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H_3 Receptors and Pain Modulation: Peripheral, Spinal, and Brain Interactions

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ABSTRACT

Histamine H_3 receptors (H_3Rs), distributed within the brain, the spinal cord, and on specific types of primary sensory neurons, can modulate pain transmission by several mechanisms. In the skin, H_3Rs are found on certain Aβ fibers, and on keratinocytes and Merkel cells, as well as on deep dermal, peptidergic Aδ fibers terminating on deep dermal blood vessels. Activation of H_3Rs on the latter in the skin, heart, lung, and dura mater reduces calcitonin gene-related peptide and substance P release, leading to anti-inflammatory (but not antinociceptive) actions. However, activation of H_3Rs on the spinal terminals of these sensory fibers reduces nociceptive responding to low-intensity mechanical stimuli and inflammatory stimuli such as formalin. These findings suggest that H_3R agonists might be useful analgesics, but these drugs have not been tested in clinically relevant pain models. Paradoxically, H_3 antagonists/inverse agonists have also been reported to attenuate several types of pain responses, including phase II responses to formalin. In the periaqueductal gray (an important pain regulatory center), the H_3 inverse agonist thioperamide releases neuronal histamine and mimics histamine's biphasic modulatory effects in thermal nociceptive tests. Newer H_3 inverse agonists with potent, selective, and brain-penetrating properties show efficacy in several neuropathic and arthritis pain models, but the sites and mechanisms for these actions remain poorly understood.

Histamine and Pain

Histamine, found throughout the body in both neuronal and non-neuronal sources, can modify pain transmission by actions at multiple receptors in the skin, spinal cord, and brain. Rapidly expanding information on the distribution and functions of H_3 receptors (H_3Rs) and the recent development of new H_3R ligands have heightened interest in the possible modulation of pain by H_3R-acting drugs. The present article provides a short, integrative overview of relevant studies (for review see also Sander et al., 2008; Tiligada et al., 2009; Gemkow et al., 2009).

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Measuring Pain in the Laboratory

Pain, which can be defined as the central representation of tissue-damaging stimuli with sensory-discriminative, motivational, and cognitive components (Besson and Chaouch, 1987), is readily understood by humans as a sensory experience. Ideally, evaluation of the pain-relieving properties of drugs should directly measure reductions in pain perception. Instead, assessment of analgesic drug action in nonverbal subjects relies heavily on measures of behavioral, often reflexive, responses. Because drugs can modify these responses (but not necessarily the underlying perceptions), results from pain testing in laboratory animals can be misleading. The present limitations of preclinical methodologies for identifying pain-relieving drugs have been discussed previously (Rice et al., 2008; Vierck et al., 2008).

Notwithstanding the limitations in state-of-the-art analgesic testing, many factors must be considered when evaluating

ABBREVIATIONS: H_3R, H_3 receptor; H_3RLI, H_3R-like immunoreactivity; H_3KO, H_3R knockout; DRG, dorsal root ganglia; PAG, periaqueductal gray; RAMH, *R*-α-methylhistamine; CGRP, calcitonin gene-related peptide; CNS, central nervous system; BP 2-94, (*R*)-(−)-2-[[*N*-[1-(1*H*-imidazol-4-yl)-2-propyl]imino]phenylmethyl] phenol; GSK189254, 6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridinecarboxamide; Thio, thioperamide; HA, histamine.

the literature on the analgesic potential of H_3R-acting drugs. As discussed below, the nature of the nociceptive stimulus (heat, pressure, chemicals), the characteristics of the stimulus (location, intensity, chronicity), and pathophysiological status of the subject all are critical variables (Le Bars et al., 2001). Finally, pharmacological variables add additional layers of complexity. These include receptor selectivity, sites of action (peripheral, spinal, brain), and dose-response characteristics.

Pharmacology of H_3R-Acting Drugs

Initially discovered as the CNS histaminergic autoreceptor (Arrang et al., 1987), the H_3R is now known to be a Gi/o-coupled receptor that functions as both an auto- and heteroreceptor in the brain. Details of H_3R signaling, splice variants, and pharmacology have been described previously (Hough, 2001; Leurs et al., 2005; Esbenshade et al., 2006; Bongers et al., 2007). Table 1 summarizes the H_3R ligands that have been most commonly used in pain research. Early studies of H_3Rs and pain used the H_3 agonist *R*-α-methylhistamine (RAMH) and the H_3 blocker thioperamide. Subsequent discovery of the potent H_3 agonists imetit and immepip (Vollinga et al., 1994) facilitated ensuing pain studies. Even though thioperamide has been pivotal in H_3R–pain studies (below), pharmaceutical interest in H_3Rs has accelerated the development of potent, selective, brain-penetrating, H_3R-blocking drugs (Leurs et al., 2005; Esbenshade et al., 2006; Sander et al., 2008; De Esch et al., 2009; Gemkow et al., 2009; Tiligada et al., 2009). As mentioned below, investigations of the pain-modulating properties of these newer compounds are only just beginning.

