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Report

Gender Differences in the Pharmacodynamics of Barbiturates in Rats

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There is considerable evidence of gender differences in the pharmacokinetics of numerous drugs, particularly in rodents, but very limited information concerning the effect of gender on pharmacodynamic characteristics (concentration-activity relationships). In this study, heptabarbital or phenobarbital was administered to male and female rats and the concentrations of these drugs in the brain, cerebrospinal fluid and serum at onset or offset of loss of righting reflex were determined. For heptabarbital, offset concentrations were determined in Lewis rats and onset concentrations in Wistar rats. Onset concentrations of phenobarbital were determined in Wistar rats. In all cases, the barbiturate concentrations in males were significantly lower than those in females at the pharmacologic endpoint. The biologic (serum) half-life of heptabarbital is much shorter in males (~10 min) than in females (~90 min) and this pharmacokinetic difference is reflected by the considerably longer duration of effect of the drug in females.

KEY WORDS: gender difference; heptabarbital; phenobarbital; sleeping time; concentration-effect relationship.

INTRODUCTION

There are appreciable gender differences in the pharmacokinetics of many drugs (1–3). Most of the evidence for such differences comes from animals, particularly rats, but some is also from humans. On the other hand, little is known about the effect of gender on the concentration-pharmacologic activity relationship (pharmacodynamics) of drugs. There have been a number of comparative pharmacologic effect studies in males and females without concomitant determination of drug concentrations. This practice usually makes it impossible to distinguish pharmacokinetic from pharmacodynamic alterations. Other investigations have produced apparently conflicting results. As early as 1937, it was observed that female rats slept longer than males after administration of certain barbiturates (4). It is now held that this difference is a consequence of gender differences in the elimination kinetics of these drugs (1). On the other hand, it has been reported that the induction dose (which is usually minimally affected by elimination kinetics) of the general anesthetic agent thiopental was 2.75 ± 0.11 mg/kg (mean ± S.E.) in men and only 2.16 ± 0.10 mg/kg in women ($p < 0.001$) (5). A more recent investigation (6) yielded much smaller (~6 percent) and not statistically significant differences between men and women but here too the men required a nominally larger induction dose of thiopental on the average.

There are a number of other examples of apparent gender differences in pharmacodynamics. Using mice that have been selectively bred for maximum differences in sensitivity to ethanol as measured by sleep time, Smolen and Smolen have found that the brain and blood concentrations of ethanol on return of the righting reflex did not differ between male and female "long sleep" mice but were lower in male than in female "short sleep" mice (7). Trenk et al. demonstrated that the serum concentration of unbound phenprocoumon needed to decrease the synthesis rate of prothrombin complex activity was about twice as high in female than in male Wistar rats (8). Whitfield and Levy found that the slope of the logarithm of anticoagulant effect (activated partial thromboplastin time) versus heparin concentration in plasma relationship is significantly steeper in women than in men (9). Male mice were reported to be significantly more sensitive than female mice to experimental pain produced by an electrical stimulus to the tail (10). On the other hand, no significant gender difference was found when tooth pulp stimulation was used to determine pain perception in human subjects (11). Gender had no effect on the minimum alveolar concentration of the inhalation anesthetics halothane, isoflurane, and enflurane in rats and mice (12). However, doses of picrotoxin that are subconvulsive in male rats were found to be almost 100 percent convulsive in females (13).

The cited evidence indicates that it is insufficient to focus determinations of possible gender differences only or predominantly on pharmacokinetics; there are a number of indications that pharmacodynamic gender differences can occur also. This is not surprising inasmuch as there are morphological, physiological and biochemical gender differences in the nervous system of rats (and presumably also of other

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species) as well as gender differences in the characteristics of various types of receptors in the rat central nervous system (reviewed in reference 14). In the present investigation, we have determined the effect of gender on the hypnotic (general anesthetic) action of two barbiturates, the long-acting (slowly eliminated) phenobarbital and the relatively short-acting (rapidly eliminated) heptabarbital, in two strains of rats, the Lewis and the Wistar. The major focus of the investigation was on drug concentrations in the cerebrospinal fluid (CSF) at onset or offset of sleep (as reflected by the loss of righting reflex) because a) protein binding of barbiturates in the CSF is virtually nil, and b) the concentrations of the two barbiturates in the CSF equilibrate very rapidly with corresponding concentrations at the site of action (15,16).

