Received: 10 April 2019 | Revised: 12 April 2020 | Accepted: 13 April 2020

DOI: 10.1111/1440-1681.13326

CEPP Clinical and Experimental Pharmacology and Physiology WILEY

ORIGINAL ARTICLE

Neurobiology

Evidence for altered insulin signalling in the brains of genetic absence epilepsy rats from Strasbourg

Sathiya Sekar[1] | Wendie N. Marks[1] | Venkat Gopalakrishnan[1] | Quentin Greba[1] | Terrance P. Snutch[2] | John G. Howland[1] | Changiz Taghibiglou[1]

[1]Department of Anatomy, Physiology, Pharmacology, College of Medicine, University of Saskatchewan, Saskatoon, SK, Canada

[2]Michael Smith Laboratories and Djavad Mowafaghian Centre for Brain Health, University of British Columbia, Vancouver, BC, Canada

Correspondence
Changiz Taghibiglou, Department of Anatomy, Physiology, Pharmacology, College of Medicine, University of Saskatchewan, 107 Wiggins Road, Saskatoon, SK S7N 5E5, Canada.
Email: changiz.taghibiglou@usask.ca

Funding information
Canadian Institutes of Health Research, Grant/Award Number: 10677 and 227397; Saskatchewan Health Research Foundation, Grant/Award Number: 3543

Abstract

Insulin-mediated signalling in the brain is critical for neuronal functioning. Insulin resistance is implicated in the development of some neurological diseases, although changes associated with absence epilepsy have not been established yet. Therefore, we examined the major components of PI3K/Akt-mediated insulin signalling in cortical, thalamic, and hippocampal tissues collected from Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and Non-Epileptic Control (NEC) rats. Insulin levels were also measured in plasma and cerebrospinal fluid (CSF). For the brain samples, the nuclear fraction (NF) and total homogenate (TH) were isolated and investigated for insulin signalling markers including insulin receptor beta (IRβ), IR substrate-1 and 2 (IRS1 & 2), phosphatase and tensin homologue (PTEN), phosphoinositide 3-kinase phospho-85 alpha (PI3K p85α), phosphatidylinositol 4,5-bisphosphate, phosphatidylinositol (3,4,5)-trisphosphate, protein kinase B (PKB/Akt1/2/3), glucose transporter-1 and 4 (GLUT1 & 4) and glycogen synthase kinase-3β (GSK3β) using western blotting. A significant increase in PTEN and GSK3β levels and decreased PI3K p85α and pAkt1/2/3 levels were observed in NF of GAERS cortical and hippocampal tissues. IRβ, IRS1, GLUT1, and GLUT4 levels were significantly decreased in hippocampal TH of GAERS compared to NEC. A non-significant increase in insulin levels was observed in plasma and CSF of GAERS rats. An insulin sensitivity assay showed decreased p-Akt level in cortical and hippocampal tissues. Together, altered hippocampal insulin signalling was more prominent in NF and TH compared to cortical and thalamic regions in GAERS. Restoring insulin signalling may improve the pathophysiology displayed by GAERS, including the spike-and-wave discharges that relate to absence seizures in patients.

KEYWORDS

absence epilepsy, brain insulin signalling, GAERS, GSK3β, PI3K p85, PTEN

The peer review history for this article is available at https://publons.com/publon/10.1111/1440-1681.13326

1 | INTRODUCTION

Insulin acts as a neurotrophic factor in the regulation of various neuronal functions.[1] Growing evidence supports the notion that brain insulin resistance contributes to the progression of Alzheimer's disease, Parkinson's disease, neuronal injury, traumatic brain injury, brain ischaemia, and epilepsy.[2,3] Hyperglycaemia and insulin resistance are associated with seizure activity and their resulting metabolic alterations modulate the outcome of seizures and brain injury.[4,5] For example, reduced glucose availability and metabolism increases the vulnerability of neurons to death following seizures.[5] Absence epilepsy is a common type of genetic generalized epilepsy in which patients show non-convulsive seizures generally characterized by hypersynchronization of thalamocortical circuits.[6,7] Studies have also demonstrated the involvement of hippocampal neuronal firing prior to thalamocortical paroxysmal discharges, which may contribute to cognitive dysfunction in absence epilepsy.[8] Glutamatergic excitatory neuronal cell death, alterations in sleep-wake cycle, and cognitive deficits have been reported in patients with absence seizures.[9-12] Hyperinsulinemia has been noted in female patients with generalized seizures including absence seizures [13] and obesity is a risk factor in paediatric epilepsy patients with untreated or newly diagnosed epilepsy.[14]

Given these observations, and a lack of data regarding insulin signalling in rodent models of absence epilepsy, we studied the insulin signalling cascade, especially PI3K/Akt signalling components, in Genetic Absence Epilepsy Rats from Strasbourg (GAERS), a well-established rat model of childhood absence epilepsy that possess a gain-of-function missense mutation in the Cav 3.2 T-type calcium channel gene.[15-20] By 8–10 weeks of age, GAERS exhibit spike-and-wave discharges on cortical electroencephalogram recordings. In contrast, the Non-Epileptic Control (NEC) strain, which was developed at the same times as GAERS, has not been reported to develop spike-and-wave discharges in previously published studies.[18,19,21] Recently, we reported that the nuclear translocation of sterol regulatory elementary binding protein 1 (SREBP1), GluA2 subunit of AMPA receptor and pro-death signalling proteins in GAERS.[22] In the present study, we examined levels of key insulin signalling proteins in both whole tissue homogenate (TH) and nuclear-enriched fraction (NF) of cortex, hippocampus, and thalamus isolated from 8–10 week old GAERS and age-matched NEC rats.

