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Aberrant cytosolic acyl-CoA thioester hydrolase in hippocampus of patients with mesial temporal lobe epilepsy

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Summary. A series of enzyme alterations has been shown to be associated with several forms of epilepsy, in mesial temporal lobe epilepsy (MTLE), however, information is limited. It was therefore the aim of the study to determine brain enzyme protein expression using a proteomic screening approach. Hippocampi of controls and patients with drug-resistant MTLE were used for evaluation of protein expression. We applied two-dimensional electrophoresis (2-DE) with mass spectrometrical identification and immunoblotting. 2-DE revealed a remarkably decreased spot identified as cytosolic acyl-CoA thioester hydrolase (BACH; EC 3.1.2.2) in patients with MTLE. Western blotting showed absence of bands at 37 kDa in MTLEs using an antibody against mouse BACH and at 140 kDa in MTLEs using anti-rat BACH. This study demonstrates that BACHs were deranged in hippocampus of MTLE patients. This finding may well contribute to the understanding of the still elusive pathomechanisms involved in MTLE.

Keywords: Acyl-CoA thioester hydrolase – Epilepsy – Hippocampus – Proteomics

Introduction

Mesial temporal lobe epilepsy (MTLE) is a common epilepsy syndrome and hippocampal sclerosis is specific for MTLE in humans, and is characterised by tissue shrinkage, cell loss and reactive gliosis in all hippocampal areas. Typically, neurons of the hippocampal area CA1, CA3 and the hilus of the dendate gyrus become severely damaged (Dalby and Mody, 2001; Pirker et al., 2001).

Although enzyme deficiencies have known to be linked to temporal lobe epilepsy (TLE), pathomechanisms are still elusive. The specific involvement of respiratory chain – rotenone sensitive NADH:CoQ_1 oxidoreductase (mitochondrial complex I) in human hippocampal area CA3 was reported (Kunz et al., 2000) and may be contributing to altered excitability and selective neuronal vulnerability. In their study of TLE patients with a hippocampal epileptic focus complex I deficiency was found in CA3 in contrast to TLE patients presenting with a parahippocampal epileptic focus that showed reduced complex I activity only in the parahippocampal region. Brines and coworkers (1995) observed another link to respiratory chain-mitochondrial-energy metabolism in TLE by the determination of the sodium pump Na^+,K^+-ATPase and cytochrome c oxidase. This ATPase is an enzyme consuming ATP to maintain an adequate neuronal transmembrane electrical potential necessary for brain function and to dissipate ionic transients. Reductions in sodium pump function augment the sensitivity of neurons to glutamate, increasing excitability and neuronal damage *in vitro*. [^{3}H]ouabain was applied to determine the density of hippocampal Na^+,K^+-ATPase by *in vitro* autoradiography. TLE exhibited significant increases in sodium pump density/unit mass of protein in the dentate molecular layer, CA2, and subiculum as compared with autopsy controls and greater hilar sodium pump density was also observed in sclerotic hippocampi. In contrast, cytochrome c oxidase activity was reduced in TLE throughout hippocampus suggesting that although sodium pump protein in surviving neurons appeared to be upregulated in epilepsy, sodium pump capacity may be limited by the reduced

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levels of cytochrome oxidase activity. Functional reduction in sodium pump capacity may be an important factor in hyperexcitability and neuronal death. Further evidence for the importance of energy metabolism and ion pumps in TLE associated with the decrease of Na^+,K^+-ATPase comes from experimental epilepsy studies (Anderson et al., 1994; Grisar et al., 1992). Leite and coworkers (2002) described the decrease of another enzyme, the neuronal nitric oxide synthase (nNOS) in hippocampal CA1–CA4 of patients with TLE. They described loss of nNOS-immunoreactive neurons that may affect major neural transmission systems and as NO can influence the dynamics of ionic channels and neurotransmitter release, neuronal membrane potential may be modulated and thus contributing to abnormal hippocampal excitability. Lie and coworkers (1998) reported that up-regulation of calcium/calmodulin kinase II (CaMKII) and down-regulation of calcineurin in the dentate gyrus of patients with Ammon's horn sclerosis may cause a pathogenetically relevant imbalance of neuronal Ca^{2+}/calmodulin-dependent phosphorylation/dephosphorylation system and this result was comparable to other studies describing an increase of CaMKII activity in an animal model of TLE (Lee et al., 2001). In animal models of TLE a couple of enzyme deficiencies or derangements have been described that may help to understand pathomechanisms of TLE (Bonan et al., 2000; Okada et al., 2001).

