EXPERIMENTAL GENETICS

Audiogenic Epilepsy in Mice with Different Genotypes after Neonatal Treatments Enhancing Neurogenesis in Dentate Gyrus

T. V. Timoshenko, O. V. Perepelkina, N. V. Markina, A.V. Revischin*,, G. V. Pavlova*,**, I. I. Poletaeva, and G. T. Sukhih****

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Pups of Wistar and KM rats (with predisposition to audiogenic epilepsy) were daily injected with neuropeptide semax (50 mg/kg) or NO-synthase inhibitor L-NAME (50 mg/kg) on days 7-11 of life. Alterations of audiogenic seizures pattern were revealed in rats of both strains at the age of 1 month, while changes in seizure severity were genotype-dependent. Both agents enhance neurogenesis in the dentate gyrus of the hippocampus and the delayed effect in the form of altered seizure pattern seems to be determined by this factor. Genotype-dependent alterations of seizure severity after administration of semax and L-NAME were differently directed. These effects are suggested to be underlined by physiological and biochemical mechanisms not related to the intensity of postnatal neurogenesis.

Key Words: *audiogenic epilepsy; semax, L-NAME; neonatal exposure; neurogenesis*

Normal development of CNS in the early postnatal period can be altered by external influences (of chemical of physical modalities) in such a way that their consequences can be revealed in the brain of adult animal. It was confirmed by numerous recent experimental data on delayed effects of administration of some pharmacological agents to rat and mouse pups [3-5].

There is multiple experimental evidence for the phenomenon of delayed effects of neonatal administration of biologically active compounds on CNS function in adult organism [1,2]. However, the possible role of genotype in the formation of delayed effects of early exposure is practically not taken into account in these studies. There are few studies that analyze the interstrain differences of such effects [1,2,7]. Apart from the necessity to find specific ways of implementation of delayed effects of early exposures, the possibility of detecting a common mechanism responsible for these effects should be analyzed. One of possible mechanisms is alterations of postnatal neurogenesis, *i.e*. proliferation of precursor cells in two major proliferative forebrain regions, formation of new neurons, and their incorporation into neuronal circuits [10,11]. The intensity of cell proliferation in mammalian brain can be modulated by regulating activity of NO-synthases, which are known to inhibit proliferation. For

Biological Faculty, M. V. Lomonosov Moscow State University; *Institute of Gene Biology, Russian Academy of Sciences; **V. I. Kulakov Research Center of Obstetrics, Gynecology, and Perinatology, Federal Agency of High-Technology Medical Service, Moscow, Russia. ***Address for correspondence:*** inga@protein.bio.msu.ru. I. I. Poletaeva

example, cell proliferation can be stimulated with L-NAME (N-nitro-L-arginine methyl ester) which inhibits NO-synthases [13]. Specific mechanism underlying the stimulating effect of reduced NO production on proliferation of neuronal and glial precursor cells are still unknown. On the other hand we have recently demonstrated [7] that heptapeptide semax also enhances cell proliferation in the dentate gyrus of rat hippocampus.

In this study we used these compounds as agents enhancing postnatal cell proliferation in the brain in order to estimate to which extent the delayed effects of their administration would affect the development of audiogenic epileptic seizure in rats of two genotypes.

MATERIALS AND METHODS

Male and female KM (highly predisposed to audiogenic epilepsy) and Wistar (low predisposition) rats were used in the study. At the age from 7 to 11 days the animals daily received 50 mg/kg L-NAME (32 KM and 14 Wistar rats) or 50 mg/kg semax (9 KM and 12 Wistar rats); 39 KM and 13 Wistar rats were used as intact controls. Upon reaching the age of 27-28 days, male and female rats were separated and kept in T3 cages (5-6 animals per cage) with free access to water and food (Laboratorkorm). All manipulations were conducted according to EU Directive 86 rules.

Predisposition to audiogenic epilepsy was detected at the age of 1 month by exposure to a buzzer (120 dB). Animal reaction to the buzzer switches on, *i.e.* the presence of startle reaction (wincing) and AS, were registered visually. The intensity of AS was scored in arbitrary points: for the startle reaction 0: no reaction, 1: weak startle reaction, 2: wince of all muscles of the body, 3: startle and jump, for AS 0: no AS, 1: motor excitement, 2: clonic convulsion, 3: clonicotonic seizures, 4: tonic seizures [6]. AS latency was also recorded, in the absence of AS the buzzer was switched off in 60 sec.

