J. Pharm. Pharmac., 1969, **21**, 109–113 *Received July* 15, 1968

Relation of brain sensitivity and hepatic metabolism of hexobarbitone to dose-response relations in infant and young rats*

SARAH C. KALSER, ELEANORE FORBES AND ROSEMARIE KUNIG

Department of Pharmacology, School of Medicine, University of Pittsburgh, Pittsburgh, Pennsylvania, U.S.A.

The dose-response relation to hexobarbitone of infant (5 day) and young (44 day) male rats was examined, and the relative contribution of hepatic metabolism (measured *in vitro*) and changes in brain sensitivity to the overall response were evaluated. The infant rat shows a parallel shift to the left in its dose-response curve with the relative potency of hexobarbitone almost 5 times greater than for the 44 day old animal. The slope of the curves show marked changes at the first lethal dose level of drug. This and other evidence suggest that death may not be merely an extension of the mechanism causing hypnosis. Infant rats exhibited a shorter increment in sleep time for increasing doses of hexobarbitone than is predictable from their low rate of *in vitro* metabolism. Although this is also true for the young rats, the two values are in much closer agreement than for the infant animals. Brain concentrations of hexobarbitone, measured upon regaining of the righting reflex, were lower in the infant than in the young rat. This suggests the central nervous system of the infant rat has an increased sensitivity to the drug.

The low activity of microsomal liver enzymes of newborn guinea-pigs, mice and rabbits was shown by Jondorf, Maikel & Brodie (1958) and Fouts & Adamson (1959). Kato, Vassanelli & others (1964) surveyed the response to, and metabolism of, several drugs including pentobarbitone, in female rats 1–250 days old. They found that hepatic drug-metabolizing activity increased with increasing age to a maximum at 30 days and then slowly declined. This was opposite to the duration of drug response. Catz & Yaffe (1967) observed that the plasma level of hexobarbitone was the same in 2, 3 or 4 week old mice upon awakening even though the duration of hypnosis differed. In contrast, brain pentobarbitone levels were lower in young than in adult rats at time of death (Bianchine & Ferguson, 1967) which they interpreted as indicative of greater sensitivity of young rats to pentobarbitone. However, toxicity and hypnosis may be unrelated properties (Catz & Yaffe, 1967).

EXPERIMENTAL

Dose-response curves for hexobarbitone hypnosis. On each of 3 trials, 5 and 44 day old, fed, male, Sprague-Dawley rats were divided into four groups of 5 animals. For the 5 day old rats, littermates were evenly distributed between the groups; for the 44 day old rats there was even matching of weights in all groups. Hexobarbitone sodium (Winthrop Laboratories, New York, N.Y.) was injected intraperitoneally in the volume of 5 ml/kg in a dose range of 15–480 mg/kg. Since infant rats lose heat rapidly, they were kept at 35°; the older rats were kept at 22°. Sleeping time was

* Requests for reprints to Dr S. C. Kalser, Room 607, Westwood Building, National Institutes of Health, Bethesda, Maryland 20014, U.S.A.

recorded as the time interval between losing and regaining the righting reflex. Animals not showing loss of righting reflex 5 min after injection were decapitated and the brain removed and immediately frozen on dry ice. The brains of rats that died from the hexobarbitone were also frozen at the time of death. All other rats were decapitated at the time of regaining of the righting reflex and then brains were frozen. All brains were kept frozen until analysed one or two days later.

Metabolism. In vitro metabolic activity was measured using the 9000 *g* supernatant from a 20 min centrifugation (5°) of liver homogenate (1 g liver + 2 ml 1·15% KCl). Incubation was for 30 min at 37° under 5% carbon dioxide in oxygen. The final incubation mixture (molar concentrations), in the order added, consisted of: 2·3 ml pH 7·4 phosphate buffer (0·1); 1·0 ml of a mixture of glucose-6-phosphate (0·02), nicotinamide (0·1) and $MgSO_4$ (0·05); 0·2 ml NADP (0·0025); 0·5 ml hexobarbitone sodium (0·006) and 1·0 ml 9000 *g* supernatant. The amount of unchanged hexobarbitone at the end of the incubation was assayed by the method of Cooper & Brodie (1955). Ten livers, each assayed in duplicate, were used for 44 day old rats; 6 pairs of livers, each assayed in duplicate, were used for 5 day old rats.