Performing protein screening by proteomics we found a manifold decreased spot in MTLE hippocampus that was unambiguously identified as cytosolic acyl-CoA thioester hydrolase (BACH; EC 3.1.2.2), present in all living organisms and localised in cellular compartments such as cytosol, mitochondria, endoplasmic reticulum, and peroxisomes (Hunt and Alexson, 2002; Waku, 1992). The enzyme catalyses the hydrolysis of acyl-CoAs to free fatty acids and coenzyme A (CoASH), however, the physiological roles of this enzyme are not fully elucidated. It appears to serve as a main regulator of acyl-CoA, CoASH and free fatty acid levels in the cell, thus providing a powerful regulation potential for many cellular processes (Hunt and Alexson, 2002). We therefore were interested to study hippocampal protein expression pattern of BACH in MTLE.

Materials and methods

Patients and control cases

Approval for this study was obtained from the Institutional Board of the University of Vienna. Specimens were obtained at surgery from 8 patients with drug-resistant MTLE with typical imaging features (Jackson et al., 1993) and pathological confirmation of hippocampal sclerosis (Babb and Brown, 1987) who had unilateral selective amygdalohippocampectomy or anteromedial temporal lobe resection. The decision for surgery was based on convergent evidence of clinical and EEG recordings during prolonged video-EEG monitoring and high-resolution magnetic resonance imaging (MRI) indicating a mesial temporal lobe seizure onset. Informed consent was obtained from patients providing specimens. Surgical specimens were examined by routine pathology. In accordance with presurgical MRI, hippocampal sclerosis was diagnosed in all patients. As control tissue, 5 normal hippocampal samples were obtained at autopsy from patients with no prior history of brain disease. Clinical information on MTLE patients and patients used as controls is presented in Table 1.

Preparation of hippocampal specimens

Specimens were taken from the hippocampal body (middle segment) of surgical and control tissues. A tissue block of 5- to 10-mm thick perpendicular to the hippocampal axis was further dissected in order to remove any cortical tissue medial to subiculum, as well as most of the white matter of the parahippocampal gyrus. A 3-mm-thick section of this block was processed for routine neuropathological evaluation. The remaining tissue of the dissected block was used for our study. Demonstration of clinical variables between MTLE group and control group is given in Table 2. Due to the limited availability of tissue, experiments could not be carried out on identical specimen but were taken as given in Table 2. Hippocampi were immediately taken and stored into liquid nitrogen and the freezing chain was never interrupted until analysis.

Two-dimensional gel electrophoresis

Hippocampal tissue was powderised in liquid nitrogen and suspended in 1 ml of sample buffer consisting of 7 M urea, 2 M thiourea, 4% CHAPS, 10 mM DL-dithiothreitol (DTT), 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride (PMSF) and a mixture of protease inhibitors. After sonication for approximately 15 sec, the suspension was left at room temperature for 1 hr and centrifuged at $14{,}000 \times g$ for 60 min at 12°C. Desalting was done with Ultrafree-4 centrifugal filter unit (Millipore, Bedford, MA, USA). The protein content of the supernatant was determined by the Coomassie blue method (Bradford, 1976). Two-DE was performed essentially as reported (Fountoulakis et al., 2000). Samples of 1 mg protein were applied on immobilized pI 3–10 nonlinear gradient strips in sample cups at their basic and acidic ends. Focusing started at 200 V and the voltage was gradually increased to 8000 V at 4 V/min and kept constant for a further 3 hrs (approximately 150,000 Vhr totally). The second-dimensional separation was performed on 9–16% gradient sodium dodecyl sulfate (SDS) polyacrylamide gels. The gels ($180 \times 200 \times 1.5$ mm) were run at 40 mA per gel. After protein fixation for 12 hrs in 50% methanol and 10% acetic acid, the gels were stained with colloidal Coomassie blue (Novex, San Diego, CA, USA) for 8 hrs. Molecular masses were determined by running standard protein markers (Bio-Rad Laboratories, Hercules, CA, USA), covering the range 10–250 kDa. pI values were used as given by the supplier of the immobilized pH gradient strips (Amersham Bioscience, Uppsala, Sweden). Excess of dye was washed out from the gels with distilled water and the gels were scanned with ImageScanner (Amersham Bioscience). The level of BACH was quantified using the Image Master 2D Elite software (version 3.1, Amersham Bioscience) and determined as a percentage volume of total protein in the area of interest.

