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Activation of spinal histamine H_3 receptors inhibits mechanical nociception

Keri E. Cannon[a], Julia W. Nalwalk[a], Rebecca Stadel[a], P. Ge[b], D. Lawson[b], I. Silos-Santiago[b], Lindsay B. Hough[a,*]

[a] *Center for Neuropharmacology and Neuroscience, Albany Medical College MC-136, 47 New Scotland Ave., Albany, NY 12206, USA*
[b] *Millennium Pharmaceuticals Inc., Cambridge, MA, USA*

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Abstract

Previous studies have suggested a possible pain-modulatory role for histamine H_3 receptors, but the localization of these receptors and nature of this modulation is not clear. In order to explore the role of spinal histamine H_3 receptors in the inhibition of nociception, the effects of systemically (subcutaneous, s.c.) and intrathecally (i.t.) administered histamine H_3 receptor agonists were studied in rats and mice. Immepip (5 mg/kg, s.c.) produced robust antinociception in rats on a mechanical (tail pinch) test but did not alter nociceptive responses on a thermal (tail flick) test. In contrast, this treatment in mice (immepip, 5 and 30 mg/kg, s.c.) did not change either mechanically or thermally evoked nociceptive responses. When administered directly into the spinal subarachnoid space, immepip (15–50 μg, i.t.) and R-α-methylhistamine (50 μg, i.t.) had no effect in rats on the tail flick and hot plate tests, but produced a dose- and time-dependent inhibition (90–100%) of nociceptive responses on the tail pinch test. This attenuation was blocked by administration of thioperamide (10 mg/kg, s.c.), a histamine H_3 receptor antagonist. Intrathecally administered thioperamide also reversed antinociceptive responses induced by systemically administered immepip, which demonstrates a spinal site of action for the histamine H_3 receptor agonist. In addition, intrathecally administered immepip (25 μg) produced maximal antinociception on the tail pinch test in wild type, but not in histamine H_3 receptor knockout (H_3KO) mice. These findings demonstrate an antinociceptive role for spinal histamine H_3 receptors. Further studies are needed to confirm the existence of modality-specific (i.e. mechanical vs. thermal) inhibition of nociception by these receptors, and to assess the efficacy of spinally delivered histamine H_3 receptor agonists for the treatment for pain.

Keywords: H_3 receptor; Immepip; Antinociception

1. Introduction

Histamine is released from neuronal and non-neuronal (i.e. mast cell) sources and exerts its actions through several receptors, including the histamine H_3 receptor (Hough, 2001). Although histamine H_3 receptors were initially discovered to be presynaptic inhibitory autoreceptors in the brain (Arrang et al., 1983), lesion studies revealed the majority of histamine H_3 receptors are inhibitory heteroreceptors located on postsynaptic neurons (Pollard et al., 1993). Biochemical studies confirmed that the histamine H_3 receptor is a pertussis toxin-sensitive, G_i/G_o-coupled, transmembrane receptor linked to the inhibition of adenylate cyclase and to other pathways (Clark and Hill, 1996; Morisset et al., 2000; Lovenberg et al., 1999; Drutel et al., 2001). Histamine H_3 receptor binding sites are found in high densities in the cerebral cortex, hippocampus, olfactory nucleus, and striatum, while lower binding densities are found in the noradrenergic and serotonergic neurons of the brainstem and superficial dorsal horn of the spinal cord (Pollard et al., 1993). The cloning of human (Lovenberg et al., 1999), rat (Lovenberg et al., 2000), and guinea pig (Tardivel-Lacombe et al., 2000) histamine H_3 receptor genes has led to the discovery of multiple isoforms in each species (Drutel et al., 2001; Tardivel-Lacombe et al., 2000; Coge et al., 2001; Wellendorph et al., 2002).

Several pharmacological agents have allowed the role of histamine H_3 receptors in physiological processes to be elucidated. One such tool is thioperamide, the first selective

* Corresponding author. Tel.: +1-518-262-5785; fax: +1-518-262-5799.
E-mail address: houghl@mail.amc.edu (L.B. Hough).

