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RAPID COMMUNICATION

Failure of the 5-HT_2 Receptor Antagonist, Ritanserin, to Alter Preference for Alcohol in Drinking Rats

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MYERS, R. D. AND M. F. LANKFORD. *Failure of the 5-HT_2 receptor antagonist, ritanserin, to alter preference for alcohol in drinking rats.* PHARMACOL BIOCHEM BEHAV **45**(1) 233–237, 1993.—The purpose of this study was to determine whether the 5-HT_2 receptor antagonist, ritanserin, possesses the same sort of efficacy as another central 5-HT_2 antagonist, amperozide, in reducing the pharmacologically induced preference for ethyl alcohol in the rat. Following the repeated administration of the inhibitor of aldehyde dehydrogenase, cyanamide, the preference for alcohol vs. water was determined in each of 20 Sprague-Dawley rats by a standard test using 3–30% concentrations. Then, each rat was offered water and its maximally preferred concentration of alcohol, which ranged from 9–15% and was consumed at a mean of 5.02 ± 0.44 g/kg per day. After a 4-day predrug control test, either the saline control solution or 0.1, 0.3, or 1.0 mg/kg ritanserin was administered SC at 1600 h over 3 days. The daily intakes of alcohol of rats both during and after treatment with ritanserin were unchanged in terms of absolute g/kg and proportion of alcohol to total fluid consumed. Similarly, the control saline also was without any effect on alcohol consumption. Neither the consumption of food and total fluids nor the level of body weight was affected by these doses of ritanserin. Because our findings fail to coincide with previous reports on the effect of ritanserin on alcohol preference, it is envisaged that a methodological difference in earlier experimental procedures, such as the use of a weak 3% concentration of alcohol, could explain the discrepancy. Further, the present results contrast with the prolonged reduction in drinking produced by another 5-HT_2 receptor antagonist, amperozide, which also acts centrally on dopaminergic neurons in the limbic system. Thus, it is concluded that the antagonism of 5-HT_2 receptors alone is not sufficient to ameliorate an aberrant level of alcohol intake or counteract an irreversible preference for the fluid induced by the inhibition of aldehyde dehydrogenase.

Alcohol drinking Ritanserin Amperozide Serotonin receptors Alcohol preference Dopaminergic systems Mesolimbic system Alcoholism 5-HT_2 receptors Therapeutic treatment Aberrant drinking

A number of compounds that affect serotonin (5-HT) systems in the brain were examined over two decades ago for their effect on preference for ethyl alcohol (24). These substances included tryptophan hydroxylase inhibitors, tryptophan, 5-hydroxytryptophan, and 5-hydroxytryptamine (5-HT) itself (19,20,23). Later, several drugs that attenuate the presynaptic reuptake of 5-HT, including zimelidine, fluoxetine, and sertraline, were found to reduce the self-administration of alcohol (6,15,25). In each case, however, interference with the functioning of 5-HT in the brain by each of these compounds, including inhibitors of 5-HT reuptake, causes an impairment of caloric regulation and appetite. Thus, the nonspecific nature of these classes of drug on ingestive behavior could preclude their consideration for the therapeutic amelioration of alcohol drinking (6,25).

Another more recent experimental approach to ameliorating voluntary selection of alcohol has been to block different 5-HT receptor subtypes (5,14). Recently, it was reported that ritanserin, a 5-HT_2 receptor antagonist, diminished the intake of 3% alcohol by rats in a self-selection situation and 5% alcohol in rats bred for a catecholamine response to stress (14,27,30). Because of the low test concentrations of alcohol used in these studies, the present experiments were undertaken to determine whether ritanserin would alter preference for

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alcohol in concentrations that are not only maximally preferred but also of potential pharmacological significance to the animal. In this investigation, Sprague-Dawley rats, which normally avoid alcohol, were induced pharmacologically to prefer this fluid by administration of an aldehyde dehydrogenase inhibitor, cyanamide (2,4). Then, one of three efficacious doses of ritanserin was administered over a 3-day period to the rat in the midpoint of an 11-day preference sequence. During the self-selection tests, animals were offered their maximally preferred concentration of alcohol in the presence of water.

METHOD

Male Sprague-Dawley rats (n = 20), 30 days old at the beginning of the experiments, were housed in stainless steel wire cages and kept on a 12 L : 12 D cycle with lights on at 0700 h. Each rat was maintained on a daily regimen of Purina Rat Chow and tapwater ad lib. The respective intakes of food and fluids as well as body weights were recorded at 0830 h on each day throughout the experiments.

