalin-induced flinching responses (A) and paw swelling (B). Rats received saline (sal, s.c.), followed 20 min later by an intrathecal injection of saline (sal) or immepip (imm, 50 μg). Ten minutes later, subjects received intraplantar formalin. The experiment proceeded and data are shown as in Fig. 2. *, **$P<0.05$, $P<0.01$ *vs.* sal/sal at same time interval, respectively.

($P<0.01$). Fig. 2A and C confirm a significant reduction in flinching responses during both phases produced by immepip. Systemic administration of thioperamide (15 mg/kg, i.p.) significantly reversed the attenuation of flinching produced by immepip during both phases (Fig. 2A). Intrathecal administration of thioperamide (50 μg) also significantly reversed immepip-induced attenuation of flinching responses during both phases (Fig. 2C). Thioperamide alone (i.p. or i.t.) had no effect on formalin-induced flinching responses (Fig. 2A and C). In a parallel experiment, a lower intrathecal dose of thioperamide (20 μg) completely abolished the inhibition of formalin-induced flinches by 5 mg/kg immepip during both phases of the formalin test (data not shown).

### *3.5. Effects of thioperamide on immepip-induced inhibition of paw swelling*

Repeated measures ANOVAs of paw diameter data from combinations of saline, immepip, and thioperamide (i.p. and i.t.) treatments revealed significant main effects of drug ($P<0.01$), time ($P<0.01$), and drug by time interactions ($P<0.01$). Systemic (Fig. 2B), but not intrathecal (Fig. 2D), administration of thioperamide completely reversed the reduction in paw diameter produced by immepip. Thioperamide alone (administered i.p. or i.t.) had no effect on formalin-induced paw swelling (Fig. 2B and D). To summarize the results of Fig. 2, systemic immepip reduced both flinching and swelling following formalin treatment, effects which were both antagonized by systemic administration of thioperamide. In contrast, intrathecal thioperamide antagonized immepip's suppression of flinching but not swelling.

### *3.6. Effects of intrathecal immepip on formalin-induced flinching and paw swelling*

To confirm the spinal localization of immepip antinociception, intrathecal administration of immepip (50 μg) reduced flinching responses during both phases of the formalin test (Fig. 3A; repeated measures ANOVA, drug by time interaction, $P<0.01$). This suppression was similar to that produced by systemic administration of immepip (Figs. 1 and 2). However, as predicted from the findings of Fig. 2, intrathecal immepip had no effect on formalin-induced paw swelling (Fig. 3B).

## 4. Discussion

The present study offers several lines of evidence showing that activation of $H_3$ receptors attenuates both nociceptive behaviors and edema associated with inflammatory pain. First, the inhibition of formalin-induced flinching by the $H_3$ agonist immepip was dose-dependent (Fig. 1). Second, this inhibition was reversed by systemic and intrathecal administration of the $H_3$ antagonist thioperamide (Fig. 2A and C). Finally, $H_3$ agonist-induced attenuation of paw swelling was dose-dependent and was reversed by systemic administration of thioperamide (Figs. 1D and 2B). In addition, the present study has located the sites for both the anti-inflammatory and antinociceptive actions of this drug.

### *4.1. A spinal site of antinociceptive action for intrathecal immepip*

The attenuation of formalin-induced flinching by intrathecal immepip (Fig. 3) makes a spinal site of action for this drug likely. In an earlier study, the same intrathecal dose of immepip as used presently (50 μg) was shown to attenuate nociceptive responses to tail pinch in rats (Cannon et al., 2003). Since systemic administration of this dose had no effect on tail pinch responses, the effect of intrathecal immepip must be a localized spinal effect (Cannon et al., 2003). Consistent with this, autoradiography experiments have revealed low but detectable levels of $H_3$ receptor binding sites in the superficial dorsal horn of rat spinal cords (Pollard et al., 1993).

$H_3$ receptors located postsynaptically on dorsal horn neurons are a possible target for the antinociceptive actions of intrathecal immepip. However, *in situ* hybridization studies of $H_3$ mRNA found very little to no $H_3$ message in dorsal horn neurons (Heron et al., 2001). In addition, immunohistochemical studies found little to no anti-$H_3$ receptor labeling on dorsal horn neurons (Cannon et al., 2007).

