GSK3β Regulates Brain Energy Metabolism

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Abstract

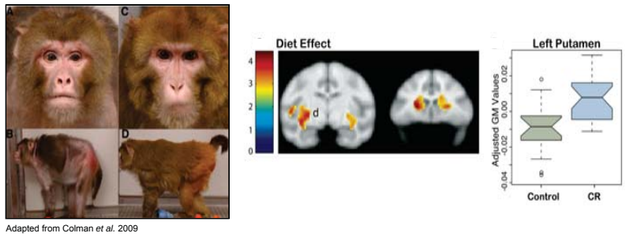
Age is the greatest risk factor for Alzheimer's disease (AD) and countless studies have linked growth and energy metabolism to age-related disease vulnerability. Indeed, the growth signaling-sensitive kinase, glycogen-synthase-kinase-3beta (GSK3β) has been directly linked to the principle biochemical features of AD, tau tangles and beta-amyloid plaques. We previously identified a novel metabolic pathway whereby GSK3β regulates the stability and activity of peroxisome proliferator-activated receptor gamma coactivator 1-alpha (PGC-1α), a master regulator of mitochondrial function (Anderson et al. 2008). Here, we characterize the extent to which GSK3β regulates metabolism at the cellular level using lithium, a widely-used inhibitor of GSK3β.

Lithium treatment resulted in upregulation of mitochondrial metabolism in cell culture models of astrocytes and mature neurons. This shift in metabolism involved increases in basal and maximal oxygen consumption, mitochondrial membrane potential, and lengthening of NAD(P)H fluorescent lifetime in-situ, suggesting higher levels of protein bound NAD(P)H. Co-incident with these changes was an increase in the stability of PGC-1α protein, which rapidly localized to the nucleus upon lithium administration. Overexpression of GSK3b in cells reveals a significant role for GSK3β in the regulation of growth and metabolism, further supporting the idea that GSK3β deregulation with age could contribute to disease vulnerability. Finally, mice fed a diet of lithium carbonate over four months exhibited lengthening of NAD(P)H fluorescence lifetime in key areas of the hippocampus and alteration in cytochrome c oxidase activity in a highly region and cell-type specific manner, suggesting that GSK3β operates similarly in regulating metabolism of the whole-brain.

Altogether, these results suggest a role for GSK3β as a driver of metabolic dysfunction with age. It also appears that metabolism itself, and the GSK3β/PGC-1α axis in particular, may be an ideal target for the prevention and treatment of age-related neurodegeneration.

Background

Caloric Restriction (CR) is a well established model of delayed aging that prolongs lifespan and reduces the incidece of disease in diverse organisms ranging from C. elegans to humans. The current project explores the extent to which GSK3β contributes to energy metabolism, a function that is highly responsive to CR.



Adapted from Colman *et al.* 2009

GSK3β regulates mitochondrial function

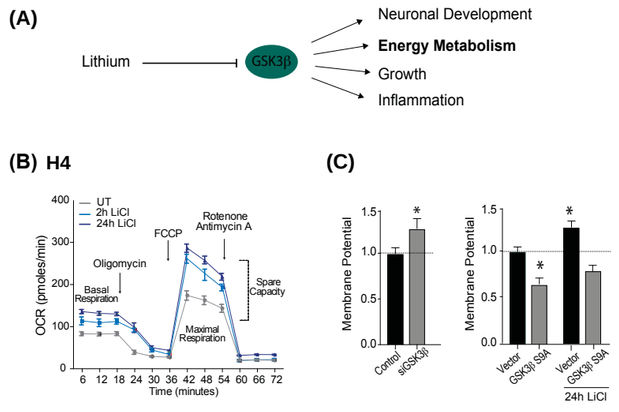


Figure 1: **Lithium works through GSK3β to increase mitochondrial function** (A) GSK3β has several roles related to neuronal function and is sensitive to direct inhibition by lithium (B) Seahorse XF Cell Mito Stress Kit; sequential injection of ETS inhibitors reveals basal and maximal respiration in addition to respiratory capacity. (B) JC-1 Assay; measurement of mitochondrial membrane potential in cells transfected with antisense RNA to GSK3β or cDNA to GSK3β[S9A], a constitutively active mutant

Lithium response is cell-type specific

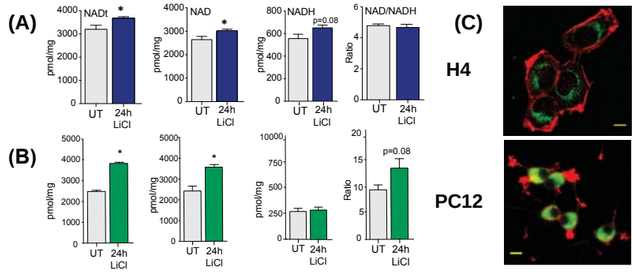


Figure 2: The impact of lithium is cell-type specific between H4 glioblastoma and PC12 neuron-like cells. (A,B) Whole-cell NAD(H) assay of lithium treated H4 cells. (C) Immunofluorescent staining of the mitochondrial marker Tomm20 and tubulin. Scale bar = 10 μm