The extensive use of thioperamide, immepip, and imetit in H_3R-related pain studies has been complicated by the more recent discovery that these three drugs have H_4 activity. Even though the H_4 affinity is lower than the respective H_3 value for all three drugs (Lim et al., 2005), the former may be pharmacologically significant in some cases, especially considering the more recent interest in the H_4 receptor as a target for anti-inflammatory or analgesic drug development (Hsieh et al., 2010a). Initial H_4 receptor research focused on hematopoietic cells (which have the highest receptor densities), but more recent studies have identified the H_4 receptor in the brain (Connelly et al., 2009; Strakhova et al., 2009). Thus, pain-related H_3R functions implicated by the use of these drugs require additional validation with pharmacological and/or molecular genetic tools. The opposite point also needs to be made: all H_3R-related findings with these drugs cannot be dismissed a priori based only on H_4 properties. For example, immepip antinociception (discussed below) cannot be assumed to be caused by H_4 receptors (Gemkow et al., 2009) when the effect is mimicked by other H_3 agonists that lack H_4 affinity (e.g., RAMH; Lim et al., 2005), especially when the effect is abolished in H_3R knockout mice (H_3KO) mice (Cannon et al., 2003).

TABLE 1
H_3R ligands that have been commonly used in pain research

Drug	Classification	Reference
BP 2–94	H_3 agonist prodrug	Rouleau et al., 1997
GSK189254	H_3 inverse agonist[a]	Medhurst et al., 2008
Imetit	H_3 agonist[b]	Vollinga et al., 1994
Immepip	H_3 agonist[b]	Vollinga et al., 1994
RAMH	H_3 agonist	Arrang et al., 1987
Thioperamide	H_3 inverse agonist[a]	Arrang et al., 1987

[a] Because nearly all H_3 antagonists have inverse agonist properties in vitro, the terms "antagonist" and "inverse agonist" are used interchangeably in the present work.
[b] Has weaker, but possibly relevant, histamine H_4 receptor affinity (Lim et al., 2005).

Histamine and H_3Rs in the Nervous System

Histamine in the CNS is found in both histaminergic neurons and mast cells (Hough and Leurs, 2006). The latter are bone marrow-derived secretory cells found within diencephalic structures and dura mater. The close proximity of mast cells to blood vessels has led to the suggestion that they regulate blood flow, permeability, and/or immunological access to the brain, but other physiological and pathological roles have been proposed (Hough and Leurs, 2006). In adult mammals, histaminergic neurons are localized exclusively in the tuberomammillary region of the posterior hypothalamus. Both ascending and descending projections from these cells account for the widespread distribution of fibers throughout the brain and spinal cord (Haas and Panula, 2003; Hough and Leurs, 2006). Outside the CNS, histamine is stored in mast cells and other types of cells.

Radioligand binding (Pollard et al., 1993; Medhurst et al., 2008), immunohistochemistry (Chazot et al., 2001), and in situ hybridization studies show the existence of H_3Rs throughout the CNS (Pillot et al., 2002). The highest densities of binding sites were seen in the striatum, cerebral cortex, and olfactory tubercles. Moderate levels of H_3R mRNA were seen in the periaqueductal gray (PAG), a pivotal region for the supraspinal control of nociceptive transmission.

Early measurements of inflammatory peptide release suggested that H_3Rs are present on sensory nerves (Delaunois et al., 1995; Ohkubo et al., 1995; Imamura et al., 1996; Rouleau et al., 1997; Poveda et al., 2006), but the localization of H_3Rs within the peripheral nervous system and spinal cord (Pollard et al., 1993; Héron et al., 2001; Medhurst et al., 2008) was only recently confirmed by immunohistochemistry (Cannon et al., 2007a). In rat and mouse skin, H_3R-like immunoreactivity (H_3RLI) was absent in all of the thin-caliber epidermal and superficial dermal innervation, but present in a subset of thin-caliber fibers associated with arterioles in the deep dermis (Fig. 1). By both anatomical location and immunochemical properties, these fibers were classified as peptidergic (i.e., containing both substance P and CGRP), Aδ-like sensory fibers (Fundin et al., 1997; Cannon et al., 2007a; Bowsher et al., 2009). H_3KO mice showed a complete loss of the H_3RLI on this innervation, confirming the presence of neuronal H_3Rs in the deep dermis of normal mice. The perivascular localization of these fibers is consistent with well known functional and anatomical associations between histamine-containing mast cells, blood vessels, and peptidergic afferent fibers (Imamura et al., 1996; Dimitriadou et al., 1997). H_3RLI was found on Aβ fibers that terminate in Meissner's corpuscles in glabrous skin and as lanceolate endings in hairy skin, as well as on epidermal keratinocytes and Merkel cells (Cannon et al., 2007a), all of which coexpress CGRP immunoreactivity (Paré et al., 2001; Khodorova et al., 2003) (Fig. 1). The presence of H_3RLI (Cannon et al., 2007a) in dorsal root ganglia (DRG) and the spinal dorsal horn is consistent with an H_3 localization on Aδ and Aβ sensory