2 | RESULTS

2.1 | Alterations in insulin-related receptor markers

The levels of PI3K/Akt signalling molecules were assessed in cortical, hippocampal and thalamic regions of NEC and GAERS rats. We observed that IRβ ($P < .01$; 3-fold) and IRS1 ($P < .05$; 1.3-fold) levels were significantly decreased in GAERS hippocampal TH when compared to NEC (Figure 1A-C). Decreased hippocampal IRS2 levels ($P > .05$ 1.5-fold) were also observed in GAERS together with a decrease in the catalytic subunit of PI3K, although this change did not reach significance. In addition, the levels of GLUT1 ($P < .05$; 18-fold) and GLUT4 ($P < .05$; 2.4-fold) were significantly decreased in GAERS hippocampal TH when compared to levels in the NEC strain (Figure 1A, D-G). In contrast, no significant alterations in any of these signalling factors were observed in cortical and thalamic tissues of GAERS when compared to NEC rats.

2.2 | Alterations in insulin-related cytosolic proteins

Key negative regulatory markers of the PI3K/Akt signalling cascade nuclear PTEN (cortex: 1.6-fold ($P < .05$) and hippocampus: 7.7-fold ($P < .01$)) and GSK3β (cortex: 1.2-fold ($P > .05$) and hippocampus: 2.1-fold ($P < .05$)), were significantly increased when compared to samples from the NEC strain. A significant decrease in PI3K p85α (cortex: 1.9-fold ($P < .01$); hippocampus: 1.8-fold ($P < .05$)) and pAkt1/2/3 (cortex: 1.8-fold ($P < .05$); hippocampus 2.1-fold ($P < .05$)) levels were observed in NF of GAERS cortical and hippocampal tissues when compared to tissues from the NEC strain (Figure 1A, G-H). No significant differences were detected in either TH or NF isolated from the thalamus of the GAERS and NEC strains. Dot blotting analysis also revealed a significant increase in PIP2/PIP3 ratio ($P < .05$; 3.4-fold) in NF of GAERS hippocampal homogenate compared to NEC strain (Figure 2). A non-significant increase in PIP2/PIP3 ratio was observed in NF of GAERS cortical (2.5-fold) and thalamic (2.3-fold) tissues (Figure 2).

A significant decrease in cortical PI3K p85α ($P < .05$; 1.8-fold) and hippocampal pAkt1/2/3 ($P < .05$; 1.4-fold) levels in TH of GAERS was found when compared to the NEC strain (Figure 1A, G-I). PTEN levels in GAERS cortical, thalamic, and hippocampal TH were comparable to those of the NEC strain (Figure 1A, J). No alterations in TH insulin signalling proteins were observed between GAERS and NEC thalamic tissues.

2.3 | Glucose and insulin levels in blood, plasma, and CSF of GAERS and NEC rats

Peripheral blood glucose levels in GAERS (6.73 ± 0.39 mmol/L) and NEC rats (6.31 ± 0.37 mmol/L) were comparable. Further, insulin levels in plasma and CSF were measured. Although they were not statistically different, insulin levels were increased in plasma (1.2-fold) and CSF (1.8-fold) of GAERS, when compared to NEC rats (Figure 3). Levels of insulin reported in the NEC rats are similar to those typically reported for outbred Wistar rats.[23,24]

2.4 | Decreased phosphorylated Akt level in cortical, thalamic, and hippocampal tissues from GAERS rats

In addition, an insulin sensitivity assay was performed on cortical, thalamic, and hippocampal tissues from GAERS and NEC

