Pharmacology Biochemistry & Behavior, Vol. 16, pp. 815–818, 1982. Printed in the U.S.A.

Duration of Response to Pentobarbital of Female vs Male Albino and Pigmented Rats[1]

IRWIN S. WESTENBERG AND JEFFREY M. BOLAM

Institute for the Study of Developmental Disabilities, 1640 West Roosevelt Road, Chicago, IL 60608

Received 15 August 1981

WESTENBERG, I. S. AND J. M. BOLAM. *Duration of response to pentobarbital of female vs male albino and pigmented rats.* PHARMAC. BIOCHEM. BEHAV. **16**(5) 815–818, 1982.—We measured responses to pentobarbital of females and males of Sim:(LE) ancestry to test Long-Evans rats for a sex difference in drug response, i.e., higher pentobarbital susceptibility in females. We studied littermate quadruplets made up of an albino male and female and a black-hooded male and female to determine if the sex difference, if any, would be seen in both pigmented and albino rats. The rats were injected weekly IP with sodium pentobarbital; each rat's dosage increased 10 mg/kg per week to the dose that was lethal. In no case was the onset of the drug's effects consistently more rapid in the females. In both albino and hooded rats the drug effects' duration was significantly longer in the females. The females' lethal doses were lower than the males', but not significantly. Thus for at least one drug response measure there is a sex difference in drug response in pigmented and albino Long-Evans rats.

Male vs female Albino vs pigmented Long-Evans rats Pentobarbital Sleeptime
Drug response onset Drug response duration Lethal dose

SINCE 1935 [10] it has been found frequently and consistently that in albino rat strains females are more susceptible than males to pentobarbital sodium. Pigmented strains have been studied much less frequently, and higher pentobarbital susceptibility has not always been found [4]. A problem with most of the earlier studies is the lack of clear genealogical lines from the stocks then in use to currently available stocks. Of experiments on rats related to currently available stocks, many have involved albinos of Wistar or Sprague-Dawley derivation, and the usual sex differences have been found [3,14]. However, in a study on pigmented rats related to a currently available stock, higher pentobarbital susceptibility in females was not found in hooded Long-Evans rats of Sim:(LE) ancestry [7].

This difference in results between albino strains and a pigmented strain, which is not surprising [18, 20, 21], is subject to alternative explanations: (A) the females' higher pentobarbital susceptibility is not seen in certain strains, e.g., Long-Evans strains; (B) the females' higher pentobarbital susceptibility is seen only in albinos; (C) the measure of drug response used with the Long-Evans rats [7] is not sensitive enough to detect consistently the females' higher pentobarbital susceptibility. We considered each of these alternatives: (A) to determine if the females' higher susceptibility to pentobarbital can be seen in Long-Evans rats, we compared females vs males of Long-Evans ancestry; (B) to ascertain if the females' higher pentobarbital susceptibility, if any, can be found only in albinos, we compared albino as well as pigmented Long-Evans females vs albino as well as pigmented Long-Evans males; (C) to see if detection of the females' higher pentobarbital susceptibility, if any, depends on the measure of drug response employed, we used several measures of drug response.

METHOD

Subjects were Long-Evans rats of Sim:(LE) ancestry; they were the product of five to eight generations of inbreeding with forced heterozygosis at the albino, or c locus. Their coefficients of inbreeding were 0.67 to 0.83 [9]. There were four littermate quadruplets; each quadruplet consisted of an albino male and female and a pigmented male and female. The albinos were homozygous at the c locus with a pair of mutant genes (c/c); the pigmented rats were black-hooded, heterozygous at the c locus with one normal (+) gene and one mutant c gene (+/c). There were two quadruplets from each of two breeding lines provisionally designated WLE-3 and WLE-4. Rats were used when they were no longer needed in our breeding program; thus, when they entered the experiment quadruplets WLE-3-6 and WLE-4-5 were 16 to 17.5 months of age, and quadruplets WLE-3-7A and WLE-4-8 were 10 to 11 months of age. Rats in this age range are not "old" [2]. For example, the 50% survival age for a

[1]Supported by NIH grant EY 01888 and EY 03013 (I. S. W.), grant G-599, awarded by Fight for Sight, Inc., New York, NY (I. S. W.), and the Veterans Administration Career Development Program.

