Proteomic analysis of dorsal and ventral subiculum isolated from the pilocarpine model of Mesial Temporal Lobe Epilepsy

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Abstract

Mesial temporal lobe epilepsy (MTLE) is the most frequent type of epilepsy in adults and it is usually refractory to clinical treatments. In most patients with MTLE a characteristic histopathological lesion is observed, including hippocampal sclerosis (HS). The subiculum is an important area which connects the hippocampus with the enthorinal cortex. In the present work we aim to use laser-capture microdissection (LCM) to isolate dorsal and ventral subiculum from epileptic rats induced by the classic pilocarpine protocol. With this material we will perform proteomics analysis to identify molecular and biochemical changes that could be involved in the context of MTLE.

Key words:

Epilepsy, Subiculum, Proteomics

Introduction

Epilepsy is a neurological disorder characterized predominantly by spontaneous and recurrent seizures. In the histopathological aspect the most frequent feature is hippocampal sclerosis (HS), which basically is characterized by the loss of pyramidal neurons in CA1 and CA3 regions [1]. The hippocampus is a structure localized in the mesial temporal lobe and its function is related with memory formation and emotional control. The hippocampus is divided into dorsal and ventral regions. Electrophysiological analysis in rodent shows that the dorsal region is related to spatial learning, and the ventral region to emotional responses. The subiculum forms the transition that connects the hippocampus with the enthorinal cortex, which allows for high amplification and modulation of the neuronal response, and it is involved in the recovered short-term memory and spatial memory codification. In this study, we aim to isolate dorsal and ventral subiculum from an epilepsy animal model induced with pilocarpine. Isolation of the subiculum regions will be achieved by LCM and used to perform proteomic analysis.

Methodology

Results and Discussion

Total proteins	Differentially expressed protein	Enriched pathways	Altered proteins in enriched pathways
180	7	9	24

Table 1. Number of proteins identified, total and differentially expressed, from the ventral subiculum of Sham controls and induced animals, number of enriched pathways and their respective proteins. Data by MaxQuant/Perseus and Metacore softwares

Fig 1. **A** Hippocampus from control Sham. Green coloring indicates sub-region CA1, red coloring indicates sub region CA2, blue coloring

indicates sub region CA3, orange coloring indicates the granular layer of the dentate gyrus and purple indicates the molecular layer of the dentate gyrus. Circulated areas in black indicates the dorsal subiculum region. **B** Control sham hippocampus marked to the same colors, and circulated in black is the ventral subiculum region.

Enriched Pathways	Altered Proteins	Altered proteins/ total proteins
Cytoskeleton organization/Neurofilament	GFAP, MUNC18, Tubulin (in microtubules), Tubulin alpha, Tubulin beta	7/25
Proteolysis, Role of Parkin in the Ubiquitin-Proteasomal Pathways/ Proteasomal degradation	HSP70, Tubulin alpha, Tubulin beta	5/24
Cell adhesion, Gap junctions/ Microtubules in contacted cells	Tubulin (in microtubules), Tubulin alpha, Tubulin beta	4/30
Cell cycle, Role of Wee1 in cell cycle regulation/ Chromosome condensation and separation	Tubulin (in microtubules), Tubulin alpha, Tubulin beta	4/30
Cytoskeleton remodeling/actin filaments/ Cytoskeleton organization	Tubulin (in microtubules), Tubulin alpha, Tubulin beta	4/26
Development, Regulation of cytoskeleton proteins in oligodendrocyte differentiation and myelination/ It through TAU, Fyn and Myelinic basic protein	Tubulin (in microtubules), Tubulin alpha, Tubulin beta	4/58
Development, Sirt-Riboz signaling/ Cytoskeleton organization and axon guidance	CRMP2, Tubulin (in microtubules)	4/30
Nicotinic signaling in glutamatergic neurons/ Cytoskeleton organization and Synaptic glutamate release	CaMKII alpha, Synapsin I	3/33
Neurophysiological process, Receptor-mediated axon growth repulsion/ Electron transport chain	CaMKII alpha, Synapsin I	3/45
Neurophysiological process, Synaptic vesicle fusion and recycling in nerve terminals/ Synaptic Vesicles exocytosis	MUNC18, Synapsin I	4/52

Table 2. Main enriched pathways identified in the ventral subiculum and their respective altered proteins.

Conclusions

We have identified some altered proteins in ventral subiculum that are related with important biological pathways. As the dorsal subiculum is still under evaluation we expect that it will present different altered biological pathways as previously described in literature. Therefore we presented new biological pathways of the ventral subiculum that can be involved with the epileptogenic process of the pilocarpine model of MTLE.

Acknowledgement

This work was supported by Cepid-BRAINN/ FAPESP and the National Counsel of Technological and Scientific Development (CNPq). All animal procedures were approved by the UNICAMP Ethics Committee on Animal Experimentation (ceua 4062-1).

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