საქართველოს მეცნიერებათა ეროვნული აკადემიის მოამბე, ტ. 19(193), №4, 2025
BULLETIN OF THE NATIONAL ACADEMY OF SCIENCES OF GEORGIA, vol. 19(193), no. 4, 2025

Cytology

Kainic Acid Model of Temporal Lobe Epilepsy, Ultrastructural Alterations in the Rat Hippocampus

Irina Sharikadze[*], Mzia Zhvania[*], Giorgi Lobzhanidze[**]

[*] *School of Natural Sciences and Medicine, Ilia State University, Tbilisi, Georgia*
[**] *Department of Brain Ultrastructure and Nanoarchitecture, Ivane Beritashvili Center of Experimental Biomedicine*

(Presented by Academy Member David Mikeladze)

Abstract. Kainic acid (KA) model of temporal lobe epilepsy (TLE) is one of the most reliable tools for understanding the mechanisms of epileptogenesis. Numerous data show that systemic, intrahippocampal, intraamygdalar, intraperitioneal or intranasal administration of KA in rats, induces behavioral, electrophysiological, chemical and histological effects that are similar to those observed in patients with TLE. While physiological, chemical and histological effects of KA treatment have been studied extensively, much lesser is known about KA-induced ultrastructural alterations that develop in brain regions involved in TLE. In the present research, we describe the ultrastructure of hippocampal CA1 area of adult male Wistar rats 24 h, 7 days and 21 days after KA-induced status epilepticus (SE). Electron microscopic analysis revealed that KA-induced SE produces structural pathologies at all stages of epileptogenesis, but the most substantial (in some cases, irreversible) – 24 h after initial insult. At all three time-points the most common were destructed mitochondria and damaged dendrites. Besides, electron microscopic analysis shows that among hippocampal neurons the most vulnerable to SE are pyramidal cells. In general, such results demonstrated that KA-provoked SE is immediately damaging and induces continuing neuronal network reorganization that should predispose to chronic seizures. Therefore, despite transient nature, SE could be considered as a fundamental insult to the affected brain structure. © *2025 Bull. Natl. Acad. Sci. Georg.*

Keywords: kainic acid model of epilepsy, the ultrastructure of the hippocampus, Wistar rats

Introduction

Temporal lobe epilepsy (TLE) is the most common form of focal epilepsy in adults (Hines & Wu, 2023). About 80% of temporal lobe epilepsy arises from the medial part of the temporal lobe. Usually, TLE involves the hippocampus, parahippocampal gyrus, and amygdala—brain regions related to emotions, memory, and the processing of sensory information (Meng et al., 2023). The understanding of the mechanisms of TLE largely rests on the use of corresponding animal models. No model fully reproduces all the features of TLE; however, some exhibit a high level of similarity with human epilepsy. One of these is the kainic acid (KA) model, introduced by Ben-Ari and colleagues (Ben-Ari et al., 1979). KA is a cyclic analogue of L-glutamate and an agonist of ionotropic KA receptors. It is well known that systemic, intrahippocampal, intra-amygdalar, intraperitoneal, or intranasal

administration of KA in rats induces behavioral, electrophysiological, chemical, and histological effects similar to those observed in patients with TLE (Ben-Ari et al., 1979; Rusina et al., 2021). Physiological, chemical, and histological effects of KA treatment have been studied extensively. However, much less is known about KA-induced ultrastructural alterations that develop in brain regions involved in TLE. In the present research, the ultrastructure of the hippocampal CA1 area of adult male Wistar rats at various stages of KA-induced epileptogenesis is decsribed. Epileptogenesis was induced by multiple low-dose intraperitoneal injections of KA (Hellier et al., 1998).

