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# Commentary: Chloride Regulation: A Dynamic Equilibrium Crucial for Synaptic Inhibition

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## A commentary on

### Chloride Regulation: A Dynamic Equilibrium Crucial for Synaptic Inhibition

by Doyon, N., Vinay, L., Prescott, S. A., and De Koninck, Y. (2016). *Neuron* 89, 1157–1172.

The recent review by Doyon et al. (2016) is for the main part an excellent description of many important aspects of neuronal chloride regulation and will be of good use to many scientists interested in synaptic function. Nevertheless, some information given on the role of the  $\text{K}^+ \text{Cl}^-$  cotransporter 2 (KCC2) is likely to be misleading. While proposing an explanation for the controversial findings by Glykys et al. (2014) that blockers of cation-chloride cotransporters did not affect the basal intracellular  $\text{Cl}^-$  concentration ( $[\text{Cl}^-]_i$ ), contrary to expectations from previous transporter manipulations (see e.g., references in Ben-Ari, 2014; Kaila et al., 2014), Doyon et al. (2016) suggest that this may be due to the small degree of inhibitory synaptic activity in the preparation used, with extremely low  $\text{Cl}^-$  load. In their explanation, they give an equation for the equilibrium relation between  $\text{Cl}^-$  flux through an inhibitory ( $\text{Cl}^-$ ) conductance ( $g_{\text{inh}}$ ) and the  $\text{Cl}^-$  transported by KCC2. On basis of this equation, the conclusion is that the  $\text{Cl}^-$  equilibrium potential “ $E_{\text{Cl}}$  is sensitive to changes in KCC2 activity ( $g_{\text{KCC2}}$ ) only when  $\text{Cl}^-$  load ( $g_{\text{inh}}$ ) is large.” This conclusion, however, cannot be justified on basis of the relation between  $\text{Cl}^-$  flux through channels and  $\text{Cl}^-$  transported by KCC2. (The equation given by Doyon et al. is not correctly formulated, although the reason for their claim may not depend on this mistake).

For an explanation of our point of view, consider a hypothetical cell with  $\text{Cl}^-$  transport across the outer membrane only via  $\text{Cl}^-$  selective channels and KCC2. At equilibrium, the amount (mol/s) of  $\text{Cl}^-$  transported by the channels must be equal, but opposite, to that transported by KCC2. We thus formulate the relation:

$$I_{\text{Cl}}/F = g_{\text{KCC2}}U_{\text{KCC2}} \quad (1)$$

where  $I_{\text{Cl}}$  is  $\text{Cl}^-$  current,  $F$  is the Faraday constant and  $U_{\text{KCC2}}$  is the driving force for transport by KCC2.  $g_{\text{KCC2}}$  is a proportionality factor that may be thought of as an “apparent conductance,” and should reflect the number of transporters in the membrane as well as the transport rate of the individual transporter molecules at fixed  $\text{K}^+$  and  $\text{Cl}^-$  concentrations, similarly as  $I_{\text{Cl}}$  depends on the  $\text{Cl}^-$  conductance ( $g_{\text{Cl}}$ ) which reflects the number of  $\text{Cl}^-$  channels as well as the conductance of individual channels. ( $g_{\text{KCC2}}$  is, however, not a conductance in the usual electrical sense). Equation (1) may be reformulated, in several steps, for clarity:

$$g_{\text{Cl}}(V_m - E_{\text{Cl}})/F = g_{\text{KCC2}}(RT \ln([\text{Cl}^-]_i/[\text{Cl}^-]_o) + RT \ln([\text{K}^+]_i/[\text{K}^+]_o)) \quad (2)$$

