Jumping Activity Induced by Sodium 5-(1,3-Dimethylbutyl)-5-Ethyl Barbiturate

II. The Effects of Age Difference (34570)

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Sodium 5-(1, 3-dimethylbutyl)-5-ethyl barbiturate (DMBEB) is a potent convulsant barbiturate which is closely related in structure to two commonly used depressant barbiturates, pentobarbital and amobarbital. We have previously reported (1) that DMBEB given (in appropriate doses) to 1-month-old mice produced jumping activity which was not accompanied or followed by convulsions. In higher doses, however, the drug produced jumping together with clonic and eventually tonic convulsions.

In these experiments, it was noted that doses of DMBEB which produced jumping in young mice were relatively ineffective in older animals. The present study is a comparison of the effects of DMBEB in 1-month- and 9-month-old mice.

Methods and Materials. Experiments were performed on male mice from two random-bred strains, ICR (Hazleton Research Animals, Inc., Burtonsville, Md.) and NIH. Mouse weights were: ICR strain, 15–28 g at 1 month and 30–55 g at 9 months; NIH strain, 15–22 g at 1 month and 24–40 g at 7.5 to 9 months. Mice were fasted for 3 hr before administration of drugs.

Jumps were counted while the mice were in individual compartments of an apparatus originally designed for measuring confinement motor activity of rats (2), as described in a previous publication (1). In this apparatus, counts were recorded when a mouse interrupted either of two photoelectric beams 8.5 cm above the floor of its compartment. For small mice, the recorded counts were corrected by adding visually-observed jumps which failed to interrupt either photoelectric beam. For large mice, the recorded counts were corrected by subtracting counts made by exploratory up and down movements, without jumping, which interrupted one of the photoelectric beams. Jumping scores represent the total number of jumps during the first 20 min after administration of DMBEB.

All comparisons were made in balanced randomized blocks of 12 mice, 6 young and 6 old. The data in Table I were obtained in 12 blocks, with doses of 12.5, 15.0, 17.5, 20.0, 22.5, and 25.0 mg/kg compared in 1-month- and 9-month-old NIH mice in each block. The data in Table II were obtained in 4 blocks; in each block, two 1-month- and two 7.5-month-old NIH mice received each of the following treatments: saline 10 min before DMBEB, 22.5 mg/kg; propranolol, 30 mg/kg, 15 min before DMBEB; and chlorisondamine, 3 mg/kg, 5 min before DMBEB.

For statistical analyses, the jumping scores were transformed into their square roots, in order to stabilize the variance, as required for the analysis of variance. The mean values of the square roots of the jumping scores were tested for statistically significant differences by Tukey's method of multiple comparisons, as explained by Scheffé (3). The 2 $\times$ 2 contingency tables were tested for association between age and effect (number of jumping mice, number of convulsing mice, number of deaths), using chi-square when more than 40 animals were in each age group and using Fisher's exact test (4) when fewer animals were involved.

Brain levels of DMBEB were measured by

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TABLE I. The Effect of Age on Jumping, Convulsions, and Death After DMBEB.

Age (months)	12.5–20.0 mg/kg of DMBEB					22.5–25.0 mg/kg of DMBEB				
			Percentage of mice					Percentage of mice		
	n	Square root of jumping score[a]	Jumps	Convulsions	Deaths	n	Square root of jumping score[a]	Jumps	Convulsions	Deaths
1	48	4.17	72.9	12.5	10.4	24	5.72	70.8	54.1	33.3
9	48	1.54[b]	45.8[c]	0[c]	0	24	7.83	91.7	66.7	66.7[c]

[a] Jumping scores were measured in 4 blocks, each containing 6 young and 6 old mice. Each mouse in each block was injected with one of the following doses of DMBEB (mg/kg): 12.5, 15.0, 17.5, 20.0, 22.5, and 25.0.

[b] $p < .01$.

[c] $p < .05$.

the method described for pentobarbital by Brodie *et al.* (5), as modified by Vesell (6). Each determination was made on one mouse brain.

All drugs were injected intraperitoneally. Doses are expressed in terms of the salts.

Samples of DMBEB were gifts of Dr. Irwin H. Slater (Eli Lilly Research Labs) and the late Dr. Gordon Alles. The two samples were shown to be identical chemically and in biological action, as reported elsewhere (1). The manufacturers kindly supplied the blocking drugs as follows: propranolol hydrochloride, Ayerst Labs., and chlorisondamine chloride, Ciba Pharmaceutical Co.

Results. Saline-treated young and old mice did not jump under our experimental conditions. Therefore, Tables I and II do not include saline-treated controls. Table I shows that DMBEB produced jumping in both young and old mice of the NIH strain, but the scores after the smaller doses (12.5–20.0 mg/kg) were greater in the 1-month-old mice than in 9-month-old mice. Similar results were observed with another random-bred strain (ICR). With higher doses (22.5–25.0 mg/kg), however, the jumping scores were about the same in young and old mice (Table I). This indicates that 9-month-old mice were able to jump as well as 1-month-old mice.

A number of mice did not jump at all. However, jumping occurred in more than 70 % of the young mice, with both smaller doses (12.5–20.0 mg/kg) and larger doses (22.5–25.0 mg/kg). In contrast, only 46 % of the old mice jumped after the smaller doses, but after the larger doses, the percentage of jumpers doubled (Table I).

With doses from 12.5–20.0 mg/kg in 48 young mice, DMBEB produced convulsions in 6 mice and 5 deaths. The same doses in 48

TABLE II. Inhibition by the *Beta*-Adrenergic Blocking Agent, Propranolol and the Ganglionic Blocking Agent, Chlorisondamine, of the Effects of DMBEB in Young and Old Mice.

