Neuropsychopharmacology (2004) 29, 441–449

www.neuropsychopharmacology.org

Role of H_3-Receptor-Mediated Signaling in Anxiety and Cognition in Wild-Type and *Apoe*$^{-/-}$ Mice

Gerald Bongers[1,2], Rob Leurs[2], Jennifer Robertson[3] and Jacob Raber*[1,3,4,5,6]

[1]Gladstone Institute of Neurological Disease, University of California, San Francisco, CA, USA; [2]Leiden/Amsterdam Center for Drug Research, Division of Medicinal Chemistry, Faculty of Chemistry, Vrije Universiteit, Amsterdam, The Netherlands; [3]Department of Behavioral Neuroscience, Oregon Health and Science University, Portland, OR, USA; [4]Department of Neurology, University of California, San Francisco, CA, USA; [5]Department of Neurology, Oregon Health and Science University, Portland, OR, USA; [6]Division of Neuroscience, ONPRC, Oregon Health and Science University, Portland, OR, USA

Increasing evidence supports a role for histamine as a neurotransmitter and neuromodulator in emotion and cognition. The H_3 receptor was first characterized as an autoreceptor that modulates histamine release and synthesis via negative feedback. Mice deficient in apoE (*Apoe*$^{-/-}$) have been used to define the role of apoE in brain function. In the present study, we investigated the possible role of histamine H_3-receptor-mediated signaling in anxiety and cognition in mice *Apoe*$^{-/-}$ and wild-type mice. H_3 antagonists increased measures of anxiety in wild-type, but not *Apoe*$^{-/-}$, mice. In contrast, H_3 antagonists similarly impaired object recognition in wild-type and *Apoe*$^{-/-}$ mice. In *Apoe*$^{-/-}$ mice, reduced negative feedback via H_3 receptors could contribute to increased signaling of H_1 receptors. *Apoe*$^{-/-}$ mice showed higher sensitivity to the anxiety-reducing effects of the H_1 receptor antagonist mepyramine than wild-type mice. These effects were dissociated from effects of mepyramine on the HPA axis. Compared to saline controls, mepyramine reduced plasma ACTH and corticosterone levels in wild-type, but not *Apoe*$^{-/-}$, mice. These data support a role for apoE in H_3 receptor signaling. H_3 antagonists were proposed as a treatment for cognitive disorders such as Alzheimer's disease, which is associated with increased anxiety and cognitive impairments. As H_3 antagonists increase measures of anxiety and impair object recognition in wild-type mice, the use of H_3 antagonists in cognitive disorders may be counterproductive and should be carefully evaluated.
Neuropsychopharmacology (2004) **29,** 441–449, advance online publication, 12 November 2003; doi:10.1038/sj.npp.1300352

Keywords: histamine; H_3 receptor; H_1 receptor; anxiety; HPA axis; apoE-deficient mice

INTRODUCTION

Increasing evidence supports a role for histamine as a neurotransmitter and neuromodulator in various brain functions, including cognition, emotion, and feeding (Timmerman, 1990; Onodera *et al*, 1994; Leurs *et al*, 1998). Histamine is also released by activation of heteroreceptors (Hill and Straw, 1988; Gulat-Marnay *et al*, 1989a, 1990; Prast *et al*, 1991; Ono *et al*, 1992; Chikai *et al*, 1994; Laitinen *et al*, 1995). Histaminergic neurons are concentrated in the tuberomammillary nucleus of the posterior basal hypothalamus (Panula *et al*, 1984; Watanabe *et al*, 1984) and project to various brain regions (Inagaki *et al*, 1988; Panula *et al*, 1989).

*Correspondence: J Raber, Departments of Behavioral Neuroscience and Neurology, L470, Oregon Health and Science University, 3181 S.W. Sam Jackson Park Road, Portland, OR 97201, USA, Tel: +1 503 494 1524, Fax: +1 503 494 6877, E-mail: raberj@ohsu.edu
Received 02 June 2003; revised 30 September 2003; accepted 03 October 2003
Online publication: 10 October 2003 at http://www.acnp.org/citations/Npp10100303246/default.pdf

H_3 receptors were first characterized as autoreceptors that modulate histamine synthesis and release through negative feedback (Arrang *et al*, 1983, 1987; Van der Werf *et al*, 1987; Jansen *et al*, 1998). They are highly expressed throughout the brain (Arrang *et al*, 1998; Lovenberg *et al*, 2000; Drutel *et al*, 2001) on presynaptic terminals of histaminergic and nonhistaminergic neurons and also modulate the release of other neurotransmitters (Schlicker *et al*, 1988, 1989, 1993; Fink *et al*, 1990; Clapham and Kilpatrick, 1992). As H_3 receptors display high constitutive activity, previously known antagonists were reclassified as inverse agonists (Morisset *et al*, 2000; Wieland *et al*, 2001).

Apolipoprotein E (apoE), which is important in lipoprotein and cholesterol metabolism (Mahley, 1988), has been implicated in nerve development and regeneration, neurite outgrowth, and neuroprotection (Weisgraber and Mahley, 1996). Mice deficient in apoE (*Apoe*$^{-/-}$) (Piedrahita *et al*, 1992; Plump *et al*, 1992) have been used to define the role of apoE in brain function. Cardiac and serum histamine levels are higher in *Apoe*$^{-/-}$ than wild-type mice and *Apoe*$^{-/-}$ mice have 37% more cardiac mast cells (Huang *et al*, 2002). Mast cells migrate from blood to brain (Silverman *et al*, 2000)

and brains of *Apoe*$^{-/-}$ mice might also be exposed to higher histamine levels than wild-type mice. In the present study, we analyzed *Apoe*$^{-/-}$ mice to investigate the possible role of apoE in the regulation of the H_3-receptor-mediated signaling.

In the present study, we demonstrate that H_3 antagonists increase anxiety in wild-type, but not *Apoe*$^{-/-}$, mice. The differential effects of H_3 antagonists on measures of anxiety were not seen on nonspatial and emotional learning and memory. As *Apoe*$^{-/-}$ mice showed higher sensitivity to the anxiety-reducing effects of the H_1 receptor antagonist mepyramine than wild-type mice, reduced negative feedback via H_3 receptors could contribute to increased signaling of H_1 receptors in *Apoe*$^{-/-}$ mice.

Experimental Procedures

Animals. Male *Apoe*$^{-/-}$ (C57BL/6J-Apoe^{tm1Unc}) and wild-type C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Young (3–5 months old) *Apoe*$^{-/-}$ and wild-type mice were used in all the experiments, as indicated ($n = 5$–19 mice per group). Young *Apoe*$^{-/-}$ mice were studied to evaluate a potential role of apoE on H_3-receptor-mediated signaling, as adult *Apoe*$^{-/-}$ mice show age-dependent structural and functional alterations in the cortex and hippocampus (Masliah *et al*, 1995; Raber *et al*, 1998; Buttini *et al*, 1999). Such alterations could cause secondary changes in H_3-receptor-mediated signaling. Mice were housed under conditions of constant temperature (18°C), light from 06:00 to 13:00, and free access to food and water. To minimize the effects of social influences on behavior, mice were housed individually 24 h before behavioral testing. Otherwise, they were group-housed. To avoid circadian variation, the mice were tested and killed between 10:00 and 14:00. All animal experiments were carried out in accordance with the National Institute of Health Guide for the Care and Use of Laboratory Animals.

Drugs. The H_3 ligands thioperamide and clobenpropit, the H_1 ligand mepyramine maleate (pyrilamine maleate), and the H_2 ligand zolantidine were gifts from Dr H Timmerman and Dr R Leurs (Department of Pharmacochemistry, Free University of Amsterdam, The Netherlands). Drugs were dissolved in saline and administered by intraperitoneal injection 1 h before behavioral testing at a dose of 5 mg/kg (thioperamide), 5.6 mg/kg (mepyramine), or 10 mg/kg (clobenpropit and zolantidine). The person testing the mice was blinded to genotype and treatment. The doses were selected based on reported studies.