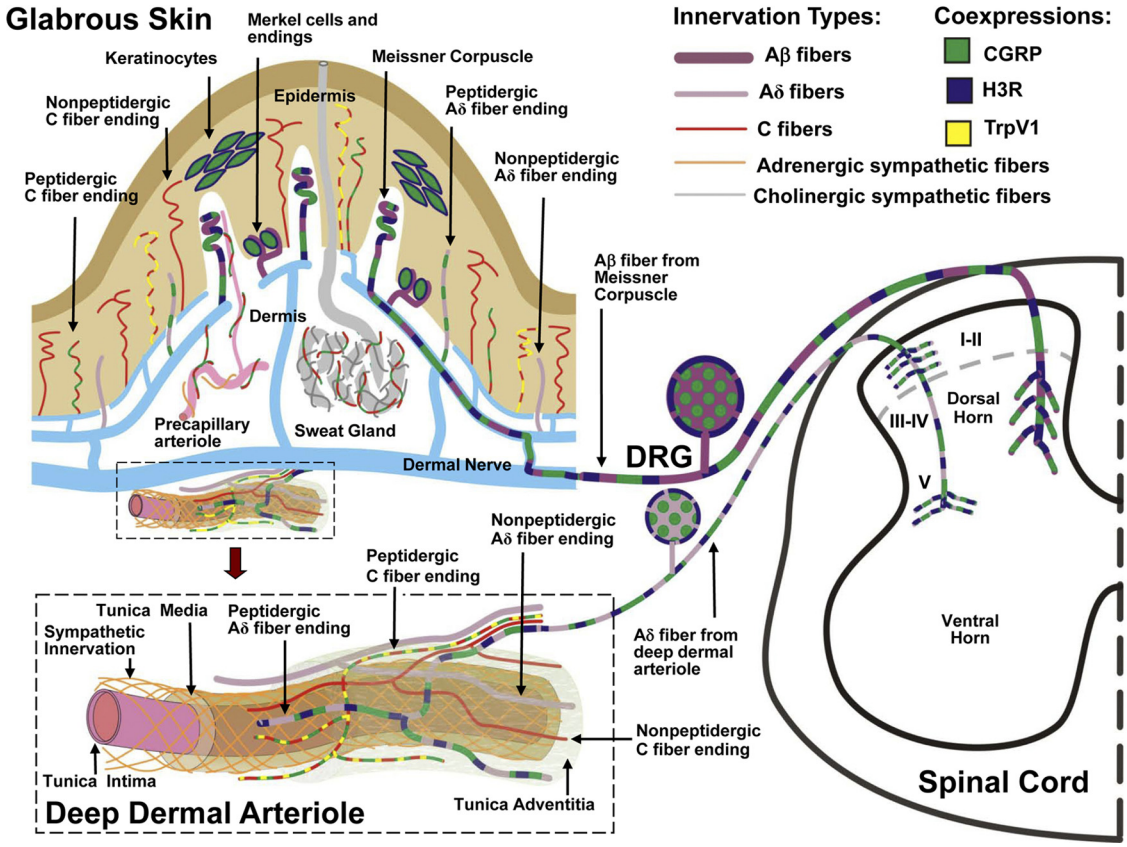

Fig. 1. Schematic localization of H_3Rs in the rat glabrous skin, DRG, and spinal cord. The innervation of glabrous skin is shown (top left), along with an enlargement of a deep dermal arteriole (bottom lower left). Terminations of H_3R-expressing Aβ and Aδ innervation are shown in the dorsal horn of the spinal cord (right). Large-caliber (Aβ) and small-caliber (Aδ) myelinated fibers express 200-kDa neurofilament (purple). Smaller-caliber C fibers (red) lack neurofilament. CGRP (green) is expressed in certain types of C fibers, Aδ fibers, and keratinocytes. TrpV1 channels (yellow) are shown on certain CGRP-containing C fibers. Noradrenergic sympathetic fibers (orange) and cholinergic sympathetic fibers (gray) supply blood vessels and sweat glands, respectively. H_3Rs (blue) are coexpressed with CGRP on Meissner Aβ fibers, Merkel cells, and keratinocytes (Cannon et al., 2007a). Although these components may be related to pain, the distribution of H_3Rs on classically defined pain fibers is limited to the deep dermal perivascular Aδ fibers, which are closely associated with blood vessels. In the skin, activation of H_3Rs on peptidergic Aδ fibers presumably suppresses neuropeptide release and attenuates inflammation-related edema (Ohkubo et al., 1995). In the spinal cord, activation of H_3Rs reduces nociceptive responses elicited by low-intensity mechanical stimulation (Cannon et al., 2003) and proinflammatory agents such as formalin (Cannon et al., 2007b). Inhibition of transmitter release at the spinal terminations of these deep dermal fibers has been proposed to account for this antinociceptive activity. Although the figure is based on immunohistochemical studies of the skin, DRG, and spinal cord, H_3R activation is known to reduce inflammatory peptide release the heart (Imamura et al., 1996), lung (Delaunois et al., 1995), and dura mater (Dimitriadou et al., 1997). Other spinal nerve terminals and spinal neurons may also have H_3Rs.

fibers (see also Medhurst et al., 2008). Although the ability of H_3 agonists to reduce peptide release from the skin and elsewhere has been clearly established, the proposed existence of these receptors on sensory C fibers or sympathetic terminals was not confirmed in studies of the skin (Cannon et al., 2007a).

