Psychopharmacology (1999) 142 : 215–220

ORIGINAL INVESTIGATION

Carmen Pérez-García · Lydia Morales
M. Victoria Cano · Isabel Sancho
Luis F. Alguacil

Effects of histamine H_3 receptor ligands in experimental models of anxiety and depression

Received: 1 July 1998/Final version: 8 September 1998

Abstract Histamine H_3 receptor ligands have been proposed to be of potential therapeutic interest for the treatment of different central nervous system disorders; however, the psychopharmacological properties of these drugs have not been studied extensively. In this work, we investigated the possible involvement of histamine H_3 receptor function in experimental models of anxiety (elevated plus-maze) and depression (forced swimming test). Male Sprague-Dawley rats were treated IP with the histamine H_3 receptor agonist *R*-α-methylhistamine (10 mg/kg) or the histamine H_3 receptor antagonist thioperamide (0.2, 2 and 10 mg/kg) and 30 min afterwards the time spent in the open arms of an elevated plus-maze was registered for 5 min. The immobility time of male OF1 mice in the forced swimming test was recorded for 6 min, 1 h after the IP administration of *R*-α-methylhistamine (10 and 20 mg/kg), thioperamide (0.2, 2, 10 and 20 mg/kg) or another histamine H_3 receptor antagonist, clobenpropit (5 mg/kg). The locomotor activity of mice was checked in parallel by means of an activity meter. Both saline controls and active drug controls were used in all the paradigms. Neither thioperamide nor *R*-α-methylhistamine significantly changed animal behaviour in the elevated plus-maze. *R*-α-methylhistamine and the higher dose of thioperamide assayed (20 mg/kg) were also inactive in the forced swimming test. By contrast, thioperamide (0.2–10 mg/kg) dose-dependently decreased immobility, the effect being significant at 10 mg/kg (33% reduction of immobility); clobenpropit produced an effect qualitatively similar (24% reduction of immobility). None of these histamine H_3 receptor antagonists affected locomotor activity. These preliminary results suggest that the histamine H_3 receptor blockade could be devoid of anxiolytic potential but have antidepressant effects. Besides, the stimulation of these receptors does not seem to be followed by changes in the behavioural parameters studied.

Key words Histamine H_3 receptor · *R*-α-methylhistamine · Thioperamide · Clobenpropit · Anxiety · Depression

C. Pérez-García · L. Morales · M.V. Cano · I. Sancho
L.F. Alguacil (✉)
Laboratory of Pharmacology,
University of San Pablo CEU, P.O. Box 67,
E-28660 Boadilla, Madrid, Spain
e-mail: laguacil@ceu.es, Fax: +34-1-3510475

Introduction

The role of histamine as a cellular mediator has been known for a long time, whereas the knowledge of its role as a neurotransmitter is more recent (Schwartz et al. 1991). In addition to the classical histamine receptors termed H_1 and H_2, Arrang et al. (1983) proposed a third receptor subtype that was initially described as a presynaptically located autoreceptor. Thus, activation of the histamine H_3 receptor led to inhibition of histamine synthesis and decreased release of histamine from neuronal synaptic vesicles (Garbarg et al. 1989). Specific agonists (i.e. *R*-α-methylhistamine) and antagonists, such as thioperamide, were available soon after the description of this new receptor (Arrang et al. 1987).

Histamine H_3 receptors were also shown to behave as heteroreceptors, modulating the release of several neurotransmitters such as noradrenaline (Schlicker et al. 1994), acetylcholine (Vollinga et al. 1992), serotonin (Schlicker et al. 1988), dopamine (Schlicker et al. 1993) and neuropeptides from nonadrenergic noncholinergic nerves (Burgaud et al. 1993). Taking into account the multiple roles of those neurotransmitters and the presence of histamine H_3 receptors in several areas of the central nervous system (CNS), such as the ventral striatum, substantia nigra and hypothalamus (Ligneau et al. 1994), the use of histamine H_3

receptor ligands could be expected to have therapeutic potential in the treatment of different CNS disorders. At present, however, neither the psychopharmacology of histamine H_3 receptors nor the therapeutic value of their ligands is clear enough. The aim of this work is to study these questions further by investigating the possible involvement of histamine H_3 receptor function in experimental models of anxiety and depression.

