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Development of Physical Dependence on t-Butanol in Rats: An Examination Using Schedule-Induced Drinking[1]

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GRANT, K. A. AND H. H. SAMSON. *Development of physical dependence on t-butanol in rats: An examination using schedule-induced drinking.* PHARMAC. BIOCHEM. BEHAV. **14**(5) 633–637, 1981.—Rats were exposed to various concentrations of t-butanol as their only available fluid in either the home cage or a schedule-induced drinking situation previously shown to induce overdrinking of ethanol. When animals consumed at least 3 g/kg/day of t-butanol for 90 days, independent of the condition, withdrawal symptoms were observed. This daily intake occurred only when the concentration of t-butanol was 3% (v/v) or greater. The schedule-induction procedure did not induce t-butanol overdrinking at any concentration tested as it does with ethanol, but its use did result in increased probability of the occurrence of withdrawal symptoms over the home cage condition. In no cases was the severity of withdrawal from t-butanol as great as previously reported for ethanol. When the concentration of t-butanol was increased to 3.5% (v/v), severe toxic reactions were found, that included anorexia, self-mutilation, and deaths from no specific determinable causes.

t-Butanol Ethanol Physical dependence Schedule-induction

FOLLOWING prolonged exposure to ethanol a well documented sequence of withdrawal symptoms occur in man and laboratory animals [1, 2, 3, 6, 7, 13, 14]. The withdrawal hyperactivity is taken as evidence for physical dependence upon the alcohol. However, the mechanisms by which ethanol results in physical dependence have not been completely identified. In an attempt to separate the metabolic consequences from direct action of ethanol, several investigators have used tertiary butanol to produce physical dependence in rats and mice [5, 12, 16, 17]. Tertiary butanol, unlike ethanol, is not oxidized to an aldehyde but instead is largely detoxified by conjugation to glucuronic acid and passed through the urine [15]. Thus t-butanol has been considered as a possible control for the relative contribution which the oxidative metabolites of ethanol might have in producing physical dependence.

A withdrawal syndrome, similar to that seen with ethanol, has been observed following exposure to t-butanol using either a liquid diet [16,17] or intragastric intubation [12] with rats, and forced inhalation with mice [5]. Although these exposure methods found qualitatively similar withdrawal signs, the severity of the abstinence syndrome differed depending on the exposure technique.

This study was designed to test if another method known to produce physical dependence upon ethanol, psychogenic polydipsia [2,3], would produce physical dependence upon t-butanol in rats.

METHODS

Animals

Twenty-nine adult male Long-Evans rats were used: 15 animals were in one of four different schedule-induced conditions and 14 were home-cage controls.

Experimental Environment

Schedule-induced condition: Details of the experimental environment have been described previously [2,3]. Basically, the subjects were housed in Plexiglas chambers connected to an automatic food dispenser (Gerbrands Corp, Arlington, MA) designed to deliver 45 mg food pellets (J. P. Noyes, Inc., Lancaster, NH). During each one hour delivery session one food pellet was delivered every two minutes. There were 3 hours between each session, resulting in 6 food delivery sessions every 24 hours. Attached to each chamber was a calibrated drinking tube with a ball-bearing spout. In the room containing the chambers, lighting was on a 12 hour on/off cycle.

Home cage conditions: Subjects were housed individually in standard steel rat cages. Attached to each cage was a drinking tube with a ball-bearing spout. Lighting was identical to the schedule-induced condition.

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Procedure

Schedule-induced condition: All subjects were slowly reduced to 80% of their free feeding body weight by restricting food intake. After reaching goal weight, the animals were placed in the experimental chambers and given an initial 5 days to habituate to the schedule with water as the available fluid. All animals in the schedule-induced condition had fluid intakes and body weights recorded at the same time daily. Food supplements, when necessary to maintain at least 80% body weight, were given in two portions 8 hours apart.

