Neuropharmacology 71 (2013) 141–147

Contents lists available at SciVerse ScienceDirect

Neuropharmacology

journal homepage: www.elsevier.com/locate/neuropharm

Pleiotropic effect of histamine H4 receptor modulation in the central nervous system

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ARTICLE INFO

Article history:
Received 24 December 2012
Received in revised form
15 February 2013
Accepted 21 March 2013

Keywords:
Histamine H4 receptors
Central nervous system
Pain
Anxiety
Memory
Feeding

ABSTRACT

The histamine H4 receptor (H4R) is expressed primarily on cells involved in inflammation and immune responses. Recently, it has been reported the functional expression of H4R within neurons of the central nervous system, but their role has been poorly understood. The present study aimed to elucidate the physiopathological role of cerebral H4R in animal models by the intracerebroventricular administration of the H4R agonist VUF 8430 (20–40 μg per mouse). Selectivity of results was confirmed by the prevention of the effects produced by the H4R antagonist JNJ 10191584 (3–9 mg/kg p.o.). Neuronal H4R activation induced acute thermal antinociception, indicating that neuronal histamine H4R might be involved in the production of antinociception in the absence of an inflammatory process. An anxiolytic-like effect of intensity comparable to that exerted by diazepam, used as reference drug, was produced in the light–dark box test. VUF 8430 reversed the scopolamine-induced amnesia in the passive avoidance test and showed anorexant activity in food deprived mice. Conversely, the H4R activation did not modify the immobility time in the tail suspension test. Rotarod performance test was employed to demonstrate that the effects observed following the administration of VUF 8430 and JNJ 10191584 were not due to impaired motor function of animals. Furthermore, both compounds did not alter spontaneous mobility and exploratory activity in the hole board test. These results show the antinociceptive, antiamnesic, anxiolytic and anorexant effects induced by neuronal H4R agonism, suggesting that H4 modulators may have broader utility further the control of inflammatory and immune processes.

1. Introduction

Multiple receptors exist for histamine in mammalian tissues and these have been classified into 4 distinct receptor types (H1R, H2R, H3R, and H4R), all of which are G-protein coupled receptors (GPCRs) (Schneider et al., 2002). The four histamine receptor subtypes are distinct in terms of their pharmacology and molecular biology and have been implicated in diverse biological effects of the neurotransmitter histamine (Haas et al., 2008).

The human histamine H4 receptor (hH4 receptor) is the most recently discovered member of the G protein-coupled receptor subfamily of histamine receptors (Nakamura et al., 2000; Oda et al., 2000; Liu et al., 2001). The H4 receptor mediates its effects by coupling to Gαi/o G-proteins and has low homology with other histamine receptors, sharing only 35% amino acid identity with the H3R (58% homology in its transmembrane regions) and a much lower homology to H1R and H2R (Oda et al., 2000; Nakamura et al., 2000; Liu et al., 2001).

This receptor has a distinct expression profile on immune cells including mast cells, eosinophils, dendritic cells, and T cells and has modulatory functions of these cells, such as, activation, migration, and production of cytokines and chemokines (O'Reilly et al., 2002; Hofstra et al., 2003). The characterization of the H4 as the immune system histamine receptor directed growing attention toward its therapeutic exploitation in chronic inflammatory disorders, such as allergy, asthma (Dunford et al., 2006), chronic pruritus (Dunford et al., 2007; Dijkstra et al., 2007; Bäumer et al., 2008), inflammatory bowel disease (Varga et al., 2005; Dunford et al., 2007), and autoimmune diseases, such as rheumatoid arthritis (Ikawa et al., 2005) and multiple sclerosis (Jadidi-Niaragh and Mirshafiey, 2010).

Interestingly, the recently reported functional expression of H4 receptors on human and rodent neurons highlights their implication in neuronal functions. H4R expression has been reported in peripheral nerves and in the neurons of the submucous plexus (Nakaya et al., 2004; Breunig et al., 2007). More recently it has been observed the presence of H4R receptors in numerous areas of the

Abbreviation: H4R, histamine H4 receptors.

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http://dx.doi.org/10.1016/j.neuropharm.2013.03.026

central nervous system (CNS), such as hippocampus, thalamus, amygdala, cortex, striatum and spinal cord (Strakhova et al., 2009; Connelly et al., 2009). Electrophysiological studies showed that H4R directly hyperpolarized cortical neurons (Connelly et al., 2009). More recent studies on the potential roles for H4R in the human brain from its predicted functional interactions in the human proteome suggested the participation of H4R in circadian cycle modulation, neuronal firing inhibition and, in cooperation with H3R, in thyroid function and appetite coordination (Moya-Garcia et al., 2011).

The elucidation of the neuronal processes modulated by neuronal H4 receptors might help clarify the physiological and pathological role of this receptor subtype within the central nervous system. For these reasons, the present study used VUF 8430, a H4 agonist (Lim et al., 2009), and JNJ 10191584 (also known as VUF 6002), a potent and selective H4 antagonist (Terzioglu et al., 2004), to explore the cerebral role of the H4R.

2. Materials and methods

2.1. Animals

Male Swiss albino mice (20–22 g) from the Morini (San Polo d'Enza, Italy) breeding farm were used. Ten mice were housed per cage (26 × 41 cm). The cages were placed in the experimental room 24 h before the test for acclimatization. The animals were fed a standard laboratory diet and tap water ad libitum and kept at 23 ± 1 °C with a 12 h light/dark cycle, light on at 7 a.m. All experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC). All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Behavioral testing

Animals were habituated to the experimental room and randomly assigned to each treatment group. Mice were investigated by observers blinded for treatment of the animals. Ten animals per group were used.

2.2.1. Tail suspension test

A piece of tape was adhered to the upper middle of the tail of each animal, creating a flap with the overlap of tape. Mice were suspended from a plastic rod mounted 50 cm above the surface by fastening the tail to the rod with adhesive tape. The duration of the test was 6 min and immobility was measured the last 4 min to facilitate comparison with the forced swimming test. Immobility was defined as the absence of any limb or body movements, except those caused by respiration.

2.2.2. Evaluation of food consumption

Mice did not have access to food for 12 h but water was available ad libitum. A weighed amount of food (standard laboratory pellets) was given and the weight consumed (evaluated as the difference between the original amount and the food left in the cage, including spillage), was measured 15, 30, 45 and 60 min after injection, to an accuracy of 0.1 g. An arbitrary cut-off time of 60 min was used.