Cyanamide Treatment

Each rat was given a series of SC injections of cyanamide (Sigma Chemical Co., St. Louis, MO) according to experimental procedures described previously (2). A dose of 10 mg/kg cyanamide was selected based upon previous dose-response data to induce an elevated and stable preference for alcohol (4). An injection of cyanamide was given b.i.d. at 1000 and 1600 h for 3 consecutive days.

Alcohol Preference Tests

A standard three-bottle-two-choice procedure was used to determine the maximally preferred concentration of alcohol (10). One 100-ml Kimax drinking tube contained water, the second was empty, and a third tube was filled with a v/v solution of alcohol. On the first day of the test sequence, a 3% concentration of alcohol was offered to each animal for 1 day; thereafter, the concentration was increased over the next 10 days as follows: 4, 5, 6, 7, 9, 11, 13, 15, 20, and 30%. The tubes were rotated daily according to a predetermined random schedule to prevent the development of a position habit (17).

A maximally preferred concentration of alcohol was determined subsequently for each animal as based upon the highest intake of one of the solutions offered during the 3–30% preference test (10,21). This procedure required a 4- to 6-day interval for the intake of alcohol to stabilize. The individual solution of alcohol consumed by the rat in the largest volume above the 50% level in the proportion of alcohol to total fluid consumed was then selected as the test concentration.

Administration of Ritanserin

Following a 4-day predrug control interval, rats were given ritanserin or 0.9% saline control solution on 3 consecutive days. To enhance its dissolution, ritanserin was prepared daily in sterilized water acidified in a 1 : 2,000 dilution of 1.0 normal acetic acid to a pH of 3.5–4.0. Injections of the drug or saline (n = 6) were given SC once a day at 1600 h as reported previously (14). On the basis of previous pharmacological reports (1,3,8,11,33) as well as receptor binding data that show that 0.63 mg/kg saturates 5-HT_2 receptors in cortical tissue (11), three doses of ritanserin were used as follows: 0.1 mg/kg (n = 4); 0.3 mg/kg (n = 5); and 1.0 mg/kg (n = 5). The sequence of injection of these doses and the control saline was randomized after the intake of the preferred concentration of alcohol of each rat had stabilized. During the 3-day period of injections and for 4 days thereafter, testing of alcohol preference continued.

The data were analyzed using the InStat software program and a one-way analysis of variance followed by posthoc Student–Newman–Keuls test when appropriate. A p value of <0.05 was considered statistically significant.

RESULTS

The consumption of alcohol over 11 days expressed as the mean proportional and g/kg intakes is presented in Fig. 1 top and bottom, respectively, for the pre- and postcontrol and treatment intervals. As shown in Fig. 1 (top), during the 3-day period of injections of each of the doses of ritanserin, the proportions of alcohol to total fluid consumed by rats were unchanged in comparison to the precontrol levels. Similarly, as presented in Fig. 1 (bottom), no significant differences in the g/kg intakes of alcohol from the precontrol mean level of 5.0 g/kg were produced by the 5-HT_2 receptor antagonist either during or after the treatment with the three doses of the drug. Although the intermediate dose of 0.3 mg/kg given similarly reduced the daily g/kg intake of alcohol from the

