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Rodent antinociception following acute treatment with different histamine receptor agonists and antagonists

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Abstract

The effects of different histamine receptor agonists and antagonists on the nociceptive threshold were investigated in mice by two different kinds of noxious stimuli: thermal (hot plate) and chemical (acetic acid-induced abdominal writhing). Intracerebroventricular (icv) injection of the histamine H_1 receptor agonist, HTMT (6-[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl) heptanecarboxamide) (50 μg/mouse), produced a hypernociception in the hot plate and writhing tests. Conversely, intraperitoneal (ip) injection of dexchlorpheniramine (30 and 40 mg/kg) and diphenhydramine (20 and 40 mg/kg) increased the pain threshold in both tests. The histamine H_2 receptor agonist, dimaprit (50 and 100 μg/mouse icv), or antagonist, ranitidine (50 and 100 μg/mouse icv), raised the pain threshold in both hot plate and writhing tests. In the mouse hot plate test, the histamine H_3 receptor agonist, imetit (50 mg/kg ip), reduced the pain threshold, while the histamine H_3 receptor antagonist, thioperamide (10 and 20 mg/kg ip), produced an antinociception. The hypernociceptive effects of HTMT and imetit were antagonized by dexchlorpheniramine (20 mg/kg ip) and thioperamide (5 mg/kg ip), respectively. The results suggest that histaminergic mechanisms may be involved in the modulation of nociceptive stimuli.

Keywords: Hot plate test; Writhing test; Pain; Histamine; Mouse

1. Introduction

Histamine, which is regarded as a neurotransmitter or modulator in the mammalian brain (Prell and Green, 1986; Schwartz et al., 1991a), has been shown to play a role in the modulation of pain transmission. For example, intracerebroventricular (icv) administration of histamine induces antinociception or hypernociception, depending on the site of cerebral injection or on the dose, in various analgesic tests in rodents. The injection of histamine into the rat dorsal raphe nucleus and periaqueductal grey region produces an antinociception, while its injection into the median raphe nucleus causes hyperalgesia (Glick and Crane, 1978; Thoburn et al., 1994). Intracerebroventricular administrations of low doses of histamine elicit hyperalgesia, while high doses of histamine produce antinociception (Chung et al., 1984; Malmberg-Aiello et al., 1994). The results of above studies suggest that the opposite effects of histamine on pain threshold may be mediated through different subtypes of receptors (Lamberti et al., 1996; Malmberg-Aiello et al., 1994; Thoburn et al., 1994). In order to clarify the possible role of the histaminergic mechanism(s) in the modulation of nociceptive stimuli, the effects on the pain threshold of several histamine receptor agonists and antagonists were studied in tests inducing two different kinds of noxious stimuli.

2. Methods and materials

2.1. Animals

All experiments were carried out on male Swiss–Webster mice (20–25 g). The animals were housed nine per plastic cage in an animal room maintained at 21 ± 2 °C on a 12-h light/dark cycle (lights on 0700–1900 h). Food and water were available at all times except during the experiments. Each animal was used once only.

2.2. Hot plate test

The thermal nociceptive threshold in mice was assessed using a hot plate apparatus (Harvard, UK). The hot plate temperature thermostatically set at 52.5 ± 0.5 °C. The latency to licking or kicking of the fore or hind paws was recorded at

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various times after drug injection. An cut-off time of 45 s was imposed to avoid tissue damage.

2.3. Acetic acid-induced writhing test

Mice were injected intraperitoneally (ip) with 0.6% aqueous solution of acetic acid (10 ml/kg). The number of writhes was counted for 30 min, starting 5 min after acetic acid injection.

2.4. Rota rod test

The integrity of motor coordination was assessed with a rota rod apparatus (Harvard, UK) on the basis of the endurance time of the mice on the rotating rod, at a rotating speed of 16 rpm. One day before the test, the animals were trained twice. On the day of the test, only mice able to stay balanced on the rotating rod between 100 and 300 s (cut-off time) were selected. The performance time was measured before and at various times after treatment.

2.5. Intracerebroventricular injection

The intracerebroventricular injection was performed during short ether anesthesia, according to the method of Haley and McCormick (1957), with a constant volume of 5 μl. To ascertain the exact point into which drugs were administered, some mice were deeply anesthesized and injected intracerebroventricular with 5 μl of diluted 1:10 Indian ink and their brains were examined macroscopically after sectioning. The experimental protocol was approved by the Research and Ethics Committee of Mazandaran University of Medical Sciences (No. 77/8).

2.6. Drugs

The following drugs were used: *S*(+)-dexchlorpheniramine maleate (Research Biochemicals, Natick, MA, USA), dimaprit dihydrochloride (ICN Biomedicals, Oxfordshire, UK), diphenhydramine hydrochloride (Research Biochemicals, Natick, MA, USA), HTMT dimaleate ((6-[2-(4-imidazolyl)ethylamino]-*N*-(4-trifluoromethylphenyl) heptanecarboxamide; Tocris, Bristol, UK), imetit dihydrobromide (ICN Biomedicals, Oxfordshire, UK), ranitidine hydrochloride (Sigma, St. Louis, MO, USA), and thioperamide maleate (ICN Biomedicals, Oxfordshire, UK). In all cases, the drug doses reported are for the base. The drugs were dissolved in saline, except for HTMT, which was dissolved in a drop of ethanol and then diluted with saline. The vehicle control was ethanol in saline. Drug concentrations were prepared so that the necessary dose could be injected in a volume of 10 ml/kg by intraperitoneal route. Owing to the reportedly poor ability of trifluoromethylphenyl or-toluidide derivatives of histamine to cross the blood–brain barrier (Malmberg-Aiello et al., 1998; Qiu et al., 1990), the intracerebroventricular route of administration was used for the histamine H_1 receptor agonist, HTMT (Khan et al., 1986; Qiu et al., 1990). For HTMT, the doses were chosen, on a molar basis, as those at which histamine 2HCl exerts its pharmacologic actions on target cells in central nervous system tissue (Chung et al., 1984; Malmberg-Aiello et al., 1994; Oluyomi and Hart, 1991). The intracerebroventricular route of administration was also used for ranitidine and dimaprit, as most H_2 receptor ligands are polar compounds and penetrate poorly into the CNS (Hill et al., 1997). The intraperitoneal route of administration was chosen for thioperamide and imetit because following peripheral injection, both thioperamide and imetit penetrate the brain, where they can subsequently interact with H_3 receptors (Garbarg et al., 1992; Taylor et al., 1992). In general, the doses of drugs and pretreatment time were usually those used previously and shown to be pharmacologically active (Chung et al., 1984; Farzin and Attarzadeh, 2000; Lamberti et al., 1996; Malmberg-Aiello et al., 1994; Netti et al., 1984; Oluyomi and Hart, 1991; Rumore and Schlichting, 1985; Taylor et al., 1992).

