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Research report

Changes in intracellular protein expression in cortex, thalamus and hippocampus in a genetic rat model of absence epilepsy

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ABSTRACT

Epilepsy is a chronic disorder characterized by repeated seizures resulting from abnormal activation of neurons in the brain. Although mutations in genes related to Na^+, K^+, Ca^{2+} channels have been defined, few studies show intracellular protein changes. We have used proteomics to investigate the expression of soluble proteins in a genetic rat model of absence epilepsy "Genetic Absence Epilepsy Rats from Strasbourg (GAERS)". The advantage of this technique is its high throughput quantitative and qualitative detection of all proteins with their post-translational modifications at a given time. The parietal cortex and thalamus, which are the regions responsible for the generation of absence seizures, and the hippocampus, which is not involved in this activity, were dissected from GAERS and from non-epileptic control rat brains. Proteins from each tissue sample were isolated and separated by two-dimensional gel electrophoresis. Spots that showed significantly different levels of expression between controls and GAERS were identified by nano LC–ESI-MS/MS. Identified proteins were: ATP synthase subunit delta and the 14-3-3 zeta isoform in parietal cortex; myelin basic protein and macrophage migration inhibitory factor in thalamus; and macrophage migration inhibitory factor and 0-beta 2 globulin in hippocampus. All protein expressions were up-regulated in GAERS except 0-beta globulin. These soluble proteins are related to energy generation, signal transduction, inflammatory processes and membrane conductance. These results indicate that not only membrane proteins but also cytoplasmic proteins may take place in the pathophysiology and can be therapeutic targets in absence epilepsy.

1. Introduction

Epilepsy is a common serious neurological condition that is characterized by recurrent seizures and affects more than 0.5% of the world population [7]. Although earlier studies have defined mutations and polymorphisms in genes related to Na^+, K^+ and Ca^{2+} ion channels and to neuronal signalling in some types of epilepsy, there are few studies showing intracellular protein changes [24,51]. Proteomics that is a technique enables one to find protein changes responding to different states in cells [17], may be useful to understand the mechanisms underlying the diseases [10].

Absence epilepsy is a particular epileptic syndrome in which patients show generalized non-convulsive seizures characterized by a brief unresponsiveness to environmental stimuli and a cessation of motor activity [31,46]. Spike-and-wave discharges in the electroencephalogram (EEG) are the hall mark of the seizures. Several studies have pointed out that hypersynchronization in thalamo-cortical circuits is the major mechanism underlying absence epilepsy [47]. Recently, experimental studies of genetic rat models of absence epilepsy have indicated that the perioral region of somatosensory cortex initiates the seizure activity in the first milliseconds of a seizure and then entrains the thalamus to sustain the activity in the thalamo-cortical circuit and produce generalized spike-and-wave activity [36,48]. One of the most studied genetic rat models are the Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a fully inbred strain of rats, with 100% of animals displaying the EEG and behavioural characteristics similar to those observed in human absence epilepsy [12]. No structural changes were observed in these animals but several changes at the subcellular level have been shown, such as changes in receptor subunits and ion channel expressions. For example, the mRNA of the alpha1G subunit of low-voltage activated calcium channel was elevated in the neurons of ventral posterior relay nuclei of the thalamus in GAERS compared to control animals [56], mRNA and protein

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expression of voltage-gated sodium channels Nav1.1 and Nav1.6 were increased in the perioral somatosensory cortex of another rat absence epilepsy model [27].

In this study, we analyzed the protein profiles from three areas of the rat brain to investigate any differences in intracellular protein expressions in absence epilepsy, using two-dimensional electrophoresis (2-DE). Proteins that showed different level of expression between the non-epileptic control and the GAERS rat groups were detected and identified by nano LC–ESI-MS/MS. These brain areas were: (1) the parietal cortex that includes the perioral region of somatosensory focus for absence seizures; (2) the thalamus that plays an important role in the generation of spike-and-wave activity; and (3) the hippocampus where no spike-and-wave activity has been recorded. Although the hippocampus does not reveal spike-and-wave activity, studies using 2-deoxyglucose utilization and functional magnetic resonance imaging showed that the hippocampus is affected in absence epilepsy [38,37].

2. Materials and methods

2.1. Materials

Immobilized pH-gradient (IPG) strips, tributyl phosphine, ampholyte pH 3–10 were purchased from BioRad (BioRad Laboratories, Hercules CA, USA). Dithiothreitol (DTT), acrylamide, *N*,*N*′-methylenebisacrylamide, TEMED (*N*,*N*,*N*′,*N*′-tetramethyl-ethane-1,2-diamine), iodoacetamide, protease inhibitor cocktail, Tris, urea, thiourea, ASB-14, alfa-naphtol, anti-rabbit IgG (whole molecule) – alkaline phosphatase antibody produced in goat, fast red, nitrocellulose membranes were all from Sigma Chemical Co., St. Louis, MO, USA. Myelin basic protein antibody was from Abcam Inc., Cambridge, MA, USA. Anti-14-3-3 zeta was from Anaspec, San Jose, CA, USA. All the chemicals used were analytical grade.