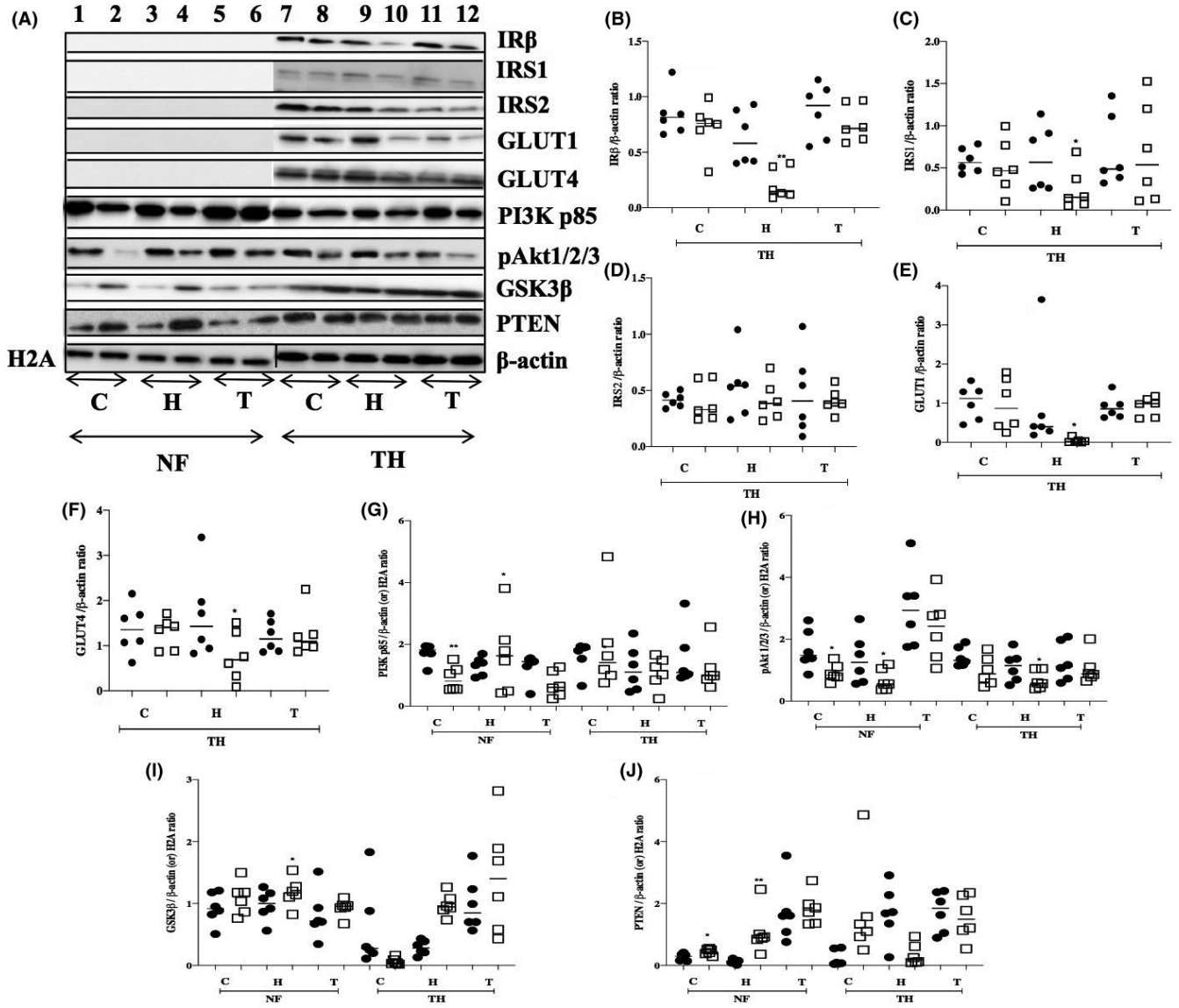

FIGURE 1 A, Western blot analyses for insulin signalling markers in nuclear fraction (NF) and total homogenate (TH) of cortex (C), hippocampus (H) and thalamus (T) from 8–10 week old male Genetic Absence Epilepsy Rats from Strasbourg (GAERS, bands 2, 4, 6, 8, 10 and 12) and their Non-Epileptic Control (NEC, bands 1, 3, 5, 7, 9 and 11) strain are presented above and the loading controls for NF (Histone 2A, H2A) and TH (beta-actin) are presented below. B-J. The dot diagrams compare the mean ± SEM levels in C, H and T brain tissues isolated from GAERS and NEC groups [n = 6]. *, ** $P < .05$ and 0.01, respectively compared to the NEC group. Insulin signalling markers include insulin receptor beta subunit (IRβ), insulin receptor substrate 1 (IRS1), insulin receptor substrate 2 (IRS2), glucose transporter 1 (GLUT1), glucose transporter 4 (GLUT4), phosphoinositide 3-kinase P85α (PI3K), protein kinase B (PKB/pAkt1/2/3), glycogen synthase kinase 3 beta (GSK3β) and phosphatase and tensin homologue (PTEN). (B-J) ●, NEC; □, GAERS

rats. The results showed that insulin incubation increased the insulin signalling cascade in NEC rats. Further, the phosphorylated ser473 Akt was decreased in cortical (2.1-fold), thalamic (1.5-fold), and hippocampal (1.8-fold) tissues of GAERS rats compared to their respective NEC controls (Figure 4). These data clearly show that addition of insulin did not stimulate the phosphorylation of insulin signalling proteins in GAERS brain, resulting in the existence of insulin resistance in cortical and hippocampal tissues of GAERS.

3 | DISCUSSION

The present study provides the first evidence for aberrant insulin-mediated signalling in cortical and hippocampal tissues in a genetic model of absence epilepsy. In addition, we observed increased nuclear translocation of PTEN and GSK3β, both negative regulators of insulin signalling, in cortical and hippocampal regions, while no differences were observed in thalamic tissues of GAERS when they were compared to NEC rats. Together, these data suggest alterations

in the insulin signalling cascade in GAERS and/or nuclear translocation of negative regulators in cortical, thalamic, and hippocampal tissues of this established model of childhood absence epilepsy.

