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Effects of Ethyl Alcohol on Forced Consumption of an Acclimated Saline Solution[1]

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WAYNER, M. J., F. C. BARONE AND F. B. JOLICOEUR. *Effects of ethyl alcohol on forced consumption of an acclimated saline solution.* PHARMAC. BIOCHEM. BEHAV. 8(4) 417–420, 1978. – Forced consumption of a 1.7% sodium chloride solution was determined in 3 groups of male hooded rats, 4 in each group, over a period of 115 days. A control group received 1.7% NaCl for the duration of the experiment. An ethyl alcohol experimental group received gradually increasing concentrations of ethyl alcohol, 0.5 to 6.0%, in the 1.7% NaCl drinking fluid for 30 days. For the next 25 days 6% ethanol was mixed with the 1.7% sodium chloride solution. A second experimental group was treated similarly except that varying concentrations of citric acid were added to the saline drinking fluid in place of the ethyl alcohol. At the end of the 55 day period, the ethyl alcohol and citric acid were removed from the drinking fluid of both groups. Dramatic and copious amounts of drinking occurred only in the group which had previously drunk the ethyl alcohol. Since excessive drinking of the 1.7% sodium chloride solution did not occur in the other experimental group when the citric acid was removed, the copious drinking can be attributed specifically to the prolonged ingestion and withdrawal of the ethyl alcohol. Possible significance to an animal model for alcoholism is discussed.

Sodium chloride Ethyl alcohol Citric acid Drinking Ethanol withdrawal Forced saline consumption Acclimation of taste

MANY animal models of alcoholism have been proposed. Several models utilize the addition of taste and flavorful substances to ethanol in order to change its palatability and odor and consequently to increase its consumption. Unfortunately, even when the adulterated ethanol or cocktail is the only fluid available, consumption seems to be limited by the rate at which the ethyl alcohol is eliminated and such models fail to produce intakes which sustain a high blood alcohol concentration characteristic of alcoholism and result in physical dependence. In a recent attempt to avoid taste acclimation and renal concentration adjustments due to high fluid intakes, animals were forced to drink a 1.7% sodium chloride solution to which various increasing amounts of ethyl alcohol had been added [2]. In this model relatively large intakes of ethanol plus sodium chloride occurred. Since this was a preliminary investigation and animals were forced to drink copious amounts of the ethanol plus sodium chloride for a relatively short period of time, only one out of seven animals displayed clear withdrawal symptoms. However, after the animals had been forced to drink 1.7% saline mixed with daily increases of ethyl alcohol from one to six percent over an extensive period and then the ethyl alcohol was removed from the drinking fluid, fluid consumption increased from about 95 ml per day to approximately 218 ml per day. When the animals' drinking fluid was changed back to 1.7% NaCl plus 6% ethanol, fluid consumption decreased to the previous level of about 100 ml. The increase in fluid intake might have been a sensitive indicator of developing physical dependence and withdrawal.

The purpose of the present study was to investigate this phenomenon in greater detail. Specifically, animals were forced to drink a 1.7% sodium chloride solution to which various increasing amounts of ethyl alcohol were added. The specificity of the effect to ethyl alcohol was determined by studying the addition of various concentrations of citric acid to the 1.7% saline drinking solution. Citric acid was selected because in low concentrations it tends to be mildly aversive and produced intakes similar to those of ethanol. In addition, it is also a source of calories similar to ethyl alcohol. The data substantiate the results of the previous report [2] and indicate that the phenomenon is specific to ethyl alcohol.

METHOD

Animals

Twelve male hooded rats with an average body weight of 217 ± 24 (SD) g were selected from our colony and were adapted to individual living cages for a period of 4 weeks.