2.2. Experimental animals

Four to six months old male non-epileptic control Wistar ($n = 6$) and GAERS ($n = 8$) rats weighing 250–300 g were used in the study. All the animals were housed in a temperature-controlled room ($20 \pm 3\,^{\circ}C$) with a 12-h light–dark cycle and were allowed free access to commercial rat pellets and tap water. Experiments were carried out in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the experimental protocol was approved by the Animal Care and Use Committee of Marmara University (Protocol number: 43.2004.Mar).

2.3. Sample preparation

Animals were decapitated under ether anaesthesia, brains were quickly removed and washed twice in ice cold homogenization buffer consisting of 7 M urea, 2 M thiourea, 1% (w/v) ASB-14, 40 mM Tris base, 0.001% (w/v) bromophenol blue, 1% (v/v) tributylphosphine, 5% (v/v) protease inhibitor cocktail, 0.5% (w/v) ampholyte pH 3–10. After the brain was placed in a dish on ice, parietal cortex and hippocampal tissue were dissected from one hemisphere, thalamic tissue was dissected from the other hemisphere.

For each experimental animal about 40–60 mg wet weight of tissue from the hippocampus, thalamus and parietal cortex were separately grounded up in liquid nitrogen and the fine powder was mixed with 500 μL of homogenization buffer. After sonication on an ice for approximately 15 s, the suspension was incubated at room temperature for an hour and centrifuged at $30{,}000 \times g$ for 50 min at 25 °C. The protein content of the supernatant was determined by using the Bradford method [6].

2.4. Two-dimensional gel electrophoresis (2-DE)

Four hundred micrograms of protein from each brain region of each animal was used for 2-DE. 17 cm linear pH 3–10 IPG strips were used for the first dimension. The passive rehydration was carried out for 12 h. Isoelectric focusing was performed by using a Protean IEF cell (BioRad Laboratories, Hercules, CA, USA). Focusing was started at 250 V, and after 20 min the voltage was gradually increased to 10,000 V in a linear mode during 150 min and, finally, 10,000 V was applied until 52 kV h was reached. The temperature was kept at 20 °C. After isoelectric focusing the strips were equilibrated in equilibration buffer I and equilibration buffer II for 15 min each sequentially according to the manufacturer's instructions. The equilibrated strips were then placed onto second dimension 12.5% SDS–PAGE gels. The SDS–PAGE was conducted in a standard Tris–Glycine–SDS buffer in Protean II xi Cell (BioRad Laboratories, Hercules, CA, USA) at a constant current setting of 20 mA/gel for 1 h, then at 40 mA/gel until the bromophenol blue dye reached the end of the gel. Gels were stained by the colloidal Coomassie staining method [41].

2.5. Image analysis and determination of proteome differences

Stained gels were scanned by a GS-800 calibrated densitometer (BioRad Laboratories, Hercules, CA, USA) and the PDQuest 2D-gel analysis software (Version 8.0.1, BioRad Laboratories, Hercules, CA, USA) was used to process and analyze the gel images. For each data analysis set, we used five technical replicates for each biological replicate of GAERS and Wistar protein extracts. Following the automatic detection mode, spots were manually edited, so that the spots that were not present in all replica gels were excluded from the analysis. For quasiquantitative comparisons, protein spots observed in the GAERS and control groups were normalized for the total density of each gel after calibration by manual indication of the lowest and highest density spots. Normalized density values were used for comparisons, and spots exhibiting at least a 1.5 fold increase or decrease was identified by mass spectrometry.

2.6. In-gel digestion

Excised protein spots were subjected to in-gel trypsin digestion. The spots were briefly washed three times by adding and removing alternating 100 mM $(NH_4)_2CO_3$, 50% acetonitrile and 50 mM $(NH_4)_2CO_3$ solutions. After removing the supernatant, the protein spots were overlaid by 200 ng trypsin in 50 mM $(NH_4)_2CO_3$, 10% acetonitrile in H_2O. Incubation was carried out overnight at 37 °C and was stopped by adding 0.5 volumes of 2% formic acid. After incubation for 1 h the supernatant was transferred to a new reaction tube or directly applied to nano-LC–ESI-MS/MS analysis.