In peripheral tissues, insulin stimulates glucose uptake and metabolism through the PI3K/Akt pathway. Although the brain was once considered independent of insulin, insulin receptors and downstream signalling molecules are present in various brain regions including cortex, hippocampus, and thalamus.[3] Brain insulin signalling is essential for regulating oxidative processes and mitochondrial functions and is required for the survival of neurons.[3] Further evidence supports a role for insulin in cognitive functions such as learning and memory.[25] Brain insulin resistance is also associated with certain CNS diseases.[2,3] Increased glucose levels have been found in absence epilepsy patients and animal models and altered metabolic activity in limbic and nigral areas before the occurrence of spike-and-wave discharges have been suggested.[26-30] In the present study, we observed decreased insulin-induced phosphorylation of Akt, a prominent member of insulin signalling pathway, in GAERS, a finding that demonstrates a marked reduction in insulin action and glucose uptake in this rat model of absence epilepsy. Further, the observed decreases in insulin signalling proteins such as IRβ, IRS1, PI3K, Akt1/2/3 in GAERS may contribute to insulin resistance and other pathological conditions in absence epilepsy.

PTEN, a tumour suppressor protein, blocks the PI3K/Akt cell survival pathway by dephosphorylating PIP3 to PIP2, further contributing to cell death-promoting function.[31] Increased nuclear translocation of PTEN also contributes to excitotoxicity-induced cell death.[31] The observed increases in nuclear PTEN levels and PIP2/PIP3 ratio and decreased PI3K p85α and Akt1/2/3 levels suggest that PTEN nuclear translocation may halt nuclear insulin-mediated signalling and contribute to neuronal cell death in GAERS. Evidence supports the hypothesis that GSK3β promotes apoptosis during neuronal insult and forms a complex with p53 in the nucleus further promoting neuronal apoptosis.[32] Further, Papp et al [33] suggest that altered hippocampal and cortical interneuron densities are linked to neuronal injury in GAERS rats. Therefore, the observed increase in GSK3β in both TH and NF of cortical and hippocampal areas of GAERS would therefore be predicted to contribute to neuronal loss in GAERS rats.

GLUT1 and GLUT4 generally facilitate glucose transport via insulin-independent and insulin-dependent mechanisms, respectively. Ebeling et al showed that if glucose entering through GLUT1 increases the hexosamine pathway, decreased insulin-mediated GLUT4 transportation leads to insulin resistance.[34] The observed decrease in GLUT4 levels corroborates with the above finding and suggests the induction of insulin resistance in GAERS rats. In addition, decreased GLUT1 in the present study suggests that these alterations may relate to the pathological conditions in GAERS.

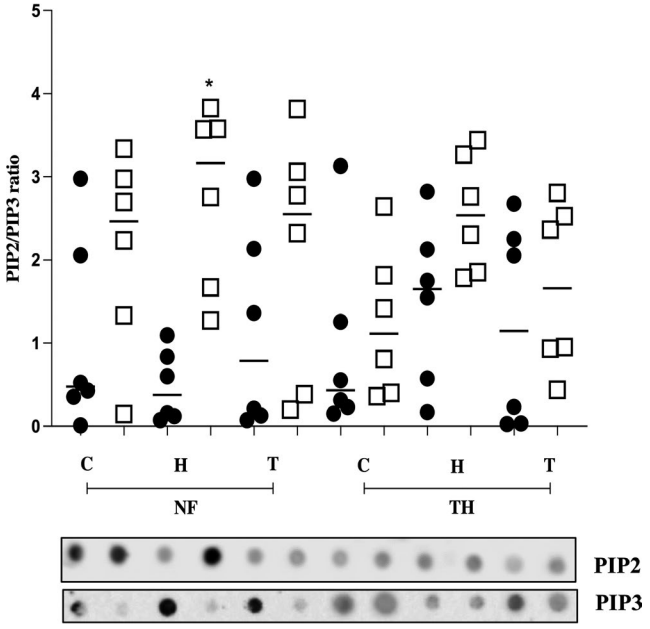

FIGURE 2 Summary of dot blot analyses for phosphatidylinositol 4,5-bisphosphate (PIP2) and phosphatidylinositol (3,4,5)-trisphosphate (PIP3). Plotted data represent PIP2/PIP3 ratios in the nuclear fraction (NF) and total homogenate (TH) of cortex (C), hippocampus (H) and thalamus (T) from 8–10 week old male Genetic Absence Epilepsy Rats from Strasbourg (GAERS, band 2, 4, 6, 8, 10 and 12) and its Non-Epileptic Control (NEC, bands 1, 3, 5, 7, 9 and 11) strain. The dot diagram compares the mean ± SEM levels in C, H and T brain tissues isolated from GAERS and NEC groups [n = 6]. * $P < .05$ compared to data of NEC group. ●, NEC; □, GAERS