2.2.3. Rotarod test

The apparatus consisted of a base platform and a rotating rod of 3 cm diameter with a non-skid surface. The rod was placed at a height of 15 cm from the base. The rod, 30 cm in length, was divided into 5 equal sections by 6 disks. Thus up to 5 mice were tested simultaneously on the apparatus, with a rod-rotation speed of 16 r.p.m. The integrity of motor coordination was assessed on the basis of the number of falls from the rod in 30 s. Performance time was measured before and 15, 30, 45 and 60 min after i.c.v. administration of saline or VUF 8430 and after p.o. administration of CMC or JNJ 10191584.

2.2.4. Locomotor activity

The locomotor activity was evaluated by using the hole-board test. The apparatus consisted of a 40 cm square plane with 16 flush mounted cylindrical holes (3 cm diameter) distributed 4 × 4 in an equidistant, grid-like manner. Mice were placed on the center of the board one by one and allowed to move about freely for a period of 5 min each. Two photobeams, crossing the plane from mid-point to mid-point of opposite sides, thus dividing the plane into 4 equal quadrants, automatically signaled the movement of the animal (counts in 5 min) on the surface of the plane (locomotor activity). Miniature photoelectric cells, in each of the 16 holes, recorded (counts in 5 min) the exploration of the holes (exploratory activity) by the mice.

2.2.5. Passive-avoidance test

The apparatus consisted of a two-compartment acrylic box with a lighted compartment connected to a darkened one by a guillotine door. As soon as the mouse entered the dark compartment, it received a punishing electrical shock (0.5 mA, 1 s). The latency times for entering the dark compartment were measured in the training test and after 24 h in the retention test. The maximum entry latency allowed in the training and retention sessions was, respectively, 60 and 180 s.

2.2.6. Hot-plate test

The hot plate test was performed as previously described (Galeotti et al., 2003). Mice were placed inside a stainless steel container, which was set thermostatically at 52.5 ± 0.1 °C in a precision water-bath from KW Mechanical Workshop, Siena, Italy. Reaction times(s), were measured with a stopwatch before and 15, 30, 45 and 60 min after administration of the analgesic drug. The endpoint used was the licking of the fore or hind paws. Those mice scoring less than 12 and more than 18 s in the pretest were rejected (30%). An arbitrary cut-off time of 45 s was adopted.

2.2.7. Light dark box

The light–dark box was made of white and black opaque apparatus (length 50 cm, width 20.5 cm, and height 19 cm) consisted of two equal acrylic compartments, one dark and one white, illuminated by a 60-W bulb lamp and separated by a divider with a 10 × 3.2 cm opening at floor level. Each mouse was placed in the middle of the light chamber facing a side away from the door and then released. Animals' behaviors were scored for 5 min and included the duration of time spent in the light chamber, number of full-body transitions between chambers. These behaviors have previously been measured as a reflection of anxiety in this apparatus (Bourin and Hascoët, 2003). After testing, subjects were removed from the light–dark box and returned to their home cage in colony room. The apparatus was cleaned with 70% ethanol after each use and allowed to dry before the next subject was tested.

This test exploited the conflict between the animal's tendency to explore a new environment and its fear of bright light.

2.3. Drug administration

VUF 8430, scopolamine hydrobromide, naloxone hydrochloride, mecamylamine hydrochloride, atropine sulfate, yohimbine hydrochloride, (Sigma, Milan, Italy) morphine hydrochloride (SALARS, Milan, Italy), amitriptyline, D-amphetamine (De Angeli, Rome, Italy), were dissolved in isotonic (NaCl 0.9%) saline solution immediately before use. JNJ 10191584 (Sigma, Milan, Italy) was dispersed in 1% sodium carboxymethylcellulose (CMC) and administered by gavage 30 min before behavioral testing. Drug concentrations were prepared in such a way that the necessary dose could be administered in a volume of 10 ml/kg by intraperitoneal (i.p.) or gavage (p.o.) administration, or in a volume of 5 μl per mouse by intracerebroventricular (i.c.v.) injection.

Animals were used a single time and were randomly assigned to the treatment groups. To evaluate the role of H4R within the central nervous system, VUF 8430 (20–60 μg per mouse) was administered i.c.v., as previously described (Galeotti et al., 2003), 15 min before the tests. Naloxone (1 mg/kg), atropine (5 mg/kg), mecamylamine (2 mg/kg) were administered 15 min before the test, diazepam (1 mg/kg), amitriptyline (10 mg/kg) 30 min before, yohimbine (2 mg/kg) 60 min before, intraperitoneally. Scopolamine (3 mg/kg i.p.) was administered immediately after the training session. Doses and administration schedule were chosen on the basis of time-course and dose–response curves performed in our laboratory.

2.4. Statistical analysis

All experimental results are given as the mean ± s.e. mean. One-way and two-way analysis of variance, followed, respectively, by Tukey and Bonferroni *post hoc* test, were used for statistical analysis.

3. Results

3.1. Antinociceptive effect of the H4 receptor agonist VUF 8430

The involvement of histamine H4 receptors in the modulation of the pain threshold following an acute thermal stimulus was evaluated in the mouse hot plate test. The H4 receptor agonist VUF 8430 induced a dose-dependent antinociceptive effect. Two-way ANOVA for repeated measures yielded a significant main effect for VUF 8430 treatment ($F(3,180)$ 66.18; $P < 0.0001$), time ($F(4,180)$ 17.25; $P < 0.0001$) and interaction ($F(12,180)$ 3.774; $p < 0.0001$). *Post hoc* comparison showed that the dose of 20 μg per mouse i.c.v. was devoid of any effect, at 30 μg per mouse VUF 8430 significantly increased the licking latency value at 30 min, and at the dose of

40 μg per mouse it produced a long lasting antinociceptive effect that peaked at 30 min and was still significant after 60 min (Fig. 1a). This increase of the pain threshold was related to the stimulation of the H4R since it was selectively prevented by the H4R antagonist JNJ 10191584 (Fig. 1b). Two-way ANOVA for repeated measures showed a significant main effect for treatment ($F(3,180)$ 46.00; $P < 0.0001$), time ($F(4,180)$ 19.84; $P < 0.0001$) and interaction ($F(12,180)$ 3.110; $p < 0.0001$). The opioid antagonist naloxone, the muscarinic antagonist atropine, the α_2-adrenoceptor antagonist yohimbine and the nicotinic antagonist mecamylamine did not modify the VUF 8430-induced antinociceptive effect (Fig. 1c), further confirming the H4R-mediated mechanism. The selectivity of JNJ 10191584 was demonstrated by the inefficacy on preventing morphine (7 mg/kg i.p.) analgesia (Fig. 1d).