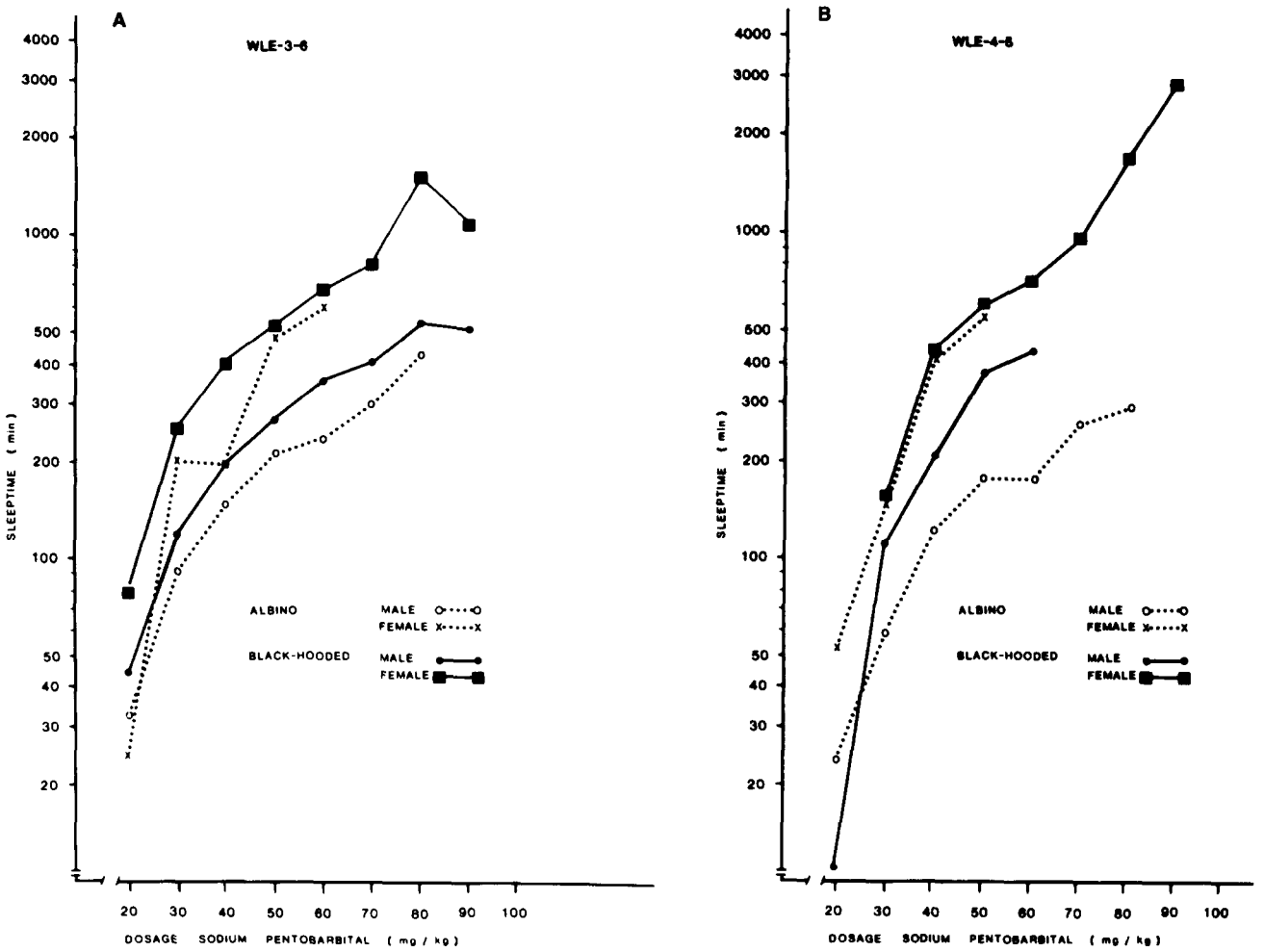

FIG. 1. Sleeptimes of black-hooded (solid lines) and albino (dotted lines) littermate quadruplets of male (open and filled circles) and female (X's and squares) rats injected weekly with pentobarbital IP; doses for each rat started at 0 mg/kg and increased 10 mg/kg per week. The last data point for each rat is for the week prior to its death. The rats were of Long-Evans Sim:(LE) ancestry; they were produced by inbreeding with forced heterozygosis at the c locus. Each set of graphs is for a separate breeding line and generation: (A) line WLE-3, generation 6; (B) line WLE-4, generation 5. There was a significant correlation between increasing doses and increasing female-male differences for the albinos and the non-albinos of both quadruplets ($p<0.05$, rank correlation coefficient [19]).

