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# Role of histamine in rodent antinociception

[1]Petra Malmberg-Aiello, Claudia Lamberti, Carla Ghelardini, Alberto Giotti & Alessandro Bartolini

Department of Preclinical and Clinical Pharmacology, University of Florence, Viale G.B. Morgagni 65, I-50134 Firenze, Italy

**1** Effects of substances which are able to alter brain histamine levels on the nociceptive threshold were investigated in mice and rats by means of tests inducing three different kinds of noxious stimuli: mechanical (paw pressure), chemical (abdominal constriction) and thermal (hot plate).

**2** A wide range of i.c.v. doses of histamine 2HCl was studied. Relatively high doses were dose-dependently antinociceptive in all three tests: 5–100 μg per rat in the paw pressure test, 5–50 μg per mouse in the abdominal constriction test and 50–100 μg per mouse in the hot plate test. Conversely, very low doses were hyperalgesic: 0.5 μg per rat in the paw pressure test and 0.1–1 μg per mouse in the hot plate test. In the abdominal constriction test no hyperalgesic effect was observed.

**3** The histamine $H_3$ antagonist, thioperamide maleate, elicited a weak but statistically significant dose-dependent antinociceptive effect by both parenteral (10–40 mg $kg^{-1}$) and i.c.v. (1.1–10 μg per rat and 3.4–10 μg per mouse) routes.

**4** The histamine $H_3$ agonist, (R)-α-methylhistamine dihydrogenomaleate was hyperalgesic, with a rapid effect (15 min after treatment) following i.c.v. administration of 1 μg per rat and 3 μg per mouse, or i.p. administration of 100 mg $kg^{-1}$ in mice. In rats 20 mg $kg^{-1}$, i.p., elicited hyperalgesia only 4 h after treatment.

**5** Thioperamide-induced antinociception was completely prevented by pretreatment with a non-hyperalgesic i.p. dose of (R)-α-methylhistamine in the mouse hot plate and abdominal constriction tests. Antagonism was also observed when both substances were administered i.c.v. in rats.

**6** L-Histidine HCl dose-dependently induced a slowly occurring antinociception in all three tests. The doses of 250 and 500 mg $kg^{-1}$, i.p. were effective in the rat paw pressure test, and those of 500 and 1500 mg $kg^{-1}$, i.p. in the mouse hot plate test. In the mouse abdominal constriction test 500 and 1000 mg $kg^{-1}$, i.p. showed their maximum effect 2 h after treatment.

**7** The histamine N-methyltransferase inhibitor, metoprine, elicited a long-lasting, dose-dependent antinociception in all three tests by both i.p. (10–30 mg $kg^{-1}$) and i.c.v. (50–100 μg per rat) routes.

**8** To ascertain the mechanism of action of the antinociceptive effect of L-histidine and metoprine, the two substances were also studied in combination with the histamine synthesis inhibitor (S)-α-fluoromethylhistidine and with (R)-α-methylhistamine, respectively. L-Histidine antinociception was completely antagonized in all three tests by pretreatment with (S)-α-fluoromethylhistidine HCl (50 mg $kg^{-1}$, i.p.) administered 2 h before L-histidine treatment. Similarly, metoprine antinociception was prevented by (R)-α-methylhistamine dihydrogenomaleate 20 mg $kg^{-1}$, i.p. administered 15 min before metoprine. Both (S)-α-fluoromethylhistidine and (R)-α-methylhistamine were used at doses which did not modify the nociceptive threshold when given alone.

**9** The catabolism product, 1-methylhistamine, administered i.c.v. had no effect in either rat paw pressure or mouse abdominal constriction tests.

**10** These results indicate that the antinociceptive action of histamine may take place on the postsynaptic site, and that its hyperalgesic effect occurs with low doses acting on the presynaptic receptor. This hypothesis is supported by the fact that the $H_3$ antagonist, thioperamide is antinociceptive and the $H_3$ agonist, (R)-α-methylhistamine is hyperalgesic, probably modulating endogenous histamine release. L-Histidine and metoprine, which are both able to increase brain histamine levels, are also able to induce antinociception in mice and rats. Involvement of the histaminergic system in the modulation of nociceptive stimuli is thus proposed.

**Keywords:** Analgesia; antinociception; pain; histamine; L-histidine; (R)-α-methylhistamine; thioperamide; metoprine; (S)-α-fluoromethylhistidine; methylhistamine

## Introduction

Modulation of nociception can occur via different neuronal systems. Many neuromediators besides enkephalins, such as acetylcholine (ACh) (Metys *et al.*, 1969; Bartolini *et al.*, 1992), γ-aminobutyric acid (GABA) (Liebman & Pastor, 1980; Malcangio *et al.*, 1992), catecholamines (Jones & Gebhart, 1986) and 5-hydroxytryptamine (5-HT) (Samanin & Valzelli, 1971) have been reported to be involved in nociception control. Recently histamine, which is regarded as an autacoid associated with cutaneous pain (Crossland, 1980), has also been shown to take part in antinociception. Intracerebroventricular (i.c.v.) administration elicits antinociception in both rats (Glick & Crane, 1978; Bhattacharya & Parmar, 1985; Parolaro *et al.*, 1989) and mice (Chung *et al.*, 1984; Oluyomi & Hart, 1991) at relatively high doses. Glick & Crane (1978) also reported that injection of histamine into the rat dorsal raphe nucleus and periaqueductal grey region caused antinociception, while its injection into the median raphe nucleus caused hyperalgesia.

Conversely, the role of endogenous histamine in antinociception has not yet been investigated. Its importance has grown in the last few years with the discovery and definition of a histaminergic neuronal system in mammalian brain. According to many research groups (Steinbusch & Mulder,

[1] Author for correspondence.

1985; Panula et al., 1989; Wada et al., 1991), in addition to two ascending histaminergic pathways, there is a minor descending pathway which arises from hypothalamic neurones. Its fibres can be found in the dorsal raphe nucleus and periaqueductal grey region, areas which are considered to be important for pain modulation (Basbaum & Fields, 1984).

Furthermore, the existence of presynaptic histamine receptors, called $H_3$, was reported by Arrang et al. (1983). According to the authors, their stimulation inhibits histamine release (Arrang et al., 1983; Van der Werf et al., 1987) and synthesis (Arrang et al., 1987b). Lately, this same group described the effects of a potent and selective $H_3$ receptor agonist, (R)-α-methylhistamine (RAMH), and an antagonist, thioperamide (Arrang et al., 1987a; Garbarg et al., 1989). The two molecules were seen to be good tools for studying the role of endogenous histamine.

Besides investigating the effects of a wide range of histamine doses, or acting on the histamine $H_3$ receptor with RAMH and thioperamide, a further way to study the role of endogenous histamine in antinociception might be to alter histamine brain levels. Different substances such as the histamine precursor, L-histidine, the histidine decarboxylase [EC 4.1.1.22] (HDC), (S)-α-fluoromethylhistidine (FMH) (Kollonitsch et al., 1978) and the histamine-*N*-methyltransferase [EC 2.1.1.8] (HMT) inhibitor, metoprine (Duch et al., 1978), have in fact been described as able to alter selectively histamine brain levels. Oluyomi & Hart (1991) recently reported an antinociceptive effect for histidine, as well as for thioperamide, in the mouse hot plate test, while there are no reports on the effects of histamine synthesis or catabolism inhibitors.

We therefore considered it worthwhile investigating the role of the histaminergic system in antinociception by using all three of the aforementioned strategies in both mice and rats, with three different kinds of antinociceptive tests.

Preliminary data were presented at the XXIth and XXIIth Annual Meetings of the European Histamine Research Society, (Lamberti et al., 1992b; Malmberg-Aiello et al., 1992; 1993) and at the IXth Meeting of the European Society for Neurochemistry (Lamberti et al., 1992a).