METHODS

Male and female inbred Lewis rats (LEW/Crl/BR), Charles River Laboratories, Wilmington, MA), 11 weeks old (all born on the same day), were used in the first part of this investigation. They were maintained on Charles River Rat-Mouse-Hamster Formula and had a cannula implanted into the right jugular vein under light ether anesthesia one day before the experiment. The next morning, the male rats received a rapid (25.5 mg/min) i.v. infusion of heptabarbital, either 46, 60 or 100 mg/kg, whereas the females received either 40, 60 or 100 mg/kg in the same manner. The rats were placed on heated pads to maintain their body temperature and the time of onset and offset of loss of righting reflex (without nociceptive stimulus) were determined. Immediately upon return of the righting reflex, the rats were rapidly anesthetized with ether and samples of CSF, blood (for serum) and the brain were obtained (15) and stored in a freezer pending assay.

Male and female Wistar rats (Crl:(WI)BR, Charles River Laboratories, Wilmington, MA), 11 weeks old and born on the same day, were used in the second part of this investigation. A jugular vein cannula was implanted under light ether anesthesia and the next day an i.v. infusion of heptabarbital, 0.306 mg/min for females and 0.871 mg/min for males, was administered while the animals were positioned on heated pads. At onset of loss of righting reflex (without nociceptive stimulus) the infusion was stopped and CSF, blood and the brain were obtained for subsequent determination of heptabarbital concentrations. This experiment was performed in conjunction with a cyclosporine-heptabarbital interaction study; the female rats also served as controls in that study (17).

The third experiment was conducted on male and female 11 week old Wistar rats (born on the same day). Two days after implantation of a jugular cannula, sodium phenobarbital was infused i.v. at a rate of 2.04 mg/min until the onset of loss of righting reflex. Other conditions and procedures were as in the preceding experiment.

The pharmacokinetics of heptabarbital were determined in male and female Lewis rats, about 13 weeks old, after i.v. infusion of 25 mg/kg over 1 minute. Blood samples (0.25 ml) were collected from a jugular vein cannula at 2, 5, 10, 15, 30, 45 and 60 minutes and for the females also at 120, 180 and 240 minutes. The concentrations were fit to a biexponential equation by the computer program NONLIN (18). Pharmacokinetic parameters were obtained by standard methods which included a mathematical correction for the 1 minute infusion period (19).

Heptabarbital and metabolite concentrations were determined by high performance liquid chromatography (20). The concentration data for the male rats obtained in the first part of this investigation (particularly the metabolite concentrations which are not reported here) were also required as background information for another study (21) and Table II therefore duplicates some data published previously (Table 3 in reference 21). Phenobarbital concentrations were determined by high performance liquid chromatography (15).

Statistical assessment of the data was by one-way analysis of variance followed by the Newman-Keuls test where appropriate. Fischer's test was used to determine the homogeneity of variances. The statistical significance of differences between 2 groups was determined by the unpaired t-test or, in case of heteroscedasticity, by the Mann-Whitney U-test.

Simulations were performed to characterize, in principle, the relationship between duration of action and dose of a drug whose site of action is in the peripheral compartment of a two-compartment system. It was assumed that drug concentrations in the peripheral compartment at onset and offset of the pharmacologic effect were equal (the "effective" concentration). The time course of the amount of drug in the peripheral compartment, A_p, is

$$A_p = \frac{k_{12}\ \text{Dose}}{\lambda_1 - \lambda_2}\left(e^{-\lambda_2 t} - e^{-\lambda_1 t}\right)$$

where λ_1 and λ_2 are the exponents of the biexponential equation, k_{12} is the apparent first-order rate constant for drug transfer from the central to the peripheral compartment, and t is time after drug injection. By rearrangement,