Materials and Methods

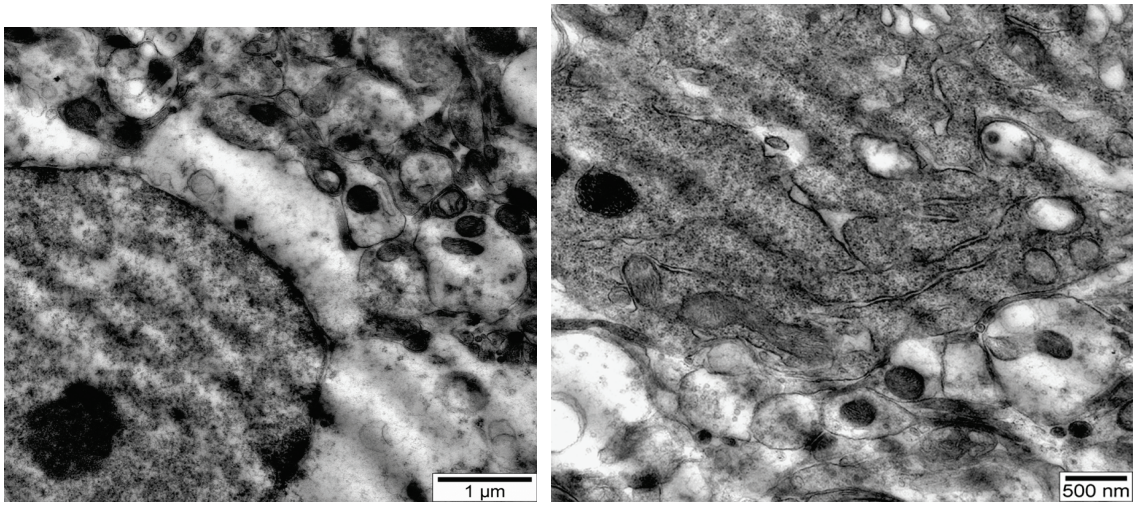
The study was performed on twenty-eight adult male Wistar rats (P30-35), weighting 185-215 g obtained from Ivane Beritashvili Center of Experimental Biomedicine. The animals were housed three per cage, in a climate-controlled environment (temperature 20-22°C, humidity – 55%-60%, 12-hr light/ dark cycle), With ad libitum access to food and water. Just before the treatment, the rats were randomly assigned to control (n = 7) and three experimental groups (n = 7 in each group). Experimental rats received an initial dose of KA (Merck, catalog number – 420318) of 7.5 mg/kg. After 45 min, repetitive injections of 2.5 mg/kg were given every 30 min. The injections were repeated until convulsive seizures were induced. The first convulsive seizure was considered as the beginning of status epilepticus (SE). Rarely more than five injections were needed and animals entered SE. Control rats were injected IP, with saline. Behavior was monitored performed from Day 1 until day 21. The material for electron microscopy (EM) was taken 24 h, 8 days and 21 days after SE – 5 animals for each case. Only those KA-treated rats, which developed the seizures of class 4 or greater, were selected. All procedures were performed in accordance with EU Directive 2010/63/EU, on the protection of animals used for scientific purposes.

EM Analysis

The conventional technique described in our earlier studies was used (Lobzhanidze et al., 2020; Zhvania et al., 2020). Briefly: after pentobarbital injection, the rats underwent transcardiac perfusion with heparinized 0.9% NaCl, followed by 500 mL of 4% paraformaldehyde and 2.5% glutaraldehyde in 0.1 M phosphate buffer, pH 7.4. The left hemispheric tissue blocks containing the hippocampus were cut into 400-micron-thick coronal slices. The coordinates for the hippocampus were 3.8 mm posterior to Bregma and lateral ±1.8 to ±2.2 mm (Paxinos & Watson, 2014). CA1 was identified with an optical microscope (Leica MM AF), cut out, dehydrated, and embedded in Araldite. Sections 35 nm thick were prepared with an ultramicrotome (Leica EM UC7), stained with lead citrate, and examined with a JEM 1400 electron microscope. From each rat, three blocks were prepared, and every fifth section was evaluated.

Results

EM analysis revealed a number of ultrastructural modifications in some neurons, glial cells and synapses of CA 1 area. The character of modifications differed at various stages of epileptogenesis. Thus, 24 hours after SE, approximately 22% of observed neurons were altered. The majority of them exhibited the peculiarities of pyramidal cells. About 9% of such cells were irreversibly modified. Specifically, the signs of late phase of apoptosis, almost total chromatolysis or significantly destroyed cytoplasmic organelles were observed (Fig. A, B). The most common were swollen or destructed mitochondria and swollen or damaged dendrites, even without tubules. The majority of synapses were normal, whereas in other presynaptic profiles, synaptic vesicles showed the tendency to be concentrated in close vicinity from an active zone. Some astrocytes were moderately swollen. On 7 and 21 days after treatment, irreversibly damaged neurons constituted about 5% and



A B

Fig. Electron microscopic micrographs of the effects of kainic acid on hippocampal CA1 area. A – Total chromatolysis on cytoplasm. B – The fragment of dark degenerated neuron with destructed mitochondria.

