Pharmacology Biochemistry and Behavior, Vol. 46, pp. 853–856, 1993
Printed in the U.S.A.

0091-3057/93 $6.00 + .00

5-HT_2 Receptor Antagonists Do Not Reduce Ethanol Preference in Sardinian Alcohol-Preferring (sP) Rats

IZABELA PANOCKA,* ROBERTO CICCOCIOPPO,†
PIERLUIGI POMPEI† AND MAURIZIO MASSI†[1]

**Department of Behavioral Physiology, Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzebiec, 05-551 Mrokow, Poland*
†Institute of Pharmacology, University of Camerino, 62032 Camerino, Italy

Received 16 February 1993

PANOCKA, I., R. CICCOCIOPPO, P. POMPEI AND M. MASSI. *5-HT_2 receptor antagonists do not reduce ethanol preference in Sardinian alcohol-preferring (sP) rats.* PHARMACOL BIOCHEM BEHAV **46**(4) 853–856, 1993.—The present study investigated the effect of the 5-$HT_{2/1C}$ receptor antagonist ritanserin, and of the 5-HT_2/D_2 receptor antagonist risperidone on ethanol preference in Sardinian alcohol-preferring (sP) rats. Rats were offered free access to both tap water and 8% (in one experiment 3%) ethanol solution. Subchronic (10 or 1 mg/kg/day, for 10 days) or chronic (1 mg/kg/day, for 30 days) subcutaneous (SC) ritanserin treatment failed to reduce 8% ethanol preference. Risperidone doses that produce marked 5-HT_2, but low dopamine D_2, receptor blockade (1 and 0.1 mg/kg/day, SC, for 9 and 10 days, respectively) did not modify 8% ethanol preference. On the other hand, a high risperidone dose (10 mg/kg/day, SC, for 14 days), which produces pronounced dopamine D_2 receptor blockade, reduced 8% ethanol preference, like the dopamine receptor antagonist haloperidol. Previous studies have shown that both ritanserin and risperidone evoke long-lasting and pronounced suppression of 3% ethanol preference in genetically nonselected rats. However, in the present study, SC ritanserin treatment (1 mg/kg/day for 10 days) did not modify 3% ethanol preference in sP rats. The failure of 5-HT_2 antagonists to reduce ethanol preference in sP rats raises the question whether genetic selection might have resulted in altered regulation of 5-HTergic mechanisms in sP rats.

Ritanserin Risperidone Haloperidol Ethanol preference Sardinian alcohol-preferring rats

A LARGE body of evidence indicates that drugs selectively interacting with 5-HT mechanisms may reduce ethanol intake both in experimental animals and in humans (27). An extensive literature shows that drugs that increase the synaptic availability of 5-HT, such as uptake inhibitors or releasers, as well as 5-HT agonists may reduce ethanol intake (1,5,16, 21,22). Reduction in ethanol intake, however, has also been recently reported in response to administration of 5-HT antagonists in rats. Fadda et al. (6) observed reduction in ethanol consumption in genetically selected alcohol-preferring rats, following treatment with the 5-HT_3 receptor antagonist MDL 72222. Moreover, inhibition of alcohol intake in genetically nonselected Wistar rats has been observed following treatment with ritanserin, a 5-$HT_{2/1C}$ receptor antagonist (18,19,23), or with risperidone (24), a 5-HT_2 and dopamine D_2 receptor antagonist (4,13,14).

The present study evaluated the effect of ritanserin and of risperidone on ethanol preference of Sardinian genetically selected alcohol-preferring (sP) rats (8). The aim was to extend our investigation on the effect of 5-HT_2 antagonists on ethanol intake to a different experimental animal model and to different experimental conditions. Our interest in genetically selected alcohol-preferring rats was also stimulated by previous studies that have shown that genetic selection for alcohol preference may be associated with altered function of central 5-HT systems (11,17,20,30).

METHOD

Animals

Male rats belonging to the 17th generation of sP rats, selected for 8% ethanol preference according to the method of Lumeng et al. (15), were used. Animals were individually housed on a 12L : 12D cycle. Food pellets (diet No. 4RF18,

[1] Requests for reprints should be addressed to Dr. Maurizio Massi, Institute of Pharmacology, University of Camerino, Via Scalzino 5, 62032 Camerino (MC), Italy.

Mucedola, Settimo Milanese, Italy) were available ad lib. Rats were offered free access to tap water and to 8% or 3% ethanol solution in graduate drinking tubes.

