Royal College of Surgeons in Ireland

repository@rcsi.com

Detection of 14-3-3zeta in cerebrospinal fluid following experimentally evoked seizures.

AUTHOR(S)

Niamh Murphy, Akitaka Yamamoto, David Henshall

CITATION

Murphy, Niamh; Yamamoto, Akitaka; Henshall, David (2008): Detection of 14-3-3zeta in cerebrospinal fluid following experimentally evoked seizures.. Royal College of Surgeons in Ireland. Journal contribution. https://hdl.handle.net/10779/rcsi.10790195.v2

HANDLE

10779/rcsi.10790195.v2

LICENCE

CC BY-NC-ND 4.0

This work is made available under the above open licence by RCSI and has been printed from https://repository.rcsi.com. For more information please contact repository@rcsi.com

URL

https://repository.rcsi.com/articles/journal_contribution/Detection_of_14-3-3zeta_in_cerebrospinal_fluid_following_experimentally_evoked_seizures_/10790195/2

Biomarkers, 2008; 00(0): 1–8

informa
healthcare

Detection of 14-3-3ζ in cerebrospinal fluid following experimentally evoked seizures

NIAMH MURPHY[1], AKITAKA YAMAMOTO[2,3], & DAVID C. HENSHALL[1]

[1]*Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland,* [2]*Robert S. Dow Neurobiology Laboratories, Legacy Research, Portland, OR, USA and* [3]*Department of Neurosurgery, Mie University School of Medicine, Mie, Tsu, Japan*

Abstract

Surrogate and peripheral (bio)markers of neuronal injury may be of value in assessing effects of seizures on the brain or epilepsy development following trauma. The presence of 14-3-3 isoforms in cerebrospinal fluid (CSF) is a diagnostic indicator of Creutzfeldt–Jakob disease but these proteins may also be present following acute neurological insults. Here, we examined neuronal and 14-3-3 proteins in CSF from rats after seizures. Seizures induced by intra-amygdala microinjection of 0.1 μg kainic acid (KA) caused damage which was mainly restricted to the ipsilateral CA3 subfield of the hippocampus. 14-3-3ζ was detected at significant levels in CSF sampled 4 h after seizures compared with near absence in control CSF. Neuron-specific nuclear protein (NeuN) was also elevated in CSF in seizure rats. CSF 14-3-3ζ levels were significantly lower in rats treated with 0.01 μg KA. These data suggest the presence of 14-3-3ζ within CSF may be a biomarker of acute seizure damage.

Keywords: *epilepsy, epileptogenesis, biomarker, hippocampus, neurodegeneration, transmissible spongiform encephalopathy*

(Received 13 December 2007; accepted 27 February 2008)

Introduction

The 14-3-3 proteins are a family of seven chaperone proteins (ζ, η, σ, ε, γ, β, θ) that are highly expressed in the nervous system where they account for 1% of soluble brain protein (Aitken et al. 1992). 14-3-3 proteins regulate enzyme activity, subcellular localization and protein–protein interactions within pathways including neuronal development, cell cycling, apoptosis and signal transduction (Berg et al. 2003).

Detection of 14-3-3 protein in the cerebrospinal fluid (CSF) has been used as a selective premortem diagnostic test for Creutzfeldt–Jakob disease (CJD), a rapidly progressive and fatal transmissible spongiform encephalopathy (Hsich et al. 1996, Wiltfang et al. 1999). However, 14-3-3 isoforms have also been detected in CSF from patients with other neurodegenerative conditions, including Alzheimer's disease, frontotemporal and vascular dementia (Burkhard et al. 2001), AIDS–dementia

Correspondence: David C. Henshall, Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, 123 St Stephen's Green, Dublin 2, Ireland. Tel: +353 1 402 8629. Fax: +353 1 402 2447. E-mail: dhenshall@rcsi.ie

ISSN 1354-750X print/ISSN 1366-5804 online © 2008 Informa UK Ltd.
DOI: 10.1080/13547500802027971

UNCORRECTED PROOF

complex (Wakabayashi et al. 2001) and multiple sclerosis (Martinez-Yelamos et al. 2001). Acute neurological insults, including ischaemic stroke can also cause appearance of 14-3-3 in CSF (Collins et al. 2000, Lemstra et al. 2000).

