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Raclopride lessens the ability of clozapine to suppress alcohol drinking in Syrian golden hamsters

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ABSTRACT

Emerging evidence suggests that the atypical antipsychotic clozapine decreases alcohol consumption in patients with schizophrenia, while typical antipsychotics, all of which are potent dopamine (DA) D2 receptor antagonists, do not. We have proposed that clozapine, through its weak DA D2 receptor blocking action, coupled with its ability to potentiate noradrenergic and serotonergic activity, may ameliorate a dysfunction in the mesocorticolimbic DA reward circuitry that underlies alcohol use disorder in patients with schizophrenia. In prior studies, we have demonstrated that clozapine also decreases alcohol drinking in the Syrian golden hamster, but haloperidol does not. The purposes of the current study were: (1) to further assess the effect of clozapine (2 or 4 mg/kg/day, s.c.) on alcohol consumption in hamsters, using a continuous access, 2-bottle choice paradigm; and (2) to examine whether clozapine's effect on alcohol drinking is affected by increasing its DA D2 blockade through adjunctive use of the potent DA D2 receptor antagonist raclopride (2, 4, or 6 mg/kg/day, s.c.). The major findings were: (1) clozapine suppressed both initiation and maintenance of alcohol drinking in hamsters; and (2) these effects of clozapine were lessened when raclopride was given adjunctively with clozapine. These data suggest that clozapine may limit alcohol drinking in the golden hamster (and possibly in patients with schizophrenia) in part because of its weak blockade of the DA D2 receptor.

1. Introduction

Alcohol use disorder occurs commonly in patients with schizophrenia and dramatically worsens their clinical course (Drake et al., 1989; Regier et al., 1990). Despite the problems presented by their alcohol use, few treatment options are available to control alcohol use in these individuals. Typical antipsychotic medications, such as haloperidol, do not appear to decrease their alcohol consumption (Green et al., 2008). However, emerging data from our group and others suggest that the atypical antipsychotic clozapine may substantially decrease alcohol use in patients with schizophrenia and co-occurring alcoholism (Albanese et al., 1994; Drake et al., 2000; Green et al., 2003, 2008; Lee et al., 1998; Marcus and Snyder, 1995; Yovell and Opler, 1994; Zimmet et al., 2000), although it is unclear what pharmacologic action of clozapine allows it to do so.

Unlike typical antipsychotics, all of which have potent dopamine (DA) D2 receptor blocking ability, clozapine is a broad-spectrum agent with relatively weak affinity for the DA D2 receptor (Ashby and Wang, 1996). Clozapine's weak affinity for the DA D2 receptor, coupled with its diverse effects on noradrenergic and serotonergic systems, has been proposed to contribute to its atypicality, i.e., its ability to ameliorate positive and negative symptoms without producing significant extrapyramidal symptoms (Deutch et al., 1991), and that its atypicality may be lost when the potency of its DA D2 receptor antagonism is increased (Green et al., 1999; Kapur and Remington, 1996; Svensson, 2003b). For instance, isoclozapine, which has equivalent affinity to clozapine for multiple receptors (e.g., 5-HT1a, 5-HT2, D1, D4, and M1), but 10-fold higher affinity than clozapine for DA D2 receptors, behaves, not like clozapine, but like typical antipsychotics in behavioral assays in rodents, e.g., in tests of catalepsy and conditioned avoidance response, as well as in molecular assays – isoclozapine increases FOS expression in both nucleus accumbens and striatum, whereas clozapine increases it preferentially in the accumbens (Kapur et al., 2002).

Studies (mostly in animals) suggest the possibility that clozapine may normalize a dysfunctional mesocorticolimbic DA reward

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circuitry in patients with schizophrenia through a mechanism involving weak DA D2 receptor blockade, coupled with blockade of noradrenergic and serotonergic receptors (Green et al., 1999). For example, clozapine-like drug combinations consisting of low doses of a D2 receptor antagonist and either a noradrenergic α-2 or a serotonergic 5-HT2 receptor antagonist have been shown to enhance the signal detection capacity of dysfunctional mesocorticolimbic circuitry by increasing DA release in the prefrontal cortex and by partially restoring the balance between burst and tonic firing modes of dopaminergic neurons in the ventral tegmental area (Svensson, 2003a; Svensson et al., 1995).