Procedure

Each cage was fitted with three graduated plastic 100 ml

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cylinders. Animals drank by means of licking a ball point stainless steel drinking spout attached to each graduated cylinder with a rubber stopper. All three cylinders contained the same solution for each animal. Three cylinders were required because of the large volumes consumed by some animals. Purina Lab Chow Pellets were available ad lib for the duration of the experiment. The body weight of each animal was measured daily. Following the adaptation period 24 hr water consumption was measured for a five day period. Animals were then divided into three groups of four rats each, G1, G2 and G3. G1, the control group, drank a 1.7% NaCl solution for 115 days. The sodium chloride solution was prepared as a w/v solution with distilled water. Body weights of the animals at the beginning of this period were 357, 360, 307 and 340 g.

The ethanol group, G2, drank a 1.7% NaCl solution for 30 days. At the end of this period small amounts of ethanol were added to the 1.7% NaCl solution in increasing concentrations for successive 5 day periods. The concentrations of ethanol solutions were prepared as v/v solutions made from 95% ethanol and distilled water. At 5 day intervals the solutions were increased as follows: 1.7% NaCl + 0.5% ethanol, 1.7% NaCl + 1.0% ethanol, 1.7% NaCl + 2.0% ethanol, 1.7% NaCl + 3.0% ethanol, 1.7% NaCl + 4.0% ethanol and 1.7% NaCl + 5.0% ethanol. For the next 25 days the ethanol was increased to 6% ethanol, 1.7% NaCl + 6.0% ethanol. Ethanol was then removed from the drinking fluid for the last 30 days of the experiment and the animals drank 1.7% NaCl. The body weights of these animals were 355, 348, 342 and 336 g.

The citric acid group, G3, drank a 1.7% NaCl solution for 30 days. Small amounts of citric acid were then added to the 1.7% NaCl solution. Citric acid solutions were prepared as w/v solutions with distilled water. The concentrations of the citric acid were varied in order to adjust the intakes of these animals to correspond to the fluid consumption of G2, the ethanol group. For the first seven days animals drank 1.7% NaCl + 0.1% citric acid. For the next three days, animals drank 1.7% NaCl + 0.05% citric acid. For the following 30 days, animals drank 1.7% NaCl + 0.025% citric acid. For the next 6 days, animals drank 1.7% NaCl + 0.037% citric acid. Finally, for the last 9 days animals drank 1.7% NaCl + 0.05% citric acid. Citric acid was then removed from the drinking solution for the remaining 30 days and animals drank 1.7% NaCl. Body weights for these animals were 365, 347, 343 and 358 g.

RESULTS

The data for fluid intakes were analyzed by means of a two factor ANOVA. The factors were groups, 3 levels consisting of G1, G2 and G3, and days, 13 levels with repeated measures consisting of the 5 day means for each animal corresponding to the last 10 days of the first 30 days of 1.7% NaCl for all animals and those data collected 30 days later on Days 45 through 95. These 5 day periods correspond to the 5 day means labeled 5, 10, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90 and 95 on the abcissa in Fig. 1. Due to the adjustment of fluid consumption in G3, the citric acid group, and consequent variations in intakes, the analysis of the data on Days 15 through 40 will not be presented. The groups were significantly different, $F(2,9) = 11.935$, $p<0.01$. The main effect for days was also significant, $F(12,108) = 32.527$, $p<0.01$. The groups by days interaction was significant, $F(24,108) = 12.664$, $p<0.01$. The fluid consumption in ml for G1, G2 and G3 is presented as a function of days in Fig. 1. Fluid consumption for G2 is indicated by circles connected by a solid line. The fluid consumption of G3 is indicated by squares connected by a solid line. Ethanol and citric acid were introduced in the 1.7% NaCl solution on Day 11 for groups G2 and G3 indicated by an arrow. On Day 66, also indicated by an arrow, both the ethanol and citric acid were removed from the drinking fluid in G2 and G3, and the animals were returned to drinking 1.7% NaCl. No changes in taste were introduced in the 1.7% NaCl drinking solution of G1, the control group.

