Modulation of KCC2 function by tyrosine phosphorylation

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Neuronal chloride homeostasis by the transporters NKCC1 and KCC2 plays a critical role in determing the response to the transmitters GABA and glycine. The expression levels of KCC2 decrease in response to neuronal injury, but less is known about the mechanisms mediating more dynamic modulation of KCC2 function. Using gramicidin-perforated patch-clamp recordings and the GABA reversal potential (E_{GABA}) as a measure of intracellular Cl^-, we investigated how tyrosine phosphorylation affects KCC2 function (Watanabe *et al.*, 2009). Application of the tyrosine kinase inhibitor genistein (100 μM) to cultured hippocampal neurons results in a positive shift of E_{GABA} by 10.5 ± 1.2 mV (n=5). Transfecting KCC2-EGFP into GT1-7 cells resulted in Cl^- efflux and a more negative E_{GABA} (-66.6 ± 2.4 mV, n=6) compared to untransfected cells (-36.6 ± 0.9 mV, n= 5). In GT1-7 cells, genistein also shifted E_{GABA} to more positive values (n=2, by 10 mV and 15 mV). Mutating the putative KCC2 tyrosine phosphorylation site (Y1087D) resulted in non-functional KCC2 (E_{GABA} was -36.6 ± 0.9 mV, n=5). This mutation also resulted in a translocation of surface KCC2 from a punctate pattern associated with lipid rafts, to a more diffuse distribution. Neuronal stress is known to induce a rapid loss of KCC2 tyrosine phosphorylation and transport function (Wake *et al.*, 2007) and we conclude that 1) GT1-7 cells represent a good cellular model for further studies of the regulation of KCC2 in models of neuronal injury, and 2) loss of KCC2 tyrosine phosphorylation is associated with loss of function and altered membrane surface localisation.

Wake H. *et al.* (2007) *J. Neurosci.* **27**: 1642-1650.
Watanabe M. *et al.* (2009) *J. Biol. Chem.* ***284****: 27980-8.*