MALDI-TOF mass spectrometry

In-gel digestion was executed as described (Dürauer et al., 2000). The mass spectrometer used in this work was a Bruker REFLEX III (Bruker

Table 1. Clinical characterisation of MTLE patients and controls

	ID number	Sex	Age (yrs)	Duration of epilepsy (yrs)	Side	Antiepileptic drugs (AED)
Patient with MTLE	1	F	42.7	36	L	CBZ, SVP, DPH, TIA
	2	M	39.9	15	R	LAM, CBZ, SVP
	3	F	38.0	34	R	OX, LEV, PB, TOP, LAM, PRIM, CBZ,VIG, SVP
	4	M	34.4	14	R	CBZ
	5	M	35.2	32	R	OX, PRIM, SVP
	6	F	21.5	13	L	SVP, TOP
	7	F	35.7	14	R	CBZ, TOP
	8	M	32.4	16	R	LEV, CBZ, TOP, LAM, SVP
	ID number	Sex	Age (yrs)	Post-mortem interval (hrs)	Side	Causes of death
Control	9	M	62.6	11	L	Coronary heart disease
	10	F	60.1	10	L	Cholangiocarcinoma
	11	M	56.4	10	R	Squamous cell carcinoma (retropharyngeal)
	12	M	73.1	6	L	Papillary renal cell carcinoma
	13	M	55.0	11	R	Squamous cell carcinoma (palate)

SVP, Sodium Valproate; *TOP*, Topiramate; *CBZ*, Carbamazepine; *DPH*, Phenytoin; *TIA*, Tiagabine; *OX*, Oxcarbazepine; *LEV*, Levetiracetam; *PB*, Phenobarbital; *LAM*, Lamotrigine; *PRIM*, Primidone; *VIG*, Vigabatrin; *M*, Male; *F*, Female; *R*, Right; *L*, Left

Table 2. Comparison of clinical variables between MTLE group and control group

	2-DE		Western blot	
	Control	MTLE	Control	MTLE
Male:Female	4:1	3:2	4:1	4:4
Age (yrs) ± SD	61.4 ± 7.2	32.9 ± 6.9	61.4 ± 7.2	35.0 ± 6.4
Duration of epilepsy (yrs)	–	18.0 ± 7.9	–	21.8 ± 10.2
Post-mortem interval (hrs)	9.6 ± 2.1	–	9.6 ± 2.1	–
ID number (Table 1)	9–13	2, 5–8	9–13	1–8

Daltonics, Bremen, Germany) MALDI-TOF instrument, equipped with a standard nitrogen laser (337 nm). Spectra were recorded in reflectron mode, positive ionisation and with 25 kV acceleration voltage. The laser power was varied on a relative scale of 0–100 and was kept at the threshold value to obtain appropriate signal intensity. Calibration of the instrument was performed externally with $[M+H]^+$ ions of des-Arg-Bradykinin, angiotensin I, Glu-Fibrinopeptide B, and adrenocorticotropic hormones (clip 1–17 and clip 18–39). Samples were prepared with a 75:25 (v/v) mixture of α-Cyano-4-hydroxycinnamic acid (CHCA) matrix (saturated solution in acetone) and nitrocellulose (10 mg/ml solution in acetone–isopropanol 50:50 v/v) solutions. One μl of the mixture was placed onto the sample slide and allowed to dry at room temperature. The aliquot (0.5 μl) of the in-gel digestion was mixed with 0.5 μl of 0.1% trifluoroacetic acid (TFA) on this thin layer of matrix crystals and was dried by the application of vacuum. Samples were washed with ice-cold 0.1% TFA. Each spectrum was produced by accumulating data from 90–120 consecutive laser shots. Monoisotopic mass values are reported through the results. Spectra were interpreted with the aid of the Mascot (Matrix Science Ltd, London, UK) or MS-Fit software using the NR database (NCBI Resources, NIH, Bethesda, MD, USA) (Clauser et al., 1999).

