ANNOTATION

of the dissertation for the degree of Doctor of Philosophy (Ph.D.)
in the educational program 8D05101 – "Biology"
Kairat Bakytzhan Kairatuly

"The role of calcium-permeable kainate and AMPA receptors of inhibitory neurons in the control of excitation of the hippocampal neural network"

General Characteristics of the Study. The present work is devoted to investigating the role of calcium-permeable kainate and AMPA receptors of inhibitory neurons in controlling the excitation of the hippocampal neuronal network.

Assessment of the Current State of the Problem. Neuronal hyperexcitability within neural networks occurs in conditions such as epilepsy, the early stages of stroke, and other neurodegenerative disorders. Prolonged hyperexcitation leads to neuronal death. In *in vitro* conditions, hyperexcitability of the neuronal network can be induced by various physiological methods: generalized neuronal depolarization through the application of different concentrations of KCl; removal of Mg^{2+} ions from the medium to unblock NMDA receptors; compounds that increase intracellular cAMP concentration; high concentrations of ammonia; and removal of GABA(A)-mediated receptor inhibition. These interventions lead to high-frequency synchronized firing activity of neurons, which, at an initial stage, induces adaptation and preconditioning processes in glutamate receptors and voltage-gated Ca^{2+} channels, but with prolonged activity, results in neuronal death.

To suppress hyperexcitability, it is more effective to employ endogenous adaptive mechanisms, which can be pre-activated by various exogenous compounds. One such mechanism of suppressing hyperexcitability is associated with the use of selective activators of a specific subpopulation of inhibitory neurons containing calcium-permeable kainate and AMPA receptors, which respond rapidly to hyperexcitation by releasing GABA.

Relevance of the Research Topic. The hippocampus is a central structure of the limbic system that plays a crucial role in memory consolidation (the process by which short-term memory is converted into long-term memory), spatial memory formation, and the generation of emotions. The two main types of neurons in the hippocampus are excitatory pyramidal cells and inhibitory interneurons. Typically, hippocampal interneurons are GABAergic and secrete γ-aminobutyric acid (GABA), the brain's primary inhibitory neurotransmitter. To date, more than 20 subtypes of hippocampal interneurons have been identified, which differ in their morphology, physiology, and gene expression profiles. These interneurons regulate the activity of pyramidal cells and are essential at all stages of information processing.

Dysfunction of the GABAergic system is associated with a wide range of neurological disorders—from neurodegenerative to psychiatric diseases. Selective degeneration of specific GABAergic neuronal populations has been observed in epilepsy, ischemia, hepatic encephalopathy, and traumatic brain injury. In many cases, certain GABAergic neuron subpopulations are more vulnerable than

pyramidal neurons. Despite extensive research, the mechanisms underlying the selective degeneration of these GABAergic neuron subpopulations remain poorly understood.

The primary cause of neuronal death in the aforementioned conditions is believed to be excitotoxicity, which results from elevated extracellular glutamate levels. Currently, the standard treatment approach involves the use of antagonists of excitatory receptors and voltage-gated channels. However, because these channels are widely expressed across different cell types, such treatments are often associated with numerous side effects. This necessitates the development of new therapeutic strategies that offer more targeted action.

To achieve selective intervention, it is essential to identify and characterize the specific subpopulations of neurons that are most vulnerable during the progression of these pathological conditions and to elucidate the mechanisms underlying their degeneration.

Degree of Elaboration of the Problem.

It is hypothesized that the primary cause of impaired GABAergic regulation in neuronal networks during hyperexcitation may be the damage to GABAergic neurons containing calcium-permeable AMPA (CP-AMPARs) and kainate (CP-KARs) receptors. The presence of CP-AMPARs and CP-KARs on the membranes of GABAergic neurons leads to an additional influx of calcium during hyperexcitation, thereby intensifying excitotoxic stress and potentially resulting in the selective death of these neurons. This hypothesis is supported by the fact that CP-AMPARs are expressed only in specific neuronal subtypes, including GABAergic ones, while CP-KARs appear to be expressed exclusively in GABAergic neurons. Therefore, it is proposed that GABAergic neurons expressing these calcium-permeable receptors represent a particularly vulnerable population in various pathologies characterized by network hyperexcitability.

Object of the Study. Co-cultures of hippocampal neurons and astrocytes isolated from the brains of neonatal rats.

Subject of the Study. Calcium-permeable kainate and AMPA receptors of inhibitory neurons in the hippocampus and their role in controlling neuronal network excitability.

Aim and Objectives of the Study. The aim of this dissertation is to investigate the role of calcium-permeable kainate and AMPA receptors of inhibitory neurons in regulating the excitability of the hippocampal neuronal network.

To achieve this aim, the following **objectives** were addressed:

1. To identify neurons expressing CP-AMPARs and CP-KARs.
2. To determine the mechanisms underlying calcium responses in CP-AMPA and CP-KA neurons upon agonist application.
3. Investigating the role of calcium-permeable AMPA receptors in inhibitory neurons in the regulation of epileptiform activity.
4. To assess neuronal resistance under conditions of acute glutamate-induced excitotoxicity.

5. To elucidate the ionic mechanisms associated with calcium homeostasis disruption. This includes studying the dynamics of intracellular Ca^{2+}, K^{+}, Na^{+}, and pH levels during prolonged exposure to selective agonists.