Statistical analysis of the AS intensity was done using two-way and three-factor ANOVA (Statistica 6.0) with consequent *post-hoc* analysis (LSD test). In some cases a nonparametric test (median test) was used.

RESULTS

Three-factor ANOVA (strain, sex, exposure were factors 1, 2, and 3) revealed no significant effects of sex on AS parameters in KM and Wistar rats. For further analysis, pooled data on AS parameters in males and females were used. Two-way ANOVA for AS intensity (arbitrary score) demonstrated significant effect of the strain factor ($F_{1\text{-}96}$=74.585, p=0.0000) and a tendency for interaction of the strain and exposure factors ($F_{1\text{-}96}$=2.578, p=0.0804). These data indicate that neonatal administration of semax and L-NAME causes differently directed alteration of AS score in KM and Wistar rats (Fig. 1). Nonparametric median test revealed significant effects of early exposures in KM rats (χ^2=15.27, df=2, p=0.0005). LSD analysis showed that AS intensity was significantly (p=0.0008) higher in KM rats with early semax administration compared to intact control group (2.16±0.15 and 1.46±0.13, respectively). KM rats receiving L-NAME had higher AS score than intact animals, but the difference was insignificant. In Wistar rats, changes in AS intensity after neonatal exposures were insignificant. At the same time, the mean AS score in Wistar rats was significantly (p=0.0000) lower than in KM rats (in intact control groups 0.31±0.23 and 1.46±0.13, respectively).

Two-way ANOVA of AS latencies revealed significant effect of both factors (strain: $F_{1\text{-}96}$=76.01, p=0.0000, and exposure: $F_{1\text{-}96}$=18.74, p=0.0000). In KM rats only a tendency for latency reduction as a consequence of early exposure was revealed (LSD test). It should be noted that the population of Wistar rats with AS was not large, therefore these data were analyzed using a nonparametric test. Median test showed significantly longer latency (χ^2= 6.00, df=2, p=0.0498) in Wistar rats after neonatal administration of semax and L-NAME than in intact rats from this population (Fig. 2).

At the same time, AS changes in 1-month-old rats after the specified early exposures can not be reduced to changes of AS intensity. In the group

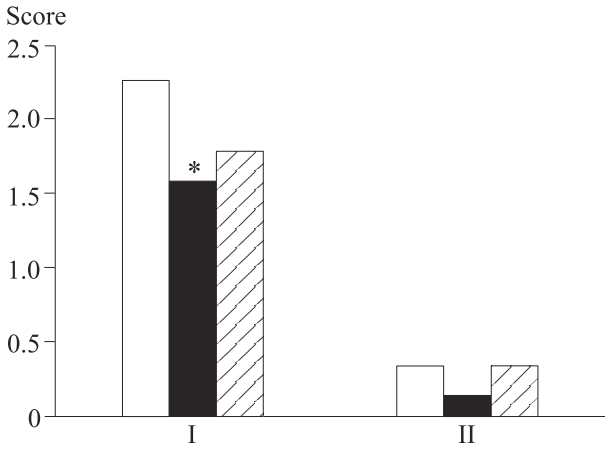

Fig. 1. AS strength in KM and Wistar rats. Here and of Fig. 2: I: KM rats, II: Wistar rats. Light bars: intact rats, dark bars: semax administration, dashed bars: L-NAME administration. *p<0.001 compared to intact rats.

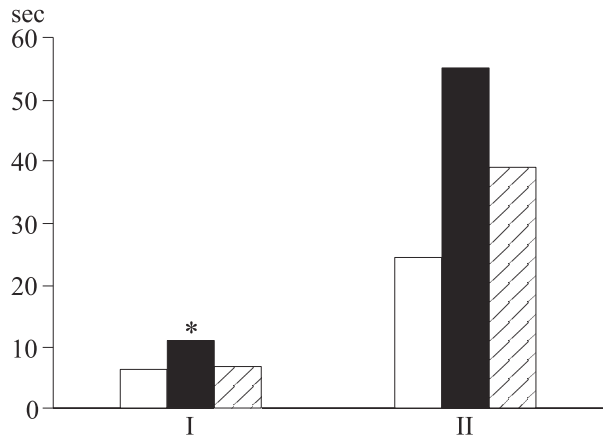

Fig. 2. Mean latency of AS in KM and Wistar rats. *$p<0.057$ compared to intact animals.

of KM rats with early semax administration, 3 of 20 animals died after seizure (during the period up to 12 h), while no deaths occured among the KM rats receiving L-NAME and control animals. There were no animal deaths among Wistar rats either.