Elevated plus maze. Anxiety levels were assessed with an elevated, plus-shaped maze consisting of two open arms and two closed arms equipped with rows of infrared photocells interfaced with a computer (Hamilton-Kinder, Poway, CA), as described (Raber *et al*, 2000). Mice were placed individually in the center of the maze and allowed free access for 10 min. They could spend their time either in a closed safe area (closed arms) or in an open area (open arms). Recorded beam breaks were used to calculate the time spent in the open arms, the distance moved in the open arms, entries into the open arms, and the number of times the mice extended over the edges of the open arms. Reductions in these measures indicate increased anxiety. After behavioral testing, the equipment was cleaned with 5% acetic acid to remove odors.

Novel object recognition. Novel object recognition was used to evaluate nonspatial learning and memory, as described (Raber *et al*, 2002) according to Rampon *et al* (2000). On each of 3 consecutive days, the mice were habituated to a Plexiglas enclosure (16 × 16 inches, open from the top) for 5 min. On day 4, they were allowed to explore the enclosure containing two objects, placed in equivalent positions (1 cm from the edge), for 15 min. On day 5, the mice were put in the enclosure for 15 min. One of the objects was replaced by a replica and the other one with a novel object. The amount of time the animals spent exploring the objects on days 4 and 5 was recorded with two stopwatches. Exploring was defined as close investigation within 1 cm of the objects, including sniffing of the objects. Between trials, the enclosure was cleaned with 5% acetic acid.

Dissection of brain regions. The mice were killed by cervical dislocation, and their brains were rapidly removed. A sagittal cut was made along the midline, and the whole hippocampus and cortex were dissected. Hypothalamic and amygdaloid regions were dissected out as described (Raber *et al*, 1994). The hypothalamus was defined as the region between two vertical cuts starting from the two lateral hypothalamic sulci and a horizontal cut through the mammillothalamic tracts and the rhomboid thalamic nucleus. The dissection of the amygdala was defined as the region between a vertical cut tangential to the external capsule and a diagonal cut along the medial border of the ipsilateral optic tract. The dissected brain regions were frozen on dry ice and stored at −80°C until use.

Histamine receptor binding. Membranes from the brain regions described above were prepared by homogenization in 10 volumes (wt/vol) of 50 mM sodium-PBS, pH 7.5, at 4°C containing EDTA (10 mM), 0.1 mMphenylmethylsulfonyl fluoride, 0.004 mg/ml chymostatin, 0.004 mg/ml leupeptin, and centrifugation at 40 000*g* for 30 min at 4°C. The pellet was resuspended in 5 ml of water and lysed for 30 min on ice. The centrifugation and lysing steps were repeated, followed by centrifugation at 40 000*g* for 30 min at 4°C. The final membrane pellets were resuspended in water and stored frozen at −80°C until use. H_3 receptor binding was determined with [^{3}H]N$^\alpha$-methylhistamine ([^{3}H]NAMH) as described (Tedford *et al*, 1995). Before use, the pellets were dissolved in distilled water and homogenized for 2 s by sonication. The homogenates (100 μg of protein) were incubated for 40 min at 25°C with increasing concentrations of [^{3}H]NAMH (82.0 Ci/mmol) in 50 mM sodium phosphate buffer, pH 7.4, in the presence or absence of 10 μM thioperamide. The reaction was terminated by rapid dilution with 3 ml of ice cold 50 mM Tris, pH 7.4, and filtration through Whatmann GF/B filters that had been pretreated with polyethylenimine (0.3%), followed by two subsequent washes with 3 ml of 50 mM Tris, pH 7.4. Retained radioactivity was determined by liquid scintillation counting. Nonspecific binding was defined with 10 μM thioperamide as competing ligand. H_1 receptor binding was

determined with [^{3}H]mepyramine (20 Ci/mmol), binding was performed similar to H_3 using conditions as described (Yanai *et al*, 1998). Briefly, the homogenates were incubated for 30 min at 25°C in a 50 mM Na^+/K^+-phosphate buffer, pH 7.4, in the presence or absence of 1 μM mianserin. The reaction was terminated by rapid dilution with 3 ml of ice cold 50 mM Na^+/K^+-phosphate buffer, pH 7.4. Protein concentrations were determined spectrophotometrically with the Bradford reagent (Bradford, 1976), with bovine serum albumin as a standard.

Plasma corticosterone and ACTH. Plasma corticosterone and ACTH were determined using commercial radioimmunoassays from ICN. The intra- and interassay variation were both 7%. Plasma ACTH was determined using commercial radioimmunoassays from Phoenix Pharmaceuticals. The intra- and interassay variation were 9 and 10%, respectively.

Statistical analysis. Data are expressed as mean ± SEM. Statistical analyses were carried out with Prism 3.0. The statistical differences between groups were determined by ANOVA, followed by a Tukey–Kramer *post hoc* test when appropriate; $p < 0.05$ was considered significant.

RESULTS

Effects of the H_3 Antagonist Thioperamide on Measures of Anxiety in Wild-Type and *Apoe*$^{-/-}$ Mice

Anxiety levels in young wild-type and *Apoe*$^{-/-}$ mice (3–5 months old) were assessed in the elevated plus maze 1 h after intraperitoneal administration of thioperamide or saline. Wild-type mice treated with thioperamide showed increased measures of anxiety as compared to wild-type mice treated with saline (Figure 1a–d). The total activity in the closed arms was comparable and not significantly different between the saline- and thioperamide-treated wild-type mice, indicating that the differences in measures in the open arms were not caused by differences in activity levels. In contrast, thioperamide had no effect on measures of anxiety in *Apoe*$^{-/-}$ mice (Figure 1a–d).

Effects of H_3 Ligands on Novel Object Recognition in Wild-Type and *Apoe*$^{-/-}$ Mice

Next we determined whether in wild-type and *Apoe*$^{-/-}$ mice H_3 antagonists also have differential effects on novel object recognition (Rampon *et al*, 2000), as described previously (Raber *et al*, 2002). During the training session, mice were allowed to explore for 15 min an open field containing two objects. For the retention session (24 h later), they were placed back into the same open field for 15 min, after one of the familiar objects had been replaced with a novel object and the other familiar object with an exact replica. The percentage of time the mice spent exploring the novel *vs* the familiar object relative to the total amount of time they explored either object in the retention session was used as a measure of object recognition memory. Wild-type and *Apoe*$^{-/-}$ mice (3–5 months old) received saline, thioperamide, or clobenpropit during the training (day 4) and retention (day 5) sessions. The recently cloned H_4 receptor

