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# Getting Excited About Chloride Cotransporters: Neuroinflammation and Inhibition

**Acute Neuroinflammation Leads to Disruption of Neuronal Chloride Regulation and Consequent Hyperexcitability in the Dentate Gyrus**

Kurki SN, Srinivasan R, Laine J, Virtanen MA, Ala-Kurikka T, Voipio J, Kaila K. *Cell Rep*. 2023;42(11):113379. doi:10.1016/j.celrep.2023.113379

Neuroinflammation is a salient part of diverse neurological and psychiatric pathologies that associate with neuronal hyperexcitability, but the underlying molecular and cellular mechanisms remain to be identified. Here, we show that peripheral injection of lipopolysaccharide (LPS) renders the dentate gyrus (DG) hyperexcitable to perforant pathway stimulation in vivo and increases the internal spiking propensity of dentate granule cells (DGCs) in vitro 24 h post-injection (hpi). In parallel, LPS leads to a prominent downregulation of chloride extrusion via KCC2 and to the emergence of NKCC1-mediated chloride uptake in DGCs under experimental conditions optimized to detect specific changes in transporter efficacy. These data show that acute neuroinflammation leads to disruption of neuronal chloride regulation, which unequivocally results in a loss of GABAergic inhibition in the DGCs, collapsing the gating function of the DG. The present work provides a mechanistic explanation for neuroinflammation-driven hyperexcitability and consequent cognitive disturbance.

## Commentary

Although the past 20 years have yielded considerable information about the initiation and consequences of neuroinflammation, the cellular and molecular mechanisms that lead to a progression of existing pathology are not clearly established. Neuroinflammation is a crucial early-stage mechanism of synaptic and cognitive impairment in numerous acute and chronic neuropathological conditions. If not resolved, the early impact of neuroinflammation can exacerbate neurological damage and prolong functional impairment. For example, inflammatory factors or neuroinflammatory processes in the brain can enhance seizure susceptibility, which can contribute to long-term progression of epilepsy and aggravation of seizures. Previous evidence indicates that acute neuroinflammation can reduce sharp-wave ripples in the CA1 area of the hippocampus but enhance slow-wave activity in the dentate gyrus (DG)[1] which may contribute to cognitive impairment and hippocampal hyperexcitability. Although there is vast evidence demonstrating the DG's critical role in seizure circuitry, whether and how (ie, by which mechanisms) neuroinflammatory processes compromise DG functions to promote hyperexcitability are still unknown.

To better understand the molecular and cellular mechanisms of DG hyperexcitability that are sensitive to inflammatory processes, Kurki et al, used the well-established peripheral lipopolysaccharide (LPS) injection model to induce acute neuroinflammation. They first, replicated the inflammation-induced neuronal hyperexcitability phenotype by evaluating the intrinsic excitability (as seen with decreases in rheobase and increases in the number of action potentials) of DG cells and stimulation-evoked excitability (as evidenced by enhancement in population spike amplitude) in the DG area of mouse acute brain slices prepared 24 and 48 hours post-peripheral LPS injection. Having established the model, they subsequently examined brain chloride regulation and transporter functions.[2] Neuronal chloride regulatory mechanisms, such as the $Na^+$-$K^+$-$Cl^-$ (NKCC1) and the $K^+$-$Cl^-$ (KCC2) cotransporters, are essential for the recovery of chloride homeostasis following GABA receptor–mediated chloride flux and can modulate GABAergic neurotransmission and contribute to seizures and epilepsy. The authors, therefore, evaluated KCC2 and NKCC1 efficacy following LPS, and found that LPS causes a reduction in chloride extrusion (following decreases in KCC2 activity and/or mRNA expression levels) and enhancement of chloride influx (by increasing NKCC1 conductance), leading to potential increases in intracellular chloride and depolarized $E_{GABA}$. This will cause decreases in the efficacy of inhibition resulting in increases in the excitability of neurons thereby contributing to hyperexcitability.

Although it is tempting to accept that a single administration of LPS has such a robust influence on GABAergic signaling and excitability, the most obvious question is whether these results are recapitulated following repeated administration of LPS or other chronic neuroinflammatory insult. Of note, while this study provides initial steps forward in our understanding of how an inflammatory environment can impact basic mechanisms of neuronal inhibition *acutely*, the direct link between neuroinflammation and the ensuing reduction in GABAergic inhibition (and hyperexcitability) remains a significant gap in our knowledge.