Thus, results of two-way ANOVA and more detailed *post-hoc* data analysis revealed both interstrain differences in the effect of early exposure and differently directed changes of AS pattern after administration of semax of L-NAME. At the same time, the pattern of the effects of both exposures had a common component: alteration of the phenotypic seizure pattern. In groups subjected to early exposures, switching on the buzzer induced typical for AS motor excitement (clonical running), and then after a short phase of ordinary clonic muscle contractions the seizures had a non-typical character: there appeared a pronounced cataleptic component when the animal froze in an unnatural posture with muscular seizures retention. In some cases, the seizure developed when the animal had "down on the back" posture (which had never been observed either in intact rats or after administration pharmacological agents).

The intensity of acoustic startle reaction was also altered as a result of early semax and L-NAME administration: two-way ANOVA revealed a significant effect of the strain factor ($F=11.30$, $p=0.001$), exposure factor ($F=9.19$, $p=0.0002$) and a significant interaction of these factors ($F=5.40$, $p=0.0058$). It was more pronounced in Wistar rats and was practically not altered by early exposures (2.08±0.28, 2.28±0.27, 2.00±0.29). In KM rats, early semax administration significantly enhanced (compared to intact group, $p=0.0089$), while administration of L-NAME significantly inhibited ($p=0.0002$) this response. It can be stated that alterations of the startle response in the studied groups were generally in parallel with differences in AS indices. This picture can be quite logically explained by the involvement of the inferior (as well as superior) colliculi of the corpora quadrigemina, which possess altered physiological properties in rats predisposed to AS [9].

We previously demonstrated that semax and L-NAME administration at different periods of early ontogeny (first 2 postnatal weeks) stimulates cell proliferation in the dentate gyrus of the hippocampus in later periods of life, after termination of direct exposure to these compounds. At the same time, no interstrain difference in the intensity of this modulatory influence was revealed [7]. This study showed that the altered seizure pattern, which has never been observed in intact animals, was the common delayed effect of the specified exposures in KM and Wistar rats. These data suggest that altered processes of proliferation and probably appearance of excessive (compared to normal) amounts of cells with glial and neuronal phenotype determine changes in the function of motor centers which participate in AS genesis. Since both occurrence of cataleptic seizure component and alteration of muscular tonus causing turn on the back were typical of animals from experimental groups, it can be expected that not only the brainstem regions important for AS development were involved in these changes, but also basal ganglion structures, in particular, the striatum. There are published data on enhanced haloperidol catalepsy after acute and chronic L-NAME administration to adult animals [12], and this effect is usually attributed to alteration of CNS excitability under conditions of reduced NO level in the brain. It can be supposed that changes in motor activity after AS development in our experiments are mediated by enhancement of neurogenesis (*i.e.* appearance of new cells) and may not be directly coupled with changes in NO level. This hypothesis is supported by the delayed effect (changes in seizure motor activity) relative to the moment of drug administration, *i.e.* from the NO level decrease, while semax was demonstrated to inhibit NO production only at the moment of its application in the course of ischemia [8].

The pattern of differently directed AS changes revealed in 1-month-old animals after early semax (increase of AS score) or L-NAME (decrease of AS score) administration indicate that there might exist some still unknown alternative pathways in the action of these substanses in developing brain. Since KM and Wistar rats differ by the level of predisposition to audiogenic epilepsy and probably in many other features [6], it cannot be excluded that the interstrain differences are responsible for the differences in the above-described effects.

Study of neurogenesis in adult brain and its possible modulation, e.g. by administration of neurogenesis-enhancing drugs, is both of theoretical and significant practical importance in the context of developing technologies of application of stem cells (including neural stem cells) in the medicine. Our findings attest to the existence of a genetic component in CNS response to such exposures as well as of delayed effects of exposures which possess neurogenesis-enhancing properties.

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