SEX AND AGE DIFFERENCES IN THE EFFECTS OF BARBITURATES

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It was reported that there was a remarkable difference in the effects of pentobarbital (1) or hexobarbital (2) between adult female and male rats and that there was a sex difference in the metabolism of these drugs (1, 2). However, most works on the effects of barbiturates had been done with either male or female animals only. Moreover, the reports on the sex difference of the effects of barbiturates were principally concerned with the single injection of drug.

The authors have reported that the sex difference was observed in the effects of some hormones on the growth and development of rats and the enzyme activity in the rat's liver (3-5).

The purpose of the present study was to compare the effects in male and female rats of continued daily injection of pentobarbital as well as phenobarbital. The development of the tolerance to drugs and the existence of sex difference in drug effects were observed.

MATERIALS AND METHODS

Adult (about 3 months of age) and immature rats (about 3 weeks of age) of Wistar-King A strain were used. Rats were bred in this department and took the compressed diet (Oriental Co.) and water *ad libitum*. All rats were kept at a temperature of $23 \pm 2^\circ\text{C}$ and $55 \pm 5\%$ in humidity.

Pentobarbital sodium in aqueous solution was injected subcutaneously or intraperitoneally in doses of 30 and 40 mg/kg. Phenobarbital in 50% propyleneglycol was injected intraperitoneally in doses of 50 and 60 mg/kg. In a supplemental series of experiments methaqualone (2-methyl-3-o-tolylquinazalone; Hyminal), 30 mg/kg, in 80% propyleneglycol was injected intraperitoneally to adult rats.

The effects of these drugs compared with respect to the duration of the ataxia (atactic time including sleeping time) and also to sleeping time (the duration of the loss of righting reflex).

The determination of phenobarbital and pentobarbital concentrations in serum was carried out according to the method described by Brodie *et al.* (6). One hour after administration of phenobarbital, the total phenobarbital concentration was assayed. Serum level of pentobarbital was determined at 30 minutes after intraperitoneal injection. The liver, kidney and adrenal were weighed at 24th hour after the last injection.

FIG. 1. Atactic and sleeping times of 30 mg/kg pentobarbital in adult rats. In this figure as well as in the following figures, the vertical line shows the standard error of the mean, and each group consists of 5 animals, unless otherwise stated.

RESULTS

1. Pentobarbital in adult rats

Durations of ataxia and sleep in female rats given 30 mg/kg pentobarbital were longer than those in males (Fig. 1). Although the atactic time in female animals showed a rapid decrease toward the 4th day, no obvious daily differences in sleeping time were observed in both sexes. Subcutaneous injection of 30 mg/kg pentobarbital showed the effect similar to that of intraperitoneal injection, i.e., the atactic time as well as sleeping time were longer in females than in males (Fig. 1).

2. Phenobarbital in adult rats

Sixty milligram per kilogram of phenobarbital was intraperitoneally injected to both sexes of adult rats. Both durations of ataxia and sleep were the longest on the 3rd day of daily injection and gradually reduced there-

FIG. 2. Atactic and sleeping times of 60 mg/kg phenobarbital in adult rats.

FIG. 3. Atactic and sleeping times of 50 mg/kg phenobarbital in immature rats.

after, rats of both sexes sleeping little on the 10th day (Fig. 2). The atactic time as well as sleeping time were longer in female rats every day as observed in case of pentobarbital administration. The time required for the onset of ataxia was not significantly altered in all cases.

3. Phenobarbital in immature rats

Fifty milligram per kilogram of phenobarbital was intraperitoneally injected daily for 7 days to both male and female of immature rats weighing 40 to 50 g. No sex difference was observed in the time of ataxia or sleep. However, the atactic time and sleeping time were the longest at the 2nd day, and then gradually decreased. One week after the last injection, 50 mg/kg of phenobarbital failed to exert the hypnotic effect in either sexes of immature rats and the time of ataxia was markedly shortened (Fig. 3). No retardation was observed in the growth

FIG. 4. Atactic and sleeping times of 30 mg/kg methaqualone in adult rats.

FIG. 5. Pentobarbital level in serum 30 minutes after intraperitoneal injection of 40 mg/kg pentobarbital in adult rats. Each group consists of 4 or 5 rats.