Within the dorsal horn, $H_3$ receptors could be localized on the terminals of descending bulbospinal fibers. For example, $H_3$ agonists were shown to reduce norepinephrine release in spinal cord slices (Celuch, 1995). However, this particular effect is not likely to account for the antinociceptive actions of intrathecal immepip, since the descending noradrenergic influence is antinociceptive, and immepip would presumably reduce this activity (Millan, 2002). In contrast, there is excellent evidence that sensory neurons possess $H_3$ receptors. $H_3$ receptor mRNA was detected in large, medium, and small diameter cell bodies of rat dorsal root ganglia (Heron et al., 2001). Furthermore, recent immunohistochemical studies showed anti-$H_3$ receptor labeling on medium-sized cell bodies in dorsal root ganglia and on small-caliber, peptidergic fibers that ramified in dorsal horn laminae I, II, and V in rats and wild type mice (Cannon et al., 2007). $H_3$ knockout mice lack this anti-$H_3$ labeling, confirming the presence of authentic $H_3$ receptors (Cannon et al., 2007). The same mice also lack antinociceptive responses to immepip, providing a critical link between the anatomically-localized receptor and the drug response (Cannon et al., 2005). The localization of $H_3$ receptors on sensory neurons and fibers, and the well-established $H_3$-mediated inhibition of transmitter release (Ohkubo et al., 1995; Delaunois et al., 1995; Arrang et al., 1995; Imamura et al., 1996; Blandina et al., 1996; Garcia et al., 1997; Nemmar et al., 1999; Molina-Hernandez et al., 2001) strongly suggest that immepip produces antinociception by reducing sensory fiber transmitter release at spinal presynaptic sites.

### *4.2. A spinal site of antinociceptive action for systemic immepip*

Due to the wide distribution of $H_3$ receptors throughout the central (Pollard et al., 1993) and peripheral (Cannon et al., 2007) nervous systems, systemically-administered immepip could be acting on supraspinal, peripheral, or spinal $H_3$ targets

to attenuate formalin-induced flinching. The existence of $H_3$ receptors in brain stem areas (Pollard et al., 1993) contributing to the descending control of pain transmission (Millan, 2002) means that supraspinal sites should be considered carefully. The degree of spinal and brain penetration by immepip has not been explored, but systemic dosing with this drug was reported to inhibit the release of histamine in the anterior hypothalamus (Jansen et al., 1998). However, because an intrathecal dose of immepip mimics systemically-administered immepip in reducing both flinching (Fig. 1A) and nociceptive responses to mechanical pain (Cannon et al., 2003), a spinal site of action is the simplest explanation for these antinociceptive actions of immepip.

The reversal of the effects of systemic immepip on flinching responses by intrathecal thioperamide (Fig. 2C) strongly argues that systemic immepip produces its antinociceptive effects solely at spinal $H_3$ receptors. This result precludes the notion that immepip reduces flinching responses by acting peripherally on an $H_3$-receptor-containing sensory fiber. If systemic immepip also activated peripheral $H_3$ receptors by reducing peripherally-initiated nociceptive transmission to attenuate flinching responses, then the afferent nociceptive signal would have never reached the spinal cord. Under these conditions, blocking spinal $H_3$ receptors with intrathecal thioperamide would have had no effect on the attenuation of formalin-induced flinching responses.

### 4.3. Different nociceptive circuits may be activated by formalin to evoke different behaviors

The selective attenuation of flinching (*vs.* licking) suggests that distinct nociceptive circuits may underlie these behaviors. Clarification of the nociceptors evoking these behaviors following formalin has been difficult because: 1) inflammatory peptides are expressed on multiple afferent subtypes (Ishida-Yamamoto et al., 1989; Fundin et al., 1997; Pare et al., 2001b), and 2) many studies have either not distinguished between the two behaviors (Yaksh, 1997), or not made simultaneous measurements (Sawynok and Reid, 2003). By measuring both types of behaviors, the present work offers insight into the fibers that provoke flinching responses following formalin.