3.2. Anxiolytic-like effect following H4 receptor stimulation

Diazepam was used as a reference molecule. It was able to prolong the time spent in the lighted compartment, thus evidencing its anxiolytic-like properties and validating our experimental approach. Similarly, the H4R agonist VUF 8430 dose-dependently prolonged the time spent in the light chamber. At 20 μg it was ineffective, at 30 μg it lightly increased the permanence in the light without reaching the statistical significance whereas the dose of 40 μg markedly increased the time spent in the light compartment producing an anxiolytic effect comparable to that induced by diazepam (Fig. 2a). In order to confirm that the anxiolytic properties of VUF 8430 were related to the stimulation of H4 receptors, we performed experiments in the presence of increasing concentrations of the H4 antagonist JNJ 10191584. The H4 antagonist never modified the time spent in the light when administered alone, but it was able to completely prevent the VUF 8430-induced anxiolytic effect when orally administered at the dose of 2 mg/kg (Fig. 2b).

A second behavioral parameter detected to evaluate the anxiolytic efficacy of the H4 receptor agonist was the number of transitions from the two chambers. The reference drug diazepam markedly increased the transitions in comparison with the control group. In agreement with data on the time spent on the light, JNJ10195184 was devoid of any effect when administered alone. Surprisingly, the H4R agonist, at the dose able to prolong the permanence in the light compartment, significantly reduced the number of transfers. This effect was not prevented by pre-treatment with the H4R antagonist (Fig. 2c).

3.3. Antiamnesic activity of a H4 receptor agonist

The administration of scopolamine induced amnesia in the passive avoidance, as showed by the reduction of the latency to enter the dark compartment in the retention session. Pre-treatment with the H4 receptor agonist VUF 8430 (40 μg per mouse) enhanced the entrance latency to values comparable to the control group showing a complete prevention of the scopolamine-induced memory impairment. No ameliorative effect on memory performance was detected when VUF 8430 was administered alone (Fig. 3).

3.4. Anorexant effect by H4R stimulation

The cumulated amount of food eaten by mice which had no access to food for 12 h before the test is reported in Fig. 3. Mice showed a constant increase in the amount of food consumed in the 60 min test. Two-way ANOVA for repeated measures yielded a significant main effect for treatment ($F(2,108)$ 107.6; $P < 0.0001$), time ($F(3,108)$ 366.9; $P < 0.0001$) and interaction ($F(6,108)$ 8.76; $p < 0.0001$). *Post hoc* comparison showed that VUF 8430 (40 μg per mouse i.c.v.) significantly reduced food consumption 45 and 60 min after administration. No effect was detected at 15 and 30 min.

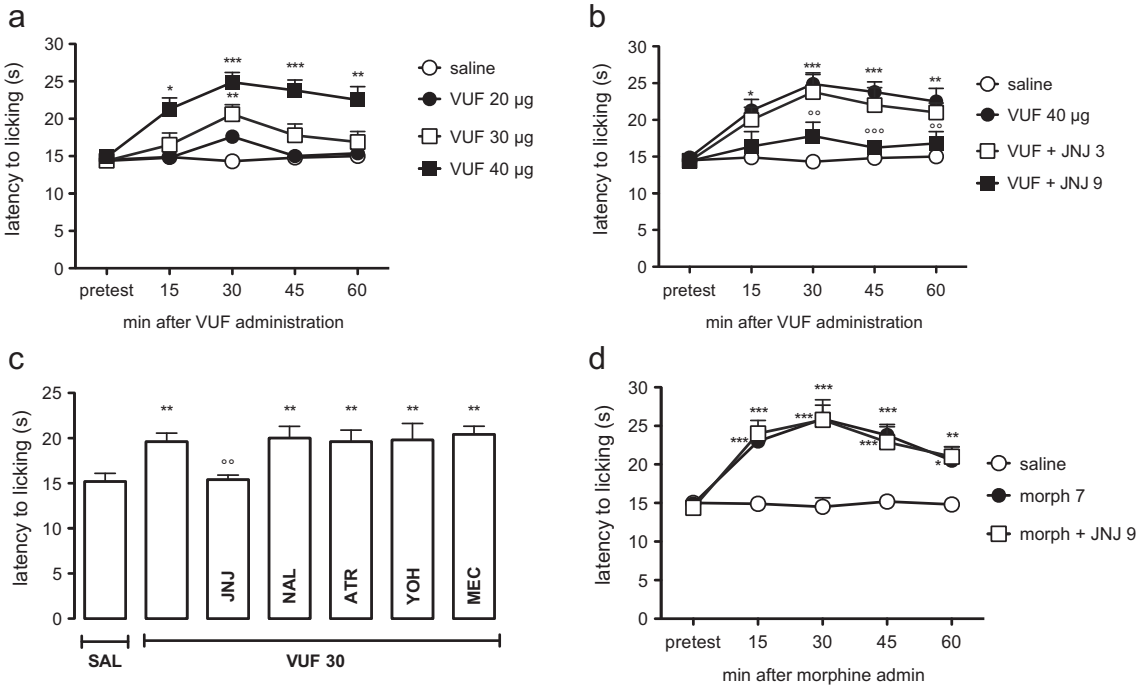

Fig. 1. Antinociceptive effect following H4R activation. **(a)** The H4R agonist VUF 8430 (20–40 μg per mouse i.c.v.) dose-dependently increased the pain threshold in the mouse hot-plate test **(b)** the antinociceptive effect of VUF 8430 was prevented by the H4R antagonist JNJ 10191584 (3–9 mg/kg p.o.) **(c)** Lack of effect of naloxone, atropine, mecamylamine, yohimbine on VUF 8430-induced antinociception (one-way ANOVA, $F(6,69)$ 3.577). **(d)** JNJ 10191584 (9 mg/kg p.o.) did not modify morphine (7 mg/kg i.p.) analgesia. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ vs control group; $^{\circ\circ}P < 0.01$, $^{\circ\circ\circ}P < 0.001$ vs VUF 8430-treated mice.