Group 1: After habituation, four animals had 5 days with 0.5% t-butanol as the only fluid available. The t-butanol solution was then increased to 1% for the next 60 days. On the 61st day, seven hours after replacing the t-butanol with water, each animal was subjected to a maximum of 30 seconds of key shaking as a test for susceptibility to audiogenic seizures (see below). The subjects were then returned to their chambers and observed for an additional hour.

Group 2: Four animals were exposed to increasing concentrations of t-butanol (0.25%, 0.50%, 1.0%, 2.0%, 2.5%, 3.0% and 3.5%). Each solution was available as the sole fluid for 5 consecutive days starting with 0.25% and going stepwise up to 3.0%. The 3.0% and the 3.5% concentrations were each available for 10 days. Following 10 days of the 3.5% solution, the animals were placed on 3.0% t-butanol for the next 40 days. Following this 40 day chronic exposure, water was substituted for the t-butanol and the animals were observed for withdrawal signs. Seven hours after the water substitution the animals were tested for audiogenic seizures. They were then returned to the chambers and given an additional 30 days exposure to 3.0% t-butanol. Following this additional exposure, the animals were again withdrawn and tested for audiogenic seizures 8 hours into the withdrawal period.

Group 3: Originally the 5 animals in this group were exposed to 1.0% t-butanol for 5 days following the habituation phase. Then, 2.0% t-butanol replaced the 1.0% solution for the next 5 days. During the next 90 days, 3.0% t-butanol was the only fluid available. Following this chronic period of t-butanol exposure, the animals were placed on water and observed for signs of withdrawal. Eight hours into the withdrawal period the subjects were tested for susceptibility to audiogenic seizures.

Group 4: Two animals given water as their only available fluid were maintained in the schedule-induced condition simultaneously with Group 3. They were tested for audiogenic seizures at the same time as Group 3.

Home cage condition: The home cage animals were divided into three groups. They were serviced daily at the same time as in the schedule-induced condition.

Group 1: Four animals were slowly reduced to 80% of free feeding body weight by restricting food intake. After reaching that criteria, they were exposed to 0.5% t-butanol as the only available fluid for 5 days. The drinking fluid was then changed to 1% t-butanol for 90 days. Following this 3 month exposure, water replaced the t-butanol and the animals were tested for audiogenic seizures 8 hours after alcohol removal.

Group 2: Six animals with free access to food throughout the experiment were first given 1% t-butanol as the only available drinking fluid for 5 days. Then, 2% t-butanol was available for the next 10 days and finally 3.0% t-butanol for the remaining 90 days. Then the t-butanol was replaced by water and the animals tested for audiogenic seizures 8 hours later.

Group 3: Four animals were slowly reduced to 80% of full body weight and placed on restricted food availability as in Group 1. This group was tested for susceptibility to audiogenic seizures after 95 days with water as their only available fluid.

Withdrawal Testing and Rating

All animals were tested for seizure susceptibility in a 18×36 inch open top plastic cage placed in a separate room from the experimental cages. A ring of 10 keys was held 12 inches above and in the center of the open plastic cage. The keys were shaken by hand at a rapid rate for 30 seconds. This was timed by an electronic stopwatch which was also used to time both onset and duration of any seizures. Preliminary testing with 3 rats exposed to 60 days of 3% t-butanol found the animals to be more susceptible to seizures at 8 hours withdrawal than 10 hours. This finding corresponds with an earlier study of t-butanol dependency in rats [17]. Therefore, all further withdrawal testing was performed 8 hours after the removal of t-butanol.

Withdrawal was scored on the basis of latency to seizure, duration of seizure, and the intensity of the seizure if one occurred. Latency was measured in seconds from the time key-shaking began until the occurrence of a seizure. A seizure beginning within the first 5 seconds of key shaking was given a score of 4, from 5–15 seconds a 3, from 15–25 seconds a 2 and from 25–30 seconds a 1. If a seizure did not begin within the 30 second key shaking test, the latency score was 0.

