Cover Page

Title: The $Na_v1.2$ channel is regulated by GSK3

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Abstract

Background: Phosphorylation plays an essential role in regulating the voltage-gated sodium (Na_v) channels and excitability. Yet, a surprisingly limited number of kinases have been identified as regulators of Na_v channels. Herein, we posited that glycogen synthase kinase 3 (GSK3), a critical kinase found associated with numerous brain disorders, might directly regulate neuronal Na_v channels.

Methods: We used patch-clamp electrophysiology to record sodium currents from $Na_v1.2$ channels stably expressed in HEK-293 cells. mRNA and protein levels were quantified with RT-PCR, Western blot, or confocal microscopy, and *in vitro* phosphorylation and mass spectrometry to identify phosphorylated residues.

Results: We found that exposure of cells to GSK3 inhibitor XIII significantly potentiates the peak current density of $Na_v1.2$, a phenotype reproduced by silencing GSK3 with siRNA. Contrarily, overexpression of GSK3β suppressed $Na_v1.2$-encoded currents. Neither mRNA nor total protein expression were changed upon GSK3 inhibition. Cell surface labeling of CD4-chimeric constructs expressing intracellular domains of the $Na_v1.2$ channel indicates that cell surface expression of CD4-Nav1.2-Ctail was up-regulated upon pharmacological inhibition of GSK3, resulting in an increase of surface puncta at the plasma membrane. Finally, using *in vitro* phosphorylation in combination with high resolution mass spectrometry, we further demonstrate that GSK3β phosphorylates T^{1966} at the C-terminal tail of $Na_v1.2$.

Conclusion: These findings provide evidence for a new mechanism by which GSK3 modulate Na_v channel function via its C-terminal tail.

General Significance: These findings provide fundamental knowledge in understanding signaling dysfunction common in several neuropsychiatric disorders.