Although it is widely thought that C fibers mediate formalin-induced flinching and licking responses (Tjolsen et al., 1992; McCall et al., 1996; Yaksh, 1997), the present findings require a re-examination of this hypothesis. Calcitonin gene-related peptide (CGRP) and substance P (SP), both released from afferent fibers, are known to contribute to the peripheral and spinal mechanisms of inflammatory pain (Ohkubo et al., 1990; Levine et al., 1993; Levine and Reichling, 1999; Yaksh, 1999). Since many C fibers contain these neuropeptides (Gibbins et al., 1985, 1987; Sann and Pierau, 1998), it has been thought that both are released solely from C fibers during nociception (Millan, 1999). However, it is often overlooked that some Aδ fibers contain CGRP and SP (Ishida-Yamamoto et al., 1989; Fundin et al., 1997; Pare et al., 2001a), and these fibers may also participate in nociceptive transmission by releasing neuropeptides in the spinal cord. (Yaksh et al., 1980; Go and Yaksh, 1987). In addition, the attenuation of depolarization-induced release of SP from spinal afferent terminals by intrathecal morphine (Yaksh et al., 1980; Go and Yaksh, 1987) has been used to emphasize the importance of peptidergic C fibers because μ-opioid receptors are found on these fibers (Gouarderes et al., 1991; Zhang et al., 1998) and because this treatment attenuates formalin-induced flinching and licking responses (Malmberg et al., 1993). However, μ opioid receptors are expressed on a variety of nociceptive afferents, including peptidergic, thinly myelinated fibers (Gouarderes et al., 1991; Arvidsson et al., 1995; Zhang et al., 1998). An analogous argument can be made about the effects of neonatal capsaicin pretreatment on formalin-evoked flinching and licking responses (Yaksh, 1997; Peterson et al., 1997), since this treatment destroys both unmyelinated (C fibers) and some myelinated fibers (Nagy et al., 1983). Taken with the recent report that $H_3$ receptors are on a select population of Aδ and absent on C fibers (Cannon et al., 2007), the present study suggests that C fibers do not play a critical role in mediating formalin-induced flinching responses. Instead, it is likely that $H_3$ receptor-containing, deep dermal, perivascular Aδ fibers are primary contributors to the nociceptive flinching circuit. By the same reasoning, the failure of immepip to attenuate licking responses (Fig. 1C; (Cannon et al., 2005)) argues that these $H_3$ receptor-containing fibers do not play an obligatory role in producing this particular behavior. C fibers are likely contributors to the stimuli which evoke formalin-induced licking responses. The differential modulation of flinching and licking behaviors following formalin has also been noted in earlier drug studies, emphasizing the possibility of distinct nociceptive circuits evoking these behaviors (Sawynok and Liu, 2004).

### 4.4. Site of anti-inflammatory action of $H_3$ agonists

Activation of peripheral $H_3$ receptors can reduce the release of inflammatory peptides (Ohkubo et al., 1995; Delaunois et al., 1995; Imamura et al., 1996; Nemmar et al., 1999), consistent with the reported anti-inflammatory activity of BP 2-94, an $H_3$ receptor pro-drug (Rouleau et al., 1997, 2000). However, the site of anti-inflammatory action of such $H_3$-acting drugs was unknown. The present results, which revealed that immepip-induced attenuation of paw swelling was completely reversed by systemic (but not intrathecal) administration of thioperamide (Fig. 2B and D), solidify an anti-inflammatory role for peripheral $H_3$ receptors. Activation of $H_3$ receptors on peptidergic, Aδ perivascular fibers of the deep dermis is likely to inhibit peripheral neuropeptide release, thereby reducing inflammation.

It is curious as to how activation of peripheral $H_3$ receptors might attenuate inflammation but not inhibit nociceptive flinching responses. Activation of other peripheral $G_i/G_o$-linked receptors, such as μ-opioid receptors, attenuates both nociceptive transmission and inflammation (Raja et al., 1999). The explanation for this difference may lie in the respective signaling mechanisms associated with these receptors. Whereas both μ-opioid and $H_3$ receptors attenuate the release of neuropeptides from peripheral terminals presumably *via* the inhibition of N- and P/Q-type $Ca^{+2}$ channels associated with

exocytosis (Twycross, 1999; Brown et al., 2001; Roberson and Clapham, 2002; Cesselin and Hamon, 2002), μ-opioid receptors also inhibit nociceptive transmission *via* activation of G-protein coupled, inwardly-rectifying $K^+$ channels (GIRKs) (Twycross, 1999; Cesselin and Hamon, 2002). $H_3$ receptors are not associated with this latter activity (Brown et al., 2001).

