Short Communication | Journal of Neurological Disorders | 2020 | Vol.8 No.7

Activation of K+–Cl-–Cotransporter KCC2 by Inhibiting the WNK-SPAK Kinase Signalling as a Novel Therapeutic Strategy for Epilepsy

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ABSTRACT

The Cl--extruding transporter KCC2 (SLC12A5) critically modulates GABAA receptor signaling via its effect on neuronal Cl- homeostasis. Previous studies have shown that KCC2 was downregulated in both epileptic patients and various epileptic animal models. We discovered that the in vitro dual phosphorylation of Thr906 and Thr1007 in the intracellular carboxyl (C)-terminal domain of KCC2, mediated by the Cl--sensitive WNK-SPAK serine-threonine protein kinase complex, maintains the depolarizing action of GABA in immature neurons by antagonizing KCC2 Cl- extrusion capacity. GABAAR-mediated inhibition confines KCC2 to the plasma membrane, while antagonizing inhibition reduces KCC2 surface expression by increasing the lateral diffusion and endocytosis of the transporter. This mechanism utilizes Cl- as an intracellular secondary messenger and is dependent on phosphorylation of KCC2 at threonines 906 and 1007 by the Cl- -sensing kinase WNK1. We propose this mechanism contributes to the homeostasis of synaptic inhibition by rapidly adjusting neuronal [Cl-]i to GABAAR activity. We further demonstrate here that this signaling pathway is rapidly and massively activated in an acute epilepsy model. This indicates that dephosphorylation of KCC2 at Thr906 and Thr1007 is a potent activator of KCC2 activity, and small molecular targets WNK-SAPK kinase signaling may be a novel therapeutic strategy for epilepsy.

Keywords: Epilepsy, WNK-SPAK Kinase Signalling, Homeostasis, Seuizures.

INTRODUCTION

Fast synaptic inhibition in the adult CNS is largely mediated via ionotropic GABAARs and GlyRs, ligand-gated anion channels permeable to Cl−. Upon receptor binding and channel opening, the electrochemical driving force determines whether the current carried by Cl− is hyperpolarizing or depolarizing. The strength of the inhibitory action of GABAARs and GlyRs is determined not only by the respective conductances, but also by the efficacy of Cl− extrusion. Therefore, regarding the role of ion transport in controlling the efficacy of inhibition, the determining factor is the capability of Cl−-extruding mechanisms to maintain the reversal potentials of GABAAR- and GlyR-mediated responses, EGABA and EGly, at a sufficiently negative level to prevent the neuron from firing action potentials . In the somatic and dendritic compartments of many adult neurons at rest, [Cl−]i is maintained at only a few millimoles. Therefore, EGABA and EGly are sensitive to small changes in [Cl−]i and must be tightly regulated to maintain the strength of inward Cl− flux upon GABAAR activation. Accordingly, an increasingly recognized form of regulation of GABAA function, known as 'ionic plasticity', is based on short- and long-term changes in neuronal [Cl−]i and is thus highly sensitive to changes in the functional expression of KCC2.

The ability of neurons to maintain low [Cl−]i is dependent upon the neuron-specific K–Cl cotransporter KCC2, the principal Cl− extruder in adult neurons. KCC2, a member of the cation-chloride cotransporter (CCC) SLC12A gene family, utilizes the energetically favorable plasmalemmal K+ concentration gradient to extrude Cl− beyond electrochemical equilibrium values. Because immature central neurons are characterized by a lower functional expression of KCC2 relative to the Na–K–2Cl cotransporter isoform NKCC1, which mediates Cl− uptake, [Cl−]i remains high and GABAAR activation results in a depolarizing rather than a hyperpolarizing response in these cells. These depolarizing GABAAR-mediated responses affect early network activities and activity-dependent synaptic changes required for neuronal migration and circuit formation. During postnatal brain development, increased functional expression of KCC2 is associated with a change in GABA- and/or glycinergic signaling from depolarizing to hyperpolarizing. Indeed, without KCC2, the inhibitory strength of GABAergic signaling is compromised and may result in increased membrane excitability.

