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RAPID COMMUNICATION

Zacopride, a 5-HT_3 Receptor Antagonist, Reduces Voluntary Ethanol Consumption in Rats

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KNAPP, D. J. AND L. A. POHORECKY. *Zacopride, a 5-HT_3 receptor antagonist, reduces voluntary ethanol consumption in rats.* PHARMACOL BIOCHEM BEHAV **41**(4) 847–850, 1992.—The effect of the selective 5-HT_3 receptor antagonist, zacopride, was assessed in male Sprague-Dawley rats in free choice (6% ethanol and water) experiments. In Experiment 1, single zacopride (0.01–10 mg/kg, IP) injections failed to alter ethanol (ET) consumption during 1-h restricted ET access. In Experiment 2, zacopride (5.0 and 10 mg/kg, IP) injected twice daily for 5 days significantly reduced ET intake and ET preference during 24-h free access to 6% ET and water without altering the total volume of fluid consumed. Thus, the schedule of ET access (i.e., free vs. restricted) and/or the duration of drug treatment may determine the efficacy of pharmacological agents in altering ET preference. 5-HT_3 receptor blockade may reduce serotonin/dopamine-mediated maintenance of ET preference; a process that may proceed via extinction mechanisms.

Drinking Zacopride 5-HT_3 receptors Restricted access 24-h access Ethanol intake Extinction Water intake

RECENT evidence indicates that the 5-HT_3 receptor may mediate the voluntary intake of ET. For example, Fadda et al. (4) found that 6 days of treatment with the 5-HT_3 antagonist MDL 72222 inhibited daily voluntary ET consumption in rats bred for high ET preference. Similarly, Sellers et al. (12) found that the 5-HT_3 antagonist GR38032F (ondansetron) reduced ET consumption monitored for 12 h after drug administration, an effect that has recently been replicated with human ET abusers (15).

The neural mechanism through which 5-HT_3 modifies ET consumption is unknown. Serotonin-3 receptor blockade may indirectly affect ET consumption by decreasing activity of the dopaminergic system, a probable substrate for ET's reinforcing effects (3,5). Thus, 2-methyl-5-HT, a selective 5-HT_3 agonist, stimulates dopamine release in the striatal slice preparation (1); and, in vivo, 5-HT_3 antagonists block ET-induced dopamine release (2,9,16). Furthermore, behavioral studies in pigeons indicate that 5-HT_3 antagonists reduce the discriminative stimulus properties of ET (8).

Together, these data suggest that 5-HT_3 receptor stimulation may mediate voluntary ET consumption by activating dopamine "reward" or reinforcement mechanisms. 5-HT_3 receptor blockade, accordingly, may reduce ET consumption by attenuating the dopaminergically mediated reinforcing effects of ET.

In the two experiments described below, the effects of single and multiple doses of the 5-HT_3 antagonist, zacopride, on voluntary ET consumption were assessed in a 1-h (restricted) and 24-h (free) access paradigms. The potent 5-HT_3 antagonist, zacopride, binds strongly to 5-HT_3 receptors (14), and thus it may be a more potent agent for the 5-HT_3-mediated anti-dopamine effects than MDL 72222 (11).

EXPERIMENT 1

METHOD

Subjects

Fifteen adult male Sprague-Dawley rats served as subjects in Experiment 1. A 12 L:12 D cycle with lights off at 0930 h was in effect throughout with ambient vivarium temperature controlled at 21 ± 1°C. Food and water were available ad lib.

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Apparatus and Procedure

Rats were tested in 26 × 26 × 30 cm Plexiglas test cages with two holes for sipper tubes on a wall, 19.5 cm above the cage floor. Plastic drinking tubes (100 ml) were equipped with steel-ball-tipped sipper tubes to reduce spillage. A 2-mm steel bar extended the width of the cage 13.5 cm above the cage floor and 3.5 cm medial to the wall with the two holes. Thus, animals could rear, grab the bar with the two forepaws, and commence licking. A Metlar GT4000 electronic balance equipped via an RS-232 interface to an IBM-compatible computer was used to record drinking tube weight and body weight measurements.

A 13-day, 24-h home cage ET conditioning phase initiated ET consumption. Over the course of the first 6 days, ET concentration increased from 1% to 6% v/v. Tubes were removed from the home cages approximately 1 h prior to the onset of the dark cycle. During this time, tubes were weighed and refilled, and body weights were taken. Weight of fluid consumed was converted to g/kg 6% ET consumed by the formula: g/kg ET = ml fluid × 0.794 g/ml × 0.06 × 1/kg body weight. Preference ratio was defined as ml 6% ET/(ml 6% ET + water).