### 4.5. Significance of histamine and its receptors during inflammation and pain

Endogenous histamine plays an important pro-nociceptive role during inflammatory pain (Millan, 1999; Basbaum and Jessell, 2000). Injection of formalin results in an initial burst of nociceptor activity, and the release of several chemical messengers, including histamine (Tjolsen et al., 1992; Parada et al., 2001). Pharmacological studies (Parada et al., 2001) and experiments with histamine receptor knockout mice (Owen et al., 1980; Owen and Woodward, 1980; Mobarakeh et al., 2000) show a critical pro-nociceptive role for the $H_1$ receptor in both phases of responses following formalin, although $H_2$ receptors may also be significant (Owen et al., 1980; Owen and Woodward, 1980).

Since histamine is released during inflammatory pain, it seems possible that $H_3$ receptors might be activated during the formalin test. Thus, endogenous histamine would be expected to produce pro-nociceptive actions at $H_1$ receptors, but antinociceptive actions at $H_3$ receptors. If so, then the $H_3$ antagonist thioperamide should have produced either pro-nociceptive or pro-inflammatory effects during the formalin test. However, thioperamide alone had no effect on nociceptive behaviors or inflammation (Fig. 2), suggesting that endogenous histamine is not activating peripheral or spinal $H_3$ receptors during inflammatory pain.

Given that histamine has higher affinity for the $H_3$ receptor for than the $H_1$ receptor (Hill et al., 1997), it is curious that the histamine released by formalin treatment seems to act on $H_1$ receptors, but not act on $H_3$ receptors. One potential explanation may be a difference in the localization of $H_1$ and $H_3$ receptors in the skin and spinal cord. Although no immunohistochemical studies have been performed with $H_1$ receptor antibodies, *in situ* hybridization studies in guinea pig dorsal root ganglia have revealed that $H_1$ receptor mRNA is found in small-sized cell bodies that are non-peptidergic and express the isolectin IB4 (Kashiba et al., 1999). In contrast, $H_3$ receptors are located on small-caliber, deep dermal, peptidergic, perivascular Aδ fibers (Cannon et al., 2007). Thus, $H_1$ and $H_3$ receptors appear to be located on separate subsets of small-caliber nociceptive fibers. It may be possible that histamine is released in the vicinity of $H_1$ receptor-containing fibers during inflammatory pain, whereas $H_3$ receptor-containing fibers may be located out of the range of the released histamine. Further studies are necessary to confirm or refute this hypothesis.

## Acknowledgements

This work was supported by grants from the National Institute on Drug Abuse (DA-03816, DA-015915, and DA-07307). We would like to thank Dr. Frank Rice (Albany Medical College, Albany, NY) for valuable discussions.

## References


Arrang JM, Drutel G, Schwartz JC. Characterization of histamine H3 receptors regulating acetylcholine release in rat entorhinal cortex. Br J Pharmacol 1995;114:1518–22.

Arvidsson U, Riedl M, Chakrabarti S, Lee JH, Nakano AH, Dado RJ, et al. Distribution and targeting of a mu-opioid receptor (MOR1) in brain and spinal cord. J Neurosci 1995;15:3328–41.

Basbaum AI, Jessell TM. The perception of pain. In: Kandel ER, Schwartz JH, Jessell TM, editors. Principles of Neural Science. New York: McGraw-Hill; 2000. p. 472–91.

Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R, et al. Inhibition of cortical acetylcholine release and cognitive performance by histamine $H_3$ receptor activation in rats. Br J Pharmacol 1996;119:1656–64.

Brown RE, Stevens DR, Haas HL. The physiology of brain histamine. Prog Neurobiol 2001;63:637–72.