Seizure duration was recorded until the subject no longer showed visible signs of seizure activity. A seizure lasting over 60 seconds was rated 4. Seizures from 30–60 seconds long were scored 3. Those from 15–30 seconds were given a 2 and those less than 15 seconds, 1. Again, if no seizure occurred, the duration score was 0.

The intensity of the seizures had to be measured somewhat subjectively within the following guides: If the animal went into a full clonic-tonic fit, it was given a rating of 4. Animals showing a running-hopping fit ending in a clonic position were rated 3. Those seizures in which the animals had a running fit were rated 2 and a strong startle response with initial jumping or tumbling was rated 1. Animals that showed only increased activity in an attempt to escape the noise were rated 0.

The final score was the sum of the three values. Thus the highest possible overall withdrawal score was 12 and the lowest, 0. Note that a score of 1 or 2 is not possible on this scale because any occurrence of a seizure must have at least a rating of 1 in each of the 3 categories.

RESULTS

An overall profile of the daily t-butanol intakes for the four t-butanol exposed conditions is given in Table 1. The first 30 days for groups maintained on 3% t-butanol has been split into two time periods: days 1–10 and 11–30. This was necessary because the concentrations of t-butanol given over the first 30 days were increasing, thus the g/kg intakes were also rising and a single mean value does not reflect the jump in intake. Groups 1 and 2 of the schedule-induced condition had only 3 and 2 animals respectively complete the study. The 5 animals lost from these groups were removed due to self-withdrawal and/or death (see below). Since there was no difference in the g/kg intakes of the 5 remaining animals, they

TABLE 1

MEAN DAILY g/kg INTAKES PER GROUP DURING t-BUTANOL EXPOSURE

		Days Exposed to t-Butanol				
Condition	N	1–10	11–30	31–60	61–90	91–120
1% S-I	4	0.8 ± 0.05	0.8 ± 0.05	0.8 ± 0.03		
1% HC	4	0.7 ± 0.5	0.7 ± 0.5	0.7 ± 0.03	0.7 ± 0.04	0.7 ± 0.04
3% S-I	5	0.9 ± 0.15	1.9 ± 0.15	2.4 ± 0.13	2.8 ± 0.09	2.7 ± 0.21
3% HC	5	1.0 ± 0.04	1.7 ± 0.16	2.3 ± 0.29	2.4 ± 0.35	

S-I=Schedule-induced condition.
HC=Home cage condition.

TABLE 2

MEAN BODY WEIGHT (g) AND FLUID INTAKES (ml) FOR LAST 20 DAYS OF EXPOSURE PRIOR TO WITHDRAWAL

			Mean Daily Intakes*	
Condition	N	Weight($\bar{x}$)	mls	g/kg
HC—H_2O	4	297 ± 6	35 ± 4	—
S-I—H_2O	2	287 ± 1	32 ± 1	—
HC (1% t-butanol)	4	303 ± 8	26 ± 1	0.7 ± 0.04
S-I (1% t-butanol)	4	442 ± 22	42 ± 1	0.7 ± 0.03
HC (3% t-butanol)	5	342 ± 30	33 ± 2	2.4 ± 0.3
S-I (3% t-butanol)	5	292 ± 4	33 ± 3	2.8 ± 0.2

*±S.E.M.
HC=Home cage.
S-I=Schedule induced.

were grouped together as the 3% schedule-induced condition.

The daily intakes (g/kg) for animals given 1% t-butanol did not differ in either condition. However, at increasing concentrations with final maintenance on 3% t-butanol, the schedule-induced condition appears to produce slightly increased intakes over that of the home cage animals.

