



Seizing the Alzheimer's Brain: A Role for Sirtuin 3 in Hyperexcitability

Epilepsy Currents
2020, Vol. 20(4) 224-226

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DOI: 10.1177/1535759720932157

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SIRT3 Haploinsufficiency Aggravates Loss of GABAergic Interneurons and Neuronal Network Hyperexcitability in an Alzheimer's Disease Model¹

Cheng A, Wang J, Ghena N, Zhao Q, Perone I, King TM, Veech RL, Gorospe M, Wan R, Mattson MP. *J Neurosci.* 2020;40(3):694-709. doi:10.1523/JNEUROSCI.1446-19.2019

Impaired mitochondrial function and aberrant neuronal network activity are believed to be early events in the pathogenesis of Alzheimer disease (AD), but how mitochondrial alterations contribute to aberrant activity in neuronal circuits is unknown. In this study, we examined the function of mitochondrial protein deacetylase sirtuin 3 (SIRT3) in the pathogenesis of AD. Compared to AppPsl mice, Sirt3-haploinsufficient AppPsl mice (Sirt3^{+/-} AppPsl) exhibit early epileptiform EEG activity and seizure. Both male and female Sirt3^{+/-} AppPsl mice were observed to die prematurely before 5 months of age. When comparing male mice among different genotypes, Sirt3 haploinsufficiency renders GABAergic interneurons in the cerebral cortex vulnerable to degeneration and associated neuronal network hyperexcitability. Feeding Sirt3^{+/-} AppPsl AD mice with a ketone ester-rich diet increases SIRT3 expression and prevents seizure-related death and the degeneration of GABAergic neurons, indicating that the aggravated GABAergic neuron loss and neuronal network hyperexcitability in Sirt3^{+/-} AppPsl mice are caused by SIRT3 reduction and can be rescued by increase of SIRT3 expression. Consistent with a protective role in AD, SIRT3 levels are reduced in association with cerebral cortical A β pathology in AD patients. In summary, SIRT3 preserves GABAergic interneurons and protects cerebral circuits against hyperexcitability, and this neuroprotective mechanism can be bolstered by dietary ketone esters.

Commentary

Alzheimer disease (AD) is a neurodegenerative disorder characterized by a progressive decline in cognitive function that results in severe memory loss and comorbidities that include epilepsy. The prevalence of epilepsy in AD is 10% to 22%, with the occurrence of unprovoked seizures being higher in younger individuals with early-onset AD (<65 years old) linked to autosomal dominant mutations in the amyloid precursor protein (App) and presenilin 1 (Ps1) genes.^{2,3} These genes are commonly associated with deposition of beta amyloid peptides (A β) in the brain, which together with the formation of neurofibrillary tangles from hyperphosphorylated tau protein, are histopathological features of AD. Rodent models carrying App gene mutations often show accumulation of A β and cognitive impairments that can be accompanied by unprovoked seizures.^{3,4} Interestingly, evidence from both human and preclinical models of AD indicate that seizures can precede cognitive decline and happen even without apparent A β deposition in the brain.²⁻⁴ This suggests that the neuronal hyperexcitability and seizures seen early in AD may potentially aggravate the associated memory impairments. However, how

this neuronal hyperexcitability develops, and whether it plays a role in the seizure or cognitive pathology of AD is not known.

It is possible that the neuronal hyperexcitability in AD may be associated with neurodegeneration of GABAergic neurons, which has been observed along with A β and cognitive pathology in both human and experimental AD.²⁻⁴ The study by Cheng et al focused on the mitochondrial NAD⁺-dependent protein deacetylase sirtuin 3 (Sirt3) as a potential mechanism modulating GABAergic interneuronal loss and seizures in a mouse model of AD.¹ Sirtuin 3 is localized in the mitochondria, where it plays an integral role in the regulation of enzymes that control metabolic processes including the tricarboxylic acid cycle, the electron transport chain, and ketogenesis.⁵ Sirtuin 3 also participates in the regulation of reactive oxygen species. Therefore, reductions or deficiency of Sirt3 are associated with oxidative stress-induced neurodegeneration and cell death.⁵

To study the role of Sirt3, mice with a chimeric human/mouse App gene and a mutant human Ps1 gene in neurons (AppPsl), which develop A β deposits, cognitive decline and unprovoked seizures, were crossed with Sirt3 knockout (KO) mice to generate Sirt3 haplo insufficient Sirt3^{+/-} AppPsl mice.



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The $Sirt3^{+/-}$ AppPs1 mice displayed a significantly higher number of epileptiform discharges relative to the AppPs1 mice implicating reduced Sirt3 signaling in the worsened epileptic phenotype of the $Sirt3^{+/-}$ AppPs1 mice. While behavioral and electrographic seizures occurred in both genotypes, more $Sirt3^{+/-}$ AppPs1 mice had unprovoked seizures compared to AppPs1 mice, suggesting that lower Sirt3 signaling may boost epileptogenic processes. The article also reported that the seizure susceptibility to the chemoconvulsant kainic acid (KA) was significantly higher in $Sirt3^{+/-}$ AppPs1 mice when compared to AppPs1 or the other 2 groups (Wild type [WT], Sirt3 KO), suggesting that Sirt3 is necessary to control neuronal hyperactivity. However, a lower seizure susceptibility to KA in the Sirt3 KO mice compared to the $Sirt3^{+/-}$ AppPs1 mice indicates that Sirt3 deficiency only aggravated the seizure phenotype in an already vulnerable system with underlying AD pathology such as in the AppPs1 mice. This raises the question of whether Sirt3 deficiency is only epileptogenic under AD conditions, thereby requiring A β deposition or other AD neuropathology to exacerbate neuronal hyperexcitability. Still, another study reported high seizure severity in response to KA in Sirt4 KO mice compared to WT mice,⁶ suggesting potential roles of the Sirtuin family of proteins in modulating neural activity under physiological conditions.

