Neuroscience and Biobehavioral Reviews 90 (2018) 260–271

Contents lists available at ScienceDirect

Neuroscience and Biobehavioral Reviews

journal homepage: www.elsevier.com/locate/neubiorev

Chloride transporters and GABA polarity in developmental, neurological and psychiatric conditions

Joran T. Schulte[a], Corette J. Wierenga[b,1], Hilgo Bruining[a,*,1]

[a] Department of Psychiatry, Brain Center Rudolf Magnus, University Medical Center, Heidelberglaan 100, 3508 GA Utrecht The Netherlands
[b] Division of Biology, Faculty of Science, Utrecht University, Padualaan 8, 3584 CH, Utrecht, The Netherlands

ARTICLE INFO

Keywords:
Chloride
Chloride regulation
GABA
GABAergic inhibition
GABA polarity
Chloride cotransporters
KCC2
NKCC1
Developmental disorders
Epilepsy
Stroke
Autism
E/I balance

ABSTRACT

Neuronal chloride regulation is a determinant factor for the dynamic tuning of GABAergic inhibition during and beyond brain development. This regulation is mainly dependent on the two co-transporters K^+/Cl^- co-transporter KCC2 and $Na^+/K^+/Cl^-$ co-transporter NKCC1, whose activity can decrease or increase neuronal chloride concentrations respectively. Altered expression and/or activity of either of these co-transporters has been associated with a wide variety of brain disorders including developmental disorders, epilepsy, schizophrenia and stroke. Here, we review current knowledge on chloride transporter expression and activity regulation and highlight the intriguing potential for existing and future interventions to support chloride homeostasis across a wide range of mental disorders and neurological conditions.

1. Introduction

The principle component in shaping neural activity is the neuronal synapse, where electric signals from the presynaptic neuron are transmitted to the postsynaptic cell via specialized chemical signaling. At the synapse, activation of a presynaptic neuron causes the release of neurotransmitters into the synaptic cleft, which can subsequently activate target receptors on the postsynaptic membrane. Many postsynaptic receptors are ion channels whose activation alters permeability for specific ions resulting in a positive or negative change on the membrane potential of the postsynaptic neuron. For instance, glutamate receptors at excitatory synapses are permeable for sodium (Na^+) and potassium (K^+) ions inducing postsynaptic depolarization (towards the firing threshold). At inhibitory synapses, the neurotransmitter, γ-aminobutyric acid (GABA) activates chloride-permeable $GABA_A$ receptors. The direction of the resulting chloride (Cl^-) ion flow will depend on the intracellular chloride concentration ($[Cl^-]_i$) of the postsynaptic neuron. As we will review, the regulation of this intraneuronal chloride concentration through cation-chloride cotransporters is indeed essential for neural activity homeostasis in the brain. In the mature brain, $[Cl^-]_i$ is generally low and postsynaptic GABAergic signaling leads to chloride influx causing a postsynaptic hyperpolarizing effect (away from firing threshold). However, in certain pathophysiological conditions fine-tuning of $[Cl^-]_i$ can become dysregulated and affect GABAergic inhibition (Ben-Ari, 2017). An increasing body of evidence shows that altered chloride regulation is a mechanistic factor in a wide variety of neurological and psychiatric conditions (Doyon et al., 2016). It should be noted that, besides regulating chloride levels, neuronal cation-chloride cotransporters also regulate cell volume in the central nervous system (Glykys et al., 2017; Kahle et al., 2015) and spine morphology (Fiumelli et al., 2013; Gauvain et al., 2011; Li et al., 2007), which will not be discussed here.

In this review, we introduce the main chloride transporters in relation to GABA signaling and discuss their regulatory dynamics in general and in particular contexts such as in development. Following these accounts, we will review which of these mechanisms are known to be dysregulated in developmental, neurological, and psychiatric conditions. Finally, we discuss how chloride transporter dysregulations can serve as pharmacotherapeutic targets based upon existing or yet to be discovered agents.

2. Chloride concentration homeostasis in neuronal cells

In mature neurons, baseline levels of $[Cl^-]_i$ are maintained at a relatively low concentration (~5 mM), while the extracellular chloride level is typically

* Corresponding author.
E-mail address: h.bruining@umcutrecht.nl (H. Bruining).
[1] Equal contribution

https://doi.org/10.1016/j.neubiorev.2018.05.001
Received 9 January 2018; Received in revised form 20 March 2018; Accepted 1 May 2018
Available online 02 May 2018

around ~110 mM. The resulting chloride equilibrium potential is more negative than the membrane potential so that chloride currents will have a hyperpolarizing (inhibitory) effect on the membrane potential. The maintenance of the $[Cl^-]_i$ is performed by cation-chloride cotransporter proteins in the neuronal membrane.