Eight simple main effects comparisons were made to evaluate differences between the fluid consumption of the three groups illustrated in Fig. 1. A comparison across groups on the last 5 days of the first 30 days of 1.7% NaCl consumption indicated groups were not different, $F(2,42) = 0.096$, $p>0.50$. This comparison was made on the data corresponding to Day 10 in Fig. 1. Another comparison was made on the data collected just prior to the withdrawal of the ethanol and citric acid in the fluids offered to G2 and G3 and indicated that groups were not different, $F(2,42) = 2.98$, $0.05<p<0.01$. This comparison was made on the data corresponding to Day 65 in Fig. 1. The third comparison, Day 70 in Fig. 1, indicated that there were differences between the 3 groups when ethanol and citric acid were withdrawn from the 1.7% NaCl drinking solution in G2 and G3, $F(2,42) = 4.056$, $p<0.05$. The Tukey A test was then used to determine specific differences between groups and indicated that G2>G1>G3, $p<0.05$. The fourth comparison, Day 75 in Fig. 1 indicated that the groups were different, $F(2,42) = 5.674$, $p<0.01$. Subsequent testing using the Tukey A test indicated again that G2>G1>G3, $p<0.01$. The fifth comparison, Day 80 in Fig. 1 indicated that the groups were different, $F(2,42) = 4.5$, $p<0.05$. The Tukey A test indicated again that G2>G1>G3, $p<0.05$. Comparison 6, corresponding to Day 85 in Fig. 1, indicated differences between the groups $F(2,42) = 5.25$, $p<0.01$. Tukey A tests indicated that both G1 and G2>G3, $p<0.01$. Differences were no longer observed between G1 and G2. Comparison 7, corresponding to Day 90 in Fig. 1, $F(2,42) = 3.23$, and Comparison 8, corresponding to Day 95 in Fig. 1, $F(2,42) = 4.03$, were both significant, $p<0.05$. The Tukey A test was utilized for both periods and indicated that G1 and G2>G3, $p<0.01$, and that G1 and G2 were not different for the remainder of the experiment. These results for between group comparisons indicate that the significant differences between the groups in Fig. 1 were for the 5 day periods corresponding to Days 70, 75 and 80 when the fluid consumption of G2 was significantly increased above G1 and G3, and that G1 drank significantly more than G3. For the remainder of the experiment, corresponding to Days 85, 90 and 95, the fluid consumption of G3 was significantly less than G1 and G2. The fluid consumption of G1 and G2 were no longer different at this time.

A simple main effects analysis of days at each group indicated days for G1, $F(12,108) = 2.762$, and days for G2, $F(12,108) = 11.418$ were significant, $p<0.01$. Days for G3 were not significant, $F(12,108) = 0.283$, $p>0.50$. Two tailed Dunnett tests were performed for G1 and G2 using Day 65 in Fig. 1 as the control treatment. Table 1 indicates with arrows each 5 day period in which fluid intakes in ml were increased or decreased relative to the period just prior to the removal of ethanol and citric acid from the 1.7% NaCl solution in G2 and G3, $p<0.01$. Successive 5 day