Drug Administration

Haloperidol (Haldol, Janssen Farmaceutici, Rome, Italy), purchased from commercial sources, was diluted with distilled water. Risperidone and ritanserin (a generous gift of Janssen Pharmaceutica, Beerse, Belgium) were dissolved in a vehicle containing 20% propylene glycol and a few drops of lactic acid. The pH of the solutions was adjusted to 5 by adding 2 N NaOH. All the doses of the three drugs were injected subcutaneously (SC) in a volume of 1 ml/kg of body weight at 6:00 p.m. The rather long half-lives of ritanserin (20 h) and of risperidone (8 h) in rats (9,12) allowed a single daily administration of both drugs. The same treatment was shown to be clearly effective in reducing alcohol intake in nongenetically selected rats (23,24).

Experiment 1. Effect of Ritanserin, Risperidone, and Haloperidol on 8% Ethanol Preference

Ritanserin was tested at the doses of 10 mg/kg/day (eight rats for 10 days) and 1 mg/kg/day (six rats for 10 days and six rats for 30 days). Control groups of eight, six, and six animals, respectively, received SC injections of the vehicle according to the same protocol.

Risperidone was tested at the doses of 10 mg/kg/day (eight rats for 14 days), 1 mg/kg/day (five rats for 9 days), and 0.1 mg/kg/day (six rats for 10 days). Control groups of eight, five, and six animals, respectively, receiving SC injections of the vehicle were employed.

Haloperidol was tested in five rats for 10 days at the daily dose of 0.0625 mg/kg. Another five animals served as controls.

Experiment 2. Effect of Ritanserin on 3% Ethanol Preference

Twelve sP rats, previously selected for 8% ethanol preference, were offered 3% ethanol solution and water for a week before the experiment began. Six animals were treated with SC ritanserin 1 mg/kg/day (for 10 days). Six control rats received SC injections of the vehicle according to the same protocol.

Statistical Analysis

Statistical analysis of data was performed by means of split-plot analysis of variance (ANOVA) with between-group comparisons for drug treatment and within-group comparisons for time (treatment day). Individual comparisons were performed by means of *t*-test. Statistical significance was set at $p < 0.05$. Ethanol solution intake (ml/rat), total fluid intake (ml/rat), and percent ethanol preference (percentage of daily total fluid intake drank as ethanol solution) were submitted to statistical analysis. Results are reported in the figures as percent ethanol preference ± SEM.

RESULTS

Experiment 1. Effect of Ritanserin, Risperidone, and Haloperidol on 8% Ethanol Preference

Ten-day SC treatment with ritanserin 10 mg/kg/day or 1 mg/kg/day did not modify either ethanol intake [$F(1, 14) = 0.811$, $p > 0.05$ and $F(1, 10) = 0.671$, $p > 0.05$, respectively] or ethanol preference [$F(1, 14) = 0.483$, $p > 0.05$ and $F(1, 10) = 0.576$, $p > 0.05$, respectively] (Fig. 1A and B).

Moreover, the chronic (for 30 days) treatment with ritanserin 1 mg/kg/day (Fig. 1C) also failed to alter ethanol intake and ethanol preference.

During the periods of ritanserin administration, the total fluid intake of ritanserin-treated rats ranged between 39 and 50 ml/rat/day and was never significantly different from that of controls (data not shown).

Figure 2B and C shows the alcohol preference of sP rats treated, respectively, with 1 or 0.1 mg/kg/day of risperidone. The overall ANOVA revealed that both treatments did not significantly modify either alcohol intake or alcohol preference. On the other hand, 10 mg/kg/day of risperidone produced a significant inhibition of 8% ethanol intake, $F(1, 14) = 9.209$, $p < 0.01$, and of 8% ethanol preference, $F(1, 14) = 14.037$, $p < 0.01$ (Fig. 2A). The effect was statistically significant from the 3rd to the 9th day of treatment.

None of the risperidone doses significantly altered total fluid intake, which ranged between 36 and 45 ml/rat/day and was never significantly different from that of controls (data not shown).

Figure 3 shows the effect of 0.0625 mg/kg/day of haloperidol on 8% alcohol preference of sP rats. The overall ANOVA revealed a highly significant haloperidol effect on both 8% ethanol intake, $F(1, 8) = 61.390$, $p < 0.001$, and 8% ethanol preference, $F(1, 18) = 49.532$, $p < 0.001$. Individual comparisons showed that the effect was statistically significant in the first 8 days of treatment.