Lithium alters NAD(P)H fluorescence decay

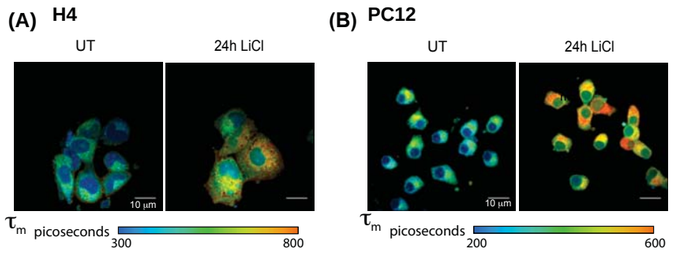


Figure 3: Lithium inreases the proportion of protein-bound NAD(P)H as revealed by multiphoton fluorescence lifetime microscopy (A,B) False-color images of NAD(P)H mean fluorescent lifetime (τm) in 15mM lithium-treated H4 and 7-day NGF-differentiated PC12 cells (exλ780nm)

PGC-1α responds dynamically to lithium

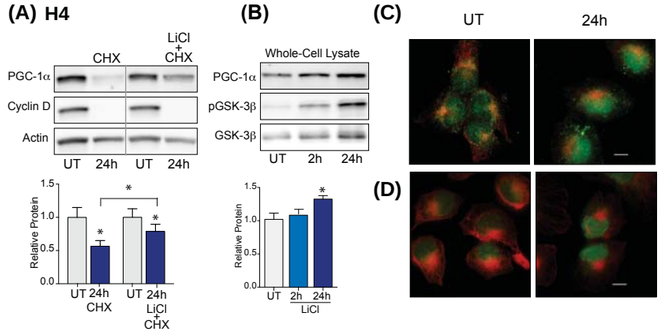


Figure 4: Lithium increases the stability of the transcriptional co-activator PGC-1α, which rapidly localizes to the nucleus upon administration (A) Representative immunoblot of whole-cell PGC-1α protein. Cells were pre-treated with lithium for 1 hour followed by treatment with cycloheximide, an inhibitor of protein synthesis. Cyclin D was used to confirm cycloheximide function. (C) Immunofluorescent detection of PGC-1α and tubulin in lithium-treated H4 cells. Scale bar = 10 μm (D) Immunofluorescent detection of GSK3β and tubulin in lithium-treated H4 cells

References:

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GSK3β regulates growth and metabolism

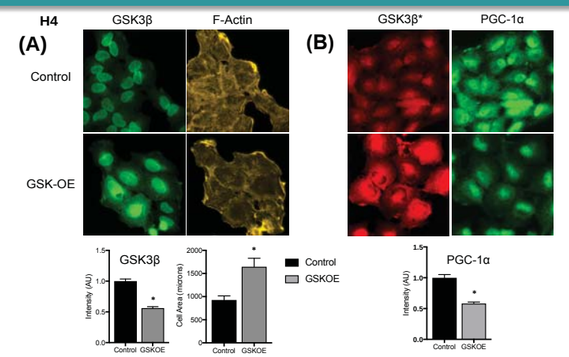


Figure 5: GSK3β overexpression changes cell morphology and depletes PGC-1α protein (A) Immunofluorescent detection of GSK3β and F-actin in H4 cells containing a doxycycline-inducible GSK3β transgene (H4[iGOE]) (B) Immunofluorescent detection of GSK3β and PGC-1α protein in H4[iGOE] cells. *brightness enhanced in controls

GSK3β regulates brain metabolism

Figure 6: GSK3β regulates energy metabolism in a cell-type and region-specific manner in the mouse brain (A) False-color images displaying NAD(P)H τm within the dentate gyrus of the hippocampus (exλ780nm). Mice were treated with dietary lithium carbonate four months prior to collection. (B) Cytochrome c oxidase activity staining (C) Immunostaining of PGC-1α

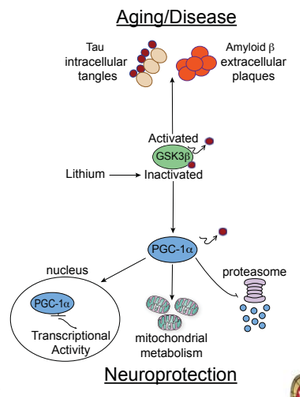
Conclusions:

GSK3β regulates multiple facets of cellular energy metabolism:

- Basal and maximal respiration
- Mitochondrial membrane potential
- Redox function
- NAD(P)H metabolism

Importantly, these effects appear to be conserved from the level of individual cells to the whole brain, where the impact of GSK3β inhibition is highly cell-type and region specific.

We propose a model of the aging brain in which GSK3β drives multiple, convergent aspects of neurodegeneration, particularly energy metabolism:



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