UNIVERSITY OF MEDICINE, PHARMACY, SCIENCES AND TECHNOLOGY
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SUMMARY OF DOCTORAL THESIS

Changes of calcium-binding proteins and glial cells in epileptic circuitry: implications for epileptogenesis and antiseizure therapy

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Background/introduction

Despite the numerous studies on its pathomechanism and treatment, epilepsy remains to date a disease of many faces that creates a significant global health burden. Epilepsy substantially reduces the quality of life and uncontrolled seizures can pose a significant risk to the patient's life. Mesial temporal lobe epilepsy (TLE) is among the most common forms of focal epilepsy, and approximately 30% of cases are resistant to treatment with anti-seizure medications (ASMs), creating a challenge for both clinicians and patients. Epileptogenesis involves complex cellular and molecular changes that affect the structure and function of neural circuits. Among these, alterations in calcium signaling pathways have emerged as important contributors to both the initiation and progression of epileptic activity. Given the critical role of calcium-buffering mechanisms in neuronal function, we hypothesized that both classical and novel calcium-binding proteins (CaBPs) undergo alterations in hippocampal and extrahippocampal neurons during epileptogenesis, as well as during treatment with novel antiseizure medication. Additionally, TLE is known to be associated with focal inflammatory processes leading to gliosis in very specific brain regions, possibly with a distinct vulnerability, and which can further contribute to the dysfunctional activity of the pathological network

To investigate this, we designed a series of three complementary studies that collectively examine previously underexplored changes in CaBPs within the context of epileptogenesis and investigate the effects of new ASMs on CaBPs, neurons and glia, with results that may contribute to the development of antiepileptogenic therapies.

These investigations were conducted through a comprehensive, multidisciplinary analysis of the molecular, cellular, and behavioral aspects of TLE using the intracerebroventricular (ICV) kainic acid (KA) model, a well-established experimental paradigm for studying pharmacoresistant TLE and screening novel ASMs. While the KA model is widely recognized for its utility in mimicking the pathophysiology of human TLE, our study employed the ICV administration route to achieve more precise targeting and reproducibility of epileptogenic damage. This methodological refinement enabled the detailed evaluation of both hippocampal and extra-hippocampal structures involved in the epileptic circuitry.

Study I – aimed to characterize the spatial distribution and cell-type specificity of functionally relevant CaBPs in the central nervous system (CNS). The kinetic properties and variable buffering capacity of CaBPs contribute to the modulation of synaptic responses and neuronal excitability, adapting to the specific needs of the cell populations that express them. From a methodological perspective, we performed a selection of relevant genes based on biological function and we cross-referenced our gene-function based selection with publicly available, high-quality RNA-sequencing and *in situ* hybridization databases, including the Human Protein Atlas (HPA), Brain RNA-seq database and the Allen Brain Atlas (as integrated into the HPA). Based on these resources, we generated gene expression heatmaps that visualize the regional and cell type-specific expression levels of the selected CaBPs.

We created a tool to predict and investigate different expression patterns and functions of the less-known CaBPs of the CNS. The various kinetic and buffering properties of CaBPs help modulate the synaptic responses and the excitability of neurons therefore they are adapted to the specific needs of the cell populations that express them. The examples presented in the thesis show that databases can be used to further predict and investigate different expression patterns of the little known CaBPs.

This approach facilitates a better understanding of the specific spatiotemporal distribution and potential functional relevance of CaBP genes expressed in the brain. By summarising quantitative regional and cell-specific data in a visually clear way, our study aimed to find relevant information regarding the functionally important CaBPs in the brain.

Study II - CaBPs are known to modulate neuronal excitability and calcium signaling and they may contribute the imbalance between excitation and inhibition observed in TLE. While parvalbumin (PV) and calretinin (CR) are well-characterized CaBPs, NECAB1 (N-Terminal EF-Hand Calcium Binding Protein 1) remains understudied in epilepsy, despite its reported association with neurodegenerative conditions. In this study, we used fluorescent immunolabeling to determine the distribution of NECAB1, and assess its coexpression with PV and CR, in brain regions associated with the epileptic circuitry using the KA-induced TLE model. Additionally, we investigated the effects of the ASMs levetiracetam and brivaracetam on NECAB1 expression.

We have shown, that in healthy brain, in the areas otherwise involved in the epileptic circuitry, NECAB1-positive cells were most abundant in the paraventricular nucleus of the thalamus (PVT), endopiriform nucleus (EPN), and amygdala (AMY). A subpopulation of cells co-expressing CR was found in the amygdala (AMY), PVT, and hippocampus but was nearly absent in the EPN. In the chronic phase of KA-induced epilepsy, NECAB1 expression was significantly upregulated in the PVT, and bilaterally in the amygdala. In epileptic rats NECAB1-CR co-expressing cells were absent in the right EPN but had a high density on the left, whereas the NECAB1-PV colocalizing cells showed an asymmetry between the right and left amygdala. We also found that levetiracetam and brivaracetam treatments partially reduced the NECAB1 density increase in TLE, indicating a modulatory effect on NECAB1 expression.

These findings suggest that NECAB1 upregulation may be a compensatory response to epileptic hyperexcitability. Given its involvement in thalamocortical regulation and interneuron diversity, NECAB1 may play a critical role in circuit remodeling.

Study III - In this study we compared the effects of brivaracetam and levetiracetam on histological alterations in key brain regions involved in the epileptic circuitry, namely, the hippocampus, AMY, piriform cortex (PC), EPN and PVT, using the KA rat model of TLE. Given the less known role of glial cells in the mechanisms of TLE as well as their changes due to ASMs this study focused, besides neurons, on microglia and astrocyte changes.

We have shown that, after three weeks of treatment with novel ASMs, there has been a significant difference between treatment groups in microglia cell density. Brivaracetam-treated animals showed an increased microglia density in anatomical structures contributing to epileptic activity (such as hippocampal CA3, CA1 regions, AMY, PC, EPN and PVT) compared to levetiracetam-treated animals. Furthermore, a prominent astrogliosis could be observed in the hippocampus particularly in the right CA3, ipsilateral to the KA injection, which was alleviated by levetiracetam compared to brivaracetam treatment. Also, a significant difference was observed in neuronal cell density between the epileptic and ASM treated groups, with a relative decrease in neuron numbers in the ASMs treated groups.

General conclusions - Altogether, this work supports the growing recognition of calcium-binding proteins as critical regulators of neuronal excitability and potential therapeutic targets. Additionally, our results emphasize the relevance of glial cell changes in epilepsy pathogenesis and drug response.

Originality of the thesis - By using the parallel examinations of changes in CaBPs (e.g., NECAB1, PV, CR), glial activation and neuronal reorganization, the study bridges gaps between molecular neurobiology and clinical epileptology. The findings highlight the role of calcium dysregulation in TLE and propose mechanisms through which ASMs may modulate disease progression.