FUNCTIONAL REORGANIZATION OF NEURONAL CIRCUITS IN THE HIPPOCAMPUS DURING EPILEPTOGENESIS

-abstract of PhD thesis-

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Introduction

Temporal lobe epilepsy is a common neurological disorder characterized by recurrent seizures caused by synchronized, excessive neuronal activity, often initiated in the hippocampus. Over 30% of patients are drug-resistant, with a major impact on the life of the patient and relatives and a severe socio-economic burden.

The mechanisms of epileptogenesis are not fully elucidated, and the causes of drug resistance are multiple and complex, partly due to the pharmacokinetic characteristics of available anticonvulsant drugs (ATCs). Improving drug bioavailability by creating inclusion complexes using beta-cyclodextrin (β-CD) is an under-tested method for ATCs. Also, the effects of this oligosaccharide on neuronal cells have not been investigated.

Objectives

The aim of our research is to better understand the mechanisms underlying seizure initiation and propagation in an *in vitro* $0Mg^{2+}$ model of epilepsy and the effects of four β-CD-solubilized voltage-gated sodium channel (VGSC) blockers on seizure-like event (SLE) parameters.

General methodology

300 μm thick hippocampal sections were obtained from male Wistar P7-13 rats. Field potentials in the CA3 region of the hippocampus were recorded using a microelectrode. Baseline activity was recorded in normal artificial cerebrospinal fluid (nACSF) and epileptiform activity was triggered using magnesium-free, high potassium ACSF ($0Mg^{2+}$ ACSF). The individual effect of β-CD and the effect of carbamazepine (CBZ, 100 μM), lacosamide (LAC, 100, 50, 25 μM), rufinamide (RUF, 100, 50 μM) and phenytoin (PHT, 100, 50, 25 μM) complexed with β-CD dissolved in $0Mg^{2+}$ ACSF was tested. The recording protocol was terminated by washout with $0Mg^{2+}$ ACSF and nACSF (Figure 1.). SLE phases were delineated on recordings based on neuronal discharge frequency.

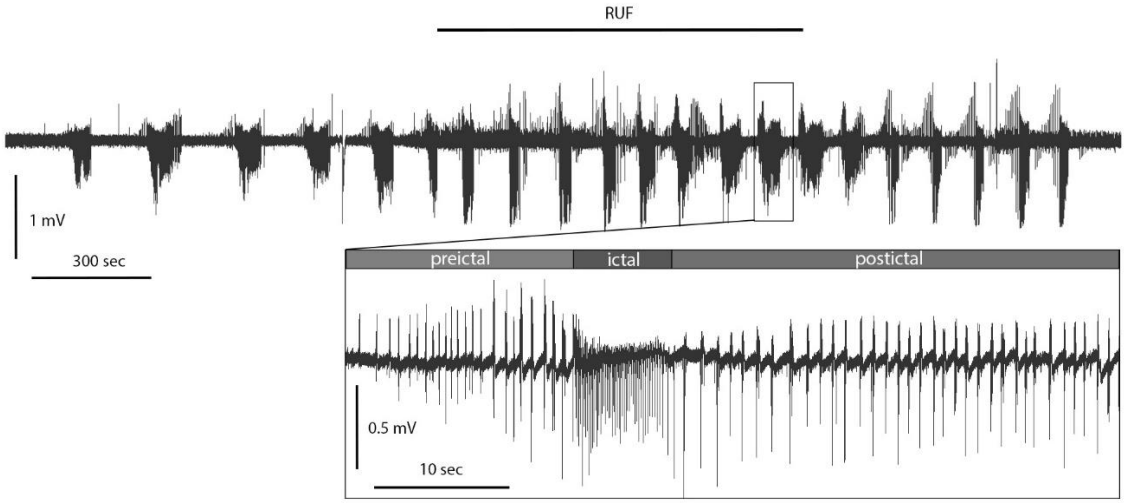

Figure 1. Complete recording with SLE phase delineation based on discharge frequency.

In **Study 1**, the impact of β-CD, and the four β-CD complexed drugs on the duration and characteristics of the different phases of SLE was investigated, and a comparative analysis between different concentrations was performed for PHT, LAC, RUF, to understand their dose-dependent effect.