2.7. Statistical analysis

One-way analysis of variance (ANOVA) (for the writhing test) or repeated-measures ANOVA (for the hot plate and rota rod tests), followed by Newman–Keuls multiple comparisons test, was used for statistical analysis. Differences with $P<.05$ between experimental groups at each point were considered statistically significant. All data were analyzed with the computer program, GRAPHPAD software (V2.01$^+$).

Table 1
Effects of HTMT, dexchlorpheniramine (DEX), and diphenhydramine (DIP) on paw licking or kicking latency in the hot plate test (52.5 °C)

		Licking or kicking latency (s)			
Treatment	*n*	Pretest	20 min	30 min	40 min
μg/mouse icv					
Vehicle	6	14.7 ± 0.8	15.5 ± 2.2	16.6 ± 3.5	17.6 ± 2.8
HTMT, 50 μg	6	13.5 ± 0.7	7 ± 0.6*	7.2 ± 0.6**	6.5 ± 0.7**
HTMT, 100 μg	6	12.3 ± 1.5	10.7 ± 1	10.2 ± 0.8	12 ± 1.3
mg/kg ip					
Saline	8	12.7 ± 1.4	13.7 ± 1.6	12.5 ± 1.1	12 ± 1.8
DEX 20	8	12 ± 1.8	22.5 ± 4.8	23.2 ± 4.3	21.6 ± 4
DEX 30	8	12.3 ± 1.1	30 ± 5.6***	37 ± 4.7***	34.6 ± 4.3***
DEX 40	8	11.4 ± 1.2	37.2 ± 3.3***	38 ± 3.3***	32.1 ± 4***
DIP 20	8	11.3 ± 1	26.6 ± 4.5**	26 ± 3.8**	27.2 ± 4**
DIP 40	8	13.1 ± 1.7	37.1 ± 3***	34.7 ± 3.5***	34.1 ± 3.4***

In the hot plate test, mice were tested for baseline nociception (pretest) and received different doses of HTMT (50 and 100 μg/mouse icv), dexchlorpheniramine (20, 30, and 40 mg/kg ip), diphenhydramine (20 and 40 mg/kg ip), and vehicle or saline (5 μl/mouse icv or 10 ml/kg ip, respectively). Animals were then retested at various times after drug injection. Results are expressed as mean ± S.E.M.

* $P<.05$, different from control groups.

** $P<.01$, different from control groups.

*** $P<.001$, different from control groups.

3. Results

3.1. Effects of HTMT, dexchlorpheniramine and diphenhydramine on pain threshold

Pretreatment with 50 [$F(7,5)=6.689$, $P<.0001$], but not 100 μg/mouse HTMT icv [$F(7,5)=1.907$, $P>.0980$], produced a hypernociception in the mouse hot plate test (Table 1). Similarly, intracerebroventricular injection of HTMT (50 μg/mouse) reduced the pain threshold in the writhing test [$F(2,15)=4.280$, $P<.0338$] (Fig. 1). The dose of 100 μg/mouse HTMT icv, which was ineffective in both hot plate and writhing tests, significantly produced a strong motor impairment in the rota rod test [$F(7,5)=10.309$, $P<.0001$] (Fig. 1).

In the mouse hot plate test, the doses of 30 [$F(7,7)=14.832$, $P<.0001$] and 40 [$F(7,7)=19.959$, $P<.0001$] mg/kg dexchlorpheniramine ip, which were ineffective in the rota