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antagonist developed for the histamine H_3 receptor (Arrang et al., 1987). Thioperamide inhibits radiolabeled histamine H_3 receptor binding in rat cerebral cortex membranes with a K_i of 2.1 nM (Arrang et al., 1987) and remains the prototypical histamine H_3 receptor antagonist used for in vivo and in vitro research (Hill et al., 1997). The first selective histamine H_3 receptor agonist R-α-methylhistamine has been widely used as a potent in vivo tool, as well as a useful radioligand for characterizing the role of histamine H_3 receptors in physiological processes (Hill et al., 1997). R-α-methylhistamine has high affinity for the histamine H_3 receptor, giving a pD_2 of 7.8 on electrically evoked twitches of the guinea pig jejunum (Vollinga et al., 1994). However, several limitations of R-α-methylhistamine exist, including poor blood–brain barrier penetration due to its highly polar structure (Taylor et al., 1992; Rouleau et al., 1997, 2000). In addition, at higher concentrations R-α-methylhistamine can act at histamine H_1 receptors (Hey et al., 1992). Finally, R-α-methylhistamine is also a substrate for histamine *N*-methyltransferase, the histamine-metabolizing enzyme, causing rapid inactivation of the drug in humans (Rouleau et al., 1997, 2000). Therefore, the pro-drug (*R*)-(−)-2-[[*N*-[1-(1 *H*-imidazol-4-yl)-2-propyl]imino]phenylmethyl]phenol (referred to as BP 2-94) was developed to slowly metabolize into the desired active metabolite, R-α-methylhistamine (Krause et al., 1995). BP 2-94 succeeded in achieving sustained plasma levels of R-α-methylhistamine in humans, but this drug failed to penetrate the brain in rodents (Rouleau et al., 1997).

Other highly selective histamine H_3 receptor agonists have been developed, including immepip (Vollinga et al., 1994). Immepip is a potent histamine H_3 receptor agonist (pD_2 of 8.0 on electrically evoked twitches of the guinea pig jejunum). Unlike R-α-methylhistamine, immepip is not a substrate for histamine *N*-methyltransferase, and it lacks activity at the histamine H_1 receptor (Hough and Leurs, 2002). Furthermore, immepip may penetrate the brain (Jansen et al., 1998) and spinal cord after systemic administration, thus providing a better tool for studying the role of histamine H_3 receptors in vivo.

Localization of histamine H_3 receptors on sensory fibers has been suggested by several laboratories (Imamura et al., 1996; Ohkubo et al., 1995; Delaunois et al., 1995; Nemmar et al., 1999). Imamura et al. (1996) demonstrated a histamine H_3 receptor-induced inhibition of bradykinin-induced or capsaicin-induced release of calcitonin gene-related peptide from cardiac tissues. Delaunois et al. (1995) showed that histamine H_3 receptor agonists inhibited acetylcholine- and capsaicin-induced edema in isolated rabbit lungs, thus suggesting the presence of prejunctional histamine H_3 receptors on C fibers. In addition, the histamine H_3 receptor agonist R-α-methylhistamine was shown to inhibit the release of substance P from peripheral sciatic nerve endings of rats (Ohkubo et al., 1995).

Since nociceptive (i.e. pain) transmission is conveyed by Aδ and C sensory fibers, and histamine H_3 receptors have been suggested to inhibit the activity of sensory fibers, then histamine H_3 receptor agonists could inhibit nociceptive transmission and show promise as analgesics. However, relatively few studies (Rouleau et al., 1997, 2000) have focused on antinociceptive effects of histamine H_3 receptor agonists. Oral administration of the prodrug BP 2-94 in mice reduced capsaicin-induced licking in mice (Rouleau et al., 2000) and inhibited nociceptive responses on the phenylbenzoquinone writhing test and on both phases of the formalin test (Rouleau et al., 1997). However, BP 2-94 failed to produce antinociceptive effects on the hot plate test (Rouleau et al., 1997). Because neither BP 2-94 nor its active metabolite R-α-methylhistamine were detectable in the brain (Rouleau et al., 1997), the mixed antinociceptive actions of BP 2-94 could be due to poor central nervous system penetration or to a limited role for the histamine H_3 receptor in pain modulation. The present work addresses the blood–brain barrier problem by assessing the antinociceptive activity of intrathecally (i.t.) and systemically (subcutaneously, s.c.) administered histamine H_3 receptor agonists.

2. Methods and materials

2.1. Animals

All experiments were approved by the Institutional Animal Care and Use Committee of Albany Medical College. Male Sprague–Dawley rats (300–350 g, Taconic Farms, Germantown, NY), housed in groups of two or three per cage, and female wild type or histamine H_3 receptor knockout mice (H_3KO, 10–14 weeks old, 20–30 g), housed up to seven per cage, 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.

