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Intensity of the Withdrawal Syndrome Varies With Duration of Pentobarbital Administration

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YUTRZENKA, G J *Intensity of the withdrawal syndrome varies with duration of pentobarbital administration* PHARMACOL BIOCHEM BEHAV **34**(1) 49–51, 1989 —The effect of three dosage schedules on the expression of a withdrawal syndrome indicative of physical dependence on pentobarbital was determined in male Sprague-Dawley rats Rats were prepared with an intraperitoneal cannula and were continuously infused with either saline (control) or pentobarbital sodium, using an escalating drug dosage schedule, for either 5 (PB-5), 13 (PB-13) or 20 (PB-20) days Final doses reached were 500 mg/kg/day (PB-5) and 1000 mg/kg/day (PB-13) PB-20 rats reached 1000 mg/kg/day on day 13 and were maintained at this dose for an additional 7 days Body weight, water consumption and assessment of CNS depression were obtained daily Following the last day of pentobarbital infusion all rats were infused with saline for a 72-hour drug-free period Water consumption, body weight and assessment of overt behavioral signs indicative of a drug withdrawal syndrome were obtained at specific times during the drug-free period PB-5 rats showed little evidence of withdrawal while PB-20 rats demonstrated the greatest degree of withdrawal Peak withdrawal scores were observed to be 1, 3 8 and 5 for PB-5, PB-13, and PB-20, respectively Withdrawal scores for group PB-13 and PB-20 were found to be significantly greater than either control or PB-5 but were not significantly different from each other Body weight for PB-13 and PB-20 mice declined slightly (nonsignificant) during the drug-free period while a significant decrease (40% decline) in water consumption was demonstrated by 24 hours of this period Taken together, this data indicates an increased degree of severity of the withdrawal syndrome as a result of both an increased dose of pentobarbital as well as an increased duration of exposure to pentobarbital

Pentobarbital Withdrawal syndrome Physical dependence Rat

THERE is an increased interest in defining the various mechanisms which may underlie the establishment of physical dependence on central nervous system (CNS) depressant agents. To this end, several models of physical dependence on CNS depressants have been described Typically these models utilize one of several methods for the chronic administration of the drug including incorporation of drug into the diet (1,2) or drinking water (3); implantation of either drug-impregnated pellets (5) or osmotic minipumps (11), or the use of multiple daily administration techniques (4, 6, 8, 9). Recently, this laboratory has reported on a continuous intraperitoneal infusion technique for the chronic administration of pentobarbital to rats (14). This model utilizes an escalating dosage schedule and reliably produces physical dependence on pentobarbital as evidenced by the occurrence of a withdrawal syndrome following cessation of drug administration

The current study is designed to further define this model and offer insight into the relative roles of dosage and duration of exposure in the production of physical dependence on pentobarbital.

METHOD

Male, Sprague-Dawley rats (Sasco, Omaha, NE) weighing 150–175 g at the start of the experiment were individually housed in stainless steel cages with ad lib access to food and water All rats were maintained under a 12 hour on/off light cycle with lights on at 7 00 a m. All rats were acclimated to the colony for at least five days prior to the start of the study

Rats were surgically prepared with a PE-90 polyethylene intraperitoneal cannula under halothane/nitrous oxide anesthesia using a previously described procedure (14) Following surgery, rats were returned to their home cage and allowed three days of recovery before being placed in an infusion harness and attached to the infusion apparatus Rats were acclimated to the infusion system for an additional three days during which time they received a constant infusion of 0 9% saline The apparatus and drug dosing protocol have been previously described (14)

At the start of each study rats were randomly assigned to either a saline control group (9 rats/group) or to a pentobarbital treatment group (12 rats/group) Control rats were infused concurrently with pentobarbital-infused rats. In Study 1, rats received a continuous infusion of pentobarbital sodium for five consecutive days (PB-5) beginning at a dose of 100 mg/kg/day which was increased, daily, by 100 mg/kg to a final dose of 500 mg/kg/day In Study 2, rats were infused with pentobarbital for 13 days (PB-13) beginning at 100 mg/kg/day and reaching a final dose of 1000 mg/kg/day Similarly, in Study 3, rats were infused with pentobarbital so as to reach 1000 mg/kg/day after 13 days and were then maintained at this dosage for an additional 7 days (PB-20) In all studies rats received 9 ml of infusate per day All infused solutions were filtered through 0 2 μm Metricel® membrane filters (Gelman Science Inc) prior to infusion into the rats