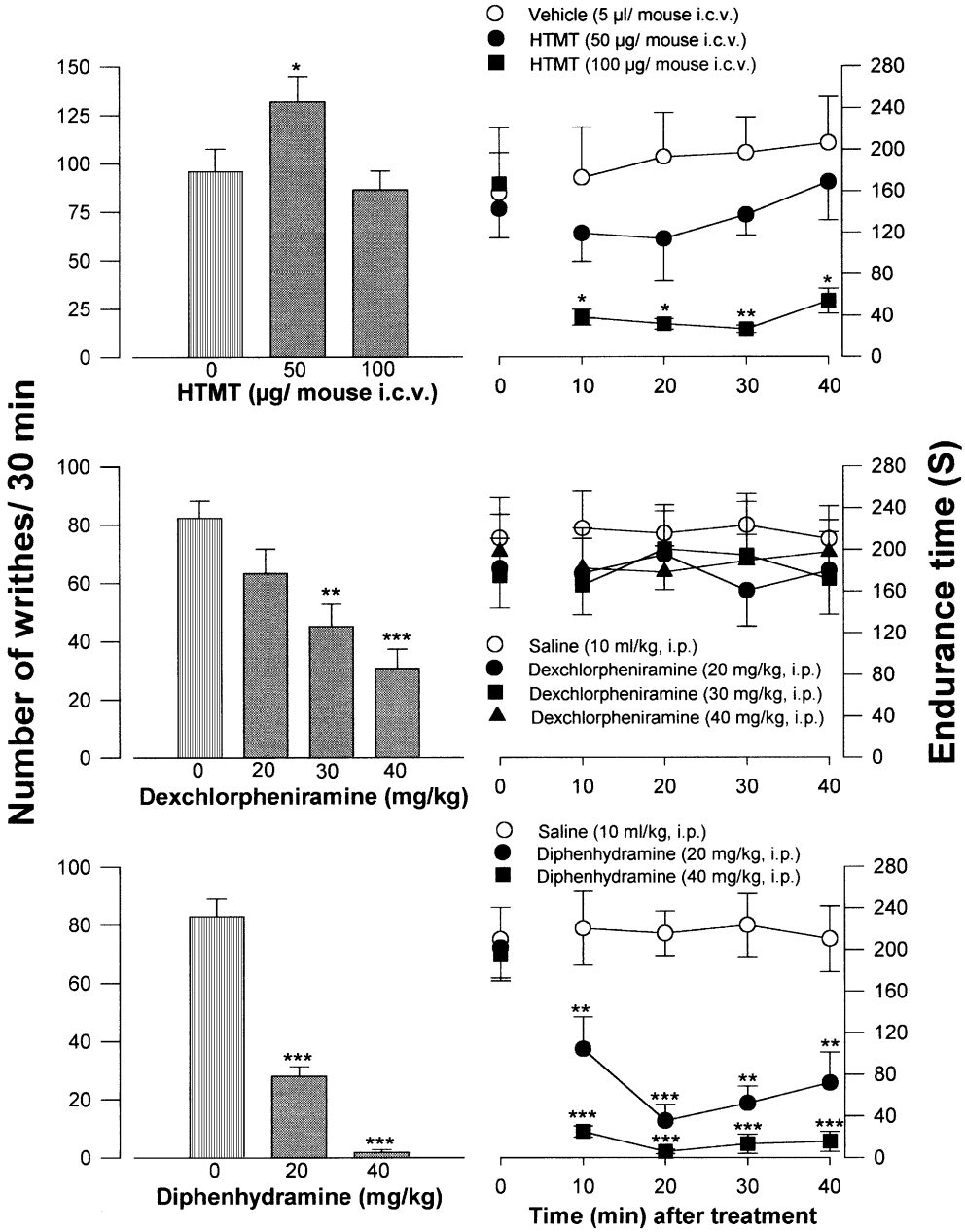

Fig. 1. Effects of HTMT, dexchlorpheniramine, and diphenhydramine on the nociceptive threshold and endurance time in the mice writhing and rota rod tests, respectively. In the writhing test, HTMT was administered by intracerebroventricular injection 15 min before test; dexchlorpheniramine (20, 30 and 40 mg/kg ip) and diphenhydramine (20 and 40 mg/kg ip) were also administered 15 min before test ($n=6-9$ mice/group). In the rota rod test, endurance time of mice on the treadmill was measured before treatment and then starting 10 min after treatment, up 40 min ($n=6$ mice/group). Results are expressed as mean ± S.E.M. *$P<.05$, ** $P<.01$, ***$P<.001$, different from control groups.

Table 2
Effect of HTMT alone or in combination with dexchlorpheniramine (DEX) on paw licking or kicking latency in the hot plate test (52.5 °C)

		Licking or kicking latency (s)			
Treatment	*n*	Pretest	20 min	30 min	40 min
μg/mouse icv + mg/kg ip					
Vehicle + saline	6	15.3 ± 1	15.2 ± 1.8	14.2 ± 1.1	14.3 ± 1.9
Vehicle + DEX 20	6	14.8 ± 1.2	21.7 ± 3.2	22.5 ± 3.3	20.5 ± 2.6
HTMT 50 + saline	6	13.8 ± 1.5	8.3 ± 0.7*	8 ± 0.9*	8.2 ± 0.6*
HTMT 50 + DEX 20	6	14.2 ± 1.3	10.2 ± 0.9	12.5 ± 2.2	11.3 ± 1.3

In the hot plate test, mice were tested for baseline nociception (pretest) and received vehicle (5 μl/mouse icv), in combination with saline (10 ml/kg ip) and dexchlorpheniramine (20 mg/kg ip), or HTMT (50 μg/mouse icv), in combination with saline and dexchlorpheniramine. Animals were then retested at various times after drug injection. Results are expressed as mean ± S.E.M.

* $P < .01$, different from control groups.

rod test, significantly increased the pain threshold (Table 1). No antinociceptive effect for the dose of 20 mg/kg dexchlorpheniramine ip was observed [$F(7,7) = 0.672$, $P > .649$]. The doses of 20 [$F(7,7) = 6.634$, $P < .0001$] and 40 [$F(7,7) = 27.712$, $P < .0001$] mg/kg diphenhydramine ip also significantly increased the pain threshold. The endurance time on the rota rod for mice treated with these doses of diphenhydramine was significantly reduced (Fig. 1).

In the mouse writhing test, dexchlorpheniramine [$F(3,26) = 10.752$, $P < .0001$] or diphenhydramine [$F(2,20) = 90.053$, $P < .0001$] caused a dose-dependent elevation in the pain threshold (Fig. 1).

In the mice hot plate and writhing tests, dexchlorpheniramine (20 mg/kg ip) was able to antagonize the hypernociception induced by HTMT (50 μg/mouse icv) (Table 2, Fig. 2).

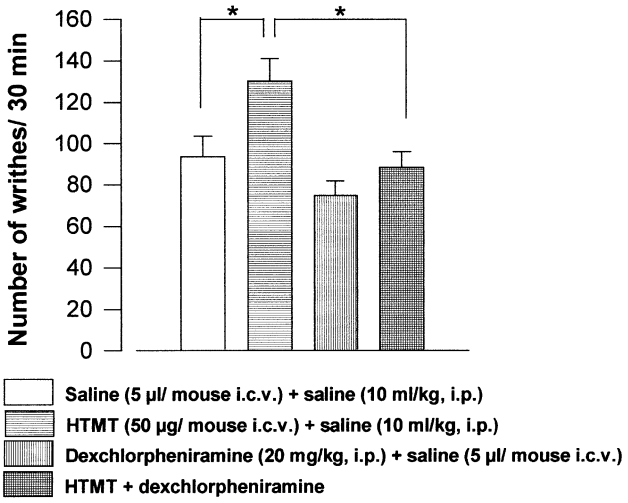

Fig. 2. Antagonism of HTMT hypernociception in the writhing test. Drugs and saline were injected 15 min before test. Results are expressed as mean ± S.E.M. ($n = 7$ mice/group). *$P < .01$, different from control groups.