## Methods

Male Swiss-Webster mice (22–28 g) and Wistar rats (120–180 g) were used. Fifteen mice or four rats were housed per cage. The cages were taken to the experimental room 24 h before the experiment, for acclimatization. The animals were fed *ad libitum* a standard laboratory diet and tap water.

### *Hot plate test*

The method described by O'Callaghan & Holtzman (1976) was adopted, using a stainless steel container (36 × 28 × 30 cm), thermostatically set at 52.5 ± 0.1°C, in a precision water-bath. Mice with a licking latency below 12 and over 18 s in the test before drug administration (30%) were rejected. An arbitrary cut-off time of 45 s was adopted.

### *Abdominal constriction test*

The test was performed in mice according to Koster et al. (1959). The number of stretching movements was counted for 10 min, starting 5 min after 0.6% acetic acid injection.

### *Paw pressure test*

The nociceptive threshold in rats was determined with an analgesymeter (Ugo Basile, Varese, Italy) according to the method described by Leighton et al. (1988). Rats scoring below 50 g or over 80 g during the test before drug administration (25%) were rejected. An arbitrary cut-off value of 250 g was adopted.

### *Rota-rod test*

The integrity of motor coordination was assessed on the basis of the endurance time of the animals on the rotating rod according to Kuribara et al. (1977). On the day of the test, the performance time was measured before and 15 min after treatment.

### *Drugs*

The following drugs were used: histamine dihydrochloride, L-histidine monohydrochloride monohydrate and 1-methylhistamine dihydrochloride (Sigma); (R)-α-methylhistamine dihydrogenomaleate (Bioprojet), (S)-α-fluoromethylhistidine monohydrochloride (Merck Sharp & Dohme Research Lab.), morphine hydrochloride (USL 10/D), metoprine (Burroughs Wellcome) and thioperamide maleate (RBI). The doses given in the text are expressed as salts. All drugs except metoprine were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. Metoprine was dissolved in 10% aqueous lactic acid and then diluted with saline (1:30). Drug concentrations were prepared in such a way that the necessary dose could be injected in a volume of 10 ml $kg^{-1}$ by both s.c. and i.p. route.

I.c.v. administration was performed in two different ways. For the first method a short ether anaesthesia was adopted. Substances were injected in the necessary dose dissolved in 5 μl for mice and in 10 μl for rats, according to the method described by Haley & McCormick (1957) for mice and extended to rats by us. The second approach consisted of injecting the substances in conscious rats with permanent i.c.v. polyethylene cannulae (5 μl of drug solution + 2 μl air + 5 μl saline) implanted according to the method described by Altaffer et al. (1970), in order to avoid false responses due to the effect of ether. To ascertain the exact site of i.c.v. injection, some mice or rats were injected i.c.v. with 5 μl or 10 μl of 1:10 diluted Indian ink and their brains were examined macroscopically after sectioning.

### *Statistical analysis*

Results are given as the mean ± s.e. Student's two-tailed *t* test was used to verify significance between two means. *P* values of less than 0.05 were considered significant. Multiple comparisons with appropriate controls were made with ANOVA, followed by the multiple range test for least significant differences (LSD). Means with 95% confidence intervals that did not overlap were considered significantly different. $ED_{50}$ and $ED_{30}$ values are the doses which produced respectively the 50% and 30% of the maximum possible effect with 95% confidence limits. Data were analyzed with computer programmes (Tallarida & Murray, 1984, and STATGRAPHICS, 1986, STSC Inc. U.S.A.).

## Results

### *Histamine antinociceptive and hyperalgesic effects*

Histamine was administered i.c.v. in doses ranging from 0.05 to 100 μg per mouse and from 0.1 to 100 μg per rat. Very low doses (0.1, 0.5 and 1 μg per mouse) induced significant hyperalgesia 15 min after treatment, while high doses (50 and 100 μg per mouse) elicited antinociception which was still evident 45 min after treatment in the hot plate test (Figure 1a). The $ED_{30}$ for the antinociceptive effect (15 min after treatment) was 96.0 (71.9–144.7) μg per mouse i.c.v.

The same biphasic effect was observed in the paw pressure test on rats. Histamine caused statistically-significant hyperalgesia 30 min after treatment at 0.5 μg per rat administered i.c.v. during ether anaesthesia and at both 0.5 and 1 μg per rat i.c.v., 45 min after treatment; antinociception was detectable at doses of 5–50 μg per rat (Figure 2a). In order to

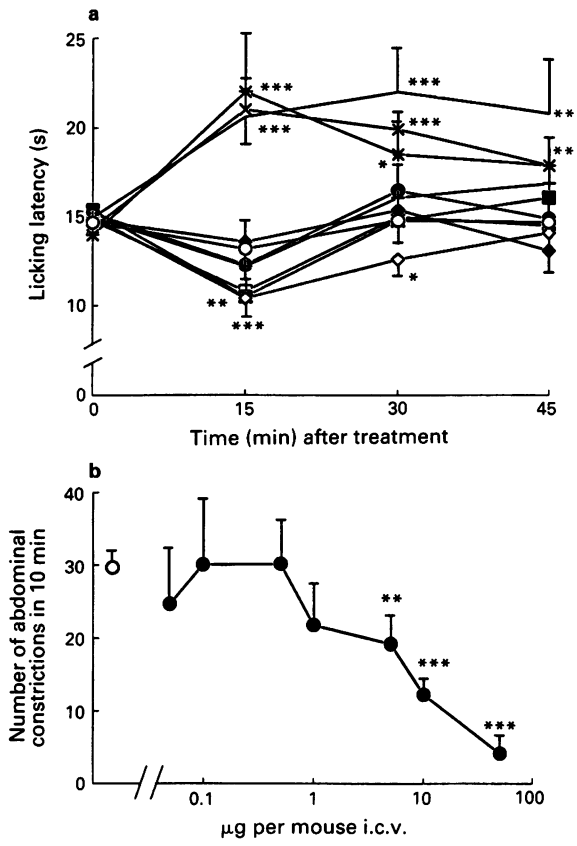


**Figure 1** Effect of i.c.v. histamine 2HCl on the nociceptive threshold in mice. (a) Biphasic effect in the hot plate test. Groups were treated (μg per mouse i.c.v.) as follows: saline 5 μl (○); histamine 0.05 (●); 0.1 (□); 0.5 (■); 1 (◇); 5 (◆); 10 (+); 50 (×) and 100 (*). In comparison, the effect of morphine HCl 5 mg kg$^{-1}$, s.c. (no symbol) is shown. (b) Antinociceptive effect in the abdominal constriction test. Saline (○) and histamine (●) were injected 15 min before the test. $*P<0.05$; $**P<0.01$; $***P<0.001$ versus saline controls. Each point represents the mean (with s.e.mean) of 11–79 mice.

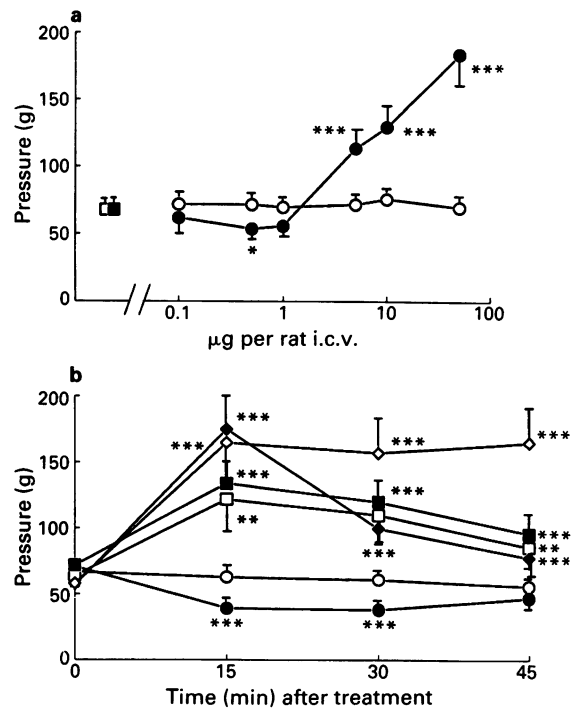


**Figure 2** Biphasic effect of histamine on the nociceptive threshold in the rat paw pressure test. (a) Dose-response curve of histamine effect. Saline 10 μl per rat (○) and histamine 2HCl (●) were administered by i.c.v. injection during short ether anaesthesia, 30 min before test. Symbols (□) and (■) indicate pretest values of saline and histamine-treated groups respectively. (b) Effects on rats with permanent i.c.v. cannulae. Groups were (dose per rat i.c.v.): saline 5 μl (○), histamine 2HCl 0.5 μg (●), 10 μg (□), 50 μg (■), 100 μg (◆). In comparison the effect of morphine 5 mg kg$^{-1}$, s.c. (◇) is shown. $*P<0.05$; $**P<0.01$; $***P<0.001$ versus saline controls. Each point represents the mean (with s.e.mean) of 7–23 rats.