Neuroimaging and neuropathology suggest ongoing seizures in patients with drug-refractory temporal lobe epilepsy may cause progressive damage to brain within vulnerable structures such as the hippocampus (Kalviainen et al. 1998, Mathern et al. 2002, Liu et al. 2003). Additionally, prolonged seizures (status epilepticus) are profoundly damaging to the brain and a potential cause of cognitive deficits and epilepsy. Elevated neuron specific enolase (NSE) is detected in CSF following epileptic seizures and status epilepticus in patients (DeGiorgio et al. 2006). Whether or not 14-3-3 is present in CSF after seizures is unknown but hippocampal levels of certain 14-3-3 isoforms decline following damaging seizures (Schindler et al. 2004, 2006). Additionally, levels of a subset of 14-3-3 isoforms, including ζ, are altered in hippocampus from patients with intractable epilepsy (Schindler et al. 2006). In the present study we investigated whether seizures in rats cause the appearance of 14-3-3 in CSF as a potential surrogate marker of seizure-induced hippocampal injury.

Methods

Surgical model

All animal procedures were performed in a facility accredited by the Association for Assessment and Accreditation of Laboratory Animal Care in accordance with protocols approved by the Legacy Institutional Animal Care and Use Committee and the principles outlined in the National Institute of Health *Guide for the Care and Use of Laboratory Animals*. Studies were performed using adult male Sprague–Dawley rats (280–350 g) according to previously described techniques with modifications (Schindler et al. 2006). Following anaesthesia and femoral vein catheterization, animals were placed in a stereotaxic frame. Three recording electrodes were affixed for cranial EEG and a craniectomy was performed for placement of an injection cannula. The animal was then removed from the frame, anaesthesia was discontinued, EEG recordings were commenced and a 31 gauge internal cannula (Plastics One Inc., Roanoke, VA, USA) was inserted into the lumen of the guide to inject kainic acid (KA, at 0.1 or 0.01 μg in 0.5 μl saline vehicle) into the right amygdala (Schindler et al. 2006). Non-seizure control rats underwent the same surgical procedure but received intra-amygdala vehicle injection. The EEG was monitored until lorazepam (6 mg kg^{-1} intravenously) was administered 40 min later to curtail seizure activity. CSF was sampled from rats 4 h following lorazepam administration, according to previously described techniques (Fujiki et al. 2001). Briefly, rats were deeply anaesthetized and the head of the rat was held in place by the experimenter. A microsyringe was introduced percutaneously into the cisterna magna and CSF of approximately 10 μl volume was aspirated and subsequently stored at $-80^{\circ}C$.

CSF analysis

For analysis of CSF, samples were subject to Western blotting as described previously with modifications (Schindler et al. 2006). Samples were boiled in gel loading buffer and then separated on 12% sodium dodecylsulphate–polyacrylamide electrophoresis gels. Proteins were transferred to nitrocellulose membranes and then incubated with

UNCORRECTED PROOF

antibodies against 14-3-3ζ (sc-1019) and 14-3-3β (sc-25276) (Santa Cruz, Biotechnology, Santa Cruz, CA, USA) and neuron-specific nuclear protein (NeuN; Chemicon, MA, USA). Membranes were then incubated with appropriate secondary antibodies (Jackson Immunoresearch, Plymouth, PA, USA) followed by chemiluminescence detection (Pierce, UK) and exposure under a Fuji-film LAC-300 gel imager. Gel bands were digitized and analyzed in a semiquantitative manner using time-matched exposures by optical densitometry (AlphaEase V4.0; Alpha Innotech Corporation, San Leandro, CA, USA). Data are presented as mean ± SEM of n independent experiments and analyzed using Student's t-test and analysis of variance (ANOVA) with *post hoc* Fisher's PLSD test (StatView software, SAS Institute, Inc., Cary, NC, USA). Significance was accepted at $p < 0.05$.

Histopathology

For examination of seizure damage, fresh frozen coronal sections at the level of the dorsal hippocampus were prepared from a separate group of rats killed 4 h following anticonvulsant. Sections (12 μm) were air-dried, post-fixed in 4% paraformaldehyde for 30 min, hydrated, immersed in a 0.06% potassium permanganate solution (Sigma-Aldrich, Ireland), rinsed and then incubated for 30 min in 0.001% Fluoro-jade B solution (Chemicon, Temecula, CA, USA). Slides were then rinsed again, dried and mounted and visualized using a Hamamatsu Orca 285 camera attached to a Nikon 2000s epifluorescence microscope (Micro-optica, Ireland).