3.2. Effects of dimaprit and ranitidine on pain threshold

In the mouse hot plate test, the high dose of 100 μg/mouse dimaprit icv increased the pain threshold [$F(7,5) = 4.945$, $P < .0006$] with a motor impairment in the rota rod test (Table 3, Fig. 3). The dose of 50 μg/mouse dimaprit icv was ineffective in both hot plate and rota rod tests. Dimaprit (50 and 100 μg/mouse icv) also increased the pain threshold [$F(2,15) = 4.349$, $P < .0324$] in the mouse writhing test (Fig. 3).

Pretreatment with 50 and 100 μg/mouse ranitidine icv increased the pain threshold in the mice hot plate and writhing tests (Table 3, Fig. 3). A reduction in the mouse endurance time on the rota rod was induced by ranitidine at dose of 100 μg/mouse icv [$F(7,5) = 8.274$, $P < .0001$] (Fig. 3). The combination of dimaprit (100 μg/mouse icv) and ranitidine (50 μg/mouse icv), at doses which alone produced some antinociception, resulted in an increase in the pain threshold not significantly greater than with either drug alone (data not shown).

In the mice hot plate [$F(15, 6) = 24.481$, $P < .0001$] and writhing [$F(3,26) = 10.479$, $P < .0001$] tests, imetit (50 mg/kg ip) completely antagonized the antinociception induced by dimaprit (100 μg/mouse icv) (Table 4, Fig. 4).

3.3. Effects of imetit and thioperamide on pain threshold

In the mouse hot plate test, the dose of 50 mg/kg imetit ip, which was ineffective in the rota rod test, caused a reduction in the pain threshold [$F(7,6) = 17.2$, $P < .0001$] (Table 5, Fig. 5). In contrast, the doses of 10 and 20 mg/kg thioperamide ip potentiated the pain threshold [$F(11,6) = 9.366$, $P < .0001$] (Table 5, Fig. 5). Motor coordination as measured with the rota rod test was unaffected when mice were treated with these doses of thioperamide [$F(15,5) = 0.1413$, $P > .999$]. The combination of thiopera-

Table 3
Effects of dimaprit (DIM) and ranitidine (RAN) on paw licking or kicking latency in the hot plate test (52.5 °C)

		Licking or kicking latency (s)			
Treatment	*n*	Pretest	20 min	30 min	40 min
μg/mouse icv					
Saline	6	15.8 ± 0.9	14.5 ± 1.4	15 ± 1.6	16.2 ± 0.5
DIM 50	6	14.3 ± 2.1	14.8 ± 2.5	11.2 ± 1.2	11 ± 1.9
DIM 100	6	13.7 ± 1.3	19 ± 1.6	22 ± 2.1*	22.2 ± 2.2*
RAN 50	6	15.5 ± 1.5	30.2 ± 4.8*	32.2 ± 5.8*	31.3 ± 5.9*
RAN 100	6	16 ± 1.1	41.2 ± 2.8**	43.8 ± 1.2**	44 ± 1**

In the hot plate test, mice were tested for baseline nociception (pretest) and received different doses of dimaprit (50 and 100 μg/mouse icv), ranitidine (50 and 100 μg/mouse icv,) and saline (5 μl/mouse icv). Animals were then retested at various times after drug injection. Results are expressed as mean ± S.E.M.

* $P < .05$, different from control groups.

** $P < .001$, different from control groups.

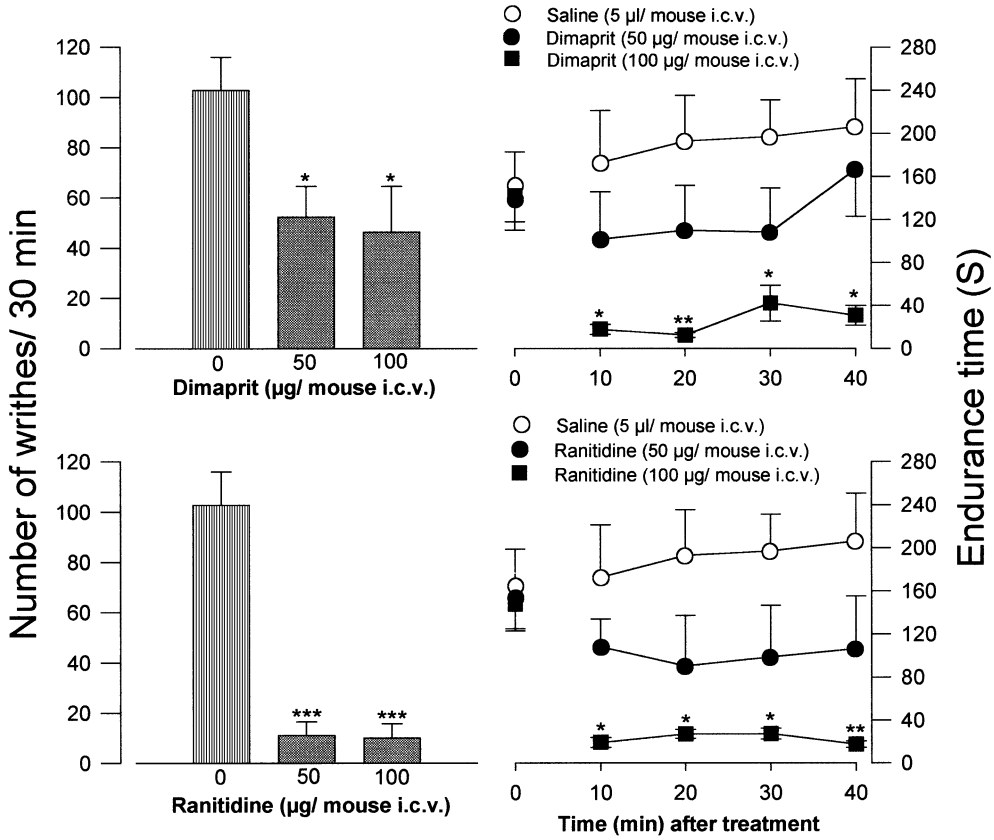

Fig. 3. Effects of dimaprit and ranitidine on the nociceptive threshold and endurance time in the mice writhing and rota rod tests, respectively. In the writhing test, dimaprit (50 and 100 µg/mouse) and ranitidine (50 and 100 µg/mouse) were administered by intracerebroventricular injection 15 min before test ($n=6$ mice/group). In the rota rod test, endurance time of mice on the treadmill was measured before treatment and then starting 10 min after treatment, up 40 min ($n=6$ mice/group). Results are expressed as mean ± S.E.M. *$P<.05$, **$P<.01$, ***$P<.001$, different from control groups.

mide (5 mg/kg ip) and imetit (50 mg/kg ip) did not modify the pain threshold [$F(7,6)=0.786$, $P>.605$] in the mouse hot plate test (Table 6).