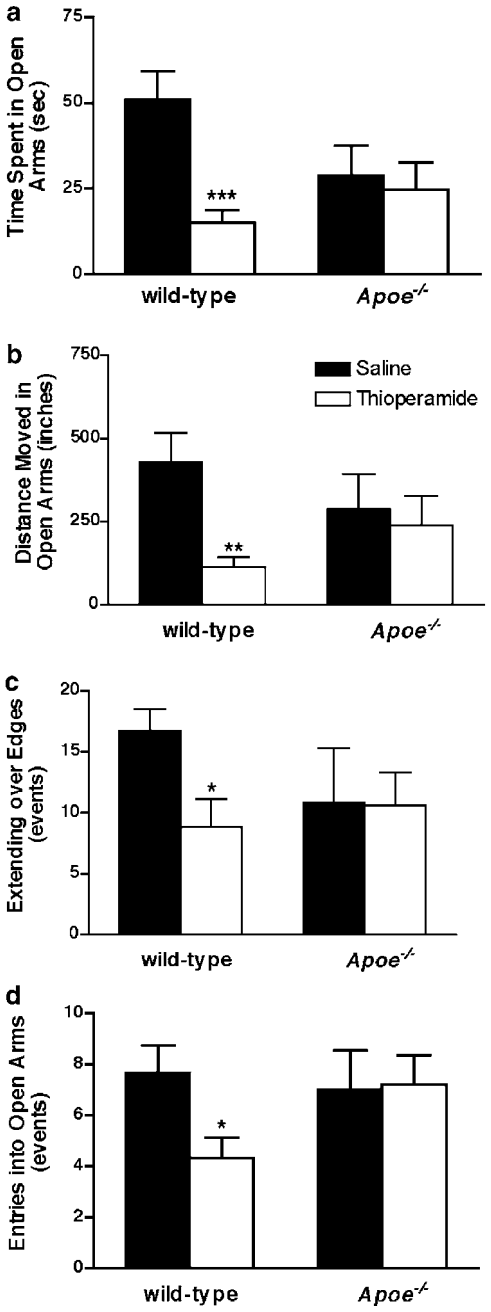

Figure 1 Measures of anxiety levels in thioperamide-treated male wild-type and *Apoe*$^{-/-}$ mice in the elevated plus maze. Compared with saline controls, wild-type mice that received thioperamide showed significant reductions in time spent in the open arms (a), in distance moved in the open arms (b), in the number of extensions over the edges of the open arms (c), and in the number of entries into the open arms (d), indicating increased measures of anxiety after treatment with thioperamide. *Apoe*$^{-/-}$ mice showed no significant change in any of these measures of anxiety. None of the differences between wild-type and *Apoe*$^{-/-}$ mice were significant. ***$p < 0.001$, **$p < 0.01$, *$p < 0.05$ *vs* saline controls; $n = 5$–15 mice per group. Wild-type mice saline, $n = 15$; thioperamide, $n = 15$; *Apoe*$^{-/-}$ mice: saline, $n = 7$; thioperamide, $n = 8$.

(Oda *et al*, 2000; Liu *et al*, 2001; Morse *et al*, 2001; Nguyen *et al*, 2001; Zhu *et al*, 2001) was found to have an affinity for H_3-specific ligands. To rule out the possible contribution of the H_4 receptor to the effects of thioperamide, we also treated wild-type and *Apoe*$^{-/-}$ mice with clobenpropit,

a H_3-specific antagonist that was reported to be an H_4 receptor agonist as well (Oda *et al*, 2000). In the training session, all groups of mice spent a comparable amount of time exploring each object. In the retention session, the saline-treated wild-type and *Apoe*$^{-/-}$ mice spent significantly more time exploring the novel object (wild type: 8.37 ± 0.93 s; *Apoe*$^{-/-}$: 9.34 ± 2.76 s) than the familiar object (wild type: 5.43 ± 0.69 s; *Apoe*$^{-/-}$: 5.15 ± 1.25 s) (Figure 2a, b), whereas the thioperamide- and clobenpropit-treated wild-type and *Apoe*$^{-/-}$ mice spent equal amounts of time exploring both objects. The similar effects of thioperamide and clobenpropit on novel object recognition suggest that these effects are mediated by the H_3 receptor and not the H_4 receptor. Thus, the H_3 antagonists similarly impaired object recognition in wild-type and *Apoe*$^{-/-}$ mice (Figure 2a, b).

H_3 Expression Levels in Wild-Type and *Apoe*$^{-/-}$ Mice

To determine whether there are differences in H_3 receptor expression in young *Apoe*$^{-/-}$ and wild-type mice (3–5 months old), which could have contributed to their differential response to H_3 antagonists on measures of anxiety, we performed saturation analysis with [^{3}H]NAMH in brain regions that have been implicated in cognition or emotion. The total number of receptors (B_{max} in fmol/mg protein) in the amygdala (wild type: 87.3 ± 2.5; *Apoe*$^{-/-}$: 81.8 ± 2.3), cortex (wild type: 119.9 ± 3.0; *Apoe*$^{-/-}$: 56.8 ± 5.8), and hippocampus (wild type: 108.4 ± 10.5; *Apoe*$^{-/-}$: 29.1 ± 1.7) was significantly lower in *Apoe*$^{-/-}$ than in wild-type mice (Figure 3). In the hypothalamus, B_{max} was not significantly different between the groups. No significant difference was found in the binding affinities (K_d) of [^{3}H]NAMH in any of the brain regions. Thus, compared to wild-type controls, *Apoe*$^{-/-}$ mice have a region-specific downregulation of the H_3 receptor, without a change in affinity for H_3-specific ligands in brain regions implicated in cognition and emotion.

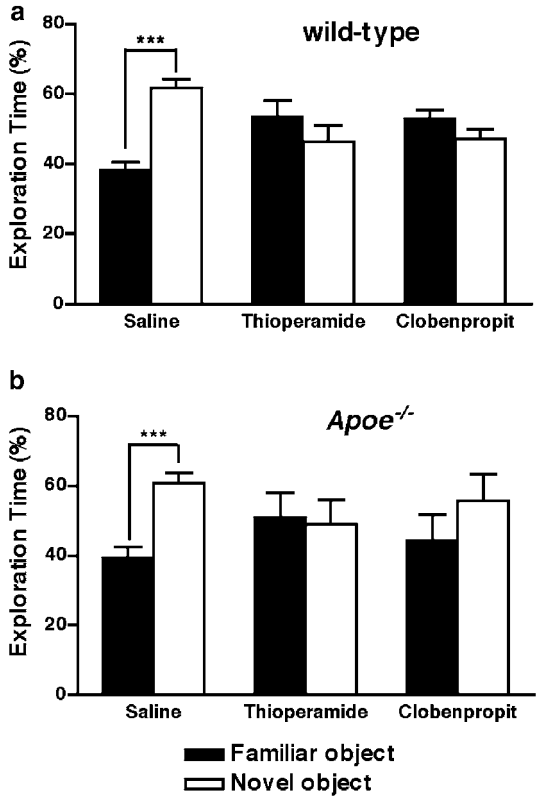

Figure 2 Novel object recognition in thioperamide- and clobenpropit-treated wild-type and *Apoe*$^{-/-}$ male mice. The percentages of time spent exploring the novel and familiar objects on day 5 of testing are shown. Only saline-treated mice spent significantly more time exploring the novel object. ***$p<0.001$ novel *vs* familiar object; $n=5$–19 mice per group. Wild-type mice saline, $n=19$; thioperamide, $n=15$; clobenpropit, $n=6$; *Apoe*$^{-/-}$ mice: saline, $n=10$; thioperamide, $n=5$; clobenpropit, $n=6$.