Results

Restricted damage to the ipsilateral CA3 following seizures induced by intra-amygdala KA

Intra-amygdala microinjection of 0.1 μg KA induced polyspike paroxysmal seizure discharges on EEG until termination by lorazepam, as previously described (Henshall et al. 2000, Schindler et al. 2006). Examination of Fluoro-jade B staining, which identifies irreversibly damaged cells, revealed seizures mainly caused damage to CA3 pyramidal neurons of the ipsilateral hippocampus (Figure 1b, d). Fluoro-jade B stained cells were not found in the contralateral hippocampus (Figure 1a). A small number of scattered Fluoro-jade B-stained cells with neuronal morphology were present within cortex ipsilateral (Figure 1c, e) but not contralateral (data not shown) to the side of seizure elicitation.

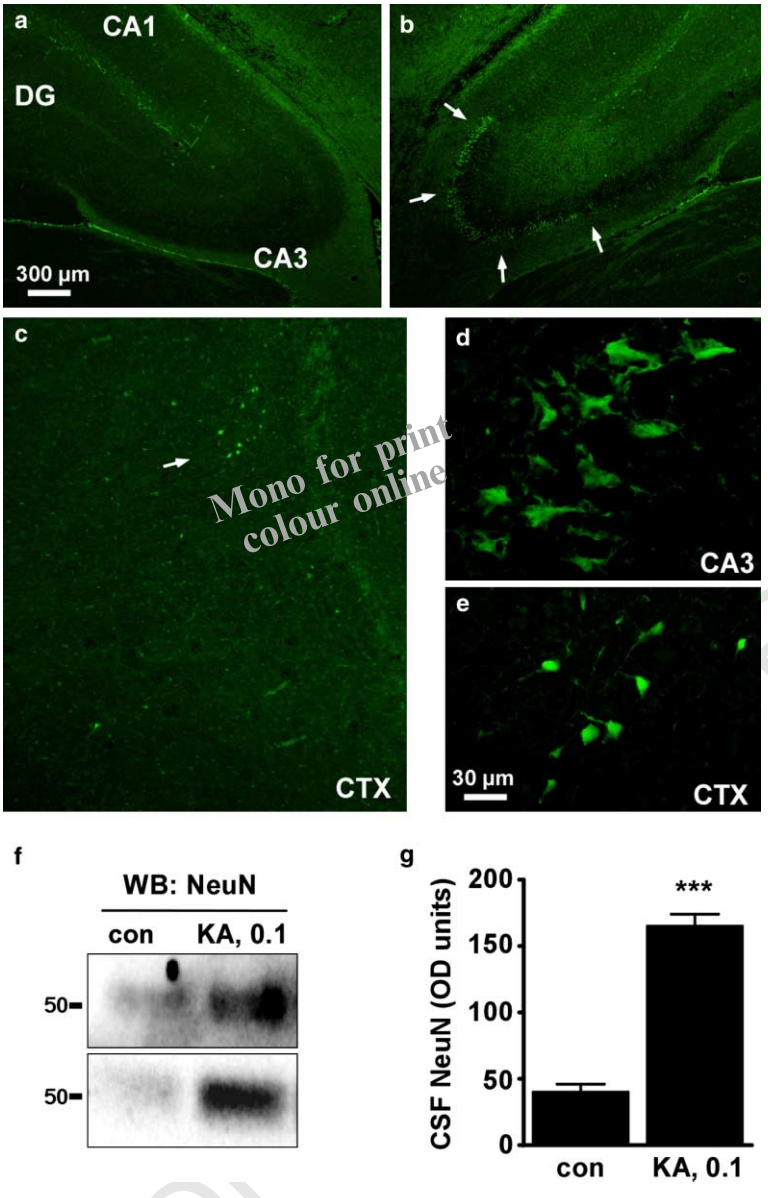
Detection of NeuN within CSF following seizures

While seizures are known to provoke release of NSE into CSF we examined whether additional neuron-specific proteins are released to CSF. Accordingly, we investigated whether seizures induced release of NeuN. Western blot analysis of CSF from rats determined NeuN was largely undetectable in control CSF but was increased by ~4-fold in rats subject to seizures induced by 0.1 μg KA (Figure 1f, g).

