

# Role of H<sub>3</sub>-Receptor-Mediated Signaling in Anxiety and Cognition in Wild-Type and *ApoE*<sup>-/-</sup> Mice

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Increasing evidence supports a role for histamine as a neurotransmitter and neuromodulator in emotion and cognition. The H<sub>3</sub> receptor was first characterized as an autoreceptor that modulates histamine release and synthesis via negative feedback. Mice deficient in apoE (*ApoE*<sup>-/-</sup>) have been used to define the role of apoE in brain function. In the present study, we investigated the possible role of histamine H<sub>3</sub>-receptor-mediated signaling in anxiety and cognition in mice *ApoE*<sup>-/-</sup> and wild-type mice. H<sub>3</sub> antagonists increased measures of anxiety in wild-type, but not *ApoE*<sup>-/-</sup>, mice. In contrast, H<sub>3</sub> antagonists similarly impaired object recognition in wild-type and *ApoE*<sup>-/-</sup> mice. In *ApoE*<sup>-/-</sup> mice, reduced negative feedback via H<sub>3</sub> receptors could contribute to increased signaling of H<sub>1</sub> receptors. *ApoE*<sup>-/-</sup> mice showed higher sensitivity to the anxiety-reducing effects of the H<sub>1</sub> 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*<sup>-/-</sup>, mice. These data support a role for apoE in H<sub>3</sub> receptor signaling. H<sub>3</sub> 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<sub>3</sub> antagonists increase measures of anxiety and impair object recognition in wild-type mice, the use of H<sub>3</sub> 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<sub>3</sub> receptor; H<sub>1</sub> 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).

H<sub>3</sub> 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<sub>3</sub> 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*<sup>-/-</sup>) (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*<sup>-/-</sup> than wild-type mice and *ApoE*<sup>-/-</sup> mice have 37% more cardiac mast cells (Huang *et al*, 2002). Mast cells migrate from blood to brain (Silverman *et al*, 2000)

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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>

and brains of *ApoE*<sup>-/-</sup> mice might also be exposed to higher histamine levels than wild-type mice. In the present study, we analyzed *ApoE*<sup>-/-</sup> mice to investigate the possible role of apoE in the regulation of the H<sub>3</sub>-receptor-mediated signaling.

In the present study, we demonstrate that H<sub>3</sub> antagonists increase anxiety in wild-type, but not *ApoE*<sup>-/-</sup>, mice. The differential effects of H<sub>3</sub> antagonists on measures of anxiety were not seen on nonspatial and emotional learning and memory. As *ApoE*<sup>-/-</sup> mice showed higher sensitivity to the anxiety-reducing effects of the H<sub>1</sub> receptor antagonist mepyramine than wild-type mice, reduced negative feedback via H<sub>3</sub> receptors could contribute to increased signaling of H<sub>1</sub> receptors in *ApoE*<sup>-/-</sup> mice.

## Experimental Procedures

**Animals.** Male *ApoE*<sup>-/-</sup> (C57BL/6J-*ApoE*<sup>tm1Unc</sup>) and wild-type C57BL/6J mice were obtained from The Jackson Laboratory (Bar Harbor, ME). Young (3–5 months old) *ApoE*<sup>-/-</sup> and wild-type mice were used in all the experiments, as indicated ( $n = 5–19$  mice per group). Young *ApoE*<sup>-/-</sup> mice were studied to evaluate a potential role of apoE on H<sub>3</sub>-receptor-mediated signaling, as adult *ApoE*<sup>-/-</sup> 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<sub>3</sub>-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<sub>3</sub> ligands thioperamide and clobenpropit, the H<sub>1</sub> ligand mepyramine maleate (pyrilamine maleate), and the H<sub>2</sub> ligand zolantidine were gifts from Dr H Timmerman and Dr R Leurs (Department of Pharmacochimistry, 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 mM phenylmethylsulfonyl fluoride, 0.004 mg/ml chymostatin, 0.004 mg/ml leupeptin, and centrifugation at 40 000g 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 000g for 30 min at 4°C. The final membrane pellets were resuspended in water and stored frozen at -80°C until use. H<sub>3</sub> receptor binding was determined with [<sup>3</sup>H]N<sup>z</sup>-methylhistamine ([<sup>3</sup>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 [<sup>3</sup>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<sub>1</sub> receptor binding was