2.7. Nano-LC–ESI-MS/MS analysis

Protein identification using Nano LC–ESI-MS/MS was performed by Proteome Factory (Proteome Factory AG, Berlin, Germany). The MS system consists of an Agilent 1100 NanoLC system (Agilent Technologies, Waldbroon, Germany), a PicoTip emitter (New Objective, Woburn, MA, USA) and an Esquire 3000 plus ion trap MS (Bruker, Bremen, Germany). Protein spots were in-gel digested by trypsin (Promega, Mannheim, Germany) and applied to nano LC–ESI-MS/MS. After trapping and desalting the peptides on an enrichment column (Zorbax SB C18, 0.3 mm × 5 mm, Agilent Technologies, Waldbroon, Germany) using 1% acetonitrile, 0.1% formic acid solution for 5 min, peptides were separated on a Zorbax 300 SB C18, 75 μm × 150 mm column (Agilent Technologies, Waldbroon, Germany) using an acetonitrile, 0.1% formic acid gradient from 5% to 40% acetonitrile within 40 min. MS spectra were automatically taken by Esquire 3000 plus according to the manufacturer's instrument settings for nano-LC–ESI-MS/MS analyses. Proteins were identified using the MS/MS ion search of Mascot search engine (Matrix Science, London, UK) and nr protein database (National Center for Biotechnology Information, Bethesda, MD, USA). Search parameters used for MS/MS ion search of the Mascot Search engine are as follows: variable modifications; oxidation, mass values; monoisotopic, protein mass; unrestricted, peptide mass tolerance: ±0.1%, fragment mass tolerance; ±0.5 Da, maximum missed cleavages; 1. Instrument type is ESI-QUAD-TOF, number of queries is 300. Probability-based Mowse scores above the calculated threshold value ($p < 0.05$) were considered for protein identification.

2.8. Western blotting

Western blotting was carried out according to Towbin et al. [57]. After completion of the 2-DE, the polyacrylamide gels were soaked in transfer buffer (100 mM Tris, 135 mM glycine) and then transferred onto nitrocellulose membranes (Sigma Chemical Co., St. Louis, MO, USA). The membranes were washed three times in TBST (50 mM Tris, pH 7.5, 150 mM NaCl, 0.1% Tween 20) and then blocked in 2% BSA in TBS (50 mM Tris, pH 7.5, 150 mM NaCl) for at least 2 h. The membranes were probed with the primary antibody specific for 14-3-3 zeta (1:1000), myelin basic protein (1:1000) in blocking buffer, respectively. The membranes then were washed five times in TBST and incubated with a goat anti-rabbit IgG alkaline phosphatase (1:30,000) in blocking buffer for 1 h. After that the membranes were washed three times in TBST and stained with fast red/α naphtol solution for 1 min.

Analyze-it 2.20 software (Analyse-it Software Ltd., Leeds, UK) was used for the statistical analyses of the 2D-Gel data. Student's *t*-test was applied to compare the intensity of spots from the control and GAERS groups. Changes in protein expression were considered significant if $p < 0.05$.

3. Results

3.1. 2-DE of proteins in parietal cortex, thalamus and hippocampus

Protein extracts from the parietal cortex, thalamus and hippocampus of GAERS ($n = 8$) and control animals ($n = 6$) were separated by 2-DE and the protein spots were visualized by colloidal Coomassie staining and compared between the GAERS and the controls using the PDQuest 2D-gel analysis software as