Detection of 14-3-3ζ but not 14-3-3β in CSF following seizures

We next examined whether the CSF contained 14-3-3 isoforms, focusing on the ζ and β isoforms that we have previously shown to be regulated in hippocampus following

UNCORRECTED PROOF



Mono for print colour online

UNCORRECTED PROOF

Figure 1. Seizure damage and detection of NeuN in CSF. (a) Representative photomicrograph (4 × lens) of Fluoro-jade B staining of the contralateral hippocampus of a rat 4 h following seizures induced by 0.1 μg intra-amygdala KA. Note absence of Fluoro-jade B-stained cells in any subfield. (b) Ipilateral hippocampus 4 h following seizures induced by 0.1 μg KA showing Fluoro-jade B-positive CA3 neurons (arrows). (c) Representative field view (4 × lens) of the ipsilateral cortex (CTX) of a rat 4 h after seizures, showing presence of only limited numbers of Fluoro-jade B-stained cells (arrow). (D, E) Higher power (20 × lens) views of Fluoro-jade B-positive neurons within (d) CA3 and (e) ipsilateral cortex. (f) Representative Western blots (WB) ($n=1$ per lane) of CSF samples at 4 h from two independent sets of animals, comparing presence of NeuN in 0.1 μg KA-injected rats (KA, 0.1) with controls (con). (g) Graph ($n=4$ per group) showing semiquantitative optical densitometry (OD) analysis of CSF NeuN. ***$p<0.0001$ compared with control. CA, cornu ammonis; DG, dentate gyrus.

C:/3B2WIN/temp files/tbmk302965_S100.3d[x] Friday, 28th March 2008 18:34:53

seizures in rats and in human temporal lobe epilepsy (Schindler et al. 2004, 2006). The 14-3-3ζ isoform was largely undetectable in CSF from vehicle-injected control rats (Figure 2a). In contrast, significant amounts of 14-3-3ζ were present in CSF from rats subjected to seizures induced by 0.1 μg KA (Figure 2a, c). To investigate whether CSF 14-3-3 levels were related to the extent of seizure damage we examined 14-3-3 levels in CSF from a second group of rats treated with 0.01 μg KA. We have previously reported seizures at this dose are mild and do not cause overt lesions to CA3 and only minor changes to expression of cell death-regulatory genes (Henshall et al. 2000, 2002, 2003). CSF levels of 14-3-3ζ were significantly lower in 0.01 μg KA-injected rats compared to 0.1 μg KA-injected rats (Figure 2a, c).

To gain insight into the specificity of the 14-3-3ζ response we examined the CSF samples for the presence of 14-3-3β which is also expressed in rat and human hippocampus (Schindler et al. 2004, 2006). In contrast to 14-3-3ζ, seizures induced by KA did not cause the appearance of 14-3-3β in the CSF (Figure 2b, d).

