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Schedule-Induced Ethanol Polydipsia: Function of Ethanol Concentration[1]

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ROEHRS, T. A. AND H. H. SAMSON. *Schedule-induced ethanol polydipsia: Function of ethanol concentration.* PHARMAC. BIOCHEM. BEHAV. 13(2) 291–294, 1980.—Rats were maintained in cages with automatic food dispensers that provided a 24 hr feeding regimen known to produce schedule-induced ethanol polydipsia. For ten days water was the only available fluid; then for 17 days either 5% or 10% ethanol replaced water. The ethanol concentrations were then switched between groups for a final 13 days. Ethanol intake increased for both groups over the first seven days and then reached asymptote. The daily intake (ml) of 5% ethanol was two times that of 10%, resulting in no difference between groups in g/kg ethanol consumed. When the concentrations were switched, g/kg/day dropped, but returned to previous levels within seven days. Again intake (ml) of 5% was two times that of 10% but groups did not differ in g/kg/day. Mean blood ethanol concentration at 9:30 hr was 75.0 mg/100 ml with 5% ethanol and 127.8 mg/100 ml with 10% ethanol.

Ethanol intake Schedule induced polydipsia Ethanol concentration Blood ethanol concentration

PREVIOUS studies have shown that chronic ethanol overdrinking resulting in physical dependence on ethanol occurs in rats maintained with the behavioral technique of schedule-induction [2,5]. In their original study, Falk *et al.* [2] gradually reduced rats to 80% of their free-feeding body weights and placed them on a 24-hr feeding regimen consisting of six one-hour food delivery periods separated by three-hour intervals. During the one-hour food delivery periods a 45 mg Noyes food pellet was delivered every two minutes. After schedule-induced water polydipsia developed, a one percent ethanol solution (v/v), which was increased in one percent increments every six to eight days, replaced water as the only drinking fluid. Mean daily ethanol intake (g/kg) increased as concentration increased reaching an asymptote at 13.1 g/kg with either a five or six percent ethanol solution. The increase from five to six percent ethanol did not lead to increased daily ethanol intake (g/kg) and thus further increments in concentration were not made [2].

While five or six percent ethanol is within the preferred concentration range in most water versus ethanol preference tests, it generally is found that rats do not prefer concentrations much greater than six percent [7,8]. Studies using single daily polydipsia sessions of two to five hours in duration have presented nonpreferred ethanol concentrations. As concentration was increased beyond the preference range, ethanol intake (g/kg) continued to increase with concentrations from 10% (v/v) [3,11] up to 32% [4,6].

The present study assessed the effect of a nonpreferred ethanol concentration on chronic ethanol overdrinking obtained in the 24-hr polydipsia feeding regimen previously described [2]. Previous studies in our laboratory have found ten percent ethanol to be a nonpreferred concentration [9]. The present study compared schedule-induced ethanol polydipsia with a five percent or ten percent ethanol solution.

METHOD

Animals and Experimental Environment

Six male Long Evans strain rats, ninety days old at the start of the experiment and weighing between 290 and 380 g, were used. All rats were housed individually in 12×11×10 inch Plexiglas chambers with stainless steel bar floors. On the front wall of each chamber was a food cup and a stainless steel, ball-point drinking tube which protruded through a small aperture. The drinking tube was attached to a 250 ml graduated cylinder mounted to the outside of the chamber. Food pellets (45 mg Noyes) were delivered automatically to each cage by a pellet dispenser (Gerbrands, Model D-1). Food supplements (standard Purina laboratory chow), when necessary, were placed in a food hopper attached to the back wall. There was artificial illumination from 6:30 to 18:30 hours.

Procedure

All rats were gradually reduced to between 75 and 80% of free-feeding body weights over a ten-day period by limiting food intake. Then they were placed on the same intermittent feeding schedule as described by Falk *et al.* [2]. On this schedule, a food pellet was delivered every two minutes dur-