For days 7–14, the onset of the dark cycle signalled the immediate transfer of the animals to the test cages, where water and 6% ET was available. After 1 h, animals were returned to home cages and volumes consumed were recorded. On day 15, zacopride (0.0, 0.01, 0.1, 1.0, or 10.0 mg/kg, IP, 1 ml/kg; A.H. Robbins) was administered 45 min prior to the 1-h ethanol access session. Zacopride was administered in a counterbalanced design with 4-day intervals between treatment days, such that all animals received all drug dosages.

For statistical analyses, significance level was set at $p \leq 0.05$ (analysis of variance [ANOVA]). Comparisons of saline versus drug treatments on each day were made following the determination of significant main effects of dosage across days.

RESULTS

Zacopride failed to alter ET consumption at any of the four dosages tested, $F(4,56) < 1$. The mean (± SEM) ET consumption for each of the 0.01, 0.1, 1.0, and 10.0 g/kg groups was 6.0(0.45), 5.3(0.54), 6.2(0.38), 5.8(0.41), and 5.5(0.40) ml, respectively. Water intake during this 1-h restricted access, which was minimal and averaged approximately 1 ml, was also unaltered by the zacopride treatment. No change in ET preference was noted following this treatment.

These results indicate that zacopride fails to alter voluntary ET intake during 1-h restricted access. Experiment 2 was designed to determine whether zacopride reduces ET intake in a free-access paradigm.

EXPERIMENT 2

METHOD

Subjects

For Experiment 2, 29 male Sprague-Dawley rats, 540–640 g at the start of the experiment, served as subjects. All other conditions were similar to those in Experiment 1.

Apparatus and Procedure

Animals were tested in their home cages (standard wire-mesh-bottomed steel). Water and 6% ET tubes were attached to the front of the home cages with wire springs. As in Experiment 1, a 13-day ET conditioning phase initiated ET consumption. Four groups of rats (n = 7–8) were matched on baseline ET consumption (days 11–13, predrug phase) prior to administration of either 0.0 or 1.0, 5.0, or 10.0 mg/kg zacopride 45 min prior to the onset of the dark cycle (first injection) for a period of 5 days (days 14–18, drug phase). Identical treatments were administered 6 h following the onset of the dark cycle (second injection) for a total of two drug administrations per day. Following the drug treatment period, ET and water consumption were monitored for days 19–21 (postdrug phase).

RESULTS

Ethanol consumption during the predrug phase averaged approximately 2.5 g/kg/day (Fig. 1A). No significant change in body weight was noted during the three phases of this experiment. As depicted in Fig. 1A and 1B, ET intake, expressed as g/kg ET consumed and preference ratios, was reduced by the zacopride treatment, $F(3,200) = 11.44$, $p < 0.0001$, and $F(3,200) = 23.91$, $p < 0.0001$, respectively, over the combined course of the drug and postdrug phases. Concomitant increases in water consumption (Fig. 1C) occurred during the drug treatment period, $F(3,200) = 12.89$, $p < 0.0001$. No differences in total ml/kg fluid consumed was noted during this period, $F(3,200) < 1$.

