Proteomic analysis of rats submitted to the animal model of epilepsy induced by pilocarpine

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Background and Purpose: The temporal lobe epilepsy (TLE) is the most common type of partial complex seizure in adulthood. High doses of pilocarpine to rats induce status epilepticus (SE) and reproduce the main characteristics of TLE. This model appears to be highly isomorphic with the human disease, so it has been used to elucidate the main mechanisms involved with epileptogenesis.

Material & Methods: Here, we employed a two-dimensional gel electrophoresis (2-DE) to study differential expression of proteins in the hippocampus of rats exhibiting SRS induced by pilocarpine (360mg/kg, N=6) compared to a control group (saline, N=6). Both groups were analyzed 90 days after SE onset. Hippocampi were homogenized in a lysis buffer and used to perform 2-DE. Protein spots analyzed by PDQuest software and identified by LC-ESI-MS/MS and MASCOT MS/MS ions search.

Results: forty proteins were found differentially expressed in the hippocampus of epileptic rats compared to control animals from which thirty-seven were successfully identified. Twenty-nine of the identified proteins were up-regulated in epileptic rats while six proteins were down-regulated and two proteins were expressed only in the control animals.

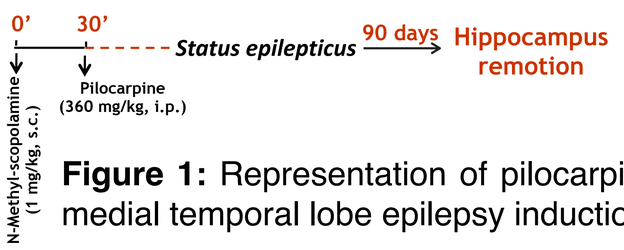

Figure 1: Representation of pilocarpine model for medial temporal lobe epilepsy induction in animals.