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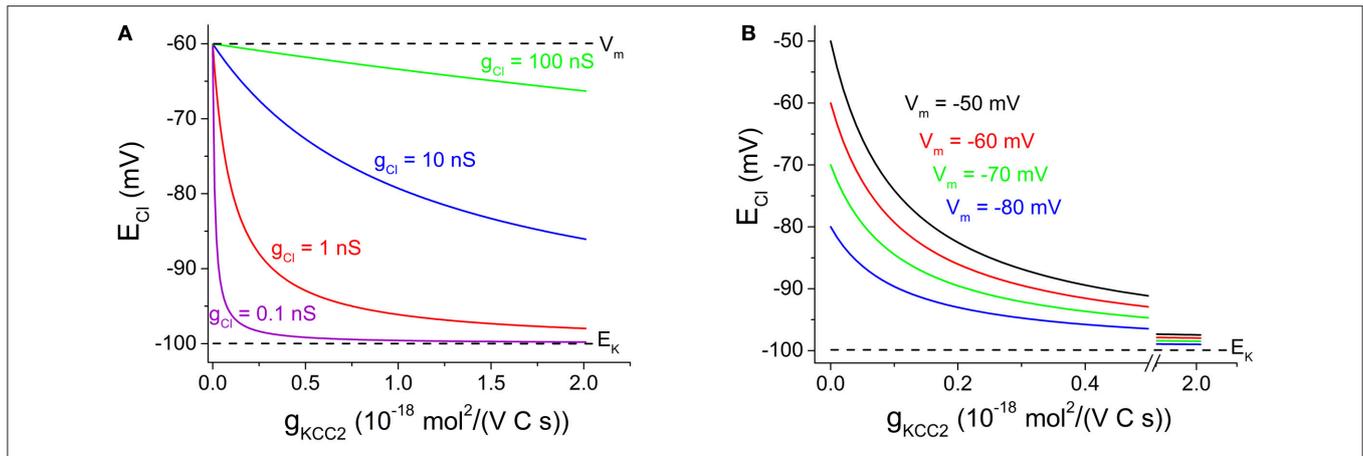
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**FIGURE 1 | Cl<sup>-</sup> equilibrium potential – dependence on KCC2 transporter capacity at various Cl<sup>-</sup> conductance and various membrane potentials. (A)** E<sub>Cl</sub> vs. g<sub>KCC2</sub> with E<sub>K</sub> fixed at -100 mV and V<sub>m</sub> at -60 mV. g<sub>Cl</sub> as indicated. Note that E<sub>Cl</sub> dependence on g<sub>KCC2</sub> is reduced with increased g<sub>Cl</sub>. **(B)** E<sub>Cl</sub> vs. g<sub>KCC2</sub> with E<sub>K</sub> fixed at -100 mV and g<sub>Cl</sub> at 1 nS. V<sub>m</sub> as indicated. Note that E<sub>Cl</sub> dependence on g<sub>KCC2</sub> increases when V<sub>m</sub> changes in positive direction. Note the x-axis break between 0.5 and 1.9 10<sup>-18</sup> mol<sup>2</sup>/(V C s), to clearer illustrate the steeply decaying region of the curves. Justification of illustrated parameter ranges: The g<sub>Cl</sub> range (in **A**) was chosen to include cells with a low g<sub>Cl</sub> as evident from the high membrane resistance (Johansson et al., 1995) as well as cells with a high g<sub>Cl</sub> (very low input resistance dominated by inhibitory conductances; Destexhe et al., 2003). The g<sub>KCC2</sub> range (in **A,B**) shown likely covers the capacity for most central neurons: When g<sub>KCC2</sub> = 1 10<sup>-18</sup> mol<sup>2</sup>/(V C s), KCC2-mediated transport modeled as described by Karlsson et al. (2011) may reduce [Cl<sup>-</sup>]<sub>i</sub> from 20 mM to ~5 mM with approximated time constants of 0.85, 6.8, and 55 s for spherical cells of radius 5, 10, and 20 μm, respectively, assuming 50% cytosolic volume and no other Cl<sup>-</sup> transport/leak. Experimentally observed [Cl<sup>-</sup>]<sub>i</sub> recovery is slower or comparable (Berglund et al., 2006; Lee et al., 2011; Pellegrino et al., 2011).

$$g_{Cl} (V_m - E_{Cl})/F = g_{KCC2} F (E_{Cl} - E_K) \tag{3}$$

$$E_{Cl} = (g_{KCC2} F^2 E_K + g_{Cl} V_m) / (g_{KCC2} F^2 + g_{Cl}) \tag{4}$$

where V<sub>m</sub> is membrane potential, R the gas constant, T temperature (in K), E<sub>K</sub> the K<sup>+</sup> equilibrium potential and Cl<sup>-</sup> and K<sup>+</sup> concentrations are given within brackets with subscripts i and o for inside and outside, respectively.