During the 10-day period of haloperidol administration, the total fluid intake of treated rats ranged between 37 and 42

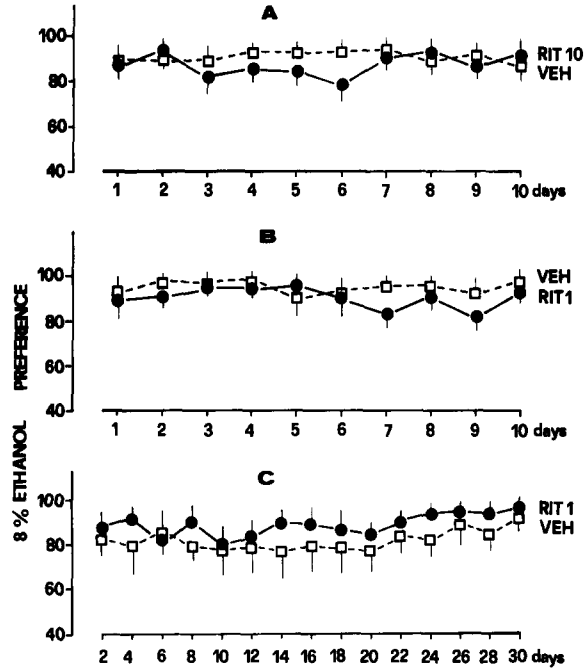

FIG. 1. The 8% ethanol preference in sP rats during (A) subchronic treatment with 10 mg/kg/day of ritanserin (RIT) or with its vehicle (VEH); (B) subchronic treatment with 1 mg/kg/day of RIT or with its VEH; (C) chronic treatment with 1 mg/kg/day of RIT or with its VEH. Difference from controls was never significant.

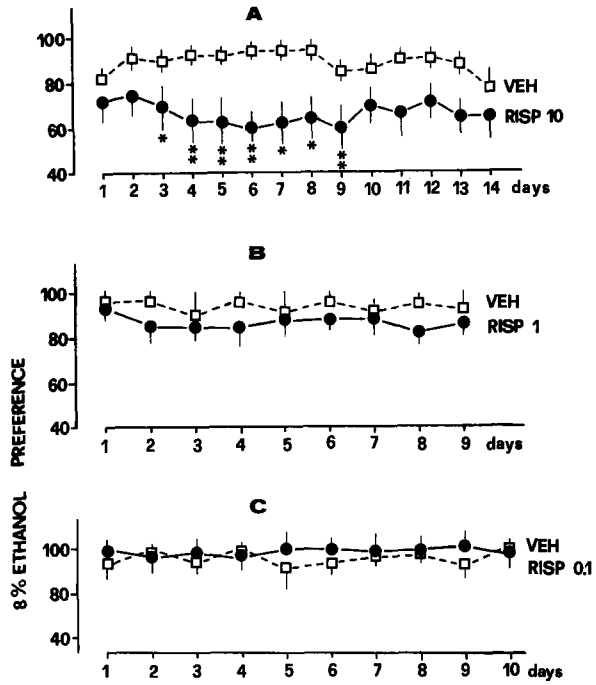

FIG. 2. The 8% ethanol preference in sP rats treated with (A) 10 mg/kg/day; (B) 1 mg/kg/day; and (C) 0.1 mg/kg/day of risperidone (RISP) or with its vehicle (VEH). Statistical difference from controls: $*p < 0.05$; $**p < 0.01$; where not indicated, difference from controls was not significant.

ml/rat/day and was never significantly different from that of controls (data not shown).

Experiment 2. Effect of Ritanserin on 3% Ethanol Preference

The sP rats employed in the present experiment were previously selected on the basis of their 8% ethanol preference, which ranged between 80% and 90%, their total fluid intake ranging between 42 and 50 ml/rat/day. When they were offered 3% ethanol solution and water, they maintained their usual total fluid intake, as well as a percentage of ethanol preference of about 90%. Owing to the lower concentration of the ethanol solution offered, this resulted in substantial reduction of the amount of ethanol taken per day.

As shown in Fig. 4, 3% ethanol preference was not affected by the ritanserin treatment, $F(1, 10) = 1.129$, $p > 0.05$. The ANOVA revealed that ethanol intake also was not significantly modified.

During the 10-day period of drug administration, the total fluid intake of ritanserin-treated rats ranged between 36 and 43 ml/rat/day and was never significantly different from that of controls (data not shown).

DISCUSSION

The results of the present study clearly show that the selective 5-HT$_{2/1C}$ receptor antagonist ritanserin does not modify either 3% or 8% alcohol preference in sP rats, not even in response to a 30-day treatment with 1 mg/kg/day. This finding is in sharp contrast with the results obtained in genetically nonselected rats, in which ritanserin produces a marked and long-lasting 3% ethanol preference suppression (23).