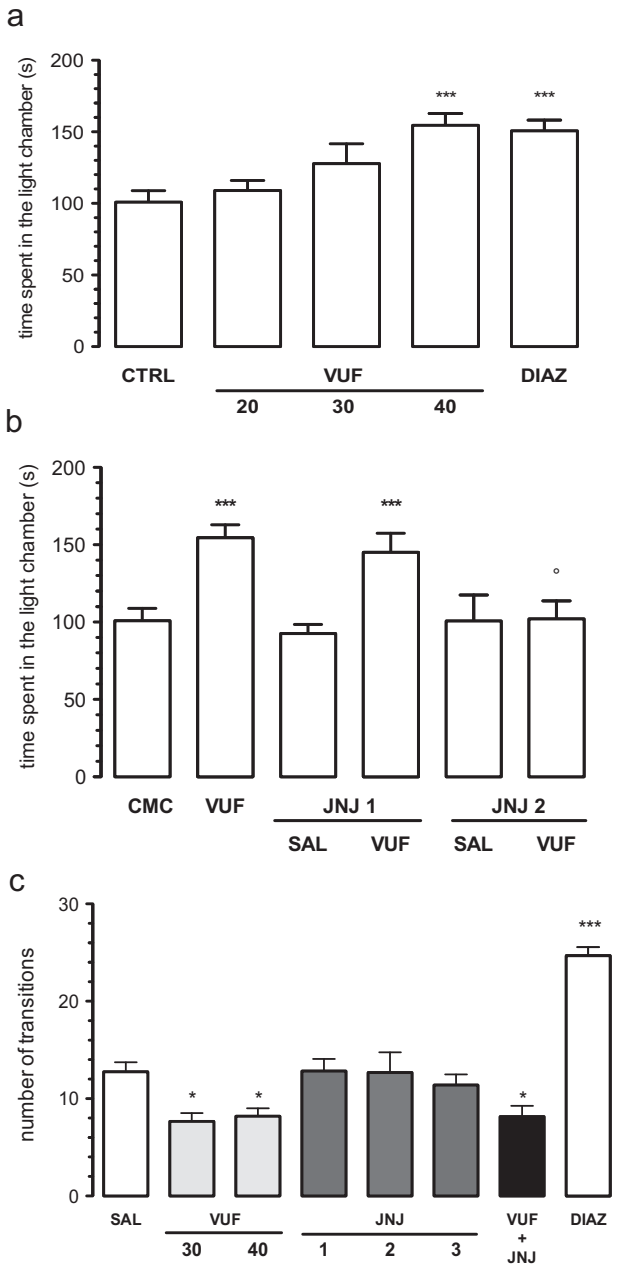

Fig. 2. Anxiolytic-like effect of VUF 8430 in the mouse light/dark box test. **(a)** The H4R agonist VUF 8430 (20–40 μg per mouse i.c.v.) increased the permanence in the light compartment ($F(4,49)$ 10.23). Diazepam (1 mg/kg i.p.) was used as reference drug. **(b)** Administration of the H4R antagonist JNJ 10191584 (1–2 mg/kg p.o.) prevented the anxiolytic-like effect of VUF 8430 ($F(5,60)$ 10.02). **(c)** VUF 8430 reduced the number of transitions. JNJ 10191584 (1–3 mg/kg p.o.) did not prevent this effect ($F(7,79)$ 30.39). $^{*}P < 0.05$, $^{***}P < 0.001$ in comparison with the control group; $^{\circ}P < 0.05$ in comparison with VUF 8430-treated mice by one-way ANOVA.

D-Amphetamine (1 mg/kg i.p.) was used as anorexant reference drug (Fig. 4).

3.5. Lack of antidepressant-like activity following H4 receptor stimulation

VUF 8430, at all doses investigated that resulted effective in the above-mentioned behavioral paradigms, did not modify the immobility time in the tail suspension test (TST), showing the lack of antidepressant-like activity. The administration of amitriptyline,

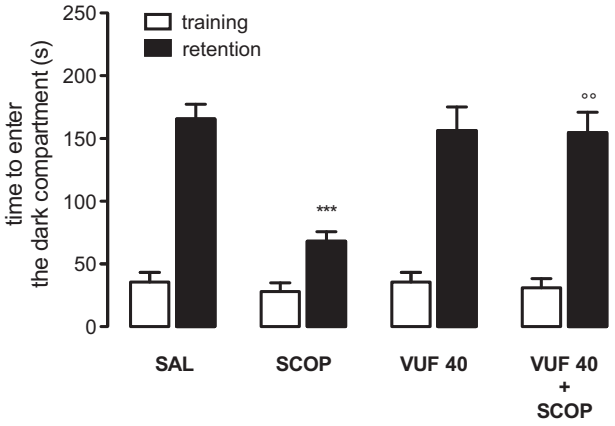

Fig. 3. Antiamnesic effect following H4R activation in the mouse passive avoidance test. The administration of scopolamine (SCOP) produced amnesia that was prevented by VUF 8430 (40 μg per mouse i.c.v.) ($F(3,39)$ 22.35). $^{***}P < 0.001$ in comparison with the control group; $^{\circ\circ}P < 0.01$ in comparison with scopolamine-treated mice by one-way ANOVA.

used as reference drug, decreased the immobility time values in the mouse TST, thus validating our experimental results (Fig. 5).

3.6. Effect of H4 receptor modulation on locomotor behavior

The H4 receptor agonist VUF 8430 and the H4 receptor antagonist JNJ 10191584 were tested in order to assess their effect on mouse locomotor behavior. Mice pretreated with the above-mentioned compounds were evaluated for motor coordination by use of the rotarod test and for spontaneous mobility and exploratory activity by use of the hole board test.

Two-way ANOVA for repeated measures yielded a significant main effect for treatment ($F(3,180)$ 32.49; $P < 0.0001$), time ($F(4,180)$12.60; $P < 0.0001$) and interaction ($F(12,180)$ 2.22; $p < 0.0125$). *Post hoc* comparison showed that the lack of any impairment in the motor coordination after administration of VUF 8430 at the dose of 40 μg per mouse. At the dose of 50 μg there was a trend to higher values of number of falls without reaching the statistical significance whereas at the dose of 60 μg VUF 8430 produced a significant impairment in the motor coordination (Fig. 6A). The H4R antagonist JNJ 10191584 (9 mg/kg p.o.) did not

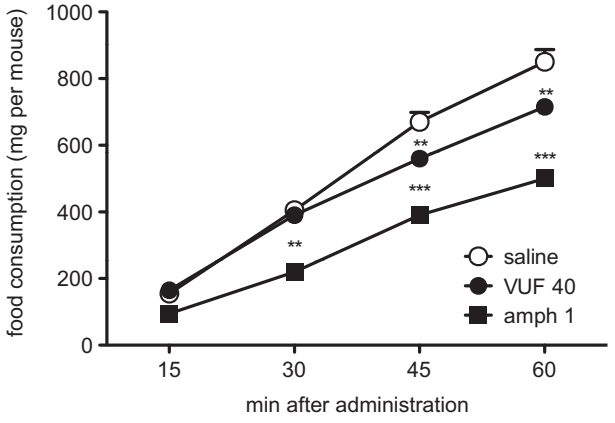

Fig. 4. Anorexant effect of VUF 8430 in 12 h food-deprived mice. The food intake values were evaluated as the cumulated amount of food eaten 60 min after the beginning of the test. D-Amphetamine (amph) was used as reference drug. $^{**}P < 0.01$ in comparison with the control group.