Brain concentration of hexobarbitone. Brains from all animals used in the 3 trials were assayed for their hexobarbitone content. Each brain was analysed individually for the 44 day old rats; 2–3 brains were needed for each measurement at time of regaining righting reflex for the 5 day old animals. The brain levels at death were high enough to allow single brains to be analysed in all groups. Brains were weighed, homogenized with 0·001 N NaOH (2 ml/g brain), and 3 ml of the homogenate was immediately added to 4·0 ml pH 5·5 citrate buffer (0·5 M). Hexobarbitone was then extracted into heptane containing 1·5% isoamyl alcohol by the same method used for the liver homogenates (Cooper & Brodie, 1955).

RESULTS

Dose-response relation of hexobarbitone. The 5 day old rat showed major differences from the 44 day old rat in its dose-response relation to hexobarbitone

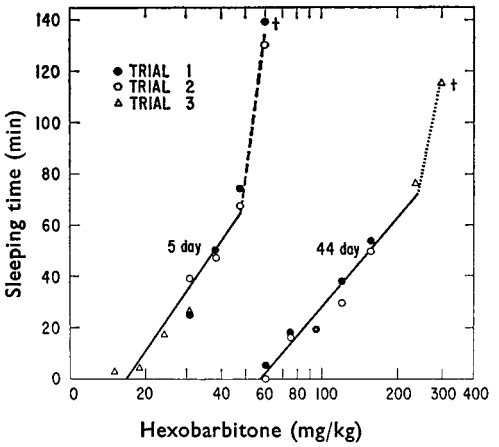

FIG. 1. Dose-response relation for hexobarbitone in 5 and 44 day old rats. Fed, male Sprague-Dawley rats were injected i.p. with 5 ml/kg hexobarbitone sodium in the doses indicated. Sleeping time was measured as the interval between loss and regaining of the righting reflex. Slopes of 140 ± 7·5 (s.e.) and 120 ± 3·7 were obtained for the 5 and 44 day old rats by regression line calculation in the hypnotic range. † Indicates some deaths occurred at that dose. Time till death is not included.

(Fig. 1). Linear regression lines (fitted by least squares; Burn, Finney & Goodwin, 1952) show that the response curves of the two groups are parallel up to the minimal dose causing death, the relative potency of hexobarbitone is about 5 times greater for infant than for young rats, and a distinct change in slope occurs for both age groups at the minimum lethal dose level. Although among 5 day old rats one rat from each litter was in each dose level, there was no rank correlation between littermates for duration of the hypnotic response and no correlation of duration of hypnosis with body weight. The 44 day old rats do not show the latter correlation, either.

Table 1. *Sleeping time*[a] *and brain concentrations*[b] *of hexobarbitone in* 5 *and* 44 *day old rats*

		5 day		44 day	
Dose (mg/kg)	Trial	Sleep time (min ± s.e., n)	Brain concn (μg/g ± s.e., n.)	Sleep time (min ± s.e., n)	Brain concn (μg/g ± s.e., n)
15·0	3	3·2 ± 0·6 (5)	17·6 ± — (1)		
18·9	3	4·4 ± 3·0 (5)	14·9 ± 0·9 (2)		
23·8	3	17·4 ± 3·3 (5)	14·9 ± 2·1 (2)		
30·0	1	25·0 ± 4·0 (4)	19·8 ± 1·4 (4)		
	2	38·8 ± 6·8 (5)			
	3	26·0 ± 1·1 (4)	20·1 ± 1·4 (2)		
37·8	1	50·0 ± 15·2 (5)	21·6 ± 1·7 (4)		
	2	47·0 ± 10·6 (5)			
47·6	1	74·2 ± 13·5 (5)	22·0 ± 1·5 (4)		
	2	67·0 ± 9·8 (5)			
60·0	1	†138·8 ± 14·6 (4)	21·7 ± 1·8 (4)	0 ± 0 (5)	20·6 ± 0·5 (2)
	2	130·4 ± 8·9 (5)		5·4 ± 3·0 (5)	
75·6	1	†227·0 ± 57·0 (2)	25·7 ± 2·6 (2)	17·7 ± 6·7 (3)	28·6 ± 1·8 (7)
	2	†287·3 ± 23·0 (3)		16·4 ± 5·4 (5)	
95·2	1			19·0 ± 1·0 (3)	29·9 ± 0·9 (8)
	2			*32·0 ± 5·9 (5)	
	3			19·2 ± 2·1 (5)	32·9 ± 1·2 (5)
120·0	1			38·0 ± 5·9 (4)	28·8 ± 1·0 (8)
	2			29·0 ± 2·9 (4)	
155·9	1			53·5 ± 4·5 (4)	31·2 ± 1·6 (10)
	2			49·8 ± 3·7 (5)	
240·0	3			75·8 ± 10·1 (5)	31·4 ± 0 (2)
302·4	3			†115·8 ± 23·5 (4)	40·3 ± 3·2 (4)
480·0	3			†55·0 ± — (1)	31·5 ± — (1)