Western blot

Hippocampal tissues were homogenised for Western blotting in lysis buffer consisting of 10 mM Tris-HCl (pH 7.5), 150 mM NaCl, 0.05% Tween 20, 1% SDS and 1 mM PMSF and centrifuged at 12,000 × g for 10 min at 4°C. The protein concentration of the supernatant was measured with a BCA protein assay kit (Pierce, Rockford, IL, USA) using a standard curve constructed from a range of known bovine serum albumin standards from 1.25 μg/ml to 20 μg/ml. After mixing samples with sample buffer (60 mM Tris-HCl, 2% SDS, 0.1% bromophenol blue, 25% glycerol, and 14.4 mM 2-mercaptoethanol, pH 6.8) and incubation at 95°C for 5 min, samples were separated by 12.5% homogenous ExcelGel SDS gels (Amersham Bioscience) and electrotransferred onto PVDF membranes. The following antibodies were used for Western blot analysis. The C-terminal 15 amino acid sequence of rat BACH (rBACH; Yamada et al., 1996) polypeptide is identical to the corresponding mouse BACH (mBACH; Kuramochi et al., 2002). After blocking (10 mM Tris-HCl, pH 7.5, 150 mM NaCl, 0.05% Tween 20 and 5% non-fat dry milk), membranes were incubated with mBACH, rBACH, NSE (dilution 1:1000) and actin (dilution 1:2000) antibodies in blocking solution for 2 hrs at room temperature. After washing 3 times for 10 min with washing

solution (0.3% Tween 20 in tris-buffered saline), membranes were incubated with a horseradish peroxidase conjugated goat anti-rabbit IgG (H + L) (Southern Biotechnology Associates, Inc., Birmingham, AL, USA) for 1 hr at room temperature. Membranes were washed 3 times for 10 min and antigen-antibody complexes were visualised by an enhanced chemiluminescence reagent (PerkinElmer Life Sciences, Inc., Boston, MA, USA) on an X-ray film according to the manufacturer's protocol. Densities of immunoreactive signaling bands were measured by RELPscan version 2.1 software program (Scanalytics, Billerica, MA, USA).

Statistical analysis

Data obtained from quantifying protein spots on 2-D map and immunoblotting are presented as mean ± standard deviation (SD). The statistical analysis was evaluated by unpaired Student's t-test using GraphPad Instat2 program and statistical significance was considered at the $P < 0.05$ level.

Results

To screen the differentially expressed proteins in hippocampus of MTLE, we compared the protein expression pattern of hippocampi of controls and patients with MTLE using 2-DE.

Figure 1A shows the 2-DE pattern of hippocampus of a control and a TLE patient with arrowheads indicating spots in the enlarged partial 2-DE images (Fig. 1B) that were remarkably decreased in MTLE group ($n = 5$) as compared to controls ($n = 5$).

Protein spots were excised from the 2-D gel and subjected to in-gel digestion using trypsin. After digestion, the masses of the resulting peptide mixtures were determined by MALDI TOF-MS. Search in the Mascot database resulted in the identification of the protein as cytosolic acyl-CoA thioester hydrolase (EC 3.1.2.2; synonym: acyl-CoA hydrolase and acyl-CoA thioesterase) (Hunt and Alexson, 2002). The relative expression levels of BACH on 2-D gels were 2.01 ± 0.69 in controls ($n = 5$) and 0.27 ± 0.39 in patients with MTLE ($n = 5$) (Fig. 1C). When levels of BACH were normalised with those of NSE or actin used as reference proteins to rule out an effect of neuronal or overall cell loss, the significant decrease was also observed between the two groups (Fig. 1D).