4% of observed cells. Damaged organelles are also relatively rare. However, altered mitochondria in different parts of cells, including presynaptic terminals as well as swollen dendrites were still relatively numerous.

Discussion

The hippocampal CA1 area represents the main output point of the hippocampal tri-synaptic circuit. Therefore, CA1 is a special site of epileptogenesis (Aksoz et al., 2020; Núñez-Ochoa et al., 2021; Su et al., 2002). Recently, it was shown that status epilepticus (SE), as a transient insult, induces a transcriptional program which differs in different regions of the hippocampus. Genetic testing also indicates that SE induces highly variable gene expression in different cell types of CA1 (Galvis-Montes et al., 2023; Pfisterer et al., 2020). According to other data, only a few days after SE, CA1 may acquire the properties of an epileptogenic focus (Su et al., 2002). Our electron microscopy analysis indicates that KA-induced SE produces structural pathologies in CA1 at all stages of epileptogenesis, with the most substantial alterations observed 24 hours after the initial insult. Additionally, data show that among neurons, pyramidal cells are the most vulnerable to KA-induced SE. These results provide additional evidence that, despite its transient nature, SE can be considered a fundamental insult to affected brain structures (Núñez-Ochoa et al., 2021).

In our material, mitochondria and dendrites were the most altered structures at all stages of epileptogenesis. Mitochondrial impairments were described in several human imaging studies (Rho & Boison, 2022). Experimental data also suggest that mitochondrial reactive oxygen species occur during different phases of epileptogenesis (Ignacio-Mejía et al., 2023; Xing et al., 2019). Moreover, it was proposed that mitochondrial dysfunction and oxidative stress not only result from SE, but may also contribute to epileptogenesis (Mercado-Gómez et al., 2023; Rowley & Patel, 2013). The most reviewed potential links are bioenergetic failure and fuel utilization (Mercado-Gómez et al., 2023; Rowley & Patel, 2013). The mitochondrial alterations described in the present study provide additional evidence supporting this suggestion; however, further research emphasizing the effects of energy failure and ATP depletion is needed.

Dendrite pathologies in brain epileptogenic zones are also described in multiple studies and are thought to be associated with the reorganization of neuronal networks. Mechanistically targeted approaches to limit dendritic alterations may represent novel therapeutic strategies for treating epilepsy (Wong & Guo, 2013). However, the exact etiology and functional implications of such disorders are not yet established (Kumari & Brewster, 2023). Therefore, a better understanding of the functional significance of these pathologies is needed.

Conclusion

The results show that KA-induced SE is immediately damaging and induces continuing neuronal network reorganization that should predispose to chronic seizures.

Acknowledgements

This research was funded by Shota Rustaveli National Science Foundation of Georgia (SRNSFG) [grant number #PHDF-21-1592].

ციტოლოგია

საფეთქლის წილის ეპილეფსიის კაინის მჟავას მოდელი, ვირთაგვას ჰიპოკამპის ულტრასტრუქტურული ცვლილებები

ი. შარიქაძე*, მ. ჟვანია*, გ. ლობჟანიძე**

** საბუნებისმეტყველო მეცნიერებისა და მედიცინის ფაკულტეტი, ილიას სახელმწიფო უნივერსიტეტი, თბილისი, საქართველო*
*** თავის ტვინის ულტრა- და ნანოარქიტექტონიკის ლაბორატორია, ივ. ბერიტაშვილის ექსპერიმენტული ბიომედიცინის ცენტრი, თბილისი, საქართველო*

(წარმოდგენილია აკადემიის წევრის დ. მიქელაძის მიერ)