Given that neuronal H_3Rs are found in skin, DRG, spinal dorsal horn, and brain, it is not surprising that confusing (even apparently contradictory) results have been reported with H_3R-acting drugs in pain assays. The potential for multiple sites of action is enhanced when brain- penetrating and spinally penetrating drugs are administered systemically. This complexity can be reduced by local, intrathecal, or intracerebral drug administration.

Formalin-Induced Nociception

Injection of dilute formalin into the rodent footpad elicits nociceptive motor responses and other signs of inflammation. The former include paw shaking ("flinching"), paw licking, and vocalization. Measurements of any of three end points reveal two well recognized phases of formalin action: "early-phase" responses (0–10 min) are thought to result from direct nociceptor activation; "late-phase" responses (e.g., 15–60 min) follow a brief quiescent period and are considered to be reflective of spinal sensitization mechanisms (Abbott et al., 1995). The ability of H_3 agonists to reduce inflammatory peptide release prompted the development of (*R*)-(−)-2-[[*N*-[1-(1*H*-imidazol-4-yl)-2-propyl]imino]phenylmethyl] phenol (BP 2-94) (an H_3 agonist prodrug) as a possible analgesic or anti-inflammatory agent (Rouleau et al., 1997). In mice, oral BP 2-94 reduced both phases of formalin-induced licking and biting, but did not modify thermal (hot plate) responses (Rouleau et al., 1997). In a more recent rat study, systemic (subcutaneous) administration of the H_3 agonist immepip produced dose-dependent reductions (up to 70%) in both phases of formalin-induced flinching (Cannon et al., 2007b). These effects were mimicked by intrathecal immepip and blocked by systemic and intrathecal thioperamide. Although subcutaneous immepip reduced formalin-induced flinching, this treatment had no effect on formalin-induced vocalizations in

rats (Cannon et al., 2007b). Thus, studies in mice and rats with two different H_3 agonists (Rouleau et al., 1997; Cannon et al., 2007b) suggest that inhibition of formalin flinching or licking is mediated by activation of spinal H_3Rs on peptidergic afferent fibers. If this is correct, then the absence of H_3Rs on C fibers in the skin (Fig. 1) and on small DRG neurons (Cannon et al., 2007a) seems to challenge the commonly held view that C-fiber activation evokes formalin-induced flinching (Cannon et al., 2007b). Instead, H_3-containing peptidergic Aδ fibers (Cannon et al., 2007a) may be the critical elements for provoking this behavior (Cannon et al., 2007a,b). Alternatively, C-fiber involvement in formalin-induced flinching could involve an indirect mechanism initiated through keratinocyte activation (Dussor et al., 2009). The inhibitory effects of H_3 agonists on formalin-induced responses need to studied in H_3KO mice to positively confirm the proposed role for spinal H_3Rs.

In contrast to the above-cited results with BP 2-94 and immepip, another study reported that systemic administration of the H_3 agonist imetit enhanced formalin-induced vocalization responses (Farzin and Nosrati, 2007). These apparently contradictory findings could be caused by the use of vocalization (versus flinching, biting, or licking) as the nociceptive-dependent variable, but other explanations are also possible. Although formalin studies in mice often rely on vocalization responses, there is evidence that different underlying mechanisms may account for supraspinally organized versus spinally organized responses to formalin (Sawynok and Liu, 2004). Additional experiments using multiple routes of injection and measuring both motor and vocalization responses to formalin in wild-type and H_3KO mice are needed.

Studies with H_3 inverse agonists on formalin nociception have introduced additional complexities. In rats, a moderate dose of thioperamide given alone (15 mg/kg i.p.) had no effect on either phase of formalin-induced flinching; this dose blocked immepip's antiflinching activity (Cannon et al., 2007b). However, a more recent study in rats reported dose-dependent reductions in late-phase, formalin-induced flinching responses after systemic administration of the highly selective, potent H_3 inverse agonist 6-[(3-cyclobutyl-2,3,4,5-tetrahydro-1*H*-3-benzazepin-7-yl)oxy]-*N*-methyl-3-pyridine carboxamide (GSK189254) (Hsieh et al., 2010b). Although a spinal action was not shown for this antiformalin effect, other findings in the same article suggest that blockade of spinal H_3Rs may produce antinociception by enhancing the release of spinal norepinephrine (Hsieh et al., 2010b). Along the same lines, systemic thioperamide (10–15 mg/kg) antagonized both phases of formalin-induced vocalizations in mice (Farzin and Nosrati, 2007). None of the published findings on formalin nociception by H_3R drugs have been validated with H_3KO mice.