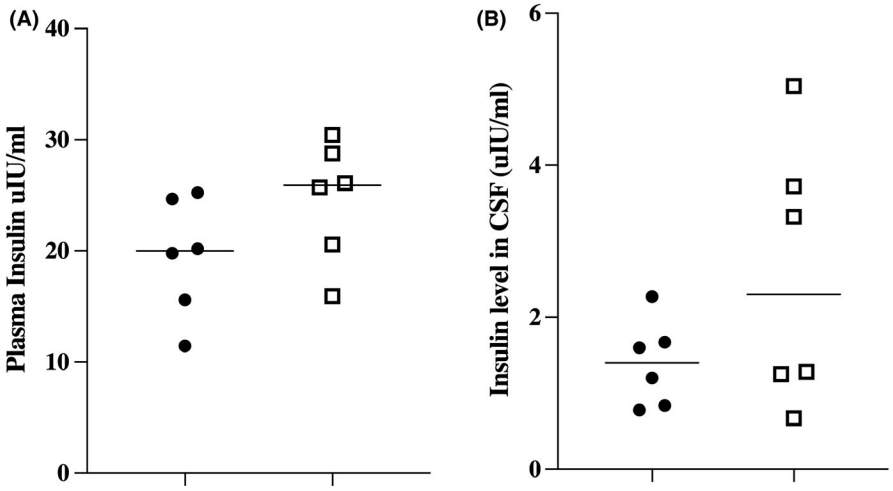

FIGURE 3 Insulin level in plasma (A) and cerebrospinal fluid (CSF; B) from 8-10 week old male Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and its Non-Epileptic Control (NEC) strain were represented. The dot diagram compares the mean ± SEM levels from GAERS and NEC groups [n = 6]. (A,B) ●, NEC; □, GAERS

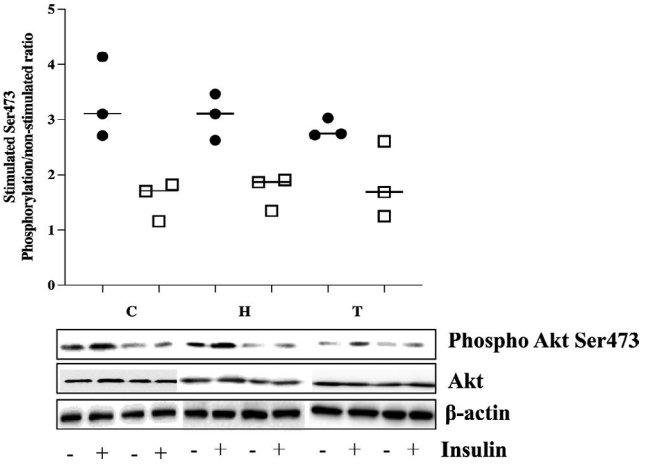

FIGURE 4 A representative assay of insulin sensitivity in cortex (C), hippocampus (H) and thalamus (T) from 8–10 week old male Genetic Absence Epilepsy Rats from Strasbourg (GAERS) and its Non-Epileptic Control (NEC) strain. Plotted data representing stimulated ser473 phosphorylation to non-stimulated ratios in the presence and absence of insulin in cortex C, H and T regions of GAERS (band 3, 4, 7, 8, 11 and 12) and NEC (bands 1, 2, 5, 6, 9 and 10). Representative blot of pAkt ser 473, mass Akt and their loading control β-actin was shown below the dot graph. The dot diagram compares the mean ± SEM levels in C, H and T brain tissues isolated from GAERS and NEC groups [n = 3]. ●, NEC; □, GAERS

Surprisingly, alterations in hippocampal insulin signalling proteins were more prominent than in cortical or thalamic tissues from GAERS. Evidence supports the involvement of hippocampal neuronal firing in absence epilepsy.[8,35] Previously, we reported that increased nuclear translocation of sterol regulatory elementary binding protein 1 and GluA2 subunit of AMPA receptor triggers a pro-death signalling cascade in the hippocampus of GAERS.[22] Taken together, these data suggest the possibility that the alterations of insulin signalling proteins may contribute to the pathologies observed in GAERS.

Our study provides direct evidence for altered insulin-mediated signalling in cortical and hippocampal tissues from GAERS. In addition, the associated increased nuclear translocation of PTEN and GSK3β may contribute to neuronal cell death in GAERS. Further studies should examine different age groups of GAERS, other metabolic alterations, and drug treatments that attenuate absence seizures to clearly elucidate the contribution of altered brain insulin signalling in the pathologies of GAERS. Restoring insulin signalling may provide novel and effective treatment options in the management of absence epilepsy and also warrants further investigation.

4 | MATERIALS AND METHODS

4.1 | Chemicals and reagents

Anti-mouse insulin receptor beta (IRβ), anti-rabbit IR substrate-1 (IRS1), Anti-goat IR substrate-2 (IRS2), anti-mouse phosphatase and tensin homologue (PTEN), anti-rabbit protein kinase B (PKB/Akt1/2/3), anti-rabbit phospho Akt ser473, anti-mouse glycogen synthase kinase-3β (GSK3β), anti-goat glucose transporter-4 (GLUT4), anti-mouse β-actin and anti-goat IgG were obtained from Santa Cruz Biotechnology. Anti-rabbit glucose transporter-1 (GLUT1), anti-rabbit histone 2A (H2A), anti-mouse IgG and anti-rabbit IgG were purchase from Abcam. Phosphoinositide 3-kinase p-85 alpha (PI3K p85α) was procured from Cell Signaling. Phosphatidylinositol 4,5-bisphosphate (PIP2), phosphatidylinositol (3,4,5)-trisphosphate (PIP3) were purchased from Echelon Bioscience. All other chemicals and reagents were from Sigma Aldrich. Rat insulin ELISA kit was obtained from Ray Biotech.