The duration of preictal, ictal, postictal and interictal phases was measured and SLE frequency was calculated. The results revealed that β-CD causes shortening of preictal and ictal phases and increases SLE frequency by shortening of interictal phases. All tested drugs decreased the duration of ictal phase, at the same time increased the frequency of SLE. LAC in 100 μM concentration completely suppressed ictal activity. In lower concentrations besides shortening ictal phase and increasing SLE frequency, it influenced the duration of pre- and postictal phase in a dose-dependent manner. CBZ increased the duration of pre-ictal phase and decreased the duration of ictal and postictal phase, SLE frequency was increased.

In **Study 2** we looked into variations of neuronal discharge frequency at the onset of the ictal phase, by which we studied how rapid oscillations contribute to seizure development. We calculated the average discharge frequency for the first 10 (SF1) and next 10 (SF2) ictal spikes.

In this study the power spectral density (PSD) was also measured in the preictal-ictal transition period. The last 2 preictal spikes, the ictal onset *burst* and the next 2 ictal spikes were analysed for the following frequencies: subripple (1-100 Hz), ripple (100-200 Hz), fast ripple I. (200-300 Hz), fast ripple II. (300-400 Hz).

In control SLEs there was no change in discharge frequency between the two compared segments. Of all the substances tested CBZ was the only one that caused a decrease in frequency in both phases and a reduction of discharge rate in the second phase compared to the first. β-CD did not influence the discharge rate compared to the control, but caused it to slow down in the second phase compared to the first.

Spectral density in the subripple band increased progressively during the preictal-ictal transition period. For the other frequencies the increase was present only in the ictal burst. All tested substances influenced PSD at variable times and frequencies. Their main effects were predominantly to increase spectral density. RUF in 100 μM concentration was the only VGSC blocker that caused only spectral density decrease.

In **Study 3** we investigated the complex interactions between oscillatory brain rhythms. Phase-amplitude coupling (PAC) is a form of interaction in which the phase of low-frequency oscillations modulates the amplitude of high-frequency oscillations. PAC was computed in 5 s windows at the end of the preictal phase, the beginning of the ictal phase, and the end of the postictal phase for the modulating frequency of 1-12 Hz and the modulated frequencies gamma (30-100 Hz), ripple (100-200 Hz), and fast ripple (200-400 Hz).

The results demonstrated that the coupling strength is lower at the beginning of the ictal phase compared to the pre- and postictal phases for the three frequencies studied. The tested molecules sporadically had effects on PAC, CBZ caused the most changes by increasing PAC in the ictal and postictal phase for gamma and ripple frequencies.

Discussion, conclusions

In vitro models of epilepsy provide a controlled environment for ATC studies, allowing rapid testing of different drug concentrations. The use of β-CD as an excipient improved the solubility of anticonvulsant drugs, suppressing epileptiform activity at lower concentrations than those used with other solubilization methods.

It has been shown that β-CD is not an inert excipient. When applied alone, it demonstrated both anticonvulsant and moderate proconvulsant effects, reducing the duration of preictal and ictal phases, but increasing the frequency of SLE. The exact mechanisms by which β-CD influences neuronal excitability are not completely understood.

The VGSC blockers tested - lacosamide, carbamazepine, rufinamide and phenytoin - showed different and dose-dependent effects in suppressing epileptiform activity.

Our analysis revealed power spectral density variations in the preictal-ictal transition phase. The effects of VGSC blockers on PSD were variable and concentration-dependent, reflecting complex mechanisms of action. PAC strength varied in different phases of SLE, with a decrease in the ictal phase relative to the preictal phase and partial recovery in the postictal phase.

Our results can only partially be compared with those in the literature because of differences between *in vitro* and *in vivo* experimental models.

Our study makes an important contribution to understanding the effects of β-CD and VGSC blockers on epileptiform neuronal activity in an *in vitro* model. The effects of β-CD on neuronal activity have not been studied previously, although it has been used in clinical trials in combination with different drugs. The effects of VGSC blockers on SLE have not been tested in combination with β-CD. Our observations on dose-dependent effects, variability in drug efficacy, and detailed analysis of PSD and PAC parameters provide a deeper understanding of the mechanisms of action and therapeutic potential of these substances.