In the mouse writhing test, the two doses used of imetit (25 and 50 mg/kg ip) [$F(2,20)=2.058$, $P>.154$] were

Table 4
Effect of dimaprit (DIM) alone or in combination with imetit (IME) on paw licking or kicking latency in the hot plate test (52.5 °C)

		Licking or kicking latency (s)			
Treatment	*n*	Pretest	20 min	30 min	40 min
µg/mouse icv + mg/kg ip					
Saline + saline	6	12.5 ± 1.1	13.4 ± 1.3	13.6 ± 0.8	14.1 ± 1.3
Saline + IME 50	6	12.3 ± 0.5	6.7 ± 0.3*	7 ± 0.5*	6.8 ± 0.5*
DIM 100 + saline	6	10.6 ± 1.4	20.4 ± 2.2**	26.6 ± 1.7**	25.7 ± 1.8**
DIM 100 + IME 50	6	11.1 ± 1.2	12.8 ± 1	11.3 ± 0.9	11.1 ± 1.1

In the hot plate test, mice were tested for baseline nociception (pretest) and received saline (5 µl/mouse icv), in combination with saline (10 ml/kg ip) and imetit (50 mg/kg ip), or dimaprit (100 µg/mouse icv), in combination with saline and imetit. Animals were then retested at various times after drug injection. Results are expressed as mean ± S.E.M.

* $P<.01$, different from control groups.

** $P<.001$, different from control groups.

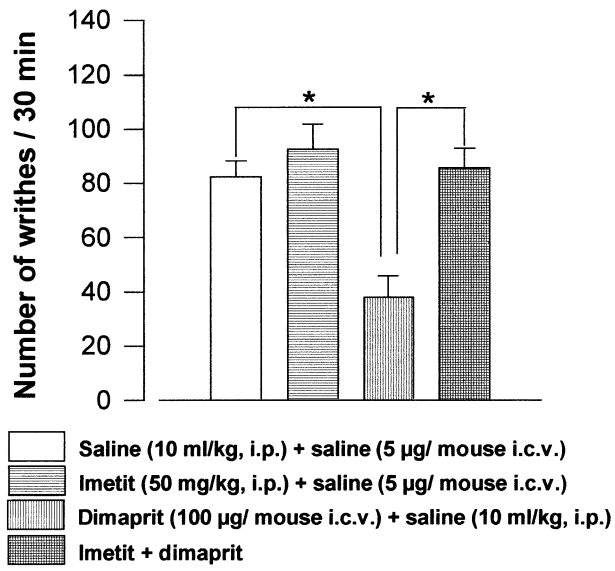

Fig. 4. Antagonism of dimaprit antinociception in the writhing test. Drugs and saline were injected 15 min before test. Results are expressed as mean ± S.E.M. ($n=7$–9 mice/group). * $P<.001$, different from control groups.

Table 5
Effects of imetit (IME) and thioperamide (THI) on paw licking or kicking latency in the hot plate test (52.5 °C)

		Licking or kicking latency (s)			
Treatment	*n*	Pretest	20 min	30 min	40 min
mg/kg ip					
Saline	7	12.6 ± 1.1	13.4 ± 1.2	13.6 ± 0.8	14.1 ± 1.3
IME 25	7	13 ± 1	11.8 ± 1.3	10.6 ± 2.2	9 ± 2.2
IME 50	7	12.3 ± 0.6	6.7 ± 0.3**	7 ± 0.5**	6.8 ± 0.5**
THI 5	7	11.7 ± 1.6	13.8 ± 1.7	13.4 ± 1.4	13.5 ± 0.9
THI 10	7	11.1 ± 0.8	17.1 ± 1	18.3 ± 1.5*	18.5 ± 0.8*
THI 20	7	11.4 ± 1.5	17 ± 1.1	19.1 ± 1.1*	21.3 ± 0.9**

In the hot plate test, mice were tested for baseline nociception (pretest) and received different doses of imetit (25 and 50 mg/kg ip), thioperamide (5, 10, and 20 mg/kg ip), and saline (10 ml/kg ip). Animals were then retested at various times after drug injection. Results are expressed as mean ± S.E.M.

* $P < .05$, different from control groups.

** $P < .001$, different from control groups.

Table 6
Effect of imetit (IME) alone or in combination with thioperamide (THI) on paw licking or kicking latency in the hot plate test (52.5 °C)

		Licking or kicking latency (s)			
Treatment	*n*	Pretest	20 min	30 min	40 min
mg/kg ip + mg/kg ip					
Saline + saline	7	13.3 ± 1.5	12.7 ± 1.1	12.3 ± 0.8	13.7 ± 0.9
IME 50 + saline	7	11.7 ± 0.3	7.1 ± 0.5*	7.2 ± 0.4*	6 ± 0.3*
THI 5 + saline	7	12.5 ± 1.7	13.7 ± 1.3	14.8 ± 1.9	17.3 ± 1.5
IME 50 + THI 5	7	11.1 ± 0.6	12.5 ± 1	12.8 ± 1.3	14 ± 0.9

In the hot plate test, mice were tested for baseline nociception (pretest) and received saline (10 ml/kg ip), in combination with imetit (50 mg/kg ip) and thioperamide (5 mg/kg ip), or imetit, in combination with thioperamide. Animals were then retested at various times after drug injection. Results are expressed as mean ± S.E.M.