Increased Sensitivity of *Apoe*$^{-/-}$ Mice to the Effects of the H_1 Antagonist Mepyramine on Measures of Anxiety

In *Apoe*$^{-/-}$ mice, reduced negative feedback via H_3 receptors could increase signaling of H_1 and H_2 receptors. To

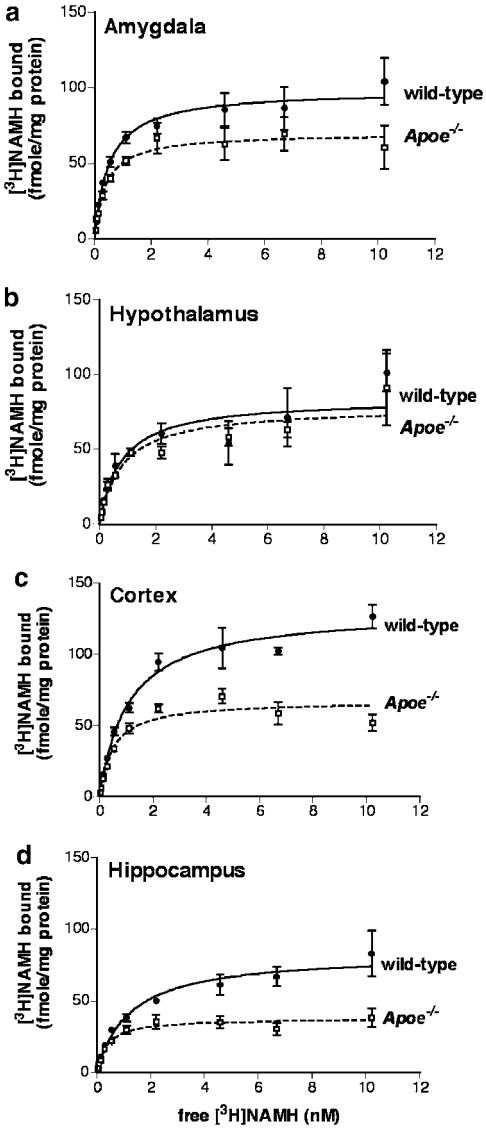

Figure 3 Saturation curve of [^{3}H]NAMH in amygdala (a), hypothalamus (b), cortex (c), and hippocampus (d), of male wild-type (solid line, ●) and *Apoe*$^{-/-}$ (dashed line, □) mice. Nonspecific binding was determined in the presence of 10 μM thioperamide. Significant differences in total number of receptors were seen in amygdala ($p<0.05$), cortex ($p<0.001$), and hippocampus ($p<0.001$). $n=7$ pooled mice per brain region.

determine whether in *Apoe*$^{-/-}$ mice there is increased H_1- or H_2-receptor-mediated signaling, wild-type and *Apoe*$^{-/-}$ mice were assessed in the elevated plus maze 1 h after intraperitoneal administration of the H_1 antagonist mepyramine, the H_2 antagonist zolantidine, or saline. *Apoe*$^{-/-}$ mice treated with mepyramine, but not with zolantidine, showed reduced measures of anxiety as compared to *Apoe*$^{-/-}$ mice treated with saline (Figure 4a–d). The total activity in the closed arms was comparable and not significantly different between the saline-, mepyramine-, and zolantidine-treated *Apoe*$^{-/-}$ mice, indicating that the differences in measures in the open arms were not caused by differences in activity levels. In contrast, mepyramine and zolantidine had no effect on measures of anxiety in wild-type mice (Figure 4a–d). Thus, compared to wild-type controls, *Apoe*$^{-/-}$ mice show an increased sensitivity to the effects of H_1 receptor blockade on measures of anxiety. To determine whether in *Apoe*$^{-/-}$ the effects of mepyramine on measures of anxiety in the plus maze were associated with an altered HPA axis response (Knigge *et al*, 1999), we also measured plasma ACTH and corticosterone levels directly after plus maze testing, as described previously (Raber *et al*, 2000). Compared to saline controls, mepyramine reduced the plasma corticosterone levels in wild-type (saline: 179 ± 38 pg/ml, $n=6$; mepyramine: 89 ± 26 pg/ml, $n=6$; $p<0.05$ Tukey–Kramer), but not in *Apoe*$^{-/-}$ (saline: 206 ± 30 pg/ml, $n=8$; mepyramine: 224 ± 10 pg/ml, $n=9$), mice. Mepyramine also reduced plasma ACTH levels in wild-type (saline: 121 ± 20 pg/ml, $n=6$; mepyramine: 77 ± 9 pg/ml, $n=6$; $p<0.05$ Tukey–Kramer), but not in *Apoe*$^{-/-}$, mice (saline: 57 ± 5 pg/ml, $n=8$; mepyramine: 62 ± 11 pg/ml, $n=9$). Thus, in the plus maze, the effects of mepyramine on measures of anxiety are dissociated from those on the HPA axis.

H_1 Expression Levels in Wild-Type and *Apoe*$^{-/-}$ Mice

To determine whether there are differences in H_1 receptor expression in young *Apoe*$^{-/-}$ and wild-type mice (3–5 months old), which could have contributed to their differential response to mepyramine on measures of anxiety, we performed saturation analysis with [^{3}H]mepyramine in brain regions that have been implicated in cognition or emotion. There was no difference in the total number of H_1 receptors or H_1 receptor binding affinity in the amygdala, cortex, hippocampus, or hypothalamus (Figure 5 and Table 1).

DISCUSSION

This study shows that H_3 antagonists increased measures of anxiety in wild-type, but not in *Apoe*$^{-/-}$, mice. In contrast, *Apoe*$^{-/-}$ mice showed a higher sensitivity to the anxiety-reducing effects of H_1 receptor blockade than wild-type mice. The effects of H_1 receptor blockade on measures of anxiety were dissociated from those on the HPA axis response. These differential effects of H_3 antagonists on measures of anxiety were not seen on object recognition.

H_1 receptor blockade did not reduce measures of anxiety in wild-type C57Bl/6J mice. This is consistent with the lack of effect of H_1 receptor blockade on measures of anxiety in wild-type ddY mice. The reduced measures of anxiety in *Apoe*$^{-/-}$ mice after H_1 receptor blockade might be caused by increased levels of histamine release. H_1 receptors become activated at levels of histamine release higher than normal

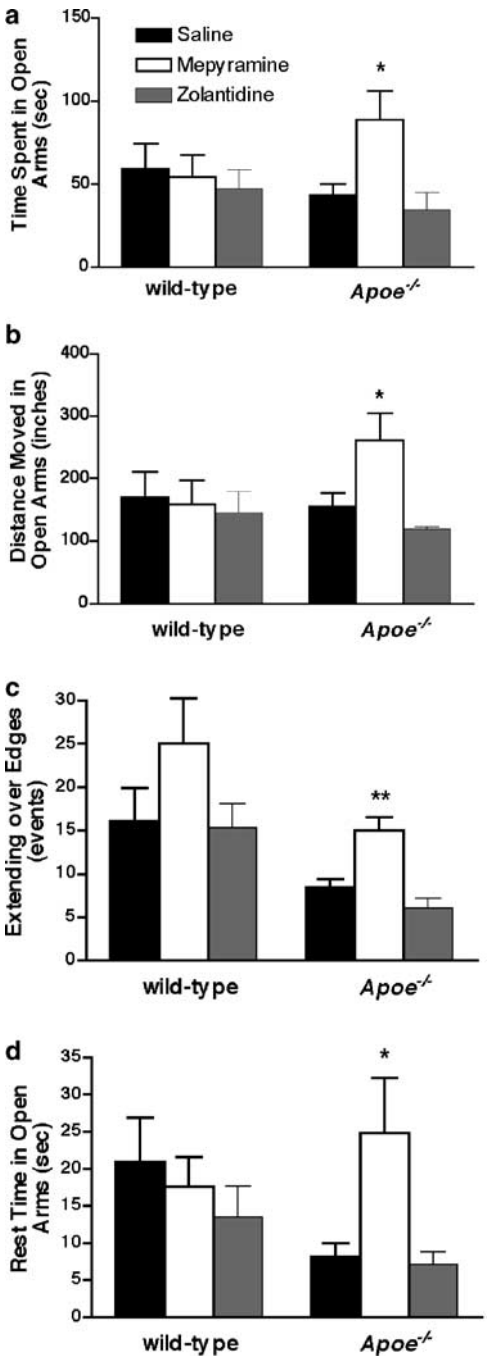

Figure 4 Measures of anxiety in mepyramine- and zolantidine-treated male wild-type and *Apoe*$^{-/-}$ mice in the elevated plus maze. Compared with saline controls, *Apoe*$^{-/-}$ mice that received mepyramine showed significant increases in time spent in the open arms (a), in distance moved in the open arms (b), in the number of extensions over the edges of the open arms (c), and in the rest time in the open arms (d), indicating decreased measures of anxiety after treatment with mepyramine. Wild-type mice showed no significant change in any of these measures of anxiety. None of the differences between wild-type and *Apoe*$^{-/-}$ mice were significant. $^{**}p<0.01$, $^{*}p<0.05$ *vs* saline controls. Wild-type mice: saline, $n=9$; mepyramine, $n=8$; zolantidine, $n=8$; *Apoe*$^{-/-}$ mice: saline, $n=7$; mepyramine, $n=7$; zolantidine, $n=5$.