Long-Evans strain of rats is a little over 27 months [5], a value comparable to that of many rat strains [2]. Our oldest rat was almost 20 months of age at the end of the experiment; this rat's age was several months below typical 50% survival ages and mean life-spans for rats [5] and in the age range of rats used in previous experiments on sex differences in response to pentobarbital [15]. The males weighed about 550 g, and the females weighed about 350 g; their weights generally fluctuated in a range of less than 40 g. Each rat was housed with its littermate of the same sex on wood shavings in a clear polycarbonate cage with a stainless steel lid with food (Purina rat chow) and water ad lib. The light-dark cycle was L13–D11, with (fluorescent) lights on at 11:00 p.m. and off at 12:00 noon.

Rats were moved in their home cages to a laboratory room weekly and injected IP between 7:00 a.m. and 12:00 noon. The first injection was 0.9% saline (0.2 cc/kg). On subsequent weekly injection days they were injected with pentobarbital sodium (Nembutal, 50 mg/cc). The first dose was 10 mg/kg (0.2 cc/kg), and the dosage for each rat was cumulative, increasing 10 mg/kg (0.2 cc/kg) per week until the rat died.

To measure the onset of drug effects we recorded the post-injection times to fall off a walkway (droptime) and to lose righting reflexes (LRR-time). To measure the duration of drug effects we recorded the time of the return of righting reflexes (RRR-time) and calculated the sleeptime (RRR-time–LRR-time).

RESULTS AND DISCUSSION

The onset measures, droptimes and LRR-times, decreased as dosage increased. Typically, droptimes and LRR-times were more than three minutes at lower doses and less than two minutes at higher doses. Usually, LRR-times exceeded corresponding droptimes by about one minute. Neither an albino female's nor a pigmented female's measures ever became systematically lower than those of its male counterpart as dosage increased. Thus, in the onset meas-

ures there was no evidence of greater pentobarbital susceptibility in females.

Sleeptimes generally increased steadily with increasing dosage (Fig. 1A,B). In all quadruplets the female rats consistently slept longer than their respective male littermates. At the 40 mg/kg dose, the first at which all rats slept, the females' sleeptimes were significantly longer than the males' ($p=0.001$, Mann-Whitney U test, [19]). At all subsequent doses, all of the surviving females slept longer than all of the surviving males (p's<0.0005 to <0.05 at doses 50 mg/kg to 80 mg/kg, Mann-Whitney U test, [19]; at higher doses there were too few surviving rats for meaningful statistical tests). This sex difference in sleeptimes was obvious in albinos and non-albinos; the possibility of an interaction between this sex effect and an albinism effect will be considered below. In summary, the sleeptime measure clearly showed the females' greater pentobarbital susceptibility.

Lethal doses ranged from 60 to 160 mg/kg. The median lethal dose for the males was about 96 mg/kg vs about 75 mg/kg for the females. Although the females tended to have lower lethal doses, there was some overlap between the males' and females' distributions of lethal doses. Consequently, the female-male difference was not significant ($p>0.05$, Mann-Whitney U test, [19]). Similar female-male differences were observed in the albino rats and in the pigmented rats; in both cases the differences were not significant. Thus, the females' higher pentobarbital susceptibility was seen less clearly in the lethal dose measure than in the sleeptime measure.

The results show a clear sex difference in the response of rats of Long-Evans Sim:(LE) ancestry to pentobarbital; the females are more susceptible than the males to pentobarbital. However, the sex difference does not appear with equal consistency in all measures of drug response. The difference was most clearcut in the sleeptime measure. On the other hand, the difference in lethal doses was less consistent, and there was no evidence of the females' higher pentobarbital susceptibility in the onset measures. It is conceivable that others [7] would have found a sex difference more consistently in their stock of Long-Evans rats had they used a sleeptime measure. The inconsistency of the measure used earlier [7] is pointed up by a follow-up study [6]. The stock of rats used was the same as before [7], but significantly different results were obtained [6]. The present results complement and extend past findings on sex differences in the drug response of Long-Evans rats.

A problem inherent in any study of female-male differences in response to pentobarbital is that, by the age such a sex difference appears (2–3 months [12]), there is a substantial female-male weight difference. Thus, the variables of weight and sex are confounded. For example, in rats about 90 days old females weigh 225–250 g, while males weigh 325–350 g, a difference of about 30%. The female-male weight difference of our rats was slightly higher, about 40%. However, our rats' weight differences were stable throughout the experiment; as noted above, the rats' weights generally fluctuated in a range of less than 40 g. Had we used younger rats, their female-male weight difference would have been smaller, but it would have been changing progressively as the rats matured during the course of the experiment. This would have complicated the problem caused by the confounded variables of weight and sex.