NMDA receptor-dependent KCC2 serine 940 dephosphorylation: implications for seizures

Lee et al. demonstrated using dissociated HC neurons that an increase in NMDA receptor (NMDAR) activation, experimentally induced by elevating ambient glutamate, triggers a Ca2+-dependent KCC2 dephosphorylation at S940 and downregulation of both the surface expression and function of KCC2. Both of these events were sensitive to the protein phosphatase 1 (PP1) inhibitor okadaic acid, which prevented the loss of hyperpolarizing GABAergic inhibition triggered by glutamate exposure. Thus, PKC-dependent phosphorylation of S940 enhances [58], whereas PP1-mediated dephosphorylation of S940 appears to inhibit KCC2. Lee et al. proposed that the KCC2 functionality is strongly influenced by the phosphorylation state of S940 which is, in turn, controlled by the relative activities of PKC and PP1. These results are compelling, considering the occurrence of elevated glutamate signaling in the CNS during numerous pathophysiological states associated with a decrease in the functional expression of KCC2. Lee et al. also highlighted the therapeutic potential of NMDAR antagonists to limit damage to the Cl− homeostatic

mechanism during the acute phase of neuronal injury. In this context, it is not unlikely that the recently reported C-terminal truncation and functional inactivation of KCC2 by the Ca2+-dependent cysteine protease calpain triggered by sustained NMDAR activation, observed in HC and spinal cord neurons is regulated by a KCC2 phosphorylation state-dependent mechanism (Figure 2B). For example, the GlyR and GABAAR scaf-folding protein gephyrin and the canonical calpain substrate spectrin are notable examples of substrate phosphorylation state-dependent targeting to calpain-mediated cleavage.

CONCLUDING REMARKS AND FUTURE DIRECTIONS

Recent evidence suggests that rapid and reversible phosphorylation and/or dephosphorylation of critical serine, threonine, and tyrosine residues in the KCC2 cytoplasmic carboxyl terminus constitutes a potent and dynamic set of mechanisms to modulate KCC2 activity. PKC-dependent S940 phosphorylation increases KCC2 activity and plasma membrane accumulation by slowing transporter endocytosis. By contrast, WNK kinase-dependent phosphorylation of T906 and T1007 appears to reduce the intrinsic rate of KCC2-mediated ion transport, and dephosphorylation of these residues is an important activator of KCC2. Y1087 phosphorylation promotes KCC2 activity by increasing the membrane insertion of transporters. The role of Y903 in KCC2 regulation is currently unclear, but it is intriguing that this residue is positioned close to T906, raising the possibility of tyrosine kinase modulation of Y903 having an impact on adjacent threonine phosphorylation, or vice versa.

Alterations in the phosphorylation state of these residues are variably altered across neurodevelopment and in models of different neurological diseases. Normally, these phosphoevents probably function not so much as on/off switches of KCC2 activity, but more as modulators, conferring quantitatively graded changes in KCC2 activity in response to physiological signals and perturbations, thus matching fluctuations in neuronal Cl^- loads, or increased metabolic demands. Selective modulation of these phosphoresidues, either directly or indirectly, could represent novel therapeutic strategies for the treatment of epilepsy, neuropathic pain, and spasticity, conditions that have been linked to the functional downregulation of KCC2 (or to enhancement in the functional expression of NKCC1), and, in some instances, have shown an altered phosphorylation profile.

An important future direction will be to relate functional modulation, produced by phosphorylation, to changes in the 3D structure of KCC2, which has 12 transmembrane domains. Unfortunately, structural studies of membrane proteins are difficult and, to date, there is no high-resolution structure available for KCC2. Another major area of focus in the near future will be a proteomic analysis of other KCC2-associated proteins, including protein phosphatases. Moreover, upstream signaling elements, including hormones, peptides, membrane receptors, scaffolding proteins, kinases, and phosphatases that transmit signals to effect changes in KCC2 (de)phosphorylation at the important residues described here are essentially unknown. Understanding the normal and pathological cues that trigger the phosphorylation events to elicit changes in KCC2 function could be an important step in facilitating or preventing these processes for therapeutic benefit.

