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Ciproxifan, an H₃ Receptor Antagonist, Alleviates Hyperactivity and Cognitive Deficits in the APP_{Tg2576} Mouse Model of Alzheimer's Disease

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Abstract

Previous research has indicated that the blockade of H₃-type histamine receptors may improve attention and memory in normal rodents. The purpose of this study was to determine if ciproxifan, an H₃ receptor antagonist, could alleviate the hyperactivity and cognitive deficits observed in a transgenic mouse model (APP_{Tg2576}) of Alzheimer's disease. APP_{Tg2576} mice displayed significantly greater locomotor activity than wild-type mice, but APP_{Tg2576} mice provided with daily ciproxifan treatment showed activity levels that did not differ from wild-type mice. In the swim maze, APP_{Tg2576} mice exhibited significantly longer escape latencies, but the APP_{Tg2576} mice treated daily with ciproxifan had latencies that were indistinguishable from controls. In probe trials conducted one hour after the last training trial, ciproxifan-treated APP_{Tg2576} mice spent more time near the previous platform location and made more crossings of this area than did saline-treated APP_{Tg2576} mice. APP_{Tg2576} mice also demonstrated a significant impairment in the object recognition task that was reversed by acute treatment with ciproxifan (3.0 mg/kg). These data support the idea that modulation of H₃ receptors represents a novel and viable therapeutic strategy in the treatment of Alzheimer's disease.

Keywords

Locomotor activity; swim maze; object recognition; histamine; amyloid-precursor protein; cognitive enhancement

1.1 Introduction

Over the past decade, preclinical research has identified the H₃ histamine receptor as a possible target for cognitive-enhancing drugs (Bonaventure, Letavic, Dugovic, Wilson, Aluisio, et al., 2007; Esbenshade, Fox, & Cowart, 2006). The H₃ receptor exists as a presynaptic autoreceptor that is expressed in relatively high densities in brain regions associated with memory function, such as the frontal cortex and hippocampus (Pillot, Heron, Cochois, Tardivel-Lacombe, Ligneau et al. 2002). Antagonism (or inverse agonism) of the receptor leads to the release of histamine as well as neurotransmitters involved in learning

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and memory, such as acetylcholine and dopamine, in the hippocampus and prefrontal cortex (Bacciottini, Passani, Giovannelli, Cangioli, Mannaioni et al., 2002; Clapham & Kilpatrick, 1992; Fox, Esbenshade, Pan, Radek, Krueger, et al., 2005; Ligneau, Lin, Vanni-Mercier, Jouvet, Muir, Ganellin, et al., 1998; Ligneau, Perrin, Landais, Camelin, Calmels, et al., 2007b; Medhurst, Atkins, Beresford, Brackenborough, Briggs, et al., 2007). Moreover, H₃ antagonists can generate theta rhythms in the brain (Hajós, Siok, Hoffmann, Li, & Kocsis, 2008) – a form of activity that predicts the onset of new learning (Berry & Seager 2001). On a behavioral level, drugs that act as H₃ antagonists, such as the prototypical imidazole-containing compounds, ciproxifan and thioperamide, have been shown to improve memory function in several tasks – in normal rats and mice, as well as in animals treated with anti-cholinergic or anti-glutamatergic drugs (Bardgett, Points, Kleier, LaMontagne, & Griffith, 2010; Fox, Pan, Radek, Lewis, Bitner, et al., 2003; Bernaerts, Lamberty, & Tirelli, 2004; Galici, Boggs, Aluisio, Fraser, Bonaventure, et al., 2009; Ligneau et al., 1998). In addition to their effects on learning and memory, H₃ antagonists have also been shown to modulate the elevating effects of psychostimulants on locomotor activity in rats and mice (Clapham & Kilpatrick, 1994, Fox et al., 2005; Ligneau, Landais, Perrin, Piriou, Uguen, et al., 2007a; Morisset, Pilon, Tardivel-Lacombe, Weinstein, Rostene, et al., 2002).

The ability of H₃ antagonists to enhance memory in normal animals and in pharmacological models of memory impairment raises the possibility that such compounds may represent an effective treatment strategy for Alzheimer's disease. As a way of addressing this possibility, we tested the effects of the H₃ antagonist, ciproxifan (Ligneau et al., 1998), on the learning and memory deficits and hyperlocomotion observed in the amyloid precursor protein (APP_{Tg2576}) transgenic mouse model of Alzheimer's disease. Developed by Hsiao and colleagues (1996), APP_{Tg2576} mice express a mutant form of the human APP gene associated with early-onset, familial Alzheimer's disease. These mice exhibit a phenotype that includes the formation of amyloid plaques with increasing age as well as deficits in spatial learning and memory, and object recognition (Hsiao, Chapman, Nilsen, Eckman, Harigaya, et al., 1996; Taglialetela, Hogan, Zhang, & Dineley, 2009). Some studies of APP_{Tg2576} mice have also reported that mutant mice demonstrate elevated locomotor activity (Golub, Germann, Mercer, Gordon, Morgan, et al., 2008; Tabuchi, Yamaguchi, Iizuka, Imamura, Ikarashi, et al., 2009).