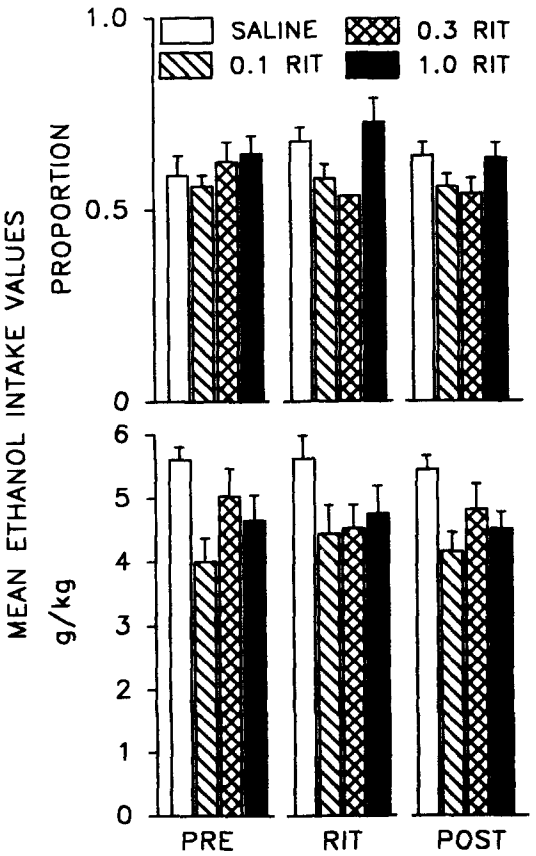

FIG. 1. Mean ± SE intakes of alcohol in proportion of alcohol to total fluid (top) and absolute g/kg (bottom). Preference was tested for 4 control days before (PRE), 3 days during the administration of saline control vehicle (n = 6) or of ritanserin b.i.d. in a dose of 0.1 mg/kg (n = 4), 0.3 mg/kg (n = 5), or 1.0 mg/kg (n = 5), and 4 control days after ritanserin injections (POST).

mean precontrol level of 5.02 ± 0.44 g/kg to a mean of 4.51 ± 0.37 g/kg during treatment, this decline was not statistically significant (Fig. 1, bottom).

A composite analysis of the mean effects of the three doses of ritanserin on the intakes of food, water, and alcohol as well as on body weights of treated rats is presented in Table 1. Both during and after the course of the drug treatment, no significant effects were produced by any of the doses of ritanserin in terms of a change in body weight nor in the amounts of food and water consumed by the rats. As shown in Table 1, the saline control vehicle also was without any significant effects on the intake of alcohol.

DISCUSSION

Historically, central serotonergic synapses have been implicated in the selection as well as rejection of alcohol offered in a free choice situation with water (13,17,24). The present experiments address the issue of the efficacy of ritanserin, a drug that has a high affinity for 5-HT_2 receptors in the brain, on the self-selection of alcohol. The results show that ritanserin administered in one of three doses fails to alter the volitional intake of alcohol offered in individually determined, maximally preferred concentrations. These findings do not coincide with earlier reports in which higher doses attenuated the intake of a single low concentration of 3% alcohol (14,27). However, they do confirm two other reports in which ritanserin administered in a range of doses also was without effect on the preference for pharmacologically significant concentrations of alcohol [(35); Nichols et al., 1993, personal communication]. Several factors may explain, at least in part, the source of the discrepancy between all of these data and the earlier findings with ritanserin.

First, the doses used in the present study may have been too low to bring about a change in serotonergic function in the rat and, subsequently, its preference for alcohol. This explanation is unlikely because doses of ritanserin in the range of 0.037–1.0 mg/kg are pharmacologically efficacious in a number of diverse experimental conditions. For example, 0.037 mg/kg blocks convulsions in the forepaws of the rat induced by tryptamine, whereas 0.11 mg/kg inhibits twitches of the head caused by 5-hydroxytryptophan or mescaline (1); 0.5 mg/kg attenuates significantly the anorexic effect of quipazine (8); and 0.63 mg/kg, which saturates maximally the 5-HT_2 receptors in cortical tissue (11), markedly alters slow-wave sleep, paradoxical sleep, and the period of waking in the rat (31), as well as reduces in vivo portal venous blood pressure, which is potentiated by propranolol (29); 0.7 mg/kg lowers portal blood pressure without altering hepatic or renal blood flow (26); and 1.0 mg/kg ritanserin antagonizes the impaired performance caused by LSD or quipazine (3), blocks the inhibitory effect of amphetamine on the firing rates of mesencephalic dopaminergic neurons (33), and antagonizes the inhibition of sexual responses produced by the 5-HT agonist DOI (36). Thus, the doses of ritanserin used in the present experiments not only are in the range of pharmacological efficacy but also are concordant with its binding characteristics to 5-HT_2 receptors in cerebral tissue (11).