საფეთქლის წილის ეპილეფსიის კაინის მჟავას მოდელი ერთ-ერთი გავრცელებული მოდელია ეპილეპტოგენეზის მექანიზმების გასაშუქებლად. მთელი რიგი კვლევების თანახმად, ვირთაგვებში კაინის მჟავას ინტრაჰიპოკამპალური, ინტრაამიგდალარული, ინტრაპერიტონეული ან ინტრანაზალური ადმინისტრირება საფეთქლის წილის ეპილეფსიის მქონე პაციენტებისთვის დამახასიათებელ ქცევით, ელექტროფიზიოლოგიურ, ქიმიურ და ჰისტოლოგიურ ეფექტებს იწვევს. კაინის მჟავას ადმინისტრირებით გამოწვეული ფიზიოლოგიური, ქიმიური და ჰისტოლოგიური ეფექტები მრავალი კვლევების საგანია; ამასთანავე, გაცილებით მცირე ინფორმაციაა იმ ულტრასტრუქტურული ცვლილებების შესახებ, რომლებსაც კაინის მჟავა საფეთქლის წილის ეპილეფსიაში ჩართულ თავის ტვინის უბნებში იწვევს. წარმოდგენილ კვლევაში,

ვისტარის ხაზის ზრდასრული მამრი ვირთაგვების ჰიპოკამპის CA1 ველში აღწერილია ის ულტრასტრუქტურული ცვლილებები, რომლებსაც კაინის მჟავათი გამოწვეული ეპილეფსიური სტატუსი 24 საათის, 8 და 21 დღის შემდეგ იწვევს. ეპილეპტოგენეზის სხვადასხვა სტადიაზე მთელი რიგი სტრუქტურული პათოლოგიები განვითარდა, მათ შორის, შეუქცევადი, განსაკუთრებით გამოხატული ეპილეფსიური სტატუსიდან 24 საათის შემდეგ იყო. ამასთანავე, დროის სამივე წერტილში, ულტრასტრუქტურულ პათოლოგიებს შორის, დესტრუქტურირებული მიტოქონდრიები და დაზიანებული დენდრიტები ჭარბობდა. გარდა ამისა, კაინის მჟავას ზემოქმედების მიმართ, ჰიპოკამპის უჯრედებს შორის, განსაკუთრებით მგრძნობიარე პირამიდული ნეირონები აღმოჩნდა. ჯამში, ასეთი მონაცემები მიუთითებს, რომ კაინის მჟავას ადმინისტრირებით გამოწვეული ეპილეფსიური სტატუსი, უმოკლეს ხანში, ჰიპოკამპის სტრუქტურულ დაზიანებას და ნეირონული ქსელის ხანგრძლივ რეორგანიზაციას იწვევს. ასეთმა ცვლილებებმა, გარკვეული წვლილი უნდა შეიტანოს ქრონიკული კრუნჩხვების მიმართ ორგანიზმის მიდრეკილებაში. ამგვარად, ტრანზიტული ბუნების მიუხედავად, კაინის მჟავათი გამოწვეული ეპილეფსიური სტატუსი საფეთქლის წილის ეპილეფსიის პათოგენეზში ჩართული ერთ-ერთი წამყვანი უბნის, ჰიპოკამპის უნატიფესი სტრუქტურული აღნაგობის ფუნდამენტურ დაზიანებას იწვევს.

REFERENCES

Aksoz, E., Sara, Y., & Onur, R. (2020). T-type Ca^{2+} channel activity increases in rat hippocampal CA1 region during kindling epileptogenesis. *Synapse, 74*(9), e22155. https://doi.org/10.1002/syn.22155

Ben-Ari, Y., Lagowska, J., & Tremblay, E. (1979). A new model of focal status epilepticus: Intra-amygdaloid application of kainic acid elicits repetitive secondarily generalized convulsive seizures. *Brain Research, 163*(1), 176-179. https://doi.org/10.1016/0006-8993(79)90106-9

Galvis-Montes, D. S., van Loo, K. M. J., van Waardenberg, A. J., et al. (2023). Highly dynamic inflammatory and excitability transcriptional profiles in hippocampal CA1 following status epilepticus. *Scientific Reports, 13,* 22187. https://doi.org/10.1038/s41598-023-49214-w

Hellier, J. L., Patrylo, P. R., Buckmaster, P. S., et al. (1998). Recurrent spontaneous motor seizures after repeated low-dose systemic treatment with kainate: Assessment of a rat model of temporal lobe epilepsy. *Epilepsy Research, 31*(1), 73-84. https://doi.org/10.1016/S0920-1211(98)00011-8

