Modification of Ethanol Effects by Chlorcyclizine in the Rat (34929)

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It has been reported by our group (1) and other investigators (2) that replacement of water by dilute alcohol leads to a significant reduction in liquid consumption by rats. However, it is not clear at this time whether the reduced consumption of alcohol is due to the natural aversion to alcohol by rats, as has been suggested by Lieber and Rubin (3), or whether alcohol-treated animals reduce their fluid intake because their capacity to metabolize alcohol is temporarily overloaded. In order to investigate this question we decided to treat animals with chlorcyclizine, a known inducer of drug-metabolizing enzymes (4), which was reported to enhance ethanol metabolism and to reduce blood alcohol levels (5). If the amount of alcohol consumption is determined by blood alcohol levels, administration of chlorcyclizine should allow higher intake of a dilute alcohol solution. Since the original observation of the alcohol consumption enhancement by chlorcyclizine was made in an acute study (5), we decided to investigate whether this effect of chlorcyclizine could be maintained over a prolonged period of time in rats chronically given alcohol as the sole drinking fluid.

Furthermore, since chlorcyclizine in the above study also prevented the increase in hepatic triglycerides, usually seen after administration of a single large dose of alcohol (6, 7), we decided to study whether long-term administration of chlorcyclizine and alcohol affects the hepatic triglyceride levels of the animals.

Material and Methods. Forty male rats of the Sprague-Dawley strain, weighing 225–260 g each at the start of the experiment, were caged individually and randomly assigned to 4 groups of 10 animals. A dry mixture of *Instant Breakfast*[1] and nonfat dry milk, in a ratio of 35.5–23.0 g, was offered *ad libitum* to all animals. Group 1 received tap water in calibrated drinking bottles; Group 2 received a 15% (w/v) solution of ethyl alcohol; Group 3 received tap water and chlorcyclizine HCl[2] at a dosage of 12.5 mg/kg/day; Group 4 received the 15% ethanol solution and the same concentration of chlorcyclizine.

Food and liquid intake was measured, and weight changes were determined during a 31-day experimental period. Drug concentration was adjusted every second day to compensate for weight change and changes in liquid intake noted at the previous measurement. The mean dosage over the entire experiment was calculated on the basis of the amount actually consumed by each group. These averaged 13.2 mg chlorcyclizine/kg/day for Group 3 and 12.3 mg/kg/day for Group 4.

After 31 days the rats were anesthetized with sodium pentothal, and blood was drawn from the abdominal aorta for alcohol and lipid analysis (8, 9). Livers were removed, rinsed, weighed, and frozen for liver triglyceride determinations (10).

Results. The results shown in Table I confirm our previous observations (1) that dilute ethanol reduces total liquid consumption in animals. However, when the alcohol solution contained chlorcyclizine, the alcohol-induced depression in liquid consumption was less pronounced. The difference in liquid consumption between the groups receiving alcohol with or without chlorcyclizine was statistically significant ($p < .01$). Chlorcyclizine alone significantly reduced water consumption from 58.4 to 38.0 ml/day.

[1] Carnation Instant Breakfast was obtained from commercial sources.

[2] Chlorcyclizine was provided through the courtesy of Dr. Allen H. Conney of the Burrows-Wellcome Company.

TABLE I. Liquid and Food Consumption, Weight Changes, and Liver Triglyceride Levels of Rats Receiving Ethanol and Chlorcyclizine.

	Treatment	N	Liquid intake/day (ml)	Food intake/day (g)	Weight gain (g)	Liver triglycerides (mg/g)
1	Water	10	58.4 ± 2.3[a]	17.1 ± 0.7	50 ± 11.9	7.9 ± 0.5
2	Ethanol[b]	10	18.7 ± 0.5	9.0 ± 0.2	1 ± 4.3[c]	7.6 ± 0.6
3	Water + CL[d]	10	38.0 ± 1.3[c]	18.8 ± 0.4	68 ± 8.3	10.4 ± 0.4[c]
4	Ethanol + CL	10	23.2 ± 1.1[c]	9.3 ± 0.3	1 ± 5.7[d]	55.9 ± 5.5[c]

[a] Mean ± standard error. Duration of the experiment was 31 days.