Histamine H_3 receptors have been thought to play a role in sleeping (Monti et al. 1993) and convulsions (Yokoyama et al. 1994). These processes are also modulated by anxiolytic compounds of the benzodiazepine type; accordingly, it would be advisable to check the effect of histamine H_3 receptor ligands in models of anxiety such as animal behaviour in the elevated plus maze.

It has been suggested that the central histaminergic system may be involved in the effects of antidepressants (Schwartz et al. 1981). Furthermore, histamine-induced catalepsy has been proposed as a model for the evaluation of antidepressant drugs (Onodera et al. 1991). The effect of some antidepressants could be mainly related to modifications of histaminergic mechanisms. Thus, levoprotyline-induced decrease of immobility time in the forced swimming test has been directly related to its action as a histamine H_1 receptor antagonist (Noguchi et al. 1992). The possible involvement of histamine H_3 receptor function in stress and depression has been previously studied by Ghi et al. (1995a,b), who showed that the tricyclic antidepressant amitriptyline counteracted the stress-induced decrease of histamine H_3 receptor density in rat brain cortex and increased the number of receptors in non-stressed animals. Lamberti et al. (1998) have recently found that thioperamide exhibited an antidepressant-like activity in the mouse forced swimming test, but their results could not rule out a possible contribution of increased locomotor activity to the effect observed (a common cause of false positive results in this test); moreover, a possible action of thioperamide on other pharmacological targets such as 5-HT_3 receptors (Leurs et al. 1995) could be involved. We have tried to verify the possible effect of the histamine H_3 receptor blockade on mouse forced swimming by running parallel experiments with thioperamide in a locomotor activity paradigm. Furthermore, the effect of another histamine H_3 receptor antagonist, clobenpropit, has been studied in the same experimental conditions. Preliminary results were presented at the VII Meeting of the Spanish Society of Neuroscience (Pérez-García et al. 1997).

Materials and methods

Elevated plus-maze test

Animals

Male Sprague-Dawley rats (180–220 g; San Pablo-CEU University bred) were housed under a 12:12 dark/light cycle (lights on at 8:00 a.m.), at an ambient temperature of 22 ± 1°C and fed with standard diet. Two hours before the test the animals were moved to the experimental room where they had free access to food and water.

Drugs

Diazepam (2 mg/kg; Valium®, Roche, Spain), *R*-α-methylhistamine dihydrochloride (10 mg/kg; RBI, USA) and thioperamide maleate (0.2, 2 and 10 mg/kg; RBI, USA) were dissolved in physiological saline and injected IP in a volume of 10 ml/kg, 30 min before the test. Control injection consisted of an equivalent volume of saline.

Apparatus and procedure

The elevated plus maze was made of black Plexiglas and consisted of two opposing open arms (50 × 10 cm^2) and two enclosed arms (50 × 10 × 40 cm^3) united by a central platform (10 × 10 cm^2) and elevated to a height of 60 cm above the floor level. The experimental room was lit by red fluorescent lamps.

For behavioural testing, the rats were placed individually on the central platform facing a closed arm and allowed 5 min of free exploration. The behaviour was recorded by a videocamera linked to a videotracking system (San Diego Instruments, USA) which automatically quantified the time spent in the open arms. The tests were always run between 1400 and 1900 hours.

Forced swimming test

Animals

Male OF1 mice (IFFA-CREDO) weighing 25–30 g were used. Animals were kept at 22 ± 1°C, with commercial diet and tap water ad libitum and under a 12:12 dark/light cycle.