* $P < .001$, different from control groups.

ineffective in modifying the pain threshold (Fig. 5). Conversely, in mice treated with thioperamide (10 and 20 mg/kg ip), the number of writhes was significantly reduced [$F(3,26) = 5.260$, $P < .0057$] (Fig. 5).

In the mice hot plate and writhing tests, dexchlorpheniramine (20 mg/kg ip) and ranitidine (50 μg/mouse icv) were not able to antagonize the antinociception induced by thioperamide (20 mg/kg ip) (Tables 7 and 8, Fig. 6).

4. Discussion

In the present experiment, the effects of different histamine receptor agonists and antagonists on the pain threshold

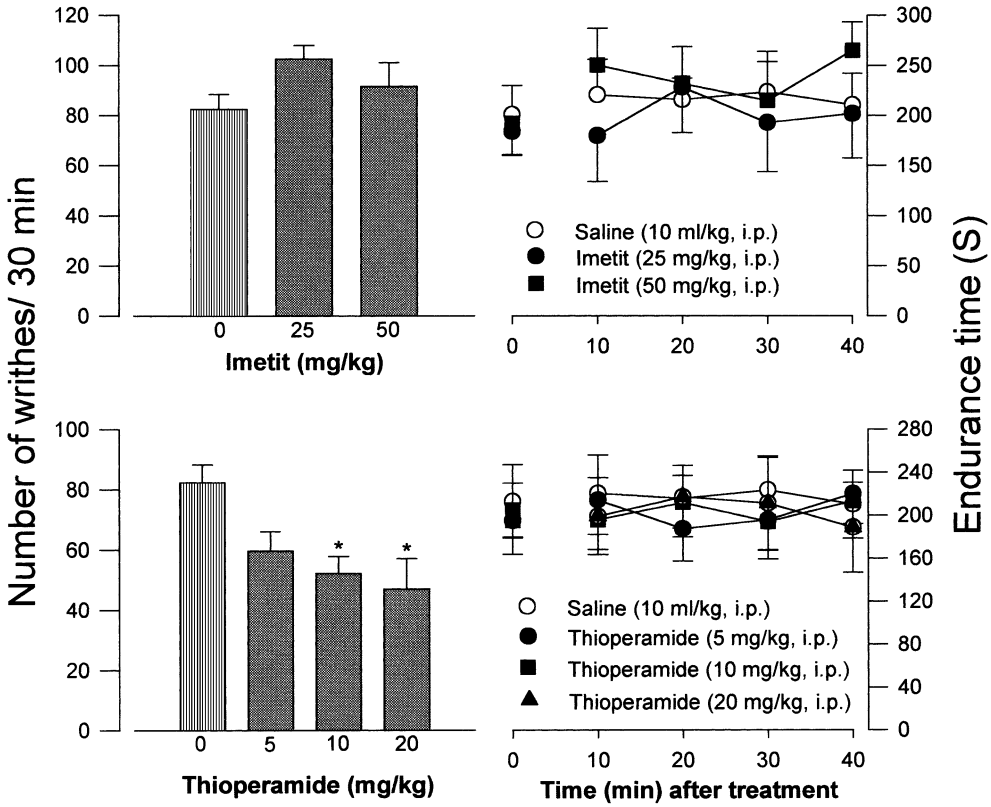

Fig. 5. Effects of imetit and thioperamide on the nociceptive threshold and endurance time in the mice writhing and rota rod tests, respectively. In the writhing test, imetit (25 and 50 mg/kg ip) and thioperamide (5, 10, and 20 mg/kg ip) were administered 15 min before test ($n = 7–9$ mice/group). In the rota rod test, endurance time of mice on the treadmill was measured before treatment and then starting 10 min after treatment, up 40 min ($n = 6$ mice/group). Results are expressed as mean ± S.E.M. * $P < .05$, different from control groups.

Table 7
Effect of thioperamide (THI) alone or in combination with dexchlorpheniramine (DEX) on paw licking or kicking latency in the hot plate test (52.5 °C)

		Licking or kicking latency (s)			
Treatment	*n*	Pretest	20 min	30 min	40 min
mg/kg ip + mg/kg ip					
Saline + saline	6	14.7 ± 1.1	14.3 ± 1.3	15.2 ± 0.6	14.3 ± 1.9
THI 20 + saline	6	11.5 ± 1.3	21.8 ± 1.1*	23.8 ± 1.9**	26.5 ± 2.1***
DEX 20 + saline	6	11.3 ± 1.3	16.8 ± 1.2	18.8 ± 2.7	16.2 ± 1.4
THI 20 + DEX 20	6	13.7 ± 1.1	22.5 ± 1.8*	24 ± 1.9**	24.2 ± 1.6**

In the hot plate test, mice were tested for baseline nociception (pretest) and received saline (10 ml/kg ip), in combination with saline (10 ml/kg ip) and dexchlorpheniramine (20 mg/kg ip), or thioperamide (20 mg/kg ip), in combination with saline and dexchlorpheniramine. Animals were then retested at various times after drug injection. Results are expressed as mean ± S.E.M.

* $P < .05$, different from control groups.

** $P < .01$, different from control groups.

*** $P < .001$, different from control groups.

were examined in mice using tests inducing two different kinds of noxious stimuli: thermal for the hot plate, and chemical for the writhing. The main findings are as follows.

(a) The histamine H_1 receptor agonist, HTMT, induced a hypernociception in both tests. Dexchlorpheniramine significantly antagonized the hypernociceptive effect of HTMT.

(b) In both tests, the pain threshold was significantly increased by the histamine H_2 receptor agonist and antagonist, dimaprit and ranitidine, respectively. The effect of dimaprit was blocked by the histamine H_3 receptor agonist, imetit, but not by ranitidine.

(c) In the mouse hot plate test, the pain threshold was significantly decreased by the histamine H_3 receptor agonist, imetit. Thioperamide significantly antagonized the response induced by imetit.