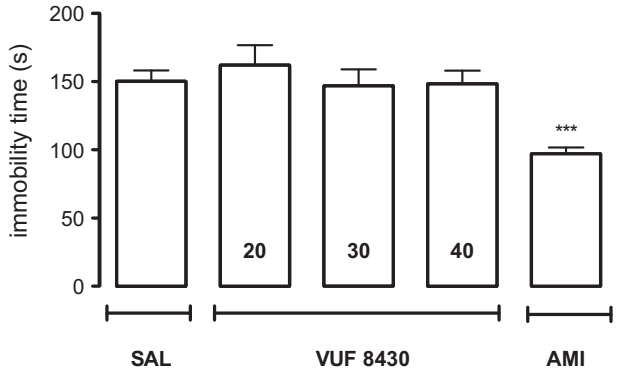

Fig. 5. Lack of effect of H4R modulation in the mouse tail suspension test. VUF 8430 (20–40 μg per mouse i.c.v.) did not modify the immobility time of animals. Amitriptyline (AMI) was used as antidepressant reference drug. $^{***}P < 0.001$ in comparison with control mice.

modify the number of falls in comparison with the CMC-treated control group (Fig. 6B).

The spontaneous mobility and exploratory activity of mice treated with VUF 8430 (40 μg per mouse i.c.v.) or JNJ 10191584 (9 mg/kg p.o.) were unmodified in comparison with the control group (Fig. 6C).

4. Discussion

The central histaminergic system has been implicated in the regulation of a wide range of physiological functions. The role of H1, H2 and H3 receptor subtypes in the modulation of neuronal processes has been widely investigated. Conversely, the role of neuronal H4 receptors is poorly understood. The present study investigated the involvement of H4 receptors in the histaminergic regulation of neuronal functions, such as locomotor activity, memory, pain perception, feeding behavior, depressive behavior, anxiety, by using a potent H4 agonist, VUF 8430 (Lim et al., 2009), in mice. Since H4 receptors have a broader distribution than areas of the central nervous system, VUF 8430 was administered intracerebroventricularly (i.c.v.) to avoid any interference by peripheral H4R activation.

VUF 8430 produced a dose-dependent increase of pain threshold against an acute thermal stimulus. This modulation of the pain perception was related to an interaction with histamine H4 receptors since the antinociception was antagonized by JNJ 10191584, a potent and selective H4 receptor antagonist (Terzioglu et al., 2004), at a concentration devoid of any effect on pain threshold when administered alone. The involvement of other neurotransmission systems can be ruled out since the VUF 8430-induced thermal antinociception was unmodified by the treatment with the opioid antagonist naloxone, the α_2-adrenoceptor antagonist yohimbine, the muscarinic antagonist atropine, the nicotinic antagonist mecamylamine. Furthermore, the selectivity of JNJ 10191584 was demonstrated by the lack of any effect of the H4 receptor antagonist on analgesia unrelated to the histaminergic system. These results, indicating the involvement of neuronal H4 receptors in acute thermal antinociception, are in agreement with previous studies reporting the decrease in mechanical hyperalgesia produced by VUF 8430 in a model of neuropathic pain (Smith et al., 2007). However, in contrast with our findings, it has been observed that the systemic administration of the H4 antagonists JNJ7777120 and JNJ 10191584 exhibited anti-hyperalgesic activity in different models of inflammatory pain and anti-allodynic effects in models of neuropathic pain (Coruzzi et al., 2007; Hsieh et al., 2010). This discrepancy might be explained considering that this anti-

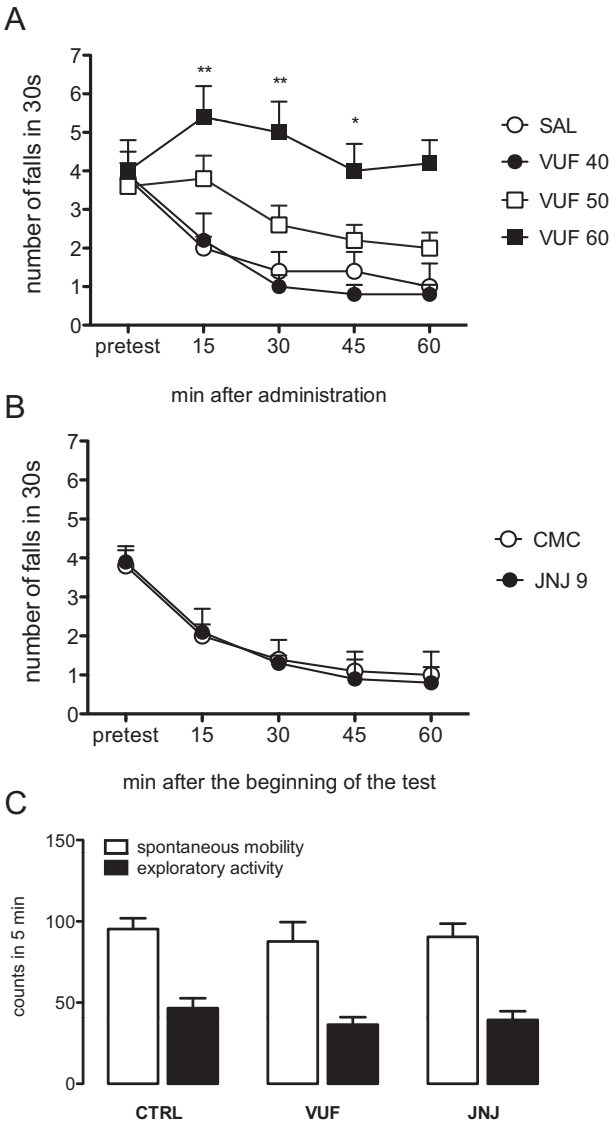

Fig. 6. Effect on mouse locomotor activity by H4R modulation. **(A)** Doses of VUF 8430 higher than dose effective in behavioral tests (50–60 μg per mouse i.c.v.) induced motor incoordination in the mouse rotarod test. **(B)** JNJ 10191584 did not alter motor coordination. **(C)** The absence of any alteration of spontaneous mobility and exploratory activity by the H4R agonist VUF 8430 and the H4R antagonist JNJ 10191584 was observed in comparison with control (CTRL) mice. $^{*}P < 0.05$, $^{**}P < 0.01$ in comparison with control group.

hyperalgesic effect is obtained following systemic administration of the H4 antagonists and, as stated by the authors, it appears to be secondary to the anti-inflammatory activity induced by the antagonism of the H4 receptor on immune system cells. Similarly, the anti-allodynic effect, which is weaker than the anti-inflammatory one, origins from the reduction of the inflammatory response at the site of injury in the animal model of neuropathic pain. This hypothesis is further supported by the observations that the H4 antagonist JNJ7777120 did not produce any antinociceptive effect on the contralateral non-inflamed paw (Hsieh et al., 2010). Present results indicate that neuronal histamine H4 receptor activation might be involved in the production of an acute thermal antinociception in the absence of an inflammatory process. H4 receptor modulation might have a broader utility in pain states than those secondary to inflammation.