Histamine H_3 receptor knockout (H_3KO) mice were generated by targeted deletion of exon 3 of the mouse histamine H_3 receptor gene. The H_3KO targeting vector, constructed by homologous recombination in yeast (Storck et al., 1996), consisted of a lacZ-neo-URA3 cassette that replaced all but the first 45 base pairs of exon 3 and 300 base pairs of the intronic sequence downstream of exon 3. The targeted embryonic stem cell clones (129/SvEv cells) were produced by electroporation, confirmed by Southern blots, and injected into C57BL/6J blastocysts to generate chimeras. Male chimeras were mated with C57BL/6J females, and their heterozygous offspring were interbred to generate F2 progeny. Polymerase chain reaction (PCR) was performed to genotype the resulting progeny. Mendelian inheritance patterns were seen in offspring from heterozygous matings. H_3KO mice showed no detectable histamine H_3 receptor mRNA in the brain or the spinal cord by in situ hybridization. Brains from these subjects also had no detectable histamine H_3 receptor binding as assessed with [^{3}H]R-α-methylhistamine (Harper et al.,

1999). Further characterization of these H_3KO mice is being reported elsewhere (manuscript in preparation). In addition, two other laboratories have recently generated and characterized H_3KO mice (Toyota et al., 2002; Takahashi et al., 2002).

2.2. Drugs

Immepip 2 HBr (kindly provided by Professor R. Leurs, Vrije University, Amsterdam, Netherlands), R-α-methylhistamine 2 HCl (kindly provided by Dr. C. Tedford, Gliatech, Cleveland, OH), and thioperamide maleate (Tocris, Ellisville, MO) were dissolved in saline and neutralized with 1 N NaOH to pH 6.5. All doses are expressed as bases.

2.3. Surgery

For rat intrathecal injections, the spinal subarachnoid space was chronically cannulated under general anesthesia as described previously by Yaksh and Rudy (1976) with modifications (Hammond, 1988). The animals were then placed in separate cages and allowed to recover for a minimum of 10 days.

2.4. Nociceptive tests

Three nociceptive tests were performed in rats. For the hot plate test (Eddy and Leimbach, 1953), the rats were placed on a 52 °C metal surface and the latency for a hind paw lift or lick was recorded. Baseline latencies were between 8 and 14 s. Animals not responding within 60 s (cutoff) were removed from the heat source. For the tail flick test (D'Amour and Smith, 1941), the ventral surface of the rat's tail (approximately 2–5 cm from the tip) was exposed to radiant heat and the latency of tail removal from the heat was recorded. Baseline latencies were between 3 to 4 s with a 15-s cutoff. For the tail pinch test (Bianchi and Franceschini, 1954), rats were placed in a clean cage and an alligator clip exerting force equal to 625 g of weight was placed on the tail towards the tip (approximately 5–10 cm from the tip). The latency for vocalization, biting, or flicking the clip was recorded. Baseline latencies fell between 15 and 25 s with a 90-s cutoff.

Two nociceptive tests were performed in mice. In the tail immersion test (Sewell and Spencer, 1976), mice were loosely restrained in a conical polypropylene tube. The tip (2–3 cm) of the tail was immersed into a 55 °C water bath and the latency to the flick or removal of the tail from the water was recorded. Baseline latencies fell between 1 and 3 s with an 8-s cutoff. For the tail pinch test (Bianchi and Franceschini, 1954), a microserrafine arterial clip (Fine Science Tools, Foster City, CA) was placed at the base of the tail. The latency for vocalization, biting, or flicking the clip was recorded. Baseline latencies fell between 0.5 and 2 s with a 30-s cutoff.

2.5. Rat test procedure

Rats were tested at 60 s intervals with a single hot plate test, three tail flick tests, and a single tail pinch test. The sequence of nociceptive tests was performed with 60 s intervals separating the tests. Subjects were then secured in a laboratory pad for intrathecal injection. The seal at the free ending of the chronic catheter was removed and a 10 μl manual injection was made over 1 min followed by a 10 μl flush of saline over 1 min. Movement of an air bubble between the tubing and the syringe, as well as absence of leakage, ensured successful intrathecal injections. Following intrathecal injection, the catheter was heat-sealed, and rats were tested at 60 s intervals with single hot plate, tail flick, and tail pinch tests. Following the experiments, successful cannulations were verified by observing cut-off responses on the tail flick test after a 10 μg morphine sulfate intrathecal injection. Some rats were baseline tested and received systemically administered drug prior to intrathecal injection. For systemic injections in rats, subjects received a single baseline tail flick and tail pinch tests.

2.6. Mouse test procedure

Intrathecal injections were made exactly as described by Hylden and Wilcox (1980). Mice were first baseline tested on the tail immersion and tail pinch tests. Intrathecal injections were performed with a total of 5 μl of solution injected over 30 s. Following injections, subjects were tested on the tail immersion and tail pinch test. The intrathecal method used presently was independently validated by demonstrating maximal nociceptive activity of intrathecal morphine in at least 95% of injected subjects.