To investigate the expression pattern of BACH in hippocampus, we performed Western blot analysis using BACH antibodies. On Western blots several immunoreactive bands were detected including a 40 kDa BACH protein and additional bands in controls that were undetectable in MTLEs. The major band at approximately 40 kDa was comparable between MTLE and control group. An

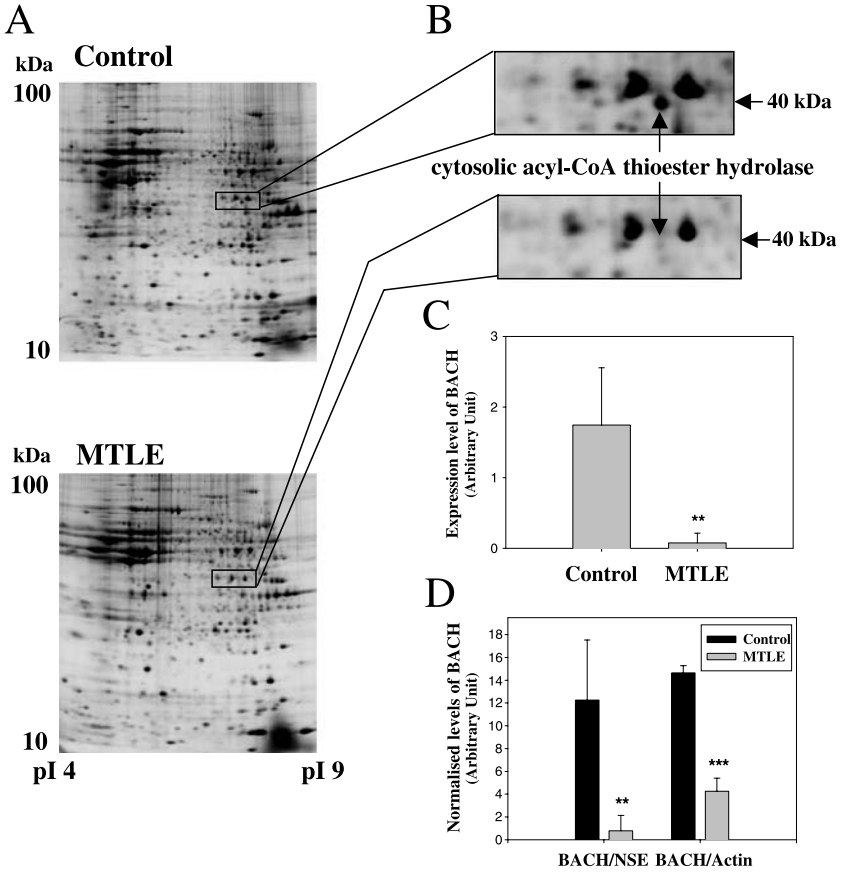

Fig. 1. The representative 2-D gels of human hippocampal tissue from control and patient with MTLE (**A**) and enlarged partial 2-DE images demonstrate the decrease of BACH in hippocampus of patients with MTLE (**B**). MALDI-TOF-MS detection and identification unambiguously assigned spots to cytosolic acyl-CoA thioester hydrolase. The expression level of BACH that significantly decreased in MTLE was determined as the percentage volume of total proteins presented in the gel part considered (**C**). Normalised levels of BACH versus NSE and actin were significantly decreased in MTLE group (**D**). The columns and bars indicate means and SD of the expression levels of proteins in control ($n = 5$) and MTLE ($n = 5$) group. In comparison with controls, $^{**}P < 0.01$ and $^{***}P < 0.001$ values were considered as significantly decreased