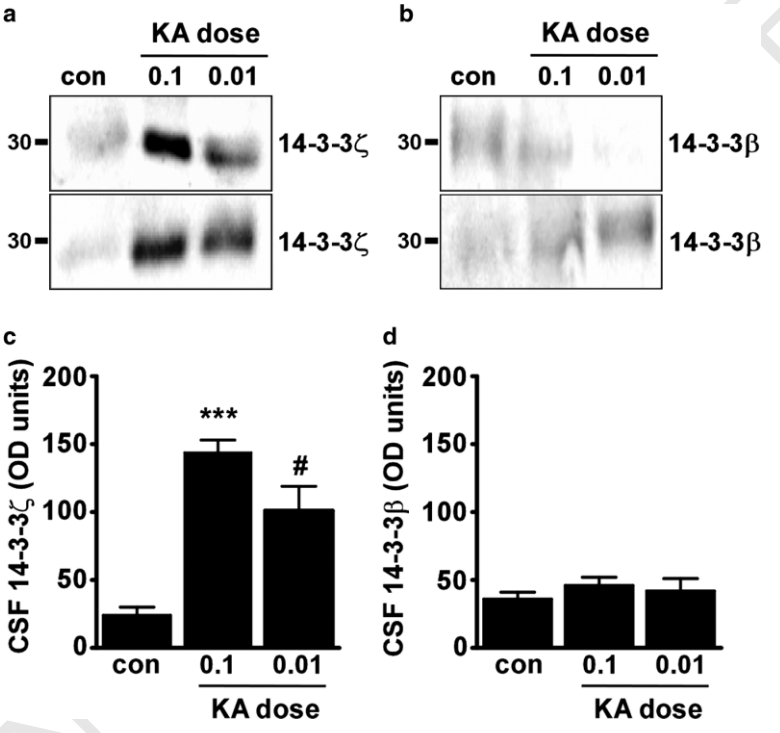


Figure 2. Presence of 14-3-3ζ in CSF following seizures. (a) Representative Western blots ($n = 1$ per lane) of CSF samples at 4 h from two independent sets of animals, comparing presence of 14-3-3ζ in seizure rats (KA, 0.1 μg and 0.01 μg treated) to controls (con). (b) Representative Western blots ($n = 1$ per lane) of CSF samples at 4 h from two independent sets of animals, comparing presence of 14-3-3β in seizure rats to controls. Graphs ($n = 4$ per group) show semiquantitative analysis of (c) 14-3-3ζ and (d) 14-3-3β levels. ***$p < 0.0001$ for comparison of 0.1 μg to control. #$p < 0.05$ for comparison of 0.01 μg to 0.1 μg groups, and $p < 0.01$ for comparison of 0.01 μg group to control.

Discussion

Surrogate markers are required to assess potentially subtle brain injury caused by repeated brief or single prolonged seizures. The present study was undertaken to determine whether seizures caused the appearance of 14-3-3 within CSF. Our data show that 14-3-3ζ, but not 14-3-3β is present in the CSF of rats shortly after seizures, and its abundance was related to the severity of the seizure insult (KA dose). These data suggest CSF 14-3-3ζ measurement may be of value as a surrogate marker of seizure damage and perhaps epileptogenesis in studies of acquired epilepsy or in evaluating therapeutic efficacy of interventions.

There is significant interest in identifying surrogate markers of brain injury after seizures, which may complement imaging approaches (Pitkanen et al. 2007). The present study shows for the first time that prolonged seizures result in an isoform-selective appearance of 14-3-3 within CSF. Detection of 14-3-3ζ in CSF just 4 h after seizures suggests it could serve as an early marker of seizure damage. We also found CSF 14-3-3ζ levels were significantly higher in rats given 0.1 μg compared with 0.01 μg KA. Thus, 14-3-3ζ levels in CSF after seizures like NSE (DeGiorgio et al. 2006), may reflect the degree of damage. This contrasts clinical findings on dementias associated with 14-3-3 in CSF, where correlations between abundance and diagnosis are not found (Burkhard et al. 2001). However, the difference in CSF levels of 14-3-3ζ were rather smaller than would be expected based on the disparity in seizure intensity and hippocampal damage between the low and high KA doses used (Henshall et al. 2000, 2002). Thus, the sensitivity of 14-3-3ζ in CSF for discriminating between subtle and overt hippocampal neuronal injury requires further investigation.

Experimental models of neuronal trauma or ischaemia are associated with transition of 14-3-3 proteins into the extracellular milieu (Siman et al. 2004). In the present study, the appearance of 14-3-3ζ in the CSF is most likely a result of material transiting from seizure-damaged neurons (Siman et al. 2004). Indeed, hippocampal 14-3-3ζ levels drop rapidly following damaging seizures (Schindler et al. 2006) and ~97% of dying cells in this model are NeuN-positive (Henshall et al. 2001). Why 14-3-3ζ and not 14-3-3β was present in CSF after seizures is uncertain, but may reflect greater abundance of this isoform in the rat hippocampus (Schindler et al. 2006). The particular enrichment of the ζ isoform within the membrane-containing fraction of cells (Schindler et al. 2006) may further increase the likelihood of it being detected as a result of damage to neurons.

Whether 14-3-3ζ release into CSF occurs following briefer, epileptic seizures is uncertain, although it has been reported for NSE (DeGiorgio et al. 2006). Of note, 14-3-3 isoform-specific differences are found in hippocampus from patients with intractable epileptic seizures, although levels of 14-3-3η rather than 14-3-3ζ were lower in that study (Schindler et al. 2006). It may be useful to ascertain whether isoform or abundance profiles change over time post-seizure, along with whether animals display changes to CSF 14-3-3 during epilepsy development.

