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Dependence on Phenobarbital But Not Pentobarbital Using Drug-Adulterated Food

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YUTRZENKA, G. J. AND K. KOSSE. *Dependence on phenobarbital but not pentobarbital using drug-adulterated food.* PHARMACOL BIOCHEM BEHAV **32**(4) 891–895, 1989.—A drug-adulterated food (DAF) method was used in an attempt to establish physical dependence on either pentobarbital or phenobarbital in male CF1 mice. The mice were acclimated to the control diet for four days and then assigned to treatment groups. Group I continued to receive the control diet; Group II received pentobarbital, 5 mg/g food, increased by 5 mg, daily, for 14 days; Group III received pentobarbital, 5 mg/g food, increased by 5 mg, daily, for 7 days; Group IV received pentobarbital, 10 mg/g food, increased by 10 mg, daily, for 7 days; Group V received phenobarbital at 2.5 mg/g food for 5 days and then 3.0 mg/g food for 2 days. During drug administration, all mice were monitored daily for signs of intoxication, change in body weight, and food consumption. At the end of the drug exposure period all mice received control diet and were observed for signs of withdrawal. Mice in Group II and Group IV demonstrated significant declines in body weight and food consumption and an apparent increase in the degree of intoxication but no signs of withdrawal. Group III mice demonstrated little sign of impairment during exposure to pentobarbital and no withdrawal syndrome was observed. Mice presented phenobarbital were found to exhibit a significant degree of intoxication and a withdrawal syndrome was demonstrated. The data suggest, with respect to the drug administration schedules used, that the DAF method was not suitable for the establishment of physical dependence on pentobarbital in mice.

Pentobarbital Phenobarbital Drug-adulterated food

THE drug-adulterated food (DAF) method has been previously utilized for the establishment of physical dependence on several classes of drugs in laboratory rodents (1, 8, 10). Belknap and colleagues (1,2) first reported the use of this method for the chronic administration of the long-acting barbiturate, phenobarbital, to mice. Mice exhibited both a significant degree of intoxication as well as a withdrawal syndrome during a subsequent drug abstinence period. Furthermore, there existed strain differences in sensitivity to the effects of the phenobarbital with DBA/2J mice being more sensitive to the effects than were the C57Bl/6J strain. It was also demonstrated that the severity of the withdrawal syndrome in the DBA/2J strain increased with increasing duration of administration of the DAF diet and that the degree of development of both physical dependence and of functional tolerance were reduced when a discontinuous schedule (incorporating one or two drug "holidays," each of 24 hours duration) of phenobarbital administration was utilized.

Other investigators have extended the use of the method for the chronic administration of barbiturates, benzodiazepines, and opiates to rats (8–10). Physical dependence to these classes of drugs was demonstrated following drug exposure of from 1 week to up to 40 days. Rats administered barbiturates were noted to exhibit impaired locomotor activity and sedation along with evidence of tolerance to these effects. Withdrawal signs included loss of body weight, decreased food intake, increased irritability, ataxia and convulsive activity. Most withdrawal signs could be reversed following readministration of the drug-adulterated diet. Martin and colleagues (6) also presented evidence for the ability to produce dependence on pentobarbital, in rats, using the DAF techniques. The subsequent withdrawal syndrome was rapid in onset and of a relatively short duration (approximately 10 hours). Problems with overdosage and a lack of palatability of the diet were noted.

There is currently no published data which indicate the successful use of the DAF technique for the administration of the short-acting barbiturate, pentobarbital, to mice. This technique could be advantageous in that there is a relative ease of administration of drug, elimination of multiple drug injections and, presumably, a lesser degree of handling-induced stress experienced by the animals. The advantage of using pentobarbital is that the withdrawal syndrome is typically rapid in onset with a relatively short duration which may be of benefit in the laboratory setting.

METHOD

Male, CF1 mice (Sasco, Omaha, NE) weighing 25–30 g at the start of the study were housed individually in wire cages with food and water available ad lib. All mice were maintained under a 12 hour on:12 hour off light cycle (on at 7:00 a.m.) and were allowed to acclimate to the colony for at least 7 days prior to the start of the study.

