Membrane transporters regulating inhibitory neurotransmitter signaling in health and disease

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The efficacy of inhibitory signalling at GABA and glycinergic synapses depends on a number of factors including the permeability properties of the GABA and glycine-activated receptor channels, and also the electrochemical gradients for the ions that permeate through these channels. The chloride ion (Cl^-) is most permeant through these channels. The neuronal isoform of the K^+-Cl^- cotransporter (KCC2) is a major determinant of the intracellular Cl^- concentration in neurons, and hence a key determinant of the equilibrium potential for Cl^-. The expression levels and function of KCC2 has a large impact on GABA responses. The conversion of GABAA receptor responses from depolarizing to hyperpolarizing that occurs in some neurons during development is due to an upregulation of KCC2 expression. More recent data has indicated that a range of neuronal injuries and stress applied to the adult nervous system, both *in vivo* and *in vitro*, results in loss of KCC2 transport and a reversion of GABA responses towards a depolarizing phenotype (Moorhouse & Nabekura, 2011). This downregulation occurs in two stages, an early phase of loss of function and a later phase where expression levels decrease (Wake *et al.*, 2007). The early phase is associated with reduced tyrosine phosphorylation of KCC2, which is needed for robust transport function and a punctuate distribution in the plasma membrane (Watanabe *et al.*, 2009).

To further address the functional and cellular consequences of KCC2 expression on GABAergic responses and neuronal inhibition we have utilized a conditional transgenic mouse in which KCC2 expression is under the control of a tetracycline responsive element. Expression levels of KCC2 are increased by withdrawal of doxyclycline (DOX) from the diet. Neuronal inhibition was evaluated by susceptibility of mice to induction of status epilepticus (SE) in response to systemic injection of pilocarpine or kainic acid. All procedures were approved by the Ethics Review Committee for Animal Experimentation of the National Institutes for Natural Sciences. Systemic injection of pilocarpine (290-400 mg/kg) resulted in SE in four out of 6 control transgenic mice (with normal KCC2) and that was associated with some modest subsequent histological sequelae. SE was stopped after about 1 hour by diazepam (10-20 mg/kg, ip). The incidence of SE was reduced in mice in which DOX had been withdrawn from the diet to upregulate KCC2. A ramp-up dosing schedule using kainic acid (25-35 mg/kg, ip) induced SE with greater reproducibility and less mortality. Five out of five control mice (with DOX) had SE that was associated with typically six to ten stage 5 seizures in each mouse. When DOX was withdrawn from the diet prior to testing (to upregulate KCC2 expression), none of an additional five mice injected with kainic acid (30-50 mg/kg) showed any SE, and only one mouse had any stage 5 seizures. Hence switching on KCC2 is strongly preventing seizures and SE *in vivo.* Preliminary data in hippocampal slices isolated from DOX-on (control) and DOX-off (KCC2 upregulation) mice suggests only modest changes in the sensitivity of the population spike field potential to inhibition by the GABA receptor agonist muscimol. The data demonstrate that upregulating KCC2 enhances neuronal inhibition during seizure activity, but may not cause major changes in basal levels of neuronal inhibition in quiescent neuronal circuits.

Moorhouse AJ, Nabekura J. (2011) Cellular Mechanisms of Neuronal Cl^- Homeostasis and its Modulation by Neuronal Injury. In *Inhibitory Synaptic Plasticity*. Eds. Maffei A, Woodin MA. Contemporary Neuroscience Series. Springer, Ch. 9, pp. 123-136.

Wake H, Watanabe M, Moorhouse AJ, Kanematsu T, Horibe S, Matsukawa N, Asai K, Ojika K, Hirata M, Nabekura J. (2007). Early changes in KCC2 phosphorylation in response to neuronal stress result in functional downregulation. *Journal of Neuroscience* **27,** 1642-1650.

Watanabe M, Wake H, Moorhouse AJ, Nabekura J. (2009) Clustering of neuronal K^+-Cl^--cotransporters in lipid rafts by tyrosine phosphorylation. *Journal of Biological Chemistry* **284,** 27980-27988.