In the first study, the effects of ciproxifan on locomotor activity and performance in the swim maze were compared between APP_{Tg2576} mice and wild-type (WT) littermates of both genders at 12–14 months of age. Using a between-subjects design, approximately half of the mice of each genotype received daily intraperitoneal injections of ciproxifan (3 mg/kg) and the other half received injections of saline. The dose of ciproxifan chosen for study was based on previous research demonstrating its ability to improve attention and memory in normal rats (Bardgett et al., 2010; Fox et al., 2003; 2005; Ligneau et al., 1998) and inhibit stimulant-induced hyperactivity in mice (Morisset et al., 2002). Mice received daily injections for one week prior to testing and daily injections 30 minutes prior to testing over the subsequent three weeks. In a second study using a within-subjects design, a separate cohort of 12–14 month old APP_{Tg2576} and WT mice was tested in a novel object recognition task. Ciproxifan or saline was injected 30 minutes prior to testing in this study.

1.2 Materials and methods

1.2.1. Animals and housing

Offspring of an original crossing of APP_{Tg2576} (Taconic Labs, Hudson, NY) male mice with B6SJLF1/J (Jackson Laboratory, Bar Harbor, ME) female mice were backcrossed with C57Bl6/J mice (Jackson Laboratory, Bar Harbor, ME) for five generations. APP_{Tg2576} mice and their wild-type (WT) littermates of each gender representing the sixth generation of this

breeding protocol were used in the present studies. They were housed three to four per cage with free access to food and water. Lighting in the animal colony was maintained on a 12-hour light/dark schedule with lights on at 07:00. All experimental procedures were performed according to the Current Guide for the Care and Use of Laboratory Animals (USPHS) under a protocol approved by the Northern Kentucky University Institutional Animal Use and Care Committee.

1.2.2. Genotyping

PCR genotyping was performed using DNA isolated from post-weaning tail biopsies. Forward (5'-GTGGATAACCCCTCCCCAGCCTAGACCA-3') and reverse (5'-CTGACCACTCGACCAGGTTCTGGGT-3') primers amplified a 410-base pair product from the amyloid precursor protein transgene (APPTg2576). DNA isolated from non-transgenic littermates did not give a PCR product and served as negative controls.

1.2.3. Ciproxifan dosing and injections

Ciproxifan was kindly provided by the National Institute of Mental Health's Chemical Synthesis and Drug Supply program and dissolved in saline prior to each injection. In each experiment, mice received intraperitoneal injections 30 minutes prior to testing of either saline or ciproxifan (3.0 mg/kg of body weight). This dose of ciproxifan was based on earlier studies demonstrating that it effectively improved attention, inhibitory avoidance, and social and spatial memory in rats (Bardgett et al., 2010; Fox et al., 2003;2005; Ligneau et al., 1998) and antagonized stimulant-induced hyperactivity in mice (Morisset et al., 2002). Using a between subject design, the first cohort of mice received daily injections beginning one week before locomotor testing and 30 minutes prior to testing each day over the subsequent three weeks. Using a within-subjects, repeated-measures design, the second cohort of mice received injections 30 minutes prior to each object recognition test in a counter-balanced manner.

1.2.4. Locomotor testing

A total of 39 twelve - fourteen month old mice were tested for locomotor activity: 10 WT and 9 APP_{Tg2576} mice treated with saline, and 9 WT and 11 APP_{Tg2576} mice were treated with ciproxifan. Of the 39 mice tested, nine were male (4 WT and 5 APP) and 30 were female (15 WT and 15 APP). Testing equipment consisted of 12 Hamilton-Kinder (HK) Cage Rack - high density activity frames with 12 polypropylene cages (28cm x 17cm x 12.5cm h) tracked by (HK) MotorMonitor software (Kinder Scientific, Poway, CA). Drug injections occurred 30 minutes prior to testing. Mice were placed in the testing apparatus with the lights off for 60 minutes. Testing was conducted between 8:00 a.m. and 2:00 p.m. for 5 consecutive days.