determined with [<sup>3</sup>H]mepyramine (20 Ci/mmol), binding was performed similar to H<sub>3</sub> using conditions as described (Yanai *et al*, 1998). Briefly, the homogenates were incubated for 30 min at 25°C in a 50 mM Na<sup>+</sup>/K<sup>+</sup>-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<sup>+</sup>/K<sup>+</sup>-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.

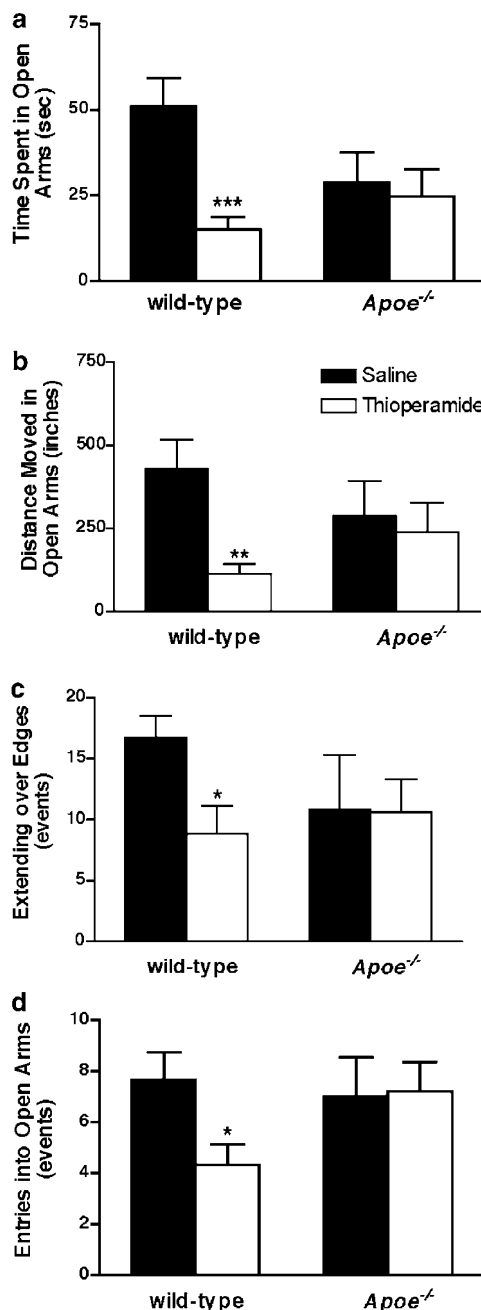
## RESULTS

### Effects of the H<sub>3</sub> Antagonist Thioperamide on Measures of Anxiety in Wild-Type and *Apoe*<sup>-/-</sup> Mice

Anxiety levels in young wild-type and *Apoe*<sup>-/-</sup> 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*<sup>-/-</sup> mice (Figure 1a–d).

### Effects of H<sub>3</sub> Ligands on Novel Object Recognition in Wild-Type and *Apoe*<sup>-/-</sup> Mice

Next we determined whether in wild-type and *Apoe*<sup>-/-</sup> mice H<sub>3</sub> 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*<sup>-/-</sup> mice (3–5 months old) received saline, thioperamide, or clobenpropit during the training (day 4) and retention (day 5) sessions. The recently cloned H<sub>4</sub> receptor