Drugs

Imipramine (10 and 15 mg/kg; Sigma, Spain), *R*-α-methylhistamine dihydrochloride (10 and 20 mg/kg; RBI, USA), thioperamide maleate (0.2, 2, 10 and 20 mg/kg; RBI, USA) and clobenpropit dihydrobromide (5 mg/kg; RBI, USA) were dissolved in physiological saline and injected in a volume of 10 ml/kg, 60 min prior to the test. Control animals received an equivalent volume of saline.

Procedure

Animals were individually introduced for 6 min in a glass cylinder (11 cm in diameter and 14 cm in height), which was filled to a depth of 7.5 cm with water maintained at 25°C. The immobility time of each animal was measured during the last 4 min.

Locomotor activity

Mice (as used in the forced swimming test) were placed in individual cages (25 × 25 × 14 cm^3) and allowed to habituate themselves to the novel environment for 30 min. The animals were then treated (IP 10 ml/kg) with saline, thioperamide maleate (10 mg/kg), clobenpropit dihydrobromide (5 mg/kg) or amphetamine sulphate (3 mg/kg) and immediately returned to the cages. The locomotor activity of the animals was continuously recorded for 90 min by a

Table 1 Effect of thioperamide and *R*-α-methylhistamine on the time spent in the open arms of the elevated plus maze (*$P < 0.05$ with respect to saline)

Treatment	*n*	Time spent in open arms (s) mean ± SEM	% Change with respect to saline
Saline	32	32.55 ± 4.50	–
Diazepam 2 mg/kg	10	96.79 ± 35.95*	197.30
R-methylhistamine 10 mg/kg	12	61.04 ± 14.52	87.53
Thioperamide 0.2 mg/kg	7	55.48 ± 19.59	70.44
Thioperamide 2 mg/kg	11	18.07 ± 8.85	−44.48
Thioperamide 10 mg/kg	8	35.74 ± 6.39	9.8

Table 2 Effect of thioperamide and *R*-α-methylhistamine on the immobility time in the forced swimming test (*$P < 0.05$ with respect to saline)

Treatment	*n*	Immobility time (s) mean ± SEM
Trial 1		
Saline	11	150.10 ± 10.22
Imipramine 15 mg/kg	11	89.36 ± 8.36*
Thioperamide 10 mg/kg	11	100.91 ± 9.78*
Trial 2		
Saline	21	166.24 ± 6.48
Imipramine 10 mg/kg	11	130.54 ± 12.68*
Thioperamide 0.2 mg/kg	10	160.00 ± 9.90
Thioperamide 2 mg/kg	10	142.30 ± 29.78
Trial 3		
Saline	26	166.27 ± 9.68
Thioperamide 20 mg/kg	10	144.70 ± 13.78
R-α-methylhistamine 10 mg/kg	10	159.80 ± 12.18
R-α-methylhistamine 20 mg/kg	17	180.53 ± 12.15

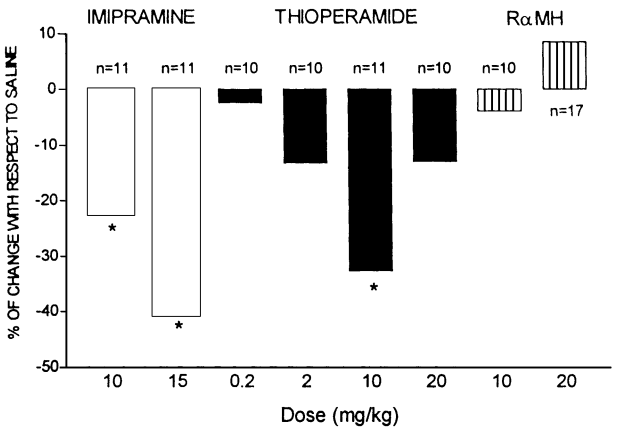

Fig. 1 Forced swimming test. Effect of thioperamide (0.2, 2, 10 and 20 mg/kg) and *R*-α-methylhistamine (*RαMH*; 10 and 20 mg/kg) on the immobility time. Imipramine (10 and 15 mg/kg) was used as a positive control (*$P < 0.05$ with respect to saline)

magnetic activity meter (Panlab, Spain) placed at the bottom of the cages. This device transformed locomotion-induced changes of magnetic field into arbitrary activity counts.