TABLE 1

RATING SCALE FOR ASSESSMENT OF WITHDRAWAL SIGNS*

A) "High" posture
   0—No "high" posture evident
   1—"High" posture evident (standing on all four feet, back arched, with or without piloerection)
B) Response to prodding (flank)
   0—No response
   1—Vocalizes
C) Response to air puff (head)
   0—No response
   1—Jumps
   2—Jumps and vocalizes
D) Response to being grasped and held
   0—No response
   1—Struggle *or* vocalizes
   2—Struggle *and* vocalizes
   3—Struggle, vocalizes, claws and/or bites

*Rats are assessed on each parameter in the order indicated Scores for each parameter are added together and a total score (maximum = 7) obtained for each rat

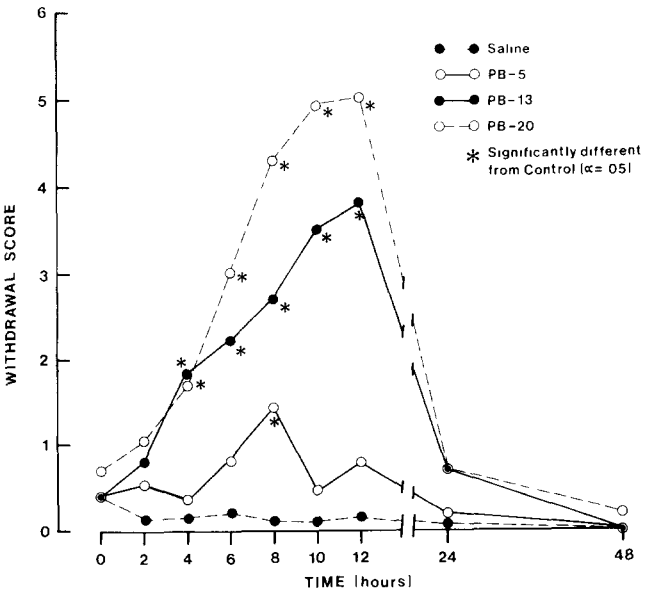

FIG 1 Mean withdrawal score for control and pentobarbital-treated rats Control groups were assessed concurrently with drug-treated groups There was no significant difference between control groups and thus all control values were combined In the pentobarbital treatment groups, n = 12 for PB-5, n = 9 for PB-13 and n = 7 for PB-20 The combined control group represented 27 rats

During each study the body weight, 24-hour water consumption and the degree of CNS depression were determined at 9 00 a m daily The degree of CNS depression exhibited by each rat was evaluated following observation of the rat for approximately one minute and was determined by use of a CNS depression rating scale described previously (14)

Following the final day of drug infusion, rats were subjected to a 72-hour drug-free period during which all rats received a continuous infusion of 0 9% saline Body weight and water consumption was determined at the start of this period and at 24, 48 and 72 hours In addition, each rat was observed for signs indicative of a drug withdrawal syndrome (Table 1) Observation of each rat was carried out at the beginning of the drug-free period and every 2 hours for the first 12 hours and at 24 and 48 hours All observations were conducted, with the rat in its home cage, by a single trained observer who was blind to the treatment each rat had received Rats were observed only once in each observation period A withdrawal score was assigned to each rat based on the degree of expression of this overt behavior No attempt was made to observe convulsive activity during the withdrawal period

Changes in body weight and water consumption between drug-treated and concurrently-infused control rats was tested for significant difference by use of analysis of variance (10) Withdrawal scores were analyzed for significant differences using the Mann-Whitney U-test (7)

RESULTS

The continuous administration of pentobarbital sodium to rats for either 13 or 20 days resulted in the establishment of physical dependence This was evidenced by observation of changes in overt behavior (Fig 1), body weight and water consumption (Fig 2) which, when taken together, are indicative of a drug withdrawal syndrome

Rats in groups PB-13 and PB-20 demonstrated a rapid increase in withdrawal scores with a peak score observed at 10–12 hours of this period (Fig 1) The withdrawal scores were observed to return to control values by 24 hours of the drug-free period While the withdrawal scores of rats in groups PB-13 and PB-20 were not significantly different from each other at any time point, these scores were significantly increased over the scores obtained for PB-5 rats following the fourth hour of the drug-free period

Neither body weight nor water consumption of pentobarbital-infused rats was significantly different from concurrently infused saline control rats during the drug infusion phase of the experiment (data not shown) During the drug-free period body weight tended to be slightly, but not significantly, decreased in PB-13 and PB-20 rats (Fig 2B,C) On the other hand, water consumption was significantly decreased at 24 hours of the drug-free period in groups PB-13 and PB-20 (Fig 2E,F) While water consumption in PB-13 rats returned to control levels by 48 hours, water consumption in PB-20 rats was still depressed at 72 hours of the drug-free period