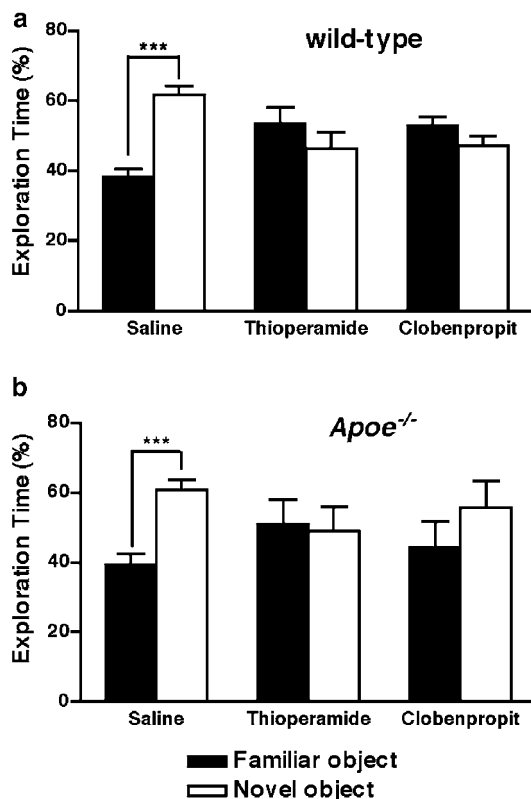
**Figure 1** Measures of anxiety levels in thioperamide-treated male wild-type and *Apoe*<sup>-/-</sup> mice in the elevated plus maze. Compared with saline controls, wild-type mice that received thioperamide showed significant reductions in time spent in the open arms (a), in distance moved in the open 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*<sup>-/-</sup> mice showed no significant change in any of these measures of anxiety. None of the differences between wild-type and *Apoe*<sup>-/-</sup> 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*<sup>-/-</sup> 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<sub>3</sub>-specific ligands. To rule out the possible contribution of the H<sub>4</sub> receptor to the effects of thioperamide, we also treated wild-type and *Apoe*<sup>-/-</sup> mice with clobenpropit,

a H<sub>3</sub>-specific antagonist that was reported to be an H<sub>4</sub> 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*<sup>-/-</sup> mice spent significantly more time exploring the novel object (wild type: 8.37 ± 0.93 s; *Apoe*<sup>-/-</sup>: 9.34 ± 2.76 s) than the familiar object (wild type: 5.43 ± 0.69 s; *Apoe*<sup>-/-</sup>: 5.15 ± 1.25 s) (Figure 2a, b), whereas the thioperamide- and clobenpropit-treated wild-type and *Apoe*<sup>-/-</sup> 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<sub>3</sub> receptor and not the H<sub>4</sub> receptor. Thus, the H<sub>3</sub> antagonists similarly impaired object recognition in wild-type and *Apoe*<sup>-/-</sup> mice (Figure 2a, b).

### H<sub>3</sub> Expression Levels in Wild-Type and *Apoe*<sup>-/-</sup> Mice

To determine whether there are differences in H<sub>3</sub> receptor expression in young *Apoe*<sup>-/-</sup> and wild-type mice (3–5 months old), which could have contributed to their differential response to H<sub>3</sub> antagonists on measures of anxiety, we performed saturation analysis with [<sup>3</sup>H]NAMH in brain regions that have been implicated in cognition or emotion. The total number of receptors (*B*<sub>max</sub> in fmol/mg protein) in the amygdala (wild type: 87.3 ± 2.5; *Apoe*<sup>-/-</sup>: 81.8 ± 2.3), cortex (wild type: 119.9 ± 3.0; *Apoe*<sup>-/-</sup>:

