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Effects of Phenobarbital on Ethanol Intake in Fluid Deprived Rats[1]

DANIEL B. RONDEAU[2,3], FRANCOIS B. JOLICOEUR[3]

Département de Psychologie, Université du Québec à Montréal

R. KACHANOFF, P. SCHERZER AND M. J. WAYNER

Douglas Hospital, Verdun, Québec; Université du Québec à Montréal; Brain Research Laboratory, Syracuse University

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RONDEAU, D. B., F. B. JOLICOEUR, R. KACHANOFF, P. SCHERZER AND M. J. WAYNER. *Effects of phenobarbital on ethanol intake in fluid deprived rats.* PHARMAC. BIOCHEM. BEHAV. 3(3) 493–497, 1975. – Rats were adapted to a 23 hr fluid deprivation schedule. Every third day animals were offered either 3 or 7 percent ethanol in place of water during the 1 hr drinking session. Three doses of sodium phenobarbital were administered subcutaneously to 3 groups of animals – 20, 40, and 60 mg/kg. Results indicate that the drug increases ethanol consumption following the injection but decreases consumption of ethanol on subsequent postdrug days. There was an attenuation in these effects from the first to the third injection. Although a dose effect was not determined, changes in ethanol consumption were greater with the higher concentrations.

Ethanol Sodium phenobarbital Fluid deprivation Alcohol intake

THE barbiturates, especially sodium phenobarbital, injected subcutaneously in amounts below the hypnotic dose produce an increase in water intake of rats under ad lib conditions [6], on a 23 hr fluid deprivation schedule [5, 7, 9, 14, 19] and following injections of hypertonic saline solutions [14,17]. Maximal increases are obtained with moderate doses, 25–40 mg/kg, with a reduction towards baseline with higher doses [4,18]. Phenobarbital also enhances consumption of solutions that are considered aversive in taste. Increased intakes of various saline solutions were noted in water deprived rats following administration of sodium phenobarbital [5, 15, 16]. As the concentration of a saline solution is increased above isotonicity, it becomes less palatable to rats and although the magnitude of facilitation of drinking by sodium phenobarbital is at a maximum in the vicinity of optimal solution acceptability, i.e. around 0.9 percent NaCl, increased intakes of solutions up to 3 percent NaCl have been reported [16]. Ethanol solutions above 5 percent in concentration are less palatable than water for the rat since ethanol consumption in these animals declines rapidly in preference for water in the range of 5–7 percent [10, 13, 21].

Effects of various doses of sodium phenobarbital on the consumption of 4, 6, and 8 percent ethanol solutions have been examined in some detail [7]. In this unpublished study 23 hr water deprived rats received a given dose of phenobarbital 15 min prior to a 1 hr drinking session in which an ethanol solution was presented. Drug-ethanol treatments were given four times. Each treatment was interspersed by two days when water alone was presented during the drinking session. Results demonstrated that sodium phenobarbital increased the intakes of all ethanol solutions. Of special interest was the finding that postdrug intakes of the ethanol solutions were significantly lower than the predrug intakes. This decrease occurred after animals received four successive phenobarbital-ethanol treatments and drank different alcohol solutions. The purpose of the experiment was to determine the effects of three doses of sodium phenobarbital – 20, 40, and 60 mg/kg – on fluid deprivation produced drinking of 3 or 7 percent ethanol. Results demonstrate a clear increase in the drinking of 3 or 7 percent ethanol solutions by sodium phenobarbital and a subsequent postdrug decrease in the consumption of the same solutions.

METHOD

Animals

Thirty-six male hooded rats from Ferme et Laboratoire

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[2] Reprint requests to D. B. Rondeau, Brain Research Laboratory, Syracuse University, 601 University Avenue, Syracuse NY 13210.

[3] Present address: Brain Research Laboratory, Syracuse University, 601 University Avenue, Syracuse NY 13210.

Canadien d'Elevage, St-Constant, Que., were used. Upon their arrival, animals were weighed and injected with 0.05 cc Bicilin to prevent infections. For 4 days the animals were maintained on tap water and Purina Rat Chow ad lib. They were weighed and handled daily. At the beginning of the experiment their mean age was 44 days and their mean weight was 184.4 g, range 164–200 g. The rats were separated into 6 groups of 6 animals each.