The idea that both H_3 agonists and H_3 inverse agonists could have antinociceptive properties in the spinal cord is counterintuitive and requires considerable additional studies. If, as the literature suggests, H_3Rs in the spinal cord have multiple locations [e.g., on primary afferent terminals (Cannon et al., 2007a) and spinal noradrenergic terminals (Celuch, 1995)], then there may be an anatomical basis for such effects. It should also be noted that H_3 agonists suppressed both early- and late-phase formalin responses (Rouleau et al., 1997; Cannon et al., 2007b), whereas the H_3 inverse agonist GSK189254 acted only on the late phase (Hsieh et al., 2010b). The late phase of formalin responding is thought to represent central spinal sensitization. It is also possible that constitutively active H_3Rs within the spinal cord might be relevant in understanding these drug actions, because the activity of inverse agonists can depend on the degree of constitutive receptor activity. Although there is compelling evidence that H_3Rs in the brain are constitutively active in vivo (Morisset et al., 2000), no such studies have been performed in the spinal cord.

Peripheral H_3Rs and Inflammation

Many studies have reported that H_3R activation inhibits peptide release (CGRP and substance P) from sensory nerves in skin and other organs. Such effects are thought to account for the well established anti-inflammatory properties of systemically and locally administered H_3 agonists. Active compounds include BP 2-94 (Rouleau et al., 1997, 2000), RAMH (Ohkubo et al., 1995; Dimitriadou et al., 1997; Poveda et al., 2006), imetit (Delaunois et al., 1995; Imamura et al., 1996), and immepip (Cannon et al., 2007b). Inflammation provoked by many treatments (including antidromic nerve stimulation, formalin, capsaicin, zymosan, complete Freud's adjuvant, and bradykinin) is reduced by these drugs; end points measured include edema, plasma extravasation, and increases in vascular permeability. Local application of the agonists to heart (Imamura et al., 1996), lung (Delaunois et al., 1995), and dura mater (Dimitriadou et al., 1997) effectively reduced these inflammatory responses, strongly suggesting that H_3Rs on local afferent fibers can attenuate peptide-mediated inflammation. The H_4 agonist properties of immepip and imetit (Lim et al., 2005) may or may not be relevant for inflammation research. H_4 antagonists have potent anti-inflammatory activity (Hsieh et al., 2010a), but assessment of anti-inflammatory actions of H_4 agonists has not been published. The target for the anti-inflammatory effects of H_3 agonists has not been confirmed with H_3KO mice experiments.

Studies of drug dose and route show the necessity of considering separately the anti-inflammatory versus antinociceptive properties of H_3 agonists. For example, in formalin-treated rats, systemically administered immepip reduced both nociceptive flinching and paw edema, but intrathecally delivered immepip reduced the former, but not the latter (Cannon et al., 2007b). Thus, systemically administered H_3 agonists seem to act directly on peripheral fibers in the skin to reduce peptide-mediated inflammation (Cannon et al., 2007b). Combinations of intradermal injections of formalin and H_3 agonists have not directly tested this hypothesis.

Acute Mechanical Nociception: Relevance of Dermal and Spinal H_3Rs

Spinal H_3Rs seem to be capable of attenuating specific types of acute nociceptive transmission. In rats, systemically administered immepip produced dose-dependent decreases in nociceptive responses to low-intensity mechanical tail pinch, but had no effect on thermally evoked (i.e., tail-flick and hot plate) responses; the effect was mimicked by two different intrathecally administered H_3 agonists and blocked by intrathecally injected thioperamide (Cannon et al., 2003).

In mice, immepip effectively attenuated mechanical nociception after intrathecal, but not subcutaneous, administration (Cannon et al., 2003). Because systemically administered immepip was effective against mechanical nociception in rats, Cannon et al. (2003) suggested the possibility of a species difference in the spinal penetration by immepip, but several explanations seem possible for the discrepancy. A subsequent detailed evaluation with a wide variety of nociceptive stimuli in rats confirmed that the acute antinociceptive profile of H_3 agonists is both modality-specific (i.e., mechanical versus thermal) and intensity-specific (low versus high mechanical) (Cannon and Hough, 2005). Collectively, the studies show that low-intensity mechanical nociception is attenuated by activation of spinal H_3Rs (Cannon et al., 2003; Cannon and Hough, 2005). Because 1) specific types of H_3R-containing peripheral afferent fibers project to the dorsal horn, and 2) dorsal horn neurons have limited expression of H_3R message (Héron et al., 2001) and H_3RLI (Cannon et al., 2007a), it was suggested that the antinociceptive effects of intrathecal H_3 agonists are mediated by inhibition of transmitter release from afferent fibers in the spinal cord, a presynaptic effect (Cannon et al., 2003). Whereas such a presynaptic H_3 action may explain the attenuation of mechanical nociception, newer findings suggest that H_3Rs elsewhere in the spinal cord may also have pain-related functions (see below).

The mechanical antinociceptive effects of intrathecally delivered H_3 agonists in rats and mice, and the attenuation of these effects with the H_3 inverse agonist thioperamide, suggest that spinal H_3 receptors are the targets for these drugs (Cannon et al., 2003). Although immepip and thioperamide have H_4 and H_3 affinity, the intrathecal effects of immepip on mechanical nociception were completely abolished in H_3KO mice, confirming the significance of the proposed target receptor (Cannon et al., 2003). Based on a review of current literature, the mechanical antinociceptive properties of intrathecal immepip are the only H_3R-related pain results to have been validated with knockout mice studies.