The reduced levels of Sirt3 also resulted in an increased mortality rate in the group of $Sirt3^{+/-}$ AppPs1 mice (60%) compared to the AppPs1 mice (25%) by ~5 months. Interestingly, the authors captured the death of one $Sirt3^{+/-}$ AppPs1 mouse which occurred minutes following intense seizure activity evidenced by electroencephalogram (EEG). This suggests the possibility of a case of sudden unexpected death in epilepsy (SUDEP), which may be a comorbidity in this mouse model of AD that contributes to the high mortality rate in both $Sirt3^{+/-}$ AppPs1 and AppPs1 mice seen in comparison to WT mice. It can be speculated that the increased seizure severity in $Sirt3^{+/-}$ AppPs1 mice may contribute to their premature death, and potential SUDEP, because unexpected deaths have been reported in epilepsies with mitochondrial dysfunction.⁷


At the neuropathological level, Sirt3 haploinsufficiency was associated with a significant loss in the number of parvalbumin (PV) and calretinin (CR) GABAergic interneurons in the cortex which was evident at 4 months but not at 4 weeks of age in $Sirt3^{+/-}$ AppPs1 mice relative to AppPs1 mice. While this evidence implicates Sirt3 haploinsufficiency in neuronal loss in $Sirt3^{+/-}$ AppPs1 mice, interneuronal loss could also be due to and/or exacerbated by underlying epilepsy of which progression was not determined between 4 weeks and 4 months with continuous EEG monitoring. Furthermore, episodes of status epilepticus in models of acquired epilepsy promote reductions in the levels of Sirt3 in the brain,⁸ and Sirt3 deficiency exacerbates seizure-induced neuronal loss by oxidative stress.⁹⁻¹¹ Thus, it is also possible that seizures further reduce Sirt3 levels thereby aggravating the loss of GABAergic interneurons and epileptiform activity in the $Sirt3^{+/-}$ AppPs1 mice. While the study showed evidence that KA provoked drastic increases in c-Fos expression in cortical neurons as well as DNA damage of

PV cells in $Sirt3^{+/-}$ AppPs1 mice relative to AppPs1 or WT mice, a comparison was not made with KA versus non-KA-treated mice. This limits our understanding on whether the observed neuropathological effects are a direct result of an increased susceptibility to KA-induced neurodegeneration in Sirt3 haploinsufficient mice or whether the AD-related neurodegeneration was already exacerbated in non-KA-treated $Sirt3^{+/-}$ AppPs1 mice.

Interestingly, $Sirt3^{+/-}$ AppPs1 mice that were fed a ketone ester (KE) diet, which increases the levels of Sirt3 protein, showed significantly increased numbers of PV and CR cells in the cortex, and reductions in both mortality rate and seizure susceptibility to KA. These findings argue in favor that reduced Sirt3 could be causal in the processes leading to PV and CR cell loss in the $Sirt3^{+/-}$ AppPs1 mice. However, the ketogenic diet has been shown to have multiple beneficial effects in epilepsy through the modulation of a myriad of molecular mechanisms including mitochondrial functions, ion channel activity, DNA methylation, and expression of neurotrophic factors,¹² suggesting that additional mechanisms may be part of the neuroprotection seen after KE treatment in the $Sirt3^{+/-}$ AppPs1 mice.

Overall, this is an interesting study that provides evidence that Sirt3 signaling may be needed for the protection of GABAergic inhibitory interneurons and that Sirt3 may be potentially anti-epileptogenic under conditions associated with AD. In the context of epilepsy, a limitation of this study is that continuous EEG monitoring was not performed. As seizures can directly modulate Sirt3 levels and interneuronal loss, identifying the extent at which seizure progression/severity evolves over time could help define what influences this neuropathology. In addition, it would have been interesting to know whether Sirt3 haploinsufficiency as well as the KE diet had an impact on the A β and cognitive pathology and how these parallel epileptiform activity in the $Sirt3^{+/-}$ AppPs1 mice. A temporal analysis of these pathological events could help shed light into whether A β or neuronal hyperactivity may influence Sirt3, and if Sirt3 deficiency exacerbates the pathology of AD and epilepsy in parallel, or if one precedes the other. This is important because mechanistic studies support that Sirt3 deregulation directly impairs basal mitochondrial functions including energy metabolism, oxygen consumption, and oxidative capacity leading to neurodegeneration in AD^{10,11,13} but also in experimental epilepsy.⁸⁻¹¹ Thus, it is possible that similar Sirt3-dependent mitochondrial mechanisms may underlie comorbidities such as seizures and memory deficits, as well as neuronal hyperexcitability and cell loss in the 2 disorders. There is evidence that augmenting Sirt3 levels through KE diets¹ and even intermittent fasting¹¹ promotes neuroprotection and reduces neuronal hyperexcitability and seizures in models of AD. This supports the possibility that Sirt3 may be a potential novel therapeutic target for reducing seizures, which may be worth investigating in epilepsies that are not comorbid with AD.

**ORCID iD**

Amy L. Brewster  <https://orcid.org/0000-0002-3677-410X>

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