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Evidence for tolerance following repeated dosing in rats with ciproxifan, but not with A-304121

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Abstract

Blockade of presynaptic histamine H_3 receptors with potent and selective ligands improves cognitive function in rodents and there is significant interest in developing such drugs for long-term symptomatic treatment of CNS disorders such as attention deficit hyperactivity disorder (ADHD). Unfortunately, little is known about the effects of repeated exposure to H_3 receptor antagonists/inverse agonists. We therefore investigated the effects of acute and repeated daily administration of two potent, brain penetrating H_3 receptor antagonists/inverse agonists, ciproxifan and A-304121, on rat body weight, food and water intake, core temperature and locomotor activity, as well as H_3 receptor density and gene expression levels. Methylphenidate, used clinically for the treatment of ADHD, was included as an additional comparator. Ciproxifan, an imidazole-based compound, decreased food intake over the first 10 days and locomotor activity acutely, but these effects were lost after further repeated administration. The ex vivo binding studies revealed increased H_3 receptor density in rats following repeated administration of ciproxifan for 10 or 15 days; however, H_3 receptor gene expression was not changed. In contrast, rats treated with the non-imidazole, A-304121, did not differ from controls on any measure during the observation period, while rats treated with methylphenidate exhibited hyperthermia and hyperactivity. The implications for potential long-term treatment with H_3 receptor antagonists in CNS disorders such as ADHD are discussed.

Keywords: Histamine H_3 receptor inverse agonist; Tolerance; Chronic treatment; Ciproxifan; A-304121

Introduction

The biogenic amine neurotransmitter, histamine, is released from a discrete group of neurons located in the tuberomammillary nucleus of the posterior hypothalamus, which sends projections widely throughout the central nervous system (CNS) to regions including the forebrain, hippocampus, amygdala and hypothalamus (Brown et al., 2001). In the brain, histamine exerts its activity by interacting with three separate G protein-coupled receptors, H_1, H_2 and H_3, and plays important roles in the normal functioning of the CNS including the regulation of various behaviors and physiologic processes such as arousal, learning and memory, circadian rhythm, locomotion, as well as feeding and drinking (for 2 recent reviews see Hancock and Fox, 2004; Witkin and Nelson, 2004).

The efficacy and safety profiles of marketed antagonist drugs targeting peripheral histamine H_1 receptors for allergy and histamine H_2 receptors for gastric ulceration has prompted much interest in the potential therapeutic uses for drugs targeting H_3 receptors. Accumulating evidence indicates that histamine H_3 receptors are located predominantly in the CNS, functioning as presynaptic autoreceptors to regulate the synthesis and release of histamine (Arrang et al., 1983, 1987), as well as heteroreceptors to modulate release of additional neurotransmitters such as acetylcholine and dopamine (Blandina et al., 1998). In rodents, autoradiographic, immunohistochemical or in situ hybridization studies have demonstrated H_3 receptor expression in the cerebral cortex, hippocampus,

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amygdala, hypothalamus, striatum, and nucleus accumbens (for a review, see Leurs et al., 2005) and H_3 receptor ligands have been used extensively in acute preclinical studies to establish roles for these receptors in food/water intake (Morimoto et al., 2001; Hancock et al., 2004), cognitive function (Fox et al., 2002a, 2005; Passani et al., 2000; Onodera et al., 1998; Prast et al., 1996), and sleep-wake control (for a review, see Hancock and Fox, 2004).

H_3 receptors also appear to be tonically activated in vivo and recent evidence indicates a high level of constitutive activity for both rat and human receptors (Morisset et al., 2000; Rouleau et al., 2002). Thus, some H_3 receptor ligands formerly classified as antagonists may now be better classified as inverse agonists, exhibiting negative intrinsic activity after binding to the receptor, whereas others that do not show intrinsic activity are perhaps better referred to as neutral antagonists (Leurs et al., 2005). This point is important since inverse agonists binding H_3 auto- or heteroreceptors may more effectively enhance release of histamine or other neurotransmitters than neutral antagonists, and the vast majority of H_3 receptor blockers appear to be inverse agonists. However, receptor upregulation, manifested as behavioral tolerance following repeated administration, can be potentially more of a problem with inverse agonists (Leurs et al., 2005).