The mean body weights and fluid intakes for all groups over the last 20 days of exposure are given in Table 2. The subjects in the 1% t-butanol groups (both the schedule-induced and home cage condition) had relatively low mean daily intakes. When the concentration of t-butanol available was 3%, the g/kg intakes increased, with the schedule-induced drinkers having slightly higher intakes than that of the home cage animals. However, with the higher t-butanol concentration, incidences of self-withdrawal, self-mutilation, and death resulting from unknown causes were observed. The self-withdrawal resulted in the animals refusing to drink to the point of death. This withdrawal was accompanied with tremors, strobbed tail and occasionally spontaneous seizures. Of the 15 animals given 3% t-butanol, 5 animals had to be removed from the experiment due to self-withdrawal and/or self-mutilation. Two of these 5 animals were found dead in their cages. All 5 animals showed a similar trend of increased t-butanol intake and decreased food consumption just prior to removal or death. None of these symptoms were seen in animals drinking water or 1% t-butanol.

Figure 1 gives the g/kg and ml intake of group 2 schedule-induced drinkers as a function of the t-butanol concentration. The g/kg intake shows a steady rise over all concentrations, however, the fluid intake begins to level at a t-butanol concentration of 2.5%. When the concentration of t-butanol was raised to 3.5%, one subject began to self-mutilate, ceased drinking, and was removed from the experiment. To prevent further loss of animals the concentration of t-butanol in the schedule-induced drinking condition was reduced to 3%.

The seizure activity of this group after 60 days of 3% t-butanol was relatively moderate (withdrawal scores 9, 5, and 6) compared to their seizure susceptibility after the additional 30 days of 3% t-butanol drinking (withdrawal scores of 12, 6, and 7, respectively).

Figure 2 shows the withdrawal ratings of every animal completing the experiment as a function of their mean daily g/kg intake of t-butanol over the last 20 days of exposure. As shown in the figure, at least one animal from every group had a withdrawal score of zero. All subjects having water or 1% t-butanol in either home cage or schedule-induced condition had a withdrawal score of 5 or less. Thus all animals with a withdrawal score of 6 or greater were those subjects exposed to 3% t-butanol. When the animals are arbitrarily grouped into one of 4 categories based on their intakes and withdrawal scores (daily intake above or below 2 g/kg; and withdrawal score above or below 6), animals with high daily intakes (g/kg) had significantly higher withdrawal scores (Fischer's exact $p<0.002$).

DISCUSSION

Physical dependence on t-butanol, as defined by withdrawal symptoms, can be induced by several procedures [5, 12, 16, 17]. The findings from this study support these previous studies and suggest that those procedures used to develop dependence on ethanol are also capable of producing physical dependence upon t-butanol. If daily intakes are maintained above 3 g/kg of t-butanol, then withdrawal symptoms are seen, independent of the method used to induce the intake.

In this study, the mean g/kg daily intake of the 20 day period prior to withdrawal was shown to be significantly related to withdrawal severity. This finding agrees with earlier reports using t-butanol although a ceiling effect of the withdrawal severity with increasing dose has been reported [5]. Since the schedule-induced 3% t-butanol animals had overall more severe seizure scores, it would appear that the schedule-induction paradigm slightly enhances withdrawal but is not necessary for the production of physical depend-