2.7. Statistics

Results are expressed in latencies, mean ± S.E.M. Repeated measures analysis of variance (ANOVA) and planned comparisons were used to analyze the data (Statistica, CSS, Tulsa, OK).

3. Results

3.1. Systemic studies

Systemically administered immepip (50 μg or 5 mg/kg, s.c.) had no effect on tail flick latencies in rats (Fig. 1A). However, the larger dose (5 mg/kg) produced significant antinociception on the tail pinch test 30 and 60 min post-injection (Fig. 1B). The smaller dose (50 μg), shown below to be active intrathecally, was inactive after subcutaneous administration. In mice, large systemic doses of immepip (5 and 30 mg/kg, s.c.) failed to alter nociceptive responses on

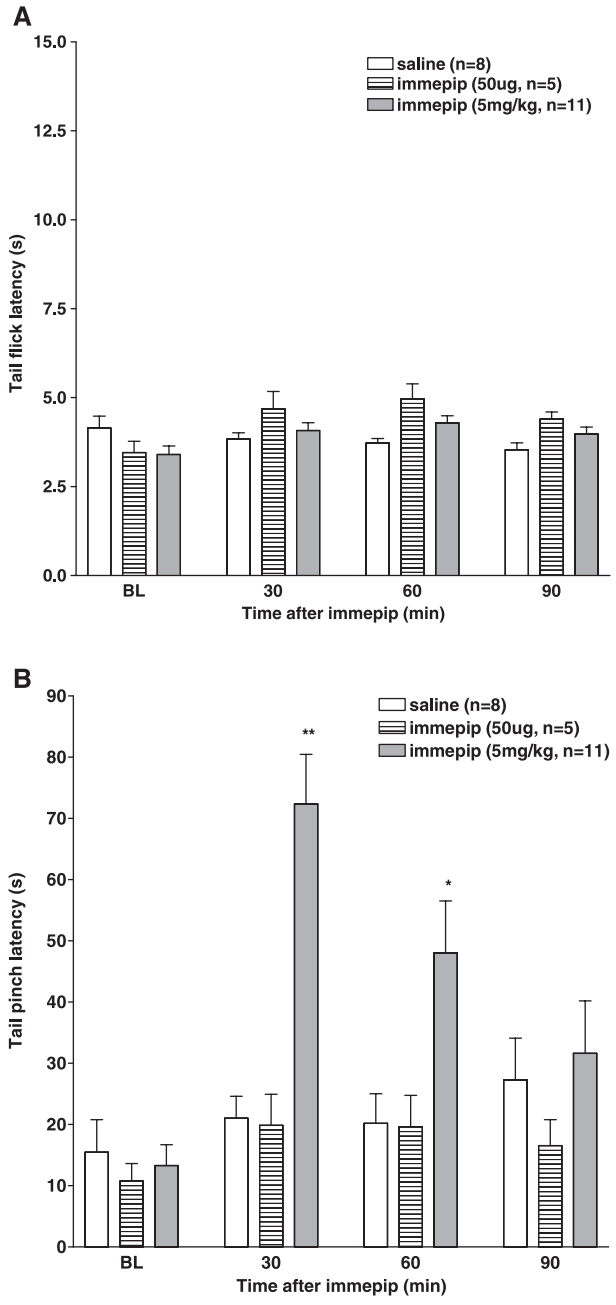


Fig. 1. Effect of systemically administered immepip on nociceptive responses. Rats were tested for baseline responses (BL), then received immepip (50 μg or 5 mg/kg, s.c.) or saline vehicle. Tail flick (A) and tail pinch (B) latencies (ordinate, s, mean ± S.E.M., number of subjects in parentheses) were recorded at the times shown after immepip injection (abscissa, min). *,**$P<0.01$, $P<0.001$ vs. saline at same time interval, respectively.

either the tail immersion or the tail pinch test (data not shown).

3.2. Rat intrathecal studies

When injected intrathecally, histamine H_3 receptor agonists did not change nociceptive responses to the tail flick test (data not shown) or the hot plate test (data not shown). In contrast, intrathecal administration of immepip (50 μg) produced nearly maximal antinociception on the tail pinch test (Fig. 2). Significant effects, seen as early as 5 min post-injection, remained significant over the course of 60 min, and declined thereafter. A lower dose of immepip (15 μg) produced mean nociceptive responses greater than those produced by saline; however, this effect was not statistically significant. Similar to the effect of immepip (50 μg), R-α-methylhistamine (50 μg), produced significant antinociception on the tail pinch test from 10 to 120 min post-injection.