Ample evidence has shown that histamine release is a sensitive indicator of stress and the involvement of the histaminergic system

in the modulation of anxiety-like behaviors in animals has been suggested. The administration of histamine or L-histidine induces an anxiogenic-like effect (Kumar et al., 2007) whereas the destruction of the rat tuberomammillary rostroventral E-2 subregion, from which histaminergic neuron fibers arise, can induce anxiolytic-like effects (Frisch et al., 1998). Furthermore, clinically effective anxiolytic drugs, such as diazepam and buspirone, have been found to decrease the turnover rate of brain histamine in mice and rats (Oishi et al., 1992; Chikai et al., 1993). Concerning the receptor subtype involved in the anxiogenic-like effect of histamine, it has been observed that the activation of H1 receptors appears to have a prominent role (Malmberg-Aiello et al., 2002; Kumar et al., 2007). This hypothesis is supported by the observation that mice lacking histamine H1 receptors showed prolonged transfer latency in the light/dark box test, indicating that mutant mice were less fearful than wild-type mice (Yanai et al., 1998). However, the role of neuronal H4 receptors in the modulation of anxiety by the histaminergic system has not been elucidated. In the present study we evaluated the effect of the activation of neuronal histamine H4 receptors in an anxiety-like condition, using the light–dark box test. Surprisingly, the i.c.v. administration of the H4 receptor agonist VUF 8430 produced a dose-dependent increase of the time spent in the light compartment, indicating the induction of an anxiolytic-like effect of intensity comparable to that exerted by diazepam, used as reference drug. Pre-treatment with the H4 receptor antagonist JNJ 10191584 prevented this effect, further confirming the selective involvement of H4 receptor activation. In contrast to anxiolytic reference drugs, VUF 8430 reduced the number of transitions between the two compartments, a parameter usually considered to evaluate the anxiolytic-like efficacy of compounds along with the light/dark performance. However, the VUF 8430-induced inhibitory effect on transitions appeared at doses lower than those effective as anxiolytic and it was JNJ 10191584 insensitive. These data suggest that this might be an unspecific effect of VUF 8430, non-related to the activation of H4 receptors. A similar profile of activity in the light dark box test was seen for the H2 agonist impromidine. This compound increased the permanence in the light compartment, but also decreased the number of transitions (Malmberg-Aiello et al., 2002). Even if VUF 8430 has a very low affinity for H2 receptors, we cannot exclude that the H4-insensitive effect on transitions might be secondary to an activation of H2 receptors.

The light/dark box test is limited by its ability to yield false-positive results if a drug increases general motor function. Screening of locomotor activity appears to be necessary for eliminating false-positive results. Administration of VUF 8430 did not alter spontaneous mobility and exploratory activity in the hole board test, excluding the presence of an altered locomotor activity following H4 receptor activation that might lead to a misinterpretation of the results obtained. It should be finally taken into account that transitions in the light–dark box test have been reported to be an index of exploration activity because of habituation over time, and the time spent in each compartment to be a reflection of aversion, but the measure with the highest validity seems to be the percentage of time spent in each compartment (Bourin and Hascoët, 2003).

To investigate to the role of the neuronal H4 receptors in the modulation of memory processes, we evaluated the effects produced by VUF 8430 on a passive avoidance paradigm. The administration of the H4 agonist was able to prevent the memory impairment induced by scopolamine, showing antiamnesic properties. In our experimental conditions, VUF 8430 did not induce any procognitive effect. However, it should be taken into account that an improvement in cognition of animals that have no memory impairment is difficult to demonstrate in the passive avoidance test. As a matter of fact, well known nootropic drugs such as piracetam and aniracetam or cholinomimetics such as physostigmine and oxotremorine, do not show any memory facilitation in unamnesic animals (Gouliaev and Senning, 1994).

It has been demonstrated that histamine is involved in learning and memory. However, these studies have used many behavioral tasks and obtained contradictory results. Nevertheless, the H3 receptor has been recently implicated in learning and memory processes. Blockade of presynaptic H3 autoreceptors would enhance the release of histamine and other transmitter systems involved in cognitive processes and, indirectly, ameliorate cognitive performances (Esbenshade et al., 2008). Present results suggest a beneficial activity induced by neuronal H4 receptor stimulation on memory impairments, extending and further clarifying the role of the histaminergic system in the modulation of cognitive functions.

The modulation of eating behavior by activation of neuronal H4 receptors was evaluated in mice deprived of food for 12 h. These experimental conditions were needed to highlight a reduction in food intake. The i.c.v. administration of the H4 receptor agonist VUF 8430 significantly reduced food consumption, showing an anorexant effect. This is the first evidence of a role of neuronal H4 receptors in the modulation of food intake. These results are in agreement with the role of histamine in the regulation of appetite and satiety (Masaki and Yoshimatsu, 2010). Brain histamine appears to suppress food intake via histamine H1 receptors. Centrally administered histamine H1 receptor agonists suppressed food consumption in rats (Lecklin et al., 1998), whereas injection of H1-antagonists elicited food intake (Sakata et al., 1988; Ookuma et al., 1993). However, relatively few studies have been carried out to unequivocally establish a relationship between food consumption and H1 receptors blockade in humans (Deng et al., 2010). Both H2-agonists (Lecklin et al., 1998) and antagonists (Sakata et al., 1988) centrally injected had no effect on food intake. More recently has been postulated a role of H3 receptors in the eating behavior. H3 agonists increase feeding (Chiba et al., 2009) whereas H3 antagonists have been reported to induce weight loss (Malmlöf et al., 2005). However, a stimulation of H3 autoreceptors would reduce the histamine release increasing food consumption. The activation of H3R in the anorexant mechanism of VUF 8430 appears very unlikely.

Pharmacological or genetic loss of histamine or histamine receptor function in animals produces phenotypes that model human depression (Haas et al., 2008). Endogenous histamine reduces the time of immobility in the forced swimming test, suggesting an antidepressant-like effect, via activation of H1 receptors (Lamberti et al., 1998). More recently, the involvement of the H3 receptors has been postulated since the H3 receptor antagonist and inhibitor of the serotonin transporter JNJ 28583867 showed antidepressant-like activity in animal models of depression (Barbier et al., 2007). However, the activation of cerebral H4 receptors was devoid of any effect on the mobility time in the mouse tail suspension test, indicating the lack of any antidepressant-like effect by this histamine receptor subtype.