Our data also show CSF levels of NeuN are elevated after seizures. NSE is detectable in normal human CSF at low levels, increasing 2–5-fold following individual seizures and status epilepticus (DeGiorgio et al. 2006). However, false-negative results have been reported (DeGiorgio et al. 2006). Our study establishes a neuron-specific protein in addition to NSE is released to CSF after seizures. However, NeuN levels in CSF were not appreciably lower in rats given the 0.01 μg KA dose

UNCORRECTED PROOF

(data not shown). Thus, the sensitivity of NeuN for grades of seizure damage may be more limited than using 14-3-3ζ.

While the diagnostic value of 14-3-3 as a marker for sporadic CJD is still higher than that of other CSF markers, including NSE and s100β, its accuracy as a marker for variant CJD is more limited (Green et al. 2001). Moreover, 14-3-3 isoforms, including ζ, appear in the CSF of patients with other dementias and neurological disorders (Chapman et al. 2000, Green et al. 2001, Wakabayashi et al. 2001, Zanusso et al. 2005). Since seizures are associated with a number of acute neurological injuries and occur in patients with CJD it is possible that they can contribute to CSF results or provide potentially erroneous data (Donmez et al. 2005). In turn, this may have implications for diagnosis where CJD may present as refractory non-convulsive status epilepticus (Cohen et al. 2004).

In summary, the present study reveals prolonged seizures cause the appearance of 14-3-3ζ within CSF. Detecting seizure damage and tracking epilepsy development following trauma and other CNS insults requires surrogates and biomarkers of the pathogenic process. Accordingly, measuring CSF 14-3-3 in at-risk individuals may hold prognostic or diagnostic value in studies of epileptogenesis and neuronal damage following status epilepticus.

Acknowledgements

This work was supported by grants from Science Foundation Ireland (04/IN3/B466) and NIH/NINDS NS39016 and NS41935.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

AQ1

References

Aitken A, Collinge DB, van Heusden BP, Isobe T, Roseboom PH, Rosenfeld G, Soll J. 1992. 14-3-3 proteins: a highly conserved, widespread family of eukaryotic proteins. Trends in Biochemical Sciences 17:498–501.

Berg D, Holzmann C, Riess O. 2003. 14-3-3 proteins in the nervous system. Nature Review. Neuroscience 4:752–762.

Burkhard PR, Sanchez JC, Landis T, Hochstrasser DF. 2001. CSF detection of the 14-3-3 protein in unselected patients with dementia. Neurology 56:1528–1533.

Chapman T, McKeel DW Jr, Morris JC. 2000. Misleading results with the 14-3-3 assay for the diagnosis of Creutzfeldt–Jakob disease. Neurology 55:1396–1397.

Cohen D, Kutluay E, Edwards J, Peltier A, Beydoun A. 2004. Sporadic Creutzfeldt–Jakob disease presenting with nonconvulsive status epilepticus. Epilepsy & Behavior 5:792–796.

Collins S, Boyd A, Fletcher A, Gonzales M, McLean CA, Byron K, Masters CL. 2000. Creutzfeldt–Jakob disease: diagnostic utility of 14-3-3 protein immunodetection in cerebrospinal fluid. Journal of Clinical Neuroscience 7:203–208.

DeGiorgio CM, Rabinowicz AL, Correale J, Heck CN, Gott PS, Schreiber S. 2006. Neuron-specific enolase in status epilepticus. In: Status epilepticus: mechanisms and management. Wasterlain CG, Treiman DM, editors. Cambridge, MA: The MIT press. p. 169–175.

Donmez B, Cakmur R, Men S, Oztura I, Kitis A. 2005. Coexistence of movement disorders and epilepsia partialis continua as the initial signs in probable Creutzfeldt–Jakob disease. Movement Disorders 20:1220–1223.

Fujiki N, Yoshida Y, Ripley B, Honda K, Mignot E, Nishino S. 2001. Changes in CSF hypocretin-1 (orexin A) levels in rats across 24 hours and in response to food deprivation. Neuroreport 12:993–997.

UNCORRECTED PROOF

Green AJ, Thompson EJ, Stewart GE, Zeidler M, McKenzie JM, MacLeod MA, Ironside JW, Will RG, Knight RS. 2001. Use of 14-3-3 and other brain-specific proteins in CSF in the diagnosis of variant Creutzfeldt–Jakob disease. Journal of Neurology, Neurosurgery, & Psychiatry 70:744–748.

Henshall DC, Sinclair J, Simon RP. 2000. Spatio-temporal profile of DNA fragmentation and its relationship to patterns of epileptiform activity following focally evoked limbic seizures. Brain Research 858:290–302.