[a] Time between loss and regaining of righting reflex after i.p. administration of hexobarbitone sodium in dose indicated; 5·0 ml/kg injected into fed, male Sprague-Dawley rats.

[b] Brain concentration at time of awakening (regaining of righting reflex). 2–3 brains pooled for analysis of 5 day old; individual brains analysed in adults. Brains from rats not falling asleep analysed separately 5 min after injection; rats dying analysed at time of death.

* Group inadvertently fasted overnight, omitted from Fig. 1.

† Each experiment had 5 rats/group. Where numbers after sleeping times differ from these, either animals died or did not lose their righting reflex by 5 min after the injection.

Brain concentration. Table 1 provides figures for the sleep times and the respective brain concentrations upon regaining of the righting reflex. Six dose levels in the hypnotic range as well as two dose levels at which deaths occurred are included. At doses defining the middle two-thirds of the hypnotic range (23·8–37·8 and 95–156 mg/kg for 5 and 44 day old, respectively), brain concentrations within an age group do not differ with dose. There is a significant difference in the brain concentration of

hexobarbitone between the two age groups; the 5 day old rat awakens at significantly lower brain levels than the 44 day old rat (Table 2). Also in this Table are additional measurements of brain concentrations obtained from the few rats exhibiting only ataxia, and from those rats dying from the injected dose. The infant rat seems to exhibit ataxia and death at lower brain levels than does the 44 day old rat.

Table 2. *Brain concentration of hexobarbitone at various stages of response in 5 and 44 day old rats*

Stage[a]	Brain concentration μg/g ± s.e. (n)	
	5 day	44 day
Ataxic	14·2 ± (1)	20·8 ± 2·34 (4)
Awakening from hypnosis	19·8 ± 0·81 (18)	**29·8 ± 0·80 (42)
Dead	77·9 ± 4·21 (6)	**256·5 ± 13·23 (5)

[a] Fed male Sprague-Dawley rats (mean body weights 11·4–14·8 and 180·8–192·6 g) injected i.p. with varying doses of hexobarbitone sodium in volume of 5 ml/kg. Animals decapitated and brain concentrations of unchanged hexobarbitone estimated: (1), 5 min after injection in animals ataxic but not showing loss of righting reflex; (2), upon awakening (regaining righting reflex), or (3) at time of death. Single brains used for analysis in 44 day old; 2–3 brains pooled for analysis in 5 day old.

** Significantly different from the corresponding concentration in the 5 day old at $P < 0·01$.

Hepatic metabolism, in vitro. Similarly enriched preparations of 9000 *g* liver supernatants from 5 and 44 day old rats were compared for their ability to metabolize hexobarbitone *in vitro*. The respective metabolic rates (μg/g liver in 30 min ± s.e.) were for 5 day rats 98 ± 27 (6 assays), 39 ± 12 (6 assays), while the 44 day old rats showed rate of metabolism 10–25 times greater: 922 ± 46 (10 assays) and 900 ± 123 (2 assays, each of the pooled livers of 3 rats).

*Approximation of LD*50. From doses over the range 60–480 mg/kg the limited data yielded approximations of LD50 values which were 75 and 375 mg/kg for the 5 and 44 day old rats, respectively. The curves appear to be parallel on a semi-log plot, but too few values are available to test for parallelism.

DISCUSSION

No single factor was exclusively responsible for the five-fold increase in potency of hexobarbitone in the 5 versus the 44 day old rats. A slower hepatic metabolism and an increased brain sensitivity exist, but do not account quantitatively for the entire prolongation of sleep time. During maturation, glial cells increase in proportion to neurons and an altered brain sensitivity on this basis is considered later.