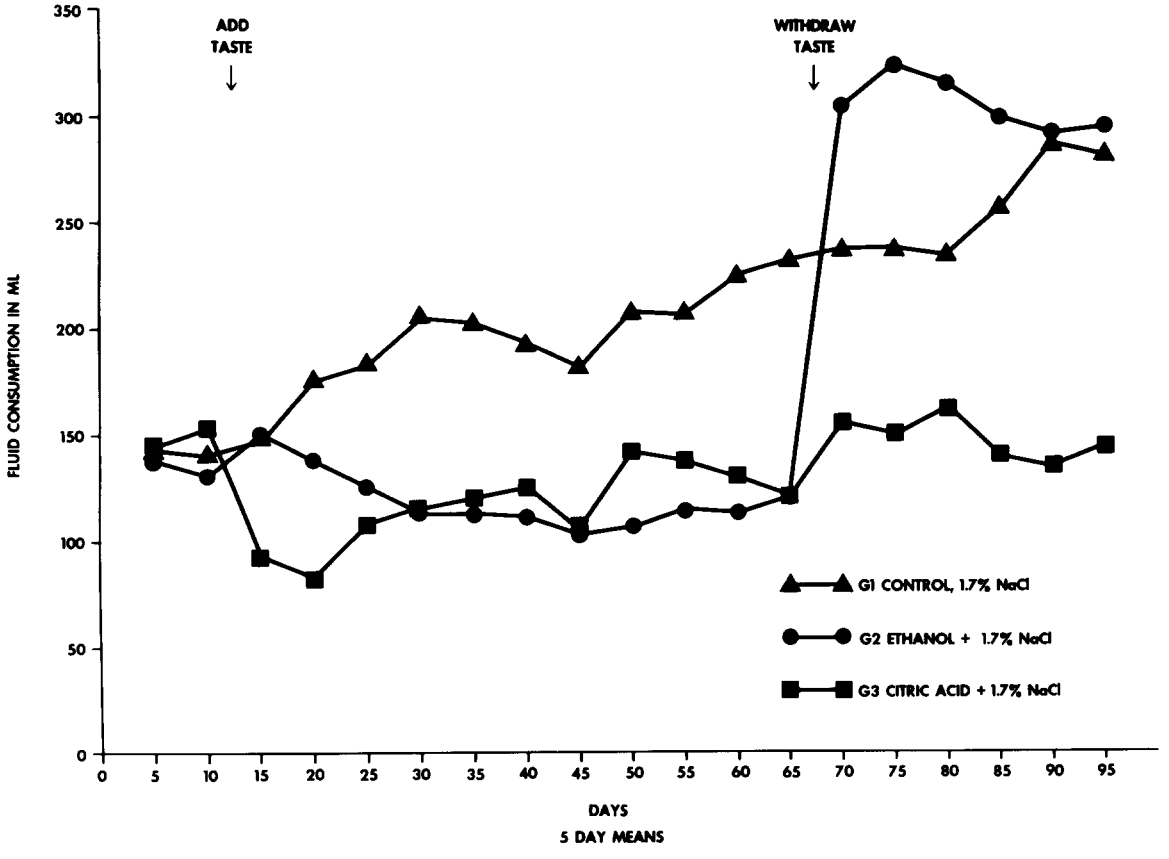

FIG. 1. Mean fluid intake in ml presented as a function of successive 5-day blocks. Arrows indicate when ethyl alcohol or citric acid were added to and withdrawn from the 1.7% NaCl drinking fluid of the 2 experimental groups, G2 and G3.

periods are indicated by increasing numbers which correspond to the days in Fig. 1. Significant decreases in consumption for the 5 day periods 5, 10 and 45 and significant increases for Days 90 and 95 occurred for G1. In G2, fluid consumption was increased significantly immediately following the removal of 6% ethanol from the 1.7% NaCl solution and for the remainder of the experiment, 5 day periods 70, 75, 80, 85, 90 and 95. In the citric acid group, G3, fluid consumption did not differ.

Body weight data were analyzed by means of a two factor ANOVA exactly as described for the analysis of fluid intake. The groups were not significantly different, $F(2,9) = 0.205$, $p>0.50$. The main effect for days was significant, $F(12,108) = 16.548$, $p<0.01$. The groups by days interaction was significant, $F(24,108) = 2.119$, $p<0.01$. A simple main effects analysis of days at each group indicated days for G1, $F(12,108) = 2.467$, and days for G2, $F(12,108) = 2.341$, were significant, $p<0.01$. Days for G3 were not significant, $F(12,108) = 0.383$, $p>0.50$. In general the only differential effects on body weight between the three groups was the fact that the control group, G1, and the G2 ethyl alcohol group gained weight at a faster rate during the course of the experiment than the citric acid group, G3. There were no other significant overall differences in body weight between the three groups.

