GSK-3β Regulates Brain Energy Metabolism

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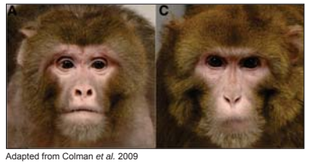
Abstract

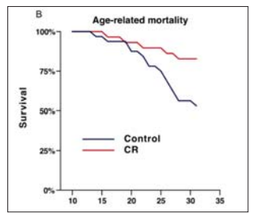
Neurodegeneration produces significant functional decline among the elderly and is the primary factor underlying late-onset Alzheimer's disease (AD). The insulin-sensitive kinase, glycogen-synthase-kinase-3beta (GSK3β) has been directly linked to the principle biochemical features of AD, tau tangles 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, and GSK3β inhibitor VIII.
Lithium treatment produced substantial upregulation of mitochondrial metabolism in cell culture models of astrocytes and mature neurons. This shift in metabolism extended to 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. Additionally, we observed transcription reprogramming with long-term lithium treatment consistent with increases in PGC-1α activity.
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. Furthermore, it 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 metabolic change and neuroprotection observed in CR.



Adapted from Colman *et al.* 2009



GSK-3β regulates mitochondrial function

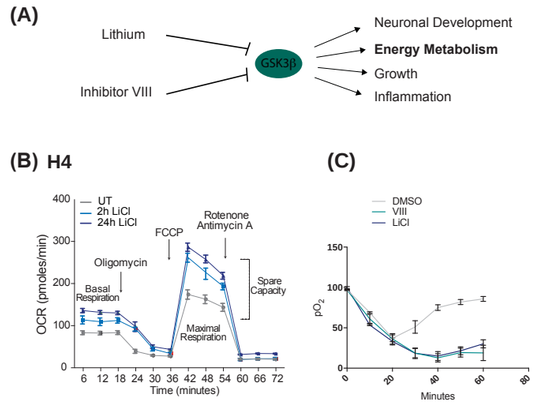


Figure 1: **GSK-3β inhibition with lithium increases basal and maximal cellular respiration in H4 glioblastoma cells.** (A) GSK3β has several roles related to cellular function and is sensitive to direct inhibition by lithium and GSK3β inhibitor VIII. (B) Seahorse XF Cell Mito Stress Kit; sequential injection of ETS inhibitors reveals basal and maximal respiration in addition to respiratory capacity. (B) Oxoplate Assay; measurement of cellular oxygen consuption of LiCl and Inhibitor VIII-treated H4 cells after a 24-hour pre-treatment

Lithium response is cell-type specific

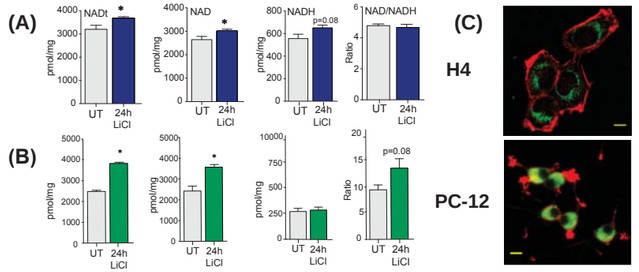


Figure 2: The impact of lithium is cell-type specific between H4 glioblastoma and PC-12 neuron-like cells. (A,B) Whole-cell NAD(H) assay of lithium treated H4 cells. (C) Immunofluorescent staining of the mitochondrial marker Tomm20 and tubulin. Scale bar = 10 μm

Lithium alters NAD(P)H fluorescence decay

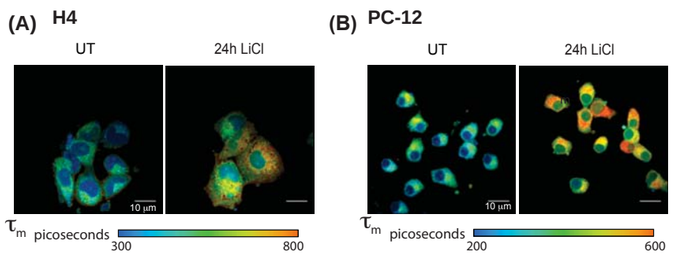


Figure 3: Lithium inreases the proportion of protein-bound NAD(P)H as revealed by multiphoton fluorescence lifetime microscopy (A,B) False-color images of NAD(P)H mean fluorescent lifetime (τm) in 15mM lithium-treated H4 and 7-day NGF-differentiated PC-12 cells (exλ780nm)

