ISSN 1607-6729, Doklady Biochemistry and Biophysics, 2018, Vol. 478, pp. 47–49. © Pleiades Publishing, Ltd., 2018.
Published in Russian in Doklady Akademii Nauk, 2018, Vol. 478, No. 6, pp. 712–715.

BIOCHEMISTRY, BIOPHYSICS, AND MOLECULAR BIOLOGY

Audiogenic Epilepsy and Structural Features of Superior Colliculus in KM Rats[1]

A. V. Revishchin[a], *, G. M. Solus[a], I. I. Poletaeva[b], and G. V. Pavlova[a]

Presented by Academician P.G. Georgiev June 20, 2017

Received October 2, 2017

Abstract– Using immunoblotting, we showed that in rats of audiogenic epilepsy (AE) prone strain (Krushinsky-Molodkina, KM) the superior colliculus tissue (SC) contains significantly less quantity of glial neurotrophic factor (GDNF), beta-tubulin and actin in comparison to the same brain region in "0" rats, non-prone to AE. This fact led to the suggestion that the histological structure of the SC in KM rats could differ significantly from that of the "0" strain. Using neuromorphology technique, we demonstrated that the total number of SC cells, as well as the number of neurons were significantly less in KM rats than in the "0" strain rats. Particularly strong differences were found in the deep layers of SC, the area of terminals from IC. Further studies of the midbrain structures, will help to identify the novel aspects of neural networks, involved in the genesis of AE in rats of KM strain.

DOI: 10.1134/S1607672918010155

Rodent refractory audiogenic epilepsy (AE) is an important and fruitful model for studying the mechanisms of epileptogenesis and for testing new antiepileptic drugs. Krushinsky–Molodkina rats (KM), which almost 100% react to loud sound by intense seizures, were successfully used in evaluation of anticonvulsant effects [1–3, 9], studies of genetic [4], physiological [6, 7] and morphology [8] foundations of this pathological trait.

Previously immunohistochemical studies demonstrated [9, 10] that in the GEPR rat strain, also selected for AE, the number of small (<15 μm in diameter), medium (15–20 μm), and large (>25 μm) GABAergic neurons in the inferior colliculus (IC) were significantly larger than that in animals of the basic strain, used for selection which had no AE [10]. In our study, the IC of Krushinskii–Molodkina (KM) rats the number of GABAergic neurons was not higher in comparison with the rats of the control "0" strain without AE [8]. At the same time, the expression level of glutamate-decarboxylase mRNA in KM rats was five times higher than in rats "0" [8]. One may conclude that the GABAergic system role in the genesis of AE proneness in KM and GEPR strains could be realized on the basis of different morphofological and physiological intricate characteristics.

According to the simplified scheme of AE seizure generation, the pathological excitation, which starts in the auditory nuclei of the brain stem, via the IC, and then the superior colliculus (SC), arrives into the pontine reticular nucleus and reaches the spinal cord by the reticulo-spinal tract, realizing as AE seizures [11]. The study of the c-fos gene expression in GEPR rats showed the participation of SC in the transmission of epileptic activity from IC [12]. The development of AE seizures involves also the periaqueductal grey matter (PAG), SC and the substantia nigra projections into the tectum [12]. Thus, the manifestation of epileptic activity may be the result of a mismatch in the activity of interconnected midbrain structures. If this is true, AE prone animals could reveal the significant deviations in the morphology and function in each of these structures.

The goal of this study was to compare the molecular and morphological characteristics of SC in KM rats and in control animals of the closely related "0" strain non-prone to AE. This "0" strain was selected for the lack of AE from the population of F2 hybrids of KM inbred strain and Wistar rats non-prone to AE [13]. This study was initiated by the quantitative (electrophoretic) evaluation of glial neurotrophic factor (GDNF), tubulin and actin in the midbrain of KM and "0" rats.

[1]The article was translated by the authors.