1.2.5. Swim maze training and testing

A total of 38 mice tested for locomotor activity were trained and tested in the hidden and visible versions of swim maze task: 9 WT and 8 APP_{Tg2576} mice treated with saline, and 9 WT and 11 APP_{Tg2576} mice treated with ciproxifan. Of the 38 mice tested, nine were male (4 WT and 5 APP) and 29 were female (15 WT and 14 APP). Mice were placed in the testing room 30 minutes prior to testing on each test day. The swim maze consisted of a 1.2 m high circular stainless steel tank measuring 114 cm in diameter. Data were collected and analyzed using San Diego Instruments SmartTrack software (San Diego, CA). The platform consisted of a weighted PVC pipe base that was 45.7 cm tall with a Plexiglas platform that was 12 cm in diameter. The platform was maintained at approximately 2 cm below the surface of the water for habituation and the hidden platform conditions. White non-toxic

poster paint was added to the pool to make the water opaque. The water was kept at room temperature. All drug injections occurred 30 minutes prior to training and testing.

Habituation—The mice were habituated to the swim maze by placing the platform in the center of the pool. Mice were placed in the water near the platform and guided to it. They were then required to climb upon the platform and stay there for 10 seconds. This procedure was repeated three times. All mice demonstrated an ability to climb and stay on the platform for 10 seconds by the end of the third trial. Following all trials, mice were placed in a holding cage that had a 60 watt lamp centered immediately above it to provide warmth and an absorbent pad placed on its floor to absorb excess water.

Hidden Platform Condition—For training, each mouse was placed in the swim maze at a randomly chosen starting point and was required to find the platform within 60 seconds. The same start point was used for each trial on a given day, but switched each day. Escape was defined as climbing onto the platform, and each mouse was left on the platform for 10 seconds after escape. If the platform was not found within 60 seconds, the mouse was manually placed on the platform for 10 seconds and then returned to a holding cage. Five days of training were conducted, and each day of testing consisted of four test trials with a five-minute intertrial interval. During each training trial, the following measures were recorded: latency to find the platform, path length, swim speed, and average distance from the platform.

Probe Trial—A probe trial was conducted one hour after training on the last day of the hidden platform condition. The platform was removed from the pool. Each mouse was placed in the pool at a starting point opposite the original platform location and allowed to swim for 60 seconds. A second probe trial was conducted 48 hours later. During each trial, the following measures were recorded: the number of crossings over the previous platform location and the time spent swimming in an area (2232.56 cm²) that included the former platform location.

Visible Platform Condition—Two days after the completion of hidden platform testing, the ability to locate a visible platform was tested in each mouse over three consecutive days. A Styrofoam ball wrapped in aluminum foil was attached to a bolt sticking 7 cm above the platform. Each day of testing consisted of four test trials with a five-minute intertrial interval. Prior to each trial, the platform was moved to a new location. At the beginning of each trial, the mouse was placed into the water at a randomly chosen starting point that remained constant within each day of testing. During each trial, the following measures were recorded: latency to find the platform, path length, swim speed, and average distance from the platform. Each mouse was given 60 seconds to find the platform. If the mouse did not find the platform within 60 seconds, it was placed on it for 10 seconds and then returned to a holding cage.

1.2.6. Novel object recognition

A within-subjects, repeated-measures design was used to assess the effects of ciproxifan on object recognition behavior in a separate cohort of 11 APP_{Tg2576} and 11 WT mice (6 females and 5 males per group). The mice in this cohort were 12–14 months old. A 60 cm × 60 cm × 60 cm gray wooden box with Plexiglas floor painted gray was used to test the mice. Four 50 ml polypropylene lids were anchored flush into the floor upside down 12 cm from each corner - these were used to secure objects to the floor. Six groups of three objects (glass or plastic) were used and each object had the threaded end (~2 cm in length) of a 50 ml polypropylene tube glued to its bottom. A digital camera mounted 6 feet above the

apparatus was connected to a video monitor in an adjacent room and was used to observe object investigation.

Habituation—The mice were habituated by placing them in the testing room for 20 minutes with the overhead lights on. The mice were then placed in the apparatus without any objects for 15 minutes. Habituation was performed for three days.

Testing—The mice were placed in the testing room 20 minutes prior to testing. Two identical objects were placed in the apparatus, diagonal from one another. The mice were then placed into the apparatus for five minutes. Investigation of each object was measured continuously every minute. A mouse was considered to be exploring an object when it was within 2 cm of the object and oriented within 45 degrees of the object or had one paw on the object. Climbing or sitting on the object was not considered exploration. After five minutes, the mice were removed from the testing apparatus and placed in a holding cage for five minutes. During this time, the original objects were removed and replaced with one identical object and one novel object. After the five-minute delay, the mice were returned to the apparatus for three minutes. A record of exploration for each object was recorded each minute. The apparatus and objects were cleaned between trials with 75% ethanol/water.