If activation of spinal H_3Rs produces mechanical antinociception, then H_3 antagonists or H_3 inverse agonists might be expected to produce the opposite (hyperalgesic) effect. However, this would occur only if the spinal presynaptic H_3Rs were normally active. H_3 blockade with thioperamide in rats (10 mg/kg s.c., a dose that blocked the intrathecal effects of immepip) had no effect on baseline mechanical nociception when given alone (Cannon et al., 2003), implying that H_3Rs are not active during acute mechanical nociception. Also consistent with this conclusion, H_3KO mice have normal baseline responses to mechanical pinch (Cannon et al., 2003). In a separate study, a larger dose of thioperamide given alone (20 mg/kg i.p.) produced antinociception on the paw pressure test (Malmberg-Aiello et al., 1994). A thioperamide action on brain (versus spinal) H_3Rs may account for this effect, consistent with the mechanical hyperalgesic actions of brain-administered RAMH in the same study (Malmberg-Aiello et al., 1994).

The discovery of H_3Rs on peptidergic, periarteriolar Aδ fibers in the deep dermis led to the suggestion that these fibers might mediate high-threshold mechanical nociception (Cannon et al., 2007a). Although not proven, the hypothesis has support from several findings: 1) H_3KO mice lack H_3RLI in the deep dermis, DRG, and spinal cord (Cannon et al., 2007a); 2) intrathecal immepip reduces mechanical nociception in control, but not in H_3KO, mice (Cannon et al., 2003); and 3) the H_3R-containing Aδ fibers in the deep dermis possess ASIC3 channels (Molliver et al., 2005), which may be transducers of mechanical nociception (Hu et al., 2006). Although the epidermis is widely held to be responsible for touch and pain perception from the skin, a study has reported that patients with a total loss of epidermal innervation could still detect cutaneous stimulation (Bowsher et al., 2009). A sensory transduction role for dermal vascular afferents is consistent with these provocative new findings.

Although intrathecal H_3 agonists suppress acute mechanical nociception in healthy subjects, the relevance of this finding for clinical pain is unknown. The effects of intrathecal H_3 agonists in chronic pain models (including mechanical allodynia after nerve injury) have not been reported. The possibility that deep dermal pain (such as seen in fibromyalgia) might be attenuated by systemic or spinally administered H_3 agonists has not been investigated. Systemically administered H_3 agonists achieve some degree of brain and spinal penetration. In rats, spinal penetration by immepip is sufficient to suppress mechanical nociception (Cannon et al., 2003); very modest doses decrease hypothalamic histamine release (Jansen et al., 1998).

Antinociceptive Profile for Brain Histamine

Histamine, a pain-enhancing substance in the skin and spinal cord (Kajihara et al., 2010), reduces nociceptive transmission when injected directly into the brain (Glick and Crane, 1978; Chung et al., 1984; Bhattacharya and Parmar, 1985; Braga et al., 1992; Sibilia et al., 1992; Malmberg-Aiello et al., 1994; Thoburn et al., 1994). H_1 and H_2 receptors are thought to mediate this effect (Thoburn et al., 1994; Braga et al., 1996; Lamberti et al., 1996). Related to pain relief, H_2 receptors in the brain are activated as part of opioid and nonopioid antinociceptive mechanisms (Gogas and Hough, 1989; Gogas et al., 1989), especially when stress elicits or enhances these responses (Nalwalk and Hough, 1995). Such findings suggest that treatments that enhance neuronal histamine release (e.g., blockade of brain H_3 autoreceptors) should relieve pain. There is also evidence that exposure to nociceptive stimuli increases neuronal histamine turnover (Itoh et al., 1989). As discussed below, drugs that reduce H_3 activity (inverse agonists and/or antagonists) act in the brain or spinal cord to modify nociceptive thresholds in several acute and chronic pain tests.

Bidirectional Modulation of Acute Thermal Nociceptive Responses

Several laboratory groups have shown that thioperamide inhibits hot-plate nociceptive responses in rodents. In mice, low systemic doses (1–5 mg/kg) are inactive (Owen et al., 1994; Suzuki et al., 1994), but higher doses (5–30 mg/kg) produce dose-dependent, mild antinociceptive effects (Malmberg-Aiello et al., 1994; Farzin et al., 2002). These treatments do not impair motor balance in the rotorod test, suggesting a pain-relieving profile (Farzin et al., 2002). Intraventricular (Oluyomi and Hart, 1991), but not intrathecal (Suh et al., 1999; Mobarakeh et al., 2009), injections of thioperamide reproduce these antinociceptive effects on the hot-plate test,

implying that the systemically administered drug targets brain H_3Rs. Although intrathecal thioperamide is inactive on acute thermal tests when given alone, it is interesting that this treatment can either enhance (Mobarakeh et al., 2009) or inhibit (Suh et al., 1999) opioid antinociceptive responses in these tests, depending on whether the opioid is given intrathecally (Mobarakeh et al., 2009) or supraspinally (Suh et al., 1999), respectively. In rats, intracerebral injections of thioperamide into the PAG reproduce the mild antinociceptive effects seen after systemic and intraventricular thioperamide (Li et al., 1996; Fig. 2).

