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

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A R T I C L E   I N F O

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

A B S T R A C T

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 anorexiant 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, antiinflammatory, anxiolytic and anorexiant effects induced by neuronal H4R agonism, suggesting that H4 modulators may have broader utility further the control of inflammatory and immune processes.

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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., 2004) 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...
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 pro-
tome suggested the participation of H4R in circadian cycle mod-
ulation, 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 ner-
vous 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 appa-
ratus consisted of a 40 cm square plane with 16 flux 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, automati-
cally 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 stop-watch 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 compart-
ments, 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 be-
haviors 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 imme-
diately before use. JNJ 10191584 (Sigma, Milan, Italy) was dispersed in 1% sodium
carboxymethylcellulose (CMC) and administered by gavage 30 min before behav-
ioral 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 intra-
cerebroventricular (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 (30 mg/kg) 30 min before, yoimbine (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 $\alpha_2$-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; $\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\cdot\·
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, 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 mg per mouse. At the dose of 50 mg there was a trend to higher values of number of falls without reaching the statistical significance whereas at the dose of 60 mg VUF 8430 produced a significant impairment in the motor coordination (Fig. 6A). The H4R antagonist JNJ 10191584 (9 mg/kg p.o.) did not
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 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.

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.

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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 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.

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.
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 sub-region, 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 compartment from which histaminergic neuron fibers originate. The pharmacological effects produced by VUF 8430 is very unlikely. 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). A main histaminergic compound 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-agonists 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 JN1 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 withdrawal 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 JN1 10191584 did not produce any motor coordination deficit of movement in rotarod performance assays. 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, antiinflammatory and anorexant effects mediated by H4R agonism.

Conflicts of interest
None.

Acknowledgments
This work was supported by grants from MIUR.

References