The present results indicate that both dexchlorpheniramine (30 and 40 mg/kg ip) and diphenhydramine (20 and 40 mg/kg ip) significantly increased the pain threshold in the hot plate and writhing tests; but such increase in pain threshold for diphenhydramine cannot be considered as real antinociceptive effect, as we observed a motor impairment in mice treated with equal doses of diphenhydramine. Most of the works so far published regarding the role of histamine H_1 receptor in modulating nociceptive stimuli have been based mainly on the use of H_1 antagonists. For example, H_1 receptor antagonists have been shown to have an antinociceptive effect (Oluyomi and Hart, 1991; Rumore and Schlichting, 1985), or to be able to antagonize histamine antinociception (Parolaro et al., 1989). There is also a report showing that diphenhydramine, hydroxyzine, and chlorpheniramine markedly potentiate analgesia of opioids (Bluhm et al., 1982). Histamine H_1 receptor antagonists are known not only to block H_1 receptors, but also to antagonize serotonergic, muscarinic, and catecholaminergic actions (Schwartz et al., 1991a). But these systems do not seem to be involved in the antinociceptive effects exerted by H_1 receptor antagonists, as specific molecules interfering with these systems were not able to prevent the antinociception induced by H_1 antagonists (Malmberg-Aiello et al., 1998). Histamine H_1 receptor antagonists also possess antagonistic properties on other subtypes of histamine receptors (H_2 or H_3 receptors) (Hill et al., 1997). It may be the case that dexchlorpheniramine increases the pain threshold by such a mechanism, as we observed an antinociception in mice treated with ranitidine or thioperamide. In order to verify whether the antinociceptive effect of dexchlorpheniramine is actually due to blockade of histamine H_1 receptors, and not to any other nonspecific effect, we have investigated the effect of HTMT on the pain threshold. The present data indicate that the dose of 50 μg/mouse icv of the histamine H_1 receptor agonist, HTMT, which was ineffective in the rota rod test, significantly decreased the pain threshold in the mice hot plate and writhing tests. Since dexchlorpheniramine significantly antagonized the hypernociceptive effect of HTMT, it may be that histamine H_1 receptor mechanisms are involved in the modulation of nociception. Such hypothesis is in agreement with previous studies. For example, Malmberg-Aiello et al. (1998) documented an H_1 receptor mechanism for increasing of sensitivity to noxious stimuli. In addition, Mobarakeh et al. (2000) reported that histamine plays an important role in both somatic and visceral pain perceptions through H_1 receptors using histamine H_1 receptor knockout mice.

The present data show that the intracerebroventricular injection of the histamine H_2 receptor agonist dimaprit (Durant et al., 1977) (100 μg/mouse), or antagonist ranitidine (50 and 100 μg/mouse) significantly increased the pain threshold in both hot plate and writhing tests. The dose of 100 μg/mouse dimaprit or ranitidine caused a motor impairment in the rota rod test. These results add further data to support a central role for histamine in nociception, but it remains difficult to explain the antinociceptive activity of histamine H_2 receptor agonists and antagonists. Although dimaprit is thought to be a selective histamine H_2 receptor agonist, it binds to H_3 receptors in the brain and antagonizes H_3 receptor activation (Arrang et al., 1983). Therefore, it

Table 8
Effect of thioperamide (THI) alone or in combination with ranitidine (RAN) on paw licking or kicking latency in the hot plate test (52.5 °C)

		Licking or kicking latency (s)			
Treatment	*n*	Pretest	20 min	30 min	40 min
mg/kg ip + μg/mouse icv					
Saline + saline	6	12.7 ± 1.5	12.5 ± 1.4	13.5 ± 1.2	12.6 ± 2.2
THI 20 + saline	6	10 ± 1.1	19.5 ± 1.4*	23.2 ± 2.1**	24.7 ± 2.5**
Saline + RAN 50	6	10.3 ± 0.9	27 ± 2.6**	29 ± 1.8**	29.7 ± 0.8**
THI 20 + RAN 50	6	12.8 ± 1.3	23.5 ± 1.9**	25.2 ± 1.7**	26.2 ± 1.2**

In the hot plate test, mice were tested for baseline nociception (pretest) and received saline (10 ml/kg ip), in combination with saline (5 μl/mouse icv) and ranitidine (50 μg/mouse icv), or thioperamide (20 mg/kg ip), in combination with saline and ranitidine. Animals were then retested at various times after drug injection. Results are expressed as mean ± S.E.M.

* $P < .05$, different from control groups.

** $P < .001$, different from control groups.

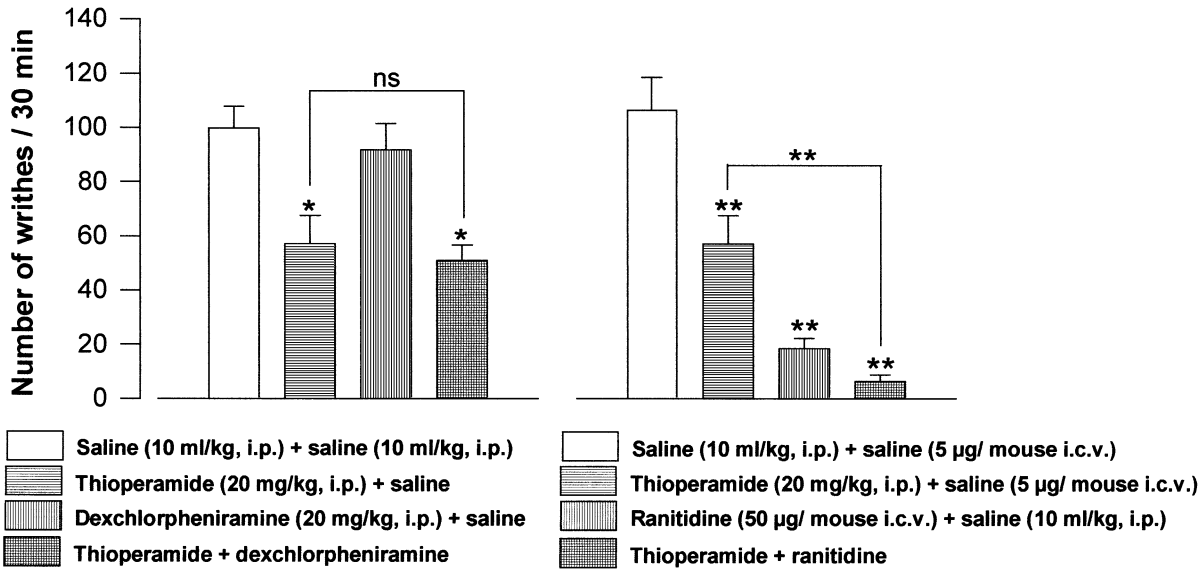

Fig. 6. Effects of H_1 or H_2 receptor antagonists on thioperamide antinociception. Drugs and saline were injected 15 min before test. Results are expressed as mean ± S.E.M. ($n = 6$ mice/group). *$P < .01$, **$P < .001$, different from control groups.

