

## SUMMARY

### **Definition of work:**

Electrophysiological models of seizure like activity for NO detection in vitro

**Supervisor:** MUDr. Jakub Otáhal, PhD.

Department of Anatomy and Biomechanics FTVS CUNI

Department of Developmental Epileptology CAS, Institute of Physiology

**Consultant:** Mgr. Renata Konopková

Department of Developmental Epileptology CAS, Institute of Physiology

**Aim:** The aim of the thesis is to introduce nitric oxide (NO) and its role in physiology and pathophysiology of central nervous system (CNS), with the intention of epileptiform activity in the nervous tissue. Our research was realized on rat hippocampal slices *in vitro* and it compares the experimental biochemical models (low-Mg<sup>2+</sup>, high-K<sup>+</sup>, Carbachol, Bicuculline and 4-Aminopyridine model). These changes in the environment of slice should evoke the epileptiform activity in the nervous tissue of the rat. The final aim of the thesis is to create an experimental model of epileptiform activity in vitro in our setup for imaging techniques and NO detection.

**Methods:** Experiments were performed in rat hippocampal slices in vitro. Rats were deeply anaesthetized with ether and decapitated. Brain was rapidly removed from the skull, cut in oxygenated (95%) and cooled (0, 5 °C) artificial cerebrospinal fluid (ACSF) using vibratome in coronal slices of 400µm of thickness. They were submerged into recording chamber where ACSF in adequate concentration was perfused (5 ml/min and temperature ± 33 °C) using a peristaltic pump. Data recordings were made in the submerged type chamber. Synaptic activation of the tissue was made with bipolar stimulating electrode placed in hillus of the dentate gyrus of hippocampus (Mossy fibers). Detection electrode was placed in pyramidal layer of CA1 region. Single shock stimulation with supramaximal stimulus was applied and then other stimuli in determined values according to stimulation protocol. Experimental models were performed by changing the concentration of ions in the ACSF (low-Mg<sup>2+</sup> and high-K<sup>+</sup>

model) or by addition of chemical into this solution (Carbachol, Bicuculline and 4-AP model).

**Results:** We evaluated eight hippocampal slices with following results. We haven't been able to elicit tonic clonic seizures in our setup. The only model whose activity resembled to seizure activity was the high-K<sup>+</sup> model (10mM). With low-Mg<sup>2+</sup> (zero Mg<sup>2+</sup>), high-K<sup>+</sup> (10mM), Bicuculline (13μM and 33μM) in combination with high-K<sup>+</sup> (7mM KCl), 4-Aminopyridine (250μM), except for Carbachol treated slices, we were able to elicit epileptic discharges.

**Key words:** nitric oxide (NO), epilepsy, hippocampus, low-Mg<sup>2+</sup>, high-K<sup>+</sup>, Carbachol, Bicuculline, 4-AP.