Statistics

Results of experiments were analysed by one-way analysis of variance (ANOVA) followed by Newman Keuls post-hoc test. Significance was considered at the 0.05 level.

Results

Results of the elevated plus maze test are given in Table 1. Intraperitoneal administration of diazepam (2 mg/kg) resulted in a significant increase in the time spent in the open arms, while neither thioperamide nor *R*-α-methylhistamine significantly changed this variable.

In the forced swimming test, thioperamide dose-dependently decreased the immobility time. The reduction was significant at 10 mg/kg and intermediate between that of the two doses of imipramine assayed. A higher dose of thioperamide (20 mg/kg) elicited a lower, non-significant reduction of immobility. The behaviour of mice treated with *R*-α-methylhistamine was similar to that of control mice, resulting in the lack of significant differences concerning immobility times in these cases. The results of three independent trials are shown in Table 2 and are globally represented in Fig. 1.

In the activity meter, locomotor activity was significantly increased by amphetamine 60 and 90 min after administration and was not modified by thioperamide at any time (Fig. 2A). Figure 2B shows the locomotor activity exhibited by mice from 60 to 66 min postinjection, which coincides with the time interval used in the forced swimming test; as above, thioperamide failed to affect locomotor activity in this interval.

Clobenpropit was also tested for its effects on swimming behaviour and locomotion. As in the case of thioperamide, this drug decreased immobility in the forced swimming test but did not significantly modify animal performance in the activity meter (Table 3, Fig. 3).

Discussion

Results from the elevated plus maze show that, at the doses tested, neither *R*-α-methylhistamine nor thioperamide affected the time that animals spent on the open arms. Since this variable is increased by drugs with anxiolytic activity, these results allow us to suggest that neither the stimulation nor the blockade of

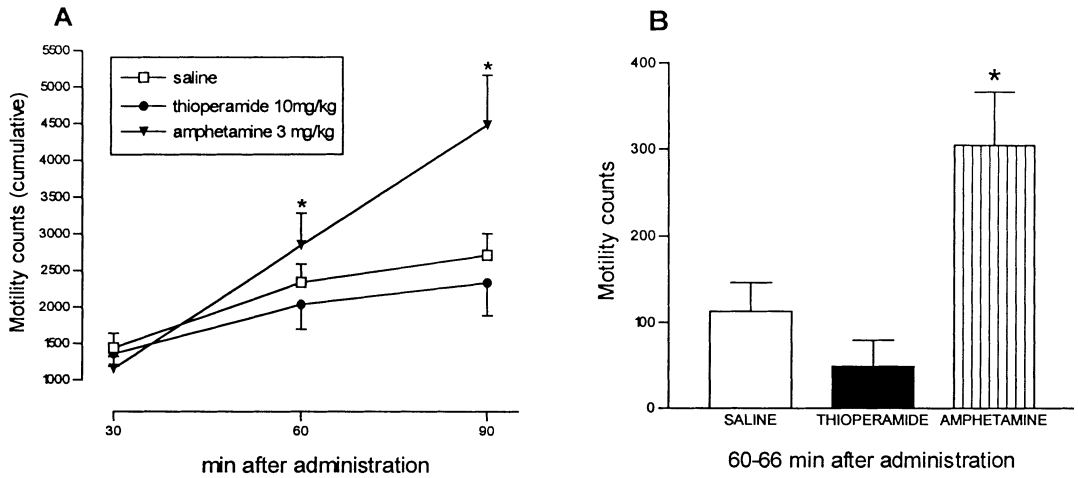

Fig. 2A, B Effect of thioperamide (10 mg/kg) and amphetamine (3 mg/kg) on the locomotor activity measured in an activity meter. Results are means ± SEM ($n = 11$) (*$P < 0.05$ with respect to saline)