[a] *Institute of Gene Biology, Russian Academy of Sciences, Moscow, 119334 Russia*

[b] *Faculty of Biology, Moscow State University, Moscow, 119992 Russia*

**e-mail: revishchin@mail.ru*

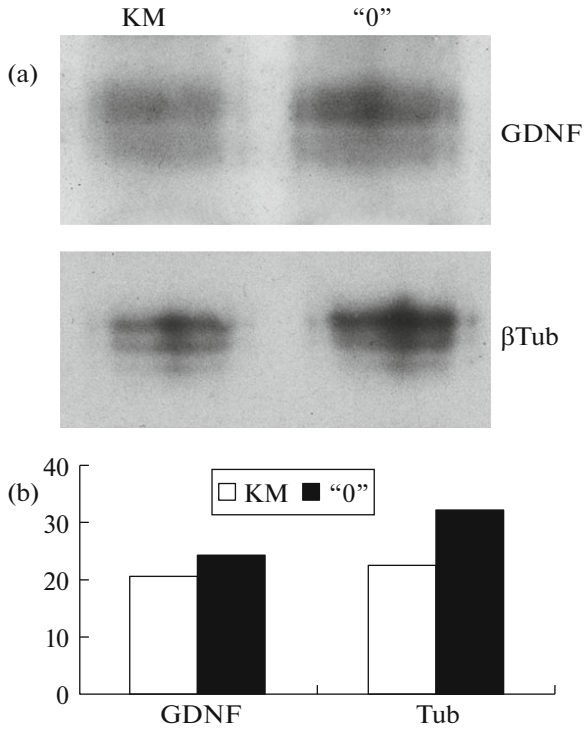

Fig. 1. The results of a comparative electrophoretic study of the midbrain SC of the KM and "0" rats on the content of the glial neurotrophic factor (GDNF), and tubulin. (a) Representative images of Western blot strips analyzing the content of GDNF and tubulin in the SC of KM and "0" rats. (b) A histogram of the mean values of the optical density of the bands, received using the "Image J" program.

Protein Western blot hybridization was performed using three male KM and three male "0" rats aging 3–4 months weighing 344–362 g. The animals were kept in standard cages in size of 36 × 50 × 16 cm with regularly alternating light (12/12 h). Water, grain and feed for rodents ("LaboratorKorm") were freely available. The room temperature varied from 20 to 22°C. The animals were anesthetized with chloral hydrate, the brain was extracted and samples containing SC were isolated. The samples were weighed and frozen in liquid nitrogen. After lysing and centrifugation at 19 000 *g*, the supernatant was used as a five-fold lysate for Western blotting. After electrophoresis, the proteins were transferred to a nitrocellulose membrane which was washed with TBS-T buffer, incubated on a shaker in 1% blocking reagent (Roche, no. 11 096 176001) and washed three times with TBS-T. For the detection of GDNF, primary antibodies Santa Cruz D-20 sc-328 (USA) with a titer of 1 : 1500 and secondary Santa Cruz sc-2030 (titer 1 : 10 000) were used. For normalization, antibodies for tubulin were used (primary—sigma T7816, titer 1 : 8000, secondary—IMTEK p-gam 60718, titer 1 : 4000), and for actin (JLA-20, Development studies hybridoma bank). After incubation with the antibodies, the membrane was washed and developed using the ECL Advance Western Blotting Detection Kit (US) according to the protocol.

The results demonstrated the significantly lower amount of GDNF, beta-tubulin, and actin in SC samples from KM rats in comparison with those from "0" strain (Fig. 1). This fact could serve as the indication that SC of KM rats contains much lower numbers of cellular elements in comparison with "0" rats.

In order to verify this assumption, the cell densities were determined, namely the densities of immunohistochemically stained cells for astrocytic and neuronal nuclei markers, as well as for the nuclei of all cells in the dorsal region of the SC (Fig. 2a).