On the other hand, rats receiving pentobarbital for 5 days demonstrated little evidence of a withdrawal syndrome While there was no change, relative to control rats, in either body weight (Fig 2A) or water consumption (Fig 2D), during the drug-free period there was a slight increase in mean withdrawal scores with a peak occurring at about 8 hours of this period (Fig 1)

With the drug dosage schedules used there was no mortality attributed to overdosage with pentobarbital However, it was necessary to remove 3 rats from the PB-13 group and 5 pentobarbital rats from the PB-20 group due to mechanical failure of the infusion system The data collected from these rats was eliminated from any further analysis

DISCUSSION

This study was designed to further characterize the time-course for the establishment of physical dependence associated with the chronic administration of pentobarbital by a continuous infusion method Consistent with a previously expressed view that physical dependence may ensue when the CNS is influenced by a sufficient level of drug for a sufficient period of time (9), the current study indicates that an increased duration of exposure to pentobarbital, once an adequate dosage has been obtained, may result in an increased intensity of the subsequent withdrawal syndrome This was especially borne out by the increased withdrawal scores noted

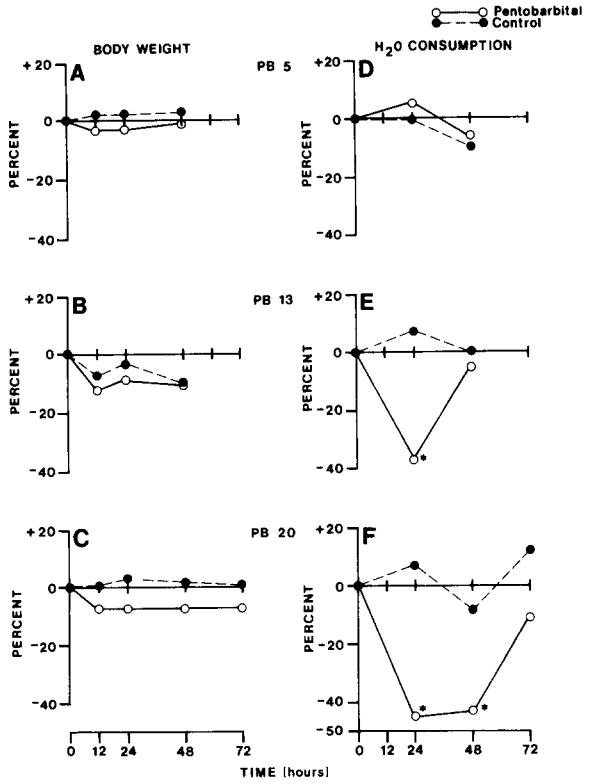

FIG 2 Percent change in body weight (A, B, C) and percent change in water consumption (D, E, F) for rats treated with pentobarbital for 5 (PB-5), 13 (PB-13) or 20 days (PB-20) *Significantly different from corresponding control value at $p \leq 0.01$ In the pentobarbital treatment groups, n = 12 for PB-5, n = 9 for PB-13 and n = 7 for PB-20 For the control groups n = 9

for PB-20 rats along with the increased duration of the hypodipsic response noted in this group of rats On the other hand, it was quite evident that exposure to pentobarbital for only 5 days, and thus reaching a maximum dose of only 500 mg/kg/day, resulted in the lack of establishment of physical dependence at least with respect to the methods used to assess the presence of physical dependence

The relative contribution of drug dosage and duration of exposure to barbiturates has been reported in previous investigations It has been noted that, following three weeks of barbital exposure to doses of either 300 mg/kg/day, 400 mg/kg/day or 500 mg/kg/day, rats exhibited a dose-dependent increase in the incidence of occurrence of withdrawal signs (12,13) It was also noted that increased duration of exposure to phenobarbital, especially at the higher doses, was associated with an increased incidence and severity of the withdrawal signs (13) This phenomena was also observed in mice continuously exposed to phenobarbital at a dose of 2 5 mg/g food for either 3, 6 or 9 days (2) This was evidenced by increased incidence and severity of the withdrawal signs as a function of increased duration of exposure It was also appreciated that the time course for development of functional tolerance correlated highly with the development of physical dependence. Finally, with the use of a chronically equivalent, maximally tolerable dosing schedule it was determined that rats exhibited a withdrawal syndrome of greater intensity following 35 days of phenobarbital exposure as compared to 10 days of exposure (4)

The results of the current study also implicate the relative roles of both dose and duration of exposure in the establishment of physical dependence on pentobarbital It was evident that once a sufficient level of drug has been delivered and is maintained within the CNS, increased duration of exposure of the CNS to that level of drug may lead to an increased severity of the withdrawal syndrome

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