Hines, K., & Wu, C. (2023). Epilepsy networks and their surgical relevance. *Brain Sciences, 14*(1), 31. https://doi.org/10.3390/brainsci14010031

Ignacio-Mejía, I., Contreras-García, I. J., Mendoza-Torreblanca, J. G., et al. (2023). Evaluation of the antioxidant activity of levetiracetam in a temporal lobe epilepsy model. *Biomedicines, 11*(3), 848. https://doi.org/10.3390/biomedicines11030848

Kumari, S., & Brewster, A. L. (2023). Exploring dendritic and spine structural profiles in epilepsy: Insights from human studies and experimental animal models. *Epilepsy Currents, 24*(1), 40-46. https://doi.org/10.1177/15357597231220276

Lobzhanidze, G., Japaridze, N., Lordkipanidze, T., et al. (2020). Behavioral and brain ultrastructural changes following the systemic administration of propionic acid in adolescent male rats: Further development of a rodent model of autism. *International Journal of Developmental Neuroscience, 80*(2), 139-156. https://doi.org/10.1002/jdn.1005

Meng, X., Deng, K., Huang, B., et al. (2023). Classification of temporal lobe epilepsy based on neuropsychological tests and exploration of its underlying neurobiology. *Frontiers in Human Neuroscience, 17,* 1100683. https://doi.org/10.3389/fnhum.2023.1100683

Mercado-Gómez, O. F., Arriaga-Ávila, V. S., Vega-García, A., et al. (2023). Daytime-restricted feeding ameliorates oxidative stress by increasing NRF2 transcriptional factor in the rat hippocampus in the pilocarpine-induced acute seizure model. *Brain Sciences, 13*(10), 1442. https://doi.org/10.3390/brainsci13101442

Núñez-Ochoa, M. A., Chiprés-Tinajero, G. A., González-Domínguez, N. P., et al. (2021). Causal relationship of CA3 back-projection to the dentate gyrus and its role in CA1 fast ripple generation. *BMC Neuroscience, 22,* 37. https://doi.org/10.1186/s12868-021-00637-y

Paxinos, G., & Watson, C. (2014). *The rat brain in stereotaxic coordinates.* Elsevier Academic Press. https://api.semanticscholar.org/CorpusID:57202076

Pfisterer, U., Petukhov, V., Demharter, S., et al. (2020). Identification of epilepsy-associated neuronal subtypes and gene expression underlying epileptogenesis. *Nature Communications, 11,* 5038. https://doi.org/10.1038/s41467-020-18844-8

Rho, J. M., & Boison, D. (2022). The metabolic basis of epilepsy. *Nature Reviews Neurology, 18*(6), 333-347. https://doi.org/10.1038/s41582-022-00671-9

Rowley, S., & Patel, M. (2013). Mitochondrial involvement and oxidative stress in temporal lobe epilepsy. *Free Radical Biology and Medicine, 62,* 121-131. https://doi.org/10.1016/j.freeradbiomed.2013.02.002

Rusina, E., Bernard, C., & Williamson, A. (2021). The kainic acid models of temporal lobe epilepsy. *eNeuro, 8*(2), ENEURO.0337-20.2021. https://doi.org/10.1523/ENEURO.0337-20.2021

Su, H., Sochivko, D., Becker, A., et al. (2002). Upregulation of a T-type Ca^{2+} channel causes a long-lasting modification of neuronal firing mode after status epilepticus. *Journal of Neuroscience, 22*(9), 3645-3655. https://doi.org/10.1523/JNEUROSCI.22-09-03645.2002

Wong, M., & Guo, D. (2013). Dendritic spine pathology in epilepsy: Cause or consequence? *Neuroscience, 251,* 141-150. https://doi.org/10.1016/j.neuroscience.2012.09.065

Xing, J., Han, D., Xu, D., et al. (2019). CREB protects against temporal lobe epilepsy associated with cognitive impairment by controlling oxidative neuronal damage. *Neurodegenerative Diseases, 19*(5-6), 225-237. https://doi.org/10.1159/000504687

Zhvania, M., Gogokhia, N., Tizabi, Y., et al. (2020). Behavioral and neuroanatomical effects of exposure to white noise in rats. *Neuroscience Letters, 728,* 134898. https://doi.org/10.1016/j.neulet.2020.134898

Received September, 2025