Cannon KE, Nalwalk JW, Stadel R, Ge P, Lawson D, Silos-Santiago I, et al. Activation of spinal histamine H3 receptors inhibits mechanical nociception. Eur J Pharmacol 2003;470:139–47.

Cannon KE, Hough LB. Inhibition of chemical and low-intensity mechanical nociception by activation of histamine $H_3$ receptors. J Pain 2005;6:193–200.

Cannon KE, Chazot PL, Hann V, Shenton F, Hough LB, Rice FL. Immunohistochemical localization of histamine $H_3$ receptors in rodent skin, dorsal root ganglia, superior cervical ganglia, and spinal cord: Potential antinociceptive targets. Pain 2007;129:76–92 http://dx.doi.org/10.1016/j.pain.2006.09.039.

Celuch SM. Possible participation of histamine $H_3$ receptors in the modulation of noradrenaline release from rat spinal cord slices. Eur J Pharmacol 1995;287:127–33.

Cesselin F, Hamon M. Opioid receptors. In: Pangalos MN, Davies CH, editors. Understanding G protein-coupled receptors and their role in the CNS. New York: Oxford University Press; 2002. p. 402–21.

Delaunois A, Gustin P, Garbarg M, Ansay M. Modulation of acetylcholine, capsaicin and substance P effects by histamine H3 receptors in isolated perfused rabbit lungs. Eur J Pharmacol 1995;277:243–50.

Esbenshade TA, Fox GB, Cowart MD. Histamine $H_3$ receptor antagonists: preclinical promise for treating obesity and cognitive disorders. Mol Interv 2006;6:77–88.

Fundin BT, Pfaller K, Rice FL. Different distribution of the sensory and autonomic innervation among the microvasculature of the rat mystacial pad. J Comp Neurol 1997;389:545–68.

Furst S. Transmitters involved in antinociception in the spinal cord. Brain Res Bull 1999;48:129–41.

Garcia M, Floran B, Arias-Montano JA, Young JM, Aceves J. Histamine $H_3$ receptor activation selectively inhibits dopamine D1 receptor-dependent [3H] GABA release from depolarization-stimulated slices of rat substantia nigra pars reticulata. Neuroscience 1997;80:241–9.

Gibbins IL, Furness JB, Costa M, MacIntyre I, Hillyard CJ, Girgis S. Co-localization of calcitonin gene-related peptide-like immunoreactivity with substance P in cutaneous, vascular and visceral sensory neurons of guinea pigs. Neurosci Lett 1985;57:125–30.

Gibbins IL, Wattchow D, Coventry B. Two immunohistochemically identified populations of calcitonin gene-related peptide (CGRP)-immunoreactive axons in human skin. Brain Res 1987;414:143–8.

Go VL, Yaksh TL. Release of substance P from the cat spinal cord 9. J Physiol 1987;391:141–67.

Gouarderes C, Beaudet A, Zajac JM, Cros J, Quirion R. High resolution radioautographic localization of [125I] FK-33-824-labelled mu opioid receptors in the spinal cord of normal and deafferented rats 163. Neuroscience 1991;43:197–209.

Hammond DL. Intrathecal administration: methodological considerations. In: Besson JM, editor. Progress in Brain Research. New York: Elsevier Science; 1988. p. 313–20.

Heron A, Rouleau A, Cochois V, Pillot C, Schwartz JC, Arrang JM. Expression analysis of the histamine H(3) receptor in developing rat tissues. Mech Dev 2001;105:167–73.

Hill SJ, Ganellin CR, Timmerman H, Schwartz JC, Shankley NP, Young JM, et al. International Union of Pharmacology. XIII. Classification of histamine receptors. Pharmacol Rev 1997;49:253–78.

Imamura M, Smith NC, Garbarg M, Levi R. Histamine H3-receptor-mediated inhibition of calcitonin gene-related peptide release from cardiac C fibers. A regulatory negative-feedback loop. Circ Res 1996;78:863–9.

Ishida-Yamamoto A, Senba E, Tohyama M. Distribution and fine structure of calcitonin gene-related peptide-like immunoreactive nerve fibers in the rat skin. Brain Res 1989;491:93–101.