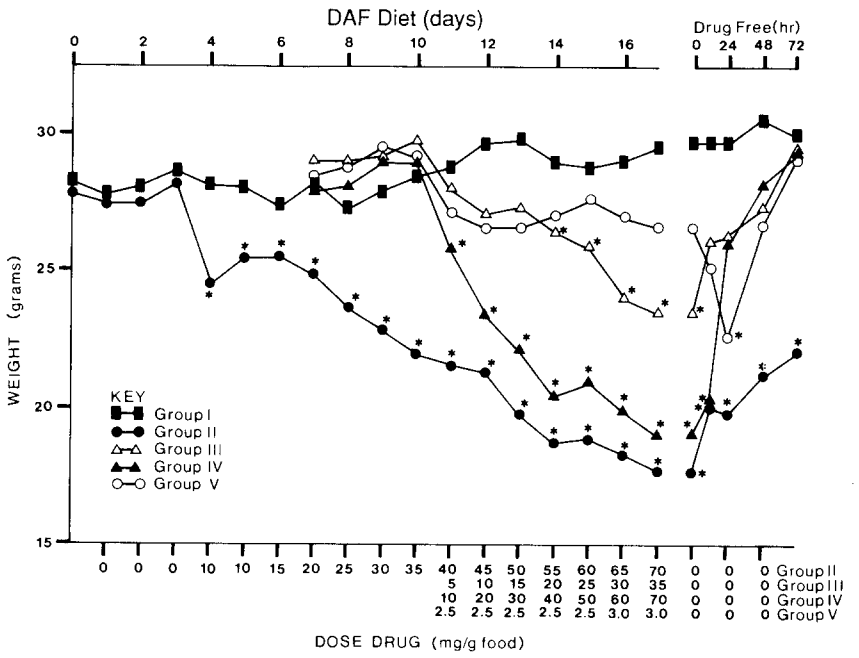

FIG. 1. Body weight of mice during the exposure to either pentobarbital sodium or phenobarbital sodium as well as during a subsequent drug-free period. Each point represents the mean body weight of 4–10 mice. Standard error bars omitted for clarity. *Significantly different from control mice (Group I) at $p<0.01$.

Mice were fed a milled food diet (Purina Lab Chow, Ralston Purina Co., St. Louis, MO) which was dispensed from a glass jar (48 × 55 mm) fitted with a plastic screw cap which had a 21 mm diameter hole drilled in the center (1). Twenty grams of the milled diet were placed into each feeding jar and fresh diet was presented daily. Each jar was placed in a plastic weigh boat to collect spillage.

Mice were randomly assigned to one of five treatment groups with 10 mice/group. All mice received the control milled diet for four days prior to administration of the drug-adulterated diet. Mice in Group I continued to receive the control diet throughout the study. Group II mice received pentobarbital sodium, at an initial concentration of 5 mg/g food, the concentration of which was increased, daily, by 5 mg to a maximum concentration of 70 mg/g food. Likewise, Group III received pentobarbital sodium in an initial concentration of 5 mg/g food, with the concentration increased daily by 5 mg until a final concentration of 35 mg/g food was attained. Group IV received pentobarbital sodium at an initial concentration of 10 mg/g food which was increased daily by 10 mg until a final concentration of 70 mg/g food was reached. Finally, Group V received phenobarbital sodium at 2.5 mg/g food for 5 days followed by 3.0 mg/g food for an additional two days (3). Immediately following the final day of drug administration, control diet was again offered and a drug abstinence period was instituted. Drug dosing schedules were arranged such that all treatment groups entered the drug abstinence period on the same day of the study period.

Each day, at 9:00 a.m., mice were weighed, fresh diet was presented and the amount of food consumed during the preceding 24 hours was determined. In addition, during the drug administration period, mice were observed, twice daily at 9:00 a.m. and again at 5:00 p.m., for signs of drug-induced intoxication. Intoxication and impairment of motor function was determined by subjecting each mouse to a battery of tests which included: gross observation of motor impairment (1); horizontal rod test (1); and the inverted screen test (4).