VUF 8430 is also endowed with some affinity for the H3R subtype showing full agonist properties (Lim et al., 2009). However, it has been supposed that H3R and H4R have distinct and contrasting role in the mammalian brain. The H3R acts as autoreceptor on histaminergic neuron endings. H3R activation moderates histamine release and synthesis and inhibits histaminergic neurotransmission. For this reason numerous H3 antagonists/inverse agonists have been synthesized and studied in order to improve histaminergic activity. On these bases, an involvement of H3R activation in the pharmacological effects produced by VUF 8430 is very unlikely. The histaminergic system is also involved in the regulation of locomotor activity. Rotarod performance test was therefore

employed to demonstrate that the effects observed following the administration of the H4 agonist VUF 8430 and the H4 antagonist JNJ 10191584 in animal models were not due to impaired motor function of these animals. Impaired motor coordination in animals can affect the ability of animals to move their paws, and therefore, may influence the paw withdraw latency during the pain efficacy testing, the movements in the light/dark box during memory and anxiety testing, the mobility time in the TST, the capability to reach the food during feeding behavior determination. However, VUF 8430 and JNJ 10191584 did not produce any motor coordination deficit of movement in rotarod performance assay. These results, along with the lack of alteration of spontaneous mobility and exploratory activity, indicate that the neuronal effects observed following the administration of the H4 modulators are likely due to specific antinociceptive, antiamnesic, anxiolytic and anorexant effects mediated by H4R agonism.

Conflicts of interest

None.

Acknowledgments

This work was supported by grants from MIUR.

References

Barbier, A.J., Aluisio, L., Lord, B., Qu, Y., Wilson, S.J., Boggs, J.D., Bonaventure, P., Miller, K., Fraser, I., Dvorak, L., Pudiak, C., Dugovic, C., Shelton, J., Mazur, C., Letavic, M.A., Carruthers, N.I., Lovenberg, T.W., 2007. Pharmacological characterization of JNJ-28583867, a histamine H3 receptor antagonist and serotonin reuptake inhibitor. Eur. J. Pharmacol. 576, 43–54.

Bäumer, W., Wendorff, S., Gutzmer, R., Werfel, T., Dijkstra, D., Chazot, P., Stark, H., Kietzmann, M., 2008. Histamine H4 receptors modulate dendritic cell migration through skin – immunomodulatory role of histamine. Allergy 63, 1387–1394.

Bourin, M., Hascoët, M., 2003. The mouse light/dark box test. Eur. J. Pharmacol. 463, 55–65.

Breunig, E., Michel, K., Zeller, F., Seidl, S., Weyhern, C.W., Schemann, M., 2007. Histamine excites neurones in the human submucous plexus through activation of H1, H2, H3 and H4 receptors. J. Physiol. 583, 731–742.

Chiba, S., Itateyama, E., Sakata, T., Yoshimatsu, H., 2009. Acute central administration of immepip, a histamine H3 receptor agonist, suppresses hypothalamic histamine release and elicits feeding behavior in rats. Brain Res. Bull. 79, 37–40.

Chikai, T., Oishi, R., Saeki, K., 1993. Microdialysis study of the effects of sedative drugs on extracellular histamine in the striatum of freely moving rats. J. Pharmacol. Exp. Ther. 266, 1277–1281.

Connelly, W.M., Shenton, F.C., Lethbridge, N., Leurs, R., Waldvogel, H.J., Faull, R.L., Lees, G., Chazot, P.L., 2009. The histamine H4 receptor is functionally expressed on neurons in the mammalian CNS. Br. J. Pharmacol. 157, 55–63.

Coruzzi, G., Adami, M., Elena Guaita, E., de Esch, I.J.P., Leurs, R., 2007. Antiinflammatory and antinociceptive effects of the selective histamine H4-receptor antagonists JNJ7777120 and VUF6002 in a rat model of carrageenan-induced acute inflammation. Eur. J. Pharmacol. 563, 240–244.

Deng, C., Weston-Green, K., Huang, X.F., 2010. The role of histaminergic H1 and H3 receptors in food intake: a mechanism for atypical antipsychotic-induced weight gain? Prog. Neuropsychopharmacol. Biol. Psychiatry 34, 1–4.

Dijkstra, D., Leurs, R., Chazot, P.L., Shenton, F.C., Stark, H., Werfel, T., Gutzmer, R., 2007. Histamine downregulates monocyte CCL2 production through the histamine H(4) receptor. J. Allergy Clin. Immunol. 120, 300–307.

Dunford, P.J., O'Donnell, N., Riley, J.P., Williams, K.N., Karlsson, L., Thurmond, R.L., 2006. The histamine H4 receptor mediates allergic airway inflammation by regulating the activation of CD4+ T cells. J. Immunol. 176, 7062–7070.

Dunford, P.J., Williams, K.N., Desai, P.J., Karlsson, L., McQueen, D., Thurmond, R.L., 2007. Histamine H4 receptor antagonists are superior to traditional antihistamines in the attenuation of experimental pruritus. J. Allergy Clin. Immunol. 119, 176–183.

Esbenshade, T.A., Browman, K.E., Bitner, R.S., Strakhova, M., Cowart, M.D., Brioni, J.D., 2008. The histamine H3 receptor: an attractive target for the treatment of cognitive disorders. Br. J. Pharmacol. 154, 1166–1181.

Frisch, C., Hasenohrl, R.U., Krauth, J., Huston, J.P., 1998. Anxiolytic-like behavior after lesion of the tuberomammillary nucleus E2-region. Exp. Brain Res. 119, 260–264.

Galeotti, N., Bartolini, A., Ghelardini, C., 2003. The phospholipase C-IP_3 pathway is involved in muscarinic antinociception. Neuropsychopharmacology 28, 888–897.

Gouliaev, A.H., Senning, A., 1994. Piracetam and other structurally related nootropics. Brain Res. Rev. 19, 180–222.

Haas, H.L., Sergeeva, O.A., Selbach, O., 2008. Histamine in the nervous system. Physiol. Rev. 88, 1183–1241.

Hofstra, C.L., Desai, P.J., Thurmond, R.L., Fung-Leung, W.P., 2003. Histamine H4 receptor mediates chemotaxis and calcium mobilization of mast cells. J. Pharmacol. Exp. Ther. 305, 1212–1221.

Hsieh, G.C., Chandran, P., Salyers, A.K., Pai, M., Zhu, C.Z., Wensink, E.J., Witte, D.G., Miller, T.R., Mikusa, J.P., Baker, S.J., Wetter, J.M., Marsh, K.C., Hancock, A.A., Cowart, M.D., Esbenshade, T.A., Brioni, J.D., Honore, P., 2010. H4 receptor antagonism exhibits anti-nociceptive effects in inflammatory and neuropathic pain models in rats. Pharmacol. Biochem. Behav. 95, 41–50.