1.2.7. Confirmation of plaque deposition in APP_{Tg2576} mice

APP_{Tg2576} and WT mice were sacrificed three months after the completion of drug and behavioral testing in order to determine if plaques were present in the brains of the APP_{Tg2576} mice. Plaque deposition was confirmed using thioflavin-S stain (Sigma, St. Louis MO). Mounted sections were rinsed with 0.1M PBS, stained with a 1% thioflavin-S solution, and differentiated in 70% ethanol. Sections were allowed to dry overnight then dehydrated and coverslipped with DPX mountant (Fluka). Plaques were visualized using an FITC fluorescent filter on a Zeiss Axioskop 40 microscope.

1.2.8. Data analyses

In the locomotor testing, data were averaged across the five daily trials for each five-minute test bin. A three-way analysis of variance (ANOVA) was conducted using drug treatment and genotype as between-groups factors and time (i.e., five minute test bin) as a repeated-measure. For the swim maze data, a three-way ANOVA was used for the training data with drug treatment and genotype as between-groups factors and test day as a repeated-measure. Probe trial was analyzed using a two-way ANOVA with drug treatment and genotype as between-groups factors. The same analysis was performed on the object recognition data with the exception that drug treatment served as a within-group factor. All post-hoc testing was conducted using Tukey/Kramer significant difference (TSD) tests. Statistical differences for all tests were considered significant if $p < .05$. Statistical testing was performed using StatView 5.0 for the Mac (SAS Institute, Inc., Cary, North Carolina).

1.3 Results

1.3.1. Confirmation of amyloid plaques in APP_{Tg2576} mice

The appearance of amyloid plaques was confirmed in the brain of each APP mouse after the completion of drug and behavioral testing (Figure 1). Numerous plaque deposits were observed in the dorsal hippocampus and cortex of the APP_{Tg2576} mice. These plaques were not observed in the brains of WT mice. In the first cohort of APP_{Tg2576} mice, statistical analyses did not indicate a difference in the number of hippocampal or cortical plaques between APP_{Tg2576} mice previously exposed to four weeks of ciproxifan treatment versus those treated with saline.

1.3.2. Ciproxifan reduces elevated locomotor activity in APP_{Tg2576} mice

The effects of ciproxifan (3.0 mg/kg) on locomotor activity were examined in the first cohort of APP_{Tg2576} and WT mice. Mice received daily intraperitoneal injections of saline or ciproxifan beginning one week prior to testing and were injected 30 minutes prior to testing on each day of locomotor testing. Locomotor testing was conducted one week prior to swim maze testing, and was performed for one hour a day for five days. APP_{Tg2576} mice treated with saline were significantly more active than WT mice treated with saline (Genotype $\times$ treatment $\times$ time interaction: $F(11, 385) = 2.0, p < .03$) (Figure 2). This effect was statistically significant over the first 25 minutes of testing (TSD test, $p \leq .05$ for each comparison). Ciproxifan reversed this effect in that APP_{Tg2576} mice treated with ciproxifan were significantly less active than the APP_{Tg2576} mice treated with saline during the first 10 minutes of testing (TSD test, $p \leq .05$ for each comparison).

1.3.3. Ciproxifan improves the performance of APP_{Tg2576} mice in the swim maze task

One week after the start of locomotor testing, the swim maze task was performed for four trials a day over five consecutive days. Ciproxifan improved performance in APP_{Tg2576} mice over the course of the training trials (Figure 3). There was a statistical interaction between ciproxifan treatment and APP genotype (Interaction term: $F(1, 34) = 6.93, p < .01$) on the time to find the platform measure. APP_{Tg2576} mice treated with saline took significantly longer to find the hidden platform relative to WT mice treated with saline on the fourth day of training (Figure 3a) (TSD test, $p \leq .05$ for each comparison). On the second, fourth, and fifth day of training, APP_{Tg2576} mice treated with ciproxifan took less time to find the platform than APP_{Tg2576} mice treated with saline (Figure 3a) (TSD test, $p \leq .05$ for each comparison). During the training trials, there was also a treatment by genotype interaction (Interaction term: $F(1, 34) = 4.46, p < .04$) as well as an overall training day effect ($F(4, 136) = 9.7, p < .0001$) on the average distance from the platform measure (Figure 3b). On the second day of training, the APP_{Tg2576} mice treated with saline swam farther away from the platform than the WT mice treated with saline (TSD test, $p \leq .05$ for each comparison). On the last four days of training, APP_{Tg2576} mice treated with saline also swam farther away from the platform when compared to APP_{Tg2576} mice treated with ciproxifan (TSD test, $p \leq .05$ for each comparison). There was a trend towards a significant treatment $\times$ genotype interaction for the distance travelled before finding the platform (Interaction term: $F(1, 34) = 4.46, p = .11$ (two-tailed)) (Figure 3c).

Ciproxifan increased swimming speed in APP_{Tg2576} mice (Interaction term: $F(1, 34) = 6.54, p < .02$) (Figure 3d). Unlike the results for the other measures, swimming speed did not distinguish APP_{Tg2576} mice treated with saline from WT mice treated with saline. For the swim speed measure, the only significant group difference found on each training trial was between the APP_{Tg2576} mice treated with ciproxifan and APP_{Tg2576} mice treated with saline (TSD test, $p \leq .05$ for each comparison).