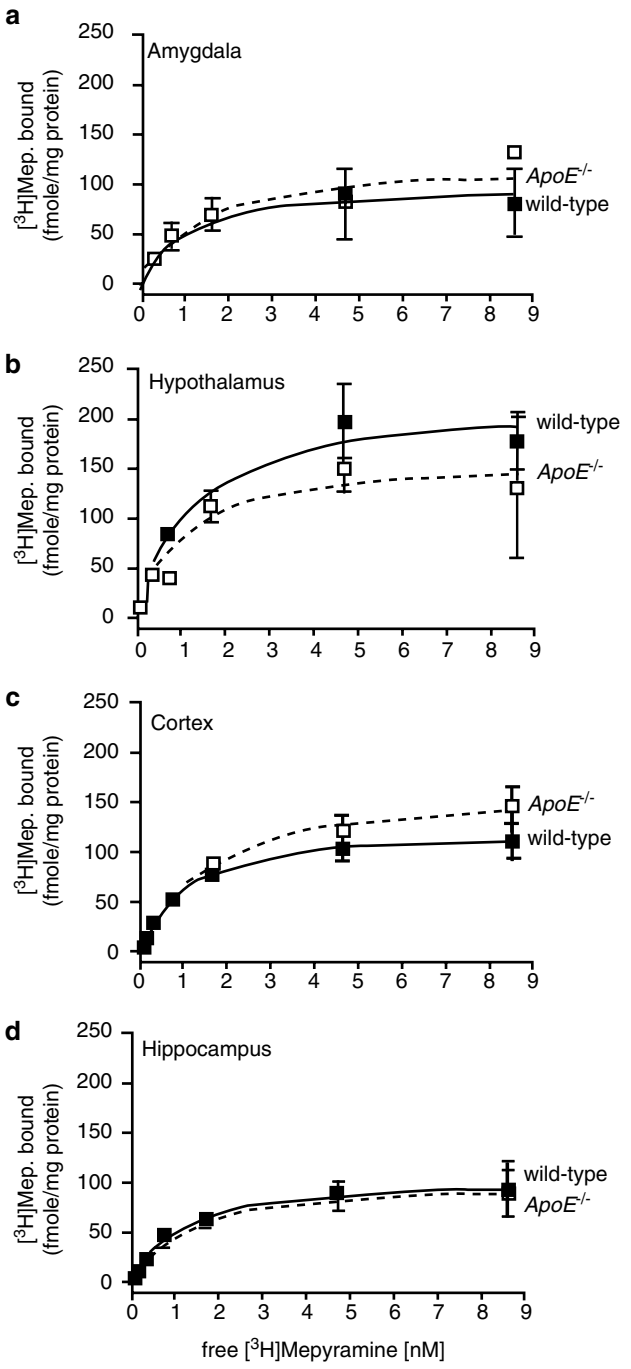

Figure 5 Saturation curve of [^{3}H]mepyramine in the amygdala (a), hypothalamus (b), cortex (c), and hippocampus (d), of male wild-type (solid line, ●) and *Apoe*$^{-/-}$ (dashed line, □) mice. Nonspecific binding was determined in the presence of 10 μM thioperamide. No Significant differences in total number of receptors or receptor affinity were seen in any brain region. $n = 7$ pooled mice per brain region.

(Yuzurihara *et al*, 2000), mepyramine has antagonizing effects of on experimental anxiety induced by histamine releasers (Imaizumi and Onodera, 1993; Yuzurihara *et al*, 2000), and the H_1 receptor agonist and H_3 receptor antagonist betahistine has anxiogenic effects (Imaizumi *et al*, 1996).

The effects of H_1 receptor blockade on measures of anxiety were dissociated from those on the HPA axis response. While mepyramine reduced measures of anxiety in *Apoe*$^{-/-}$, but not wild-type, mice, it reduced plasma ACTH and corticosterone levels in wild-type, but not *Apoe*$^{-/-}$, mice. These data suggest that in *Apoe*$^{-/-}$ mice mepyramine does not reduce measures of anxiety by inhibiting the HPA axis response. The dissociation of the effects of H_1 receptor blockade on anxiety from those on the HPA axis in *Apoe*$^{-/-}$ and wild-type mice and the differential effects of H_3 receptor blockade on novel object recognition and anxiety in *Apoe*$^{-/-}$, but not wild-type, mice suggest that differential pharmacokinetic profiles of histaminergic drugs in the two genotypes do not underlie the behavioral results.

Table 1 H_1 Expression Levels in Wild-Type and *Apoe*$^{-/-}$ Mice[a]

Mouse	Brain region	B_{max} (fmol/mg protein)	K_d (nM)
Wild type	Amygdala	103.4 ± 13.16	1.06 ± 0.40
	Hypothalamus	218.7 ± 22.33	1.26 ± 0.40
	Cortex	126.5 ± 8.472	0.978 ± 0.22
	Hippocampus	104.0 ± 10.13	1.02 ± 0.31
ApoE$^{-/-}$	Amygdala	120.9 ± 20.92	1.17 ± 0.59
	Hypothalamus	159.4 ± 29.00	0.989 ± 0.62
	Cortex	170.0 ± 13.02	1.62 ± 0.37
	Hippocampus	98.66 ± 11.03	0.976 ± 0.36

[a]B_{max} or K_d were determined as described under histamine receptor binding. There were no significant genotype differences in either the B_{max} or K_d in any brain region.

Thioperamide, at 5 mg/kg, increased measures of anxiety in wild-type mice. In a previous study in which the elevated plus maze was used to assess the anxiety of rats after the administration of 2 mg/kg thioperamide, the time spent in the open arms was reduced, but not significantly (Perez-Garcia *et al*, 1999). These data indicate that thioperamide doses higher than 2 mg/kg are required to obtain significant increased measures of anxiety.

In rodents, H_3 antagonists improved performance in the modified elevated plus maze test (Miyazaki *et al*, 1995a; Onodera *et al*, 1998; Perez-Garcia *et al*, 1999). However, in the elevated plus maze H_3 antagonists increase measures of anxiety, which could have contributed to the altered performance in the modified elevated plus maze tests.

While not statistically significant, several measures of anxiety (Figures 1 and 4) showed subtle genotype differences. It is unlikely though that the differential sensitivity of wild-type and mice to thioperamide or mepyramine are due to subtle differences in baseline behaviors. In Figure 1d, in which there were no baseline differences in wild-type and *Apoe*$^{-/-}$ mice, thioperamide decreased extending over the edges in wild-type but not *Apoe*$^{-/-}$ mice. Similarly, in Figure 4b, in which there were no baseline differences in wild-type and *Apoe*$^{-/-}$ mice, mepyramine increased the distance moved in the open arms in *Apoe*$^{-/-}$ but not in wild-type mice.