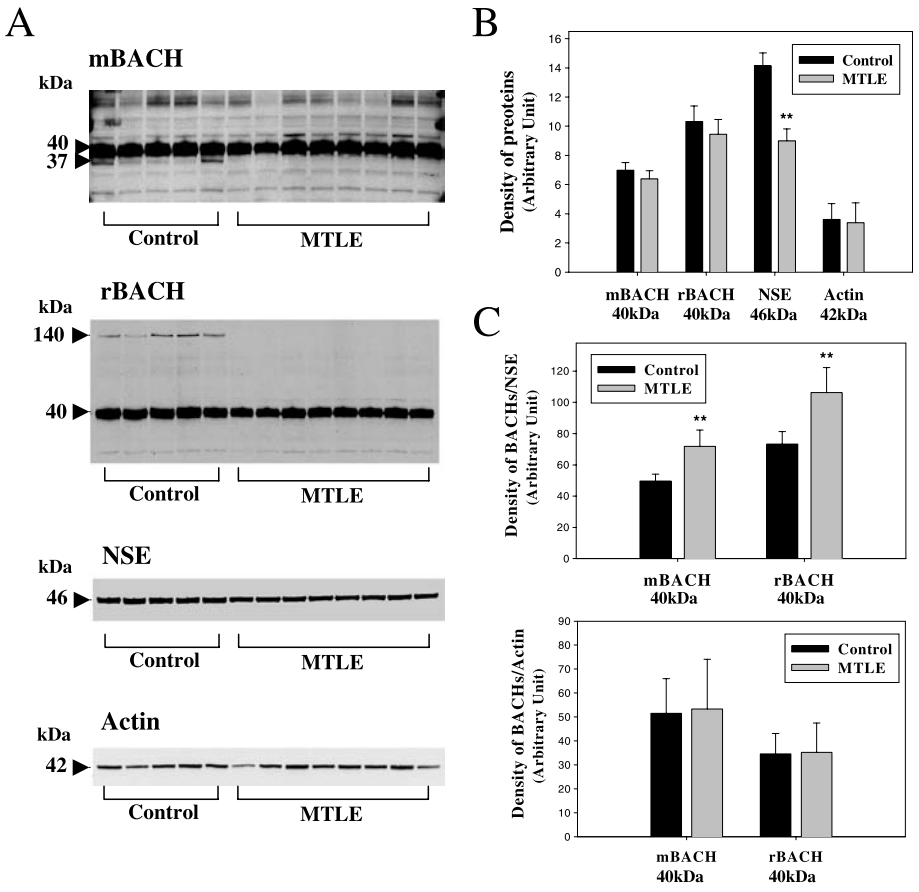

Fig. 2. Western blot patterns of mBACH, rBACH, NSE and actin in hippocampi from controls (n = 5) and patients with MTLE (n = 8) (**A**). Denatured proteins for BACHs (10 μg/lane), NSE (5 μg/lane) and actin (5 μg/lane) were separated on 12.5% homogeneous gels and transferred onto PVDF membrane and subsequently reacted with antibody against mBACH (1:1000), rBACH (1:1000), NSE (1:1000) and actin (1:2000), respectively. An additional band for mBACH at 37 kDa was absent in MTLE and another additional band for rBACH at 140 kDa was absent in MTLE. The protein levels of mBACH (40 kDa), rBACH (40 kDa), NSE (46 kDa) and actin (42 kDa) were expressed as the mean ± SD of optical density of immunoreactive bands (**B**). Normalised levels of mBACH (40 kDa) and rBACH (40 kDa) were significantly increased versus NSE in MTLE group (**$P < 0.01$) and comparable versus actin between the two groups (**C**)

additional immunoreactive band at 37 kDa in controls using an antibody against mBACH was observed, and another additional band was obtained in the control group at 140 kDa when an antibody against rBACH was applied (Fig. 2A).

Discussion

We employed proteomics with 2-DE and MALDI-TOF-MS analysis to identify differentially expressed enzyme proteins in hippocampus of patients with MTLE. We identified one enzyme with a manifold decrease on 2-D gels of hippocampus from patients with MTLE as cytosolic acyl-CoA thioester hydrolase. Although the physiological function of this enzyme remains largely unknown, it is proposed that the ability to regulate acyl-CoA concentration in the cell provides a mechanism for the control of lipid and fatty acid metabolism. Acyl-CoAs have been reported to play a role in energy production by β-oxidation in mitochondria and peroxisomes and participating in signal transduction by regulation of membrane fusion, ion fluxes, protein kinase C activity, enzymatic activity and gene expressional levels (Waku, 1992; Faergeman and Knudsen, 1997).