The main chloride 'exporter' is the K^+/Cl^- co-transporter KCC2, which can extrude chloride from the neuron against its concentration gradient (Hübner et al., 2001; Rivera et al., 1999). The ATP-dependent K^+/Na^+-ATPase maintains the high intracellular K^+ concentration required for this shuttling process (Payne et al., 2003). KCC2 therefore behaves like a chloride pump, creating the driving force for chloride entry and postsynaptic membrane hyperpolarization upon GABA release (Blaesse et al., 2009; Chamma et al., 2012; Payne et al., 2003).

In opposite direction, the $Na^+/K^+/Cl^-$ co-transporter NKCC1 is regarded as the most active chloride 'importer'. This transporter can shuttle Cl^-, K^+ and Na^+ into the neuron, using the electrochemical gradient of Na^+. Together, KCC2 and NKCC1 are the two main transporters responsible for regulating $[Cl^-]_i$ and their relative activity controls the intraneuronal chloride level, which in turn determines the postsynaptic effect of GABAergic transmission. Under normal conditions, $[Cl^-]_i$ is sufficiently low, so that $GABA_A$ receptor activation leads to an increase in membrane conductance and a chloride flow into the neuron, causing relative hyperpolarization of the postsynaptic membrane potential. This decreases the probability that the postsynaptic neuron fires an action potential and is therefore referred to as the inhibitory effect of GABA signaling.

Repeated GABA signaling induces repeated chloride influx, which imposes an increasing chloride load onto the neuron (Doyon et al., 2016). Chloride entry in the neuron can therefore be enhanced if the postsynaptic cells are depolarized during sustained excitatory activity. With increasing levels of intracellular chloride, the electrochemical gradient becomes more reduced, which in turn decreases the inhibitory effect of GABA signaling on the postsynaptic neuron. As the reversal potential for GABA and the resting membrane potential are in relatively close proximity, relatively small increases in $[Cl^-]_i$ can shift the polarity of $GABA_A$ currents from hyperpolarizing to depolarizing, emphasizing the importance of maintaining a low $[Cl^-]_i$ (Raimondo et al., 2017). To recover from high chloride load and to maintain hyperpolarizing GABA effects, chloride ions are continuously extruded from the neuron by KCC2. This chloride clearance rate through KCC2 activity thus determines the recovery rate of the $[Cl^-]_i$ of a neuron after a period of intense GABA signaling. Small reductions in KCC2 activity already result in prolonged elevation of $[Cl^-]_i$ after GABAergic signaling and compromise the inhibitory capacity of GABAergic synapses. Under normal circumstances, however, KCC2-mediated chloride extrusion provides an efficient pathway in developed neurons for chloride recovery.

In striking contrast to mature neurons, $[Cl^-]_i$ levels are kept high in developing neurons, which results in an electrochemical gradient of chloride in opposite direction. In immature neurons, activation of $GABA_A$ receptors leatds a outward flow of chloride ions, causing a depolarization effect in the postsynaptic neuron (Ben-Ari et al., 1989; Sulis Sato et al., 2017). The depolarizing effect of GABAergic signaling in immature neurons is thought to be trophic/growth promoting and essential for the early establishment of neuronal circuits (Ben-Ari et al., 2007; Wang and Kriegstein, 2010). Although the effect of $GABA_A$ receptor activation in immature neurons is predominantly depolarizing, its effect on the neuronal network is not necessarily excitatory. A recent study in the neonatal neocortex demonstrated that GABA-mediated depolarization still imposes an overall inhibitory control on cortical network activity in vivo (Kirmse et al., 2015), presumably via shunting inhibition (Heigele et al., 2016).