The numbers of objects were got from 6 KM rats and 6 "0" rats using the coronal sections of the brain at the levels of SC. Forty micrometers sections were obtained using a freezing microtome after transcardial fixation of the brain by 4% formaldehyde. Sections

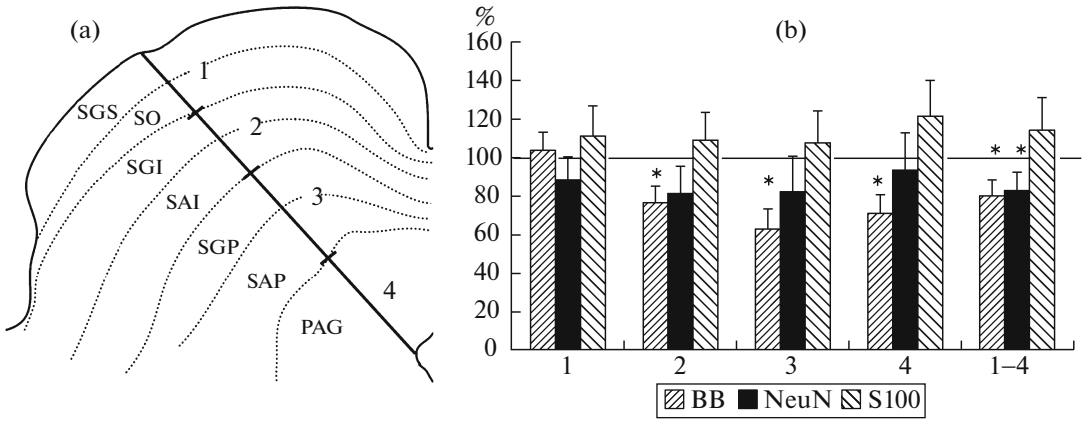

Fig. 2. The results of the comparison of the densities of cellular elements in different layers of SC in KM and "0" rats. (a) Designations of the investigated layers (1–4) relative to cytoarchitectonic layers of SC. (b) The densities of the distribution of the nuclei of all cells (BB), nerve cells, (NeuN) and astrocytes (S100) in different layers of SC of KM rats normalized to data on "0" rats. "1–4" are mean values over all layers $M \pm SD$, $n = 5$, $^*p < 0.05$.

were incubated with primary antibodies against S100 (Abcam, UK), NeuN (Millipore, USA), secondary antibodies fluorescently labeled with Texas Red or Cy2 (Jackson Immunoresearch, USA) [8]. Cell nuclei were stained with a fluorescent dye bisbenzimide (Sigma, USA).

The counting was carried out using an Olympus IX81 microscope (Japan) equipped with Märzhäuser motorized stage (FRG) controlled from a computer and a digital camera Olympus DP72 (Germany). The cells were counted from a computer monitor using the "Cell *" program (Olympus Soft Imaging Solution GmbH, Germany). At a small magnification (objective ×10), we obtained an overview image of the section of the cut, with an SC. Then, at a large magnification (objective ×100), the number of cells or nuclei was counted using the optical fractionator method [14]. The position of the 10 × 10 μm counting frame was changed in steps of 200 μm along the *X* axis and 200 μm along the *Y* axis within the SC (Fig. 2a). In each position of the frame, the uninformed operator counted the number of objects to be examined, shifting the focal plane along the *Z* axis by 30 μm. Five sections from each animal were analyzed. On the section, 130–150 points were tested.

The average densities of cells (cell nuclei) in 4 zones were calculated in SC (Fig. 2a): (1) the surface layer including stratum griseum superficiale (SGS) and stratum opticum (SO), (2) the deeper layer, including stratum griseum intermediale (SGI) and stratum album intermediale (SAI), (3) the layer, including stratum griseum profundum (SGP) and stratum album profundum (SAP), and finally (4) in the dorsolateral part of the PAG. The significance of the differences between the cell densities obtained KM-"0" rat brains was estimated using the nonparametric Mann-Whitney test.