REFERENCES

1. de Los Heros P, Alessi DR, Gourlay R, Campbell DG, Deak M, Macartney TJ, Kahle KT, Zhang J. The WNK-regulated SPAK/OSR1 kinases directly phosphorylate and inhibit the K+-Cl- co-transporters. Biochemical Journal, 2014 Mar 15;458(3):559-573

2. Friedel P, Kahle KT, Zhang J, Hertz N, Pisella LI, Buhler E, Schaller F, Duan J, Khanna AR, Bishop PN, Shokat KM, Medina I.WNK1-regulated inhibitory phosphorylation of the KCC2 cotransporter maintains the depolarizing action of GABA in immature neurons. Science Signalling, 2015 Jun 30;8(383):ra65.

3. Zhang J, Bhuiyan MIH, Zhang T, Karimy J, Wu Z, Pigott VM, Zhang J, Huang H, Hassan MN, Skrzypiec AE, Mucha M, Duran D, Huang W, Pawlak R, Foley LM, Hitchens TK, Minnigh M, Poloyac S, Alper SL, Molyneaux BJ, Trevelyan A, Kahle K, Sun D, Deng X. Modulation of brain cation-Cl- cotransport via the SPAK kinase inhibitor ZT-1a. Nature Communications, 2020 Jan 7;11(1):78.

4. Heubl M, Zhang J, Pressey J, Renner M, Moutkine I, Russeau M, Eugène E, Kahle KT, Poncer JC and Lévi S. GABAA receptor dependent synaptic inhibition rapidly tunes KCC2 activity via the Cl^--sensitive WNK1 kinase. Nature Communications, 2017, 8 (1), 1776.

5. Zhang J, Gao G, Begum G, Wang J, Khanna AR, Shmukler BE, Daubner GM, de Los Heros P, Davies P, Varghese J, Bhuiyan MI, Duan J, Zhang J, Duran D, Alper SL, Sun D, Elledge SJ, Alessi DR, Kahle KT. Functional kinomics identifies a key regulatory module of swelling-regulated Cl- transport in the mammalian brain. Scientific Reports, 2016 6, 35986

6. Wantanabe M, Zhang J, Duan J, Mansuri M, Delpire E, Lifton RP, Alper SL, Fukuda1 A, Kahle KT. Developmentally regulated KCC2 phosphorylation is essential for dynamic GABA-mediated inhibition and survival. Science Signalling, 2019, Oct 15;12(603). pii: eaaw9315.

7. Pisella L, Gaiarsa JL, Diabira D, Zhang J, Khalilov I, Duan J, Kahle KT, Medina I. Impaired regulation of KCC2 phosphorylation leads to neuronal network dysfunction and

neurodevelopmental pathology. Science Signalling, 2019 Oct 15;12(603). pii: eaay0300.

8. Tillman L, Zhang J. Crossing the Chloride Channel: The Current and Potential Therapeutic Value of the Neuronal K+-Cl- Cotransporter KCC2. BioMed Research International. 2019 May 21;2019:8941046.

9. Shekarabi M, Zhang J, Khanna AR, Ellison DH, Delpire E, Kahle KT. WNK kinase signaling in ion homeostasis and human disease. Cell Metabolism, 2017, Feb 7;25(2):285-299.

10. Zhang J, Karimy JK, Delpire E, Kahle KT. Targeting SPAK kinase in disorders of epithelial Cl- secretion. Expert Opinion On Therapeutic Targets, 2017 Aug;21(8):795-804.

11. Huang H, Song S, Banerjee S, Jiang T, Zhang J, Kahle KT, Sun D, Zhang Z. The WNK-SPAK/OSR1 kinases and the cation-chloride cotransporters as therapeutic targets for neurological diseases. Aging and Disease, 2019 Jun 1;10(3):626-636.

12. Alessi DR, Zhang J, Khanna A, Hochdörfer T, Shang Y, Kahle KT. The WNK-SPAK/OSR1 pathway: master regulator of Cation-Chloride Cotransporters. Science Signaling, 2014 Jul 15;7(334):re3.