Neurobiology of Learning and Memory 95 (2011) 64–72

Contents lists available at ScienceDirect

Neurobiology of Learning and Memory

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

Ciproxifan, an H_3 receptor antagonist, alleviates hyperactivity and cognitive deficits in the APP_{Tg2576} mouse model of Alzheimer's disease

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ARTICLE INFO

Article history:
Received 12 August 2010
Revised 26 October 2010
Accepted 31 October 2010
Available online 10 November 2010

Keywords:
Locomotor activity
Swim maze
Object recognition
Histamine
Amyloid-precursor protein
Cognitive enhancement

ABSTRACT

Previous research has indicated that the blockade of H_3-type histamine receptors may improve attention and memory in normal rodents. The purpose of this study was to determine if ciproxifan, an H_3 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 H_3 receptors represents a novel and viable therapeutic strategy in the treatment of Alzheimer's disease.

1. Introduction

Over the past decade, preclinical research has identified the H_3 histamine receptor as a possible target for cognitive-enhancing drugs (Bonaventure et al., 2007; Esbenshade, Fox, & Cowart, 2006). The H_3 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 et al., 2002). Antagonism (or inverse agonism) of the receptor leads to the release of histamine as well as neurotransmitters involved in learning and memory, such as acetylcholine and dopamine, in the hippocampus and prefrontal cortex (Bacciottini et al., 2002; Clapham & Kilpatrick, 1992; Fox et al., 2005; Ligneau et al., 1998; Ligneau, Perrin, et al., 2007; Medhurst et al., 2007). Moreover, H_3 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 H_3 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, Blankenship, & Griffith, 2010; Bernaerts, Lamberty, & Tirelli, 2004; Fox et al., 2003; Galici et al., 2009; Ligneau et al., 1998). In addition to their effects on learning and memory, H_3 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 et al., 2007; Morisset et al., 2002).

The ability of H_3 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 H_3 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 et al., 1996; Taglialatela, Hogan, Zhang, & Dineley, 2009). Some studies of APP_{Tg2576} mice have also reported that mutant mice demonstrate elevated locomotor activity (Golub et al., 2008; Tabuchi 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

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doi:10.1016/j.nlm.2010.10.008

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 min 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 min prior to testing in this study.

2. Materials and methods

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-h 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.

2.2. Genotyping

PCR genotyping was performed using DNA isolated from post-weaning tail biopsies. Forward (5′-GTGGATAACCCCTCCCCCA-GCCTAGACCA-3′) and reverse (5′-CTGACCACTCGACCAGGTTCTG-GGT-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.

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 min 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 min 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 min prior to each object recognition test in a counter-balanced manner.

2.4. Locomotor testing

A total of 39 12–14 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 (28 cm × 17 cm × 12.5 cm h) tracked by (HK) MotorMonitor software (Kinder Scientific, Poway, CA). Drug injections occurred 30 min prior to testing. Mice were placed in the testing apparatus with the lights off for 60 min. Testing was conducted between 8:00 a.m. and 2:00 p.m. for five consecutive days.

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 min 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 min prior to training and testing.

2.5.1. 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 s. This procedure was repeated three times. All mice demonstrated an ability to climb and stay on the platform for 10 s 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.

2.5.2. 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 s. 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 s after escape. If the platform was not found within 60 s, the mouse was manually placed on the platform for 10 s 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.

2.5.3. 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 s. A second probe trial was conducted 48 h 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 cm^2) that included the former platform location.