Overexpression of KCC2 reduces neuronal hyperexcitability

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Hyperpolarizing neuronal inhibition in response to activation of ionotropic γ-amino butyric (GABA) receptors depends on an electrochemical gradient to drive Cl^- into neurons. The K^+ Cl^- co-transporter, KCC2, plays an important role in keeping intracellular Cl^- concentrations $[Cl^-]_i$ low and thereby promoting GABA receptor-mediated hyperpolarizing inhibition. We have proposed that enhancing KCC2 function may enhance GABAergic inhibition, and have been using a conditional transgenic mouse to test this hypothesis. KCC2 overexpression in this transgenic mouse is restricted to pyramidal neurons of the forebrain and triggered by withdrawing doxycycline from the diet. *In vitro* experiments were done using hippocampal slices, excised from anaesthetized (pentobarbital, 130 mg/kg, i.p.) 8-14 week old mice. Overexpression of KCC2 was associated with increased neuronal membrane transport function *in vitro*, measured using a pH-sensitive fluorophore (BCECF) and the response to application of the KCC2 substrate NH_4^+. In hippocampal slices from KCC2 overexpressing mice, the field population stimulus response relationships, and the muscimol concentration-response curves, were both not significantly different from those in slices from control mice. This suggests minimal effects of KCC2 overexpression on basal synaptic and $GABA_A$ receptor responses. However, when hippocampal slices were made hyperexcitable by tetanic stimulation or by perfusion with 0 Mg^{2+} solutions, then significant differences were seen between KCC2 overexpressing mice and control mice. Tetanic afferent stimuli induced after-discharges in 7/7 slices from 5 control mice, but only in 1/7 slices from 5 KCC2 overexpressing mice. Similarly, 0 Mg^{2+} perfusion induced spontaneous spikes in 4 control slices with a mean frequency of 21.1 ± 3.9 spikes/minute, while the frequency in slices from KCC2 overexpressing mice was lower, at 9.8 ± 2.1 spikes/minute. Hence increasing KCC2 expression reduces excitability in hippocampal slices exposed to seizure-like stimuli. We next examined seizure behaviour *in vivo*. Multiple injections of low doses of kainic acid (5mg/kg, ip) resulted in escalating seizure behaviours that culminated in repeated convulsive seizures we defined as status epilepticus (SE). SE was reached in all five control mice (and at a total dose of 15 mg/kg) but was only observed in one of the five mice with overexpression of KCC2 (at a total dose of 50 mg/kg). Protection against the induction of seizures was not observed in the pentylenetetrazol model. We conclude that overexpression of KCC2 has minimal effects on basal neural network activity, but can reduce the transition into seizure behaviours when neuronal network activity is enhanced. We propose that increased KCC2 expression and membrane Cl^- transport is able to sustain efficacious inhibitory transmission during neuronal hyperactivity.

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