The difference in chloride regulation between immature and mature neurons is predominantly caused by a difference in expression and activity of the main chloride transporters NKCC1 and KCC2. Levels of NKCC1 are high in early developmental phases, while the expression of KCC2 is kept low (Ben-ari, 2002). During neuronal maturation, KCC2 expression is upregulated and NKCC1 levels are reduced, resulting in lower levels of neuronal chloride (Yamada et al., 2004). This shift in chloride level occurs during the second postnatal weeks in rodents (Ben-Ari et al., 2012). In rat hippocampi, an increased KCC2 mRNA expression is observed after postnatal week 2 (Dzhala et al., 2005; Rivera et al., 1999), whereas a decrease of NKCC1 expression is observed between P14 and P21 (Dzhala et al., 2005). In comparison, in humans, KCC2 expression increases around postconceptional week (PCW) 40, whereas NKCC1 expression reaches adult levels around PCW 50 (Dzhala et al., 2005). These findings indicate big differences of expression patterns across species, which should be kept in mind when comparing studies using different models. The characteristic upregulation of KCC2 and downregulation of NKCC1 during neurodevelopment is often referred to as the excitatory-inhibitory GABA sequence. KCC2 upregulation is crucial for functional brain development to endow mature neurons with an ability to rapidly restore $[Cl^-]_i$ after loading. The onset of developmental upregulation of KCC2 has been linked to increased postsynaptic calcium signals, as a result of activation of $GABA_A$ receptors in immature neurons (Fiumelli et al., 2005; Ganguly et al., 2001). Disturbances in the excitatory-inhibitory GABA sequence have been implicated in developmental disorders, such as childhood epilepsy and autism spectrum disorder (ASD) (Ben-Ari et al., 2012; Merner et al., 2015) but the mechanistic underpinnings of the polarity shift in GABAergic signaling during development are not fully understood.

A loss of excitatory actions of GABA in NKCC1 knockout mice has been shown to induce a compensatory increase in the intrinsic excitability of glutamatergic neurons (Sipilä et al., 2009) and delayed maturation of the glutamatergic and GABAergic synapses (Pfeffer et al., 2009). The importance of KCC2 for neuronal chloride homeostasis and GABA function was demonstrated by genetic knockout of KCC2 in animal models (Hekmat-Scafe et al., 2006; Hübner et al., 2001; Khalilov et al., 2011; Rinehart et al., 2009; Tanis et al., 2009; Zhu et al., 2008, 2005) leading to elevated neuronal $[Cl^-]_i$, depolarization of the equilibrium potential of GABA (EGABA) and reduced inhibition. Indeed, KCC2-deficient mice exhibited frequent generalized seizures and died shortly after birth (Hübner et al., 2001; Woo et al., 2002), whereas mice that were heterozygous for KCC2 deletion displayed altered seizure threshold and increased seizure susceptibility to seizure-inducing agents (Zhu et al., 2008). The effects of KCC2 or NKCC1 deficiency are reminiscent of electrophysiological phenotypes seen in multiple models of neurological and psychiatric disease, including autism (He et al., 2014; Tyzio et al., 2014), Down's syndrome (Deidda et al., 2015), schizophrenia (Hyde et al., 2011), epilepsy (Kahle et al., 2015; Silayeva et al., 2015; Woo et al., 2002), and neuropathic pain (Chen et al., 2014; Coull et al., 2003; Cramer et al., 2008; Kahle et al., 2014a). These studies underpin the importance of NKCC1 and KCC2 regulation for the homeostasis of neuronal $[Cl^-]_i$ and appropriate function of GABA signaling. As we will outline below, chloride homeostasis implies the existence of sensing and responding mechanisms to enable a dynamic and rapid equilibrium maintenance.

3. Mechanisms of chloride regulation

3.1. Regulation of KCC2 expression

KCC2 is encoded by the solute carrier family 12 member 5 (SLC12A5) gene. There are two major isoforms of KCC2. In neonatal mouse central nervous system, KCC2a and KCC2b are present in similar (low) levels, whereas in the adult brain KCC2b is the major isoform (Uvarov et al., 2009). The isoforms are differently regulated by alternative promoter and first exon usage (Uvarov et al., 2007). KCC2 is exclusively expressed in neuronal cells, a phenomenon that is mainly ascribed to the presence of a neuron-restrictive silencer element (NRSE) in the first intron of the gene (Karadsheh and Delpire, 2001; Schoenherr and Anderson, 1995). However, a 1.4 kb promoter fragment is also involved in the neuron-restricted expression of KCC2 (Uvarov et al., 2005), as KCC2 expression was still restricted to neurons in a transgene model lacking the NRSE for KCC2 but with the 1.4 kb promoter fragment active. One of the transcription factors that can bind to the 1.4 kb promotor region of *SLC12A5* is early growth response 4 (Egr4), which can regulate the expression of KCC2. This was shown by Uvarov et al. (2006), who demonstrated that co-transfection of KCC2 and Egr4 in N2a cells resulted in enhanced expression of KCC2. A second important binding site in this promoter region is the E-box region, which can bind the upstream stimulating factors