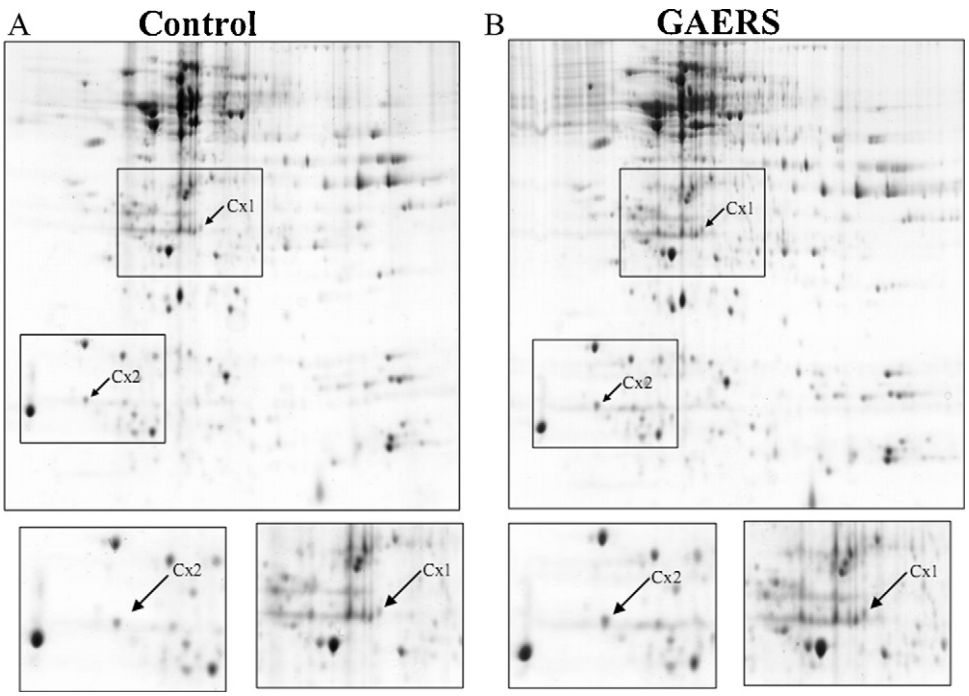

Fig. 1. Two-dimensional gel electrophoresis patterns of parietal cortex tissue of control (A) and GAERS (B) groups. Up-regulated spots in the GAERS are shown as Cx1 and Cx2 with arrows in the figures. Magnified images represent the regions defined in the rectangular boxes of each gel. Spots Cx1 and Cx2 were identified as subunit delta of ATP synthase and 14-3-3 zeta isoform, respectively, by nano LC–ESI-MS/MS analysis.

described under Section 2. For each sample, over 800 proteins were resolved in a 2-DE gel. Among the differentiated protein spots 8 reached statistically significant differences in expression level. Most significantly, two protein spots from each brain region (6 in total) were identified by nano-LC–MS/MS.

3.2. Proteins differentially expressed in absence epileptic animals

3.2.1. Parietal cortex

Fig. 1 shows 2-DE patterns for the parietal cortex of the controls (Fig. 1A) and the GAERS (Fig. 1B). Comparison of these patterns shows two protein spots (Cx1 and Cx2) expressed at significantly different levels and these were identified by nano-LC–ESI-MS/MS. Both proteins, Cx1 and Cx2 were upregulated in GAERS ($p < 0.01$). Cx1 was matched to the delta subunit of mitochondrial ATP synthase; Cx2 was matched to the 14-3-3 zeta isoform by SwissProt and non-redundant NCBI search. In the Mascot search 17 queries matched with 14-3-3 zeta and 7 of these are unique peptides, for more details see supplementary Table S1. Subcellular localization of these identified proteins was predicted with WoLF PSORT (http://wolfpsort.seq.cbrc.jp) [23]. Cx1 was suggested to be localized in mitochondria whereas Cx2 in the cytoplasm. The properties of these differentially expressed protein spots are shown in Table 1.

3.2.2. Thalamus

Fig. 2 shows the comparison of 2D gel patterns obtained from the thalamus of control (Fig. 2A) and GAERS (Fig. 2B). Two differentially expressed spots (T1 and T2) were identified as myelin basic protein and macrophage migration inhibitory factor, respectively. In the Mascot search, 7 queries matched with this T2 protein and 3 of these are unique peptides for more details see supplementary Table S1. T1 was predicted to be localized in the cytoplasm and nucleus, whereas T2 in the cytoplasm. Both were upregulated in GAERS ($p < 0.05$). The results related to these spots are shown in Table 1.

The expression levels of the differentially expressed spots are given as fold-change in Fig. 4.

3.2.3. Hippocampus

Comparison of 2D gel patterns of hippocampus tissues from control (Fig. 3A) and GAERS (Fig. 3B) spots is shown in Fig. 3. Two differentially expressed protein spots (H1 and H2) were identified as macrophage migration inhibitory factor and 0-beta 2 globin, respectively. In the Mascot search, 12 queries matched with macrophage migration inhibitory factor (H1) and 2 of these are unique peptides. In H2, 15 queries matched with 0-2-beta globin and 3 of these are unique peptides for more details see supplementary Table S1. H1 was predicted to be localized in the cytoplasm and nucleus, whereas H2 in the cytoplasm. H1 was upregulated in GAERS compared to the control group ($p < 0.01$). However, H2 was downregulated ($p < 0.05$). The information related to these spots is shown in Table 1.

All identified protein sequences were given in supplementary data.

3.3. Western blotting

The verification of myelin basic protein identified in the thalamus and the 14-3-3 zeta isoform in the parietal cortex was carried out by Western blotting. The blotting spots are shown in Fig. 5.

4. Discussion

This proteomics study showed alterations in the expression of intracellular proteins obtained from the parietal cortex, thalamus and hippocampus in rats with genetic absence epilepsy. The identified proteins were the delta subunit of ATP synthase and the 14-3-3 zeta isoform in the parietal cortex, myelin basic protein and macrophage migration inhibitory factor in the thalamus, and macrophage migration inhibitory factor and 0-beta 2 globulin in the hippocampus.