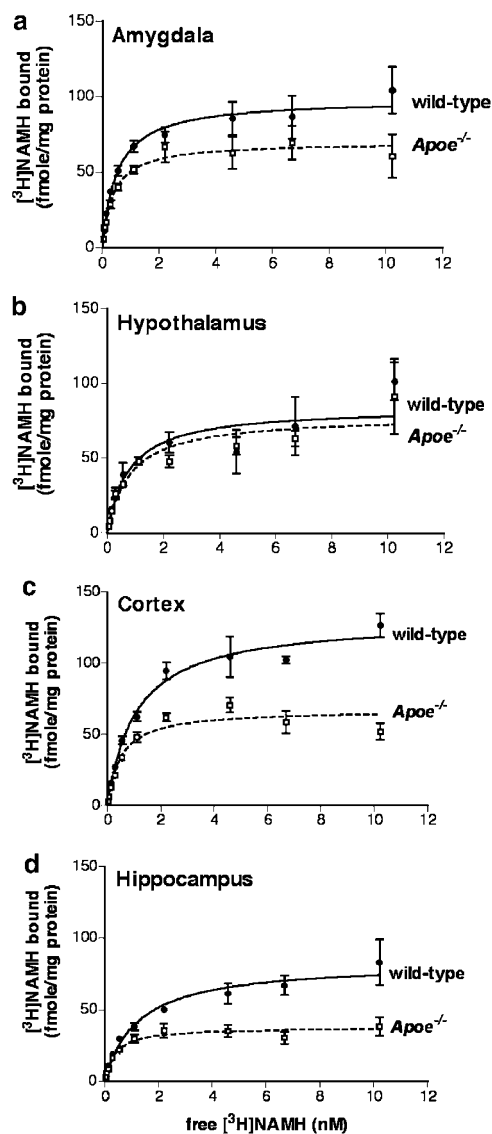


**Figure 2** Novel object recognition in thioperamide- and clobenpropit-treated wild-type and *Apoe*<sup>-/-</sup> male mice. The percentages of time spent exploring the novel and familiar objects on day 5 of testing are shown. Only saline-treated mice spent significantly more time exploring the novel 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*<sup>-/-</sup> mice: saline, *n* = 10; thioperamide, *n* = 5; clobenpropit, *n* = 6.

56.8 ± 5.8), and hippocampus (wild type: 108.4 ± 10.5; *Apoe*<sup>-/-</sup>: 29.1 ± 1.7) was significantly lower in *Apoe*<sup>-/-</sup> than in wild-type mice (Figure 3). In the hypothalamus, *B*<sub>max</sub> was not significantly different between the groups. No significant difference was found in the binding affinities (*K*<sub>d</sub>) of [<sup>3</sup>H]NAMH in any of the brain regions. Thus, compared to wild-type controls, *Apoe*<sup>-/-</sup> mice have a region-specific downregulation of the H<sub>3</sub> receptor, without a change in affinity for H<sub>3</sub>-specific ligands in brain regions implicated in cognition and emotion.

### Increased Sensitivity of *Apoe*<sup>-/-</sup> Mice to the Effects of the H<sub>1</sub> Antagonist Mepyramine on Measures of Anxiety

In *Apoe*<sup>-/-</sup> mice, reduced negative feedback via H<sub>3</sub> receptors could increase signaling of H<sub>1</sub> and H<sub>2</sub> receptors. To



**Figure 3** Saturation curve of [<sup>3</sup>H]NAMH in amygdala (a), hypothalamus (b), cortex (c), and hippocampus (d), of male wild-type (solid line, ●) and *Apoe*<sup>-/-</sup> (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*<sup>-/-</sup> mice there is increased H<sub>1</sub>- or H<sub>2</sub>-receptor-mediated signaling, wild-type and *Apoe*<sup>-/-</sup> mice were assessed in the elevated plus maze 1 h after intraperitoneal administration of the H<sub>1</sub> antagonist mepyramine, the H<sub>2</sub> antagonist zolantidine, or saline. *Apoe*<sup>-/-</sup> mice treated with mepyramine, but not with zolantidine, showed reduced measures of anxiety as compared to *Apoe*<sup>-/-</sup> 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*<sup>-/-</sup> 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*<sup>-/-</sup> mice show an increased sensitivity to the effects of H<sub>1</sub> receptor blockade on measures of anxiety. To determine whether in *Apoe*<sup>-/-</sup> 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*<sup>-/-</sup> (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*<sup>-/-</sup>, 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.