Many brain-acting analgesics produce dose-dependent 100% antinociception on thermal tests, but thioperamide's maximal activity on the rat hot-plate test is only approximately 30% of maximal responses (Fig. 2). Dose-response studies with thioperamide (given systemically, or by microinjection into the PAG) found biphasic activity (Fig. 2), with doses greater than those eliciting a 30% response producing less antinociception. Thioperamide is known to increase the release of neuronal histamine in the rat PAG (Barke and Hough, 1994), and histamine microinjections into the PAG produce biphasic antinociceptive activity that is remarkably similar to that produced by systemic or intracerebrally administered thioperamide (Fig. 2). Thus, in acute thermal nociceptive tests, thioperamide seems to reproduce both the pain-relieving and pain-enhancing actions of neuronal histamine in the PAG. Descending brainstem circuits are known to exert bidirectional control over spinal nociception (Heinricher and Ingram, 2008), and the biphasic actions of thioperamide and histamine (Fig. 2) may be indicative of separate activation of pain-inhibiting and pain-enhancing circuits. The target for thioperamide's effects in these studies is likely to be the brain H_3R but pharmacological studies are limited. For example, centrally (Oluyomi and Hart, 1991) and systemically (Malmberg-Aiello et al., 1994) administered H_3 agonists have pronociceptive actions on the hot-plate test. As noted above, the H_4 activity of thioperamide shows the need for confirmatory studies in H_3KO mice and additional testing with more H_3R-selective compounds.

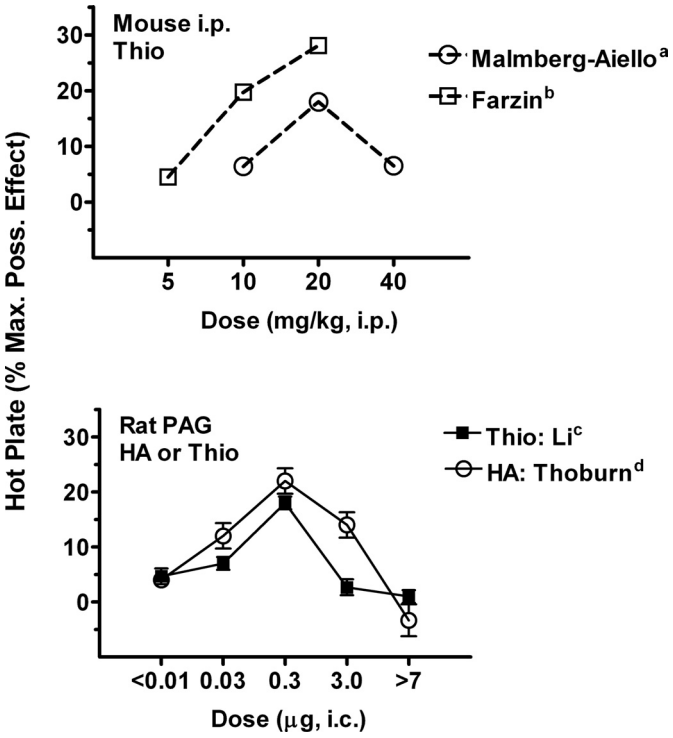

Fig. 2. Biphasic modulation of acute nociceptive responses by thioperamide (Thio) and histamine (HA). Data from published studies show dose-response curves for Thio and HA in mice (top) and rats (bottom) on the hot-plate test. Antinociceptive scores (ordinate, maximal possible effect; see Li et al., 1996) range from 0 to 100%. Thio was administered by systemic (intraperitoneal; top) or intracerebral injections into the PAG (bottom). The figure shows that: 1) the biphasic actions of systemically injected Thio can be replicated by microinjections of the same drug into the PAG, and 2) intracerebral HA injections in the PAG can closely mimic the effects of Thio, consistent with the known HA-releasing properties of this H_3 inverse agonist. Data are plotted: a, 15 min after Thio (Malmberg-Aiello et al., 1994); b, 40 min after Thio (Farzin et al., 2002); c, 10 min after intra-PAG Thio (Li et al., 1996); d, 5 min after intra-PAG HA (Thoburn et al., 1994).

