Epilepsy Currents
2020, Vol. 20(1) 43-44

DOI: 10.1177/1535759719890333
journals.sagepub.com/home/epi
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KCC2 Much Chloride Might Not Be the Only Problem

KCC2 Regulates Neuronal Excitability and Hippocampal Activity via Interaction With Task-3 Channels

Goutierre M, Al Awabdh S, Donneger F, et al. *Cell Rep.* 2019;28(1):91-103.e7. doi:10.1016/j.celrep.2019.06.001. PMID: 31269453.

KCC2 regulates neuronal transmembrane chloride gradients and thereby controls GABA signaling in the brain. KCC2 downregulation is observed in numerous neurological and psychiatric disorders. Paradoxical, excitatory GABA signaling is usually assumed to contribute to abnormal network activity underlying the pathology. We tested this hypothesis and explored the functional impact of chronic KCC2 downregulation in the rat dentate gyrus. Although the reversal potential of GABAA receptor currents is depolarized in KCC2 knockdown neurons, this shift is compensated by depolarization of the resting membrane potential. This reflects downregulation of leak potassium currents. We show KCC2 interacts with TASK-3 (KCNK9) channels and is required for their membrane expression. Increased neuronal excitability upon KCC2 suppression altered dentate gyrus rhythmogenesis, which could be normalized by chemogenetic hyperpolarization. Our data reveal KCC2 downregulation engages complex synaptic and cellular alterations beyond GABA signaling which perturb network activity, thus offering additional targets for therapeutic intervention.

Commentary

The chloride in all that salt you've been adding to your food has to go somewhere. Some of it probably makes its way into your neurons. But if your KCC2 is doing its job, then your neurons will have just the right amount. And that matters to your GABA receptors.

Why? Because the efficacy of GABAergic neurotransmission in regulating the excitability of neurons is largely dependent upon chloride ion (Cl^-) flow across the neuronal membrane. Cl^- is a negatively charged ion, so flow into the neuron will hyperpolarize the membrane potential and flow out of the neuron will depolarize the membrane potential. The direction of flow is determined by the difference between the membrane potential and the Cl^- reversal potential, which is set by relative concentration of Cl^- on either side of the membrane. Although once to be passively distributed, physiologists later realized that this must not be true because $GABA_A$ receptor activation can change the resting membrane potential.

The subsequent identification of electroneutral cation–chloride cotransporters, such as NKCC1 and KCC2, and their expression in neurons provided a molecular mechanism to explain why Cl^- concentrations are not at the thermodynamic equilibrium potential.[1,2] Later, it was shown that the K^+–Cl^- cotransporter KCC2, which functions to transport Cl^- out of neurons, shows reduced expression in several rodent epilepsy models, including kindling, traumatic brain injury, and pilocarpine-induced status epilepticus,[3-5] as well as other disease states such as neuropathic pain.[6] This downregulation of KCC2 raises the intracellular Cl^- concentration and Cl^- equilibrium potential, which can then lead to neuronal hyperexcitability as GABA-mediated opening of chloride channels causes chloride efflux and membrane depolarization.

A new study by Goutierre et al has challenged the hypothesis that neuronal hyperexcitability following KCC2 downregulation is due solely to altered effects of GABA signaling. The authors delivered a short hairpin RNA (shRNA) via lentivirus to focally reduce (knockdown) KCC2 expression in the dentate gyrus and then explored the physiological and biochemical effects of this manipulation in dentate granule neurons. First, they performed whole-cell electrophysiology recordings from granule neurons and found that the reversal potential for GABA-gated currents (E_{GABA}) was shifted by about 8 mV in the positive direction. This shift was expected but smaller than reported by other KCC2 knockdown and knockout experiments.[7-9] Surprisingly, the resting membrane potential (V_{rest}) of the granule neurons was also depolarized, by about 6 mV, meaning that the driving force for chloride through the $GABA_A$ receptor was essentially unchanged at rest. In addition to the depolarization of V_{rest}, the input resistance (R_{in}) of the granule neurons was higher and they fired more action potentials in response to small current injections, suggesting that they were hyperexcitable due to a change in their intrinsic membrane properties.

How could loss of KCC1 lead to increases in intrinsic membrane excitability? Because changes in V_{rest} and R_{in} often reflect altered potassium conductances, the authors measured K^+ currents and found a reduction in an outwardly rectifying K^+ current in KCC2 knockdown neurons. Expression patterns of outwardly rectifying K^+ channels in the dentate gyrus provided the next clue, that TASK-3 channels were involved, and biochemical experiments showed that KCC2 interacts with TASK-3 and promotes its membrane expression. This set of experiments provides strong evidence that the reason that KCC2 loss alters the membrane excitability of neurons is because it normally helps traffic TASK-3 channels to the membrane, where they lower V_{rest} and R_{in} by providing a leak K^+ conductance.

Finally, the authors explored the network-level consequences of their focal KCC2 knockdown. Mice with KCC2 knockdown did not have spontaneous seizures, epileptiform events, or increased sensitivity to pilocarpine. Despite this absence of epileptic pathology, the authors did observe an increase in the amplitude and frequency of dentate spikes during slow-wave sleep, suggesting that KCC2 knockdown had functional consequences for the dentate circuit. They were then able to rescue this abnormality by using chemogenetics to reduce V_{rest} in granule neurons, providing proof of principal that dampening the intrinsic excitability of dentate neurons without directly affecting GABA neurotransmission is sufficient to block the pathological effect of KCC2 knockdown.

So, is altered GABAergic signaling the mechanism linking KCC2 downregulation to seizures and other network abnormalities, or are the increases in membrane excitability more important? As with many biological questions, the answer probably lies somewhere in between. As noted earlier, the KCC2 knockdown in this study appears to have milder effects on E_{GABA} than other recent studies, which is in accord with the lack of epileptic pathology in this study but its presence in others.[7,8] Thus, it is possible that smaller reductions in KCC2 raise V_{rest} and E_{GABA} in tandem and cause more subtle network disturbances, while larger reductions cause a rise in E_{GABA} beyond V_{rest}, more severe disruption in GABAergic transmission, and epileptic activity. Decades of research have established the importance of KCC2 in regulating E_{GABA}, the effectiveness of GABA in neuronal inhibition, and seizure susceptibility.[10] However, Goutierre et al provide solid evidence and a molecular mechanism linking KCC2 to intrinsic excitability, which is exciting because it forces a reevaluation of the link between KCC2 dysregulation and pathology, and serves as a reminder that there is value in looking at noncanonical effects of molecules when assessing their contribution to disease states.

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