Gross observation of motor impairment was carried out by observing each mouse in the home cage for 30 seconds and assigning a score based on the following criteria: 0 = no gross signs of intoxication; 1 = impaired gait and pronounced staggering; 2 = falling onto side or back; 3 = impaired righting reflex (longer than 2 seconds). The horizontal rod test assessed the ability of the mouse to hang suspended by its front feet from a wooden dowel (7 mm diameter) positioned, horizontally, 25 cm above the bedding covered floor of an opaque plastic cage. The length of time the mouse remained suspended was subtracted from 10 (the maximum time in seconds). A score of 10 was assigned if the mice failed to hold themselves on the dowel at all, thus higher scores represent a greater degree of impairment. Finally, the mouse was tested on the inverted screen. In this test the mouse is placed on a horizontally positioned screen (13 × 13 cm; 6 mm mesh) and the screen is then inverted. The task is for the mouse to climb to the upper side of the screen within a 60-second time period with the time to accomplish this task being noted for each mouse. Either failure to climb to the upper side of the screen, or falling from the screen is scored as a 60. Thus, a higher score is indicative of a greater degree of impairment.

During drug abstinence, body weight was determined at the start of the period and at 12, 24, 48 and 72 hours while food consumption was determined at 24, 48 and 72 hours of this period. A withdrawal score was assigned to each mouse using a procedure previously described (1). Each mouse was suspended by its tail for 10 seconds, returned to its cage and observed for one minute. Scores were assigned as follows: 0 = no effect; 1 = presence of one or more of the following; marked tremor, pronounced "jumpiness" when touched, Straub tail; 2 = wild running and/or convulsions. The severity of the withdrawal syndrome was assessed every four hours for the phenobarbital group and hourly for the

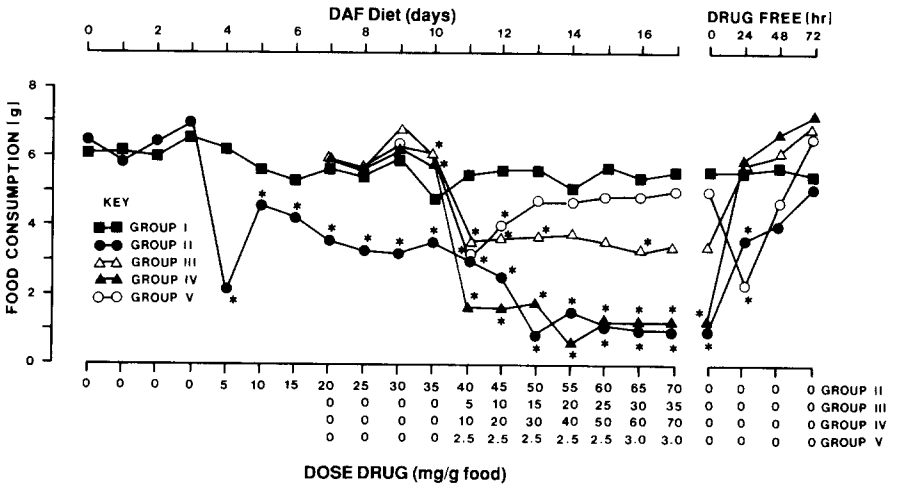

FIG. 2. Food consumption of mice during the exposure to either pentobarbital sodium or phenobarbital sodium and during the subsequent drug-free period. Each point represents the mean body weight of 4–10 mice. Standard error bars are omitted for clarity. *Significantly different from control mice (Group I) at $p<0.01$.

first 12 hours and at 24, 36, 48 and 72 hours for the groups receiving pentobarbital.

Body weight and food consumption data obtained from drug-treated mice were tested for significant difference from control mice by analysis of variance (ANOVA) with post hoc analysis using Scheffe's multiple comparisons test (7). Intoxication and withdrawal scores were analyzed for significant differences by use of the two-tailed Mann-Whitney U-test (5).

RESULTS

Mice in all groups maintained both body weight (Fig. 1) and food consumption (Fig. 2) during the four-day acclimation period. In general, control mice maintained their body weight at around 30 grams and consumed about 6 grams of food per day throughout the course of the study. Mice in Group II and Group IV demonstrated dramatic declines in body weight and food consumption as the concentration of pentobarbital in the food was increased. Mice in Group III and Group V experienced a more modest effect on both body weight and food consumption as the drug administration period progressed. Mortality in Groups II and IV was noted to increase with increasing concentration of drug and reached 60% mortality in both groups by the end of the drug administration period.

