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Review article

KCC2, epileptiform synchronization, and epileptic disorders

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ABSTRACT

The K^+-Cl^- co-transporter KCC2 is a neuron-specific, Cl^- extruder that uses K^+ gradient for maintaining low intracellular [Cl^-]. It is indeed well established that sustaining an outwardly-directed electrochemical Cl^- gradient across the neuronal membrane is fundamental for a proper function of postsynaptic $GABA_A$ receptor signaling. In particular, studies in the last two decades have shown that KCC2 activity is important to maintain a hyperpolarizing GABAergic neurotransmission. Conversely, low KCC2 activity should lead to depolarizing, and under specific conditions, excitatory GABAergic transmission. Not surprisingly given the critical role of KCC2 in regulating the inhibitory drive, alterations in its expression levels and activity are linked with epilepsy. Here, we will first summarize data regarding the role of KCC2 in epileptiform synchronization. Next, we will review evidence indicating that KCC2 expression and function are altered in chronic epileptic disorders, both in the developing and adult brain. We will also go through recent findings regarding the molecular mechanisms underlying the changes in KCC2 activity that occur following seizures. Finally, we will consider the modulation of KCC2 function as a potential, novel therapeutic target for the treatment of epileptic disorders.

Contents

1. Background

The maintenance of a transmembrane, outwardly-directed, electrochemical Cl^- gradient represents a key factor in sustaining the efficacy of fast synaptic inhibitory transmission resulting from the opening of ligand-gated ion channels activated through postsynaptic $GABA_A$ or glycine receptors (Farrant and Kaila, 2007) (Fig. 1A). This condition rests on the activity of cation-chloride cotransporters (**CCCs**) that are encoded by the solute

Abbreviations: CCCs, Cation-chloride cotransporters; KCC, K^+-Cl^- co-transporters; E, embryonic day; NCC, Na^+-Cl^- co-transporter; NKCC, Na^+-K^+-Cl^- co-transporters; P, postnatal day; [Cl^-], Chloride concentration.

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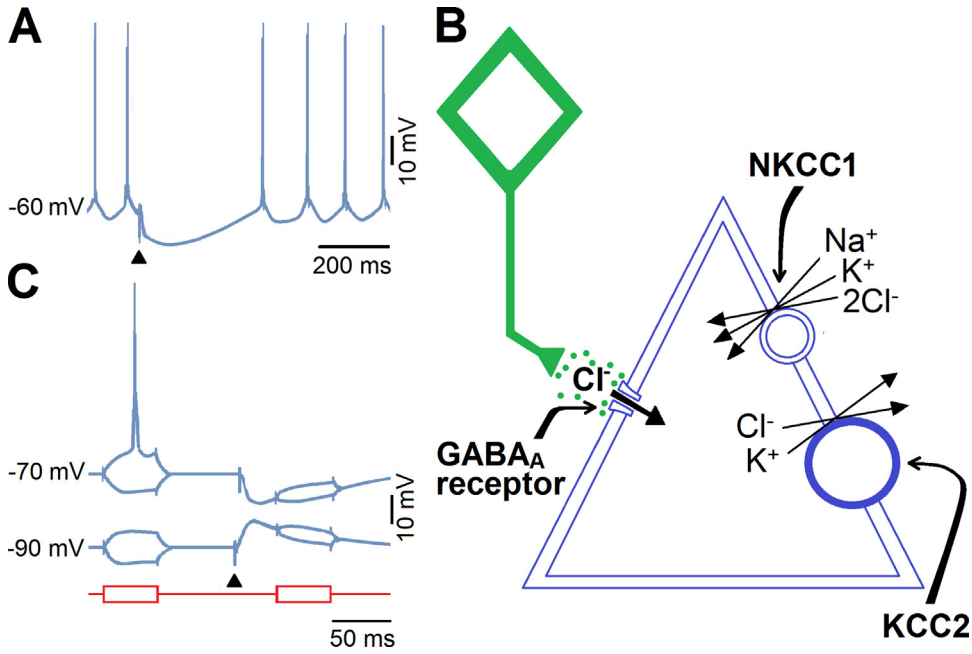