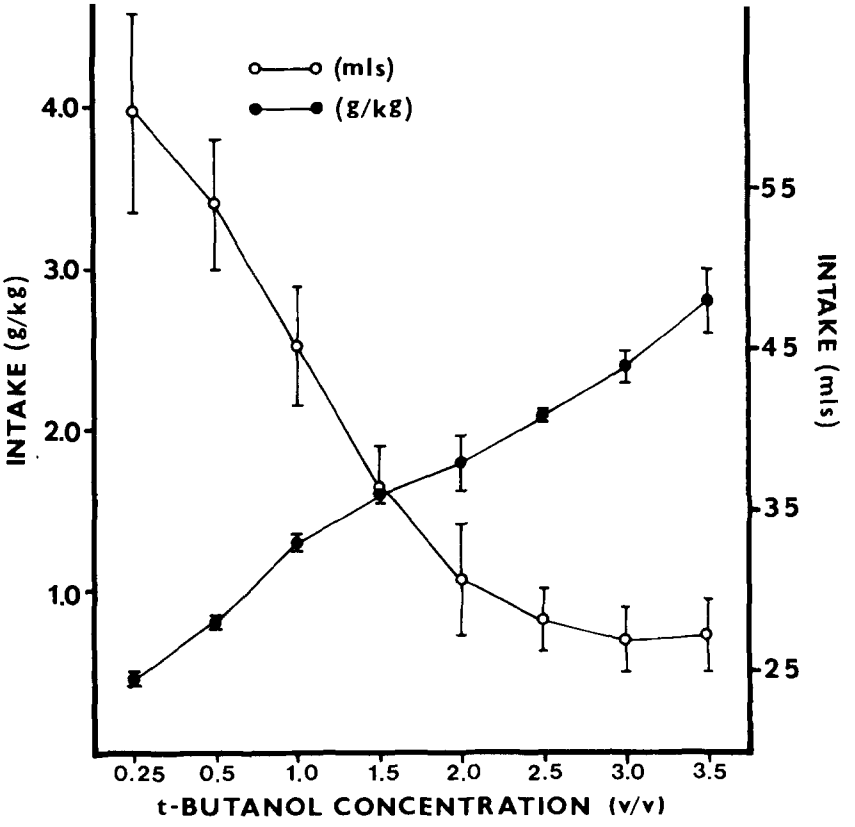

FIG. 1. Relation of concentration of t-butanol to mean (±SEM) daily intakes in the schedule-induction condition (n=4).

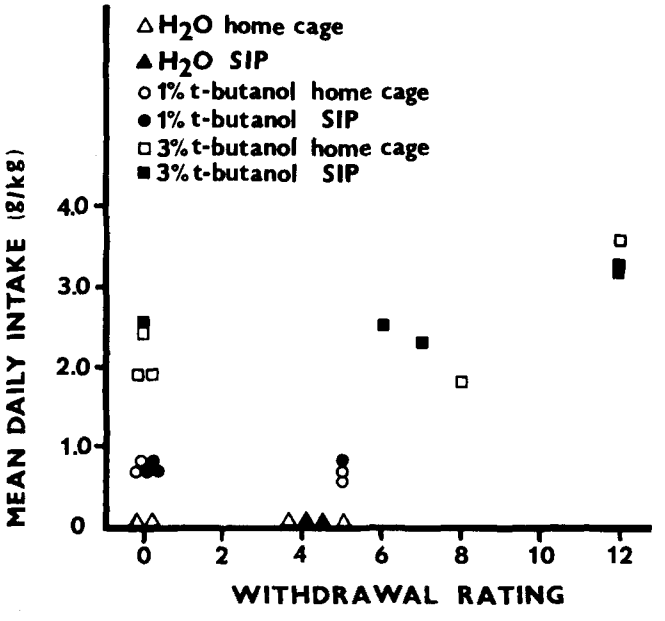

FIG. 2. Relation of mean daily t-butanol intake over the last 20 days prior to withdrawal severity. (See text for withdrawal rating procedure. SIP = schedule-induced condition.)

ence upon t-butanol. The failure of the schedule-induction regime to always produce physical dependence with t-butanol is perhaps due to the failure to induce strong polydipsic overdrinking. One aspect of the schedule-induced drinking paradigm which may underlie the increased frequency of physical dependence is the slightly higher daily g/kg intakes of the schedule-induced condition when compared to the home cage condition. Higher withdrawal scores were significantly related to higher g/kg intakes (Fig. 2).