In order to determine if the antinociceptive action of immepip is mediated by the histamine H_3 receptor, the effect of the selective histamine H_3 receptor antagonist thioperamide was determined on immepip antinociception (Fig. 3). Similar to what was seen in the previous experiment, nociceptive responses on the tail flick test were not altered by combinations of intrathecal immepip and subcutaneous thioperamide (data not shown). However, intrathecal immepip antinociception on the tail pinch test was abolished by subcutaneous pretreatment with thioperamide (Fig. 3A). In order to test the hypothesis that systemically administered immepip induces mechanical antinociception

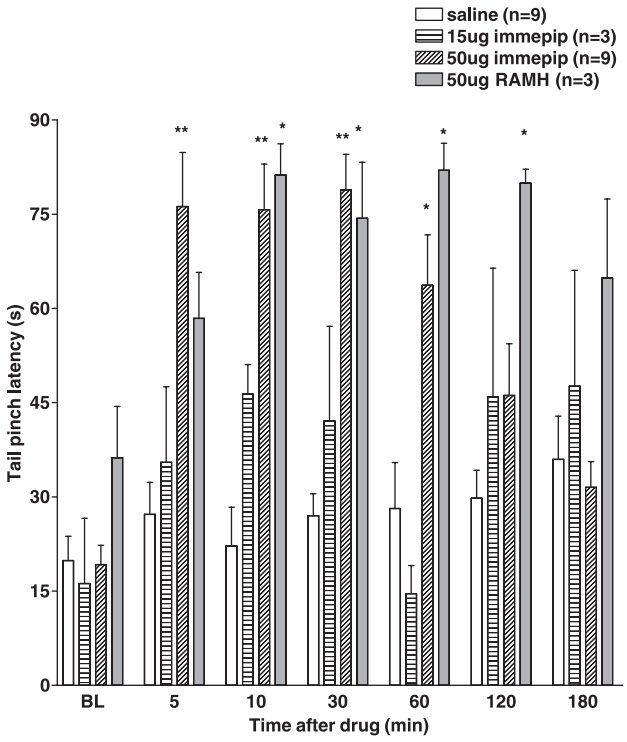
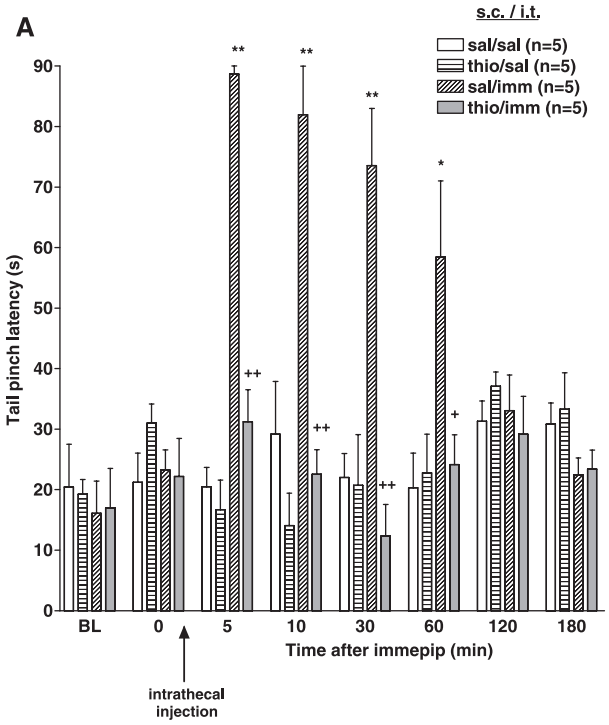


Fig. 2. Effect of intrathecal histamine H_3 receptor agonists on nociceptive responses. Rats were tested for baseline responses (BL) then received immepip (15 μg or 50 μg, i.t.), R-α-methylhistamine (RAMH, 50 μg), or saline vehicle. Tail pinch latencies (ordinate, s, mean ± S.E.M., number of subjects in parentheses) were recorded at the times shown after histamine H_3 receptor agonist injection (abscissa, min). *,**$P<0.05$, $P<0.01$ vs. saline at same time interval, respectively.

by action on spinal histamine H_3 receptors, the effects of intrathecal thioperamide were studied after subcutaneous administration of immepip. In these experiments, mechanical antinociception produced by systemically administered immepip was completely reversed by intrathecal thioperamide treatment (Fig. 3B). Thioperamide alone did not alter nociceptive responses on any test.