The main finding of the present study is the aberrant expression of BACH gene products. Yamada and coworkers (1999) purified and characterised cDNA and gene structure of a human brain acyl-CoA thioester hydrolase (hBACH). The BACH gene is well conserved in human and rodents and human BACH encodes a 338 amino acid sequence with more than 95% identity to the mouse and rat homologs (Kuramochi et al., 2002; Yamada

et al., 1999). Human BACH gene (1p36.2) is spanning about 130 kb and comprising at least 13 exons, of which an isoform named hBACHa is predominantly expressed in human brain and localised to the cytosol of neurons (Yamada et al., 2002). mRNA levels of other isoforms are less than 2% of hBACHa mRNA. For this reason Yamada and coworkers (Yamada et al., 2002) have not detected any isoform other than hBACHa at the protein level. However, molecular masses of isoforms were in order of hBACHb > c > a (~43 kDa) > c (cleaved form) > d > b (cleaved form) when expressed in bacteria, and so the major band in Western blot results was thought to be hBACHa and the bands that were undetectable in MTLEs at 37 kDa and at 140 kDa on mBACH and rBACH blots might be cleaved forms of hBACH isoforms c and hBACHb, respectively, although it is not clear whether the precursor of isoform b and c can persist at significant levels in the cell after translation.

There was no significant difference in the predominantly expressed level of BACH (40 kDa) on Western blots using antibodies against mBACH or rBACH (Fig. 2B). Considering neuronal loss in MTLE and localisation of BACH to neurons, however, the expression levels of mBACH and rBACH showed an increase by 145% and 132% when normalised versus NSE as a marker of neuronal density which is expressed in all neuronal cell types (Fig. 2C). The difference of results between the proteomic screen and Western blotting may be explained by the differential resolution, mobility and expression of each isoform depending on pI level and inherent differences between techniques. Western blot using 2-DE gel revealed that the major band (at 40 kDa) detected by one-dimensional (1D) Western blot was actually composed of at least four spots migrating with different isoelectric points ranging from 6.3 to 7.0 (data not shown). This suggested that multiple post-translational modifications are present on the protein and we identified one of isoforms of BACH using 2-DE and MALDI-TOF-MS analysis.

We conclude that BACHs were deranged in the hippocampus of patients with drug-resistant MTLE and this finding may well contribute to the understanding of the still elusive pathomechanisms involved in MTLE and studies further identifying the nature of aberrant BACH expression are in progress in our laboratory.

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References

Anderson WR, Franck JE, Stahl WL, Maki AA (1994) Na,K-ATPase is decreased in hippocampus of kainate-lesioned rats. Epilepsy Res 17: 221–231

Babb T, Brown WJ (1987) Pathological findings in epilepsy. In: Engel J Jr (ed) Surgical treatment of epilepsies. Raven Press, New York, pp 511–540

Bonan CD, Walz R, Pereira GS, Worm PV, Battastini AM, Cavalheiro EA, Izquierdo I, Sarkis JJ (2000) Changes in synaptosomal ectonucleotidase activities in two rat models of temporal lobe epilepsy. Epilepsy Res 39: 229–238

Bradford MM (1976) A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem 72: 248–254

Brines ML, Tabuteau H, Sundaresan S, Kim J, Spencer DD, de Lanerolle N (1995) Regional distributions of hippocampal Na^+,K^+-ATPase, cytochrome oxidase, and total protein in temporal lobe epilepsy. Epilepsia 36: 371–383

Clauser KR, Baker P, Burlingame AL (1999) Role of accurate mass measurement (±10 ppm) in protein identification strategies employing MS or MS/MS and database searching. Anal Chem 71: 2871–2882

Dalby NO, Mody I (2001) The process of epileptogenesis: a pathophysiological approach. Curr Opin Neurol 14: 187–192