Ikawa, Y., Suzuki, M., Shiono, S., Ohki, E., Moriya, H., Negishi, E., Ueno, K., 2005. Histamine H4 receptor expression in human synovial cells obtained from patients suffering from rheumatoid arthritis. Biol. Pharm. Bull. 28, 2016–2018.

Jadidi-Niaragh, F., Mirshafiey, A., 2010. Histamine and histamine receptors in pathogenesis and treatment of multiple sclerosis. Neuropharmacology 59, 180–189.

Kumar, K.V., Krishna, D.R., Palit, G., 2007. Histaminergic H1 receptors mediate L-histidine-induced anxiety in elevated plus-maze test in mice. Behav. Pharmacol. 18, 213–217.

Lamberti, C., Ipponi, A., Bartolini, A., Schunack, W., Malmberg-Aiello, P., 1998. Antidepressant-like effects of endogenous histamine and of two histamine H1 receptor agonists in the mouse forced swim test. Br. J. Pharmacol. 123, 1331–1336.

Lecklin, A., Etu-Seppälä, P., Stark, H., Tuomisto, L., 1998. Effects of intracerebroventricularly infused histamine and selective H1, H2 and H3 agonists on food and water intake and urine flow in Wistar rats. Brain Res. 793, 279–288.

Lim, H.D., Adami, M., Guaita, E., Werfel, T., Smits, R.A., de Esch, I.J.P., Bakker, R.A., Gutzmer, R., Coruzzi, G., Leurs, R., 2009. Pharmacological characterization of the new histamine H4 receptor agonist VUF 8430. Br. J. Pharmacol. 157, 34–43.

Liu, C., Wilson, S.J., Kuei, C., Lovenberg, T.W., 2001. Comparison of human, mouse, rat, and guinea pig histamine H4 receptors reveals substantial pharmacological species variation. J. Pharmacol. Exp. Ther. 299, 121–130.

Malmberg-Aiello, P., Ipponi, A., Bartolini, A., Schunack, W., 2002. Mouse light/dark box test reveals anxiogenic-like effects by activation of histamine H1 receptors. Pharmacol. Biochem. Behav. 71, 313–318.

Malmlöf, K., Zaragoza, F., Golozoubova, V., Refsgaard, H.H., Cremers, T., Raun, K., Wulff, B.S., Johansen, P.B., Westerink, B., Rimvall, K., 2005. Influence of a selective histamine H3 receptor antagonist on hypothalamic neural activity, food intake and body weight. Int. J. Obes. (Lond.) 29, 1402–1412.

Masaki, T., Yoshimatsu, H., 2010. Neuronal histamine and its receptors: implication of the pharmacological treatment of obesity. Curr. Med. Chem. 17, 4587–4592.

Moya-Garcia, A., Rodriguez, C.E., Morilla, I., Sanchez-Jimenez, F., Ranea, J.A.G., 2011. The function of histamine receptor H4R in the brain revealed by interaction partners. Front. Biosci. (Schol. Ed.) 3, 1058–1066.

Nakamura, T., Itadani, H., Hidaka, Y., Ohta, M., Tanaka, K., 2000. Molecular cloning and characterization of a new human histamine receptor, H4R. Biochem. Biophys. Res. Commun. 279, 615–620.

Nakaya, M., Takeuchi, N., Kondo, K., 2004. Immunohistochemical localization of histamine receptor subtypes in human inferior turbinates. Ann. Otol. Rhinol. Laryngol. 113, 552–557.

O'Reilly, M., Alpert, R., Jenkinson, S., Gladue, R.P., Foo, S., Trim, S., Peter, B., Trevethick, M., Fidock, M., 2002. Identification of a histamine H4 receptor on human eosinophils – role in eosinophil chemotaxis. J. Recept. Signal. Transduct. Res. 22, 431–448.

Oda, T., Morikawa, N., Saito, Y., Masuho, Y., Matsumoto, S., 2000. Molecular cloning and characterization of a novel type of histamine receptor preferentially expressed in leukocytes. J. Biol. Chem. 275, 36781–36786.

Oishi, R., Itho, Y., Saeki, K., 1992. Inhibition of turnover by 8-OH-DPAT, buspirone and 5-hydroxytryptophan in the mouse and rat brain. Naunyn Schmiedebergs Arch. Pharmacol. 345, 495–499.

Ookuma, K., Sakata, T., Fukagawa, K., Yoshimatsu, H., Kurokawa, M., Machidori, H., Fujimoto, K., 1993. Neuronal histamine in the hypothalamus suppresses food intake in rats. Brain Res. 628, 235–242.

Sakata, T., Ookuma, K., Fukagawa, K., Fujimoto, K., Yoshimatsu, H., Shiraishi, T., Wada, H., 1988. Blockade of the histamine H1-receptor in the rat ventromedial hypothalamus and feeding elicitation. Brain Res. 441, 403–407.

Schneider, E., Rolli-Derkinderen, M., Arock, M., Dy, M., 2002. Trends in histamine research: new functions during immune responses and hematopoiesis. Trends Immunol. 23, 255–263.

Smith, F.M., Haskelberg, H., Tracey, D.J., Moalem-Taylor, G., 2007. Role of histamine H3 and H4 receptors in mechanical hyperalgesia following peripheral nerve injury. Neuroimmunomodulation 14 (6), 317–325.

Strakhova, M.I., Nikkel, A., Manelli, A.M., Hsieh, G., Esbenshade, T.A., Brioni, J.D., Bitner, R.S., 2009. Localization of histamine H4 receptors in the central nervous system of human and rat. Brain Res. 1250, 41–48.

Terzioglu, N., Van Rijn, R.M., Bakker, R.A., De Esch, I.J.P., Leurs, 2004. Synthesis and structure–activity relationships of indole and benzimidazole piperazines as histamine H4 receptor antagonists. Bioorg. Med. Chem. Lett. 14 (21), 5251–5256.

Varga, C., Horvath, K., Berko, A., Thurmond, R.L., Dunford, P.J., Whittle, B.J., 2005. Inhibitory effects of histamine H4 receptor antagonists on experimental colitis in the rat. Eur. J. Pharmacol. 522, 130–138.

Yanai, K., Son, L.Z., Endou, M., Sakurai, E., Nakagawasai, O., Tadano, T., Kisara, K., Inoue, I., Watanabe, T., Watanabe, T., 1998. Behavioural characterization and amounts of brain monoamines and their metabolites in mice lacking histamine H1 receptors. Neuroscience 87, 479–487.