$$\text{Dose} = \frac{A_p\,(\lambda_1 - \lambda_2)}{k_{12}\left(e^{-\lambda_2 t} - e^{-\lambda_1 t}\right)}$$

which served as the basis for calculating the duration of action of different doses (i.e., the differences between the times when A_p equalled the effective concentration multiplied by the volume of the peripheral compartment during the ascending and descending concentration phases in that compartment). This simulation procedure was used for hypothetical male and female rats except that the effective concentration in males was varied empirically until a plot of duration of action versus log dose had the same extrapolated intercept on the dose axis as that for female rats. The k_{12}, λ_1 and λ_2 values (in reciprocal minutes) were 0.108, 0.2635 and 0.00759 for females and 0.161, 0.5056 and 0.0762 for males. The effective drug concentration was assumed to be 11.9 mg/L for the females. The peripheral compartment volumes were 0.278 L/kg for the females and 0.255 l/kg for the males. The reason for performing these simulations is explained in the Discussion.

RESULTS

The effect of dose of heptabarbital on the sleeping time of female and male Lewis rats is shown in Tables I and II. At any one dose, female rats slept considerably longer than

Table I. Sleeping Time and Concentrations of Heptabarbital at Return of the Righting Reflex in Female Lewis Rats as a Function of Dose[a]

Variable	Dose (mg/kg) 40	60	100
Body weight, g	183 ± 7	179 ± 8	179 ± 5
Sleeping time, min[b]	68.7 ± 29.1	173 ± 24	331 ± 58
Serum conc., mg/L			
Total drug[c]	36.4 ± 8.0	32.7 ± 5.4	28.8 ± 4.0
Free drug	15.4 ± 3.0	14.5 ± 3.0	12.6 ± 1.7
Brain conc., mg/kg	29.4 ± 5.8	28.8 ± 4.4	27.4 ± 3.2
CSF conc., mg/L	12.8 ± 2.1	12.0 ± 2.2	10.9 ± 1.3

[a] Results are reported as mean ± SD in all tables. $N = 9$.
[b] Significant differences between groups by one-way ANOVA, $p < 0.001$.
[c] Significant differences between groups by one-way ANOVA, $p < 0.05$.

male rats. Except for the serum concentration of total drug in females, there was no statistically significant effect of dose on the serum, serum water (free drug), brain and CSF concentrations of heptabarbital at the return of righting reflex (awakening). The heptabarbital concentration data in males and females, respectively, were therefore consolidated for comparison. In all cases, the concentrations were significantly higher in the female Lewis rats than in the males (Table III). Moreover, the serum protein binding of the barbiturate was significantly more extensive in females than in males (Table III).

The total clearance referenced to serum was considerably higher and the serum terminal half-life of heptabarbital was significantly shorter in male than in female Lewis rats after i.v. administration of a 25 mg/kg dose (Table IV).

The male and female Wistar rats used for the study of heptabarbital pharmacodynamics are described in Table V. As in the preceding experiments with Lewis rats, the males were heavier than the females. Body temperature was slightly higher in Wistar females than in males, a difference that was not found in the Lewis rats. The heptabarbital infusion rates were designed to produce onset of loss of righting reflex at approximately the same time in the males and females. The body weight normalized dose and the serum, serum water, brain and CSF concentrations of heptabarbital required to produce onset of loss of righting reflex in female Wistar rats were significantly higher than corresponding values in the males (Table VI). The serum protein binding of the barbiturate was slightly but statistically more pronounced in female than in male Wistar rats (Table VI).

Results of the pharmacodynamic study with phenobarbital are summarized in Table VII. The body weight normalized dose and the concentrations of phenobarbital in serum, brain and CSF at onset of loss of righting reflex were significantly higher in female than in male Wistar rats.

