Streszczenie w języku angielskim

Pharmacological modulation of epileptic activity, experiments on animals.

The aim of the presented dissertation was to develop a patch clamp animal model of epileptiform activity in the prefrontal cortex and use it to study the mechanisms of action of known medications and the therapeutic potential of novel agents. Special attention was given to short epileptiform activity, known as interictal epileptiform discharges (IEDs), which, apart from epilepsy, have been described in several other conditions including attention deficit hyperactivity disorder (ADHD), bipolar disorder, or autism spectrum disorder. Initially, the known antiepileptic medication, valproic acid, was studied, followed by the exploration of the potentially new therapeutic option, capsaicin. Lastly, using the model of IEDs, the antiepileptic properties of a known ADHD medication, guanfacine, were demonstrated.

The prefrontal cortex is a region of the brain involved in several cognitive functions, coordinating thoughts and actions to align with internal objectives. Interictal epileptiform discharges in this region have been described in several psychiatric disorders including bipolar disorder. The first article included in this thesis is an original article titled "Valproic acid potently inhibits interictal-like epileptiform activity in prefrontal cortex pyramidal neurons," published in *Neuroscience Letters*. Valproic acid is a well-known antiepileptic medication which mechanism of action involves GABA-transmission enhancement and voltage-gated sodium channels inhibition. Apart from epilepsy, valproate is commonly used in bipolar disorder, although its mechanism of action in this context is not entirely clear. In this study, we adopted a method of inducing IEDs in rat prefrontal cortex neurons. The solution in which brain slices were bathed contained no magnesium ions and an elevated concentration of potassium ions. This setup allowed us to induce depolarizations shorter than 2 seconds with firing of action potentials, stable for a period of up to an hour. We showed that the depolarization bursts were N-methyl-D-aspartic acid (NMDA) receptor-dependent, as a known NMDA blocker, AP-5, abolished this activity. Furthermore, we demonstrated that therapeutic concentrations of valproic acid abolished both the short epileptiform activity and the spontaneous excitatory postsynaptic potentials (EPSPs) in a concentration-dependent manner. Additionally, we studied the excitability of

prefrontal neurons and found that valproic acid reduced the excitability of prefrontal neurons in a dose-dependent manner.

The second study was an original article entitled “Capsaicin inhibits sodium currents and epileptiform activity in prefrontal cortex pyramidal neurons,” published in *Neurochemistry International*. Here, we investigated the therapeutic potential of capsaicin – a compound of the vanilloid family most famous for being an active ingredient of chili peppers, responsible for the hot sensation. The compound has been used in medicine for several years due to its analgesic properties. In this peripheral setting, it is believed to act via transient receptor potential vanilloid (TRPV) cation channels. We initiated our investigation by testing capsaicin's influence on neuronal excitability. We found that capsaicin significantly inhibited neuronal firing, and moreover, the amplitude of the last action potentials were markedly reduced in the presence of capsaicin. This suggested modulation of use-dependent blockade of sodium channels. To further investigate, we performed single-channel recordings in a voltage-clamp setting, and showed that capsaicin strongly inhibited sodium channels by shifting the inactivation curve of sodium channels towards hyperpolarization. Additionally, we observed that the compound strongly enhanced use-dependent blockade of sodium channels, confirming our previous observations. Furthermore, we tested the compound in three different models of epileptic activity: IEDs, long ictal events evoked with 4-aminopyridine (4-AP), a potassium channel inhibitor, and a solution without magnesium ions and intermediate-long events evoked with picrotoxin, a GABA receptor antagonist and a solution without magnesium ions. Capsaicin inhibited epileptiform activity in the first two models and shorten the duration of epileptic episodes in the third. Interestingly, the discharges evoked with 4-AP were resistant to valproate. All the findings above provide a basis for considering capsaicin as a structural framework for developing new antiepileptic medications.

The third publication, titled “Guanfacine inhibits interictal epileptiform events and sodium currents in prefrontal cortex pyramidal neurons,” was published in *Pharmacological Reports*. Several studies have described interictal discharges as prevalent in subjects suffering from ADHD and, possibly, contributing to the symptoms of the disease. At the same time, the prefrontal cortex is a center of studies investigating the pathology of ADHD.

In the presented article, we hypothesized that the known ADHD medication – guanfacine – may act by inhibiting the IEDs in the prefrontal cortex. Using the model adopted in previous studies, we showed that guanfacine potently reduced this short epileptiform activity. Furthermore, we showed that the effects were independent of the alpha-2 receptor, a nominal place of action of guanfacine, as in a series of experiments with idazoxan – an alpha-two antagonist – the effect remained unchanged. Finally, we showed that guanfacine potently inhibited sodium channels. Altogether, we point to the fact that modulating sodium channel activity may be an additional mode of action of guanfacine in ADHD.

The last publication included in the thesis is a review article entitled "Beneficial Effects of Capsaicin in Disorders of the Central Nervous System," published in *Molecules*. This article systematically summarized the available evidence on the beneficial effects of capsaicin on a wide range of neurological disorders including Parkinson's and Alzheimer's disease, stroke, and migraine. The strongest emphasis was placed on the data describing capsaicin's role in epilepsy research. Most existing research concentrates on TRPV1 actions and the hippocampal formation. Interestingly, both pro-epileptic and antiepileptic actions of capsaicin are described. In the presented article, we propose a new framework for understanding these seemingly contradictory results. In brief, we postulate that the pro-epileptic actions of capsaicin are mediated by its action on TRPV1 receptors in the hippocampal formation, whereas antiepileptic actions work via sodium channel inhibition in cortical neurons. In the few studies that used systemic application of capsaicin, the antiepileptic mechanism seemed to prevail.

In summary, the studies included in this dissertation applied several patch-clamp models of epileptiform activity to deepen our understanding of the mechanisms of action of known medications (valproate, guanfacine) and provide in vitro evidence for the therapeutic potential of a novel agent – capsaicin. Furthermore, the effects of capsaicin in the broader context of neurological disorders have been studied, with special attention given to its place in antiepileptic therapy. The obtained results may enhance our understanding of the pathologies of several disorders, as well as provide novel therapeutic options.