Fig. 1. Schematic representation of the actions exerted by $GABA_A$ receptor signaling on neuronal excitability. **A**: Intracellular recording obtained from a principal (glutamatergic) neuron during blockade of ionotropic excitatory amino acid and $GABA_B$ receptors; arrowhead identifies the time when the surrounding GABAergic interneurons are excited by an extracellular stimulus delivered close to the principal cell. Note that at a resting membrane potential of approx. −60 mV the neuron generates a hyperpolarization that causes inhibition of the spontaneous regular firing. **B**: Drawing of a GABAergic interneuron (green) synapsing on a principal cell (light blue) in the adult central nervous system. Activation of the postsynaptic $GABA_A$ receptor causes an inwardly directed Cl^- current that depends on the activity of NKCC1 and KCC2 with the latter being preponderant at this stage of maturation. In this and following figures, the actual size of the circles representing the two CCCs reflects the amount of their function. **C**: Experimental conditions similar to those illustrated in **A** but obtained while injecting intracellular pulses of depolarizing and hyperpolarizing pulses (red trace) to measure the input resistance before and after the extracellular stimulus. The two intracellular traces represent recordings obtained at membrane potentials of −70 and −90 mV. Note that the decreased input resistance caused by the postsynaptic activation of $GABA_A$ receptors leads to inhibitory shunting of the membrane thus blocking action potential generation (−70 mV sample). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

carrier 12 (SLC12) family genes. CCCs are secondary active transporters, which implies that no hydrolysis of ATP is needed for their transport cycles (Chamma et al., 2012; Medina et al., 2014). They consist of three broad groups: (i) K^+-Cl^- co-transporters (**KCC**; isoforms KCC1-4); (ii) Na^+-K^+-Cl^- co-transporters (**NKCC**; isoforms NKCC1 and NKCC2); and (iii) Na^+-Cl^- co-transporter (**NCC**) (Payne et al., 2003; Medina et al., 2014). KCC2 and NKCC1 are the two main CCCs in the central nervous system, where they maintain intracellular Cl^- concentrations. KCC2 is a neuron-specific Cl^- extruder that uses K^+ gradient for maintaining low intracellular [Cl^-], while NKCC1 causes Cl^- influx by taking advantage of the inwardly directed Na^+ gradient (Delpire, 2000; Gamba, 2005; Kahle et al., 2008) (Fig. 1B). Although these cotransporters do not require ATP to function, the K^+ and Na^+ gradients guiding their cotransporter activity are generated by the Na^+/K^+ ATPase pump (Kaila et al., 2014a, 2014b). KCC2 also has an ion-independent role on synapse formation, through it's binding to protein 4.1N to the cytoskeleton at synapses (Li et al., 2007).

Synaptic inhibition also rests on the shunting effects that occur in the neuronal membrane of the postsynaptic cell (Fig. 1C); this mechanism, which mainly depends on the duration of the increase in membrane conductance to Cl^-, can effectively reduce both ligand- and voltage-gated excitatory currents. It is also well established that $GABA_A$ receptor activation causes an efflux of HCO_3^- from the neuron (Gulledge and Stuart, 2003; Kaila et al., 2014b); this depolarizing current (with an equilibrium potential around −10 mV) leads to accumulation of Cl^- inside the neuron, and to a positive shift in $GABA_A$ equilibrium potential (Rivera et al., 2005). HCO_3^- is generated by the catalytic activity of carbonic anhydrase that converts $CO_2 + H_20$ to HCO_3^- and H^+. The rate-limiting factor for HCO_3^- generation is thus set by the extrusion of acid equivalents (e.g. H^+) from the cell. For a review of the role played by $GABA_A$-dependent HCO_3^- currents, see Kaila (1994).

KCC2 is expressed exclusively in neurons, and is found both in cortical principal (glutamatergic) cells and in multiple inhibitory interneuron subtypes, including cerebellar granule cells, thalamic relay cells as well as neurons in the auditory brain stem, olfactory bulb, and spinal cord (Kanaka et al., 2001; Gulayas et al., 2001; Barthó et al., 2004; Szabadics et al., 2006; Blaesse et al., 2006). Interestingly, dopaminergic cells of the substantia nigra and most of the neurons in the thalamic reticular nucleus lack KCC2 (Kanaka et al., 2001; Gulácsi et al., 2003; Barthó et al., 2004; Blaesse et al., 2009; Benarroch, 2013). Moreover, there are two KCC2 splice variants termed KCC2a and KCC2b; while KCC2a expression is only slightly upregulated through life, KCC2b levels increase strongly during development and represent the most abundant isoform in the adult mouse brain (Uvarov et al., 2007, 2009). Regarding human tissue, Sedmak et al. (2016) have found that KCC2 protein is already strongly expressed at birth, consistent with evidence showing that the human brain is more mature than the rodent one at birth. On the other hand, NKCC1 expression is widely distributed throughout several organs and, in the nervous system, it is found in the somato-dendritic and axonal compartments of immature and mature neurons as well as in glial and brain endothelial cells (Plotkin et al., 1997; Benarroch, 2013). Regarding the timing of NKCC1 expression, studies performed in both rodent and human cortex have reported contrasting results. Initially, it was shown that this transporter undergoes downregulation during development (Plotkin et al., 1997; Dzala et al., 2005). However, other studies have reported either no change or even a developmental upregulation of NKCC1 mRNA (Clayton et al., 1998; Hyde et al., 2011). This discrepancy is most likely due to the differential

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