Sex Differences in the Induction of Physical Dependence on Pentobarbital in the Rat

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Abstract—Sex differences in physical dependence on sodium pentobarbital in the rat were studied by the drug-admixed food (DAF) method. With male rats, the concentration of pentobarbital in the food was gradually increased from 2 to 30 mg/g over a period of 50 days. The final level of drug intake was approximately 1.7 g/kg/day. At pentobarbital concentrations of 20 and 22 mg/g of food, sedation and mild muscle relaxation were observed. At the highest drug concentration, 30 mg/g of food, marked muscle relaxation was noted. With female rats, the concentration of pentobarbital in the food was gradually increased from 1 to 16 mg/g over a period of 47 days. The final level of intake was approximately 1.0 mg/kg/day. At drug concentrations of 12 and 14 mg/g, sedation and mild muscle relaxation appeared. At 16 mg/g, female rats showed marked muscle relaxation similar to that of the male rats. To produce severe loss of muscle tone, the male rats required twice as much pentobarbital as the female rats. After substitution of normal food for the pentobarbital-admixed food, various signs of pentobarbital withdrawal occurred in both sexes. These signs included vocalization, irritability, muscle rigidity, tremors and convulsions. Onset of withdrawal was more rapid in the females, and the maximum weight loss was greater, 8.0% compared to 3.8% in the males. Physical dependence on pentobarbital was easily developed in both sexes by the DAF method. There was a marked sex difference in withdrawal which we attribute to sex differences in drug metabolizing enzyme activity.

Physical dependence on barbiturates has been reported in several species of laboratory animals (1). Barbital is the agent most frequently used to produce physical dependence, and Clossland and Leonard (2) were the first to show that withdrawal from barbital can result in convulsions in rats. Evidence for physical dependence on pentobarbital has been established in monkeys, dogs and cats (3–6). However, until recently, severe physical dependence on pentobarbital as manifested by withdrawal convulsions had not been shown in rodents. A high degree of tolerance to pentobarbital can be rapidly established by continuous exposure to the drug by means of pellet implantation (7, 8). Moreover, Ho et al. (8) reported that in mice the threshold for pentylenetetrazol-induced seizures, used by Jaffe and Sharpless (9) as the index for pentobarbital physical dependence in cats, was significantly reduced after pellet removal.

Recently, Martin et al. (10) demonstrated that after chronic treatment with pentobarbital, female rats occasionally showed spontaneous grand mal convulsions when drug administration was terminated. The rats had been given pentobarbital intragastrically four times daily, and they received pentobarbital-admixed food.

In the present study, we examined sex differences in physical dependence on pentobarbital. The drug-admixed food (DAF) procedure was used since it reliably produces severe physical dependence that is characterized by frequent spontaneous convulsions during withdrawal.
Materials and Methods

Male and female Sprague-Dawley rats that weighed 150–200 g at the beginning of the experiments were used. They were housed in individual cages in a room with artificial lighting on a 12/12 hr lighting cycle. The room temperature was maintained at 22±1 °C, and the relative humidity was maintained at 55±5%.

For preparing the drug-admixed food (11), sodium pentobarbital (Tokyokasei Kogyo, Tokyo) was mixed with a normal powder food (CA-1, Japan Clea, Tokyo) in a mortar. Each rat was allowed to eat the pentobarbital-admixed food and to drink tap water ad libitum. The concentration of pentobarbital in food was increased gradually, as shown in Fig. 1. The body weights of rats and food consumption were measured daily at 2:30 p.m. The daily pentobarbital intake was calculated as follows:

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\text{pentobarbital intake} = \frac{\text{food intake} \times \text{concentration} (\text{mg/g food})}{\text{body weight} (\text{kg})} \times (\text{mg/day})
\]

Motor incoordination in pentobarbital-treated rats was measured by the rotarod test (9 cm in diameter, 5.3 rpm, 5 min, as standards: Natsume Seisakusho Co., Tokyo). The rotarod test was carried out at intervals of 2–3 days during the treatment.

Withdrawal was performed by substituting normal food for pentobarbital-admixed food on the last day of the treatment at 2:00 p.m. Body weight and food intake were measured every 3 hr after withdrawal. After termination of drug treatment, the male rats were continuously observed over an 18 hr interval that began 6 hr after the end of drug treatment. Female rats were observed over a 15 hr interval that began 3 hr after the end of drug treatment. All instances of abnormal behavior were recorded.

Results

Pentobarbital intake during treatment is shown in Fig. 2. Food consumption remained relatively constant throughout the experiment. During the treatment of each pentobarbital concentration, daily pentobarbital intake (mg/kg/day), which was calculated from the value of food consumption, slightly decreased as body weight increased. The range of pentobarbital intake of male and female rats was 206.7–1725.1 mg/kg/day and 86.9–990.3 mg/kg/day, respectively. The final drug intake in male rats was approximately 1.7 fold greater than that in female rats.

Figure 3 presents the alterations in rotarod test performance as a function of the graded increases in pentobarbital concentration in

![Fig. 1. Gradedly increased dosage schedule of pentobarbital sodium in male and female rats.](image)
Fig. 2. Alterations in mean daily pentobarbital intake (mg/kg/day) during pentobarbital-admixed food medication on gradedly increased dosage schedule in male and female rats.

Fig. 3. Circadian alterations in motor coordination during pentobarbital-admixed food medication in male and female rats. Motor coordination measured by the rotarod test.

the drug-admixed food. Decreases in rotarod test performance in the males were observed after treatment with pentobarbital concentrations of 20 and 22 mg/g of food. Similar decreases in the test performance of the females were observed at pentobarbital concentrations of 10 and 12 mg/g of food. In both sexes, mean rotarod performance at the final pentobarbital concentration was about 100 sec. There was a close relation between the results of the rotarod test and the appearance of altered behavior in the rats. Male rats showed sedation and mild muscle relaxation at pentobarbital concentrations of
20 and 22 mg/g of food. Female rats showed similar behavioral changes at pentobarbital concentrations of 12 and 14 mg/g of food. At the highest concentrations tested, rats showed marked muscle relaxation and ataxia.