Table 1
Differentially expressed proteins in GAERS identified by Nano LC–ESI-MS/MS.

Spot no	Protein identity	Accession no (NCBI–swissprot)	Taxonomy	Subcellular localization	Function	PM*/SC/Mascot score	Change in epilepsy	Two-tailed *p* value
Cx1	ATP synthase subunit delta, mitochondrial	BAB27577–P35434	*Mus musculus*	Mitochondria	H^+ ion transport and ATP synthase	1/8%/72	↑	0.01
Cx2	14-3-3 zeta isoform	AAA80544–P63102	*Rattus norvegicus*	Cytoplasm	Protein complex binding	7/3%/402	↑	0.01
T1	Myelin basic protein	AAB59712–P04370	*Mus musculus*	Cytoplasm and nucleus	structural constituent of myelin sheath	2/14%/91	↑	0.05
T2	Macrophage migration inhibitory factor	AAA62644–P30904	*Rattus norvegicus*	Cytoplasm and nucleus	Cytokine	3/33%/115	↑	0.05
H1	Macrophage migration inhibitory factor	AAA62644–P30904	*Rattus norvegicus*	Cytoplasm and nucleus	Cytokine	2/15%/112	↑	0.01
H2	0-Beta 2 globin	CAA47877–Q62670	*Rattus norvegicus*	Cytoplasm	Heme binding	3/23%/180	↓	0.05

Spot numbers correspond to 2D gels in Figs. 1–3. Proteins were identified by MS/MS analysis and MASCOT search of MS/MS spectra with BLAST. All identifications met statistical confidence criteria according to MASCOT and BLAST scoring schemes. ↑: upregulated and ↓: downregulated. Subcellular localization is predicted by WoLF-PSORT search engine. PM: peptides matched and SC: sequence coverage.

The delta subunit of ATP synthase that was identified from the spot Cx1 in the parietal cortex, is a part of the F_1 complex of the ATP synthase. ATP synthase is an important enzyme in the mitochondrial electron transport chain. It catalyses the production of ATP, hydrolyzes ATP to ADP and to inorganic phosphate, and controls the proton gradient across the mitochondrial membrane. The delta subunit of ATP synthase exhibits hydrogen ion transmembrane transporter activity and is a part of the catalytic core of the ATPase complex [58]. Several subunits of the ATP synthase F_1 complex are related directly or indirectly to a number of diseases. Examples are:

