Histamine H3 receptor antagonist decreases cue-induced alcohol reinstatement in mice

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ABSTRACT

We have earlier found that the histamine H3 receptor (H3R) antagonism diminishes motivational aspects of alcohol reinforcement in mice. Here we studied the role of H3Rs in cue-induced reinstatement of alcohol seeking in C57BL/6 J mice using two different H3R antagonists. Systemic administration of H3R antagonists attenuated cue-induced alcohol seeking suggesting that H3R antagonists may reduce alcohol craving. To understand how alcohol affects dopamine and histamine release, a microdialysis study was performed on C57BL/6 J mice and the levels of histamine, dopamine and dopamine metabolites were measured in the nucleus accumbens. Alcohol administration was combined with an H3R antagonist pretreatment to reveal whether modulation of H3R affects the effects of alcohol on neurotransmitter release. Alcohol significantly increased the release of dopamine in the nucleus accumbens but did not affect histamine release. Pretreatment with H3R antagonist ciproxifan did not modify the effect of alcohol on dopamine release. However, histamine release was markedly increased with ciproxifan. In conclusion, our findings demonstrate that H3R antagonism attenuates cue-induced reinstatement of alcohol seeking in mice. Alcohol alone does not affect histamine release in the nucleus accumbens but H3R antagonist instead increases histamine release significantly suggesting that the mechanism by which H3R antagonist inhibits alcohol seeking found in the present study and the decreased alcohol reinforcement, reward and consumption found earlier might include alterations in the histaminergic neurotransmission in the nucleus accumbens. These findings imply that selective antagonists of H3Rs could be a therapeutic strategy to prevent relapse and possibly diminish craving to alcohol use.

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1. Introduction

The current pharmacological treatments of alcohol dependence show limited efficacy and considerable side effects limiting their utility (Hillemacher et al., 2015). Manipulation of the brain histaminergic system via histamine H3 receptor (H3R) affects alcohol reward and abuse-related behaviors (Galici et al., 2011; Nuutinen et al., 2011a, 2011b). Brain histamine is elevated in an alcohol-preferring (AA) rat line compared with the alcohol non-preferring (ANA) line and they express lower levels of H3R binding (Lintunen et al., 2001). The H3R agonist immepip and antagonists thioperamide and clobenpropit alter alcohol drinking bidirectionally in rats (Lintunen et al., 2001). Mice lacking histamine (histidine decarboxylase knock-out) display stronger alcohol-induced conditioned place preference (alcohol-CPP) than control animals (Nuutinen et al., 2010) further supporting the inhibitory role of histamine in reward. Also, the H3R knockout mice consume less alcohol in two different drinking paradigms and show a complete lack of alcohol-induced conditioned place preference (Nuutinen et al., 2011a). The H3R antagonist ciproxifan suppressed and the H3R agonist immepip increased alcohol drinking in C57BL/6 J mice (Nuutinen et al. 2011b). Similar effects have been reported in rats with an H3R antagonist (Galici et al., 2011).

Histaminergic neurons in the tuberomamillary nucleus of the hypothalamus project to most parts of the brain (Panula and

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Nuutinen, 2013). Neuronal histamine regulates e.g. sleep–wake cycle, feeding behavior and cognition. The H3R is a highly expressed G protein-coupled autoreceptor in the brain, which regulates the release and synthesis of histamine (Arrang et al., 1983). It also modulates the release other neurotransmitters (Haas et al., 2008). H3Rs are abundant in mesocorticolimbic areas of the brain in both rodents and humans (Anichtchik et al., 2001; Pollard et al., 1993; Rouleau et al., 2004). These areas are central brain regions for alcohol's reinforcing effects (Koob and Volkow, 2010). Alcohol dependence is also characterized by relapse, return to drug use after periods of abstinence. Stress (Brown et al., 1995), re-exposure to small doses of drugs (Self, 1998) and drug-associated stimuli (Epstein et al., 2006; Ludwig et al., 1974; O'Brien et al., 1992) have been shown to provoke craving and relapse in abstinent drug-users and alcoholics. The reinstatement procedure is a validated model to measure relapse to drug use (see reviews by (Epstein et al., 2006; Le and Shaham, 2002; Shaham et al., 2003; Stewart, 2003). The reinstatement models have traditionally been utilized in rats. However, C57BL/6 J mice have a genetically determined high preference for alcohol-containing solutions (Bachmanov et al., 2002; Crabbe et al., 2005) and are therefore widely used in other alcohol and reward-related studies. Although it is clear that H3R antagonists reduce alcohol drinking and place preference (Galici et al., 2011; Lintunen et al., 2001; Nuutinen et al., 2011a, 2011b), it is not known whether H3R antagonists are able to prevent alcohol seeking in animal models of relapse. Ciproxifan is an imidazole-based H3R antagonist widely used in behavioral studies. However, it is important to investigate several different H3R ligands (both imidazole and non-imidazole based) since these molecules are known to differ in their capacity to induce e.g. the release of acetylcholine in different brain areas (Munari et al., 2013). Ciproxifan is metabolized via liver cytochrome enzymes so it cannot be used in humans due to potential interactions with other drugs (Brabant et al., 2009). This being the case, we wanted to find out whether some of the newer non-imidazole based ligands more relevant to possible future therapeutic use would produce similar results. JNJ-39220675 was selected as the second drug to be tested because it has previously been shown to attenuate alcohol self-administration in a rat model (Galici et al., 2011) and in a histamine-dependent manner alcohol-CPP in mice (Vanhanen et al., 2013).