FIG. 6. Phenobarbital level in serum 1 hour after intraperitoneal injection of 60 mg/kg pentobarbital in adult rats. Each group consists of 4 or 5 rats.

of immature rats receiving daily injection of phenobarbital.

4. Methaqualone in adult rats

Thirty milligram per kilogram of methaqualone was injected intraperitoneally in both sexes of adult rats. A peak of atactic time was observed on the 3rd day of daily injection and that of sleeping time on the 2nd day in both sexes. Remarkable sex differences in the durations of ataxia and sleep were noted throughout the experimental period (Fig. 4).

5. Pentobarbital and phenobarbital levels in serum

Pentobarbital level in serum at 30 minutes after intraperitoneal injection of 40 mg/kg of pentobarbital was higher in female rats than in males on all days (Fig. 5). Phenobarbital concentration in serum was also higher in female rats at 1 hour after

TABLE 1. Sex and age differences of phenobarbital concentration in serum (1 hour after 60 mg/kg, intraperitoneal injection).

	Adult		Immature	
	Body wt. (g)	Concentration (μ g/ml serum)	Body wt. (g)	Concentration (μ g/ml serum)
♂	350 $\pm$ 27	19.9 $\pm$ 1.4	53.5 $\pm$ 1.6	31.6 $\pm$ 1.2
♀	213 $\pm$ 13	27.8 $\pm$ 1.1	55.8 $\pm$ 1.4	36.3 $\pm$ 2.2

FIG. 7. Effects of daily injection for 10 days of 30 mg/kg pentobarbital or 60 mg/kg phenobarbital on the liver, kidney and adrenal weights in adult rats. The control group received propyleneglycol.

the injection of 60 mg/kg (Fig. 6). Serum level of phenobarbital in immature rats showed no marked sex difference as was in adult animals at 1 hour after the dose of 60 mg/kg (Table 1).

6. *Organ weight*

The liver and adrenal weights in terms of weight per 100 g of body weight showed an increase in both sexes of rats given phenobarbital injection, but no changes were observed in those receiving pentobarbital (Fig. 7).

DISCUSSION

It has been reported that there was a remarkable difference in the effects of pentobarbital (1) or hexobarbital (2) between female and male adult rats, i.e., females were more susceptible to these drugs. The sex difference in the effects of pentobarbital or carisoprodol in adult rats disappeared after administration of SKF 525A and were modified by castration or treatment with testosterone and 4-chlorotestosterone (1). It has been concluded that the anabolic action of male sex hormone seems to account for the increased enzyme activities in male rats responsible for inactivation of pentobarbital or carisoprodol.

Cameron *et al.* (7) reported that the spayed male adult rats are susceptible to pentobarbital as compared with controls, and administration of testosterone is followed by break down of sensitivity. They suggested that a functional relationship exists between the sex glands and the liver, and therefore the detoxication of pentobarbital is probably under the control of sex hormones.

Present results of daily administration of pentobarbital or phenobarbital for 6 to 10 days showed a remarkable sex difference in the duration of action of these drugs throughout the course of experiments. The development of tolerance to these drugs was observed in the case of phenobarbital administration. Moreover, a remarkable tolerance to phenobarbital appeared in immature rats and the tolerance development was, however, of the same degree in both sexes of either adult and immature animals. When phenobarbital was administered 7 days after the last injection, adult rats showed a similar change in the atactic or sleeping time to that observed in the first treatment (unpublished data). In a case of immature rats, however, appeared a striking tolerance. It is interesting that the rapidly growing animals show the development of remarkable tolerance. The variation in the activity of the microsomal drug-metabolizing enzymes in rats in relation to age was observed by Kato *et al.* with hexobarbital, carisoprodol, meprobamate, strychnine and pentobarbital (8). They reported that the immature rats (30 days of age) have the highest sensitivity to the drugs and also the highest metabolic activity in the liver slices. The tolerance to pentobarbital with respect to shortening of the sleeping time was established by about 2nd day of daily administration without any further shortening from the 2nd to the 15th administration in rats (9). However, significant tolerance was not indicated in the present experiment with pentobarbital except the atactic time in female rats. If the sex difference in effects of these barbiturates

is due to their metabolic difference in the liver which is influenced by the anabolic action of androgen, it is difficult to explain that the appearance of tolerance to phenobarbital was quite similar in both sexes, and that immature rats showed marked shortening of the duration of the action as compared with adult rats.