Also, no significant suppression of ethanol preference in sP rats was obtained following SC treatments with risperidone 0.1 or 1 mg/kg/day, which markedly suppress 3% alcohol intake in genetically nonselected rats (24). Biochemical studies have shown that these risperidone doses are far larger than that required (0.0075 mg/kg, SC) to produce 50% occupancy of central 5-HT$_2$ receptors; moreover, at these doses the drug poorly affects dopamine D$_2$ receptors (27). These results are in keeping with those obtained with ritanserin in the present study and provide further evidence that 5-HT$_2$ antagonists are not able to block ethanol intake in sP rats.

On the other hand, a statistically significant inhibition of ethanol preference was observed in response to the highest dose of risperidone, 10 mg/kg/day. Binding studies have shown that this dose is four times higher than that required to get 50% occupancy of central dopamine D$_2$ receptors after SC administration (26), thus suggesting that the effect observed at this dose might be related to dopamine D$_2$ receptor blockade. This hypothesis is in keeping with the fact that the dopamine antagonist haloperidol was able to produce, like risperidone 10 mg/kg/day, a marked but short-lasting suppression of ethanol preference in sP rats.

The finding that haloperidol reduced 8% ethanol preference in sP rats confirms, once more, the idea that the dopa-

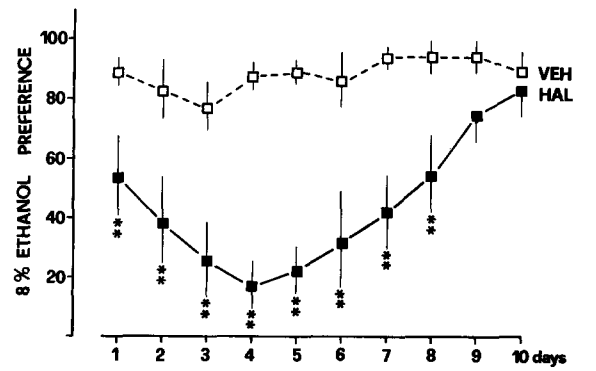

FIG. 3. The 8% ethanol preference in sP rats treated with 0.0625 mg/kg/day of haloperidol (HAL) or with its vehicle (VEH). Statistical difference from controls: $**p < 0.01$; where not reported, difference from controls was not significant.

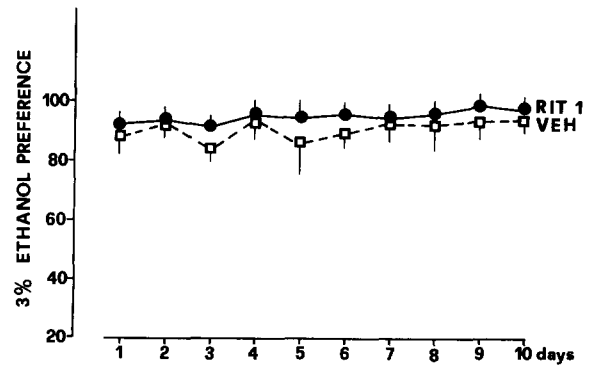

FIG. 4. The 3% ethanol preference in sP rats treated with 1 mg/kg/day of ritanserin (RIT) or with its vehicle (VEH). Difference from controls was never statistically significant.

minergic system is involved in the control of ethanol intake (2,3,10,25).

In conclusion, the present study indicates that 5-HT_2 receptor blockade does not modify alcohol preference in sP rats. At present, the reasons accounting for the discrepant results obtained with 5-HT_2 antagonists in genetically nonselected and in sP rats are unknown.

Ethanol has been shown to stimulate dopaminergic neurons in sP rats more intensely than in alcohol-nonpreferring animals (7). Thus, it might be speculated that ethanol might be more rewarding in sP rats and therefore suppression of ethanol preference might be more easily achieved in genetically nonselected than in sP rats.

On the other hand, several studies have reported alterations of 5-HTergic systems in other strains of alcohol-preferring rats (11,17,20,30) and mice (28,29). On the basis of these reports, it seems interesting to investigate whether failure of 5-HT_2 antagonists to reduce alcohol intake in sP rats might be due to alteration of 5-HTergic systems and particularly of 5-HT_2 mechanisms in the brain of sP rats.

ACKNOWLEDGEMENTS

The authors wish to thank Profs. G. L. Gessa and F. Fadda of the Department of Neurosciences, University of Cagliari, Italy, for the generous gift of Sardinian alcohol-preferring rats. They also thank Dr. Flavio Pozzi, Janssen Farmaceutici, Rome, Italy, for helpful comments and suggestions.

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