One hour after the final training session, all mice were placed in the pool for one minute without the platform. During this probe trial, the APP_{Tg2576} mice treated with ciproxifan made as many crossings of the former platform site as did the WT mice treated with saline and made significantly more crossings than the APP_{Tg2576} mice treated with saline (Interaction term: $F(1, 34) = 4.68, p < .04$, TSD test, $p \leq .05$ for each comparison) (Figure 4a). When the amount of time spent in an area of the pool that included the former platform was measured, the same pattern of results emerged. APP_{Tg2576} mice treated with ciproxifan spent as much time as each group of WT mice in the zone that formerly contained the platform. In contrast to each of these groups, APP_{Tg2576} mice treated with saline spent significantly less time in this area (Interaction term: $F(1, 34) = 6.45, p < .02$, TSD test, $p \leq .05$ for each comparison) (Figure 4b). Mice were also tested 48 hours after the last training

trial. At this time point, significant genotype or drug treatment effects were not observed for the number of platform crossings or time spent in the former platform zone (Figure 4c & 4d).

Two days after the conclusion of the probe trial, a three-day swim maze test was performed using a visual cue to mark the location of the platform. Mice were provided with four trials per day for three days. The location of the platform was made visible by attaching a ball covered in aluminum foil 7 cm above the platform, and was changed on each day of testing. There was a significant treatment by genotype interaction on the time to find the platform (Treatment $\times$ genotype interaction: $F(1, 33) = 7.05, p < .01$) (Figure 5a). APP_{Tg2576} mice treated with saline took significantly longer to find the platform on days 1 and 2 of training in comparison to the APP_{Tg2576} mice treated with ciproxifan while WT mice treated with ciproxifan also took significantly longer to find the platform on day 2 of training when compared to APP_{Tg2576} mice treated with ciproxifan (TSD test, $p \leq .05$ for each comparison). There were no significant treatment or genotype effects on the average distance from the platform during each trial (Figure 5b), but there was a significant interaction between these two factors for the path length measure (Treatment $\times$ genotype interaction: $F(1, 33) = 7.47, p < .01$) (Figure 5c). APP_{Tg2576} mice treated with saline swam farther before finding the platform than APP_{Tg2576} mice treated with ciproxifan on days 2 and 3 of training, while WT mice treated with ciproxifan swam farther than APP_{Tg2576} mice treated with ciproxifan on day 2 of training (TSD test, $p \leq .05$ for each comparison). There were no significant treatment or genotype effects on swim speed during the visible platform trials (Figure 5d).

1.3.4. Ciproxifan improves the performance of APP_{Tg2576} mice in the novel object recognition task

Novel object recognition was tested in a separate cohort of APP_{Tg2576} and WT mice. The effects of ciproxifan were assessed using a repeated-measures, within-subjects design. The order of drug treatments was counter-balanced across subjects within each genotype group. Ciproxifan improved object recognition in APP_{Tg2576} mice (Interaction term: $F(1, 20) = 11.8, p < .003$) (Figure 6a). APP_{Tg2576} mice spent significantly less time exploring a novel object relative to a familiar one in comparison to all other groups, including the APP_{Tg2576} mice treated with ciproxifan (TSD test, $p \leq .05$ for each comparison). The preference of this latter group of mice for the novel object did not differ from the WT mice treated with saline. Neither genotype or drug treatment altered the total time spent exploring both objects during testing (Figure 6b).

1.4. Discussion

These experiments are the first to demonstrate that an H₃ antagonist can alleviate hyperactivity and memory impairment in a transgenic mouse model of Alzheimer's disease. The capacity for ciproxifan to improve cognitive and behavioral outcomes across multiple tests opens the door for the pursuit of H₃ antagonism as a therapeutic strategy in the treatment of Alzheimer's disease. Obviously, more work is needed to discern the types of behavioral deficits in APP_{Tg2576} mice and other transgenic models of Alzheimer's disease that are sensitive to H₃ antagonists as well as identifying the types of H₃ antagonists that are effective in these models and the mechanism by which they work.