General Procedure

The rats were kept in individual home cages, 7 × 7 × 10 in, in a rack located in a constantly illuminated and temperature controlled room. Tap water or ethanol solutions, 3 percent w/v or 7 percent w/v, were presented in a 100 ml calibrated Richter drinking tube clipped to the front of each cage. Food, which consisted of Purina Rat Chow pellets, was placed in a holder attached to each cage. Ethanol solutions were prepared every 2 or 3 days. Distilled water was added to the given weight of ethanol desired for a particular concentration. Tap water and the ethanol solutions were presented at room temperature. Sodium phenobarbital was dissolved in physiological saline. Stock solutions of this drug were prepared the day prior to the injections and were kept under refrigeration. Dosages studied were 20, 40, and 60 mg/kg and were administered by subcutaneous injections. For all injections the animals were partially immobilized in a constraining device, Restraining Cage, 3 × 2.5 × 8 in. Fisher Scientific. None of the injection volumes exceeded 0.5 ml.

Drinking Schedule and Drug Procedures

The animals were adapted to a 23 hr fluid deprivation schedule. The volume of fluid consumed during a 1 hr drinking session was measured. During this time food was taken from the cage. For the remaining 23 hr food was present ad lib. Prior to the daily fluid consumption each rat was removed from the home cage and weighed. The specific times for weighing each of the 6 groups of animals and the hour of fluid intake for each of them were approximately the same every day.

On Days 1–14, the rats after being weighed were returned to their home cage. Fifteen min later the Richter tubes were filled with water and clipped to the cages and the animals were allowed to drink for 1 hr. On Days 9 and 12, the rats were injected subcutaneously with 0.9 percent NaCl after being weighed and before being returned to their cages. Fifteen min later tubes were replaced and animals allowed to drink as above. The volumes of the saline injections were approximately equivalent to those of the drug injections to be given later in the experiment.

On Day 15, after a 0.9 percent NaCl injection, 3 of the 6 groups of rats were presented with 1.5 percent ethanol and 3.5 percent ethanol was offered to the other three groups. These two solutions were one-half the concentration of the solutions to be used later in the experiment and were employed to provide some limited prior experience with the taste and flavor of ethanol solutions. On Days 16 and 17 tap water was the only fluid presented.

Every three days, from Day 18 to 42, 3 groups of rats were provided with a 3 percent ethanol solution and the 3 other groups with a 7 percent ethanol solution. Consequently a given group of animals was allowed to drink one of the two concentrations of ethanol throughout the experiment. Two days of water presentation were interspersed between each presentation of the ethanol solutions. Animals were forced to drink ethanol during the 1 hr session on Days 18, 21, 24, 27, 30, 33, 36, 39, and 42. Animals received injections of 0.9 percent NaCl on Days 18, 21, 24, 30, 36, 42 and injections of sodium phenobarbital on Days 27, 33, and 39.

On Days 18, 21, and 24, the animals were weighed and injected with 0.9 percent NaCl 15 min prior to the drinking session and fluid intake was measured. These data, expressed in terms of milliliters of fluid imbibed during the 1 hr drinking session, provided a saline control baseline of ethanol consumption for each group of animals. Drug administration took place on Days 27, 33, and 39. A dose of 20 mg/kg sodium phenobarbital was assigned to one of the groups that was allowed to drink the 3 percent ethanol solution. The remaining two groups for that concentration of ethanol received respectively 40 and 60 mg/kg sodium phenobarbital injections. The same three doses of the drug were employed with the three groups of rats who drank the 7 percent ethanol solution. Consequently, on each of the three drug days, a given group of animals was presented with the same ethanol solution and received the same dose of sodium phenobarbital. Otherwise the procedure was exactly the same as it was on saline injections days. On Days 30, 36, and 42, treatments of each group of rats were identical to those of predrug Days 18, 21, and 24 on which 0.9 percent NaCl injections were given. This procedure provided postdrug fluid intake measures with which it was possible to compare baseline and drug days alcohol intakes.

RESULTS

The results are based on the consumption data during the 1 hr drinking sessions on days on which ethanol solutions were presented. Water intakes on all the interdays were found to be of small variance and are not included in the detailed analysis of the results. A 3 × 3 × 2 × 3 repeated measures ANOVA [23] was carried out on the intakes for each dose, for all the treatments (predrug days, drug days and postdrug days), for each ethanol concentration and for the three different days of the same treatment.