It is important to note that, while the authors attribute deficits in KCC2 activity to LPS-induced decreases in mRNA levels, protein levels of KCC2 were not assessed. Contrary to KCC2, LPS did not influence NKCC1 transcription levels. The lack of effect on NKCC1 was acknowledged by the authors who suggest that NKCC1 may be subject to rapid changes in protein expression following LPS. Having said that the author's speculations are based on prior work that examined expression pattern changes during *development*, a time when NKCC1 protein levels are detectable in naïve animals. There is the possibility that additional mechanisms (and cell types) could also play a role. For example, LPS-induced increases in neuronal firing (due to increases in intrinsic excitability as noted in this study) and/or deficits in astrocyte reuptake mechanisms can increase extracellular potassium ($K^+$). Such changes could enhance the driving force for $Na^+$, $K^+$, $2Cl^-$ influx through NKCC1. Other possibilities could include altered phosphorylation of NKCC1 transporters and/or protein trafficking, both of which could influence NKCC1 activity. Furthermore, it is unclear what upstream mediator(s) and/or cellular source(s) are responsible for the downregulation of KCC2. One explanation could be the release of brain-derived neurotrophic factor (BDNF) by activated microglia (via LPS-evoked cytokine release), which can activate neuronal tyrosine receptor kinase B causing downregulation of KCC2.[3] Indeed, it was demonstrated that inhibition of BDNF following experimental viral infection prevented the loss of KCC2.[4]

Another finding from Kurki et al was that neuroinflammation induced increases in intrinsic excitability of DG cells—a result that may be linked to network hyperexcitability. While the authors suggest that changes in KCC2 could alter trafficking of Task-3 leak potassium channels to mediate increases in DG cell's intrinsic excitability, such changes should correspond to alterations in leak $K^+$ conductance and thus changes in input resistance, which was not observed. However, Task-3 $K^+$ channel mechanisms cannot be excluded based solely on this measure as other compensatory mechanisms may also be present. Similarly, other mechanisms, such as feedback excitatory currents within the circuit[5] (that can be activated by input currents, 500 ms in duration; used in this study) could contribute to the observed increases in action potential firing in DG cells, and therefore should not be discounted.

In addition to chloride cotransporters, some signaling molecules secreted by microglia under inflammatory circumstances (eg, matrix metalloproteinases [MMPs])[6] have the potential to influence chloride gradients and thereby serve as another possible mechanism for altered chloride flux. For example, a bacterial analog of MMP, Chondroitinase ABC, can release extracellular impermeant anions (sulfated glycosaminoglycans; $SO_4^{-2}$)—potentially causing chloride entry from the intravascular compartment (to maintain charge equilibrium) and increasing local extracellular chloride.[7] This results in increases in intracellular chloride in order to maintain the equilibrium dynamics of cation chloride cotransporters.[7] Thus, the secretion of MMPs in response to inflammation may also affect chloride homeostasis after LPS.

Several anti-inflammatory therapies (eg, non-steroidal anti-inflammatory drugs, cyclooxygenase inhibitors, glucocorticoids) have been successful in preclinical and clinical studies focusing on epilepsy. Further, multiple mediators (eg, IL-1β, IL-6, TNFα, NLRP3 inflammasome) of the inflammatory process have been proposed as potential targets in multiple neurological disease models. One tempting hypothesis is that upregulation of neuro-inflammatory processes after seizures modulate chloride currents and/or homeostatic mechanisms and thus GABAergic inhibition. Such changes in inhibition could further exaggerate seizures and/or enhance the susceptibility to the development of epilepsy. Based on the findings of this paper, prevention of NKCC1/KCC2-mediated changes in chloride conductance would diminish the loss of GABAergic inhibition, although an alternative explanation is that protective effects of anti-inflammatory cytokines contribute to the sparing of GABAergic inhibition in the face of neuroinflammatory insults.[8] Like other potential mechanisms, these hypotheses need to be evaluated more firmly in order to understand the causative relationship of inhibition (post-synaptic $Cl^-$ regulatory and/or presynaptic interneuronal GABA release mechanisms[9]) in neuroinflammation and hyperexcitability/seizures. Curiously, BDNF and its inhibition (as discussed earlier) may be an important mechanism of reducing the impact of inflammation on chloride cotransporters. Therefore, drugs that improve KCC2 function and/or reduce NKCC1 may still hold promise as novel therapies for epilepsy.

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