Table II. Sleeping Time and Concentrations of Heptabarbital at Return of the Righting Reflex in Male Lewis Rats as a Function of Dose[a]

Variable	Dose, mg/kg 46	60	100
Body weight, g	259 ± 13	254 ± 10	259 ± 16
Sleeping time, min[b]	15.1 ± 6.1	25.3 ± 7.9	50.0 ± 12.4
Serum conc., mg/L			
Total drug	18.9 ± 5.1	17.5 ± 6.2	18.3 ± 3.0
Free drug	9.1 ± 2.5	8.3 ± 2.8	8.9 ± 1.1
Brain conc., mg/kg	19.7 ± 7.4	21.8 ± 7.0	23.4 ± 4.2
CSF conc., mg/L	9.22 ± 2.49	8.99 ± 3.16	9.52 ± 1.91

[a] $N = 10$.
[b] Significant differences between groups by one-way ANOVA, $p < 0.001$.

Table III. Concentrations of Heptabarbital at Return of Righting Reflex in Male and Female Lewis Rats Independent of Dose[a]

Variable	Males	Females
Number of animals	30	27
Body weight, g	258 ± 13	180 ± 7[b]
Rectal temperature, °C	38.6 ± 0.4	39.1 ± 0.4
Serum conc., mg/L		
Total drug	18.2 ± 4.8	(32.6 ± 6.6)[b,c]
Free drug	8.8 ± 2.2	14.2 ± 2.8[b]
Serum free fraction × 100	48.1 ± 3.2	43.5 ± 2.9[b]
Brain conc., mg/L	22.3 ± 5.6	28.5 ± 4.5[b]
CSF conc., mg/L	9.24 ± 2.49	11.9 ± 2.0[b]

[a] Consolidated data from Tables I and II. The rats were 11 weeks old.
[b] Significant differences between groups by t-test, $p < 0.001$.
[c] Dose dependent.

DISCUSSION

The pronounced difference in the duration of action of heptabarbital in male and female rats observed in this investigation is consistent with the results of similar studies with other relatively short-acting barbiturates including hexobarbital (23) and pentobarbital (24). Taken alone, a difference in duration of effect can reflect gender differences in barbiturate pharmacokinetics, pharmacodynamics, or both. Ideally, mechanistically oriented studies of gender differences should include measurements of drug effects as well as concentrations. However, investigational strategies have been developed to distinguish between pharmacodynamic and pharmacokinetic differences even without chemical assays. Thus, Schnell et al. (23) used a duration of sleep versus log dose plot, as suggested by Levy (25) for drugs with one-compartment pharmacokinetic characteristics, to analyze the result of a study of the hypnotic effect of hexobarbital as a function of dose in male and female Sprague-Dawley rats. The plots were linear but had different slopes for males and females, indicative of the gender difference in elimination rate constant or half-life of the drug (25). Extrapolation of the plot to the log dose axis (i.e., to zero sleeping time) yielded almost identical estimates of apparent minimum effective hypnotic dose. Drug concentrations in the brain of male and female rats at offset of loss of righting reflex after

Table IV. Pharmacokinetics of Heptabarbital in Male and Female Lewis Rats[a]

Variable	Male	Female
Number of animals	5	7
Body weight, g	314 ± 27	195 ± 8
Total clearance, ml/(min * kg)	54.0 ± 17.0	5.27 ± 0.46
Half-life,[b] min	9.61 ± 1.78	92.8 ± 11.9

[a] A dose of 25 mg/kg was injected i.v.
[b] Terminal half-life in serum.

injection of a 100 mg/kg dose of hexobarbital were also quite similar (23). These observations can be interpreted as indicating an absence of a gender difference in the pharmacodynamics of hexobarbital, provided that the drug has "one-compartment" pharmacokinetic characteristics and the site of action is part of that single hypothetical compartment. This is unlikely in view of more recently acquired knowledge (15,16).

The pharmacokinetics of heptabarbital, the drug used in the present investigation, are definitely not of the one compartment type and its site of action is not in the hypothetical central compartment (16). Nevertheless, plots of duration of sleep versus log dose data obtained in the present investigation (Tables I and II) are also apparently linear, with different slopes for males and females (indicative of differences in elimination kinetics), and the regression lines intercept the log dose axis at essentially the same point (Fig. 1). In a one-compartment system, this intercept value reflects the minimum effective amount of drug in the body. The fact that this value was essentially the same for males and females suggests the absence of a pharmacodynamic gender difference *provided* that heptabarbital confers upon the body the pharmacokinetic characteristics of a one-compartment system (which it does not) and the apparent volume of distribution of the drug is the same in male and female rats. Based on a direct comparison of heptabarbital concentrations in the cerebrospinal fluid [which, unlike plasma or brain, reflects concentrations of the drug at the site of action (16)], male rats are more sensitive to the barbiturate than females (Tables III and VI).