A solution to the sex-weight problem is to match males and females by weight. However, this introduces a new problem, because weight-matched males and females differ in age. The variables of sex, weight, and age have not been considered jointly in a parametric study. However, the importance of the weight difference between our females and males can be assessed on the basis of available information.

The fact that our females were lighter than our males means that our females slept longer than our males (A) because they were female, or (B) because they weighed less. Two types of evidence suggest that sex, not weight, was the critical variable. First, weight has been found to be unrelated to the utilization of pentobarbital [13] or to the duration of pentobarbital-induced anesthesia [17] and sleep [4]. Similarly, in the present experiment there was no correlation between lower weight and longer sleeptime for either the males or the females. Second, the possibility that the females sleep longer than males because the females weigh less has been ruled out by demonstrations that females sleep longer than males even when the females weigh more than the males [1,15]. In summary, it appears that weight differences can be ruled out as a major factor in observed sex differences in response to pentobarbital.

It is possible that we would have found sex differences in our other drug response measures if we had used younger rats. Sex differences have been reported to be less in 600-day-old rats than in 100-day-old rats [15]. Thus, our rats' ages may have introduced a certain amount of bias against finding a sex difference. At the very least, it is quite unlikely that our rats' ages introduced a bias in favor of finding a sex difference. In experiments where age has differed from study to study, sex differences of the sort we report have been observed in rats with a broad range of ages, from young rats to rats as old as our oldest rats [12,15]. Studies that have sought the age of onset of the sex difference have found that it first appears early, around the time of the onset of sexual maturity (approximately 2 months [12]), rather than late. Studies that have included rats in a range of ages beyond sexual maturity have found that age has not been a factor in their results (2–5 months [1]; 3–13 months [6]; 7–12 months [7]; 6–10 months [11]). In our study there was an age difference of five months or more between our older rats and our younger ones. Despite this, the results were relatively consistent, suggesting a robust phenomenon. Note that at doses of 40, 50, 60, 70, and 80 mg/kg the sex difference in sleeptimes was statistically significant despite the age spread. Further, there was no correlation between age and sleeptime. While only a parametric study would answer the question of a sex-by-age interaction conclusively, there is no evidence to suggest that the sex difference we report only appears in "old age" or is amplified by our rats' ages.

Our results show that there was some interaction between the albino mutation and the sex difference in sleeptime. The possibility of such an interaction was assessable for quadruplets 3–6 and 4–5. The evidence of a sex-albinism interaction was clearer in quadruplet 4–5, where the female-male difference of the albinos and the corresponding difference of the hooded rats could be compared at the 20 mg/kg to 50 mg/kg doses (Fig. 1B). The albinos' sex difference was larger than the hooded rats' sex difference at all four doses (20 mg/kg to 50 mg/kg). Further, the albinos' sex difference grew steadily larger than the pigmented rats' sex difference as dose increased from 20 mg/kg to 50 mg/kg ($p=0.05$, rank correlation coefficient [19]). The evidence of a sex-albinism interaction was less clear in quadruplet 3–6 (Fig. 1A, $p>0.10$, rank correlation coefficient [19]). These limited data lead, at best, to the tentative conclusion that in some genetic backgrounds there may be a sex-albinism interaction. Note, however, that

the albinos were not more susceptible than their pigmented counterparts, as might have been predicted on the basis of an earlier study [18].

There have been periodic warnings [8,16] regarding the use of albino strains as "normal" experimental subjects. The use of strains that are not (possibly ábnormal) albino mutants should be encouraged by our results, as they demonstrate that phenomena such as sex differences in drug response can be observed in non-albinic rats. At the very least, results from albinos should be confirmed in concurrent experiments on non-albinos. In such experiments the albinos and non-albinos should have similar or identical genetic backgrounds (e.g., [22]); otherwise there is a risk that the variables of albinism and strain will be confounded. The importance of careful attention to the genetic background of experimental subjects cannot be overemphasized.

ACKNOWLEDGEMENTS

The authors thank V. Kennaway, Department of Psychology, Arizona State University, for establishing, maintaining, and transferring our breeding program (1977–1980).