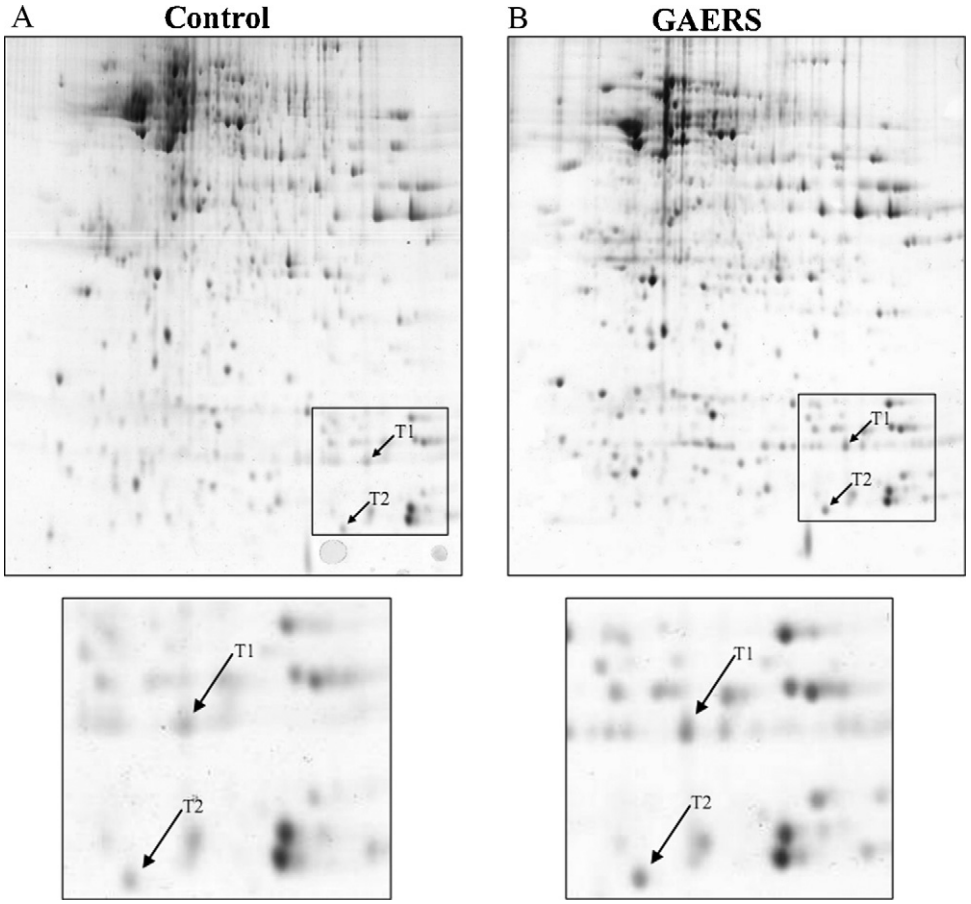

Fig. 2. Two-dimensional gel electrophoresis patterns of thalamic tissues of control (A) and GAERS (B) groups. Up-regulated spots in the GAERS are shown as T1 and T2 with arrows in the figures. Magnified images represent the regions defined in the rectangular boxes of each gel. Spots T1 and T2 were identified as myelin basic protein and macrophage migration inhibitory factor, respectively, by nano LC–ESI-MS/MS analysis.

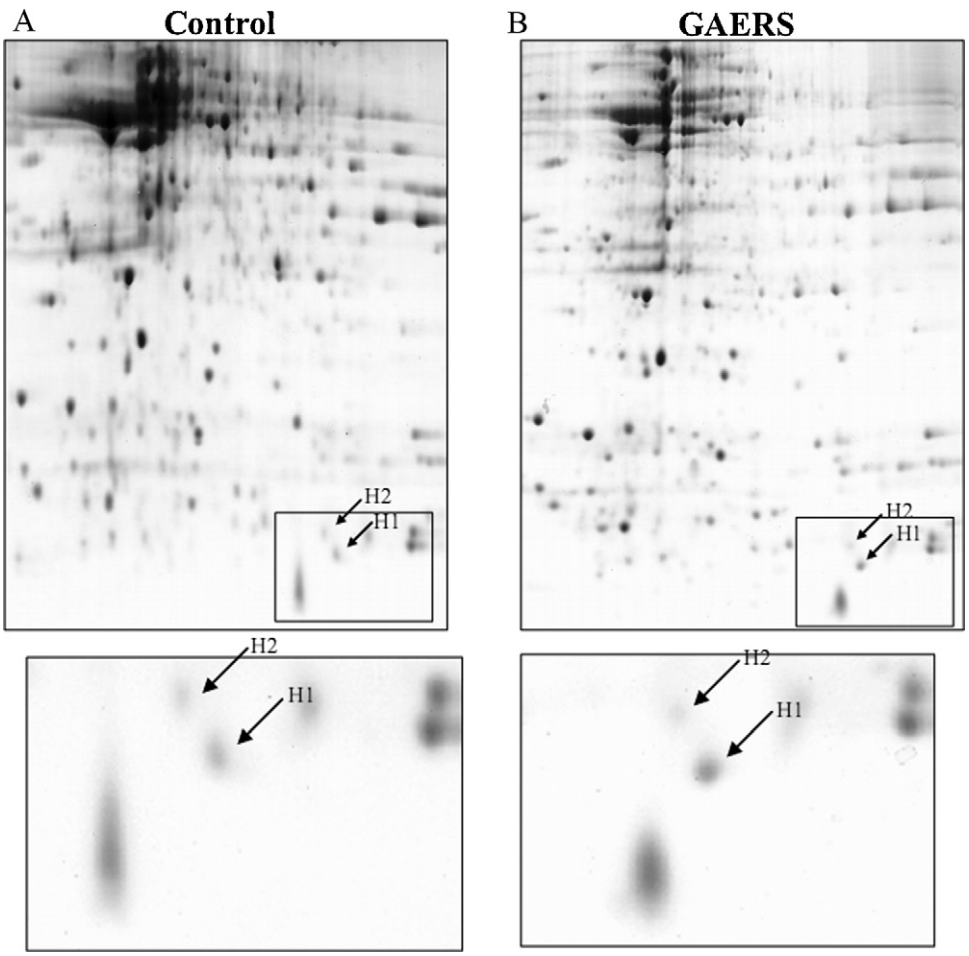

Fig. 3. Two-dimensional gel electrophoresis patterns of hippocampus tissues of control (A) and GAERS (B) groups. Differentiated spots in the GAERS are shown as H1 and H2 with arrows in the figures. H1 is found to be up-regulated in GAERS while H2 is down-regulated. Magnified images represent the regions defined in the rectangular boxes of each gel. Spots H1 and H2 were identified as macrophage migration inhibitory factor and 0-beta 2 globin, respectively, by nano LC–ESI-MS/MS analysis.