may be that dimaprit increases the pain threshold by such a mechanism, as our data demonstrated that the histamine H_3 receptor antagonist, thioperamide (Hew et al., 1990), induced an antinociception in both hot plate and writhing tests. The same mechanism of action had been previously postulated to explain the antinociception induced by impromidine (Lamberti et al., 1996). Impromidine, besides being an H_2 agonist, also showed H_3 antagonist properties (Schwartz et al., 1991b). Because antagonism on the H_3 receptor is reported for dimaprit (Arrang et al., 1983), it seemed worthwhile to verify whether imetit, a selective H_3 receptor agonist (Garbarg et al., 1992), was able to affect dimaprit antinociception. Since imetit was able to antagonize the antinociception induced by dimaprit in both mice hot plate and writhing tests, it therefore seems likely that dimaprit produces antinociception by blocking the histamine H_3 receptor.

For histamine H_2 receptor antagonists, several studies have reported that these drugs have different effects, such as antinociception (Oluyomi and Hart, 1991) or hypernociception (Lamberti et al., 1996). Moreover, a series of H_2 receptor antagonists reduced the antinociceptive effects of H_2 receptor agonists (Netti et al., 1988)—treatment that lacked antagonistic effects on histamine antinociception (Chung et al., 1984). Interaction between blockade of histamine H_2 receptors and opioid system was also observed in relation to rodent antinociception. Bluhm et al. (1982) reported that several H_2 receptor antagonists potentiate analgesia of opioids, while Gogas et al. (1989) evidenced that H_2 receptor antagonists produce a dose-related inhibition of morphine antinociception. Such discrepancies might be due to a novel brain mechanism unrelated to H_1, H_2, and H_3 receptors (Li et al., 1996), or an affinity for all three classes of histamine receptors (Schwartz et al., 1991b). The opposite effects of H_2 antagonists in various antinociceptive tests hinder their use as pharmacological tools and suggest that the antinociceptive effect of ranitidine is not caused by an action on H_2 receptor. Our results are in agreement with this hypothesis because the coadministration of dimaprit and ranitidine produced no evidence for an involvement of H_2 receptor mechanism(s).

The present data show that the histamine H_3 receptor antagonist, thioperamide (10 and 20 mg/kg ip), has analgesic activity in the hot plate and writhing tests. The rota rod test provided no motor impairment in mice treated with thioperamide. Many studies support the hypothesis that endogenous or exogenous histamine (when directly administered into the CNS) can mediate pain-relieving responses in animals (Glick and Crane, 1978; Hough et al., 1997; Lamberti et al., 1996; Onodera and Ogura, 1983; Parolaro et al., 1989). Therefore, histamine H_3 receptor antagonists were predicted to have analgesic properties, as these compounds block presynaptic autoreceptors and increase the release of neuronal histamine (Itoh et al., 1991; Mochizuki et al., 1991; Tedford et al., 1995). However, it was considered possible that blockade of histamine H_3 receptor by thioperamide could induce an antinociceptive effect, but that thioperamide's effects on other receptors might prevent expression of this response. For example, in rodents, clear effects on brain neurochemistry have been reported at doses less than 5 mg/kg for thioperamide (Garbarg et al., 1992). Therefore, the antinociceptive doses of 10 and 20 mg/kg thioperamide seem to be high. In addition, our results show that HTMT and dimaprit produce hypernociception and antinociception, respectively. Therefore, if histamine released by thioperamide acts on H_1 receptors, hypernociception might be produced, while thioperamide caused antinociception. But if histamine released by thioperamide

stimulates H_2 receptors, antinociception might be induced. Such antinociception is in agreement with thioperamide's effect on the pain threshold. In order to confirm such hypotheses, an investigation was carried out to evaluate the effects of dexchlorpheniramine and ranitidine on thioperamide antinociception. Since the results of this study indicate that dexchlorpheniramine or ranitidine did not antagonize the effect of thioperamide, the involvement of such mechanisms in the antinociceptive effect of thioperamide is unlikely. The involvement of a postsynaptic H_1 or H_2 antagonism in thioperamide antinociception is also unlikely, as thioperamide has been reported to have a K_i of 4 nM on the H_3 receptor and >10,000 nM on the H_1 or H_2 receptors (Schwartz et al., 1990). Such results provide additional support to confirm the thioperamide H_3 antinociception hypothesis. This hypothesis is also supported by our experiment. The present study indicates that the selective histamine H_3 receptor agonist, imetit (50 mg/kg ip), produced a statistically significant hypernociception in the hot plate test, but no such effect was found in the writhing test. Detection of hypernociception in the abdominal writhing test is difficult. The writhing response induced by acetic acid is unexpectedly reduced by substances that might produce a strong hypernociception in the other tests, as the high-intensity stimulus induces the activation of endogenous opioid pain suppression system (Lamberti et al., 1996; Malmberg-Aiello et al., 1998). In contrast, hypernociception induced by a low-intensity stimulus, such as a relatively low hot plate temperature (52.5 °C), allows to detect not only increase in the pain threshold, but also eventually decreases. Therefore, this may explain the noticeable contrast between those two responses of imetit in the hot plate and writhing tests. Since our results show that thioperamide (5 mg/kg ip) significantly antagonizes the hypernociceptive action of imetit, it may be that histamine H_3 receptor mechanisms are involved in the modulation of pain threshold in the mouse hot plate test.

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