make sure that ether anaesthesia did not affect the results hyperalgesic and antinociceptive doses of histamine were also administered to conscious rats via permanent i.c.v. cannulae. The results obtained confirmed our previous observations and revealed a maximum effect at 15 min (Figure 2b), with an $ED_{50}$ of 69.0 (13.3–356.9) μg per rat i.c.v.

In the mouse abdominal constriction test only the antinociceptive effect was detectable. Histamine induced a statistically-significant antinociception at 5, 10 and 50 μg per mouse with an $ED_{50}$ of 6.7 (3.8–11.7) μg per mouse i.c.v. (Figure 1b). The antinociception induced by 50 μg per mouse is comparable to that induced by 2 mg kg$^{-1}$ of morphine HCl (5.4 ± 2.0 constrictions 15 min after s.c. administration), and a comparison with the effects of morphine in the hot plate and paw pressure tests is given in Figures 1a and 2b, respectively.

The histamine dose of 50 μg per mouse was also used to study mouse rota-rod performance 15 min after treatment. No effect was seen on endurance time on the rod in histamine- and saline-treated mice (186 ± 40 s versus 229 ± 23 s of controls).

### *Thioperamide antinociceptive effect*

In all three tests thioperamide was able to induce a statistically-significant antinociception. Both i.c.v. and parenteral routes of administration were used.

In the mouse hot plate test, 20 mg kg$^{-1}$, i.p. appeared to be the optimum dose for eliciting antinociception (Table 1), which persisted up to 30 min with a maximum response at 15 min. A lower (10 mg kg$^{-1}$, i.p.) or higher (40 mg kg$^{-1}$, i.p.) dose was less effective in raising the nociceptive threshold. When given i.c.v. in doses ranging from 0.5 to 10 μg per mouse, no statistically-significant effect was observed for thioperamide (data not shown).

Conversely, in the mouse abdominal constriction test, i.c.v. administration of thioperamide showed an initial dose-dependent action with a maximum effect at 10 μg per mouse, while a higher dose (30 μg per mouse) was not effective in significantly decreasing the number of abdominal constrictions (Figure 3a). Similarly, subcutaneous administration revealed a statistically-significant antinociception only for the same dose which elicited the maximum effect in the hot plate test (Figure 3b).

Thioperamide antinociception was confirmed in the rat paw pressure test, where the time-course for the dose of 20 mg kg$^{-1}$, i.p. reflected the one observed with the hot plate test (Figure 4a). Both 1.1 and 10 μg per rat administered via permanent i.c.v. cannulae were antinociceptive; the effect of the latter was still significant 2 h after treatment (Table 2).

### *Effect of (R)-α-methylhistamine (RAMH) on nociceptive threshold*

When given at sufficiently high doses, RAMH was able to induce hyperalgesia in the hot plate and paw pressure tests.

In the mouse hot plate test, three different i.p. doses were used. The doses of 5 and 20 mg kg$^{-1}$ were ineffective in modifying the nociceptive threshold, while 100 mg kg$^{-1}$ elicited a highly significant hyperalgesia up to 60 min after treatment (Table 1). The dose 20 mg kg$^{-1}$, i.p. also had no effect in the abdominal constriction test (Figure 3b).

**Table 1** Effects of thioperamide and (R)-α-methylhistamine (RAMH) i.p. alone and combined in the mouse hot plate test

| *Pretreatment* (mg kg$^{-1}$, i.p.) | *Treatment* (mg kg$^{-1}$, i.p.) | n | *Licking latency* (s) *Pretest* | *15 min* | *30 min* | *45 min* | *60 min* |
|---|---|---|---|---|---|---|---|
| | | | | | *after treatment* | | |
| Saline 10 ml | Saline 10 ml | 47 | 14.6 ± 0.3 | 13.5 ± 0.4 | 12.9 ± 0.4 | 13.6 ± 0.4 | 13.9 ± 0.4 |
| | Thioper 10 | 14 | 14.4 ± 0.4 | 16.0 ± 1.4* | 15.3 ± 1.0* | 14.0 ± 0.9 | 13.1 ± 0.7 |
| Saline | Thioper 20 | 12 | 14.6 ± 0.3 | 19.6 ± 0.9*** | 18.3 ± 1.5*** | 15.5 ± 1.5 | 14.3 ± 0.7 |
| | Thioper 40 | 14 | 14.5 ± 0.3 | 16.0 ± 1.0* | 15.0 ± 1.1* | 13.7 ± 1.1 | 14.2 ± 0.8 |
| | RAMH 5 | 5 | 14.6 ± 1.1 | 12.4 ± 1.4 | 12.4 ± 1.1 | 12.2 ± 0.9 | 14.8 ± 0.7 |
| RAMH 20 | Saline | 30 | 14.3 ± 0.4 | 12.2 ± 0.9 | 13.1 ± 0.7 | 13.5 ± 0.6 | 14.6 ± 1.1 |
| | RAMH 100 | 11 | 14.5 ± 0.6 | 9.6 ± 0.8*** | 9.7 ± 1.0** | 9.4 ± 1.0*** | 11.6 ± 0.9* |
| RAMH 20 | Thioper 20 | 10 | 14.5 ± 0.6 | 12.7 ± 0.8^^ | 13.4 ± 0.9^ | 14.4 ± 1.2 | 13.0 ± 0.8 |

*$P<0.05$; **$P<0.01$; ***$P<0.001$ versus saline controls.
^$P$0.02; ^^$P<0.001$ versus thioperamide (20 mg kg$^{-1}$)-treated mice.
Pretreatment was performed 15 min before treatment.

A wide range of doses, from 1 to 100 μg per mouse, was tested by i.c.v. route in the hot plate test, but only 3 μg per mouse significantly lowered the latency from 13.0 ± 0.5 s of controls to 9.7 ± 0.6 s in 17 mice 15 min after treatment. The highest doses tested, 50 and 100 μg per mouse, caused immobility and convulsions.