Based on the dose of pentobarbital offered and on the estimate of daily food consumption, the daily ingestion of pentobarbital was noted to be similar for Group II and III. There was noted a steadily increased consumption of pentobarbital for several days (from initial values of about 20 mg per day consumed to a peak of 130 mg consumed per day) after which the mice in Group II showed a marked reduction in pentobarbital ingestion (approximately 40 mg pentobarbital consumed per day by day 17). Estimates of pentobarbital consumption in Group IV showed that the total amount consumed was lower than in the other groups receiving pentobarbital and ranged from approximately 20 mg consumed initially to a peak of 60 mg pentobarbital consumed per 24 hours. Mice offered phenobarbital maintained a constant low level of phenobarbital consumption of approximately 10 mg of phenobarbital consumed per day, throughout the exposure period.

An assessment of the degree of intoxication and impaired motor coordination was conducted on all mice using a battery of tests. As measured by estimation of mean intoxication scores, mice in Groups II and IV demonstrated an increasing degree of gross intoxication and incoordination with continuous exposure to increasing concentrations of pentobarbital (Fig. 3). This was especially evident when pentobarbital was present in concentrations greater than 40 mg/g food. A general debility due to a significant reduction in food consumption may also play a role in the degree of impairment noted. Group III mice failed to demonstrate any significant degree of impairment. Mice exposed to phenobarbital showed a significant degree of intoxication and impairment upon initial exposure to the drug. This was followed by a lessening of impairment until phenobarbital concentration was increased to 3.0 mg/g food, at which time a significant degree of intoxication was again apparent (Fig. 3). Testing of the mice on the horizontal rod test and the inverted screen test provided results which were similar to that provided by estimation of gross intoxication (data not shown).

Following the final day of drug exposure, the drug-adulterated diet was replaced with control diet and a drug-free period commenced. Only the mice exposed to phenobarbital were observed to exhibit the typical signs of withdrawal from barbiturates. This was characterized by a significant decrease in both body weight (Fig. 1) and food consumption (Fig. 2) at 24 hours of this period along with significantly elevated withdrawal scores occurring at 20 hours and 32 hours following the start of the withdrawal period (Fig. 4).

DISCUSSION

The previous demonstration of the successful use of the drug-adulterated food method for the production of physical dependence on long-acting barbiturates (1,2) prompted this investigation utilizing the short-acting barbiturate, pentobarbital. Use of pentobarbital may be advantageous in dependence studies since induction of physical dependence may occur more quickly while the withdrawal syndrome is often more intense and has a shorter latency to onset when compared to use of long-acting barbiturates.

In the current investigation several drug administration sched-

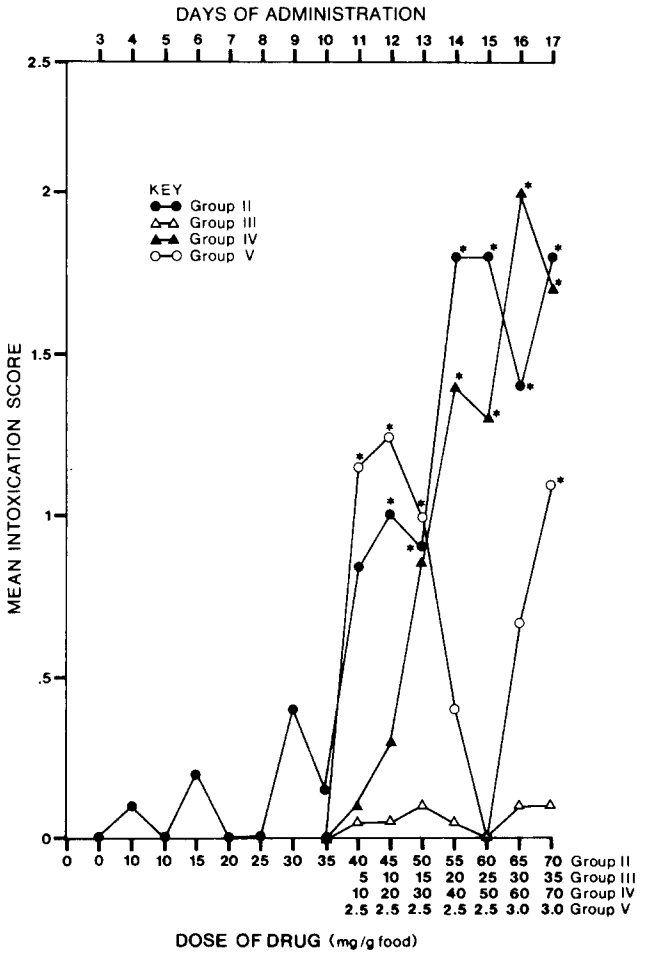

FIG. 3. Intoxication scores of mice exposed to either pentobarbital sodium or phenobarbital sodium in the diet. Each point represents the mean of 4–10 mice. Control mice (Group I) exhibited no sign of intoxication. Standard error bars are omitted for clarity. *Significantly different from control at $p<0.01$.