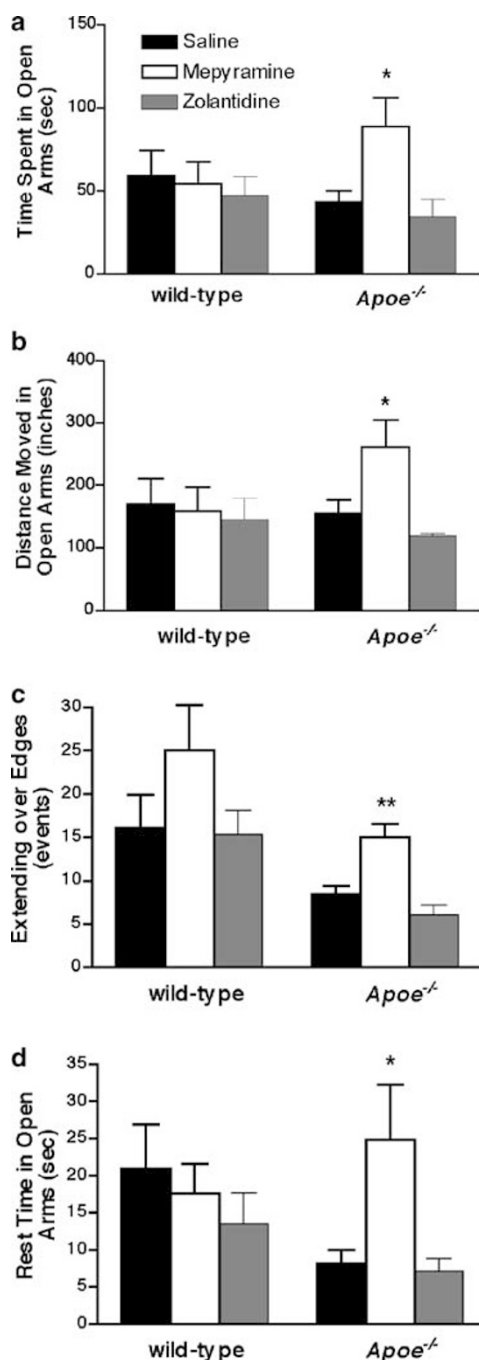
### H<sub>1</sub> Expression Levels in Wild-Type and *Apoe*<sup>-/-</sup> Mice

To determine whether there are differences in H<sub>1</sub> receptor expression in young *Apoe*<sup>-/-</sup> 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 [<sup>3</sup>H]mepyramine in brain regions that have been implicated in cognition or emotion. There was no difference in the total number of H<sub>1</sub> receptors or H<sub>1</sub> receptor binding affinity in the amygdala, cortex, hippocampus, or hypothalamus (Figure 5 and Table 1).