Apoe$^{-/-}$ mice have lower levels of H_3 expression, without a change in affinity for H_3-specific ligands, in the amygdala, hippocampus, and cortex than age-matched wild-type controls. In contrast, *Apoe*$^{-/-}$ and wild-type mice have similar levels of H_1 expression and a similar affinity for H_1-specific ligands in the amygdala, hypothalamus, hippocampus, and cortex. These data indicate that there is no simple

association between levels of H_1 and H_3 receptor expression in structures associated with anxiety *vs* cognition which could explain why H_3 antagonists impaired hippocampus- and cortex-dependent novel object recognition (Rampon *et al*, 2000) but did not increase more amygdala-dependent measures of anxiety in the plus maze or why *Apoe*$^{-/-}$ mice were more sensitive to the anxiety-reducing effects of mepyramine. Interestingly, in young *Apoe*$^{-/-}$ mice the enhancement of the α-amino-3-hydroxy-5-methyl-4-isoxazole propionate (AMPA) receptors by phosphatidylserine (PS) was abolished without genotype changes in AMPA receptor binding (Valastro *et al*, 2001). These effects on the function of specific receptors may be related to the role of apoE in cholesterol transport. In *Apoe*$^{-/-}$ mice, the distribution of cholesterol in the synaptic plasma membrane is altered (Igbavboa *et al*, 1997), which can change the function of specific membrane receptors (Sooksawate and Simmonds, 2001). For example, altered membrane cholesterol in *Apoe*$^{-/-}$ mice reduces the potency of GABA at the $GABA_A$ receptor (Sooksawate and Simmonds, 2001). Similarly, by altering the lipid environment, apoE deficiency may reduce the total number of H_3 receptor binding sites available for ligand binding in the amygdala, hippocampus, and cortex.

Recently, the H_4 receptor was cloned and characterized (Nakamura *et al*, 2000; Oda *et al*, 2000; Liu *et al*, 2001; Morse *et al*, 2001; Nguyen *et al*, 2001; Zhu *et al*, 2001). To determine whether H_4 signaling was involved in the observed effects on object recognition, we tested mice treated with the H_4 receptor agonist and H_3 receptor antagonist clobenpropit (Oda *et al*, 2000). As clobenpropit, like thioperamide, impaired object recognition test, it is likely that the effects of histamine signaling do not involve a direct histamine H_4 receptor activation.

Our data demonstrate that H_3 receptor blockade impairs novel object recognition in wild-type and *Apoe*$^{-/-}$ mice. Consistent with our data, H_3 receptor stimulation by R^{α}-methylhistamine improved memory retention of naive rats in the water maze (Rubio *et al*, 2002) and H_3 receptor blockade impaired social memory (Prast *et al*, 1996). Administration of thioperamide, histamine, or histidine, decreased investigation time of a juvenile rat by an adult rat while immepip or inhibition of histamine synthesis by α-fluoromethylhistidine prolonged recognition time (Prast *et al*, 1996). However, H_3 receptor blockade might be beneficial under condition of cognitive impairments caused by cholinergic dysfunction (Miyazaki *et al*, 1995a, b, 1997; Blandina *et al*, 1996, 1998; Gulat-Marnay *et al*, 1989a, b; Giovannini *et al*, 1999; Molinengo *et al*, 1999). In a two-arm maze using a metal box and a glass bottle as objects in the retention trial and two identical objects in the first trial, H_3 receptor blockade antagonized scopolamine-induced object recognition impairments and object recognition impairments at 90- and 120-min intertrial intervals at which male and female rats, respectively, did not exhibit object recognition anymore in this version of the test (Ghi *et al*, 1999).

Several studies have implicated the histaminergic system in cognitive tasks (Passani *et al*, 2000). Based on the effects of H_3 receptor blockade on scopolamine-induced amnesia and on retention of animals trained to avoid a footshock in repeated acquisition avoidance models of attention deficit hyperactivity disorder (ADHD) and other disorders in which vigilance and impulsivity are impaired (Fox *et al*, 2002), H_3 receptor antagonists were suggested as treatment for cognitive disorders, including Alzheimer's disease (AD) (Airaksinen *et al*, 1991; Nakamura *et al*, 1993). However, reduced brain histamine levels in AD (Mazurkiewicz-Kwilecki and Nsonwah, 1989; Panula *et al*, 1998) have not been consistently demonstrated (Cacabelos *et al*, 1989). The data reviewed above suggest that H_3 receptor blockade might only be beneficial under condition of cognitive impairments caused by cholinergic dysfunction, which could relate to the ability of thioperamide to increase the release of acetylcholine (Clapham and Kilpatrick, 1992). However, H_3 receptor agonists improved cognition and reduced muscarinic antagonist-induced cognitive deficits in the water maze (Smith *et al*, 1994). As AD patients already show cognitive deficits and increased anxiety (Ferretti *et al*, 2001), the use of H_3 antagonists may be counterproductive and should be carefully evaluated.

ACKNOWLEDGEMENTS

We thank Stephen Ordway and Gary Howard for editorial assistance. We thank Anthony LeFevour for technical assistance. This work was supported by grants from the National Alzheimer's Association (to JR), the National Institute on Aging (AG20904 to JR), the Ellison Medical Foundation (Ag-NS-0201), and Dr Saal van Zwanenbergstichting (to GB).

REFERENCES

Airaksinen MS, Paetau A, Paljarvi L, Reinikainen K, Riekkinen P, Suomalainen R *et al* (1991). Histamine neurons in human hypothalamus: anatomy in normal and Alzheimer disease brains. *Neuroscience* **44**: 465–481.

Arrang JM, Garbarg M, Schwartz JC (1983). Auto-inhibition of brain histamine release mediated by a novel class (H_3) of histamine receptor. *Nature* **302**: 832–837.

Arrang JM, Garbarg M, Schwartz JC (1987). Autoinhibition of histamine synthesis mediated by presynaptic H_3-receptors. *Neuroscience* **23**: 149–157.

Arrang JM, Morisset S, Pillot C, Schwartz JC (1998). Subclassification of histamine receptors, H_3 receptor subtypes? Localization of H_3 receptors in the brian. In: Leurs R, Timmerman H (eds). *The Histamine H3 Receptor*. Elsevier: Amsterdam. pp 1–12.

Blandina P, Bacciottini MG, Giovannini MG, Mannaoioni PF (1998). H_3 receptor modulation of the release of neurotransmitters *in vivo*. In: Leurs R, Timmerman H (eds). *The Histamine H_3 Receptor*. Elsevier: Amsterdam. pp 1–12.

Blandina P, Giorgetti M, Bartolini L, Cecchi M, Timmerman H, Leurs R *et al* (1996). Inhibition of cortical acetylcholine release and cognitive performance by histamine H_3 receptor activation in rats. *Br J Pharmacol* **119**: 1656–1664.

Bradford MM (1976). A rapid and sensitive method for the quantification of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* **72**: 248–254.

Buttini M, Orth M, Bellosta S, Akeefe H, Pitas RE, Wyss-Coray T *et al* (1999). Expression of human apolipoprotein E3 or E4 in the brains of *Apoe*–/– mice: isoform-specific effects on neurodegeneration. *J Neurosci* **19**: 4867–4880.

Cacabelos R, Yamatodani A, Niigawa H, Hariguchi S, Tada K, Nishimura T *et al* (1989). Brain histamine in Alzheimer's disease. *Methods Find Exp Clin Pharmacol* **11**: 353–360.

Chikai T, Oishi R, Saeki K (1994). Increase in the extracellular histamine concentration in the rat striatum by -opioid receptor activation. *J Neurochem* **62**: 724–729.

Clapham J, Kilpatrick GJ (1992). Histamine H_3 receptors modulate the release of [^{3}H]-acetylcholine from slices of rat entorhinal cortex: evidence for the possible existence of H_3 receptor subtypes. *Br J Pharmacol* **107**: 919–923.

Drutel G, Peitsaro N, Karlstedt K, Wieland K, Smit MJ, Timmerman H *et al* (2001). Identification of rat H_3 receptor isoforms with different brain expression and signaling properties. *Mol Pharmacol* **59**: 108.