REFERENCES

1. Buchel, L. Contribution a l'étude du métabolisme du pentobarbital chez les rongeurs, en fonction du sexe. *Archs Sci. physiol.* **23:** 109–120, 1969.
2. Burek, J. D. and C. F. Hollander. Experimental gerontology. In: *The Laboratory Rat,* vol. 2, *Research Applications,* edited by H. J. Baker, J. R. Lindsey and S. H. Weisbroth. New York: Academic Press, 1980, Chapter 7, pp. 149–159.
3. Buttar, H. S., B. B. Coldwell and B. H. Thomas. Excretion of ^{14}C-pentobarbital and its metabolites into the bile and urine of male and female rats. *Archs int. Pharmacodyn. Thèr.* **208:** 279–288, 1974.
4. Carmichael, E. B. Nembutal anesthesia III. The median lethal dose of Nembutal (pentobarbital sodium) for young and old rats. *J. Pharmac. exp. Ther.* **62:** 284–291, 1938.
5. Cohen, B. J. and N. Hulett. Aging: Rat, part I. Longevity. In: *Inbred and Genetically Defined Strains of Laboratory Animals, Part 1, Mouse and Rat,* edited by P. L. Altman and D. D. Dittmer. Bethesda, MD: Federation of American Societies for Experimental Biology, 1979, Chaper 75, pp. 277–279.
6. Collins, T. B., Jr. The toxic effects of three different dosages of pentobarbital sodium on the Long-Evans rat. *Psychon. Sci.* **15:** 276, 1969.
7. Collins, T. B. and D. F. Lott. Stock and sex specificity in the response of rats to pentobarbital sodium. *Lab. Anim. Care* **18:** 192–194, 1968.
8. Creel, D. Inappropriate use of albino animals as models in research. *Pharmac. Biochem. Behav.* **12:** 969–977, 1980.
9. Fox, R. R. *Handbook on Genetically Standardized JAX Rabbits,* 1st edition. Bar Harbor, ME: The Jackson Laboratory, 1975.
10. Holck, H. G. O. and M. A. Kanan. Sex difference in white rat in tolerance to certain barbiturates. *Proc. Soc. exp. Biol. Med.* **32:** 700–701, 1935.
11. Holck, H. G. O., D. R. Mathieson, E. L. Smith and L. D. Fink. Effects of testosterone acetate and propionate and of estradiol dipropionate upon the resistance of the rat to evipal sodium, nostal, pernoston and pentobarbital sodium. *J. Am. pharm. Ass.* **31:** 116–123, 1942.
12. Homburger, E., B. Etsten and H. E. Himwich. Some factors affecting the susceptibility of rats to various barbiturates: The effect of age and sex. *J. Lab. clin. Med.* **32:** 540–547, 1947.
13. Jarcho, L. W., C. Eyzaguirre and J. L. Lilienthal, Jr. Sex difference in the response of rats to sodium pentobarbital. *Proc. Soc. exp. Biol. Med.* **74:** 332–333, 1950.
14. Kato, R. Sex related differences in drug metabolism. *Drug Metab. Rev.* **3:** 1–32, 1974.
15. Kato, R. and A. Takanaka. Metabolism of drugs in old rats (II) Metabolism *in vivo* and effect of drugs in old rats. *Jap. J. Pharmac.* **18:** 389–396, 1968.
16. Lockard, R. B. The albino rat: A defensible choice or a bad habit? *Am. Psychol.* **23:** 734–742, 1968.
17. Matthews, H. B. A simple method for extending the period of surgical anesthesia in rats. *Lab. Anim. Sci.* **28:** 720–722, 1978.
18. Shearer, D., D. Creel and C. E. Wilson. Strain differences in the response of rats to repeated injections of pentobarbital sodium. *Lab. Anim. Sci.* **23:** 662–664, 1973.
19. Siegel, S. *Nonparametric Statistics for the Behavioral Sciences* New York: McGraw-Hill, 1956.
20. Westenberg, I. S. and J. M. Bolam. Lethal doses of pentobarbital sodium in albino versus pigmented rats: a within strain comparison. *Lab. Anim. Sci.* **31:** 360–361, 1981.
21. Westenberg, I. S. and J. M. Bolam. Onset and duration of response to sodium pentobarbital of pigmented versus albino rats: A within-strain comparison. *Behav. Neural Biol.* **33:** 391–396, 1981.
22. Westenberg, I. S. and R. Pakalnis. Response to pentobarbital of pigmented vs. albino C57BL/6J-c^{2J} mice: A within-strain comparison of sleeptimes and lethal doses. *Behav. Neural Biol.* **27:** 552–557, 1979.