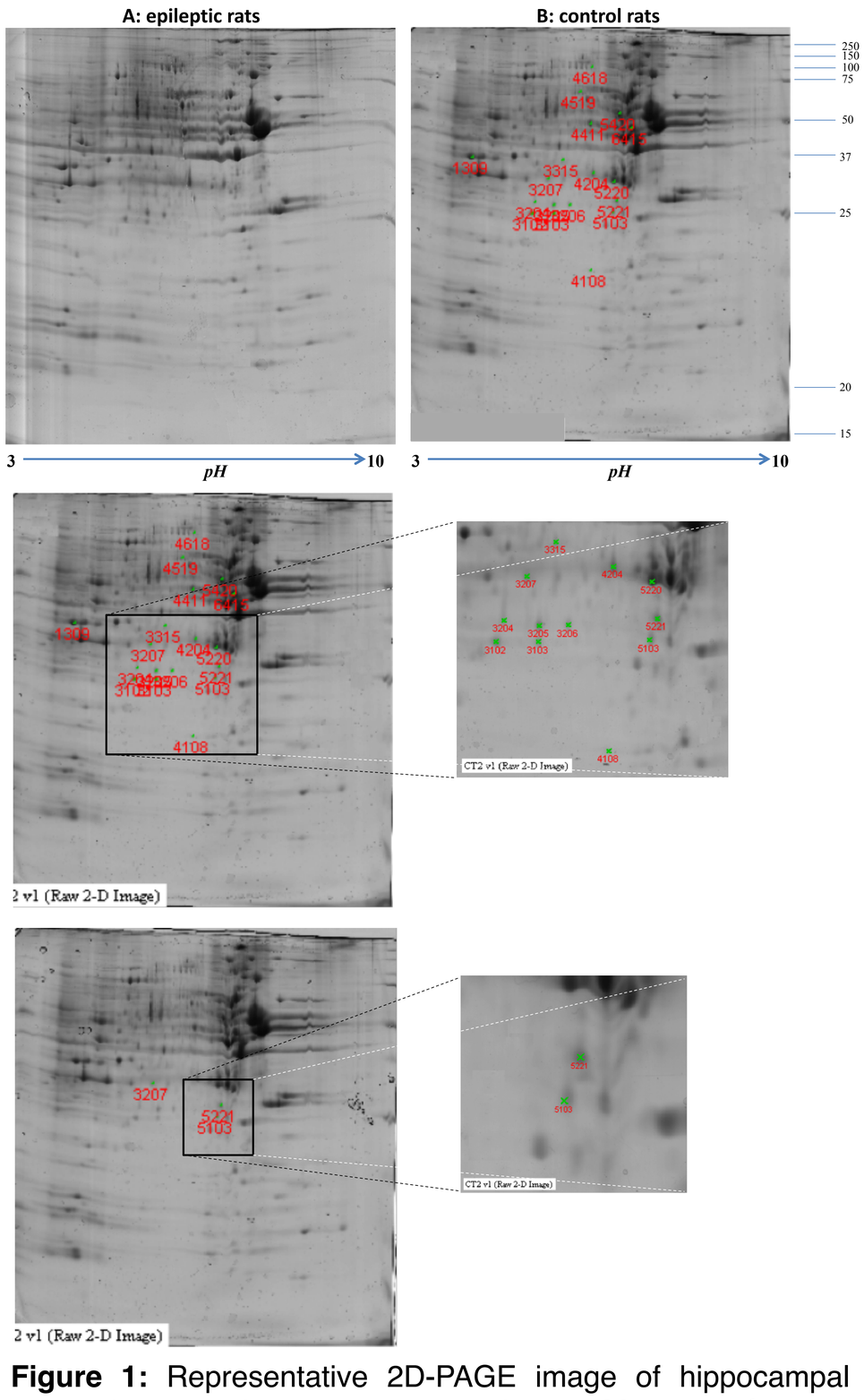

Figure 1: Representative 2D-PAGE image of hippocampal protein extracts from rats presenting chronic spontaneous seizures 90 days after status epilepticus induction by pilocarpine (A) and control rats treated with saline (B). An amount (0.5 mg) of total protein of each sample was separated by isoelectrofocusing on a pH 3-10 linear gradient followed by the second dimension ran on SDS-PAGE. Gels were stained with Coomassie brilliant blue. The peptides obtained from protein digestion of spots differentially expressed were analyzed by LC-ESI-MS/MS.

GeneCards	Protein name	Changes	IP	MW
Ldha	L-lactate dehydrogenase A chain	Ø	5.5	36874
Pebp1	Phosphatidylethanolamine-binding protein 1	Ø	5.2	20902
Aldoa	Fructose-bisphosphate aldolase A	⇩	9.3	39783
Pla2g4c	Cytosolic phospholipase A2 gamma (Fragment)	⇩	5.2	37522
Abcb6	ATP-binding cassette sub-family B member 6, mitochondrial	⇩	9.3	93305,18
Mdh1	Malate dehydrogenase, cytoplasmic	⇩	6	36631
Gnb1	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-1	⇩	5.4	38151
Gnb3	Guanine nucleotide-binding protein G(I)/G(S)/G(T) subunit beta-3	⇩	5.3	38125
Eno1	Alpha-enolase	⇧	6	47440
-	Enolase	⇧	10.3	34166
Eno3	Beta-enolase	⇧	7.9	47326
Eno2	Gamma-enolase	⇧	4.8	47510
Aldh5a1	Isoform Short of Succinate-semialdehyde dehydrogenase, mitochondrial	⇧	9.4	53391
Park7	Protein DJ-1	⇧	6.2	20190
Mapk1	Mitogen-activated protein kinase 1	⇧	6.5	41648
Tpi1	Triosephosphate isomerase	⇧	7.9	27345
Nsf	Vesicle-fusing ATPase	⇧	6.5	83170
Pgam1	Phosphoglycerate mutase 1	⇧	6,6	28928
Ywhag	14-3-3 protein gamma	⇧	4.6	28456
Ldhb	L-lactate dehydrogenase B chain	⇧	5.5	36874
RGD1565368	glyceraldehyde-3-phosphate dehydrogenase-like	⇧	9.3	36045
Dpysl2	Dihydropyrimidinase-related protein 2	⇧	5.8	62638
Dpysl3	Isoform 1 of Dihydropyrimidinase-related protein 3	⇧	5.9	62327
Atp6v1b2	V-type proton ATPase subunit B, brain isoform	⇧	5.4	56857
Car2	Carbonic anhydrase 2	⇧	6.9	29267
Gfap	Isoform 1 of Glial fibrillary acidic protein	⇧	5.1	49984
Tubb5	Isoform 1 of Tubulin beta-5 chain	⇧	4.6	50095
Tubb2a	Tubulin beta-2A chain	⇧	4.6	50274
Tubb2c	Tubulin beta-2C chain	⇧	4.6	50225
Tubb3	Tubulin beta-3 chain	⇧	4.6	50842
Atp5b	ATP synthase subunit beta, mitochondrial	⇧	4.9	56318
Tpi1	Triosephosphate isomerase	⇧	7.9	27345
LOC500959	Triosephosphate isomerase	⇧	6.4	27306
Capzb	F-actin-capping protein subunit beta	⇧	5.4	30952
Wdr1	WD repeat-containing protein 1	⇧	6.1	66824
Atp6v1a	V-type proton ATPase catalytic subunit A	⇧	5.2	68564
Cdca7l	Cell division cycle-associated 7-like protein (Cdca71)	⇧	5.8	50854

Figure 2: Proteins identified by LC-ESI-MS/MS and peptide matched by using Mascot MS/MS ion search and IPI protein database. (Filled up arrow) up-regulated proteins; (Open down arrow) down-regulated proteins; (pilocarpine versus control rats), and (Ø) proteins expressed only in the hippocampus of control rats. MW: molecular weight; IP: isoelectric point.

Gene symbol	Protein name	Changes in Pilocarpine model x control	Changes in epileptic human hippocampus x control
Park7	Protein DJ-1	⇧	+
Dpysl2	Dihydropyrimidinase-related protein 2	⇧	⇧
Atp6v1a	V-type proton ATPase catalytic subunit A	⇧	⇧

Figure 3: Proteins differentially expressed in the hippocampal samples of rats subjected to pilocarpine (90 days following SE), and of patients with MTLE. (Filled up arrow) up regulated proteins and (+) protein expressed only in the hippocampus of the patients (Persike et al, 2012; 2018).

Conclusion: Some of the proteins differentially expressed in the hippocampus of rats with SRS were also observed altered in the hippocampus of patients with mesial temporal lobe epilepsy. Such proteins are part of metabolic pathways responsible for the maintenance of vital functions for the cell. Besides, proteins potentially involved in neuronal development and plasticity, neuroprotection mechanisms and neuronal excitability were also found up-regulated in epileptic rats.

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