Ferretti L, McCurry SM, Logsdon R, Gibbons L, Teri L (2001). Anxiety and Alzheimer's disease. *J Geriatr Psychiatry Neurol* **14**: 52–58.

Fink K, Schlicker E, Neise A, Gothert M (1990). Involvement of presynaptic H_3 receptors in the inhibitory effect of histamine on serotonin release in the rat brain cortex. *Naunyn Schmiedebergs Arch Pharmacol* **342**: 513–519.

Fox GB, Pan JB, Esbenschade TA, Bennai YL, Black LA, Faghih R *et al* (2002). Effects of histamine H3 receptor ligands GT-2331 and ciproxifan in a repeated acquisition avoidance response in the spontaneously hypertensive rat pup. *Behav Brain Res* **131**: 151–161.

Ghi P, Orsetti M, Gamalero SR, Ferretti C (1999). Sex differences in memory performance in the object recognition test. Possible role of histamine receptors. *Pharmacol Biochem Behav* **64**: 761–766.

Giovannini MG, Bartolini L, Bacciottini L, Greco L, Blandina P (1999). Effects of histamine H_3 receptor agonists and antagonists on cognitive performance and scopolamine-induced amnesia. *Behav Brain Res* **104**: 147–155.

Gulat-Marnay C, Lafitte A, Arrang JM, Schwartz JC (1989a). Modulation of histamine release and synthesis in the brain mediated by 2-adrenoceptors. *J Neurochem* **53**: 513–518.

Gulat-Marnay C, Lafitte A, Arrang JM, Schwartz JC (1989b). Regulation of histamine release and synthesis in the brain by muscarinic receptors. *J Neurochem* **52**: 248–254.

Gulat-Marnay C, Latte H, Arrang JM, Schwartz JC (1990). Modulation of histamine release in the rat brain by kappa-opioid receptors. *J Neurochem* **55**: 17–53.

Hill SJ, Straw RM (1988). Alpha 2-adrenoceptor-mediated inhibition of histamine release from rat cerebral cortical slices. *Br J Pharmacol* **95**: 1213–1219.

Huang M, Pang X, Letourneau R, Boucher W, Theoharides TC (2002). Acute stress induces cardiac mast cell activation and histamine release, effects that are increased in Apolipoprotein E knockout mice. *Cardiovas Res* **55**: 150–160.

Igbavboa U, Avdulov NA, Chochina SV, Wood WG (1997). Transbilayer distribution of cholesterol is modified in brain synaptic plasma membranes of knockout mice deficient in the low-density lipoprotein receptor, apolipoprotein E, or both proteins. *J Neurochem* **69**: 1661–1667.

Imaizumi M, Miyazaki S, Onodera K (1996). Effects of betahistine, a histamine H1 agonist and H3 antagonist, in a light/dark test in mice. *Methods Find Exp Clin Pharmacol* **18**: 19–24.

Imaizumi M, Onodera K (1993). The behavioral and biochemical effects of thioperamide, a histamine H3-receptor antagonist, in a light/dark test measuring anxiety in mice. *Life Sci* **53**: 1675–1683.

Inagaki N, Yamatodani A, Ando-Yamamoto M, Tohyama M, Watanabe T, Wada H (1988). Organization of histaminergic fibers in the rat brain. *J Comp Neurol* **273**: 283–300.

Jansen FP, Mochizuki T, Yamamoto Y, Timmerman H, Yamatodani A (1998). *In vivo* modulation of rat hypothalamic histamine release by the histamine H_3 receptor ligands, immepip and clobenpropit. Effects of intrahypothalamic and peripheral application. *Eur J Pharmacol* **362**: 149–155.

Knigge U, Willems E, Kjaer A, Jorgensen H, Warberg J (1999). Histaminergic and catecholaminergic interactions in the central regulation of vasopressin and oxytocin secretion. *Endocrinology* **140**: 3713–3719.

Laitinen KS, Tuomisto L, MacDonald E (1995). Effects of a selective alpha 2-adrenoceptor antagonist, atipamezole, on hypothalamic histamine and noradrenaline release *in vivo*. *Eur J Pharmacol* **285**: 255–260.

Leurs R, Blandina P, Tedford C, Timmerman H (1998). Therapeutic potential of histamine H_3 receptor agonists and antagonists. *Trends Pharmacol Sci* **19**: 177–183.

Liu C, Ma X, Jiang X, Wilson SJ, Hofstra CL, Blevitt J *et al* (2001). Cloning and pharmacological characterization of a fourth histamine receptor (H(4)) expressed in bone marrow. *Mol Pharmacol* **59**: 420–426.

Lovenberg TW, Pyati J, Chang H, Wilson SJ, Erlander MG (2000). Cloning of rat histamine H(3) receptor reveals distinct species pharmacological profiles. *J Pharmacol Exp Ther* **293**: 771–778.

Mahley RW (1988). Apolipoprotein E: cholesterol transport protein with expanding role in cell biology. *Science* **240**: 622–630.

Masliah E, Mallory M, Ge N, Alford M, Veinbergs I, Roses AD (1995). Neurodegeneration in the central nervous system of apoE-deficient mice. *Exp Neurol* **136**: 107–122.

Mazurkiewicz-Kwilecki IM, Nsonwah S (1989). Changes in the regional brain histamine and histidine levels in postmortem brains of Alzheimer patients. *Can J Physiol Pharmacol* **67**: 75–78.

Miyazaki S, Imaizumi M, Onodera K (1995a). Effects of thioperamide, a histamine H_3-receptor antagonist, on a scopolamine-induced learning deficit using an elevated plus-maze test in mice. *Life Sci* **57**: 2137–2144.

Miyazaki S, Imaizumi M, Onodera K (1995b). Effects of thioperamide on the cholinergic system and the step-through passive avoidance test in mice. *Methods Find Exp Clin Pharmacol* **17**: 653–658.

Miyazaki S, Onodera K, Imaizumi M, Timmerman H (1997). Effects of clobenpropit (VUF-9153), a histamine H_3-receptor antagonist, on learning and memory, and on cholinergic and monoaminergic systems in mice. *Life Sci* **61**: 355–361.

Molinengo L, Di Carlo G, Ghi P (1999). Combined action of thioperamide plus scopolamine, diphenhydramine, or memethysergide on memory in mice. *Pharmacol Biochem Behav* **63**: 221–227.

Morisset S, Rouleau A, Ligneau X, Gbahou F, Tardivel-Lacombe J, Stark H *et al* (2000). High constitutive activity of native H_3 receptors regulates histamine neurons in brain. *Nature* **408**: 860–864.

Morse KL, Behan J, Laz TM, West Jr RE, Greenfeder SA, Anthes JC *et al* (2001). Cloning and characterization of a novel human histamine receptor. *J Pharmacol Exp Ther* **296**: 1058–1066.

Nakamura T, Itadani H, Hidaka Y, Ohta M, Tanaka K (2000). Molecular cloning and characterization of a new human histamine receptor, HH4R. *Biochem Biophys Res Commun* **279**: 615–620.

Nakamura S, Takemura M, Ohnishi K, Suenaga T, Nishimura M, Akiguchi I *et al* (1993). Loss of large neurons and occurrence of neurofibrillary tangles in the tuberomammillary nucleus of patients with Alzheimer's disease. *Neurosci Lett* **151**: 196–199.

Nguyen T, Shapiro DA, George SR, Setola V, Lee DK, Cheng R *et al* (2001). Discovery of a novel member of the histamine receptor family. *Mol Pharmacol* **59**: 427–433.

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.

Ono J, Yamatodani A, Kishino J, Okada S, Wada H (1992). Cholinergic influence of K(+)-evoked release of endogenous histamine from rat hypothalamic slices *in vitro*. *Methods Find Exp Clin Pharmacol* **14**: 35–40.