We may use Equation (4) to illustrate the relation between E<sub>Cl</sub> and g<sub>KCC2</sub> at various levels of g<sub>Cl</sub>. (Assume that K<sup>+</sup> concentrations and membrane potential are fixed, as controlled by other factors, such as the cellular Na<sup>+</sup>-K<sup>+</sup>-ATPase, not discussed here). As can be seen in **Figure 1A**, contrary to the claim by Doyon et al. (2016), E<sub>Cl</sub> is only weakly dependent on KCC2 transport capacity at high Cl<sup>-</sup> conductance, while it depends strongly on KCC2 when Cl<sup>-</sup> conductance is low. At the extremes, when g<sub>KCC2</sub> = 0, then E<sub>Cl</sub> = V<sub>m</sub> and when g<sub>Cl</sub> = 0, then E<sub>Cl</sub> = E<sub>K</sub>.

The relation between E<sub>Cl</sub> (and thus [Cl<sup>-</sup>]<sub>i</sub>) and transporter capacity has some bearing for the interpretation of the controversial findings by Glykys et al. (2014). Contrary to the suggestion by Doyon et al. (2016), **Figure 1A** shows that transporter block is expected to affect basal [Cl<sup>-</sup>]<sub>i</sub> especially under conditions when g<sub>Cl</sub> is low. Thus, other explanations than a low g<sub>Cl</sub> must be sought for the controversial findings of Glykys et al. (2014). An increased g<sub>Cl</sub>, perhaps due to increased non-specific leak, could in theory contribute to the limited effect of KCC2 blocker on [Cl<sup>-</sup>]<sub>i</sub>.

On the other hand, the lack of excitatory synaptic input may contribute to a reduced sensitivity to transport block. In equation (4) above, a steady excitatory input may be represented simply by a more positive V<sub>m</sub>, if we assume that E<sub>K</sub> is still maintained

(by the cellular Na<sup>+</sup>-K<sup>+</sup>-ATPase). **Figure 1B** shows that although E<sub>Cl</sub> is more positive, the dependence of E<sub>Cl</sub> on g<sub>KCC2</sub> is clearly stronger at more positive V<sub>m</sub>.

As described, an increased g<sub>Cl</sub> (**Figure 1A**) or a more positive V<sub>m</sub> (**Figure 1B**) will change E<sub>Cl</sub> in positive direction. This may be exploited experimentally e.g., by combining GABA or glycine application with depolarization to achieve a dramatic change in E<sub>Cl</sub> and rise in [Cl<sup>-</sup>]<sub>i</sub> (Karlsson et al., 2011). With such manipulations, it is obvious that the neuronal transporter capacity cannot prevent the changes in E<sub>Cl</sub> and in steady-state [Cl<sup>-</sup>]<sub>i</sub>.

Doyon et al. (2016) also note the neglected problem of apparent (illusory) conductance decrease based on recordings at different holding potentials when [Cl<sup>-</sup>]<sub>i</sub> is changing, a problem which has recently been described in more detail by Yelhekar et al. (2016). It may be noted that experimentally, the effects of a changing [Cl<sup>-</sup>]<sub>i</sub> on apparent conductance may be separated from true changes in conductance by using rapid voltage-ramp techniques (Karlsson et al., 2011; Yelhekar et al., 2016).

## AUTHOR CONTRIBUTIONS

SJ made the computations and paper writing. SJ, TY, and MD contributed to the ideas and final content.

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**Conflict of Interest Statement:** The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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