OPUS-26

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IMPORTANCE OF MITOCHONDRIAL CALCIUM UNIPORTER FOR UNIQUE RESISTANCE OF CA2 HIPPOCAMPAL NEURONS TO INJURY

The hippocampus is a brain structure responsible mainly for learning and memory. It is composed of dentate gyrus and cornu ammonis (CA) regions. The CA is divided into regions CA1, CA2 and CA3. The CA2 region, due to its small size and poorly defined boundaries with neighbouring regions, is the least known structure of the hippocampus, although it is characterized by special properties, among which low capability for long-term potentiation (LTP; a basic process involved in learning and memory), and strong resistance to damage appear to be the most prominent ones. These properties are in striking contrast to the characteristic of CA1, a region which is very prone to LTP and is very sensitive to damage.

It was shown before by other researchers that hippocampus, dependent on the region, is characterized by differential activity of calcium ions, once they get into the cell. CA2 region is equipped with the mechanisms that allow to reduce the amount of free calcium to minimum, thanks to binding it by special proteins, extruding it form the cell to the outer space and accumulating it in mitochondria, one of cellular calcium storage. Region CA1 is depleted of these mechanisms at such a level of efficiency, and it is believed that this may be a main reason why it is more prone to LTP, but also sensitive to injury in various diseases – cellular calcium is a critical mediator of both these pathways. It was partially confirmed that the way CA2 neurons are dealing with calcium is responsible for CA2-specific LTP insensitivity, but we still don't know how it really affects the response of CA2 neurons to pathological insults. My preliminary data demonstrates that functional blocking of a protein named MCU, which is absent in CA1 but present in CA2, where it mediates calcium entry into mitochondria, resulted in sensitization
of CA2 neurons to experimentally induced neurotoxicity, leading to their death. Without blocking MCU, CA2 neurons easily survived exposure to toxic stimulus, when CA1 neurons, exposed to the same neurotoxin were dying in both conditions (without and with MCU blocking). Based on these observations, I hypothesized that MCU may be a crucial factor in CA2 neurons resistance. In this project I'm planning to perform a series of experiments to confirm this hypothesis. I will compare the response of CA1 and CA2 neurons to the neurotoxic stimulus (named NMDA), by checking the changes in cellular and mitochondrial calcium, investigating mitochondrial functioning as well as determining the cellular production of another toxic compound involved in neuronal death, nitric oxide (NO; according to my preliminary study, CA2 neurons probably have exceptionally low production of NO, what may additionally help to survive them in pathological conditions). Then, using pharmacological and genetic interventions, I will check how modifying of MCU activity and NO production will affect differential survival of NMDA-treated CA1 and CA2 neurons, and how it will modify other studied parameters in these regions. I believe that this project will allow me to explain what are the relationships between regional distribution of MCU and NO-related proteins and differential calcium flux patterns, mitochondrial physiology, NO production rates and, finally, neuronal survival in hippocampal pathology. This will allow us to better understand the functioning of the hippocampus and may help to design novel therapies against hippocampus-affecting diseases including epilepsy, stroke and traumatic brain injury.

It has to be emphasized that most of my experiments will be performed using organotypic cultures of mouse hippocampus, a model where tissue is cultured on the dish, in the conditions that allow it to live many days and retain its physiological functions. Additionally, I will take an advantage of genetically modified models that allow to direct the fluorescent marker specifically to one type of cells, in my case neurons of CA2 region, what will allow me to precisely determine the area of CA2, which normally is very hardy distinguishable from neighbouring CA1 and CA3 regions. Organotypic cultures, on one hand allow for preservation of tissue structures for the experiments and, on the other, facilitate sample manipulation and analysis. Very importantly, this model allows for significant limitation of the number of living animals used for in vivo procedures in research. Thanks to such an approach I will perform only a single experiment in vivo, to confirm only prime findings from organotypic culture experiments.