The reason for the apparent conflict between the (admittedly inappropriate) graphical analysis and the direct measurement of sensitivity to heptabarbital (based on drug concentrations in cerebrospinal fluid at a defined pharmacologic endpoint) as a function of gender was explored by computer simulation. This could only be done in principle, using a two-compartment model system, since there is not enough information for meaningful physiologically based modelling of heptabarbital in male and female rats, respectively. As a matter of convenience, the pharmacokinetic constants used for the simulation were those for heptabarbital determined in the present investigation; the effective drug concentration in the peripheral (site of action) compartment of the female rats was assumed to be equal to the drug concentration in cerebrospinal fluid at the return of righting reflex as determined in this investigation. The duration of effect versus log dose plots thus obtained (Fig. 2) are essentially linear at relatively long durations of effect (larger doses) but curve downward as the dose decreases and the duration of effect approaches zero. This downward curvature is characteristic of multi-compartment type drugs with the site of action in a "deep" compartment (26). The simulation demonstrates that regres-

Table V. Description of Male and Female Wistar Rats Used in the Study of Heptabarbital Pharmacodynamics[a]

Characteristics	Male	Female
Body weight, g	292 ± 16	203 ± 14[b]
Rectal temperature, °C	38.9 ± 0.6	39.7 ± 0.5[b]
Hematocrit, %	39.7 ± 3.1	40.5 ± 3.1
Serum total protein, g/L	57.4 ± 3.4	57.2 ± 2.4
Serum total bilirubin, mg/L	2.31 ± 0.86	2.48 ± 1.33
Serum alanine aminotransferase, IU/l	24.8 ± 6.9	25.8 ± 4.3
Serum aspartate aminotransferase, IU/L	55.4 ± 30.6	43.3 ± 15.9
Serum creatinine, mg/100 ml	0.604 ± 0.191	0.444 ± 0.116
Serum urea nitrogen, mg/100 ml	12.4 ± 2.2	11.7 ± 2.1

[a] The rats were 10 weeks old. $N = 10$.
[b] Significantly different from males by t-test, $p < 0.005$.

Table VI. Total Dose and Concentrations of Heptabarbital at Onset of Loss of Righting Reflex in Male and Female Wistar Rats Receiving an Infusion of the Drug

Variable	Male	Female
Infusion rate, mg/min	0.871	0.306
Infusion time, min	27.2 ± 4.5	30.8 ± 2.7
Dose, mg/kg	81.7 ± 14.4	46.5 ± 3.2[a]
Serum conc., mg/L		
Total drug	32.2 ± 4.4	52.2 ± 4.5[a]
Free drug	14.1 ± 2.8	21.5 ± 2.0[a]
Serum free fraction × 100	43.6 ± 4.3	41.2 ± 1.5[b]
Brain conc., mg/L	34.2 ± 4.7	47.2 ± 3.2[a]
CSF conc., mg/L	15.7 ± 1.8	19.9 ± 1.7[a]

[a] Significantly different from males by t-test, $p < 0.001$.
[b] Significantly different from males by Mann-Whitney test, $p < 0.03$.

Table VII. Total Dose and Concentrations of Phenobarbital at Onset of Loss of Righting Reflex in Male and Female Wistar Rats Receiving an Infusion of the Drug[a]

Variable	Male	Female
Number of animals	12	13
Body weight, g	309 ± 11	222 ± 16[b]
Rectal temperature, °C	39.6 ± 0.4	39.9 ± 0.6
Infusion time, min	17.0 ± 2.0	14.5 ± 0.9[b]
Dose, mg/kg	112 ± 11.6	134 ± 14.9[b]
Serum total conc., mg/L	204 ± 24	250 ± 37[b]
Brain conc., mg/kg	108 ± 9	134 ± 13[b]
CSF conc., mg/L	71.2 ± 6.3	82.6 ± 8.5[b]

[a] The rats were 11 weeks old.
[b] Significantly different from males by t-test, $p < 0.001$.