Table 3 Effect of clobenpropit on the immobility time in the forced swimming test (*$P < 0.05$ with respect to saline)

Treatment	*n*	Immobility time (s) mean ± SEM	% Of change with respect to saline
Saline	12	160.30 ± 7.73	–
Imipramine 15 mg/kg	12	88.33 ± 11.69*	−44.90
Clobenpropit 5 mg/kg	12	121.00 ± 12.11*	−24.52

histamine H_3 receptors elicits a change in the level of anxiety. However, the predictive value of the elevated plus maze has some limitations, i.e. it fails to detect the anxiolytic effects of partial 5-HT_{1A} agonists such as buspirone (Dawson and Tricklenbank 1995). Therefore, it would be advisable to test *R*-α-methylhistamine and thioperamide in more robust models of anxiety.

The results obtained in the forced swimming test increase previous evidence of a possible antidepressant effect of thioperamide (Pérez-García et al. 1997; Lamberti et al. 1998). Blockade of histamine H_3 receptors seems directly involved, since clobenpropit, another histamine H_3 receptor antagonist, is also active in the test. The administration of thioperamide reduced the immobility time in a dose-dependent manner, significant at 10 mg/kg. The results from the activity meter tend to discard hyperactivity as the mechanism responsible for the effect obtained. This variable was not considered by Lamberti et al. (1998) in their studies, in spite of the fact that psychostimulation can lead to false positive results in the forced swimming test (Borsini and Meli 1988). In fact, these authors combined the forced swimming test with the study of rotarod performance, which detects changes of motor coordination but not necessarily stimulation. Previous findings from other authors also showed that thioperamide (10 mg/kg) does not increase locomotor activity in mice: by using a dose range from 0.2 to 10 mg/kg, Clapham and Kilkpatrick (1994) showed that the histamine H_3 receptor antagonist did not increase motor activity and even counteracted stimulant-induced hyperactivity. Thioperamide was only found to increase locomotor activity in mast-deficient mice at higher doses (20 mg/kg) (Sakai et al. 1991), which were devoid of significant effects in the forced swimming test.

The finding that thioperamide produces no significant effects in the forced swimming test at 20 mg/kg while being active at lower doses is consistent with the results of Lamberti et al. (1998) in this test, and also with other data from the literature. Chiechio et al. (1997) reported that thioperamide at 10 mg/kg provokes hyperalgesia and inhibits clonidine analgesia in tail-flick and hot-plate tests; at 15 mg/kg, it is less active on both parameters and at 25 mg/kg, it is analgesic and enhances the effect of clonidine. Furthermore, in the study of Sakai et al. (1991), low doses of thioperamide were found to increase locomotor activity of mast-deficient mice, whilst higher doses reduced locomotor activity and impaired motor coordination. It is possible that high doses of thioperamide could produce pharmacological effects independent of the histamine H_3 receptor blockade which could interfere with the more specific actions, i.e. 5-HT_3 antagonism (Leurs et al. 1995). However, a similar biphasic effect has been reported when the action of the H_3 receptor agonist *R*-α-methylhistamine on leptazol-induced convulsions was studied (Sturman et al. 1994). Therefore, it is more likely that H_3 receptor function could be directly involved in these biphasic effects of both agonists and antagonists. Adaptation of receptors to excessive blockade or stimulation with high doses of their ligands may also account for these results; in fact, rapid adaptations of H_3 receptors have been described in peripheral tissues (Pérez-García et al.1998). Competition between histamine H_3 receptor ligands and endogenous-released histamine could also contribute to these biphasic effects, as suggested by Lamberti et al. (1998).