Henshall DC, Bonislawski DP, Skradski SL, Meller R, Lan J-Q, Simon RP. 2001. Cleavage of Bid may amplify caspase-8-induced neuronal death following focally evoked limbic seizures. Neurobiology of Disease 8:568–580.

Henshall DC, Araki T, Schindler CK, Shinoda S, Lan J-Q, Simon RP. 2003. Expression of death-associated protein kinase and recruitment to the tumor necrosis factor signaling pathway following brief seizures. Journal of Neurochemistry 86:1260–1270.

Henshall DC, Skradski SL, Meller R, Araki T, Minami M, Schindler CK, Lan JQ, Bonislawski DP, Simon RP. 2002. Expression and differential processing of caspases 6 and 7 in relation to specific epileptiform EEG patterns following limbic seizures. Neurobiology of Disease 10:71–87.

Hsich G, Kenney K, Gibbs CJ, Lee KH, Harrington MG. 1996. The 14-3-3 brain protein in cerebrospinal fluid as a marker for transmissible spongiform encephalopathies. New England Journal of Medicine 335:924–930.

Kalviainen R, Salmenpera T, Partanen K, Vainio P, Riekkinen P, Pitkanen A. 1998. Recurrent seizures may cause hippocampal damage in temporal lobe epilepsy. Neurology 50:1377–1382.

Lemstra AW, van Meegen MT, Vreyling JP, Meijerink PH, Jansen GH, Bulk S, Baas F, van Gool WA. 2000. 14-3-3 testing in diagnosing Creutzfeldt–Jakob disease: a prospective study in 112 patients. Neurology 55:514–516.

Liu RS, Lemieux L, Bell GS, Hammers A, Sisodiya SM, Bartlett PA, Shorvon SD, Sander JW, Duncan JS. 2003. Progressive neocortical damage in epilepsy. Annals of Neurology 53:312–324.

Martinez-Yelamos A, Saiz A, Sanchez-Valle R, Casado V, Ramon JM, Graus F, Arbizu T. 2001. 14-3-3 protein in the CSF as prognostic marker in early multiple sclerosis. Neurology 57:722–724.

Mathern GW, Adelson PD, Cahan LD, Leite JP. 2002. Hippocampal neuron damage in human epilepsy: Meyer's hypothesis revisited. Progress in Brain Research 135:237–251.

Pitkanen A, Kharatishvili I, Karhunen H, Lukasiuk K, Immonen R, Nairismagi J, Grohn O, Nissinen J. 2007. Epileptogenesis in experimental models. Epilepsia 48 (Suppl. 2):13–20.

Schindler CK, Heverin M, Henshall DC. 2006. Isoform- and subcellular fraction-specific differences in hippocampal 14-3-3 levels following experimentally evoked seizures and in human temporal lobe epilepsy. Journal of Neurochemistry 99:561–569.

Schindler CK, Shinoda S, Simon RP, Henshall DC. 2004. Subcellular distribution of Bcl-2 family proteins and 14-3-3 within the rat hippocampus during seizure-induced neuronal death in the rat. Neuroscience Letters 356:163–166.

Siman R, McIntosh TK, Soltesz KM, Chen Z, Neumar RW, Roberts VL. 2004. Proteins released from degenerating neurons are surrogate markers for acute brain damage. Neurobiology of Disease 16:311–320.

Wakabayashi H, Yano M, Tachikawa N, Oka S, Maeda M, Kido H. 2001. Increased concentrations of 14-3-3 epsilon, gamma and zeta isoforms in cerebrospinal fluid of AIDS patients with neuronal destruction. Clinica Chimica Acta 312:97–105.

Wiltfang J, Otto M, Baxter HC, Bodemer M, Steinacker P, Bahn E, Zerr I, Kornhuber J, Kretzschmar HA, Poser S, Ruther E, Aitken A. 1999. Isoform pattern of 14-3-3 proteins in the cerebrospinal fluid of patients with Creutzfeldt–Jakob disease. Journal of Neurochemistry 73:2485–2490.

Zanusso G, Fiorini M, Farinazzo A, Gelati M, Benedetti MD, Ferrari S, Dalla Libera A, Capaldi S, Monaco HL, Rizzuto N, Monaco S. 2005. Phosphorylated 14-3-3zeta protein in the CSF of neuroleptic-treated patients. Neurology 64:1618–1620.

UNCORRECTED PROOF