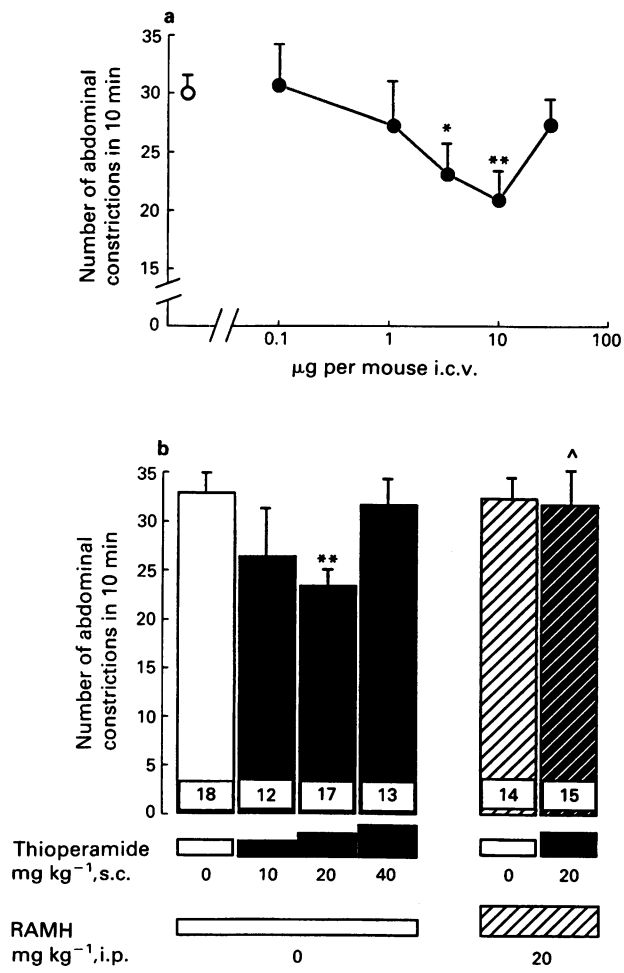


**Figure 3** Antinociceptive effect of i.c.v. and s.c. thioperamide in the mouse abdominal constriction test. (a) Thioperamide maleate was administered i.c.v. 15 min before test. Each point represents the mean of 12–19 mice. (b) Antinociceptive effect of thioperamide and its antagonism by (R)-α-methylhistamine (RAMH). RAMH dihydrogenomaleate was administered 15 min before thioperamide and thioperamide s.c. 15 min before test. Inside the columns is shown the number of mice. Vertical lines give s.e.mean. *$P<0.02$; **$P<0.01$ versus saline controls. ^$P<0.05$ versus thioperamide (20 mg kg$^{-1}$)-treated mice.

Similarly, in the rat paw pressure test 1 μg per rat administered i.c.v. via permanent cannulae was significantly hyperalgesic (Table 2). The dose of 20 mg kg$^{-1}$ was tested by parenteral administration in rats. The threshold was gradually lowered, and a statistically-significant hyperalgesia was observed from 4 to 7 h after treatment (Figure 4b).

### *Antagonism by (R)-α-methylhistamine of thioperamide antinociception*

In all three tests RAMH administered 15 min before thioperamide completely prevented the antinociception induced by the latter. When tested in mice, both RAMH and thioperamide were administered parenterally at 20 mg kg$^{-1}$ (Table 1 and Figure 3b), while in the rat paw pressure test i.c.v. administration via permanent cannulae was adopted (Table 2).

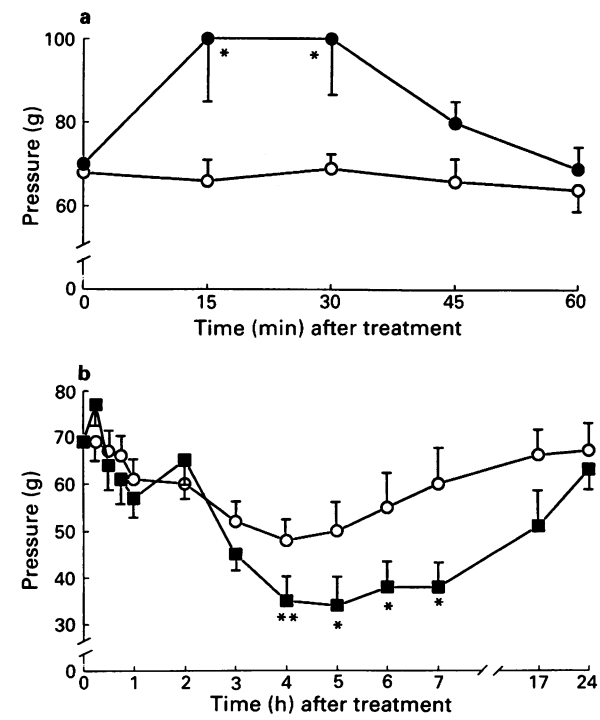


**Figure 4** Effects of thioperamide and (R)-α-methylhistamine (RAMH) on nociceptive threshold in the rat paw pressure test. (a) Antinociceptive effect of thioperamide maleate 20 mg kg$^{-1}$, i.p. (●) and (b) hyperalgesic effect of RAMH dihydrogenomaleate 20 mg kg$^{-1}$, i.p. (■) in comparison with saline-treated (○) rats. Each point represents the mean with s.e.mean of 7–11 rats. *$P<0.05$; **$P<0.01$ versus saline controls.

**Table 2** Effects of thioperamide and (R)-α-methylhistamine (RAMH) i.c.v. alone and combined in the rat paw pressure test

| *Pretreatment* (μg per rat) | *Treatment* (μg per rat) | n | *Pressure* (g) *Pretest* | *15 min* | *30 min* | *45 min after treatment* | *60 min* | *120 min* |
|---|---|---|---|---|---|---|---|---|
| Saline 10 μl | Saline 10 μl | 19 | 60 ± 2 | 60 ± 2 | 58 ± 1 | 57 ± 2 | 57 ± 2 | 57 ± 3 |
| | Thioper 1.1 | 9 | 60 ± 2 | 87 ± 6*** | 77 ± 6** | 63 ± 4 | 56 ± 2 | – |
| Saline | Thioper 10 | 10 | 60 ± 2 | 102 ± 10*** | 84 ± 6*** | 68 ± 4* | 66 ± 3* | 71 ± 3** |
| RAMH 1 | Saline | 14 | 63 ± 1 | 49 ± 3** | 54 ± 2 | 46 ± 2* | 47 ± 2** | 45 ± 2** |
| RAMH 1 | Thioper 10 | 12 | 58 ± 2 | 64 ± 2^^ | 58 ± 2^^ | 56 ± 2^ | 58 ± 2^ | – |

*$P<0.05$; **$P<0.01$; ***$P<0.001$ versus saline-treated rats.
^$P$0.05; ^^$P<0.001$ versus thioperamide (10 μg)-treated rats.
Pretreatment was performed 15 min before treatment.

### *L-Histidine antinociceptive effect*

L-Histidine was able to raise significantly the nociceptive threshold in both rats and mice in all three tests.

In the rat paw pressure test the antinociceptive effect was dose-dependent and for both doses used (250 and 500 mg $kg^{-1}$, i.p.), it lasted until 3 h after treatment, the highest one producing a peak effect 2 h after administration (Figure 5).

In the mouse abdominal constriction test also, histidine antinociception followed a pattern which was both time- and dose-dependent. Both doses used in this test, 500 and 1000 mg $kg^{-1}$ i.p., decreased the number of abdominal constrictions with a maximum effect 2 h after treatment. At this same time the percentage of inhibition was 34% and 69% respectively (Figure 6a).

In the mouse hot plate test the dose-dependent rise in the nociceptive threshold was very small but significant, and for the highest dose (1500 mg $kg^{-1}$, i.p.) was detectable from 30 to 180 min after treatment (Figure 6b). Despite this high dose, the antinociception obtained produced no visible change in the animals' normal behaviour.

### *Antagonism of histidine-induced antinociception by (S)-α-fluoromethylhistidine (FMH)*

In all three tests FMH, administered 2 h before histidine treatment, was able to prevent completely the antinociception induced by the latter (Figures 5 and 6). FMH was given at a dose (50 mg $kg^{-1}$, i.p.) which neither modified the nociceptive threshold when given alone, nor altered normal animal behaviour.