Relative to reports on learning and memory, relatively few studies have described changes in locomotor activity in APP_{Tg2576} mice. At least two studies (Golub et al., 2008; Tabuchi et al., 2009) have shown that APP_{Tg2576} mice are more active than WT controls, with hyperactivity emerging as early as four months of age and persisting through 15 months of age (Golub et al., 2008). It is not clear what neurobiological mechanism accounts for

elevated locomotor activity in APP_{Tg2576} mice, although the reported loss of synapses in the hippocampus of APP_{Tg2576} mice (Dong, Martin, Chambers, & Csernansky, 2007) could possibly lead to dysregulation of downstream targets, such as the nucleus accumbens, that modulate locomotion (e.g., David & Abbraini, 2001; White, Whitaker, & White, 2006), akin to the hyperlocomotion observed in animals with overt hippocampal damage (Bardgett, Jacobs, Jackson, & Csernansky, 1997; Bardgett, Baum, O'Connell, Lee, & Hon, 2006). If true, then the ability of ciproxifan to mitigate the elevated locomotor activity seen in APP_{Tg2576} mice may be related to the ability of H₃ antagonists to enhance neurotransmitter release (Bacciottini et al., 2002) or electrophysiological activity (Hajos et al., 2008) in the hippocampus, or induce immediate early gene expression in brain regions, such as the nucleus accumbens (Southam, Cilia, Gartlon, Woolley, Lacroix et al., 2009) or motor cortex (Bonaventure et al., 2007), that directly regulate locomotor behavior. Another possibility is that ciproxifan disrupts dopaminergic activity related to hyperactivity since H₃ antagonists have been shown decrease locomotor responses to dopamine agonists, such as apomorphine, cocaine, and methamphetamine (Clapham & Kilpatrick, 1994; Morisset et al., 2002; Fox et al., 2005; Ligneau et al., 2007a; although see Brabant et al., 2009; Ferrada et al., 2008), although enhancement of psychostimulant effects by H₃ antagonists has also been reported (Bardgett, Points, Roflow, Blankenship, & Griffith, 2009; Bardgett et al., 2010; Brabant, Alleva, Grisar, Quertemont, Lakaye, et al., 2009). Finally, it would be helpful to determine the effects of additional doses of ciproxifan on the locomotor and cognitive deficits seen in APP_{Tg2576} mice. However, it should be noted that the dose of ciproxifan (3.0 mg/kg) chosen for study binds nearly 90% of H₃ receptors (Le, Gruner, Mathiasen, Marino, & Schaffhauser, 2008) – a higher dose would likely effect increased antagonism of other receptors (alpha₂ adrenergic, 5-HT₃) with known affinity for ciproxifan (Esbenshade, Krueger, Miller, Kang, & Denny, et al., 2003).

Ciproxifan improved the performance of the APP_{Tg2576} mice in two well-characterized memory tasks: the swim maze and the object recognition test. These tasks were chosen because APP_{Tg2576} mice demonstrate clear deficits in each one (Hsiao et al., 1996; Westerman, Cooper-Blacketer, Mariash, Kotilinek, Kowarabayash, et al., 2002; Taglialatela et al., 2009). Previous studies have found that ciproxifan and other H₃ antagonists, such as ABT-239, GSK189254, GSK207040, and JNJ-10181457, enhance social memory (Fox et al., 2003; 2005), reversal learning (Gallici et al., 2009), and object recognition (Medhurst et al., 2007; Southam et al., 2009) in normal rats, improve social memory (Fox et al., 2005) and swim maze learning and memory in aged rats (Medhurst et al., 2007), and reverse the debilitating effects of scopolamine in a two-choice, swim maze-based spatial discrimination task (Fox et al., 2005), a passive avoidance task (Medhurst et al., 2007), and a delayed non-matching to sample task (Gallici et al., 2009). However, no studies have reported the effects of an H₃ antagonist on the performance of APP_{Tg2576} mice in any of these tasks. The ability of ciproxifan to improve performance in the swim maze and object recognition tasks may be related to the H₃-mediated neurotransmitter release thought to contribute to the cognitive-enhancing effects of H₃ antagonists seen in other models. For example, H₃ antagonists have been shown to elevate acetylcholine and dopamine in the prefrontal cortex (Fox et al., 2005; Ligneau et al., 2007b; Gallici et al., 2009), acetylcholine, dopamine, and norepinephrine in the anterior cingulate cortex (Medhurst et al., 2007; Southam et al., 2009), and acetylcholine in the hippocampus (Fox et al., 2005). Given these findings, it would be of interest to determine if H₃ antagonists such as ciproxifan elevate neurotransmitter release in a comparable manner in APP_{Tg2576} mice.

An alternative explanation for the ability of ciproxifan to improve performance in APP_{Tg2576} mice relates to the effects of H₃ antagonists on A β toxicity. Using a cell culture model, it has been reported that the H₃ antagonist, clobenpropit, protects cells from injury produced by A β ₄₂ toxicity via a glutamate receptor-mediated mechanism (Fu, Dai, He, Hu,

Fan, et al., 2010). Given the limited time course of ciproxifan treatment, it is unlikely that the behavioral changes produced by ciproxifan in the present study were related to H₃ receptor-mediated modification of Aβ's progressive toxic effects in APP_{Tg2576} mice. It will be important for future studies to test whether longer-term treatment with H₃ antagonists can modify these and other pathophysiological processes (e.g. synapse loss; Dong et al., 2007) observed in the APP mouse model.