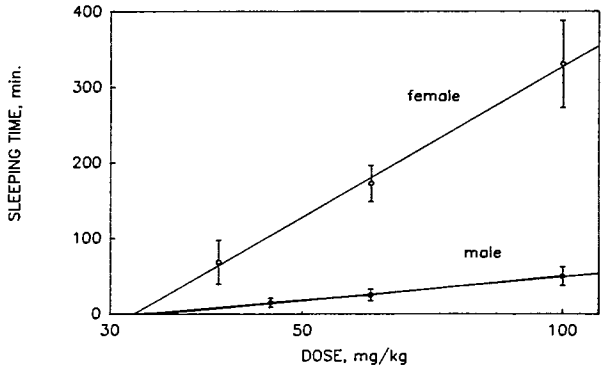

Fig. 1. Relationship between duration of sleep and i.v. dose of heptabarbital in male and female Lewis rats. Vertical bars represent ±1 SD. The intercept values are 33.5 mg/kg for males and 32.0 mg/kg for females.

sion lines for two different groups of animals that can be extrapolated to the same intercept (by disregarding the downward curvature or not having any experimental data in the region of curvature) do not necessarily signify an absence of pharmacodynamic differences between the groups. In this particular simulation, the effective drug *concentration* was 11.9 mg/L for females and 10.6 mg/L for males, i.e. of similar magnitude as the gender differences observed in this investigation. Nevertheless, the simulation should be considered only as the demonstration of a principle and not as an attempt to fit our experimental data to a model.

Considering the very rapid elimination of heptabarbital by male as compared to female rats, the possibility must be considered that the apparent gender difference in pharmacodynamics is artifactual. Specifically, the lower drug concentrations in the cerebrospinal fluid of males may be suspected to be due to the relatively more rapid decrease of concentrations in the males between the time of occurrence of the pharmacologic endpoint and the actual collection of cerebrospinal fluid one or two minutes later. In fact, this could have accounted for about one-half of the difference between male and female rats in the cerebrospinal fluid concentrations of

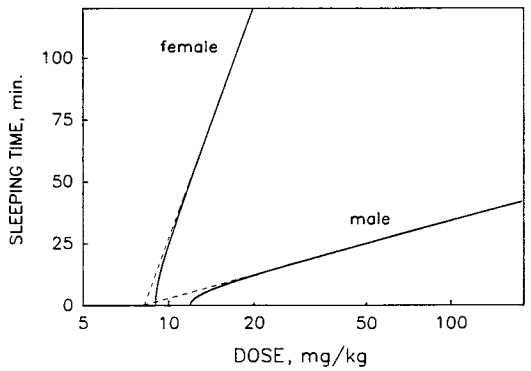

Fig. 2. Simulation of the relationship between duration of sleep and i.v. dose of a drug that confers upon the body the pharmacokinetic characteristics of a two-compartment system. It is assumed that the site of action is in the peripheral compartment. Refer to text for further discussion.

heptabarbital summarized in Table III and it could have contributed also to the corresponding gender differences in Table VI. To help clarify this uncertainty, an experiment was performed with phenobarbital, a drug with a much longer biologic half-life [about 8.5 h in male and 11 h in female Wistar rats (27,28)] than heptabarbital. The results demonstrate clearly that the effective hypnotic concentration of phenobarbital is appreciably lower in males than in females (Table VII).

In summary, a gender difference was found in the pharmacodynamics of two barbiturates, one relatively short-acting and the other long-acting, in two strains of adult rats. This study also contributes to previous reports of pronounced gender differences in the duration of action and pharmacokinetics of certain barbiturates in rats. The longer duration of action (sleeping time) of heptabarbital in female rats is due to the longer biological half-life of the drug in female as compared to male rats; the pharmacodynamic differences by themselves would have the opposite effect. The net effect reflects the pharmacokinetic differences because they are much greater than the pharmacodynamic ones.

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