[b] Ethanol in concentration of 15% (w/v) was offered as the sole drinking fluid.

[c] Values significantly different from the corresponding water or alcohol control ($p < .01$).

[d] Chlorcyclizine HCl, in doses of 12.5 mg/kg, was dissolved in the drinking fluids. The dose was adjusted for weight and fluid consumed every second day.

Alcohol reduced average daily food intake in all animals. This effect did not appear modified by the addition of chlorcyclizine. Administration of the drug itself did not seem to affect food intake in any significant way. Furthermore, the data on weight gain suggest that the alcohol-induced reduction of food and water intake interferes with the weight gains of the animals since both alcohol-treated groups failed to gain weight during the course of the experiment.

Blood alcohol levels were determined on the final day and amounted to 136.8 ± 80.7 mg/100 ml for the group receiving alcohol without the drug and 106.1 ± 87.1 mg/100 ml for the animals drinking alcohol with chlorcyclizine. This difference is not statistically significant.

The highest average levels of plasma-free fatty acids were seen in the group receiving chlorcyclizine alone and amounted to 1203 ± 117 μEq/liter. The corresponding values in the groups receiving ethanol, ethanol plus chlorcyclizine, and the controls were 767 ± 60, 763 ± 57, and 791 ± 50 μEq/liter, respectively.

Long-term administration of 15% ethanol did not seem to affect the liver triglyceride levels (Table I). Administration of chlorcyclizine in water produced a small but significant rise in liver triglycerides ($p < .01$). However, when both ethanol and chlorcyclizine were administered there was a pronounced rise in liver triglycerides. The average value amounted to 55.9 ± 5.5 mg/g which was also a significant increase ($p < .01$) over the control value.

Discussion. Animals receiving 15% ethanol with chlorcyclizine showed a higher liquid intake than the group receiving ethanol alone, indicating that the former group might have increased alcohol tolerance. These animals also tended to have a lowered blood alcohol level, suggesting an enhancement of ethanol metabolism by chlorcyclizine reported by Wooles (5). Therefore, it would seem that the ethanol-metabolizing capacity may well be a factor influencing the rat's consumption of ethanol.

An unexpected finding in this study was the pronounced increase in hepatic triglycerides in the group receiving alcohol and chlorcyclizine combined. Administration of chlorcyclizine alone produced only a slight accumulation of liver triglycerides, while ingestion of alcohol alone seemed to be without any effect in this respect. A definite mechanism for this effect of chlorcyclizine is not known at this time. Chlorcyclizine, by virtue of its atropine-like activity (11), and ethanol (12) might stimulate the sympathetic nervous system. Stimulation of sympathetic activity is usually associated with an increase in free fatty acids and frequently a rise in liver triglycerides. Such a mechanism did not seem to be operative in the present experiment since the measured plasma-free fatty acids of the animals receiving ethanol and chlorcyclizine combined were lower than the corresponding levels in the animals receiving chlorcyclizine alone, and they did not differ from the free fatty acid levels of the control group. However, it has been reported that both chlorcyclizine and ethanol may be

metabolized, to some extent, by a microsomal drug-metabolizing enzyme system (4, 13). It is feasible, under chronic conditions, that the effect of the two drugs combined is greater than the effect of either drug alone. The enhanced ethanol consumption may also have contributed to the increased liver triglyceride levels.

Summary. Rats receiving 12.5 mg of chlorcyclizine/kg of body weight and 15% ethanol (w/v) as the sole source of drinking fluid for 31 days showed a significant accumulation of hepatic triglycerides. Administration of chlorcyclizine alone led to only a slight increase in liver lipids while a group receiving alcohol failed to show any significant changes. Chronic administration of chlorcyclizine seemed to increase the tolerance to alcohol as reflected by the increased alcohol consumption in the presence of the drug.

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