Onodera K, Yamatodani A, Watanabe T, Wada H (1994). Neuropharmacology of the histaminergic neuron system in the brain and its relationship with behavioral disorders. *Prog Neurobiol* **42**: 685–702.

Onodera K, Miyazaki S, Imaizumi M, Stark H, Schunack W (1998). Improvement by FUB 181, a novel histamine H_3-receptor antagonist, of learning and memory in the elevated plus-maze test in mice. *Naunyn Schmiedebergs Arch Pharmacol* **357**: 508–513.

Panula P, Pirvola U, Auvinen S, Airaksinen MS (1989). Histamine-immunoreactive nerve fibers in the rat brain. *Neuroscience* **28**: 585–610.

Panula P, Rinne J, Kuokkanen K, Eriksson KS, Sallmen T, Kalimo H *et al* (1998). Neuronal histamine deficit in Alzheimer's disease. *Neuroscience* **82**: 993–997.

Panula P, Yang HY, Costa E (1984). Histamine-containing neurons in the rat hypothalamus. *Proc Natl Acad Sci USA* **81**: 2572–2576.

Passani MB, Bacciottini L, Mannaioni PF, Blandina P (2000). Central histaminergic system and cognition. *Neurosci Biobehav Rev* **24**: 107–113.

Perez-Garcia C, Morales L, Cano MV, Sancho I, Alguacil LF (1999). Effects of histamine H_3 receptor ligands in experimental models of anxiety and depression. *Psychopharmacology (Berl)* **142**: 215–220.

Piedrahita JA, Zhang SH, Hagaman JR, Oliver PM, Maeda N (1992). Generation of mice carrying a mutant apolipoprotein E gene inactivated by gene targeting in embryonic stem cells. *Proc Natl Acad Sci USA* **89**: 4471–4475.

Plump AS, Smith JD, Hayek T, Aalto-Setala K, Walsh A, Verstuyft JG *et al* (1992). Severe hypercholesterolemia and atherosclerosis in apolipoprotein E-deficient mice created by homologous recombination in ES cells. *Cell* **71**: 343–353.

Prast H, Argyriou A, Philippu A (1996). Histaminergic neurons facilitate social memory in rats. *Brain Res* **734**: 316–318.

Prast H, Heistracher M, Philippu A (1991). *In vivo* modulation of the histamine release in the hypothalamus by adrenoreceptor agonists and antagonists. *Naunyn Schmiedebergs Arch Pharmacol* **344**: 183–186.

Raber J, Wong D, Buttini M, Orth M, Bellosta S, Pitas RE *et al* (1998). Isoform-specific effects of human apolipoprotein E on brain function revealed in Apoe knockout mice: increased susceptibility of females. *Proc Natl Acad Sci USA* **95**: 10914–10919.

Raber J, Akana SF, Bhatnagar S, Dallman MF, Wong D, Mucke L (2000). Hypothalamic–pituitary–adrenal dysfunction in Apoe (−/−) mice: possible role in behavioral and metabolic alterations. *J Neurosci* **20**: 2064–2071.

Raber J, Bongers G, LeFevour A, Buttini M, Mucke L (2002). Androgens protect against apoE4-induced cognitive deficits. *J Neurosci* **22**: 5204–5209.

Raber J, Merlo Pich E, Koob GF, Bloom FE (1994). IL-1 potentiates the acetylcholine induced release of vasopressin from the hypothalamus *in vitro*, but not from the amygdala. *Neuroendocrinology* **59**: 208–217.

Rampon C, Yang YP, Goodhouse J, Shimizu E, Kyin M, Tsien JZ (2000). Enrichment induces structural changes and recovery from nonspatial memory deficits in CA1 NMDAR1-knockout mice. *Nat Neurosci* **3**: 238–244.

Rubio S, Begega A, Santin LJ, Arias JL (2002). Improvement of spatial memory by (*R*)–methylhistamine, a histamine H3-receptor agonist, on the Morris water-maze in rat. *Behav Brain Res* **129**: 77–82.

Schlicker E, Betz R, Gothert M (1988). Histamine H_3 receptor-mediated inhibition of serotonin release in the rat brain cortex. *Naunyn Schmiedebergs Arch Pharmacol* **337**: 588–590.

Schlicker E, Fink K, Detzner M, Gothert M (1993). Histamine inhibits dopamine release in the mouse striatum via presynaptic H_3 receptors. *J Neural Transm Gen Sect* **93**: 1–10.

Schlicker E, Fink K, Hinterthaner M, Gothert M (1989). Inhibition of noradrenaline release in the rat brain cortex via presynaptic H_3 receptors. *Naunyn Schmiedebergs Arch Pharmacol* **340**: 633–638.

Silverman AJ, Sutherland AK, Wilhelm M, Silver R (2000). Mast cells migrate from blood to brain. *J Neurosci* **20**: 401–408.

Smith CP, Hunter AJ, Bennett GW (1994). Effects of (R)–methylhistamine and scopolamine on spatial learning in the rat assessed using a water maze. *Psychopharmacology (Berl)* **114**: 651–656.

Sooksawate T, Simmonds MA (2001). Effects of membrane cholesterol on the sensitivity of the GABA(A) receptor to GABA in acutely dissociated rat hippocampal neurones. *Neuropharmacology* **40**: 178–184.

Tedford CE, Yates SL, Pawlowski GP, Nalwalk JW, Hough LB, Khan MA *et al* (1995). Pharmocological characterization of GT-2016, a non-thiourea-containing histamone H_3 receptor antagonist: *in vitro* and *in vivo* studies. *J Pharmacol Exp Ther* **275**: 598–604.

Timmerman H (1990). Histamine H_3 ligands: just pharmacological tools of potential therapeutic agents? *J Med Chem* **33**: 4–11.

Valastro B, Ghribi O, Poirier J, Krzywkowski P, Masicotte G (2001). AMPA receptor regulation and LTP in the hippocampus of young and aged apolipoprotein E-deficient mice. *Neurobiol Aging* **22**: 9–15.

Van der Werf JF, Bast A, Bijloo GJ, Van der Vliet A, Timmerman H (1987). HA autoreceptor assay with superfused slices of rat brain cortex and electrical stimulation. *Eur J Pharmacol* **138**: 199–206.

Watanabe T, Taguchi Y, Shiosaka S, Tanaka J, Kubota H, Terano Y *et al* (1984). Distribution of the histaminergic neuron system in the central nervous system of rats; a fluorescent immunohistochemical analysis with histidine decarboxylase as a marker. *Brain Res* **295**: 13–25.

Weisgraber KH, Mahley RW (1996). Human apolipoprotein E: the Alzheimer's disease connection. *FASEB J* **10**: 1485–1494.

Wieland K, Bongers G, Yamamoto Y, Hashimoto T, Yamatodani A, Menge WMBP *et al* (2001). Constitutive activity of H_3 receptors stably expressed in SK-N-MC cells: display of agonism and inverse agonism by H_3 antagonists. *J Pharmacol Exp Ther* **299**: 908–914.

Yanai K, Son LZ, Enzou M, Sakurai E, Watanabe T (1998). Targeting disruption of histamine H_1 receptors in mice: behavioral and Neurochemical characterization. *Life Sci* **62**: 1607–1610.

Yuzurihara M, Ikarashi Y, Ishige A, Sasaki H, Kuribara H, Maruyama Y (2000). Effects of drugs acting as histamine releasers or histamine receptor blockers on an experimental anxiety model in mice. *Pharmacol Biochem Behav* **67**: 145–150.

Zhu Y, Michalovich D, Wu H, Tan KB, Dytko GM, Mannan IJ *et al* (2001). Cloning, expression, and pharmacological characterization of a novel human histamine receptor. *Mol Pharmacol* **59**: 434–441.