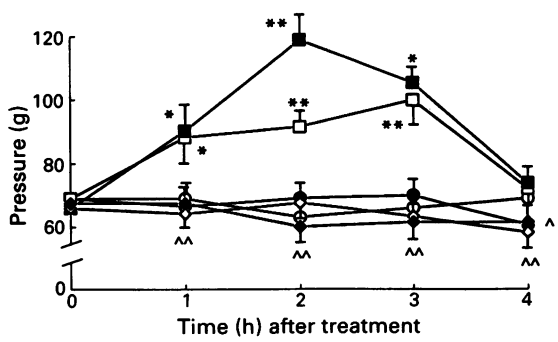


**Figure 5** Antinociceptive effect of L-histidine and its antagonism by (S)-α-fluoromethylhistidine (FMH) in the rat paw pressure test. Groups (pretreatment i.p. + treatment i.p.) were as follows: saline 0.1 ml 10 $g^{-1}$ + saline (○); FMH HCl 50 mg $kg^{-1}$ + saline (●); saline + L-histidine HCl 250 mg $kg^{-1}$ (□); saline + L-histidine 500 mg $kg^{-1}$ (■); FMH 50 mg $kg^{-1}$ + L-histidine 250 mg $kg^{-1}$ (◇); FMH 50 mg $kg^{-1}$ + L-histidine 500 mg $kg^{-1}$ (◆). Pretreatment was performed 2 h before treatment. *$P<0.01$; **$P<0.001$ versus saline controls. ^$P<0.05$; ^^$P<0.01$ versus L-histidine-treated mice. Each point represents the mean (with s.e.mean) of 9–20 rats.

### *Metoprine antinociceptive effect*

In the mouse hot plate test, doses of 5, 20 and 30 mg $kg^{-1}$, i.p. of metoprine were studied. The antinociception observed was dose-dependent: 5 mg $kg^{-1}$ was ineffective, while at 20 mg $kg^{-1}$ the antinociceptive effect was significant for 1 h and at 30 mg $kg^{-1}$ a very strong effect lasted up to 24 h (Figure 7a). Mice treated with the highest dose showed slight excitation inside their cages; this became more noticeable when they were placed on the plate. Calculations revealed an $ED_{50}$ of 19.5 (13.5–28.3) mg $kg^{-1}$, i.p. 15 min after treatment.

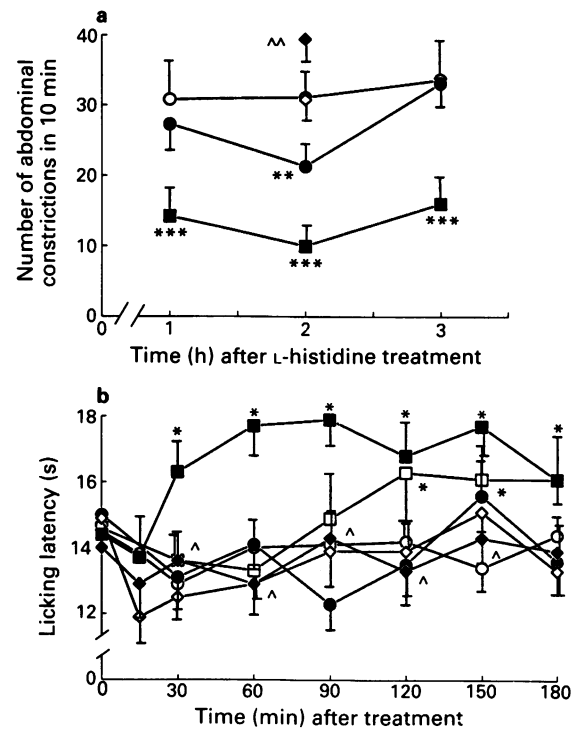


**Figure 6** Time course of L-histidine antinociception and its antagonism by (S)-α-fluoromethylhistidine (FMH) in mice. (a) Abdominal constriction test. FMH was administered 2 h before L-histidine and L-histidine 1, 2 or 3 h before test. Groups (pretreatment i.p. + treatment i.p.) were: saline 0.1 ml 10 $g^{-1}$ + saline (○); saline + L-histidine HCl 500 mg $kg^{-1}$ (●); L-histidine 1000 mg $kg^{-1}$ (■); FMH HCl 50 mg $kg^{-1}$ + saline (◇); FMH + L-histidine 500 mg $kg^{-1}$ (◆). Each point represents the mean of 9–16 mice. **$P<0.01$; ***$P<0.001$ versus saline controls. ^^$P<0.001$ versus L-histidine 500 mg $kg^{-1}$ (2 h before test)-treated mice. (b) Hot plate test. Groups (pretreatment i.p. + treatment i.p.) were as follows: saline 0.1 ml 10 $g^{-1}$ + saline (○); L-histidine 250 mg $kg^{-1}$ (●); L-histidine 500 mg $kg^{-1}$ (□); saline + L-histidine 1500 mg $kg^{-1}$ (■); FMH 50 mg $kg^{-1}$ + saline (◇); FMH 50 mg $kg^{-1}$ + L-histidine 1500 mg $kg^{-1}$ (◆). Pretreatment was performed 2 h before treatment. Each point represents the mean of 12–26 mice. *$P<0.05$ versus saline controls. ^$P<0.05$ versus L-histidine (1500 mg $kg^{-1}$)-treated mice.

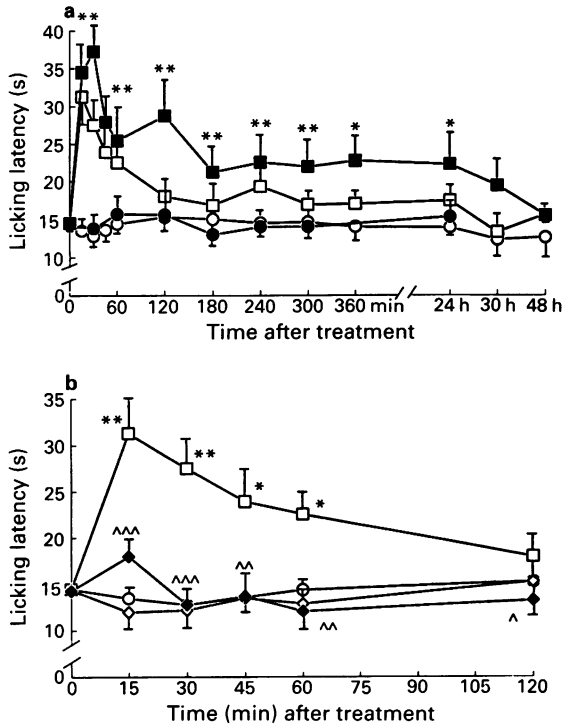


**Figure 7** Antinociceptive effect of metoprine (a) and its antagonism by (R)-α-methylhistamine (RAMH) (b) in the mouse hot plate test. Groups (pretreatment i.p. + treatment i.p.) were as follows: saline 0.1 ml 10 $g^{-1}$ + lactic acid 0.3% in saline (○); metoprine 5 mg $kg^{-1}$ (●); saline + metoprine 20 mg $kg^{-1}$ (□); metoprine 30 mg $kg^{-1}$ (■); RAMH dihydrogenomaleate 20 mg $kg^{-1}$ + lactic acid 0.3% (◇); RAMH + metoprine 20 mg $kg^{-1}$ (◆). Pretreatment was performed 15 min before treatment. $^{*}P<0.01$; $^{**}P<0.001$ versus saline controls. $^{\wedge}P<0.05$; $^{\wedge\wedge}P<0.01$; $^{\wedge\wedge\wedge}P<0.001$ versus metoprine (20 mg $kg^{-1}$)-treated mice. Each point represents the mean (with s.e.mean) of 11–24 mice.