Green and colleagues have further proposed, based on these and other data, that (1) patients with schizophrenia have a reward deficiency syndrome, secondary to a dysfunction in the mesocorticolimbic DA reward circuitry, that underlies alcohol use in this population; (2) an important biologic effect of alcohol in patients with schizophrenia may involve a transient amelioration of this brain reward deficiency; and (3) unlike typical antipsychotics, clozapine, through its varied actions on serotonergic and noradrenergic neurons (especially its antagonistic effects on α-2 adrenergic receptors and its ability to chronically elevate norepinephrine in brain and plasma), coupled with its weak dopamine D2 receptor blocking ability, may tend to have a "normalizing" effect on the signal detection capacity of the dysfunctional mesocorticolimbic DA system in such patients, thereby ameliorating the basis of their use of alcohol (Green et al., 1999).

While clozapine has been of considerable interest because of its broad-spectrum pharmacological profile, as well as its unusual clinical efficacy, including its ability to limit alcohol and substance use in patients with schizophrenia, only a few studies have assessed its effects on alcohol drinking in animals. Studies in rodents have shown that a single injection of clozapine does not decrease alcohol drinking in the limited access paradigm (Ingman and Korpi, 2006; June et al., 1991; Thrasher et al., 1999); however, other studies in rodents have shown that repeated clozapine administration decreases alcohol drinking in the continuous access paradigm (Chau et al., 2010; Green et al., 2004; Ufer et al., 1999).

To further elucidate the effects of clozapine on alcohol consumption, our laboratory has examined the comparative effects of clozapine versus the typical antipsychotic haloperidol in the Syrian golden hamster (*Mesocricetus auratus*). Our data using a continuous access paradigm have indicated that clozapine, but not haloperidol, decreases chronic alcohol drinking in this animal, as it does in patients with schizophrenia (Chau et al., 2010, 2004). In the current study, we tested the hypothesis that clozapine's ability to suppress alcohol drinking in hamsters is related in part to the fact that it is a weak, rather than strong, DA D2 receptor antagonist. Specifically, we assessed whether the effects of clozapine on the initiation and maintenance of alcohol drinking in the golden hamster would be blunted if the potent D2 receptor antagonist raclopride was added to clozapine to increase the DA D2 receptor blockade.

2. Material and methods

2.1. Experiment 1: determining the effects of raclopride and clozapine combinations on initiation of alcohol drinking

2.1.1. Animals and experimental procedures

Forty adult, male Syrian Golden Hamsters (*M. auratus*) (100–130 g) were acquired from Harlan Inc. (Indianapolis, IN) and individually housed on an altered 12 h/12 h light/dark cycle (3 AM light on/3 PM light off) in standard home cages with *ad libitum* access to food and water. To compare the effects of clozapine, raclopride, combinations of clozapine and raclopride, and vehicle on initiation of alcohol drinking, hamsters were randomly assigned to 1 of 6 treatment groups ($n = 6–7$ per group) receiving subcutaneous (s.c.) injections of: (1) 2 mg/kg/day clozapine [2CLOZ]; (2) 4 mg/kg/day clozapine [4CLOZ]; (3) 4 mg/kg/day raclopride [4RACL]; (4) 2 mg/kg/day clozapine + 4 mg/kg/day raclopride [2CLOZ + 4RACL]; (5) 4 mg/kg/day clozapine + 4 mg/kg/day raclopride [4CLOZ + 4RACL]; or (6) vehicle [VEH]. Animals from each group were injected with the respective drug(s) daily over a period of 18 days. Since studies have shown that an injection of 2 mg/kg raclopride in the rat produces peak DA D2 receptor binding >80% after 1–2 h and approximately 50% after 4–8 h (Wadenberg et al., 2000), to maintain high levels of DA D2 receptor blockade throughout the animals' most active period (i.e., the initial phase of the dark cycle), 2 mg/kg raclopride was given twice a day; the first injection occurred at 1 PM (2 h before the onset of the dark cycle) and the second injection occurred 6 h later, at 7 PM (4 h following the onset of the dark cycle), for a total of 4 mg/kg/day, as noted above. A similar twice-per-day-injection procedure has been previously used by other investigators to assess the effects of raclopride and other drugs with relatively short half-life on free-access alcohol drinking in the rat (Silvestre et al., 1996). The groups that were not given raclopride received a second injection consisting of vehicle. Since data from our prior studies demonstrated that clozapine injected in hamsters once per day, over successive days, substantially decreases their alcohol drinking (Chau et al., 2010; Green et al., 2004), in this experiment, clozapine was given once per day, at 1 PM (2 h before the onset of the dark cycle). Beginning on the 4th day of treatment, all animals were given continuous access to a 15% (v/v) ethanol solution as a second choice of drinking fluid. The positions of the water and alcohol bottles were rotated on a daily basis to prevent positional preference. A technician, blinded to the experimental conditions, measured alcohol and water intake (every 24 h), food intake (every 48 h), and body weight (every 3 days). Measurements took place at 12 noon (3 h before dark).