The cell densities data for all cell nuclei (bisbenzimide staining) showed that in all SC layers investigated (with the exception of the surface layer), the scores for KM rats' brains were lower than for "0" rats' brains (Fig. 2b). Moreover, the differences in the average total densities and in cell densities for layers 2, 3, and 4 (SGP and SAP) were statistically significant.

The average densities of NeuN-immunopositive cells (labeled neurons) in all layers of SC in KM rats brain were lower than those in "0" rats. The differences in the mean cell densities in all layers of SC (total) were statistically significant (Fig. 2b).

The average densities of S100 immunopositive cells in SC in all layers were higher in KM rat brains than in "0" strain rats', however they were not statistically significant (Fig. 2b).

The results showed that in those SC layers, where fibers from IC terminate [11], the densities of nerve cells and overall densities of cells were reliably lower in KM rats than in "0" rats. Presumably these data could explain the interstrain electrophoretic differences, which we revealed in the content of tubulin and actin proteins in the SC.

Our comparison on the architectonics of SC between AE prone and non-prone animals are very important for understanding the genesis of AE, since this midbrain structure is an important "switch point" for the excitatory flux from IC to the reticular formation of the midbrain, and, consequently, for the genesis of AE seizures [10, 11].

Further studies of the midbrain structures related to IC will help to identify new aspects of neural networks involved in the genesis of AE in KM rats.

ACKNOWLEDGMENTS

The authors are grateful to N.I. Pustogarov and D.Yu. Panteleev, the reserchers of the Institute of Gene Biology (Russian Academy of Science), for the advice and technical assistance.

The work was carried out with the funding of RFBR grant nos. 16-04-01094 and 17-29-01001.

REFERENCES

1. Fedotova, I.B., Semiokhina, A.F., Kosacheva, E.S., et al., *Exp. Clin. Pharm.*, 1996, vol. 59, pp. 6–9.
2. Fedotova, I.B., Semiokhina, A.F., Fless, D.A., et al., *Zh. Vyssh. Nerv. Deyat.*, 1996, vol. 46, pp. 1104–1108.
3. Poletaeva, I.I., Surina, N.M., Kostina, Z.A., et al., *Brain Res.*, 2015, vol. 361, pp. 324–338.
4. Malikova, L.A., Fedotova, I.B., Surina, N.M., et al., Exper. Clin. Pharm., 2010, vol. 73, pp. 2–7.
5. Fedotova, I.B., Kostina, Z.A., Poletaeva, I.I., et al., *Russ. J. Genet.*, 2005, vol. 41, pp. 1487–1494.
6. Firstova, Yu.Yu., Abaimov, D.A., Surina, N.M., et al., Bull. Exper. Biol. Med., 2012, vol. 154, pp. 196–198.
7. Sorokin, A.Ya., Kudrin, V.S., Klodt, P.M., et al., *Russ. J. Genet.*, 2004, vol. 40, pp. 846–849.
8. Solius, G.M., Revishchin, A.V., Pavlova, G.V., et al., *Dokl. Biochem. Biophys.*, 2016, vol. 466, pp. 32–34.
9. Roberts, R.C., Ribak, C.E., and Oertel, W.H. Brain Res., 1985, vol. 361, pp. 324–338
10. Ribak, C.E., *Epilepsy Behav.*, 2015. doi 10.1016/j.yebeh.2015.02.024
11. Ribak, C.E., Khurana, V., and Lien, N.T., *J. Hirnforsch.*, 1994, vol. 5, pp. 303–311.
12. Ribak, C.E., Manio, A.L., Navetta, M.S., et al., *Epilepsy Res.*, 1997, vol. 26, pp. 397–406.
13. Fedotova, I.B., Kostina, Z.A., Surina, N.M., and Poletaeva, I.I., *Russ. J. Genet.*, 2012, vol. 48, no. 4, pp. 581–586.
14. West, M.J., Ostergaard, K., Andreassen, O.A., et al., *J. Comp. Neurol.*, 1996, vol. 370, pp. 11–22.