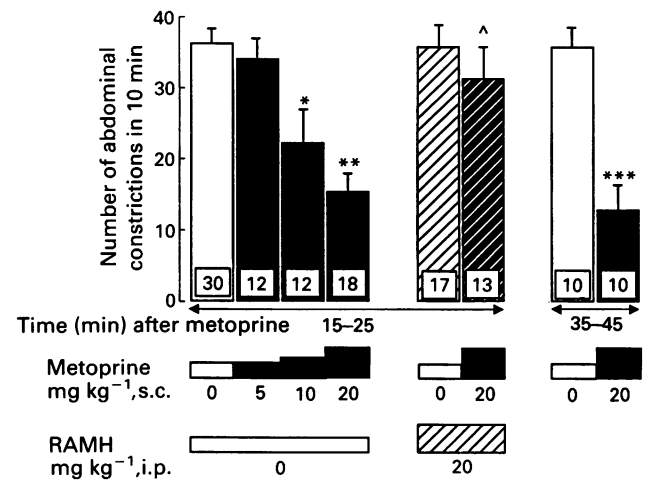


**Figure 8** Metoprine antinociception and its antagonism by (R)-α-methylhistamine (RAMH) in the mouse abdominal constriction test. RAMH dihydrogenomaleate was administered 15 min before metoprine. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ versus controls. $^{\wedge}P<0.01$ versus metoprine (20 mg $kg^{-1}$)-treated mice. Inside the columns is shown the number of mice.

The same dose-dependent antinociceptive effect was observed in the mouse abdominal constriction test. The number of stretching movements was significantly reduced with 10 and 20 mg $kg^{-1}$, i.p. 15–25 min after treatment with an $ED_{50}$ of 15.3 (10.3–22.8) mg $kg^{-1}$, i.p. and 20 min later the effect for the dose 20 mg $kg^{-1}$ was even more significant (Figure 8). In the rat paw pressure test, metoprine was studied after administration by both i.p. and i.c.v. route (Figure 9). Systemically the doses of 10 and 20 mg $kg^{-1}$ were used; both were significantly antinociceptive and at the highest dose the effect lasted up to 6 h. For i.c.v. administration in rats with permanent cannulae, the doses of 50 and 100 μg per rat were also observed to produce a dose-dependent, long-lasting (6 h) antinociception.

### *Metoprine effect in the rota-rod test*

Metoprine at 20 mg $kg^{-1}$ did not cause any impairment to performance 15 min after i.p. administration. The endurance time on the rotating rod was 282 ± 17 s before and 285 ± 14 s after treatment for control group, and 291 ± 9 s and 297 ± 3 s respectively for metoprine-treated mice (10 animals per group).

### *Antagonism by (R)-α-methylhistamine (RAMH) of metoprine-induced antinociception*

RAMH (20 mg $kg^{-1}$, i.p.) was able to prevent the antinociception induced by 20 mg $kg^{-1}$, i.p. of metoprine administered 15 min later in all three tests (Figures 7b, 8, 9a).

In both the rat paw pressure and mouse hot plate tests antinociception was significantly reduced as early as 15 min

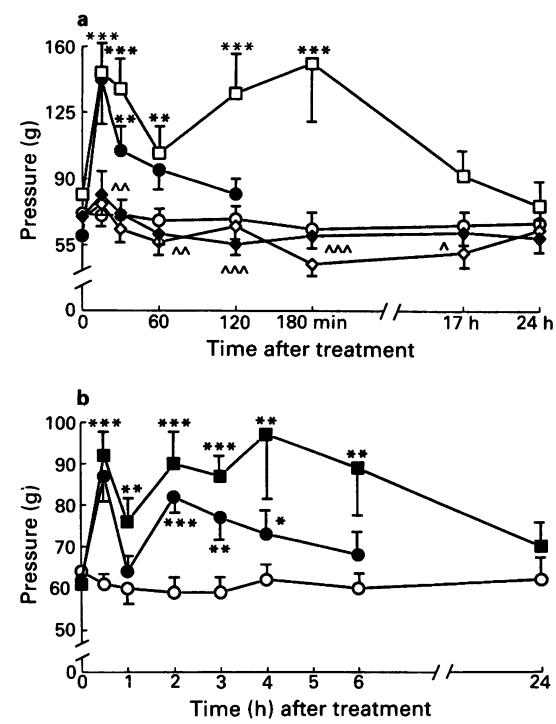


**Figure 9** Antinociceptive effect of metoprine in the rat paw pressure test. (a) Effect of i.p. metoprine and its antagonism by (R)-α-methylhistamine (RAMH). Groups (pretreatment i.p. + treatment i.p.) were as follows: saline 0.1 ml 10 $g^{-1}$ + lactic acid 0.3% in saline (○); metoprine 10 mg $kg^{-1}$ (●); saline + metoprine 20 mg $kg^{-1}$ (□); RAMH dihydrogenomaleate 20 mg $kg^{-1}$ + lactic acid 0.3% (◇); RAMH + metoprine 20 mg $kg^{-1}$ (◆). Pretreatment was performed 15 min before treatment. Each point represents the mean of 11–19 rats. (b) Effect of i.c.v. metoprine on rats with permanent cannulae. Groups were (dose per rat i.c.v.): lactic acid 1% in saline 5 μl (○); metoprine 50 μg (●); metoprine 100 μg (■). Each point represents the mean of 7–11 rats. $^{*}P<0.05$; $^{**}P<0.01$; $^{***}P<0.001$ versus control group. $^{\wedge}P<0.05$; $^{\wedge\wedge}P<0.01$; $^{\wedge\wedge\wedge}P<0.001$ versus metoprine (20 mg $kg^{-1}$)-treated rats. Vertical lines give s.e.mean.

**Table 3** Effect of i.c.v. 1-methylhistamine (MH) on pain threshold in the rat paw-pressure and mouse abdominal constriction tests

| *Treatment* (μg per animal) | *Rat paw pressure test* *Pressure* (g) *Pretest* | *30 min* after treatment | *45 min* after treatment | *60 min* after treatment | *Abdom. constr. test* *Constrictions* *in 10 min* |
|---|---|---|---|---|---|
| Saline | 59.1 ± 2.0 (6) | 58.3 ± 3.0 | 56.6 ± 3.3 | 58.3 ± 3.0 | 28.6 ± 3.3 (19) |
| MH 0.5 | 61.6 ± 3.5 (9) | 61.1 ± 3.0 | 62.2 ± 2.2 | 52.2 ± 2.7 | – |
| MH 1 | – | – | – | – | 35.5 ± 5.5 (6) |
| MH 5 | – | – | – | – | 34.8 ± 4.8 (6) |
| MH 10 | 62.2 ± 3.6 (9) | 63.3 ± 3.3 | 58.8 ± 3.8 | 58.8 ± 2.0 | 32.7 ± 1.5 (7) |
| MH 50 | – | – | – | – | 27.0 ± 3.5 (7) |

In parentheses is shown the number of animals

after metoprine treatment, and from 30 min on the antagonism was complete.

The dose of RAMH used did not modify the nociceptive threshold in any of the three tests.

### *Lack of 1-methylhistamine effect in antinociception*

1-Methylhistamine (MH) did not modify the pain threshold in either the mouse abdominal constriction test or the rat paw pressure test (Table 3).

In the abdominal constriction test, MH was studied at a wide range of doses (1–50 μg per mouse, i.c.v.). None of them reduced the number of constrictions induced by acetic acid.

In the paw pressure test also the two doses used (0.5 and 10 μg per rat i.c.v.) were ineffective in modifying the threshold pressure.

## Discussion

The present results clearly show that histamine can be either antinociceptive or hyperalgesic depending on the dose. The apparently contradictory effects of this drug might be due to its dose-related action either on the presynaptic receptor or on a postsynaptic receptor, since the selective $H_3$ receptor antagonist thioperamide caused antinociception, and the selective $H_3$ receptor agonist, RAMH caused hyperalgesia.

Owing to the reportedly poor ability of histamine to cross the blood-brain barrier (Snyder *et al.*, 1964; Schwartz *et al.*, 1971a), the i.c.v. route of administration was always used. Histamine-induced antinociception, probably due to an action on postsynaptic $H_1$ or $H_2$ receptors, was observed in all three tests, showing a dose-dependent relationship. So far, our results confirm previous observations by Glick & Crane (1978), Bhattacharya & Parmar (1985) and Parolaro *et al.* (1989) on rats and Chung *et al.* (1984) and Oluyomi & Hart (1991) on mice. The paw pressure and abdominal constriction seem to be more sensitive tests than the hot plate for detecting histamine antinociceptive effects, as can be noticed from $ED_X$ values. In fact, for the hot plate test, the same cut-off time of 45 s was maintained in order to allow comparisons with the other substances used, but an $ED_{30}$ rather than $ED_{50}$ value needed to be calculated, due to the low antinociceptive effect elicited by histamine. Such discrepancies might be due to the different kinds of noxious stimulus used, i.e. mechanical, chemical and thermal. However, a similar differential sensitivity to analgesic tests is also shared by morphine, some κ-agonists and eseroline (Tyers, 1980; Bartolini *et al.*, 1981b; Yaksh & Noueihed, 1985).