H_3Rs in Models of Chronic Pain

Many analgesics that are effective in animals and humans work extremely well in acute thermal and mechanical nociceptive tests, but these tests are not models of clinical pain. Clinically relevant human pain is usually chronic and can result from a variety of inflammatory, immunological, or tissue-injuring conditions (Argoff et al., 2009). Of special interest is neuropathic pain, in which acute nerve injury produces chronic, exaggerated, and often debilitating pain. Neuropathic pain is highly prevalent and difficult to treat (Campbell and Meyer, 2006; Dray, 2008; Argoff et al., 2009). In an attempt to model more closely these characteristics of clinical pain, several experimental pain tests in animals measure hyperalgesia (exaggerated response to a painful stimuli) and/or allodynia (nociceptive response to a nonpainful stimulus) after tissue damage, nerve injury, or inflammation. The popularity of these tests has produced an explosion of new information on biological and biochemical mediators of neuropathic pain (Jarvis and Boyce-Rustay, 2009; Milligan and Watkins, 2009). As detailed below, H_3 antagonists/inverse agonists have been evaluated in some of these models. It should also be noted that studies of human skin of patients with complex regional pain syndrome type 1 and postherpetic neuralgia and studies of rhesus monkeys with type 2 diabetes have revealed significant structural and chemical pathologies among the various components of the skin that normally express H_3Rs in rats and mice. These include not only perivascular Aδ fibers, but also epidermal keratinocytes, Aβ fiber Meissner's corpuscle endings, and lanceolate endings (Petersen et al., 2002; Albrecht et al., 2006; Cannon et al., 2007a; Paré et al., 2007; Zhao et al., 2008).

Results with thioperamide suggest the possibility that both peripheral and brain H_3Rs can modulate neuropathic pain. Mechanical allodynia was enhanced by a small, systemic dose of thioperamide (3.6 mg/kg i.p.) in rats subjected to partial ligation of the sciatic nerve (Huang et al., 2007). In a related pain model, injection of thioperamide (60 μg) into the injured hind paw had a similar effect (Smith et al., 2007). Experiments with H_4 agonists and antagonists suggest the possibility that the local thioperamide effect could be mediated through an H_4 (versus H_3) action (Smith et al., 2007). A large intraventricular dose of thioperamide (30 μg) had the opposite (antiallodynic) effect in the sciatic nerve ligation model, implying opposing roles for peripheral and central H_3Rs (Huang et al., 2007).

Dramatic progress has been reported in developing potent,

highly selective H_3 inverse agonists that lack H_4 affinity and have good brain-penetrating properties (Leurs et al., 2005; Esbenshade et al., 2006; Sander et al., 2008; Gemkow et al., 2009). Because many of these drugs are new, however, their pain-relieving properties have not been fully evaluated. Two new orally active H_3 inverse agonists had no activity on mechanical paw withdrawal thresholds in normal rats, but inhibited capsaicin-induced mechanical allodynia by 20 to 65% over a range of doses (Medhurst et al., 2007). In a follow-up study (Medhurst et al., 2008), chronic oral dosing (5–8 days) with brain-penetrating H_3 inverse agonists reversed mechanical hyperalgesia in the chronic constriction injury model. The effects were not complete, and not dose-dependent, but were comparable with those of a clinically effective, positive control (gabapentin). Two of the compounds also effectively reversed varicella zoster viral-induced mechanical allodynia in rats (Medhurst et al., 2008). These experiments used multiple measures of allodynia and demonstrated oral bioavailability, adequate brain penetration, and efficacy with chronic dosing comparable with gabapentin, but it is puzzling that the antiallodynic effects were not consistently produced by single doses of the H_3 inverse agonist drugs.

Another group has confirmed the efficacy of H_3 inverse agonists in several chronic pain tests (Hsieh et al., 2010b). Single systemic injections of GSK189254 produced dose-dependent (up to a 70%) reversal of mechanical allodynia induced by spinal nerve ligation. These effects, shown to be similar to those of gabapentin, strongly resemble those seen in the chronic constriction and viral neuropathy models discussed above (Medhurst et al., 2008). In the same study (Hsieh et al., 2010b), GSK189254 also prevented tactile allodynia after treatment with complete Freud's adjuvant, but had no effect on carrageenan-induced thermal hyperalgesia. Although these exciting new results suggest that H_3R inverse agonists could be useful in neuropathic and/or inflammatory pain, additional studies with microinjections and knockout animals are needed to establish the sites of action and confirm the identity of the drugs' target.

Selective H_3 inverse agonists are also active in other pain assays. In the monoiodoacetate arthritis model, four different drugs produced dose-dependent antinociception (Hsieh et al., 2010b). Maximal effects were 53 to 74% reversal of impairment, and none of these drugs affected grip force in the absence of the experimental arthritis. One H_3 inverse agonist (GSK189254) was also active in the arthritis model after intrathecal administration, showing the spinal cord to be an important site of action. Both the systemic and intrathecal antiarthritis effects of GSK189254 were reversed by pretreatment with the α_2 adrenergic antagonist phentolamine (Hsieh et al., 2010b), suggesting a critical role for spinal noradrenergic mechanisms in the antinociceptive effect. Supraspinally acting analgesics are known to activate spinal α_2-mediated analgesic mechanisms (Millan, 2002), but stimulation of this mechanism by an action on spinal H_3Rs is novel and potentially very important.

These recent studies with new H_3R inverse agonists suggest that these compounds could be effective in treating neuropathic, inflammatory, or arthritis pain, but additional basic and clinical studies are needed. The anticipated approval of these drugs for several nonpain indications in humans (Gemkow et al., 2009; Tiligada et al., 2009) may permit evaluation of their potential as pain medicines.

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Wrote or contributed to the writing of the manuscript: Hough and Rice.

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