Jansen FP, Mochizuki T, Yamamoto Y, Timmerman H, Yamatodani A. In vivo modulation of rat hypothalamic histamine release by the histamine $H_3$ receptor ligands, immepip and clobenpropit. Effects of intrahypothalamic and peripheral application. Eur J Pharmacol 1998;362:149–55.

Kashiba H, Fukui H, Morikawa Y, Senba E. Gene expression of histamine $H_1$ receptor in guinea pig primary sensory neurons: a relationship between $H_1$ receptor mRNA-expressing neurons and peptidergic neurons. Brain Res Mol Brain Res 1999;66:24–34.

Levine JD, Reichling DB. Peripheral mechanisms of inflammatory pain. In: Wall and PD, Melzack R, editors. Textbook of Pain. Edinburgh: Churchill Livingstone; 1999. p. 59–84.

Levine JD, Fields HL, Basbaum AI. Peptides and the primary afferent nociceptor. J Neurosci 1993;13:2273–86.

Malmberg AB, Yaksh TL. Pharmacology of the spinal action of ketorolac, morphine, ST-91, U50488H, and L-PIA on the formalin test and an isobolographic analysis of the NSAID interaction. Anesthesiology 1993;79:270–81.

McCall WD, Tanner KD, Levine JD. Formalin induces biphasic activity in C-fibers in the rat. Neurosci Lett 1996;208:45–8.

Millan MJ. The induction of pain: an integrative review 1. Prog Neurobiol 1999;57:1–164.

Millan MJ. Descending control of pain. Prog Neurobiol 2002;66:355–474.

Mobarakeh JI, Sakurada S, Katsuyama S, Kutsuwa M, Kuramasu A, Lin ZY, et al. Role of histamine H(1) receptor in pain perception: a study of the receptor gene knockout mice. Eur J Pharmacol 2000;391:81–9.

Molina-Hernandez A, Nunez A, Sierra JJ, Arias-Montano JA. Histamine $H_3$ receptor activation inhibits glutamate release from rat striatal synaptosomes. Neuropharmacology 2001;41:928–34.

Morton CR, Hutchison WD. Release of sensory neuropeptides in the spinal cord: studies with calcitonin gene-related peptide and galanin. Neuroscience 1989;31:807–15.

Nagy JI, Iversen LL, Goedert M, Chapman D, Hunt SP. Dose-dependent effects of capsaicin on primary sensory neurons in the neonatal rat. J Neurosci 1983;3:399–406.

Nemmar A, Delaunois A, Beckers JF, Sulon J, Bloden S, Gustin P. Modulatory effect of imetit, a histamine H3 receptor agonist, on C-fibers, cholinergic fibers and mast cells in rabbit lungs in vitro. Eur J Pharmacol 1999;371:23–30.

Ohkubo T, Shibata M, Takahashi H, Inoki R. Roles of substance P and somatostatin on transmission of nociceptive information induced by formalin in spinal cord. J Pharmacol Exp Ther 1990;252:1261–8.

Ohkubo T, Shibata M, Inoue M, Kaya H, Takahashi H. Regulation of substance P release mediated via prejunctional histamine $H_3$ receptors. Eur J Pharmacol 1995;273:83–8.

Owen DA, Woodward DF. Histamine and histamine $H_1$- and $H_2$-receptor antagonists in acute inflammation. Biochem Soc Trans 1980;8:150–5.

Owen DA, Poy E, Woodward DF, Daniel D. Evaluation of the role of Histamine H1- and H2-receptors in cutaneous inflammation in the guinea-pig produced by histamine and mast cell degranulation. Br J Pharmacol 1980;69:615–23.

Parada CA, Tambeli CH, Cunha FQ, Ferreira SH. The major role of peripheral release of histamine and 5-hydroxytryptamine in formalin-induced nociception. Neuroscience 2001;102:937–44.

Pare M, Elde R, Mazurkiewicz JE, Smith AM, Rice FL. The Meissner corpuscle revised: a multiafferented mechanoreceptor with nociceptor immunochemical properties. J Neurosci 2001a;21:7236–46.

Pare M, Elde R, Mazurkiewicz JE, Smith AM, Rice FL. The Meissner corpuscle revised: a multiafferented mechanoreceptor with nociceptor immunochemical properties. J Neurosci 2001b;21:7236–46.