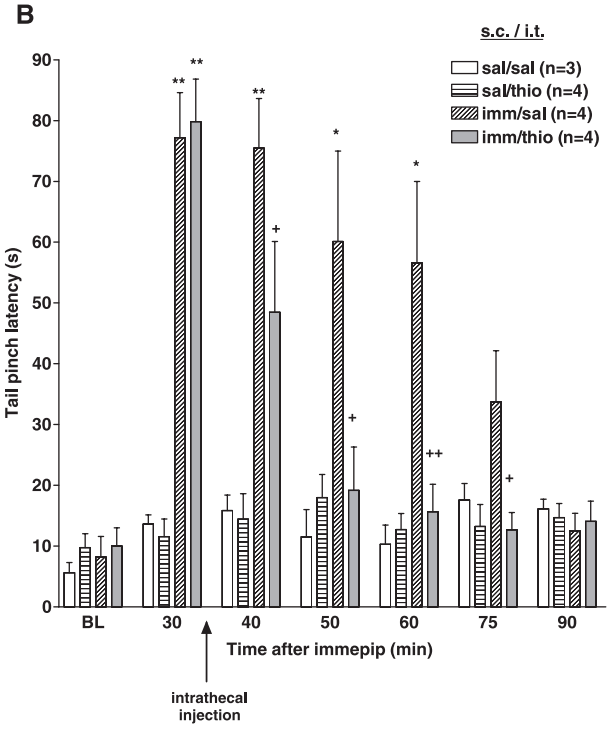


Fig. 3. Effect of thioperamide, a histamine H_3 receptor antagonist, on immepip antinociception. Rats were tested for baseline responses (BL), then immediately received a subcutaneous injection of either thioperamide (A: thio, 10 mg/kg), immepip (B: imm, 5 mg/kg), or saline (A and B: sal). Thirty minutes later, baseline testing was repeated (A: 0; B: 30), followed by intrathecal injection of immepip (A: imm, 50 μg.), thioperamide (B: thio, 20 μg), or saline (A and B: sal). Tail pinch latencies (ordinate, s, mean ± S.E.M., number of subjects in parentheses) were recorded at the times shown after immepip injection (abscissa, min). Data from A and B were from different subjects. *·**$P<0.05$, $P<0.005$ vs. sal/sal at the same time interval, respectively. +, ++ $P<0.05$, $P<0.005$ vs. imm + sal at same time interval, respectively.

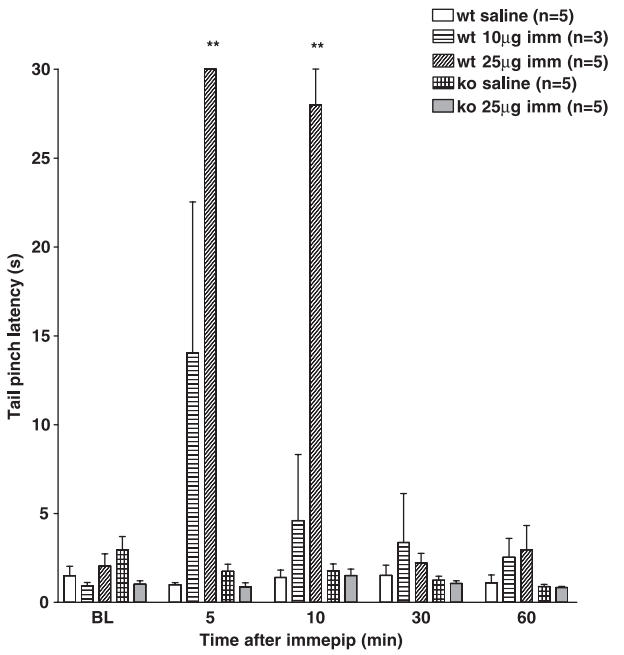


Fig. 4. Effect of intrathecal immepip on nociceptive responses in wild type (wt) and H_3 receptor knockout (ko) mice. Mice were tested for baseline responses (BL), then received immepip (imm, 10 or 25 μg) or saline vehicle. Tail pinch latencies (ordinate, s, mean ± S.E.M., number of subjects in parentheses) were recorded at the times shown after immepip injection (abscissa, min). **$P<0.001$ vs. saline of same genotype.

3.3. Mouse intrathecal studies

Intrathecal administration of immepip (25 μg) in wild type and histamine H_3 receptor knockout (H_3KO) mice produced no changes in nociceptive responses on the tail immersion test (data not shown). On the tail pinch test, however, this treatment produced maximal, reversible antinociception in wild type mice 5 and 10 min after administration (Fig. 4). On the same test, this treatment was completely without effect in H_3KO mice. A lower dose of immepip (10 μg) produced mean nociceptive latencies at 5 min post-injection in wild type mice that were greater than those produced by saline; however, this effect was not statistically significant.