2.1.2. Drug preparation

The clozapine solutions were prepared for injection by first dissolving clozapine (Sigma Chemical Inc.) in 0.5 N acetic acid, then adjusting the volumes to the desired clozapine concentrations using a vehicle solution (0.5 M sodium acetate, pH 5.5). The pH levels of the clozapine solutions were subsequently adjusted to 5.5 to match that of the vehicle. Raclopride solutions were prepared for injection by dissolving the desired amounts of raclopride hydrochloride (Sigma Chemical Inc.) in vehicle solution. The combinations of clozapine and raclopride were prepared by dissolving the desired amounts of raclopride hydrochloride in the clozapine solution. Injections were given in volumes of 2.0 ml/kg body weight.

2.1.3. Data analysis

Alcohol intake (g/kg), water intake (ml), food intake (g), and body weight (g) data were analyzed using two-way repeated measures analysis of variance (ANOVA), using time and drug treatment as independent variables. Because 2-way ANOVA did not detect significant effects of treatment group on alcohol drinking but visual inspection of the data suggested otherwise, additional analyses of the alcohol drinking data were conducted using a mixed effects model for repeated measures, implemented in SAS Proc Mixed procedure (SAS Institute, 2008), an extension of the traditional ANOVA that allows more flexible modeling of time effects and correlated observations due to repeated measurement than ANOVA (Jennrich and Schluchter, 1986; Laird and Ware, 1982). When the mixed model analysis indicated that significant differences existed among treatments, post-hoc pair-wise comparisons between groups were made based on the best-fit model of the data. Significance was determined at $p < 0.05$.

2.2. Experiment 2: determining the effects of raclopride and clozapine combinations on maintenance of alcohol drinking

2.2.1. Animals and experimental procedures

Fifty-six adult, male Syrian golden hamsters (100–130 g, Harlan Inc., Indianapolis, IN) were individually housed in standard home cages with *ad libitum* access to food and water. The room was maintained on an altered 12 h/12 h light/dark cycle (light on at 3 AM/light off at 3 PM). All animals were given continuous access to a bottle of water and a second bottle containing 15% (v/v) alcohol for 20 days prior to randomization into treatment groups. The positions of the two drinking bottles were rotated on a daily basis to prevent positional preference. A technician, blinded to the experimental conditions, measured fluid intake (every 24 h), and food intake and body weight (every 3 days). Measurements took place at the same time each day, at 12 noon (3 h before the onset of the dark cycle). On the 20th day of alcohol drinking, hamsters were divided into 8 treatment groups ($n = 7$ per group) with similar baseline alcohol intake values (g/kg/day), calculated using data from the last 4 days. Over a period of 20 days, individual treatment groups received subcutaneously (s.c.) injections with one the following: (1) vehicle [VEH]; (2) 2 mg/kg/day raclopride [2RACL]; (3) 4 mg/kg/day raclopride [4RACL]; (4) 6 mg/kg/day raclopride [6RACL]; (5) 4 mg/kg/day clozapine + 2 mg/kg/day raclopride [4CLOZ + 2RACL]; (6) 4 mg/kg/day clozapine + 4 mg/kg/day raclopride [4CLOZ + 4RACL]; (7) 4 mg/kg/day clozapine + 6 mg/kg/day raclopride [4CLOZ + 6RACL]; or (8) 4 mg/kg/day clozapine [4CLOZ]. All injections occurred once per day, at 1 PM (2 h before the onset of the dark cycle).

2.2.2. Drug preparation

The drug preparation procedures used in this experiment were identical to the procedures used in Experiment 1 (see Section 2.1.2).