We set up an operant oral alcohol administration model where mice got an oral alcohol dose via lever press combined with a light and sound cue. Next, mice went through an extinction phase during which they gave up lever presses as they no longer received alcohol. Reinstatement of alcohol responding was then studied by activating the light and sound cues when the lever was pressed and the effect of the H3R antagonist was measured. Brain *in vivo* microdialysis in freely-moving mice was used for assessing the effects of alcohol on neurotransmission in the nucleus accumbens, one of the key brain areas mediating reward, and for measuring modulation of alcohol-induced dopamine and histamine release by an H3R antagonist.

2. Materials and methods

2.1. Animals

Male JAX® C57BL/6 J mice obtained from Charles River (France) were used in the reinstatement study. At the beginning of the experiments mice were six weeks old and their body weight during the experiments ranged from 19 to 30 g. Animals were housed in groups of 3–4 in a standard ventilated Scantainer (Scanbur BK A/S, Denmark). Standard food pellets (Scanbur, Sweden) and water were available *ad libitum* except during the operant training sessions. A 12 h/12 h light–dark cycle was reversed (lights off at 0600 h) two weeks prior to the beginning of the experiments. Experiments were carried out during the dark phase between 0700 h and 1300 h. Temperature and humidity were controlled at 22 ± 2 °C and $55 \pm 15\%$, respectively.

Male C57BL/6 J mice bred at the animal facility of the University of Helsinki of age 8–10 weeks were used in the microdialysis study. Animals were single housed and microdialysis experiments were conducted during the light phase between 0700 h and 1400 h.

The principles of Finnish Act on the Use of Animals for Experimental Purposes were followed in conducting these studies and the protocol was approved by the National Animal Experiment Board in Finland. The total number of animals used in this study was 49. All efforts were made to minimize animal suffering and to reduce the number of animals used.

2.2. Drugs

Alcohol solutions were prepared from 99.5% stock solution (Altia, Rajamäki, Finland) diluted to 5%, 8% or 10% alcohol (v/v) using tap water. Alcohol solution for injections was diluted to 0.9% sterile saline (0.9% NaCl) and administered at a dose 1.5 g/kg. Saccharin (0.2%, 0.1% or 0.05% w/v) was dissolved in tap water. Ciproxifan hydrochloride (Sigma–Aldrich, St. Louis, MO) and JNJ-39220675 (Johnson & Johnson Pharmaceutical Research & Development, LLC, San Diego, CA, USA) were dissolved in sterile saline solution. The doses of ciproxifan (0.3 mg/kg, 1 mg/kg and 3 mg/kg) and JNJ-39220675 (10 mg/kg) correspond to the free bases of the drugs. Both drugs and control injections with saline were given intraperitoneally (i.p.) in volumes of 0.1 ml/10 g.

2.3. Operant self-administration apparatus

Alcohol self-administration, extinction and subsequent tests for reinstatement of alcohol seeking behavior took place in standard mouse operant conditioning chambers (21.6 × 17.8 × 12.7 cm, L × W × H) with stainless steel grid floors (ENV-307A, Med Associates, Georgia, Vermont, USA) located inside ventilated sound-attenuating cubicles. In each chamber, one wall contained two retractable levers, one termed as 'active' and the other as 'inactive', that were mounted 1 cm above the floor and extended 1 cm into the chamber. A cue light was located above each lever, but only the cue light associated with the 'active' lever was activated during the training sessions. The alcohol-paired cue light remained lit for 800 ms after each alcohol dose was delivered, during which time responses on the active lever were recorded but had no scheduled consequence. A sound cue was associated with the active lever press. One liquid receptacle was positioned between the levers and connected by a polyethylene tube to a 10-ml syringe attached to a syringe pump inside the cubicle. The top of the back wall of each chamber was equipped with a white house light that remained off at all times. The drinking receptacle was checked at the end of each session to ensure that all doses of alcohol were being consumed. Data collection and all programming functions were controlled by a computer with MED-PC software (Med Associates, Georgia, Vermont, USA).

2.4. Acquisition of alcohol self-administration

The purpose of the acquisition phase was to establish a relationship between an instrumental response (i.e., pressing the active lever) and its consequences (i.e., activation of the cue light, followed by the delivery of 10 μl of alcohol). Experiments were conducted during the dark phase between 0700 and 1300 h every day from Monday to Friday. Each mouse was always exposed to the