No significant differences were found between the effects of the three doses of the drug on ethanol consumption. Mean intakes of the ethanol solutions, with the doses combined, under all the treatment days are illustrated in Fig. 1 for the 3 percent (Panel A) and 7 percent (Panel B) ethanol concentrations. Significant differences were found between ethanol intakes under the three treatments $F(2,60) = 83.87$, $p<0.01$. A Tukey Type A analysis revealed that there were significant differences in ethanol consumption between predrug days and drug days, between drug days and postdrug days, and between predrug days and postdrug days. This is illustrated in Fig. 2 in which mean intakes of the ethanol solutions, combined for the doses and the days of a same treatment, are plotted for each treatment. It can be seen that the significant differences between treatments are reflected by an increase in ethanol intakes of both concentrations on drug days and a decrease in consumption on postdrug days when these two treatments are compared to the predrug measures.

The analysis of variance also revealed a significant difference in the consumption of the two concentrations of ethanol through all the treatments, $F(1,30) = 136.15$, $p<0.01$. On the average rats drank about 6 ml more of the 3 percent

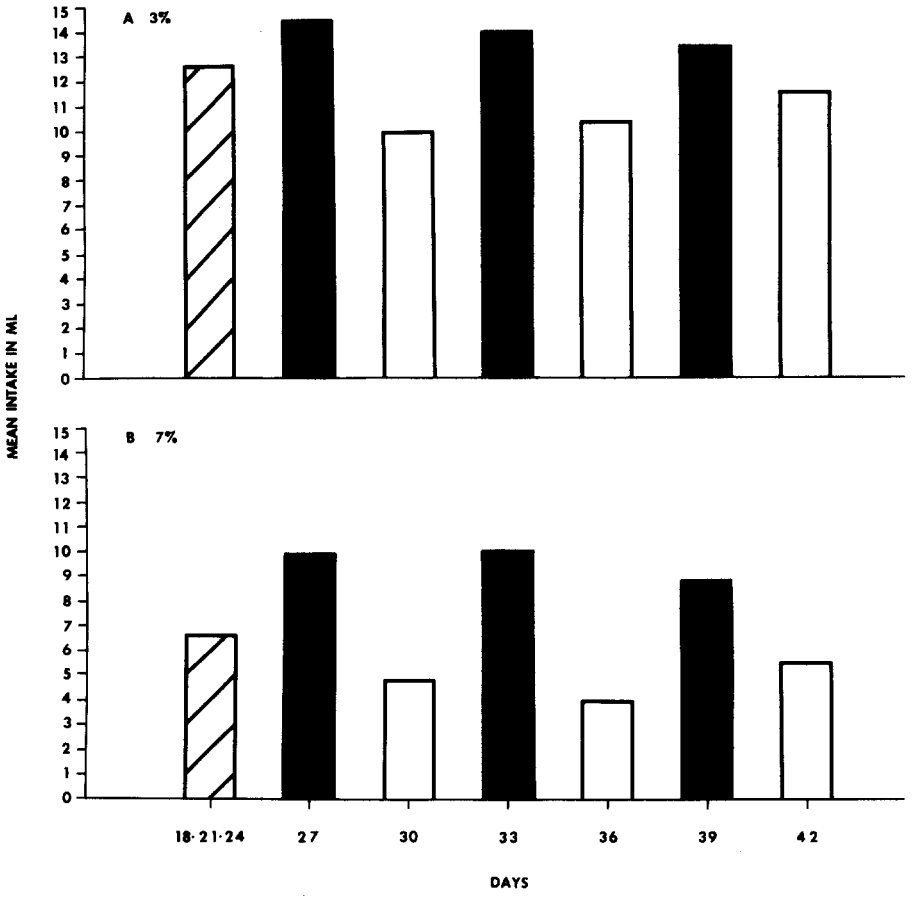

FIG. 1. Mean ethanol intake of both concentrations on combined predrug days (18, 21 and 24), on each of the drug days (27, 33 and 39), and on each of the postdrug days (30, 36 and 42). A – 3 percent; B – 7 percent. ⧄ combined predrug; ■ drug; □ postdrug.