4.2 | Animals

Male NEC and GAERS (8–10 weeks old) rats were born at the University of Saskatchewan as described previously.[16] These rats originated from the Melbourne colony. Rats were provided with food (Purina Rat chow) and water ad libitum in a standard pair housing environment. The study adhered to the current laws governing animal experimentation and was approved by the University of Saskatchewan Animal Research Ethics Board following the Canadian Council on Animal Care Guidelines.

4.3 | Isolation of nuclear-enriched fraction (NF)

Rats were anaesthetized with isoflurane, decapitated, and brains were excised. Cortical, hippocampal, and thalamic tissues were isolated and processed for nuclear extraction following the established methodology.[31] Briefly, 100 mg of tissue was homogenized with 1ml buffer A (10 mmol/L HEPES-KOH, 10 mmol/L KCl, 10 mmol/L EDTA, 1.5 mmol/L $MgCl_2$, 0.2% BSA, 1 mmol/L DTT, 0.4% NP40 and protease inhibitors), kept on ice for 15 minutes, then centrifuged at 850 x g for 10 minutes at 4°C. One ml buffer A was added to the pellet, mixed well and centrifuged at 15 000× *g* for 3 minutes at 4°C following 15 minutes incubation on ice. The supernatant thus obtained was stored as cytosolic fraction (CF). To the pellet, 150 μL buffer B (20 mM HEPES-KOH, 400 mM NaCl, 10% glycerol, 1 mmol/L DTT and protease inhibitors) was added and mixed well. The solution was incubated for 2 h on ice with shaking and centrifuged at 15 000× g for 5 minutes at 4°C. The supernatant (NF) was collected and blotted along with CF for subcellular markers (lamin B for nucleus; 14-3-3 for cytosol; insulin receptor for cell membrane; stearoyl-CoA desaturase-1 for endoplasmic reticulum and cytochrome C for mitochondria, data not shown), confirming nuclear enrichment with negligible levels of subcellular contamination.

4.4 | Isolation of whole tissue homogenate (TH)

One hundred milligrams of cortex, hippocampus and thalamus was homogenized with lysis buffer (25 mmol/L Tris, 150 mmol/L NaCl, 0.1% sodium dodecyl sulphate, 0.5% sodium deoxycholate and 1% Triton X-100, pH 7-8), kept in ice for 15 min and centrifuged at

15 000× g for 5 minutes at 4°C. The supernatant thus obtained was stored as TH for further analysis.

4.5 | Western blot analysis

Samples containing equal concentration of total protein were immunoblotted with targeted proteins as described previously.[36] The membranes were incubated with enhanced chemiluminescence reagent (Bio-Rad) and exposed to x-ray film or Bio-Rad image analyzer. Protein bands of interest were analyzed using NIH ImageJ software and expressed as the ratio to the relative level of target protein with that of loading control.

4.6 | Dot blotting

Nuclear fraction and TH containing equal concentration of total protein were spotted onto a nitrocellulose membrane using Bio-Rad microfiltration apparatus and probed with anti-PIP2 and anti-PIP3 antibodies as described previously.[31] The membranes were incubated with enhanced chemiluminescence reagent (Bio-Rad) and exposed to Bio-Rad image analyzer. Spots were analyzed using NIH ImageJ software and expressed as the percentage relative level of targeted protein with that of loading control.

4.7 | Collection of cerebrospinal fluid (CSF) and plasma

The CSF was collected following a protocol described previously.[37] Under isoflurane anaesthesia, the fur on the neck of rats was shaved and rats were placed in stereotaxic frame with the head positioned downward at approximately 45°. The shaved region was cleaned with 70% alcohol and a needle was inserted horizontally and centrally into the cisterna magna until the appearance of CSF into the needle. The required volume of CSF (100–150 µL) was collected from each animal (non-fasted) and analyzed for insulin levels using an ELISA kit (Ray Biotech Inc) following the manufacturer's instructions. In addition, insulin levels in plasma were also measured. The values were expressed in µIU/mL.

4.8 | Preparation of brain slices and insulin stimulation

Insulin stimulation assay was performed as described previously.[38] Brains from NEC and GAERS were isolated and placed in ice-cold oxygenated brain-slicing solution (2.5 mmol/L KCl, 2 mmol/L $CaCl_2$, 2 mmol/L $MgCl_2$, 1.25 mmol/L KH_2PO_4, 25.2 mmol/L sucrose, 26 mmol/L $NaHCO_3$ and 10 mmol/L glucose; pH 7.37–7.43, 310–320 mOsm). Coronal slices identifying cortex, hippocampus and thalamus tissues (400 µm thick) were then cut using Leica VT1200 Vibratome. Cortical, hippocampal and thalamus tissues were separated immediately after sectioning. The tissues were allowed to recover at room temperature for 60–90 minutes in oxygenated artificial cerebrospinal fluid [(aCSF) containing 125 mmol/L NaCl, 2.5 mmol/L KCl, 2 mmol/L $CaCl_2$, 1 mmol/L $MgCl_2$, 1.25 mmol/L NaH_2PO_4, 26 mmol/L $NaHCO_3$ and 25 mmol/L glucose; pH 7.37–7.43, 310–320 mOsm]. Tissues were then incubated with or without 500 nM insulin in aCSF for 5 minutes. The tissues were then homogenized using lysis buffer as mentioned above and processed for western blotting.