Peterson MA, Basbaum AI, Abbadie C, Rohde DS, McKay WR, Taylor BK. The differential contribution of capsaicin-sensitive afferents to behavioral and cardiovascular measures of brief and persistent nociception and to Fos expression in the formalin test. Brain Res 1997;755:9–16.

Pillot C, Heron A, Cochois V, Tardivel-Lacombe J, Ligneau X, Schwartz JC, et al. A detailed mapping of the histamine H(3) receptor and its gene transcripts in rat brain. Neuroscience 2002;114:173–93.

Pollard H, Moreau J, Arrang JM, Schwartz JC. A detailed autoradiographic mapping of histamine H3 receptors in rat brain areas. Neuroscience 1993;52:169–89.

Poveda R, Fernandez-Duenas V, Fernandez A, Sanchez S, Puig MM, Planas E. Synergistic interaction between fentanyl and the histamine $H_3$ receptor agonist R-(alpha)-methylhistamine, on the inhibition of nociception and plasma extravasation in mice. Eur J Pharmacol 2006;541:53–6.

Raja SN, Meyer RA, Ringkamp M, Campbell JN. Peripheral neural mechanisms of nociception. In: Wall PD, Melzack R, editors. Textbook of Pain. Edinburgh: Churchill Livingstone; 1999. p. 11–57.

Roberson C, Clapham DE. GPCR signalling through ion channels. In: Pangalos MN, Davies CH, editors. Understanding G protein-coupled receptors and their role in the CNS. New York: Oxford University Press; 2002. p. 108–23.

Rouleau A, Garbarg M, Ligneau X, Mantion C, Lavie P, Advenier C, et al. Bioavailability, antinociceptive and antiinflammatory properties of BP 2-94, a histamine $H_3$ receptor agonist prodrug. J Pharmacol Exp Ther 1997;281:1085–94.

Rouleau A, Stark H, Schunack W, Schwartz JC. Anti-inflammatory and antinociceptive properties of BP 2-94, a histamine H(3)-receptor agonist prodrug. J Pharmacol Exp Ther 2000;295:219–25.

Sann H, Pierau FK. Efferent functions of C-fiber nociceptors. Z Rheumatol 1998;57(Suppl 2):8–13.

Sawynok J, Liu XJ. The formalin test: characteristics and usefulness of the model. Rev Analg 2004;7:145–63.

Sawynok J, Reid A. Chronic intrathecal cannulas inhibit some and potentiate other behaviors elicited by formalin injection. Pain 2003;103:7–9.

Tjolsen A, Berge OG, Hunskaar S, Rosland JH, Hole K. The formalin test: an evaluation of the method. Pain 1992;51:5–17 [464].

Twycross RG. Opioids. In: Wall and PD, Melzack R, editors. Textbook of Pain. Edinburgh: Chruchill Livingstone; 1999. p. 1151–214.

Vollinga RC, de Koning JP, Jansen FP, Leurs R, Menge WM, Timmerman H. A new potent and selective histamine H3 receptor agonist, 4-(1H-imidazol-4-ylmethyl)piperidine. J Med Chem 1994;37:332–3.

Yaksh TL. Preclinical models of nociception. In: Yaksh TL, editor. Anesthesia Biology Foundations. Philadelphia: Lippincott-Raven Publishers; 1997. p. 685–718.

Yaksh TL. Central pharmacology of nociceptive transmission. In: Wall PD, Melzack R, editors. Textbook of Pain. Edinburgh: Churchill Livingstone; 1999. p. 253–308.

Yaksh T, Rudy TA. Chronic catheterization of the spinal subarachnoid space. Physiol Behav 1976;17:1031–6.

Yaksh TL, Jessell TM, Gamse R, Mudge AW, Leeman SE. Intrathecal morphine inhibits substance P release from mammalian spinal cord in vivo. Nature 1980;286:155–7.

Zhang X, Bao L, Shi TJ, Ju G, Elde R, Hokfelt T. Down-regulation of mu-opioid receptors in rat and monkey dorsal root ganglion neurons and spinal cord after peripheral axotomy. Neuroscience 1998;82:223–40.