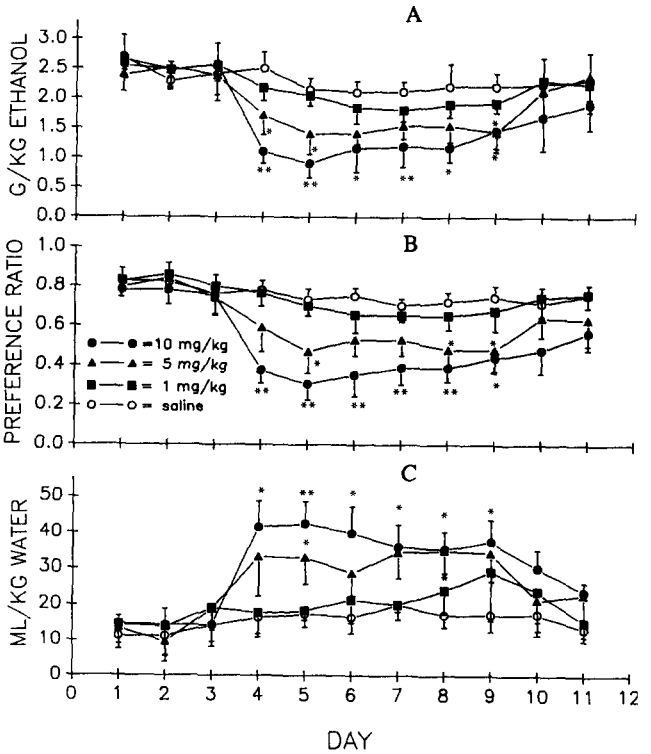

FIG. 1. The effects of zacopride on (A) g/kg ethanol consumed, (B) preference ratio, and (C) ml/kg water consumed over 11 days of the experiment. Each value represents the mean ± SEM of 7–8 animals. Zacopride was given twice daily (0.0, 1.0, 5.0, or 10.0 mg/kg, IP) from day 4 to day 8. *$p < 0.05$, **$p < 0.01$ in comparison with saline values on each day.

GENERAL DISCUSSION

The findings from these two experiments suggest that 5-HT_3 antagonism can reduce ET consumption in rats in a free-access but not in a restricted-access paradigm. The data from the free-access experiment complement the findings of other investigators who have found that 5-HT_3 antagonists decrease ET drinking behavior in rats (4,12). Furthermore, these data demonstrate that 5-HT_3 receptor antagonism can reduce ET consumption in rats not genetically selected for high ET preference.

Although zacopride reduced voluntary ET intake during free access, zacopride appears not to effectively reduce ET consumption in the context of the restricted-access paradigm. The lack of an effect of zacopride in the 1-h access paradigm could be explained in several ways. For example, the effect of 5-HT_3 antagonists may vary according to the length of drug treatment and/or the number of days of ET availability for their effects. Alternatively, the single zacopride treatment in Experiment 1 may not have been sufficient to alter 5-HT_3-mediated dopamine release. This effect is unlikely since other investigators have demonstrated that 5-HT_3 antagonists immediately reduce dopamine release (16). Thus, this neurochemical consequence may not become behaviorally manifest as reduced ET consumption in the short term. These results do not rule out, of course, the possibility that repeated zacopride treatments would have also reduced ET intake in a restricted-access paradigm. That treatments with zacopride or MDL 72222 appear to reach maximal efficacy on the first (present data) vs. third day (4), respectively, may reflect the relatively greater potency of zacopride for 5-HT_3 antagonism (11). Additionally, because the recovery of drinking behavior was not immediate following the cessation of zacopride treatment, an indirect drug effect may temporarily maintain reduced drinking in the absence of the drug.

Possibly, zacopride-mediated effects on the dopamine system account for the results of these experiments. Administration of zacopride appears to initiate a process whereby rats reduce ET consumption relatively gradually with a subsequent gradual recovery following drug removal. If 5-HT_3 antagonists reduce ET consumption by preventing serotonergically mediated dopamine release, then decreases in ET consumption (like decreased bar-pressing after haloperidol) should gradually become evident during a long (e.g., 24-h) postdrug ET access period. In contrast, decreases in ET consumption should not be evident during a short (e.g., 1 h) postdrug access period since dopaminergically mediated consummatory responses are only gradually extinguished. Thus, administration of a dopaminergic antagonist causes a gradual reduction in consummatory responding as the organism learns that a previously reinforcing substance is no longer "rewarding" or reinforcing. For example, dopamine antagonists do not immediately inhibit bar-pressing for electrical brain stimulation in rats. Rather, the bar pressing response is extinguished gradually (6,7).

Sinclair (13) demonstrated that pharmacological treatments that reduce ET consumption, like the anti-dopaminergic treatments described above, may operate via extinction. The primary information supporting this assertion appears to be that many days of naloxone treatment are required to maximally reduce ET drinking behavior. Sinclair also found that ET consumption was slow to recover after the last dose of naloxone. The extended time required for the recovery of ET drinking after naloxone suggests that animals must relearn that ET is reinforcing after many days of further drinking. Interestingly, the complementary finding that morphine-induced enhancement of ET consumption in rats remains elevated for up to 10 days following the cessation of morphine treatment (10) also suggests that the animals relearn that the ET is reinforcing only after many days of further drinking behavior.

Whether the molecular mechanism of 5-HT_3 receptor antagonism/reduced dopamine release and the behavioral process of extinction/relearning are wholly separate or related phenomena is a question that deserves further study. Inasmuch as delayed effects of pharmacological agents on ET drinking reflect extinction/relearning, our results suggest that 5-HT_3 receptor blockade may result in extinction of ET drinking.

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