## DISCUSSION

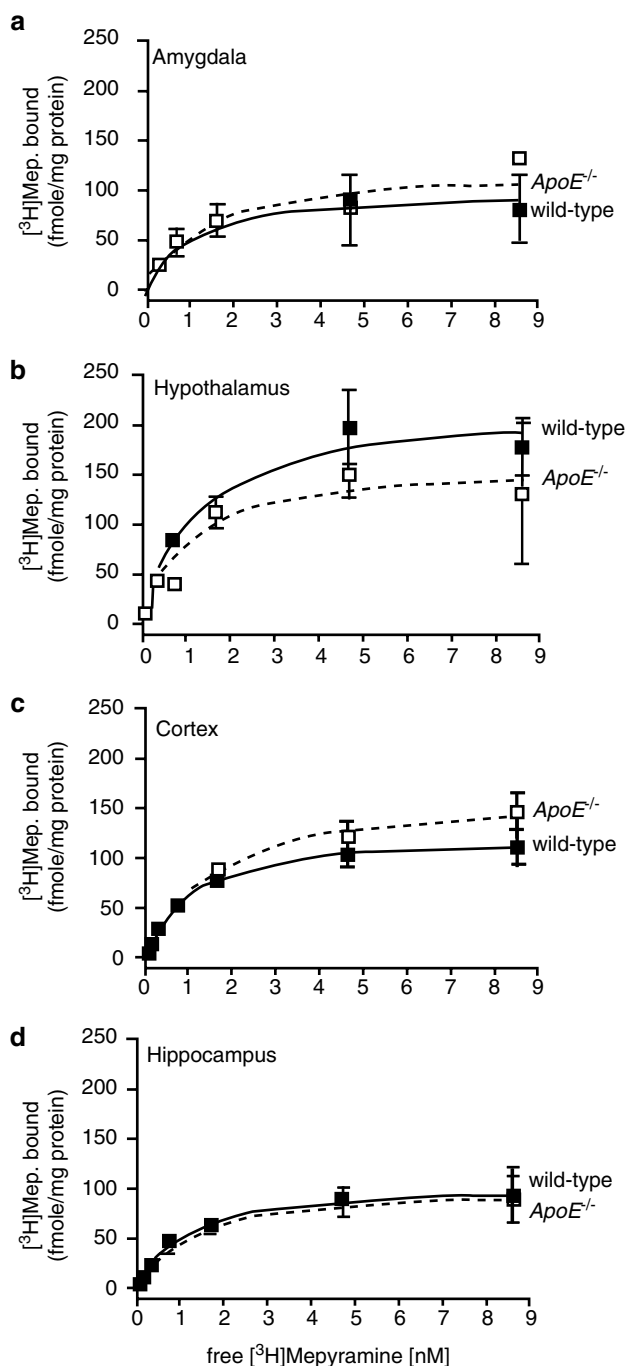
This study shows that H<sub>3</sub> antagonists increased measures of anxiety in wild-type, but not in *Apoe*<sup>-/-</sup>, mice. In contrast, *Apoe*<sup>-/-</sup> mice showed a higher sensitivity to the anxiety-reducing effects of H<sub>1</sub> receptor blockade than wild-type mice. The effects of H<sub>1</sub> receptor blockade on measures of anxiety were dissociated from those on the HPA axis response. These differential effects of H<sub>3</sub> antagonists on measures of anxiety were not seen on object recognition.

H<sub>1</sub> receptor blockade did not reduce measures of anxiety in wild-type C57Bl/6J mice. This is consistent with the lack of effect of H<sub>1</sub> receptor blockade on measures of anxiety in



**Figure 4** Measures of anxiety in mepyramine- and zolantidine-treated male wild-type and *Apoe*<sup>-/-</sup> mice in the elevated plus maze. Compared with saline controls, *Apoe*<sup>-/-</sup> 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*<sup>-/-</sup> 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*<sup>-/-</sup> mice: saline, *n* = 7; mepyramine, *n* = 7; zolantidine, *n* = 5.

wild-type ddY mice. The reduced measures of anxiety in *Apoe*<sup>-/-</sup> mice after H<sub>1</sub> receptor blockade might be caused by increased levels of histamine release. H<sub>1</sub> receptors become activated at levels of histamine release higher than normal



**Figure 5** Saturation curve of [<sup>3</sup>H]mepyramine in the amygdala (a), hypothalamus (b), cortex (c), and hippocampus (d), of male wild-type (solid line, ●) and *ApoE*<sup>-/-</sup> (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<sub>1</sub> receptor agonist and H<sub>3</sub> receptor antagonist betahistine has anxiogenic effects (Imaizumi *et al*, 1996).