neuro-degenerative diseases such as Leigh's disease [11], Batten's disease [45] and Alzheimer's disease [53]. As the subunit delta consists of part of an electrochemically driven rotor component that is essential for ATP synthesis within the F1 motor unit [25,20,59], it may be concluded that phosphorylation of the delta subunit may govern the rate of rotor rotation [28]. In the present study, the delta subunit of ATP synthase was found to be upregulated in the parietal cortex of GAERS. Upregulation in the expression of the delta subunit of ATP synthase indicates an increase in ATP synthesis that correlates with increased cellular activity in the parietal cortex [22]. Parietal cortex has been shown to be responsible for the initiation of spike-and-wave discharges [36,48] and shows increased basal levels of metabolic activity in GAERS [38,39,16]. Spike-and-wave activity in this region was associated with higher neuronal activity [40]. These findings, in parallel with the increased ATP synthesis, thus can be related to the increased level of the delta subunit of ATP synthase.

The other spot, Cx2, 14-3-3 zeta isoform (Cx2) identified in the parietal cortex is predicted to be a cytoplasmic protein by WoLF PSORT. This zeta isoform is a member of 14-3-3 protein family, which is a highly conserved eukaryotic protein, and has seven isoforms in mammals. Five of these isoforms (β, ε, γ, η, and ζ) have the highest expression level in the brain and are largely neuronal [1,21]. In recent years an increasing number of papers reporting possible physiological functions of 14-3-3 in mammalian tissues have been published, including protein trafficking, ion channel and synaptic function, cell cycle control, apoptosis, cancer, schizophrenia and neurodegenerative diseases [21,60,32,4]. Both Niu et al. [43] and Benzing et al. [3] showed that 14-3-3 controls the function of protein 'regulator of G protein signalling'. Further, Rajan et al. [49] showed that 14-3-3 has a crucial role in the membrane localization of ion channels. In line with these finding, earlier studies in genetic rat models of absence epilepsy showed upregulation of several ion channels, including voltage-gated sodium channels Nav1.1 and Nav1.6 in the somatosensory cortex [27] and hyperpolarization-activated cation (HCN1) channel expression [40]. The increased ion channel expression may result in increased expression of 14-3-3 in the parietal cortex of genetic absence epilepsy rats. An induction

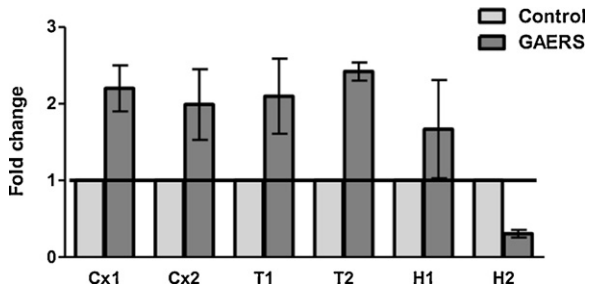

Fig. 4. The fold change of differentiated spots in parietal cortex (Cx1: subunit delta of ATP synthase; Cx2: 14-3-3 zeta isoform), in thalamus (T1: myelin basic protein; T2: macrophage migration inhibitory factor) and hippocampus (H1: macrophage migration inhibitory factor; H2: 0-beta 2 globulin).

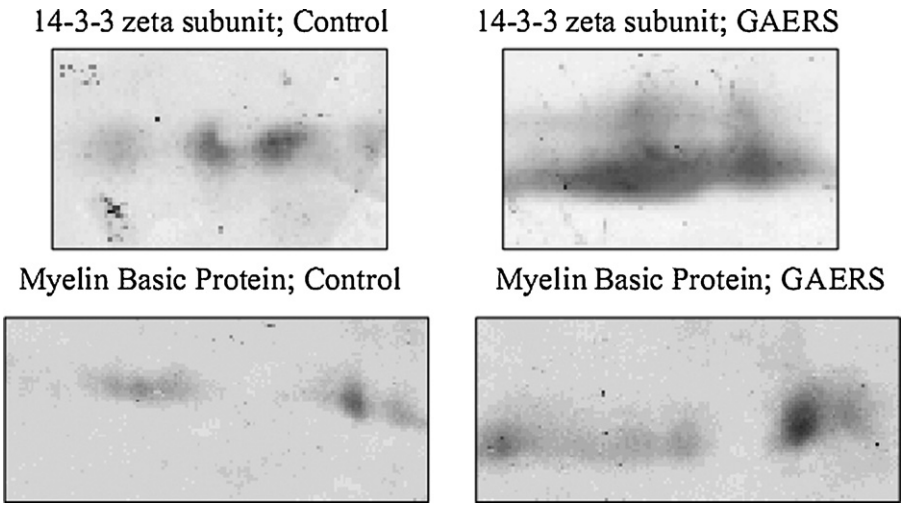

Fig. 5. 2D Gel electrophoresis and Western-blotting of parietal cortex and thalamus fractions showing immunoreactive spots of 14-3-3 zeta and myelin basic protein.

of matrix metalloprotease-9 in the WAG/Rij model has also been demonstrated [55].