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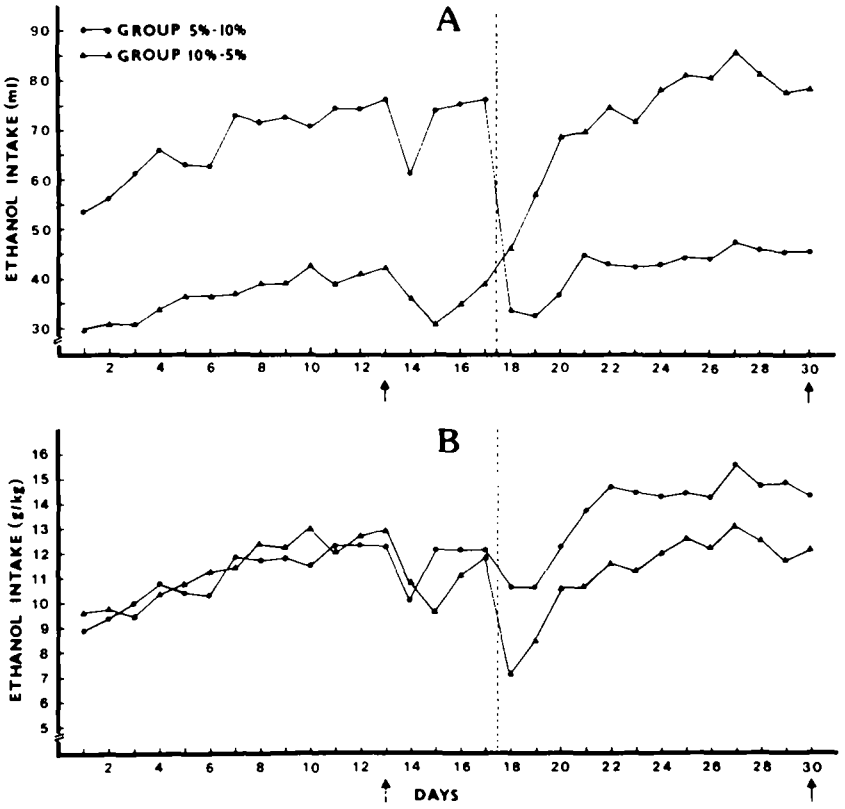

FIG. 1. Mean daily intake of ethanol in ml (Panel A) and in g/kg (Panel B) for the two groups drinking either a 5% or 10% ethanol concentration. The arrows denote days on which a blood sample was taken.

ing six one-hour periods every 24 hours. Each one-hour food delivery period was separated by a three-hour no-delivery period. Thus each rat received 30 food pellets during each one-hour food delivery period for a total 180 food pellets each day. At the same time each day (9:30 hr) fluid intakes (to the nearest ml) and body weights were recorded. Also at this time fluid reservoirs were replenished and any food supplements necessary to maintain the animals at the reduced body weights were given.

For the first ten days of the intermittent feeding schedule water was the only available drinking fluid. Thereafter, either 5% or 10% ethanol solution (v/v) replaced water. Three animals (Group 5%–10%) randomly chosen, received the 5% solution and the remaining three animals (Group 10%–5%) received the 10% solution. After 13 days on ethanol blood samples were taken from each rat. For four days following the blood samples the rats continued with the same drinking fluid. Then Group 5%–10% received a 10% ethanol solution and Group 10%–5% received a 5% ethanol solution. These ethanol solutions were presented for an additional 13 days. Blood samples were again taken from each animal on Day 13 at the same time.

To determine blood ethanol levels, blood samples were taken at 9:30 hr., 30 min after the sixth and last one-hour food delivery period of the 13th day. Each rat was lightly restrained and a 100 μl blood sample was taken from the tip of the tail. Blood ethanol levels were determined by the enzymatic method [1].

RESULTS

The two groups drank similar amounts of water on the intermittent feeding schedule. Mean water intake over the last five days of the ten-day water test was 37.7 ± 25.4 ml for the 5%–10% Group and 32.9 ± 0.92 ml for the 10%–5% Group.

The mean daily intake of ethanol in ml and g/kg during the two, 13-day ethanol drinking tests with either a five or ten percent ethanol concentration is shown in Fig. 1. For both groups intake gradually increased during the first seven days of each test period to an asymptote over the last six days of the test period. Intake of ethanol in ml and g/kg for the two groups was compared over the first seven days of each test period using a mixed design analysis of variance. Over days one to seven the ml intake of both groups increased significantly, $F(6,24)=4.76$, $p<0.001$, as did g/kg intake, $F(6,24)=2.59$, $p<0.05$. During these first seven days Group 5%–10% drank a significantly greater volume (almost two times) of five percent ethanol than Group 10%–5% drank of 10% ethanol, $F(1,4)=35.40$, $p<0.001$. Consequently the two groups did not differ significantly in g/kg of ethanol intake. When the drinking fluids of the two groups were switched, intake (g/kg) dropped on the first day of exposure to the new drinking fluid (most noticeably in the 5%–10% Group). Thereafter, over days 19 to 24, ml intake, $F(6,24)=5.43$, $p<0.001$, and g/kg of ethanol, $F(6,24)=4.98$, $p<0.001$ increased significantly for both groups. As in the first test, the group drinking 5% ethanol (Group 10%–5%) drank almost two times the

TABLE 1

ETHANOL INTAKE (g/kg) AND BLOOD ETHANOL (mg/100 ml)

Rats	5% Ethanol		10% Ethanol	
	Blood ethanol	Ethanol intake*	Blood ethanol	Ethanol intake*
Group 5%–10%				
2	73.3	13.6	162.9	18.3
3	53.9	10.1	30.5	10.0
4	102.5	13.3	198.0	15.5
X	76.6	12.3	130.5	14.6
±SEM	24.46	1.94	88.33	4.22
Group 10%–5%				
1	88.9	12.8	177.0	12.8
5	114.2	14.9	73.3	13.7
6	17.5	8.8	—†	—†
X	73.5	12.2	125.2	13.0
±SEM	50.15	3.10	73.33	0.62

*Cumulative intake over 24 hours prior to time of blood sample.
†Blood sample accidentally destroyed.

amount consumed by the group (Group 5%–10%) drinking 10% ethanol, $F(1,4)=79.21$, $p<0.001$. Again the two groups did not differ significantly in g/kg of ethanol intake.