4. Discussion

Several lines of evidence support the hypothesis that the activation of histamine H_3 receptors attenuates mechanical nociception. First, the antinociception was dose-dependent (Fig. 2). Second, the antinociception was reversible (Figs. 1B–4). Third, two structurally different histamine H_3 receptor agonists produced the effect (Fig. 2). Fourth, the antinociceptive effect of immepip was blocked by the selective histamine H_3 receptor antagonist thioperamide in rats (Fig. 3). Finally, wild type, but not H_3KO mice, demonstrated mechanical antinociception after intrathecal administration of immepip (Fig. 4).

Present results offer considerable proof for a spinal site of action for the pain-modulatory effects of histamine H_3 receptor agonists. Low doses of intrathecal immepip produced maximal mechanical antinociception in both rats (Figs. 2 and 3) and mice (Fig. 4). In rats, the intrathecal dose of immepip was not effective when administered systemically, suggesting that the effects of intrathecal immepip are localized to the spinal cord. In agreement with the idea of spinally located histamine H_3 receptors, autoradiography studies performed with [^{3}H]R-α-methylhistamine showed low-density, specific labeling of histamine H_3 receptors in the superficial layers of the rat dorsal horn (Pollard et al., 1993). Also, in situ hybridization studies in rats revealed histamine H_3 receptor mRNA in the sensory neurons of the dorsal horn and dorsal root ganglia (Heron et al., 2001). In rat spinal cord slices pre-labeled with [^{3}H]noradrenaline, R-α-methylhistamine caused a decrease in electrically evoked [^{3}H]noradrenaline release (Celuch, 1995), also suggesting localization of functional histamine H_3 receptors in the spinal cord. Finally, unpublished immunohistochemical studies in our laboratory revealed intense histamine H_3 receptor-like immunofluorescence in both the spinal cord and the dorsal root ganglia of mice (Cannon et al., unpublished).

Based on the findings of Figs. 1 and 2, systemically administered immepip could have been exerting its antinociceptive effects in rats peripherally, spinally, or supraspinally. R-α-methylhistamine does not reach the brain in measurable concentrations after systemic administration in rats and mice (Rouleau et al., 1997, 2000; Yamasaki et al., 1994), but comparable studies have not been performed with immepip. Thus, the degree of brain and spinal penetration in our experiments is unknown. However, immepip (5 mg/kg, s.c.) was shown to reduce histamine release in the anterior hypothalamus after systemic administration in rats (Jansen et al., 1998). Therefore, additional experiments were performed to directly evaluate the role of spinal histamine H_3 receptors as a target for systemically administered immepip. When administered intrathecally, thioperamide completely reversed mechanical antinociception induced by systemically administered immepip (Fig. 3B). This experiment completely rules out peripheral and supraspinal sites of action since histamine H_3 receptors at these sites could not to have been blocked by such a low intrathecal dose of thioperamide (20 μg). In addition, because subcutaneous administration of immepip induces antinociception in rats but not mice (Fig. 1), yet the same compound is effective after intrathecal treatment in both species, it is likely that systemic treatment in mice does not result in appreciable drug levels in the spinal cord. Thus, systemic administration of immepip in rats appears to induce mechanical antinociception via activation of spinal histamine H_3 receptors.

Among many possible spinal sites of action, two targets for the inhibition of mechanical nociception by histamine H_3 receptor agonists can be considered. First, histamine H_3 receptors could be located on primary afferent fiber terminals, where they could inhibit the synthesis and/or release of neurotransmitters. Based upon the present results, histamine H_3 receptors may not be located on fibers mediating thermal nociceptive transmission [i.e. polymodal C, C heat, and Aδ Type II mechanoheat fibers (Beck et al., 1974; Yeomans and Proudfit, 1996; Ringkamp et al., 2001)]. Because recent studies suggest that mechanical nociception is mediated primarily by capsaicin-insensitive Aδ high threshold mechanoreceptors and Aδ Type I mechanoheat nociceptors (Magerl et al., 2001; Ringkamp et al., 2001; Szolcsanyi et al., 1988), histamine H_3 receptors might be localized on the central terminals of these types of nociceptive fibers.

The second possible spinal target for histamine H_3 receptor agonists may be histamine H_3 receptors on dendrites or soma of second order neurons. Current studies have revealed different types of modality-specific spinothalamic tract neurons originating from lamina I of the dorsal horn (Craig and Andrew, 2002). Lamina I is the region of the dorsal horn where Aδ fibers primarily terminate. Histamine H_3 receptors may be located on mechano-specific lamina I spinothalamic neurons, but this localization has not yet been established.