Second, when a strain of rat is evaluated for its pattern of drinking it is essential that the animal drinks a concentration of alcohol in a pharmacologically significant quantity in terms of g/kg per day or other unit of time. The use of a procedure to quantitate a concentration of alcohol maximally preferred by each individual animal in a population sample fulfills this requirement (2,10). Clearly, that a single, arbitrarily selected test concentration of alcohol such as 3% (14) used vs. water may be fraught with scientific difficulties (16,17). To illustrate, a genetic line of alcohol-drinking rat typically is bred for preference of 10% alcohol over water (16). In the case of the P line of rats, the actual preferred concentration of alcohol over water is as high as 22% (10). In fact, the P line of rats (12) does represent a valid model of alcohol drinking because

TABLE 1

MEAN ± SE FOOD, WATER, ETHANOL AND TOTAL FLUID INTAKES AND BODY WEIGHT OF MALE RATS IN WHICH 0.1, 0.3, OR 1.0 MG/KG RITANSERIN OR SALINE VEHICLE WAS ADMINISTERED SC ONCE DAILY: PRECONTROL FOR 4 DAYS, INJECTIONS FOR 3 DAYS, AND POSTCONTROL FOR 4 DAYS

	Food (g)	Water(ml)	EtOH (ml)	Total (ml)	Weight (g)
Low dose (n = 4)					
Pre	13.6 ± 2.0	23.3 ± 1.5	30.5 ± 2.9	53.3 ± 3.6	448.1 ± 4.1
0.1 mg	11.8 ± 0.74	22.0 ± 1.8	31.5 ± 2.9	53.5 ± 4.7	480.9 ± 2.7
Post	12.5 ± 0.59	23.4 ± 1.4	31.2 ± 2.7	54.9 ± 3.0	473.9 ± 3.6
Intermediate dose (n = 5)					
Pre	13.4 ± 0.58	22.1 ± 3.4	33.4 ± 2.9	55.4 ± 6.4	538.9 ± 12.7
0.3 mg	12.5 ± 0.46	30.8 ± 4.1	29.7 ± 2.5	60.5 ± 6.6	531.1 ± 14.5
Post	11.6 ± 0.58	26.1 ± 2.9	30.0 ± 2.8	56.1 ± 5.7	523.6 ± 12.2
High dose (n = 5)					
Pre	13.3 ± 0.54	20.8 ± 4.4	26.3 ± 2.2	47.1 ± 6.6	494.1 ± 10.7
1.0 mg	13.7 ± 0.49	15.8 ± 5.0	31.0 ± 1.9	47.7 ± 6.9	487.9 ± 12.3
Post	13.3 ± 0.53	20.5 ± 3.5	30.1 ± 1.8	50.5 ± 5.3	481.9 ± 10.3
Saline control (n = 6)					
Pre	18.2 ± 0.62	15.4 ± 1.4	38.2 ± 1.5	52.7 ± 1.5	511.3 ± 10.1
Saline	19.8 ± 0.76	18.8 ± 2.0	38.7 ± 1.8	57.1 ± 1.6	519.2 ± 11.0
Post	16.9 ± 0.55	19.9 ± 1.9	37.4 ± 1.5	56.9 ± 2.0	521.2 ± 9.2

n = number of rats.

such concentrations are selected even when a highly palatable or nutritious solution is proffered as an alternative to water (10). Moreover, a measure of the amount of alcohol consumed, when expressed solely in terms of ml per unit time or percent of alcohol to total fluid ingested (14), may likewise be difficult to comprehend. Such a value fails to recognize the basic pharmacological precept of the efficacy of a drug in terms of the unit of weight (e.g., grams) of a drug self-administered (alcohol) per unit body weight (kilograms) of the test animal (16,17).

A third factor that could contribute to a discrepancy is the nature of the side effects of the drug. Although drugs that possess an affinity for 5-HT receptors such as the 5-HT_{1A} agonist 8-OHDPAT (9,34) and the 5-HT_3 antagonist MDL 72222 (5) can suppress alcohol preference over water, omission of measures of food intake and the lack of a maximally preferred concentration of alcohol complicate interpretation of the data (6,25). That is, it is now known that 5-HT reuptake blockers can reduce alcohol drinking but simultaneously cause anorexia (6,15). On the other hand, amperozide, a 5-HT_2 antagonist, and FG 5893, a mixed 5-HT_{1A} agonist–5-HT_2 antagonist (7,28), significantly reduce the intake of a maximally preferred concentration of alcohol without disrupting the ingestion of food, drinking of water, or body weight of the rat (21,22,32).

In conclusion, it is improbable that the a drug that blocks a single 5-HT receptor subtype or globally inhibits 5-HT reuptake in the brain will alone serve to ameliorate an aberrant intake or irreversible preference for alcohol. Rather, it is likely that the simultaneous pharmacological perturbation of combined 5-HT receptor subtypes concomitant with an interference of dopamine function (18,20) may well act to assuage craving or other component of behavior that leads to the unrestrained imbibition of alcohol.

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