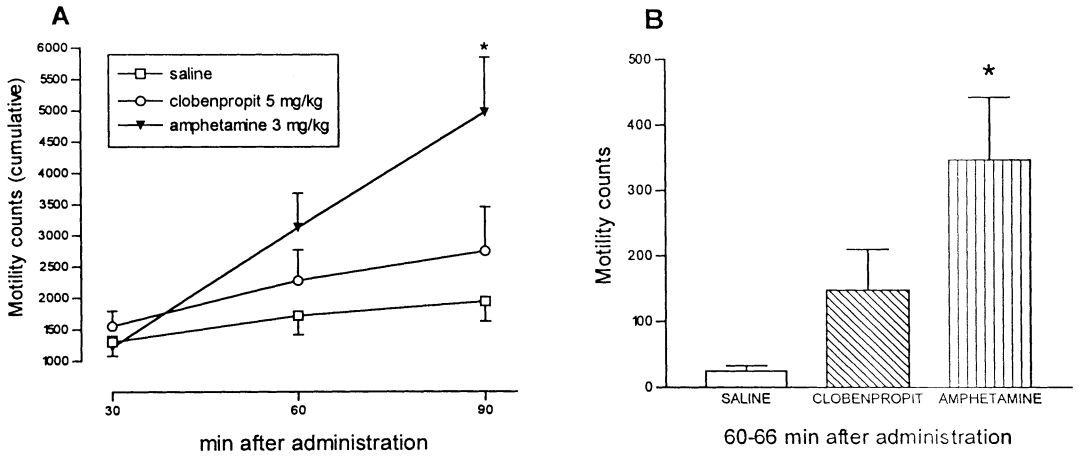

Fig. 3A, B Effect of clobenpropit (5 mg/kg) and amphetamine (3 mg/kg) on the locomotor activity measured in an activity meter. Results are means ± SEM ($n = 10$) (*$P < 0.05$ with respect to saline)

The mechanism through which thioperamide exerts its action in the forced swimming test cannot be explained easily by a H_3-mediated increase of histamine release. Lamberti et al. (1998) have suggested that thioperamide could reduce immobility by this mechanism; thus, the histamine released would be directly responsible for the antidepressant-like action by stimulating histamine H_1 postsynaptic receptors. Although these authors obtained a dose-dependent reduction of immobility with histamine H_1 receptor agonists, it should be pointed out that some data from the literature are not in agreement with this idea. Noguchi et al. (1992) have found that forced swimming increases histamine function and that histamine H_1 receptor antagonists such as mepyramine reduce immobility, which is just the opposite to the findings of Lamberti et al. (1998). In another model of depression, the olfactory bulbectomized rat, the histamine H_1 receptor antagonist terfenadine also showed an antidepressant-like effect (Song et al. 1996). Alternatively, the putative antidepressant properties of thioperamide may reside in a histamine H_3 heteroreceptor-mediated stimulation of the release of other neurotransmitters, mainly on its proved ability to increase the central levels of noradrenaline (Schlicker et al. 1994) and serotonin (Schlicker et al. 1988). As is well known, antidepressants like fluoxetin block serotonin uptake, whereas tricyclic antidepressants inhibit both serotonin and noradrenaline uptake (Baldessarini 1996).

In summary, the results from the forced swimming test indicate a possible antidepressant activity of thioperamide at 10 mg/kg, whereas *R*-α-methylhistamine did not show any significant effect. None of those histamine H_3 receptor ligands seem to modify the state of anxiety in the elevated plus maze. Further experiments using other models of depression and anxiety will be needed to confirm these findings; in the case of forced swimming, we have discarded a false positive result due to psychostimulation, but it must be pointed out that a significant number of other drugs with quite different pharmacological properties (i.e. clozapine, diphenylhydantoin, cyproheptadine, ouabain, etc.) are also active in behavioural despair paradigms (Borsini and Meli 1988).

Acknowledgements We wish to thank Linda Hamalainen for linguistic assistance. This study was supported by a grant from the University of San Pablo CEU (USP 4/96).

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