The present results confirm and extend findings by Rouleau et al. (1997) showing that activation of histamine H_3 receptors does not attenuate thermal nociceptive responses. Based on the earlier findings, it seemed possible that poor penetration of the brain or spinal cord by orally administered histamine H_3 receptor agonists might account for the absence of activity on thermal nociceptive (i.e. hot plate) responses. However, the present results, showing the same absence of activity on thermal nociceptive responses after systemically and intrathecally administered histamine H_3 receptor agonists, indicate that the degree of penetration by these drugs into the spinal cord does not account for these findings.

The absence of histamine H_3 receptor agonist and antagonist actions on thermal nociception found presently and by Rouleau et al. (1997) is in apparent contradiction with other findings. Two studies reported hyperalgesia on the mouse hot plate test after administration of very high doses of R-α-methylhistamine (100 mg/kg, i.p.; Malmberg-Aiello et al., 1994) and imetit (50 mg/kg, i.p.; Farzin et al., 2002).

Table 1
Intrathecally administered drugs that inhibit acute mechanical and thermal nociceptive transmission in animals and humans

Class	Examples of drugs	References
μ-Opioid agonist	Morphine, Endomorphin-1 and 2	(Abram, 1999)[ab] (Przewlocki et al., 1999)[a]
α_2-Adrenergic agonist	Clonidine, Oxymetazoline	(Abram, 1999)[a,b]; (Sherman et al., 1987)[a]
Muscarinic agonist	Oxotremorine	(Machelska et al., 1999)[a]
Adenosine receptor agonist	N6-cyclohexyladenosine, *N*-ethylcarboxiamidoadenosine	(Gouarderes et al., 2000)[a]
Neuropeptide FF analog	F8Famide, FMRFamide	(Gouarderes et al., 1993)[a]
COX inhibitor	Ketorolac	(Abram, 1999)[a,b]
P2Y agonist	UTP, UDP	(Okada et al., 2002)[a]
AMPA/KA receptor antagonist	LY293558	(Von Bergen et al., 2002)[a]
Acetylcholinesterase inhibitor	Neostigmine	(Abram, 1999)[a,b]

[a] Animals.
[b] Humans.

However, several studies have shown that much lower doses (e.g. 5 mg/kg, i.p.) of these histamine H_3 receptor agonists are sufficient to activate brain histamine H_3 receptors after systemic administration (Blandina et al., 1996; Farzin and Attarzadeh, 2000; Giovannini et al., 1999). Although the present studies did not measure hot plate latencies in mice after systemic administration of immepip, no effect of immepip was seen on another thermal assay (i.e. the mouse tail immersion test) after administration of histamine H_3 receptor-activating doses (5 and 30 mg/kg, s.c.). It is also noteworthy that the same studies (Malmberg-Aiello et al., 1994; Farzin et al., 2002) found slight antinociceptive activity on the mouse hot plate test after thioperamide treatment. We did not observe such effects with thioperamide in rats after systemic administration of a histamine H_3 receptor-blocking dose.

Many analgesics are effective after intrathecal administration in animals and man. A literature review of intrathecal drugs tested on both mechanical and thermal nociception has revealed that all are effective on both kinds of nociceptive stimuli in normal animals (Table 1). The present results suggest that histamine H_3 receptor agonists may differ from these other analgesics because they may attenuate mechanical but not thermal nociception. In fact, previous experiments indicate that separation of these modalities is possible (Kuraishi et al., 1985, 1991). However, results from studies with μ- and κ-opioid agonists suggest that alleged modality-specific inhibition may instead result from a difference in stimulus intensities used in the tests (Parsons and Headley, 1989). Therefore, further studies involving varying mechanical and thermal stimulus intensities are needed to confirm or refute the possibility of modality-specific inhibition of nociceptive transmission by histamine H_3 receptor agonists. The effects of histamine H_3 receptor agonists on mechanical nociceptive responses produced in tissues other than the tail also require further study. A previous study reported that systemic administration of R-α-methylhistamine (20 mg/kg, i.p.) had no effect on the rat paw pressure test up to 3 h post-injection, but produced slight hyperalgesia 4 to 7 h later (Malmberg-Aiello et al., 1994). It is unclear if using a different histamine H_3 agonist (i.e. immepip) and/or a different route of administration (i.e. intrathecal) would produce different outcomes on the rat paw pressure test. Although histamine H_3 receptor agonists seem to show antinociceptive activity on some forms of mechanical nociception, further studies are needed to evaluate the pain-relieving potential of these drugs.

Acknowledgements

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