DISCUSSION

Results on the control group, G1, demonstrate a significant continuous increase in the consumption of 1.7% NaCl, when it was the only fluid available, during the course of the study. The hyperdipsia apparently results from an increasing NaCl diuresis produced by the consumption of saline. The polyuria was obvious and except for the excessive drinking the control animals appeared normal. Body weights were normal throughout the experimental period.

The experimental ethanol group, G2, did not display an increasing hyperdipsia for the 1.7% sodium chloride solution. When the ethanol was added to the drinking fluid intakes were decreased as compared to the control group. However, it should be noted that although the consumption of the control animals increased significantly, there were no significant differences between the control, G1, and the experimental groups, G2 and G3, during the early part of the experiment from Days 10 through 65. Because of the mildly aversive qualities of the ethyl alcohol and citric acid, the intakes of the two experimental groups, G2 and G3, were very similar and were maintained consistently and relatively stable at levels below the intakes of the control group. Following Day 65, when the ethanol and citric acid were removed from the 1.7% sodium chloride drinking solution, the increased consumption of 1.7% NaCl solution by the experimental group, G2, was very dramatic. The consumption almost tripled from 120 to 302 ml per 24 hr period which might indicate a ceiling for the consumption of saline under these conditions.

Because these animals were hyperactive, drinking

TABLE 1

RESULTS OF SIMPLE MAIN EFFECTS ANALYSIS WITHIN GROUPS FOR FLUID CONSUMPTION

GROUPS	5 DAY BLOCKS												
	5	10	45	50	55	60	65	70	75	80	85	90	95
G1 CONTROL	↓	↓	↓	—	—	—	CONTROL	—	—	—	—	↑	↑
G2 ETHANOL	—	—	—	—	—	—	CONTROL	↑	↑	↑	↑	↑	↑
G3 CITRIC ACID	—	—	—	—	—	—	CONTROL	—	—	—	—	—	—

copiously, urinating voluminously, and appeared to be hyperreactive, some of the symptoms of withdrawal, we did not subject them to any physical dependence tests which might have produced convulsions and death. Since there were four animals in the group, we were concerned primarily with obtaining the intake data. Even at that, one animal died within 7 days following the withdrawal of ethyl alcohol from the 1.7% sodium chloride drinking fluid. The mean consumption of these animals of the 6% ethanol plus 1.7% sodium chloride during the final 25 days before withdrawal was 113 ml per day. The mean body weight was 406 g per day and, consequently, the mean absolute amount of ethyl alcohol consumed was 16.7 g/kg of body weight per day. Since the expected elimination of ethyl alcohol is approximately 8–9 g per kg body weight per day [1], blood alcohol must have been sustained at a relatively high level. Obviously, the possibility of severe withdrawal symptoms under these conditions should be tested with a larger number of animals in the future. The fact that the increased consumption of the experimental group, G2, remained high and seemed to be relatively permanent and similar to the intake of the control group, G1, is important because none of these effects occurred with the citric acid group, G3.

The high intakes in the control group and the ethyl alcohol group must be produced by different mechanisms. The consumption of the ethyl alcohol group was significantly elevated in comparison to the control group for 15 days following withdrawal of the alcohol. The gradual continuous increase in fluid consumption of the control group must be attributed to an increasing NaCl diuresis. The increased consumption of the ethanol group probably resulted in a withdrawal hyperreactivity and increased sensitivity of the lateral hypothalamic motor control mechanism involved in drinking [3, 4, 5] plus an increased saline diuresis. Since there were no significant effects on body weight which can be attributed to these experimental treatments and because of the expected high blood ethanol levels prior to and obvious qualitative signs of physical dependence following ethanol withdrawal, these results support the previous hypothesis that forced drinking of saline plus ethyl alcohol might be an effective means for inducing alcoholism in the rat.

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