The high reaction scores measured in the three analgesic tests are likely to be due to a real antinociception and not to the loss of ability to react to noxious stimuli, since motor coordination assessed with the rota-rod test was unaffected after the administration of 50 μg per mouse of histamine. The latter dose was preferred to the highest one tested for the rota-rod experiment, since during the hot plate experiments some mice treated with 100 μg i.c.v. developed facial convulsions when held by the tail. Moreover, also Glick & Crane (1978) described catalepsy, besides antinociception, following i.c.v. administration of 200–400 μg per rat of histamine. Further support to histamine acting as a modulator of nociception is given by the experiments of Braga *et al.* (1992). These authors demonstrated that i.c.v. injection of histamine in arthritic rats at the same doses which were antinociceptive in our experiments in the paw pressure test, inhibited the firing of nociceptive thalamic neurones after a noxious stimulus.

Conversely, low doses of histamine were hyperalgesic in the mouse hot plate and rat paw pressure tests. Doses ranging from 50 to 500 fold lower than the analgesic dose in the hot plate and 10 fold lower in the paw pressure test induced a significant decrease in nociceptive threshold (Figures 1a and 2). The dose of 0.5 μg per rat was significantly effective both 30 and 45 min after treatment in the paw pressure test on rats into which histamine was injected during short ether anaesthesia, and the same was adopted for experiments on rats with permanent i.c.v. cannulae, to confirm the data in the absence of ether. This hyperalgesic effect might not seem contradictory when one takes into account the fact that $H_3$ receptors are 100 fold more sensitive to histamine than the postsynaptic receptors in rat brain slice preparations (Arrang *et al.*, 1983). Thus, hyperalgesia induced by low doses of histamine might be due to a preferential action on $H_3$ receptors, and, consequently, to inhibition of its own release.

On the other hand, in the mouse abdominal constriction test, histamine-induced hyperalgesia was not detected, as is the case with many other drugs such as arecaidine propargyl ester and CGP 35348, which are hyperalgesic in many tests but inactive in the mouse abdominal constriction test (Bartolini *et al.*, 1992; Malcangio *et al.*, 1991). One reason for this lack might be the abdominal constriction test itself. In fact, as reported by Itoh *et al.* (1989), histamine turnover is enhanced after i.p. injection of 0.7% acetic acid. Thus, if histamine release is enhanced during the abdominal constriction test, the putative hyperalgesic effect of low doses of exogenous histamine is overcome by the augmented amount of endogenous histamine in the brain.

Subsequently, in order to verify our hypothesis that low doses of histamine act at the presynaptic receptor, we studied the effects of both an antagonist and an agonist of the $H_3$ receptor. Thioperamide, the selective histamine $H_3$ receptor

antagonist, elicited antinociception in all three tests. It has been reported that parenteral administrations of thioperamide are able to increase histamine turnover (Arrang *et al.*, 1987a; Garbarg *et al.*, 1989; Oishi *et al.*, 1989; Taylor *et al.*, 1992) or release (Itoh *et al.*, 1991) in brain tissues, indicating that it crosses the blood-brain barrier. Thus, our first approach was to study thioperamide effects following parenteral administration. Subsequently, in order to verify whether the site of action of the antinociceptive effect observed with parenteral administration was in the CNS we used the i.c.v. route. Furthermore, for this route we used thioperamide doses (1.1 μg per mouse or rat) which corresponded on a molar basis to a hyperalgesic dose of histamine (0.5 μg per mouse or rat). Despite the lack of any statistically-significant effects of i.c.v.-administered thioperamide in the hot plate test, antinociception was evident in both abdominal constriction and paw pressure tests for both administration routes. This is in agreement with the reported action of thioperamide as a histamine $H_3$ receptor antagonist, and the antinociception observed might well be due to blockade of the $H_3$ receptor and, consequently, to increased endogenous histamine release. This mechanism of action is not surprising if one bears in mind that two postsynaptic antagonists of the opioid and cholinergic systems naloxone and atropine, have also been demonstrated to induce antinociception when administered at low doses which are ineffective on the postsynaptic site, but which are capable of blocking the respective presynaptic receptors (Levine *et al.*, 1979; Ghelardini *et al.*, 1990).

In the abdominal constriction and hot plate tests it was also observed that the antinociceptive effect diminished with increasing doses of thioperamide. Since 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), the hypothesis of a postsynaptic antagonism can be ruled out. Instead, competition on the $H_3$ receptor between thioperamide and the endogenous histamine released might be postulated. When the dose of thioperamide is sufficiently strong, the amount of histamine released is high enough to compete with thioperamide, thus activating the mechanism of negative feedback on the release and, consequently, antagonizing thioperamide antinociceptive effect. This hypothesis might also explain the low maximum effect of thioperamide observed by us.

As for the histamine $H_3$ agonist, RAMH, hyperalgesia was observed at 3 μg per mouse or 1 μg per rat i.c.v. as soon as 15 min after treatment. In order to assess whether a quick hyperalgesia is also obtainable following systemic administration, a dose as high as 100 mg kg$^{-1}$ was used in the hot plate test, since 20 mg kg$^{-1}$, i.p. had no effect within 1 h of treatment. Such a rapid effect is probably due to inhibition of histamine release. Conversely, the delayed hyperalgesia observed in the rat paw pressure test following the injection of 20 mg kg$^{-1}$ i.p. (from 4 to 7 h after treatment) might be due to histamine synthesis inhibition. Garbarg *et al.* (1989) in fact reported an increase in synaptosomal histamine and a decrease in $N^{\tau}$-methylhistamine levels which reached statistical significance 3 h after oral administration of 10 mg kg$^{-1}$ of RAMH (expressed as the base), and a reduction in cortical [$^3$H]-histamine synthesis which was already significant 30 min after treatment. All these effects lasted up to 6 h. Thus, it might be postulated that the hyperalgesia observed in our conditions is due to a dose-dependent effect of RAMH on endogenous histamine release. Moreover, hyperalgesia induced by stimulation of autoreceptors is not unusual: another example is in the cholinergic system, where the presynaptic agonist, arecaidine is able to lower the nociceptive threshold in both hot plate and paw pressure tests (Bartolini *et al.*, 1992).

Pretreatment with an i.p. dose of RAMH which did not modify the pain threshold in mice completely prevented the antinociception induced by 20 mg kg$^{-1}$ of thioperamide in both abdominal constriction and hot plate tests. This antagonism might reflect the action of both substances on the same receptor, since for both of them a high selectivity on the histamine $H_3$ receptor has been reported (Arrang *et al.*, 1987b; Garbarg *et al.*, 1989). In the rat paw pressure test a similar antagonism was also observed following i.c.v. administration. Although 1 μg per rat of RAMH had an hyperalgesic effect, the antagonism is probably not due to a simple summing of the effects of the two substances, since the values obtained with double treatment are much lower than those resulting from the subtraction of the hyperalgesic effect of RAMH from the analgesic effect of thioperamide. On the other hand, the dose of 10 μg per rat of thioperamide was preferred as eliciting a clearer and longer-lasting antinociception, while for RAMH the dose of 1 μg per rat was chosen as about a ten fold lower dose (on a molar basis) than the thioperamide one. The choice was made taking into account that for RAMH and thioperamide a 1:8 affinity ratio has been reported for the $H_3$ receptor (Taylor *et al.*, 1992). Conversely, when i.p. or s.c. administration was performed (i.e. in experiments on mice), two equal doses were adopted for the two substances, since, despite its higher affinity for the $H_3$ receptor, RAMH seems to have even more difficulty in crossing the blood-brain barrier than thioperamide (Taylor *et al.*, 1992).