To date, and to the best of our knowledge, only a single report has addressed changes in H_3 receptor responsiveness following repeated administration of a H_3 receptor inverse agonist and this focused largely on biochemical changes in the mouse brain (Morisset et al., 2000). We therefore sought to investigate the effects of acute and repeated dosing in rats with representative compounds from two different classes of H_3 receptor inverse agonists on physiology (food and water intake, body weight gain and core body temperature), simple behavior (spontaneous locomotor activity), as well as H_3 receptor density and gene expression. Compounds, ciproxifan and A-304121, were chosen based on their known in vivo and in vitro pharmacological profiles: Ciproxifan, a potent inverse agonist containing the imidazole moiety found in histamine itself (Ligneau et al., 1998) and A-304121 (2-Amino-1-{4-[3-(4-cyclopropanecarbonyl-phenoxy)-propyl]-piperazin-1-yl}-propan-1-one), a non-imidazole H_3 receptor inverse agonist with a similar potency and efficacy profile to ciproxifan (Esbenshade et al., 2003; Fox et al., 2003). Since many H_3 receptor inverse agonists are targeted toward treating attention deficit hyperactivity disorder (ADHD), methylphenidate, commonly used for the treatment of ADHD (for a review, see Conners, 2002), was included as an additional comparator.

Materials and methods

Animals

Adult, male Sprague–Dawley rats (~250 g) were obtained from Charles River Laboratories (Portage, MI, USA). Rats were single housed in a quiet room under conditions of 12 h lights on/12 h lights off (on at 06:00 AM) with temperature ranging from 20 to 23 °C and relative humidity between 34% and 40%. Animals had free access to powdered food and water. Powdered food was provided in a food container with a stainless wire cover; water was presented via a modified sipper, essentially a 50 ml centrifuge tube connected to a stainless sipper with a 2.5-cm bend that protruded through the front of the cage. All care and use of animals were in compliance with Abbott Animal Care and Use Committee and National Institutes of Health Guide for Care and Use of Laboratory Animals, in facilities approved by the Association for the Assessment and Accreditation of Laboratory Animal Care.

Chemicals

Ciproxifan and A-304121 were synthesized at Abbott Laboratories (Faghih et al., 2003), while methylphenidate HCl (MPH) was purchased from Sigma (St. Louis, MO, USA). Saline (0.9% W/V, Abbott Laboratories, USA) was used as a vehicle, and drug solutions were titrated to pH 5.5–7. [^{3}H]-*N*-α-methylhistamine, 45–90 Ci/mmole, was purchased from PerkinElmer Life Science (Boston, MA, USA). TRIzol® reagent and Quantitative PCR Thermoscript buffer were purchased from InVitrogen/LifeTech (Rockville, MD, USA). The RT-PCR kits were purchased from Applied Biosystems (Foster City, CA, USA). The GC-Melt™ reagent was purchased from Clontech (Palo Alto, CA, USA).

Study design

The experiments were conducted as part of 2 studies. In the first study (Study 1), the physical and behavioral observation of rats as well as H_3 receptor binding and gene expression were conducted and analyzed. In a follow-up study (Study 2), the effects of ciproxifan were evaluated in more detail in ex vivo radioligand binding or RT-PCR studies over the time course described below.

Study 1

A total of sixty rats were randomly assigned into 10 groups. Among them, 7 groups of animals were administered ciproxifan (3 or 10 mg/kg, i.p.), methylphenidate (3 or 10 mg/kg, i.p.), A-304121 (10 or 30 mg/kg, i.p.) or saline daily for 15 days. The remaining 3 groups were not treated until day 15 (acute treatment groups), when they were administered ciproxifan (10 mg/kg, i.p.), methylphenidate (10 mg/kg, i.p.) or A-304121 (30 mg/kg, i.p.). All drugs were dosed as base weight. Body weight, food intake and water consumption were measured every morning before any treatment. Core body temperature was assessed 30 min after dosing on each day; spontaneous locomotor activity was assessed on days 1, 5, 10 and 15, immediately after recording body temperature. The same schedule was applied to animals receiving only one treatment on the last day (acute treatment groups). Designed as a pilot study, all animals were fasted after the locomotor activity test on the 15th day. After 24 h, animals were anesthetized with Nembutal (Abbott Labs, IL, USA), then perfused with 60 ml ice-cold saline; cerebral cortices from 3 brains from each group were prepared for ex vivo binding analysis, while cerebral