Thalamic tissues of the GAERS group revealed elevated myelin basic protein (spot T1) levels, the main components of the myelin membrane [44]. Myelin basic protein is responsible for adhesion to the cytosolic surfaces of multilayered compact myelin and is present in the cytosol on non-compacted regions of myelin [5]. Further, myelin basic protein is a transcriptional activator in the nucleus [13], functioning in cell signalling [18]. Martins-de-Souza et al. have been found that myelin basic protein is upregulated in postmortem mediodorsal thalamus tissues [33]. Recently Kim et al. [26] have shown that myelin basic protein interacts directly with the large conductance Ca^{2+}-activated K^+ (BK_{Ca}) channel *in vivo* and that calmodulin (CaM) interacts with it indirectly via myelin basic protein. BK_{Ca} channels are sensors for membrane voltage and intracellular Ca^{2+} that link cell excitability, signalling and metabolism. The gain of function in the BK_{Ca} channel was linked with generalized epilepsy with paroxysmal dyskinesia [15], suggesting that increased Ca^{2+} sensitivity led to a greater potassium conductance so that enhancement of inhibitory currents can switch neurons into a bursting mode as seen in absence epilepsy. Similarly, upregulation of myelin basic protein in the thalamus of GAERS can play a role in the hyperpolarization in the thalamic relay cells that are responsible for the generation and maintenance of spike-and-wave activity. Hyperpolarization of thalamo-cortical neurons and a subsequent rebound low-threshold Ca^{2+} spike are involved in the spike-and-wave oscillatory activity and physiopathology of absence epilepsy [35].

We found that the macrophage migration inhibitory factor (spots H1 and T2) is upregulated in both hippocampal and thalamic regions of GAERS relative to controls. The migration inhibitory factor is universally expressed in immune and nonimmune tissues and has extensive actions in the immune, endocrine, and nervous systems [2,42]. In the nervous system it was shown that it is constitutively expressed in neurons in the hippocampus, cortex, hypothalamus and pons [2] playing a role in the modulation of nitric oxide and prostaglandin production, catecholamine metabolism, regulation of neuronal sensitivity to glucocorticoids [19] and increases in neuronal delayed rectifier K^+ currents [34]. Migration inhibitory factor, as a proinflammatory cytokine, plays a pivotal regulatory role in the immune response and is implicated in the pathogenesis of many acute and chronic inflammatory diseases such as sepsis, acute respiratory stress syndrome, multiple sclerosis, neuro-Behcet's disease, and romatoid arthritis [9,14]. Involvement of inflammation and inflammatory cytokines in the generation of spike-and-wave discharges has been shown by Kovacs et al. [29]. In that study, injection of lipopolysaccharide, which activates inflammatory mediators in the brain, increases the number of spike-and-wave discharges in genetic absence epilepsy rats.

The other protein identified in the hippocampus, H2, was 0-beta globulin, a part of the beta-chain of the hemoglobin protein [52]. Expressions of alpha- and beta-hemoglobin proteins have been shown in neuronal tissue in rodents as well as in humans. They are suggested to be related to the oxygenation of the tissues and to oxidative stress in neurons [50,54]. In the present study 0-beta globulin was found to be down-regulated in GAERS. Interestingly, H-ferritin mRNA, which has been shown to suppress beta-globulin gene expression in the brain [8], has been found to be up-regulated in the hippocampus of GAERS [30]. This up-regulation of H-ferritin mRNA may have resulted in decreased expression of 0-beta globulin in the hippocampus. However, the functional consequence of these findings needs further research.

5. Conclusions

This study showed changes in the intracellular protein expression by proteomics techniques 2-DE combined with MS in GAERS relative to non-epileptic control rats. The changes at the level of ion channels and receptors are thought to play a principal role in the generation of spike-and-wave activity and intracellular proteins are not primarily responsible for the altered neuronal excitability during seizures. Nevertheless, there are significant experimental data suggesting these soluble proteins play an essential role in the generation of energy (delta subunit of ATP synthase), ion channel localization and signal transduction (14-3-3 zeta), inflammatory processes (macrophage inhibitory factor), membrane K^+ conductance (myelin basic protein, 0-beta globulin) that are important in neuronal function and excitability. Yet, the definite function of these proteins and their relation to the mechanisms of absence epilepsy need to be investigated in future studies.

Conflicts of interest

The author declares that there are no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.brainresbull.2011.02.002.

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