

## Roles of WNK Signaling Pathway in the Central Nervous System Development and Function.

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### Summary

We are interested in the Cl<sup>-</sup> homeodynamics regulating GABA actions. We used WNK3 KO mice, since WNK family kinases are essential for signaling cascade regulating Cl<sup>-</sup> concentrations. WNKs are known to phosphorylate KCC2 at two threonine (Thr<sup>906</sup> and Thr<sup>1007</sup>) residues via downstream kinases, SPAK/OSR1. So, we engineered mice with the missense mutations Glu<sup>906</sup> and Glu<sup>1007</sup> (Kcc2<sup>E/E</sup>) to mimic constitutive phosphorylation or with the missense mutations Ala<sup>906</sup> and Ala<sup>1007</sup> (Kcc2<sup>A/A</sup>) to mimic constitutive dephosphorylation. In WNK3 KO, [Cl<sup>-</sup>]<sub>i</sub> of layer V pyramidal neurons in medial prefrontal cortex (mPFC) was significantly increased. However, NKCC1, pT206 NKCC1, KCC2, WNK1, pWNK1, total SPAK, total OSR1 and pSPAK1/pOSR1 were all unchanged. On the other hand, a significant reduction in pre-pulse inhibition was observed in WNK3 KO mice. Thus, WNK3 was found to be important for excitation-inhibition balance of layer V pyramidal neurons and functional output from mPFC. Kcc2<sup>E/E</sup> mice demonstrated abnormal neuronal distribution but normal dendritic spine formation, status epilepticus provoked by mild physiological stimulation, normal resting [Cl<sup>-</sup>]<sub>i</sub> but with significantly impaired Cl<sup>-</sup> extrusion capacity after Cl<sup>-</sup> loading, a lack of spontaneous respiratory discharge and an altered locomotor rhythm. Thus, precisely regulated KCC2 Thr<sup>906</sup>/Thr<sup>1007</sup> phosphorylation is essential for activity-dependent Cl<sup>-</sup> extrusion required for normal brain development. Kcc2<sup>A/A</sup> showed normal body weight, normal respiration, normal somatosensory function, normal sociability and normal locomotor activity but demonstrated a tendency of less anxiety and less social novelty. Startle response test showed much less responses by Kcc2<sup>A/A</sup>, we speculate they might have hearing disability, because their muscle power was normal. What interesting is decreases in power of EEG in  $\gamma$  band. Excessive KCC2 function and excessive GABA inhibition may result in decreased sensitivity to anxiety and fear, and cognitive function.