Access to pentobarbital-admixed food was terminated at 2:00 p.m. Withdrawal signs were observed 6 to 33 hr later in the males and 3 to 30 hr later in the females. These signs included vocalization, irritability, aggression, muscle rigidity, Straub's tail, ear-twitching, tremors, and convulsions (Table 1). The signs appeared more rapidly and with greater frequency in the females. Figure 4 shows hourly changes in the incidence of convulsions. Convulsions appeared frequently from 14 to 17 hr in the males and from 5 to 8 hr in the females. Convulsions also appeared sooner and more often in the females (Table 1). Moreover, the maximum weight losses in the male and female rats were 3.8% at 12 hr and 8.0% at 21 hr, respectively (Fig. 5).

**Discussion**

The severity of physical dependence has been estimated by grading the withdrawal reaction. Yanagita and Takahashi (3) estimated the degree of physical dependence on barbiturates by rating withdrawal signs in rhesus monkeys. Boisse and Okamoto (12) assessed the severity of pentobarbital and barbital withdrawal in cats by counting the number of grand mal convulsions and by rating 20 additional motor, autonomic and behavioral signs, including tremors, twitches, myoclonic jerks, postural disturbances and motor incoordination. In these two studies, the number of animals with severe physical dependence was defined by the number of animals that manifested withdrawal convulsions. In a third study, spontaneous grand
Mal convulsions occurred in female rats after withdrawal of pentobarbital (10). The pentobarbital had been administered by the drug-admixed food method. However, no experimental details were given.

In the present study, severe physical dependence on pentobarbital was established using the drug-admixed food method (Fig. 1). The pentobarbital concentration in the food was increased every third day. The magnitude of CNS depression was evaluated by the rotarod performance test (Fig. 3). After the appearance of CNS depression, the concentration of pentobarbital continued to be increased since the rats did not show signs of toxicity such as loss of body weight and decrease in food consumption. The final concentrations were 30 mg/g of food for the males and 16 mg/g of food for the females. Both sexes were treated with the final concentration for 10 days in order to minimize the variation in subsequent withdrawal signs. The degree of physical dependence on barbiturates is a direct function of the magnitude of CNS depression and its duration (13). In the present study, the degree of CNS depression was approximately the same in both sexes. This equivalent degree of depression was important since the aim of the study was to examine sex differences in physical dependence on pentobarbital. After the termination of chronic drug treatment, the rats manifested frequent spontaneous convulsions. Thus, we were able to show that in both sexes, severe physical dependence on pentobarbital was easily developed by the drug-admixed food method.

Physical dependence on barbital can be produced in rats by the drug-admixed food method (13). In an earlier study, the concentration of barbital in the food was gradually increased from initial levels of 0.5 and 1 mg/g of food to the final levels of 6 and 8 mg/g over 36 days. Barbital intake at the highest concentration was approximately 410 mg/kg/day. In the present study, the development of physical dependence required higher levels of intake, specifically 1.7 g/kg/day, than in the former study. Thus, greater drug intake was needed to produce dependence on pentobarbital than to produce dependence on barbital. Boisse and Okamoto (12) have suggested that differences between barbital and pentobarbital in withdrawal syndromes can be best understood in terms of the slower rate of elimination of barbital compared to pentobarbital. The barbital withdrawal syndrome relative to the pentobarbital syndrome is later in onset, slower and later to peak, longer at peak, and longer in total duration. Barburate withdrawal signs may be considered to be inversely related to the residual blood barbiturate concentration.
These differences between physical dependence on pentobarbital and barbital may be due to pharmacokinetic differences.

Aston (14) reported that there were sex differences in the development of acute tolerance to pentobarbital in adult rats. Male rats appeared to develop tolerance more rapidly than the female rats, although the ultimate degree of tolerance was greater in the females than in the males. Our results show that female rats develop more severe dependence than male rats. During drug treatment, male rats were given higher concentrations of pentobarbital. Pentobarbital intake in male and female rats at the highest drug concentrations was approximately 1.7 g/kg/day and 1.0 g/kg/day, respectively. For the development of severe physical dependence on pentobarbital, the males required about twice the drug intake as the females. After the end of drug treatment, both male and female rats showed weight loss. The mean weight loss in the males and females was 4.5% and 8.0%, respectively. The most important withdrawal sign, convulsions, appeared earlier in the females and were more frequent than in the males (Fig. 4). The metabolic activity of pentobarbital was higher in the males than in the females (15), although the appearance of convulsions was earlier in the females (Fig. 4). Pentobarbital intake in females at the highest drug concentrations was less than in the males (Fig. 2). As is expected, the brain and blood pentobarbital levels of female rats is lower than male rats. After withdrawal, the levels in the females decline rapidly. We considered that the difference in brain and blood pentobarbital levels caused early occurrence of the withdrawal signs in the females. Thus, in rats we found a marked sex difference in physical dependence on pentobarbital.

Kato et al. (15) demonstrated that sex differences in the pharmacological effects of pentobarbital, carisoprodol, strychnine and octamethylylophosphoramide were due to different metabolic activities in male and female rats. Furthermore, it has been reported that sex-related differences in drug metabolizing activities in liver microsomes are due to multiple forms of cytochrome P-450 (16). Similarly, the sex differences in the development of physical dependence on pentobarbital seems to be due to sex differences in drug metabolizing enzyme activity.

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