The histaminergic system in antinociception was further studied by altering histamine brain levels. The choice of histidine as a tool for studying the role of endogenous histamine in antinociception was made on the basis that, as Schwartz *et al.* (1972) and Abou *et al.* (1973) demonstrated, its decarboxylating enzyme is not saturated in normal conditions. Systemic administrations of L-histidine are therefore able to enhance brain histamine levels.

In our experiments on rats, L-histidine induced antinociception with both doses used, with a maximum effect at 2–3 h after treatment. This time-course reflects that of endogenous brain histamine levels observed in rats by Schwartz *et al.* (1972) and Sheiner *et al.* (1985) after i.p. administration of L-histidine at the same doses: brain histidine levels were maximal within 1 h of a loading of 500 mg kg$^{-1}$, i.p., halved at 1.5 h and constantly decreased thereafter, while histamine levels reached peak values within 2–3 h of injection. This seems to support the hypothesis that antinociception is due to histamine deriving from the conversion of L-histidine, and not to histidine itself.

In mice, too, antinociception takes place slowly after L-histidine administration. In the abdominal constriction test the maximal inhibition of stretching movements was observed 2 h after treatment, while in the hot plate test antinociception reached a plateau from 1 to 2.5 h for the highest dose; in both tests the effect diminished 3 h after histidine injection. As seems also to be the case with rats, the antinociception in mice occurs parallel to, though later than, the alterations in brain histamine levels observed after a single (850 and 1000 mg kg$^{-1}$) or multiple (3 × 1000 mg kg$^{-1}$) i.p. L-histidine injection (Taylor & Sneider, 1972; Cosentin *et al.*, 1974).

With L-histidine as with histamine, the mouse hot plate is less sensitive than the mouse abdominal constriction and rat paw pressure tests. In fact, in the hot plate test, statistically-significant antinociception was found at the doses of 500 and 1500 mg kg$^{-1}$, i.p., although the effect was very small. The latter dose might seem to be high, but it should be considered that another amino acid, L-DOPA, also needs to be administered at a dose of 1000 mg kg$^{-1}$ in order to show any effect (Blaschko & Chrusciel, 1960). Nevertheless, even at the highest dose of L-histidine, the gross behaviour of animals was not modified during our experiments, as formerly reported by Schwartz *et al.* (1972) for rats and by Abou *et al.* (1973) for rabbits.

Further evidence of the fact that L-histidine-induced antinociception is due to its conversion into histamine is given by our results obtained with FMH. This substance, developed by Kollonitsch *et al.* (1978) as a highly selective irreversible

HDC inhibitor, has been demonstraed to halve brain histamine levels in mice within 1 h, up to 24 h (Garbarg *et al.*, 1980; Maeyama *et al.*, 1982; 1983), and in rats (Oishi *et al.*, 1984). The brain histamine levels which are reduced by FMH are likely to be those of neural cells, since in mutant mice devoid of mast cells brain histamine is almost totally abolished by FMH (Maeyama *et al.*, 1983). Thus, total prevention by FMH of L-histidine-induced antinociception at all the times considered was probably due to blockade of neuronal HDC activity.

The present findings partly contradict those of Oluyomi & Hart (1991), who observed histidine-induced antinociception only in the mouse hot plate test. Moreover, this effect was already present 15 min after histidine administration (400 mg $kg^{-1}$, i.p.). The authors attributed the lack of effect in the mouse abdominal constriction test to a lack of action on peripheral histamine receptors.

Our further approach to the study of endogenous histamine effects in antinociception was to raise brain histamine levels by inhibiting its catabolism. Since brain histamine catabolism occurs almost exclusively via ring methylation (Schwartz *et al.*, 1971b; Schayer & Reilly, 1973), we used for this purpose metoprine, an antifolate which has been described as a highly potent, competitive HMT inhibitor devoid of any action on HDC (Duch *et al.*, 1978) and which is able to enhance histamine release in *in vivo* microdialysis studies following i.p. administration (Itoh *et al.*, 1991).

Due to its high liposolubility (Duch *et al.*, 1978), in the present experiments metoprine was usually administered i.p. in mice and rats to obtain a more general distribution of the substance within the central nervous system. However, in some experiments with the paw pressure test, metoprine was also given i.c.v. through permanent cannulae, in order to make sure that its site of action was in fact the CNS. In both cases and species the dose-dependence of the antinociceptive action of metoprine seems to reflect the degree of inhibition of brain HMT activity. Hough *et al.* (1986) reported that 5 mg $kg^{-1}$, i.p. of metoprine caused a reduction of 70% in whole brain HMT activity, while a 90% reduction can be obtained with 20–30 mg $kg^{-1}$. The present data seem to suggest that a 70% reduction is not sufficient to induce antinociception, since at least 10 mg $kg^{-1}$ were found to be necessary to obtain a statistically significant rise in the nociceptive threshold (abdominal constriction test). A single dose (20 mg $kg^{-1}$, i.p.) was found to induce an antinociception whose potency was comparable to that induced by morphine (9 mg $kg^{-1}$, s.c. in the mouse hot plate test and 5 mg $kg^{-1}$, s.c. in the rat paw pressure test 15 and 30 min after treatment respectively). At the same dose metoprine-treated mice were able to stay balanced on the rotating rod, while morphine given at doses higher than 8.2 mg $kg^{-1}$, s.c. significantly impaired animal performance (data not shown).

Furthermore, metoprine antinociception was also longer-lasting than that of morphine; this is consistent with the reported metoprine half-life of 17 h (Duch *et al.*, 1978). In fact, a long-lasting antinociceptive effect is also shared by other neurotransmitter catabolism inhibitors, such as the enkephalinase inhibitors, thiorphan (Greenberg & O'Keefe, 1982), D-phenylalanine (Ehrenpreis *et al.*, 1983), azidothiorphan (Beaumont *et al.*, 1987) and SCH 34826 (Chipkin *et al.*, 1988), the GABA transaminase inhibitors amino-oxyacetic acid (Bartolini *et al.*, 1981a; Sawynok & LaBella, 1982) γ-acetylenic GABA and γ-vinyl GABA (Buckett, 1980; Sawynok & LaBella, 1982), and the cholinesterase inhibitor diisopropylfluorophosphate (DFP) (Costa & Murphy, 1986; Green & Kitchen, 1986).

To assess whether metoprine-induced antinociception was due to the blockade of HMT, animals were pretreated with RAMH. The complete antagonism observed is consistent with the results of Oishi *et al.* (1989), who reported a decrease in the levels of the catabolism product 1 h after RAMH treatment. Thus, since inhibition of histamine release by RAMH prevents metoprine-induced antinociception, it can be argued that the metoprine antinociceptive effect might be due to endogenous histamine which has not been catabolized.

The effect of MH was studied in the two tests most sensitive to histamine, the rat paw pressure and mouse abdominal constriction tests, at the same doses at which histamine was antinociceptive. As expected, the catabolism product was ineffective. Schwartz *et al.* (1971b) reported that 500 μg per rat intracisternally is able to inhibit HMT, but in their experiments the doses used were at least 10 fold higher than in ours.

In conclusion, our data taken together indicate that endogenous histamine is an antinociceptive neurotransmitter. All the substances used in the present work had an effect which was consistent with their reported ability to modulate endogenous histamine release or alter brain histamine levels. Thus when histamine brain levels or release are increased, antinociception can be observed, conversely, when the amount of histamine released is decreased, hyperalgesia occurs. It thus seems likely that the histaminergic system, like many other neuronal systems, plays an important role in the modulation of central perception of nociceptive stimuli.

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