Scientific Novelty of the Research. This study is the first to comprehensively examine the contribution of calcium-permeable kainate and AMPA receptors to the regulation of inhibitory neuron activity and the maintenance of excitation-inhibition balance in the hippocampal neuronal network. Specific mechanisms were identified by which calcium influx through kainate and AMPA receptors modulates the excitability of inhibitory neurons, including their effects on intracellular signaling pathways and neuronal plasticity. The study also demonstrated that dysfunction of calcium-permeable kainate and AMPA receptors disrupts excitation-inhibition balance, leading to hyperexcitability of neuronal networks—a hallmark of epilepsy and other neuropathological conditions.

Theoretical Significance of the Research. The results obtained in this study expand our understanding of the role of GABAergic neurons expressing calcium-permeable kainate and AMPA receptors in controlling the excitability of the neuronal network. The identified mechanisms linking hyperexcitability to the dysfunction of calcium-permeable kainate and AMPA receptors provide a theoretical foundation for understanding the pathogenesis of epilepsy and other neurodegenerative diseases. The findings create a basis for further research on the interaction of receptors in various types of neurons and their roles in other brain regions beyond the hippocampus.

Practical Value of the Work. The findings from this study can be applied in the development of new pharmacotherapeutic strategies for treating various neurodegenerative disorders associated with neuronal hyperexcitability in networks. The discovered neuroprotective effect of selective activation of this specific population of neurons can serve as the basis for developing new and effective approaches for the therapy of diseases characterized by neuronal network hyperexcitability, such as epilepsy, ischemia, and others. The results of this dissertation can be used in specialized courses in the educational programs "080 – Biology and Related Sciences," "Biology," "Biophysics," "Biomedicine," and "Neuroscience."

Main Findings to be Defended:

1. Calcium-permeable AMPA receptors (CP-AMPARs) of inhibitory neurons regulate the structure of paroxysmal depolarizing shift (PDS) clusters during epileptiform activity in neuronal networks. This regulation is mediated through interactions with Kv7 potassium channels, which control the hyperpolarization phases and duration of the clusters.

2. Neurons expressing CP-AMPARs play a crucial role in the transmission of excitation in neuronal networks during epileptiform activity. Activation of these receptors leads to disinhibition of glutamatergic neurons by inhibiting the activity of other GABAergic neurons through GABA(B)-receptor-dependent activation of Kv7 potassium channels.

3. Neurons containing CP-AMPARs are highly sensitive to excitotoxic damage due to their inability to effectively restore calcium homeostasis after glutamate

exposure. This makes them vulnerable to pathological processes associated with hyperexcitability.

4. Increased calcium ion influx through CP-AMPARs leads to significant disturbances in calcium homeostasis and dysregulation of intracellular concentrations of key ions, which enhances their susceptibility to damage during hyperexcitability.

Connection of the Work with the State Program Plan. The dissertation work was conducted in accordance with the PhD training program at Al-Farabi Kazakh National University, Ministry of Science and Higher Education of the Republic of Kazakhstan, in collaboration with the Institute of Cell Biophysics of the Russian Academy of Sciences (Pushchino, Russia) under the following themes: AP05133528 “Rhythmogenesis and regulation of spontaneous synchronous neural activity in the brain during hyperexcitability” (State Registration No. 0115RK00284); AP19678607 “Calcium-dependent mechanisms of rhythm control in brain neurons during hyperexcitability” (State Registration No. 0123RK00430), within the framework of the state fundamental research program.

Author's Contribution. The author's contribution to the work includes direct conduction of experiments, processing, analysis, interpretation, and generalization of the obtained scientific results.

Approval of the Work. The materials of the dissertation have been presented and discussed at the following conferences:

International Scientific Conference of Students and Young Scientists "Farabi Alemi", Kazakhstan, Almaty, 2020-2025.

International Scientific and Practical Conference "Global Science and Innovation 2021: Central Asia", Kazakhstan, Nur-Sultan, 2021.

VIII All-Russian Scientific and Practical Conference with International Participation, Russia, St. Petersburg, 2020.

International Scientific and Practical Conference "Current Problems of Biology and Biotechnology", Kazakhstan, Almaty, 2021.

25th Pushchino School-Conference of Young Scientists with International Participation "Biology – Science of the 21st Century", Russia, Pushchino, 2022.

International Interdisciplinary Congress "Neuroscience for Medicine and Psychology", Russia, Crimea, 2022, 2024.

International Scientific and Practical Conference "Integration of Sciences: Biophysics, Biomedicine, Neuroscience", Kazakhstan, Almaty, 2022-2024.

International Scientific and Practical Conference "Modern Achievements in Biomedicine and Ecology", Kazakhstan, Almaty, 2023.

International Scientific and Practical Conference of Young Scientists "Biophysics in Medicine", Uzbekistan, Tashkent, 2023.

International Symposium "Nanotechnologies in Biology and Medicine", Kazakhstan, Almaty, 2024.

IX Congress of Physiologists of Kazakhstan and Central Asia, Kazakhstan, Almaty, 2024.

Publications. The main content of the dissertation is reflected in 48 published works, including 6 articles in international journals indexed in Scopus and Web of

Science, 4 articles in national scientific journals recommended by the Committee for Science and Higher Education of the Ministry of Science and Higher Education of the Republic of Kazakhstan, 3 certificates of state registration of copyright objects, and 35 publications in the materials and proceedings of international conferences and symposia.

Structure of the Dissertation. The dissertation is presented on 125 pages and includes abbreviations and symbols, an introduction, a literature review, materials and methods of research, discussion of results, conclusions, and a list of 423 references. It contains 1 table and 39 figures.