To evaluate the role of hepatic metabolism, the *in vitro* results were taken as indicative of relative capacities under the conditions used. A 50 min increment in sleep time, from 10 to 60 min (Fig. 1) is produced by a dose increase of 24 or 120 mg/kg in the 5 or 44 day old rat, respectively. Using the *in vitro* rates of metabolism for the two groups, and the liver weights (3·0 and 4·4 g/100 g for 5 and 44 day old), a time can be calculated of 350 and 90 min to metabolize the doses causing an increased sleep time of only 50 min in the 5 and 44 day old rats, respectively. The discrepancy between the observed and calculated sleep times is obviously much greater in the 5 day old animals. A discrepancy of similar magnitude to that calculated for the 44 day old rats has been noted between *in vivo* and *in vitro* results for pentobarbitone (Kato & Takanaka, 1967).

Although Catz & Yaffe (1967) found similar plasma levels in 2, 3 and 4 week old mice at the time of their awakening from hexobarbitone, we find that the 5 day old rat awakens at a lower brain level than the 44 day old (20 *vs* 30 μg/g). Although it is possible that the lower brain concentration in the infant is due to more difficulty in determining the end point because of their slower emergence from hypnosis, we do not believe this to be so since the standard errors for both age groups are similar. We find that the water content of the infant brain is higher (89 *vs* 78%) and comprises a greater percentage of the body mass (4·4 *vs* 0·9%).

During maturation, the proportion of glial cells to neurons increases from 1 : 1 to 2 : 1 between 5 and 44 days (Brizzee, Vogt & Kharetchko, 1964). If central nervous system sensitivity is determined by the concentration at the neuron and if there is an equal distribution of drug between glial cell and neuron, then the relative brain concentrations found for the 44 and 5 day old rats (30 and 20 μg/g) would represent equivalent neuron concentrations of hexobarbitone.

The sharp change in slope at the minimal lethal dose (Fig. 1) and the differing ratios between lethal and hypnotic brain concentrations for 4 and 44 day old rats (4 : 1 and 8 : 1) suggest that the toxic effect may not be merely an extension of the hypnotic effect. Catz & Yaffe (1967) have made such a suggestion on the basis of relative differences in hypnosis and toxicity between strains of mice. The enhanced toxicity of the 5 day old seems at variance with the known resistance of infants of many species to withstand anoxia (and hence the respiratory depression caused by the barbiturates) better than the older animal (Fazekas, Alexander & Himwich, 1941). This protective effect was evident in the delayed time to death of the 5 day old (13–83 min) compared with the 44 day old (3–10 min) rather than in the brain concentration at death (78 *vs* 256 μg/g).

Acknowledgements

This study was supported in part by Grant AM—07702 from the National Institutes of Health, Bethesda, Md. We appreciate the diligent research effort of the following students who made the preliminary experiment of this series: A. Buerkle, S. Herman, R. Johnston, S. Landay, P. Simpson and R. Wasserman.

REFERENCES

Bianchine, J. R. & Ferguson, F. C., Jr. (1967). *Proc., Soc. exp. Biol. Med.*, **124**, 1077–1079.

Brizzee, K. R., Vogt, J. & Kharetchko, X. (1964). *Prog. Brain Res.*, **4**, 136–144.

Burn, J. H., Finney, D. J. & Goodwin, L. G. (1952). *Biological Standardization*, p. 51–54, Oxford Univ. Press.

Catz, C. & Yaffe, S. J. (1967). *J. Pharmac. exp. Ther.*, **155**, 152–156.

Cooper, J. R. & Brodie, B. B. (1955). *Ibid.*, **114**, 409–417.

Fazekas, J. F., Alexander, F. A. D. & Himwich, H. E. (1941). *Am. J. Physiol.*, **134**, 281–287.

Fouts, J. R. & Adamson, R. H. (1959). *Science, N.Y.*, **129**, 897–898.

Jondorf, W. R., Maickel, R. P. & Brodie, B. B. (1958). *Biochem. Pharmac.*, **1**, 352–354.

Kato, R., Vassanelli, P., Grontino, G. & Chiesara, E. (1964). *Ibid.*, **13**, 1037–1051.

Kato, R. & Takanaka, A. (1967). *Jap. J. Pharmac.*, **17**, 207–217.