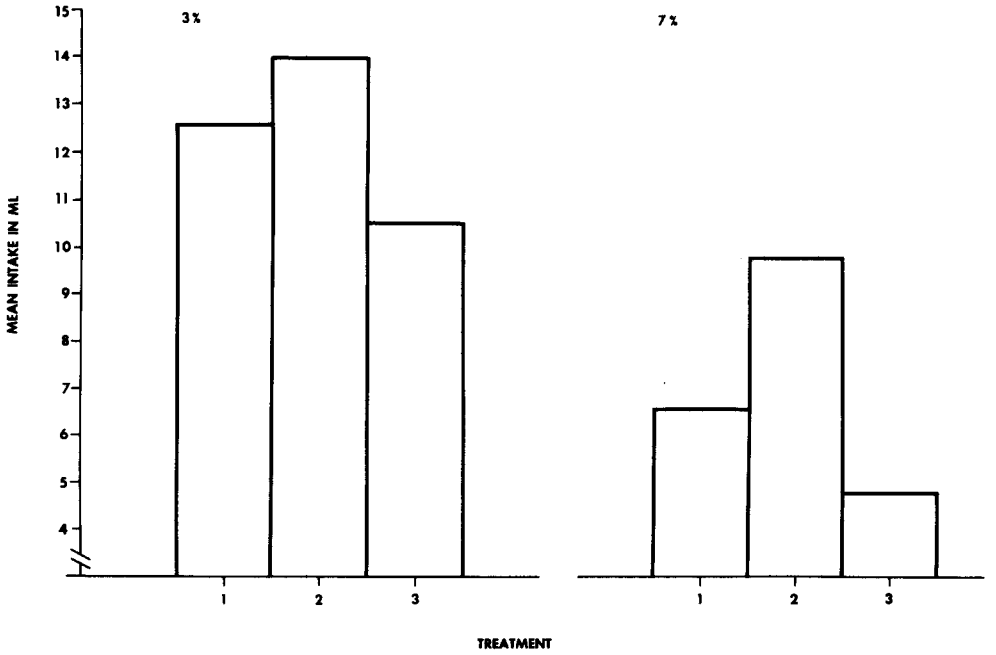

FIG. 2. Effects of sodium phenobarbital (combined doses) on 3 and 7 percent ethanol intake. Treatment 1: mean of the combined intakes for the predrug days. Treatment 2: mean of the combined intakes for the 3 drug days. Treatment 3: mean of the combined intakes for the 3 postdrug days.

ethanol solution than the 7 percent ethanol solution during the 1 hr drinking session. This is in accordance with numerous experiments dealing with the preference-aversion function of ethanol concentrations in the rat. This difference in the consumption of the two ethanol solutions is clearly shown in Figs. 1 and 2.

The treatments by ethanol concentrations interaction was significant, $F(2,60) = 4.58$, $p<0.05$. The facilitatory effect of sodium phenobarbital on drinking is much more dramatic for the animals who drank the 7 percent ethanol solution during the 1 hr session. For these rats there was a 47 percent change in consumption while the animals who drank the 3 percent ethanol solution increased their consumption by 11 percent. Also, the depressing effect of sodium phenobarbital on postdrug ethanol consumption is greater for the animals who drank the 7 percent ethanol solution. These animals decreased their consumption by 27 percent while the 3 percent ethanol rats decreased their intake by 16 percent.

There were no significant differences between the three different days of the same treatment. However, the treatments by days of the same treatment interaction was significant, $F(4,120) = 5.56$, $p<0.01$. As shown in Fig. 3, mean intakes on drug days are in the direction of a decrease from the first to the third injection of the drug indicating an attenuation of the facilitatory effect of sodium phenobarbital. On the other hand, mean intakes on postdrug days are in the direction of an increase indicating an attenuation of the depressing effect of the drug from the first to the third day of that treatment.

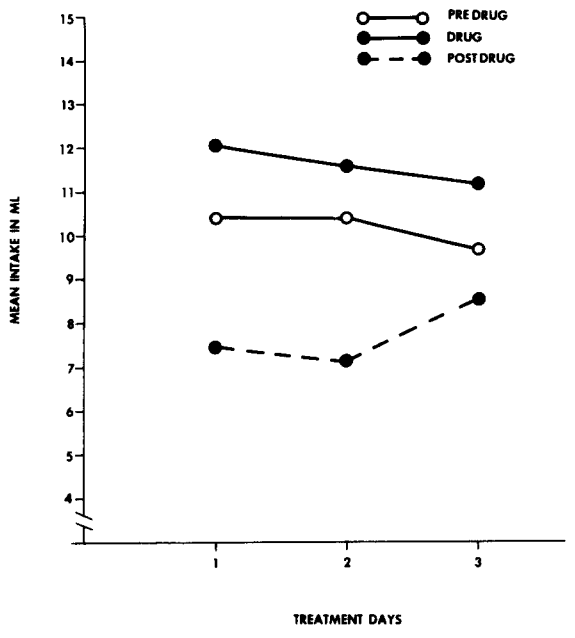

FIG. 3. Mean of the combined intakes for both ethanol solutions plotted as a function of the 3 days of the same treatment.