Another factor that could be related to the increased incidence of withdrawal in the schedule-induced animals is the daily drinking pattern. In previous studies with ethanol [10] it was found that a major factor in the production of ethanol physical dependence was the maintenance of elevated blood levels throughout each 24 hour period. The schedule-induction procedure was capable of producing distributed drinking when six, one hour feeding sessions, each separated by 3 hours, were used. Two daily one hour feeding sessions, 12 hours apart, failed to result in physical dependence even though total daily ethanol intakes were similar to the six feeding regimens. While no data were collected in the present study to determine if the distribution of t-butanol drinking in the home cage was different from the schedule-induction condition, it is probable that there were in fact different intake distributions over each 24 hour period. If the schedule-induced situation spread out the intake over the

total 24 hour period, it might have increased the rate of development of physical dependence. The rate of metabolism for t-butanol is slower than ethanol [15]. Even though large intakes of t-butanol were not generated by the schedule induction procedure, the effects of a more spread out daily intake might have an important effect upon the development of physical dependence, by maintaining higher blood levels throughout the day.

A possible explanation for the lack of strong schedule-induced drinking of t-butanol is the taste factor of t-butanol. As the concentration of t-butanol is raised above 0.5% (v/v) daily fluid intake rapidly drops, reaching a minimum 27 mls/day at 2.0% (Fig. 1). Thus, increasing concentration increased the daily g/kg but decreased total fluid consumption. With ethanol, fluid intake increased with increasing concentrations to peak at 5% [2]. While fluid intake does drop with higher ethanol concentrations, in the schedule-induction condition, animals maintain a constant daily g/kg intake independent of the ethanol concentration [3,9]. This relation was not found for t-butanol within the range tested. The actual relation of t-butanol taste to this intake pattern is unclear, but since intakes of t-butanol continued to decrease to levels necessary for fluid balance as concentration increased and do not change in order to maintain some g/kg level, it would appear that taste may be a major factor. Unlike ethanol [8], observations from a pilot study failed to find any concentration of t-butanol (as low as 0.25% was tested) that was acceptable when water was available concurrently.

All animals drinking 3% t-butanol were visibly intoxicated and frequently in a stuporous state. Many of the animals would periodically lose, for several days at a time, their grooming response, decrease their food intake, and/or self-mutilate (usually in the groin area). If the concentration of the t-butanol solution was increased above 3%, deaths occurred, apparently due to the toxicity of t-butanol. To our knowledge, there previously has been only one reported death due to voluntary ethanol consumption by animals under these conditions [11] and it would appear that the toxic qualities of t-butanol are of a different nature than those of ethanol.

While there may be some similarities between the ethanol and t-butanol schedule-induction situations, there are indications that t-butanol has qualities that are different from ethanol. When 5% ethanol is the fluid available, schedule-induction is a reliable method of inducing physical dependence as measured by several withdrawal symptoms. The studies that induced ethanol drinking for 90 days [2, 3, 11] with the schedule-induction method resulted in withdrawal sensitivities that would be scored from high moderate to severe using the system described in this paper. Clearly, the t-butanol schedule-induced animals in these studies had less severe withdrawal after the same 90 day exposure.

There have been conflicting reports of the withdrawal severity between techniques used to produce physical dependence upon t-butanol. McComb and Goldstein [5] using inhalation with mice found a less intense withdrawal from t-butanol compared to ethanol, even when t-butanol blood concentrations were near lethal. However, Wood and Laverty [16,17] using a liquid diet procedure with rats for 20 days found the withdrawal symptoms of the t-butanol animals to be more severe than ethanol controls. Our data support the finding of McComb and Goldstein [5] with withdrawal from t-butanol being less severe.

While there are similarities of withdrawal with both ethanol and t-butanol exposure, caution must be employed before making any assumptions that the metabolic products of ethanol metabolism are not responsible for the development of physical dependence. There are also a number of differences between the effects of the two alcohols, and while they both result in enhanced CNS sensitivity upon withdrawal, that an identical mechanism is responsible for this sensitivity remains to be determined.

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