4.9 | Data analysis

Data are expressed as mean ± standard error of mean (SEM). Mean differences between the groups were analyzed using non-parametric Mann-Whitney test in GraphPad Prism 5.0. *P* values less than 0.05 were considered statistically significant.

ACKNOWLEDGMENTS

This work was supported by Brain Canada Multi-Investigator Research Initiative Grant with matching support from the Genome British Columbia, the Michael Smith Foundation for Health Research, and the Koerner Foundation (PI: TPS). The authors are grateful to the Saskatchewan Health Research Foundation (SHRF) for a grant-in-aid in support of this project (SHRF establishment grant #3543). TPS is supported by an operating grant (#10677) from the Canadian Institutes of Health Research (CIHR) and the Canada Research Chair in Biotechnology and Genomics-Neurobiology. Work in the laboratory of JGH is supported by an operating grant from the CIHR (#227397) and matching funds for these experiments from the SHRF as well as postdoctoral fellowships for SS and WNM by the SHRF. Authors thank Dr Zelan Wei and Ms Nadine K. Zabder for technical assistance.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

ORCID

John G. Howland https://orcid.org/0000-0003-3326-7118
Changiz Taghibiglou https://orcid.org/0000-0002-4527-7404

REFERENCES

1. Blazquez E, Velazquez E, Hurtado-Carneiro V, Ruiz-Albusac JM. Insulin in the brain: its pathophysiological implications for States related with central insulin resistance, type 2 diabetes and Alzheimer's disease. *Front Endocrinol (Lausanne)*. 2014;5:161.
2. Karelina K, Sarac B, Freeman LM, Gaier KR, Weil ZM. Traumatic brain injury and obesity induce persistent central insulin resistance. *Eur J Neurosci*. 2016;43:1034-1043.
3. Kim B, Feldman EL. Insulin resistance in the nervous system. *Trends Endocrinol Metab*. 2012;23:133-141.
4. Lee EB, Warmann G, Dhir R, Ahima RS. Metabolic dysfunction associated with adiponectin deficiency enhances kainic acid-induced seizure severity. *J Neurosci*. 2011;31:14361-14366.