The interpretation that ciproxifan enhances task performance in APP_{Tg2576} mice due to exclusive effects on learning or memory merits caution for several reasons. First, the poor performance of the APP_{Tg2576} mice on the visible platform task may suggest that these mice harbor sensory or perceptual deficits. King and Arendash (2002) reported more significant deficits in APP_{Tg2576} mice in the visible version of the swim maze relative to the hidden version, whereas Westerman et al., (2002) found differences between APP_{Tg2576} and WT mice only in the hidden platform task. In the latter study, mice were tested in the visible platform version first, whereas in the former study and present one, mice were tested in the visible platform task after the hidden platform testing was performed, perhaps suggesting that the sequence of tests significantly alters performance. While the visible platform task has been intended to identify more basic sensorimotor deficits in mice, King and Arendash (2002) nicely argued that this task may still rely on intact learning processes by citing research showing that: 1) visual acuity is not always associated with performance in the task (Lindner, Plone, Shallert, & Emerich, 1997), 2) lesions to "cognitive" brain regions, such as the frontal cortex, impair performance in the visible, but not the hidden, platform task (de Bruin, Sanchez-Santed, Heinsbroek, Donker, & Postmes, 1994), and 3) performance in the visible platform task correlates well with performance on other cognitive tasks (King & Arendash, 2002). It is noteworthy that, like its effect in the hidden version of the swim maze task, ciproxifan improved performance in visible platform version of the task in APP mice. It is possible that ciproxifan improved attention to visual cues in APP_{Tg2576} mice, since ciproxifan has been shown to improve visual attention in other studies (Ligneau et al., 1998).

It is also possible that general changes in anxiety or activity account for the effects of ciproxifan in the swim maze task. Some studies have provided evidence that anxiety is increased in some transgenic models of AD (España, Giménez-Llort, Valero, Miñano, Rábano, et al., 2010; Le Cudennec, Faure, Ly, & Delatour, 2008), although APP_{Tg2576} mice may actually show an anxiolytic phenotype (Gil-Bea, Aisa, Schliebs, & Ramírez, 2007; Lalonde, Lewis, Strazielle, Kim, & Fukuchi, 2003). Other work has shown that stress can induce greater memory impairment in APP_{Tg2576} mice (Dong, Goico, Martin, Csernansky, Bertchume, et al., 2004; Dong, Yuede, Yoo, Martin, Deal, et al., 2008), raising the possibility that ciproxifan improves memory in APP_{Tg2576} mice by simply reducing the stress associated with the swim maze task. These explanations are unlikely, however, since H₃ agonists are known to elicit anxiolytic effects in animals (Yokoyama, Yamauchi, Oyama, Okuma, Onozawa 2009) while there are no reports of such effects after treatment with H₃ antagonists - although H₃ knockout mice demonstrate signs of reduced anxiety (Rizk, Curley, Robertson, & Raber 2004). While a significant influence of H₃ receptors over the anxiety produced by water exposure in the swim maze is unlikely, the possible contribution of the hyperactivity observed in the APP_{Tg2576} mice to the performance deficit observed in these mice is more difficult to refute. As proposed by King and Arendash (2002) and Lalonde et al., (2003), greater locomotor activity in APP_{Tg2576} mice may represent a core deficit in inhibitory control that could be expected to affect the attentional processing required to complete the swim maze task. This idea merits further investigation.

Another aspect of the data that deserves consideration is the increased swim speed observed after ciproxifan treatment in the hidden version of the swim maze. Such an increase fits with other characterizations of ciproxifan as a mild stimulant drug, since earlier studies found that

ciproxifan induces less slow-wave EEG activity and promotes greater wakefulness in rats and cats (Ligneau et al., 1998; Fox et al., 2003). To our knowledge, this is the first report of ciproxifan increasing swim speed in the swim maze, although no such effect has been seen with another H₃ antagonist, GSK189254 (Medhurst et al., 2007). While increases in swim speed could explain differences in the latency to find the platform or the distance from the platform measures, it should be noted that unlike the latter data sets, the swim speed of the APP_{Tg2576} mice treated with saline did not differ from WT mice treated with saline during the last four days of hidden platform training. These distinct patterns of data suggest that group differences in swim speed do not account completely for the significant differences found on the other measures of training performance.