Mean ethanol intake in ml and g/kg for each group at stable intake levels (Days 8–13 and Days 25–30) was determined. Both groups drank significantly greater volume (ml) of 5% ethanol than 10%, $F(1,4)=79.38$, $p<0.001$. Group 5%–10% drank 74.1 ± 1.93 ml (mean ± SEM) of 5% and 45.7 ± 5.50 ml of 10% ethanol, while Group 10%–5% drank 80.8 ± 6.50 ml of 5% and 41.2 ± 2.15 ml of 10% ethanol. However, the groups did not differ significantly in the volume of 5% or 10% ethanol consumed. Since at the asymptote, as during the acquisition periods, the intake of 5% ethanol was approximately two times that of 10% ethanol, there were no significant differences in the g/kg of ethanol intake. However, there was a trend suggesting that the group which received 5% and then 10% ethanol (Group 5%–10%) consumed a greater g/kg of ethanol (14.8 ± 2.96 versus 12.6 ± 0.38 g/kg) when 10% ethanol was the available drinking fluid than the group which received 10% ethanol first (Group 10%–5%).

Table 1 presents the blood ethanol concentrations (mg/100 ml) and corresponding ethanol intakes for the two groups while drinking either a 5% or 10% ethanol solution. At the 9:30 hr mean blood ethanol concentration with 5% ethanol as the available fluid was 76.6 mg/100 ml for Group 5%–10% and 73.5 mg/100 ml for Group 10%–5%. With the 10% ethanol solution mean blood ethanol concentration was higher, 130.5 and 125.2 mg/100 ml respectively, but not significantly.

DISCUSSION

The results of the present study confirm previous demonstrations of high daily ethanol intake under a 24 hr polydipsia feeding regimen. Other reports have found mean ethanol intakes of between 10.3 and 13.1 g/kg/day [2,5]. In the present study mean daily intake was 12.2 ± 0.80 (± SEM) g/kg with 5% ethanol as the available drinking fluid. The present study extends this finding to include a nonpreferred ethanol concentration, 10% ethanol. With 10% ethanol mean daily intake was 13.7 ± 1.30 g/kg.

It is interesting that for both groups, ml intake of 5% ethanol was approximately two times that of 10% ethanol. As a consequence the mean daily g/kg of ethanol was similar for the two concentrations. Previous studies using single daily polydipsia sessions have found that the g/kg intake of ethanol increased as the concentration was increased, in some cases up to a 32% w/v) ethanol concentration [6]. In contrast under the 24 hr polydipsia feeding regimen of the present study g/kg intake of ethanol did not increase with the increased ethanol concentration. This difference suggests that ethanol intake over a 24 hr situation is limited by the metabolic capacity of the organism. It has been shown that blood ethanol levels in the 24 hr polydipsia situation remained elevated above 75 mg/100 ml throughout the 24 hr period [2,10], while with a single daily feeding regimen one daily peak in blood ethanol level was produced with values back to zero by the start of each daily session [10]. Thus in the 24 hr polydipsia situation an animal begins any of the six feeding sessions during a 24 hr period with elevated levels of ethanol in the blood, but in single daily polydipsia situations, minimal blood ethanol levels are present at the start of the session. While it might appear that greater daily intakes in g/kg with concentration increases occur under the single daily polydipsia sessions, when 24 hr polydipsic drinking occurs, metabolic limits override schedule-induction, regardless of ethanol concentration.

Since only one blood sample was taken at 9:30 hr during each of the ethanol drinking tests (5% or 10% ethanol), it can not be determined clearly whether the daily pattern of blood ethanol elevation was the same or different for the two ethanol concentrations. With 5% ethanol the mean blood ethanol concentration at 9:30 hr was 75 mg/100 ml. This finding is comparable to earlier reports of blood levels at 9:30 hr and represents one of the lower points in the 24 hr pattern of blood ethanol elevation which was observed [2,10]. With

10% ethanol, blood levels were somewhat higher (127 mg/100 ml), although not reliably so. This trend toward a difference in blood ethanol levels at 9:30 hr suggests that the diurnal pattern of blood ethanol elevation may have been slightly different with the 10% ethanol, especially since the daily g/kg intake for the two concentrations was similar.

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