5. Schauwecker PE. The effects of glycemic control on seizures and seizure-induced excitotoxic cell death. *BMC Neurosci*. 2012;13:94.
6. Cheong E, Shin HS. T-type Ca(2)(+) channels in absence epilepsy. *Biochim Biophys Acta*. 2013;1828:1560-1571.
7. Lakaye B, Thomas E, Minet A, Grisar T. The genetic absence epilepsy rat from Strasbourg (GAERS), a rat model of absence epilepsy: computer modeling and differential gene expression. *Epilepsia*. 2002;43(suppl 5):123-129.
8. Arcaro J, Ma J, Chu LK, et al. The hippocampus participates in a pharmacological rat model of absence seizures. *Epilepsy Res*. 2016;120:79-90.
9. Holmes GL. Cognitive impairment in epilepsy: the role of network abnormalities. *Epileptic Disord*. 2015;17:101-116.
10. Kobylarek D, Iwanowski P, Lewandowski Z, et al. Advances in the potential biomarkers of epilepsy. *Front Neurol*. 2019;10:685.
11. Steriade M. Sleep, epilepsy and thalamic reticular inhibitory neurons. *Trends Neurosci*. 2005;28:317-324.
12. Dufour F, Koning E, Nehlig A. Basal levels of metabolic activity are elevated in Genetic Absence Epilepsy Rats from Strasbourg (GAERS): measurement of regional activity of cytochrome oxidase and lactate dehydrogenase by histochemistry. *Exp Neurol*. 2003;182:346-352.
13. Verrotti A, Basciani F, De Simone M, et al. Insulin resistance in epileptic girls who gain weight after therapy with valproic acid. *J Child Neurol*. 2002;17:265-268.
14. Daniels ZS, Nick TG, Liu C, Cassedy A, Glauser TA. Obesity is a common comorbidity for pediatric patients with untreated, newly diagnosed epilepsy. *Neurology*. 2009;73:658-664.
15. Farrell JS, Greba Q, Snutch TP, Howland JG, Teskey GC. Fast oxygen dynamics as a potential biomarker for epilepsy. *Sci Rep*. 2018;8:17935.
16. Henbid MT, Marks WN, Collins MJ, et al. Sociability impairments in genetic absence epilepsy rats from strasbourg: reversal by the T-type calcium channel antagonist Z944. *Exp Neurol*. 2017;296:16-22.
17. Marescaux C, Vergnes M. Genetic Absence Epilepsy in Rats from Strasbourg (GAERS). *Ital J Neurol Sci*. 1995;16:113-118.
18. Snead 3rd OC, Depaulis A, Vergnes M, Marescaux C. Absence epilepsy: advances in experimental animal models. *Adv Neurol*. 1999;79:253-278.
19. Brailowsky S, Montiel T, Boehrer A, Marescaux C, Vergnes M. Susceptibility to focal and generalized seizures in Wistar rats with genetic absence-like epilepsy. *Neuroscience*. 1999;93:1173-1177.
20. Powell KL, Cain SM, Ng C, et al. A Cav3.2 T-type calcium channel point mutation has splice-variant-specific effects on function and segregates with seizure expression in a polygenic rat model of absence epilepsy. *J Neurosci*. 2009;29:371-380.
21. Danober L, Vergnes M, Depaulis A, Marescaux C. Nucleus basalis lesions suppress spike and wave discharges in rats with spontaneous absence-epilepsy. *Neuroscience*. 1994;59:531-539.
22. Sekar S, Omran E, Gopalakrishnan V, et al. Elevated sterol regulatory elementary binding protein 1 and GluA2 levels in the hippocampal nuclear fraction of genetic absence epilepsy rats from Strasbourg. *Epilepsy Res*. 2017;136:1-4.
23. Balbaa M, Abdulmalek SA, Khalil S. Oxidative stress and expression of insulin signaling proteins in the brain of diabetic rats: role of Nigella sativa oil and antidiabetic drugs. *PLoS ONE*. 2017;12:e0172429.
24. Bathina S, Das UN. Dysregulation of PI3K-Akt-mTOR pathway in brain of streptozotocin-induced type 2 diabetes mellitus in Wistar rats. *Lipids Health Dis*. 2018;17:168.
25. Lee CC, Huang CC, Hsu KS. Insulin promotes dendritic spine and synapse formation by the PI3K/Akt/mTOR and Rac1 signaling pathways. *Neuropharmacology*. 2011;61:867-879.
26. Ari C, Murdun C, Koutnik AP, et al. Exogenous ketones lower blood glucose level in rested and exercised rodent models. *Nutrients*. 2019;11(10):2330.
27. Nehlig A, Vergnes M, Boyet S, Marescaux C. Local cerebral glucose utilization in adult and immature GAERS. *Epilepsy Res*. 1998;32:206-212.
28. Nehlig A, Vergnes M, Boyet S, Marescaux C. Metabolic activity is increased in discrete brain regions before the occurrence of spike-and-wave discharges in weanling rats with genetic absence epilepsy. *Brain Res Dev Brain Res*. 1998;108:69-75.
29. Reid CA, Kim TH, Berkovic SF, Petrou S. Low blood glucose precipitates spike-and-wave activity in genetically predisposed animals. *Epilepsia*. 2011;52:115-120.
30. Ross DL, Swaiman KF, Torres F, Hansen J. Early biochemical and EEG correlates of the ketogenic diet in children with atypical absence epilepsy. *Pediatr Neurol*. 1985;1:104-108.
31. Zhang S, Taghibiglou C, Girling K, et al. Critical role of increased PTEN nuclear translocation in excitotoxic and ischemic neuronal injuries. *J Neurosci*. 2013;33:7997-8008.
32. Jacobs KM, Bhave SR, Ferraro DJ, et al. GSK-3beta: a bifunctional role in cell death pathways. *Int J Cell Biol*. 2012;2012:930710.
33. Papp P, Kovacs Z, Szocsics P, Juhasz G, Magloczky Z. Alterations in hippocampal and cortical densities of functionally different interneurons in rat models of absence epilepsy. *Epilepsy Res*. 2018;145:40-50.
34. Ebeling P, Koistinen HA, Koivisto VA. Insulin-independent glucose transport regulates insulin sensitivity. *FEBS Lett*. 1998;436:301-303.
35. Sirvanci S, Meshul CK, Onat F, San T. Immunocytochemical analysis of glutamate and GABA in hippocampus of genetic absence epilepsy rats (GAERS). *Brain Res*. 2003;988:180-188.
36. Taghibiglou C, Martin HG, Lai TW, et al. Role of NMDA receptor-dependent activation of SREBP1 in excitotoxic and ischemic neuronal injuries. *Nat Med*. 2009;15:1399-1406.
37. Nirogi R, Kandikere V, Mudigonada K, et al. A simple and rapid method to collect the cerebrospinal fluid of rats and its application for the assessment of drug penetration into the central nervous system. *J Neurosci Methods*. 2009;178:116-119.
38. Mielke JG, Taghibiglou C, Liu L, et al. A biochemical and functional characterization of diet-induced brain insulin resistance. *J Neurochem*. 2005;93:1568-1578.

How to cite this article: Sekar S, Marks WN, Gopalakrishnan V, et al. Evidence for altered insulin signalling in the brains of genetic absence epilepsy rats from strasbourg. *Clin Exp Pharmacol Physiol*. 2020;47:1530–1536. https://doi.org/10.1111/1440-1681.13326