APP_{Tg2576} mice treated with ciproxifan spent more time near the former platform location relative to APP_{Tg2576} mice treated with saline when tested one hour after the last training trial. This same difference was not observed two days after the last training trial, nor was there a genotype effect observed during this latter trial. These data would seem to suggest that ciproxifan can at least enhance short-term memory processes in APP_{Tg2576} mice. The idea that ciproxifan improves shorter-term memory also is buttressed by the finding that ciproxifan alleviated object recognition deficits in APP_{Tg2576} mice when a five minute delay was used. Overall, the results point to the likelihood that ciproxifan acts as a mild stimulant drug that provides short-term alleviation for the cognitive deficits and hyperactivity observed in APP_{Tg2576} mice.

1.4.1. Conclusions

Current palliative treatments for Alzheimer's disease include acetylcholinesterase inhibitors and NMDA antagonists, yet these treatments possess only limited efficacy and significant side effects. Receptor binding data has shown that H₃ receptor densities are preserved in the brains of people with Alzheimer's despite disease progression (Medhurst et al., 2007), indicating that the target for H₃ antagonists remains a viable one throughout the disease process. Therefore, antagonism of H₃ receptors may represent an alternative pathway to cognitive enhancement and remediation of other behavioral symptoms in Alzheimer's disease, or at least an approach that can be coupled with current treatments (Bembenek, Keith, Letavic, Apodaca, Barbier, et al., 2008).

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Figure 1. Amyloid plaques in APP^{Tg2576} mouse brain as revealed through Thioflavin-S staining. Numerous plaque deposits were observed in the hippocampus and cortex of the APP^{Tg2576} mice. The photomicrograph in B. shows a higher magnification image of the boxed image in panel A. Scale bar = 1 mm in A. and 0.1 mm in B.

Figure 2.

Locomotor testing in APP_{Tg2576} and WT mice treated with 3.0 mg/kg of ciproxifan (Cpx) or saline. APP_{Tg2576} mice treated with saline were significantly more active than the WT mice treated with saline during the first 25 minutes of testing (indicated by +) and significantly more active than the APP_{Tg2576} mice treated with ciproxifan during the first 10 minutes of testing (indicated by *). All data represent means ± s.e.m.

Figure 3.

Hidden platform swim maze training in APP_{Tg2576} and WT mice treated with 3.0 mg/kg of ciproxifan (Cpx) or saline. (a) APP_{Tg2576} mice treated with saline took longer to find the escape platform in comparison to saline-treated WT mice on day 4 of training (indicated by *) and APP_{Tg2576} mice treated with ciproxifan (3.0 mg/kg) on days 2, 4, & 5 of testing (indicated by +). (b) APP_{Tg2576} mice swam a greater distance away from the platform in comparison to saline-treated WT mice on day 2 of training (indicated by *) and APP_{Tg2576} mice treated with ciproxifan (3.0 mg/kg) on days 2–5 of testing (indicated by +). (c) There was a trend ($p = .11$) towards a treatment × genotype interaction on the path length measure during the hidden platform training trials. (d) The APP_{Tg2576} mice treated with ciproxifan swam significantly faster than APP_{Tg2576} mice treated with saline (indicated by +). All data represent means ± s.e.m.

Figure 4.

Hidden platform probe trials in APP_{Tg2576} and WT mice treated with 3.0 mg/kg of ciproxifan (Cpx) or saline. (a) APP_{Tg2576} mice treated with saline displayed fewer crossings of the former platform location one hour after the last training trial in comparison WT mice treated with saline (indicated by *) and APP_{Tg2576} mice treated with ciproxifan (indicated by +). (b) APP_{Tg2576} mice treated with saline spent less time near the former location of the platform one hour after the last training trial in comparison to all other groups (indicated by #). There were no statistically significant effects of treatment, genotype, or treatment × genotype on (c) the number of crossings or (d) time spent near the platform when the mice were tested 48 hours after the last training trial. All data represent means ± s.e.m.

Figure 5.

Visible platform swim maze training in APP_{Tg2576} and WT mice treated with 3.0 mg/kg of ciproxifan (Cpx) or saline. (a) APP_{Tg2576} mice treated with saline took significantly longer to find the platform on the first two training days relative to the APP_{Tg2576} mice treated with ciproxifan (indicated by +). (b) There were no significant treatment or genotype effects on the average distance from the platform during each trial. (c) Path length prior to finding the platform was greater for APP_{Tg2576} mice treated with saline than APP_{Tg2576} mice treated with ciproxifan on the last two training days (indicated by +). (d) There were no significant treatment or genotype effects on swim speed. All data represent means $\pm$ s.e.m.

Figure 6.

Novel object recognition in APP_{Tg2576} and WT mice treated with 3.0 mg/kg of ciproxifan (Cpx) or saline. (a) APP_{Tg2576} mice treated with saline spent less time exploring a novel object relative to the time spent exploring a familiar one when compared to all other groups, including the APP_{Tg2576} mice treated with ciproxifan (indicated by #). (b) The total time spent exploring both objects during the test trial did not differ between groups. All data represent means $\pm$ s.e.m.