Age (months)	Saline 10 min before DMBEB			Propranolol (20 mg/kg) 15 min before DMBEB			Chlorisondamine (3 mg/kg) 5 min before DMBEB		
	Jumps[a]	Convulsions[b]	Deaths[b]	Jumps[a]	Convulsions[b]	Deaths[b]	Jumps[a]	Convulsions[b]	Deaths[b]
1	6.39	6/8	4/8	2.23[c]	1/8[c]	0/8	0.48[c]	0/8[c]	0/8
7.5	7.32	2/8	1/8	0.58[c]	0/8	0/8	1.26[c]	0/8	0/8

[a] Mean of square roots of jumping scores. The three treatments (saline + DMBEB, propranolol + DMBEB, and chlorisondamine + DMBEB) were compared in 4 blocks, each containing 6 young and 6 old mice.

[b] The numerators give the number of mice showing the effect. The denominators give the number of mice receiving the treatment.

[c] Significantly less than value for saline + DMBEB, $p < .05$.

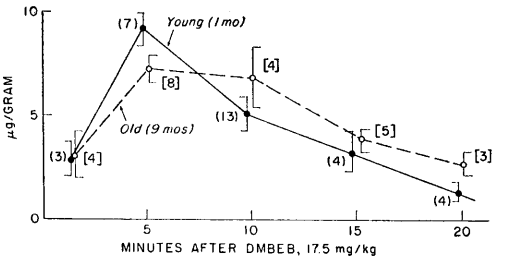

FIG. 1. Brain levels of DMBEB in 1-month- and 9-month-old mice during the first 20 min after ip injection of 17.5 mg/kg. The number of brains analyzed is indicated in parentheses. The brackets show ± SE.

old mice produced neither convulsions nor deaths. The jumping induced by doses from 22.5 to 25.0 mg/kg was intermixed with convulsions in 50–70 % of the mice, regardless of age (Table I). In the older animals, the convulsions were almost always followed by death, but about ⅓ of the convulsing young mice survived.

The failure of many old mice to jump after the smaller doses of DMBEB (Table I) was not due to lower levels of the barbiturate in the brain, since the DMBEB levels in the brain were similar in 1-month- and 9-month-old mice, following the administration of 17.5 mg/kg (Fig. 1).

We have previously reported that *beta*-adrenergic and ganglionic blocking agents greatly diminish or abolish the jumping activity induced by DMBEB in 1-month-old mice (1). Propranolol, a *beta*-adrenergic blocking agent, and chlorisondamine, a ganglionic blocking agent, were effective in both young and old mice (Table II). In addition to blocking jumping, propranolol and chlorisondamine also effectively prevented convulsions and deaths from a dose of 22.5 mg/kg of DMBEB.

Discussion. The lesser frequency of jumping in old mice after the smaller doses (12.5–20.0 mg/kg) of DMBEB cannot be explained by the sluggishness of their neuromuscular performance, since with larger doses (22.5–25.0 mg/kg) their jumping scores did not differ significantly from those of 1-month-old mice (Table I).

Other investigators have reported changes in drug responses with age, which they usually attributed to changes in drug metabolism or in receptor sensitivity (7). Since the side-chain oxidation of barbiturates by the microsomal enzymes is well-developed at the age of 1 month (8), one would not expect DMBEB metabolism to be different in young and old animals. The fact that DMBEB brain levels are similar in 1-month- and 9-month-old mice also shows that there is no difference in the blood–brain barrier between the two age groups. The difference in performance between the age groups does not reflect an incomplete differentiation of the central nervous system in the 1-month-old animals. "Taking both chemical and structural features into consideration, it appears that the critical period for neuronal maturation falls within 10 to 15 days postpartum" in mice (9). The changes with age reported in this paper cannot be explained, therefore, in terms of the incomplete development in the younger animals of the nervous system, the blood–brain barrier, or of drug metabolism.

Carmichael (10) reported that seasonal variations in the response of rats to a depressant barbiturate, vinbarbital, were more important than variations due to age; however, his data indicate that 9-month-old rats are more susceptible to this drug than young adults. We found that the younger animals often survived convulsions, while the older mice died. Pylkko and Woodbury (11) demonstrated an increase in the convulsant and lethal doses of strychnine and brucine with increasing age. In our experiments, convulsions and deaths occurred after the smaller (12.5–20.0 mg/kg) doses of DMBEB in more than 10 % of the young mice, but in none of the old mice.

The reduction in jumping scores and protection against convulsions and deaths by the *beta*-adrenergic blocking agent, propranolol, and by the ganglionic blocking drug, chlorisondamine, implicate the sympathetic nervous system in all the effects produced by DMBEB. The lesser jumping activity of old mice after the smaller doses of DMBEB indicates a decline of sympathetic functioning with aging.

Summary. In small doses (12.5–20.0 mg/kg ip), DMBEB produced greater jumping activity in 1-month- than in 9-month-old mice, both in the rates of jumping as well as in number of jumping mice. There were no appreciable differences between young and old mice with large doses (22.5–25.0 mg/kg). The brain levels of DMBEB were similar in young and old mice. Jumping was markedly reduced in old and young mice by the *beta*-adrenergic blocking agent, propranolol, and the ganglionic blocking agent, chlorisondamine. Both propranolol and chlorisondamine prevented convulsions and death from a large dose of DMBEB (22.5 mg/kg).

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