PGC-1α responds dynamically to lithium

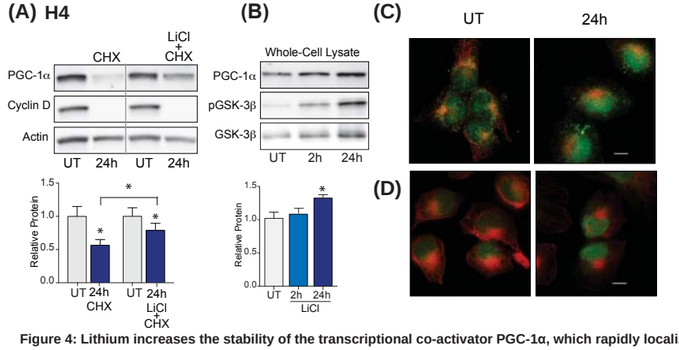


Figure 4: Lithium increases the stability of the transcriptional co-activator PGC-1α, which rapidly localizes to the nucleus upon administration (A) Representative immunoblot of whole-cell PGC-1α protein. Cells were pre-treated with lithium for 1 hour followed by treatment with cycloheximide, an inhibitor 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:

Anderson R, Weindruch R: Dynamic regulation of PGC-1a localization and turnover implicates mitochondrial adaptation in caloric restriction and the stress response. *Aging Cell* (2008) 7:101-111

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Lithium increases PGC-1α transcripts

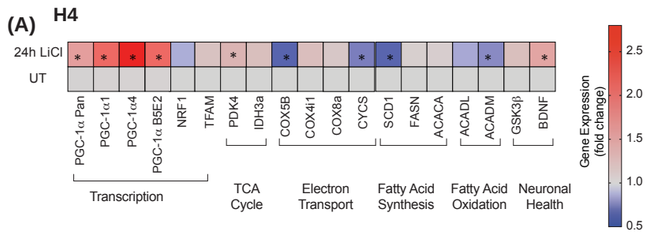


Figure 5: Lithium induces a transcriptional response in H4 cells consistent with activation of PGC-1a. (A) Heatmap displaying the fold change in expression of transcripts detected by quantitative PCR. Targets are separated by funtional category and normalized to ribosomal 18S

GSK-3β regulates brain metabolism

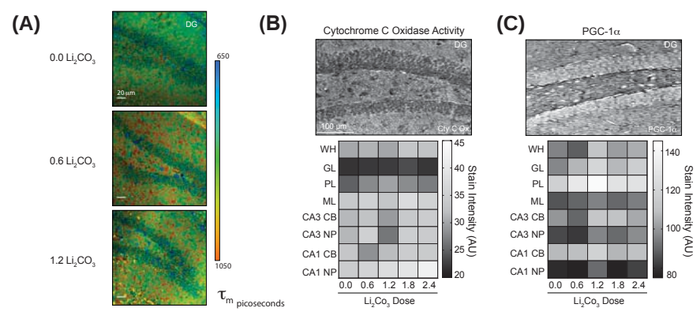


Figure 6: GSK3β regulates energy metabolism in a cell-type and region-specific manner in the mouse brain (A) False-color images displaying NAD(P)H τm within the dentate gyrus of the hippocampus (exλ780nm). Mice were treated with dietary lithium carbonate four months prior to collection. (B) Cytochrome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

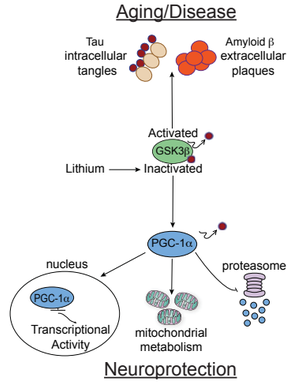
Conclusions:

GSK3β regulates multiple facets of cellular energy metabolism that converge at the level of the mitochondria:

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

Moreover, 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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