ules were employed in an attempt to induce dependence on barbiturates. Three drug administration schedules involved exposure of mice to a daily increasing concentration of pentobarbital in the diet. Group III mice, which received pentobarbital at an initial concentration of 5 mg/g food and which was subsequently increased to a final dose of 35 mg/g food after 7 days, were found to exhibit no significant signs of either intoxication or dependence on pentobarbital. Mice in Group II and Group IV, which were exposed to pentobarbital in increasing concentration for either 14 or 7 days, respectively, showed indications of intoxication during the drug administration phase. However, during a subsequent drug-free period there was no indication of withdrawal. In addition, it was evident that the palatability of the diet may have been a factor of some importance as both food consumption (Fig. 2) and body weight (Fig. 1) declined significantly as the concentration of pentobarbital was increased. Mortality was noted to be 60 percent in each of these two groups. Therefore, it would appear that the mice in Groups II and IV were, in fact, not dependent on pentobarbital but instead may have been severely debilitated by these schedules of drug administration. Thus, a general debility of the animals coupled with possibly toxic concentrations of pentobarbital seemingly limits the usefulness of these particular drug administration schedules.

On the other hand, phenobarbital administration for 7 days resulted in both a significant degree of intoxication (Fig. 3) as well as the demonstration of a characteristic withdrawal syndrome (Fig. 4). The intensity and pattern of the withdrawal syndrome was similar to that previously reported (1).

Several factors may play a role in the results noted. Among these are that the short duration of action of pentobarbital may not provide a consistent level of drug in the central nervous system (CNS). This is especially important since it has been estimated that from 60% to 90% of food intake may occur during the lights "off" part of the cycle (2). Thus, there may be several hours of the day during which mice are not feeding and are therefore, presumably, not maintaining a constant level of the drug in the CNS. In addition, the decreased palatability of the diet and a probable induction of the hepatic metabolism of pentobarbital may also tend to inhibit the consistent maintenance of sufficient levels of pentobarbital in the CNS for a sufficient duration of time. All of these factors then may underlie the lack of establishment of physical dependence on pentobarbital.

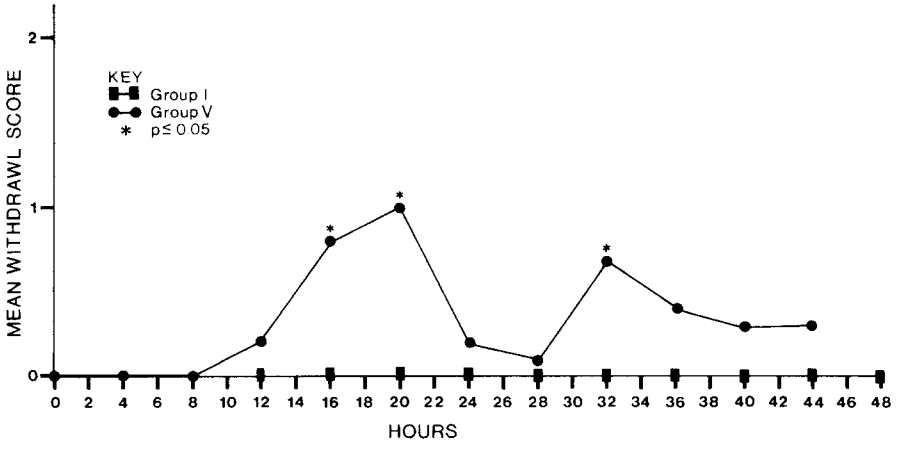

FIG. 4. Withdrawal score for mice exposed to phenobarbital sodium in the diet. Each point represents the mean withdrawal score of 10 mice. *Significantly different from control at $p<0.05$.

In conclusion, this study failed to demonstrate the establishment of dependence on the short-acting barbiturate, pentobarbital, using the drug-adulterated food method. On the other hand, dependence on phenobarbital, a barbiturate with a longer duration of action, was established and did confirm the observations of previous investigators.

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