Dürauer A, Csaszar E, Mechtler K, Jungbauer A, Schmid E (2000) Characterisation of the rubber elongation factor from ammoniated latex by electrophoresis and mass spectrometry. J Chromatogr A 890: 145–158

Faergeman NJ, Knudsen J (1997) Role of long-chain fatty acyl-CoA esters in the regulation of metabolism and in cell signalling. Biochem J 323: 1–12

Fountoulakis M, Hardmaier R, Schuller E, Lubec G (2000) Differences in protein level between neonatal and adult brain. Electrophoresis 21: 673–678

Grisar T, Guillaume D, Delgado-Escueta AV (1992) Contribution of Na^+,K^+-ATPase to focal epilepsy: a brief review. Epilepsy Res 12: 141–149

Hunt MC, Alexson SE (2002) The role Acyl-CoA thioesterases play in mediating intracellular lipid metabolism. Prog Lipid Res 41: 99–130

Jackson GD, Berkovic SF, Duncan JS, Connelly A (1993) Optimizing the diagnosis of hippocampal sclerosis using MR imaging. AJNR Am J Neuroradiol 14: 753–762

Kunz WS, Kudin AP, Vielhaber S, Blumcke I, Zuschratter W, Schramm J, Beck H, Elger CE (2000) Mitochondrial complex I deficiency in the epileptic focus of patients with temporal lobe epilepsy. Ann Neurol 48: 766–773

Kuramochi Y, Takagi-Sakuma M, Kitahara M, Emori R, Asaba Y, Sakaguchi R, Watanabe T, Kuroda J, Hiratsuka K, Nagae Y, Suga T, Yamada J (2002) Characterization of mouse homolog of brain acyl-CoA hydrolase: molecular cloning and neuronal localization. Brain Res Mol Brain Res 98: 81–92

Lee MC, Ban SS, Woo YJ, Kim SU (2001) Calcium/calmodulin kinase II activity of hippocampus in kainate-induced epilepsy. J Korean Med Sci 16: 643–648

Leite JP, Chimelli L, Terra-Bustamante VC, Costa ET, Assirati JA, de Nucci G, Martins AR (2002) Loss and sprouting of nitric oxide synthase neurons in the human epileptic hippocampus. Epilepsia 43 [Suppl 5]: 235–242

Lie AA, Blumcke I, Beck H, Schramm J, Wiestler OD, Elger CE (1998) Altered patterns of Ca^{2+}/calmodulin-dependent protein kinase II and calcineurin immunoreactivity in the hippocampus of patients with temporal lobe epilepsy. J Neuropathol Exp Neurol 57: 1078–1088

Okada K, Yuhi T, Tsuji S, Yamashita U (2001) Cyclooxygenase-2 expression in the hippocampus of genetically epilepsy susceptible El mice was increased after seizure. Brain Res 894: 332–335

Pirker S, Czech T, Baumgartner C, Maier H, Novak K, Furtinger S, Fischer-Colbrie R, Sperk G (2001) Chromogranins as markers of altered hippocampal circuitry in temporal lobe epilepsy. Ann Neurol 50: 216–226

Waku K (1992) Origins and fates of fatty acyl-CoA esters. Biochim Biophys Acta 1124: 101–111

Yamada J, Furihata T, Tamura H, Watanabe T, Suga T (1996) Long-chain acyl-CoA hydrolase from rat brain cytosol: purification, characterization, and immunohistochemical localization. Arch Biochem Biophys 326: 106–114

Yamada J, Kurata A, Hirata M, Taniguchi T, Takama H, Furihata T, Shiratori K, Iida N, Takagi-Sakuma M, Watanabe T, Kurosaki K, Endo T, Suga T (1999) Purification, molecular cloning, and genomic organization of human brain long-chain acyl-CoA hydrolase. J Biochem 126: 1013–1019

Yamada J, Kuramochi Y, Takagi M, Watanabe T, Suga T (2002) Human brain acyl-CoA hydrolase isoforms encoded by a single gene. Biochem Biophys Res Commun 299: 49–56

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