The effects of H<sub>1</sub> receptor blockade on measures of anxiety were dissociated from those on the HPA axis

**Table 1** H<sub>1</sub> Expression Levels in Wild-Type and *ApoE*<sup>-/-</sup> Mice<sup>a</sup>

Mouse	Brain region	B <sub>max</sub> (fmol/mg protein)	K <sub>d</sub> (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
<i>ApoE</i> <sup>-/-</sup>	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

<sup>a</sup>B<sub>max</sub> or K<sub>d</sub> were determined as described under histamine receptor binding. There were no significant genotype differences in either the B<sub>max</sub> or K<sub>d</sub> in any brain region.

response. While mepyramine reduced measures of anxiety in *ApoE*<sup>-/-</sup>, but not wild-type, mice, it reduced plasma ACTH and corticosterone levels in wild-type, but not *ApoE*<sup>-/-</sup>, mice. These data suggest that in *ApoE*<sup>-/-</sup> mice mepyramine does not reduce measures of anxiety by inhibiting the HPA axis response. The dissociation of the effects of H<sub>1</sub> receptor blockade on anxiety from those on the HPA axis in *ApoE*<sup>-/-</sup> and wild-type mice and the differential effects of H<sub>3</sub> receptor blockade on novel object recognition and anxiety in *ApoE*<sup>-/-</sup>, but not wild-type, mice suggest that differential pharmacokinetic profiles of histaminergic drugs in the two genotypes do not underlie the behavioral results.

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<sub>3</sub> 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<sub>3</sub> 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*<sup>-/-</sup> mice, thioperamide decreased extending over the edges in wild-type but not *ApoE*<sup>-/-</sup> mice. Similarly, in Figure 4b, in which there were no baseline differences in wild-type and *ApoE*<sup>-/-</sup> mice, mepyramine increased the distance moved in the open arms in *ApoE*<sup>-/-</sup> but not in wild-type mice.

*ApoE*<sup>-/-</sup> mice have lower levels of H<sub>3</sub> expression, without a change in affinity for H<sub>3</sub>-specific ligands, in the amygdala, hippocampus, and cortex than age-matched wild-type controls. In contrast, *ApoE*<sup>-/-</sup> and wild-type mice have similar levels of H<sub>1</sub> expression and a similar affinity for H<sub>1</sub>-specific ligands in the amygdala, hypothalamus, hippocampus, and cortex. These data indicate that there is no simple

association between levels of H<sub>1</sub> and H<sub>3</sub> receptor expression in structures associated with anxiety *vs* cognition which could explain why H<sub>3</sub> 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*<sup>-/-</sup> mice were more sensitive to the anxiety-reducing effects of mepyramine. Interestingly, in young *ApoE*<sup>-/-</sup> mice the enhancement of the  $\alpha$ -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*<sup>-/-</sup> 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*<sup>-/-</sup> mice reduces the potency of GABA at the GABA<sub>A</sub> receptor (Sooksawate and Simmonds, 2001). Similarly, by altering the lipid environment, apoE deficiency may reduce the total number of H<sub>3</sub> receptor binding sites available for ligand binding in the amygdala, hippocampus, and cortex.

Recently, the H<sub>4</sub> 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<sub>4</sub> signaling was involved in the observed effects on object recognition, we tested mice treated with the H<sub>4</sub> receptor agonist and H<sub>3</sub> 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<sub>4</sub> receptor activation.

Our data demonstrate that H<sub>3</sub> receptor blockade impairs novel object recognition in wild-type and *ApoE*<sup>-/-</sup> mice. Consistent with our data, H<sub>3</sub> receptor stimulation by R<sup>2</sup>-methylhistamine improved memory retention of naive rats in the water maze (Rubio *et al*, 2002) and H<sub>3</sub> 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 immpip or inhibition of histamine synthesis by  $\alpha$ -fluoromethylhistidine prolonged recognition time (Prast *et al*, 1996). However, H<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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<sub>3</sub> 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).

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