DISCUSSION

The results of the present experiment confirm the previous observation of the effect of sodium phenobarbital on ethanol intake in 23 hr fluid deprived rats [7]. A significant increase in liquid consumption was found when injections of sodium phenobarbital were paired with either 3 or 7 percent ethanol solutions intakes. The combination of phenobarbital and ethanol has some interesting implications. It has been reported that phenobarbital stimulates aldehyde dehydrogenase activity in rat liver [2]. Increased ethanol consumption could then be interpreted as being related to a facilitation of alcohol catabolism. However, since phenobarbital produces an increased consumption of water and saline solutions, it seems unlikely that the enhanced ethanol ingestion observed in this experiment could be solely attributable to this interaction of the drug with ethanol. An attenuation of the increasing effect of sodium phenobarbital on ethanol intake was observed from the first to the third injection. In terms of percentages of change over baseline, the facilitatory effect of the drug was approximately four times more effective for the 7 percent ethanol concentration than for the 3 percent concentration. The large increase in the consumption of the 7 percent ethanol solution is another indication that sodium phenobarbital can produce its dipsogenic action with aversive solutions. Punishment-attenuating properties of the drug have been proposed elsewhere as the behavioral explanation of this effect [5]. According to this hypothesis, the drug would counteract the aversiveness of some solutions in such a way that animals drink larger quantities of these solutions after administration of the drug than under baseline conditions. Contrary to the results on water intake, where the 40 mg/kg dose of sodium phenobarbital has been shown to be the most effective [18], the present data do not indicate that the magnitude of increased ethanol consumption is related to the amount of the drug injected. The reasons for these differences concerning the dose effect of the drug on drinking are not clear at the present time. In addition to the increases in ethanol intake on drug days, results reveal significant decreases in ethanol intakes on postdrug days when compared to predrug days or baseline data. This depressing effect was observed after each sodium phenobarbital treatment. Apparently, only a single combination or pairing of phenobarbital and ethanol is sufficient to produce a decrease in ethanol intake and that such an effect can be obtained in animals given only one ethanol solution to drink. The depressing effect of sodium phenobarbital was much greater for the 7 percent ethanol solution than for the 3 percent solution. No dose related decreases in ethanol consumption were observed.

Several drugs have been found to reduce alcohol intake in the rat [22]. Among them are p-chlorophenylalanine, which depletes brain serotonin, and n-Butyraldoxime, an aldehyde dehydrogenase inhibitor. However, it has been demonstrated that the action of these two drugs is not specific to alcohol since they produce learned taste aversions to saccharin solutions [12]. Up to now, only morphine has been unequivocally shown to induce a specific reduction in ethanol consumption [20]. The decreases in ethanol intake observed in the present experiment might be interpreted as a learned taste aversion produced by the sodium phenobarbital. The report that another barbiturate, sodium pentobarbital, causes a taste aversion to saccharin solutions [1] tends to indicate that barbiturates can produce aversions to sapid fluids and that the observed depressing effect of sodium phenobarbital might not be specific to alcohol solutions. Sodium phenobarbital might have produced a learned taste aversion to the 3 percent ethanol solution and could have reduced even more the consumption of the 7 percent ethanol solution which is already

aversive to rats. It should be mentioned that in the present experiment the reduction in intake was not found to be dose related whereas the degree of aversion to a solution is proportional to the amount of the toxic substance administered [11]. Besides the possible involvement of a learned taste aversion created by sodium phenobarbital, another factor could contribute to the depressing effect of the drug on postdrug days ethanol intakes. Sodium phenobarbital produces intakes of ethanol solutions in quantities greater than that which an animal accepts under baseline conditions. The postabsorptive effects of such higher ingestion would have an influence on subsequent intakes. This is plausible since ethanol has a systemic aversion producing effect. Intraperitoneal injections of ethanol in various doses have been found to be effective noxious stimuli inducing taste aversion to sweetened solutions [3,8]. It would be interesting to determine if increases in ethanol ingestion produced by other dipsogenic agents would reduce subsequent ethanol intake.

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