The authors observed that the striking sex difference of succinic dehydrogenase and cytochrome c oxidase activities in the liver of rats after 40 days of age, i.e., activities in adult females were higher than those in adult males. Moreover, these sex difference did not completely disappeared by castration (10). Goldenthal *et al.* (11) also reported that sex difference of monocrotaline toxicity is unaffected by gonadectomy. These facts suggest that the hormonal regulation of drug metabolism is very much complicated.

It was reported that the tolerance development to phenobarbital is very slow, and the drug, being only slowly oxidized, tends to be accumulated in association with decreased sensitivity of the central nervous system, i.e., the plasma concentration of phenobarbital is continuing to rise during the period in which the hypnotic effects are rapidly diminishing (12). The authors also did not obtain that the remarkable relationship between the duration of phenobarbital action and its concentration in serum with respect to the daily variation of the atactic and sleeping times. However, sex difference in the serum level of phenobarbital and pentobarbital in adult rats indicates that adult male animals have a higher activity of drug elimination than adult females, though a precise mechanism is unknown. Tolerance to phenobarbital in the present experiment developed after a delay of about 3 days, and increased gradually toward the 9th day. This evidence must be explained on the basis of the changes of sensitivity of central nervous system as well as metabolic activity in the tissues.

Another question arosed is that the tolerance only appeared to phenobarbital, a long-acting barbiturate, which is considered to be inactivated only to a slight extent by the liver and almost entirely excreted through the kidney (18). On the other hand, drug tolerance did not appear in the case of pentobarbital, which is mainly metabolized by the liver (14).

Conney and Klutch (15) observed that the activity of the drug metabolizing enzyme was increased by phenobarbital administration in rats. The decreased durations of zoxazolamine paralysis and hexobarbital hypnosis were noticed by pretreatment with phenobarbital. Conney *et al.* (16) also have shown that the demethylation activity in the liver microsomes was accelerated after phenobarbital treatment in rats. Their conclusion stated that phenobarbital induces the formation of various drug metabolizing enzymes and stimulates the liver protein synthesis. The increased weight of the liver in those developed tolerance to phenobarbital in the present study suggests that the development of the tolerance, different from the sex difference, is likely to be connected with an increase of the metabolic activity in the liver.

The sex difference in the effect of hexobarbital was observed in adult rats, but this is not the case in dogs, rabbits, guinea pigs and mice (2). It is generally considered

that the physiological status of sex hormones in mice is not so different from that in rats. Species difference observed in the sex difference of barbiturate effects was difficult to explain on only the basis of sex hormones.

What plays a major role in limiting the duration of action of barbiturates and the mechanism of development of the tolerance to phenobarbital with respect to sex, age or species difference must be elucidated by further experimental studies.

SUMMARY

1. The durations of ataxia and sleep after pentobarbital administration, intraperitoneal or subcutaneous injection of 30 mg/kg daily, determined on consecutive 10 days, were always longer in female than in male adult rats. No remarkable tolerance developed in both male and female rats.

2. Daily administration of phenobarbital, intraperitoneal injection of 60 mg/kg, caused marked increase in the atactic and sleeping times on the 3rd day in both sexes of adult rats and rapid decrease thereafter. The duration of ataxia or sleep was, however, always longer in females throughout the experiment for 10 days.

3. Immature rats showed no sex difference in the effects of phenobarbital. Peaks of the duration of ataxia and sleep appeared on the 2nd day and striking tolerance was still observed in 1 week after the last injection of the consecutive 6 daily injections.

4. Methaqualone given to adult male and female rats produced the changes in atactic or sleeping time similar to those observed in the animals received phenobarbital.

5. Pentobarbital or phenobarbital concentration in serum was higher in adult female rats than in male throughout 1 week of continued daily injection. Phenobarbital level in serum of immature rats showed no sex difference.

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