GENETICS

Audiogenic Epilepsy in Young Mice of Different Strains after Neonatal Semax Treatment

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Neonatal (from day 2 to day 7 of life) injection of neuropeptide semax to mice of 5 inbred strains significantly reduced predisposition to audiogenic epilepsy in only one-month-old DBA/2J mice, which manifested in changes in the mean audiogenic sensitivity score and percentage of animals dead as a result of acoustic stimulation.

Key Words: *mice; inbred strains; audiogenic epilepsy; semax; neonatal exposure; delayed effects*

Neuropeptide semax (synthetic analog of $ACTH_{4-10}$ fragment) administered to newborn rats (KM, WAG/Rij, and Wistar strains) significantly (though slightly) modified the pattern of audiogenic seisures in adult animals [1]. Such a sign as seizure in response to a loud sound is also characteristic of some mouse genotypes, for example, DBA/2J and 101/HY [4,8]. Seizures of this kind are widely used as a model of convulsive states in humans. It is known that genetic determination of liability to audiogenic epilepsy is different in mice and rats [5,8]. The study of the possibility of modulating an audiogenic convulsive attack in mice by semax treatment during the neonatal period can provide information about the mechanisms of the development of audiogenic seizure in animals of different genotypes, as the picture of its inheritance is different in mice of different strains [4,8].

We studied the severity of audiogenic attacks after neonatal semax treatment in mice of several genotypes, specifically, in inbred mice with different age-specific dynamics of the formation of audiogenic epilepsy [4].

MATERIALS AND METHODS

The study was carried out on 220 mice (42 DBA/2J mice: 30 males, 12 females, 9 litters; 38 CBA/Lac/Sto mice: 23 males, 15 females, 6 litters; 62 101/HY mice: 30 males, 32 females, 11 litters; 45 C3H mice: 19 males, 26 females, 9 litters; and 33 C57Bl/6J mice: 22 males, 11 females, 5 litters. The animals received standard fodder (MEST) and water *ad libitum*. All manipulations on mice were carried out in accordance with "Regulations for Studies with the Use of Experimental Animals" (Order of the Ministry of Health of the USSR No. 755 of August 12, 1977).

Starting from days 2 or 3 of life, the rats were subcutaneously (in the withers) injected with semax (Center of Thermobiotechnology and Molecular Diagnosis, Institute of Medical Genetics, Russian Academy of Sciences) in a dose of about 3.5 mg/kg (7 μg in 7 μl saline, 5 days). Intact animals or animals injected with the same volume of saline (neonatal painful stimulation control) served as controls.

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Predisposition to audiogenic epilepsy was detected at the age of 1 month. Each animal was placed into a box with noise-proof walls and subjected to audioexposure (100 dB). The duration of the signal was 20 sec for animals exhibiting no audiogenic seizure (AS). If AS developed, the sound was switched off as soon as the convulsions emerged. The intensity of AS was evaluated in points: 0 points: startle reaction, no AS; 1: motor excitement phase of AS, interrupted by "inhibitory pause", which could be followed by repeated motor excitement; 2: motor excitement without inhibitory pause; 3: muscle convulsions after motor excitement phase; and: 4 AS eventuating in death.

The data were processed by bifactorial aþ"lysis of dispersions (Statistica 6.0) with inserted LSD function. Alternative subgroups in the groups were compared using Fisher's φ method [3].

RESULTS

Three-factor ANOVA analysis showed that the gender was inessential for AS. Hence, the results in males and females were analyzed together. Two-way ANOVA (factor 1: genotype, factor 2: exposure) detected a significant effect of factor 1 ($F_{1,4}$= 32.33; p=0.0000) and a trend to a significant effect for factor 2 ($F_{1,2}$=2.92; p=0.056), as well as a significant effect of interaction of the factors ($F_{1,8}$=6.86; p=0.0000). This means that the type of delayed effect of neonatal semax treatment on AS is determined by the genotype.

The means of the studied sign in mice of 15 groups are presented in Table 1. Despite the fact that of the five studied strains predisposition to audiogenic epilepsy is described for DBA/2J and 101/HY [4], neonatal semax and painful stimulation sharply modified it only in DBA/2J mice, while in 101/HY animals the mean seizure score was virtually the same in 3 groups. In CBA mice, neonatal injection of semax stimulated (negligibly, at the level of a trend) seizure predisposition, in other words, the effect of this neonatal treatment was opposite to that in DBA/2J mice, which was seen also from the significance of the effect of interaction of two factors according to ANOVA (F=13.4; p=0.0000).

AS in response to a loud acoustic stimulus caused death of some animals (though the sound was switched off directly after the start of convulsions). The mortality was significantly higher in the DBA/2J group (n=21) than in groups of other strains (n=6). Higher mortality in DBA/2J mice seems to be due to the fact that testing was carried out at the age when the analyzed sign was maximally pronounced. Of 21 dead mice of this strain, 11 (58%) were from the group exposed to painful stimulation, 7 (26%) from intact group, and 3 (16%) were injected with semax neonatally. The differences in mortality (in %) between the groups were highly significant (p<0.01 and p<0.001, φ method). In DBA/2J mice exposed to painful stimulation alone during the neonatal period, mortality was very high, but semax injection (also associated with painful sensations in a newborn animal) reduced significantly this value. Delayed effects of neonatal painful stimulation on threshold painful sensitivity and behavior of mice of different genotypes were described previously [2].

Mice of DBA/2J strain differ from animal strains insensitive to acoustic signal by several neurochemical characteristics [8]. One of these characteristics is the level of cerebral neurotransmitter metabolism [7]. On the other hand, 101/HY mice are little studied as the object of research in physiological experiments, in contrast to DBA/2J strain. Animals of 101/HY strain differ from CBA mice (resistant to acoustic exposure) by the content of dopamine and particularly serotonin in the hippocampus and brain stem. Differences in cerebral monoamine metabolism indexes [6] and in reaction of these parameters to neonatal injection of an $ACTH_{4-10}$ fragment were detected. Presumably, the neuroprotec-

TABLE 1. Severity of AS (Mean Score) in 1-Month-Old Intact Mice and in Animals Neonatally Injected with Saline or Semax

Strain	Intact		Saline		Semax	
	n	AS score	*n*	AS score	*n*	AS score
DBA/2J	12	2.00±0.29	11	4.00±0.30*	19	1.16±0.23*+
CBA	28	0.84±0.24	7	0.08±0.26	6	1.21±0.18
101/HY	15	0.95±0.19	18	0.96±0.18	29	1.21±0.19
C3H	14	1.21±0.26	16	0.08±0.26	15	0.85±0.25
C57Bl/6	7	0.00±0.38	14	0.00±0.27	12	0.08±0.26

Note. p<0.001 compared to: *intact group, +animals injected with saline.

tive effect of semax on the developing brain of young DBA/2J and 101/HY mice differ, because the neurophysiological (and presumably neurochemical) substrata of AS formation in response to a loud acoustic signal differ in these strains. Presumably, the main role in AS origination in 101/HY mice belongs not to the monoaminergic groups of neurons involved in its formation, but to some other neurons (for example, GABA- and glutamatergic). The disorders in the stem GABAergic system in 101/HY animals are confirmed by their abnormal reaction (development of strong tonic convulsions) in response to barbiturate injection. Hence, it seems that the neurological substrates maintaining high excitability of stem mechanisms responsible for AS development in rodents of the two strains are different.

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