



## CLC-3 Takes a Quantum Leap Toward Understanding Mechanisms of GABA Release

### Presynaptic CLC-3 Determines Quantal Size of Inhibitory Transmission in the Hippocampus.

Riazanski V, Deriy LV, Shevchenko PD, Le B, Gomez EA, Nelson DJ. *Nature Neuroscience* 2011;14(4):487–494. Epub 2011 Mar 6.

The absence of the chloride channel CLC-3 in *Clcn3*<sup>-/-</sup> mice results in hippocampal degeneration with a distinct temporal-spatial sequence that resembles neuronal loss in temporal lobe epilepsy. We examined how the loss of CLC-3 might affect GABAergic synaptic transmission in the hippocampus. An electrophysiological study of synaptic function in hippocampal slices taken from *Clcn3*<sup>-/-</sup> mice before the onset of neurodegeneration revealed a substantial decrease in the amplitude and frequency of miniature inhibitory postsynaptic currents compared with those in wild-type slices. We found that CLC-3 colocalized with the vesicular GABA transporter VGAT in the CA1 region of the hippocampus. Acidification of inhibitory synaptic vesicles induced by Cl<sup>-</sup> showed a marked dependence on CLC-3 expression. The decrease in inhibitory transmission in *Clcn3*<sup>-/-</sup> mice suggests that the neurotransmitter loading of synaptic vesicles was reduced, which we attribute to defective vesicular acidification. Our observations extend the role of Cl<sup>-</sup> in inhibitory transmission from that of a postsynaptic permeant species to a presynaptic regulatory element. PMID: 21378974

### Commentary

Hippocampal sclerosis is a neuropathologic feature in more than half of the patient tissue resected because of intractable seizures in mesial temporal lobe epilepsy (MTLE). A similar phenotype was unexpectedly discovered in knockout mice deficient for the voltage-gated chloride ion channel called CLC-3. Many of the CLC-3 knockout mice (*Clcn3*<sup>-/-</sup>) developed spontaneous seizures that resembled MTLE. Strikingly, young *Clcn3*<sup>-/-</sup> mice developed hippocampal sclerosis and enlarged ventricles, and adults showed nearly complete bilateral destruction of the hippocampus (1). Moreover, the hippocampal neurons died sequentially, according to their locations within hippocampal circuits (2). First cells begin degenerating in the dentate gyrus; CA3 cell death follows, with those in CA1 dying last. Secondary to the loss of hippocampal neurons, pyramidal neurons in the entorhinal cortex also degenerate, suggesting that denervation may trigger some of the degeneration.

While replicating many of the characteristic features of hippocampal sclerosis in patients with severe MTLE, the phenotype of these mice is even more puzzling and complex. They also develop retinopathy, similar to human neuronal ceroid lipofuscinosis, or Batten disease, characterized by neurodegeneration, seizures, and blindness. In the intervening years, evidence accumulated showing that the CLC-3 family of voltage-gated chloride ion channels plays fundamental roles in regulating neuronal excitability in the hippocampus.

Various theories accounting for CLC-3's role in synaptic function are proposed to explain why loss of function triggers seizures and neurodegeneration. Identification of CLC-3 channels on endosomes and synaptic vesicles led to the hypothesis that the CLC-3 channels somehow regulate uptake or loading of neurotransmitter into synaptic vesicles. One speculation is that these chloride ion channels control acidification of synaptic vesicles by controlling pH changes in the lumen of synaptic vesicles. An alternate theory is that CLC-3 channels control chloride ion trafficking out of synaptic vesicles.

Now, a new study by Riazanski and colleagues elegantly tests these hypotheses in mice deficient for CLC-3 (*Clcn3*<sup>-/-</sup>) (3). Using immunohistochemistry and high-resolution confocal microscopy, the authors determined the synaptic locations of CLC-3 in hippocampal slices from wild-type mice (*Clcn3*<sup>+/+</sup>). Although they found CLC-3 in some glutamatergic synapses, it predominated in perisomatic synaptic endings on CA1 pyramidal neurons—typically sites of inhibitory synapses. At these sites, CLC-3 colocalizes with the vesicular GABA transporter (VGAT), confirming that these perisomatic sites are most likely inhibitory presynaptic terminals. Subsequent biochemical experiments by Riazanski and colleagues confirmed that CLC-3 is enriched in purified inhibitory synaptic vesicles from the hippocampus.

These findings suggest that in *Clcn3*<sup>-/-</sup> mice, loss of CLC-3 reduces inhibitory synaptic transmission in the pyramidal cell layer. To test this, Riazanski and colleagues isolated and compared miniature inhibitory postsynaptic currents (mIPSCs) on CA1 pyramidal neurons in hippocampal slices taken from *Clcn3*<sup>-/-</sup> versus *Clcn3*<sup>+/+</sup> mice. They found that both the frequency and amplitudes of mIPSCs are lower in the *Clcn3*<sup>-/-</sup> slices,



consistent with the view that CLC-3 expression in inhibitory synaptic vesicles dictates quantal release of GABA. Loss of CLC-3 apparently reduces GABA release at inhibitory synapses.

One way to explain these findings is that in *Clcn-3<sup>-/-</sup>* mice, the pH of inhibitory synaptic vesicles is altered. By experimentally buffering the pH inside inhibitory synaptic vesicles, the team led by Riazanski reduced the level of acidification in the vesicles and found this was sufficient to lower quantal release of GABA. They concluded that in these experiments, buffering lowered the amount of transmitter refilled into the synaptic vesicles during neurotransmitter reuptake. To further test their hypothesis, Riazanski and colleagues examined paired-pulse depression (PPD) in hippocampal slices. This form of short-term synaptic plasticity occurs on the presynaptic side of inhibitory synapses as a result of increased probability of synaptic vesicle release with repeated synaptic stimulation. As they predicted, PPD was significantly lower in knockout slices.

These results are consistent with the idea that CLC-3 channels help the process of filling or readying synaptic vesicles for release; CLC-3 deficiency seems to reduce the available pool of presynaptic vesicles that are refilled with GABA during synaptic firing. As the amount of neurotransmitter loaded into a vesicle is proportional to the proton electrochemical gradient formed by vesicular ATPase, measuring changes in the level of acidification of vesicles was another way to probe this theory. Riazanski and colleagues used acridine orange fluorescence to examine vesicle acidification and found that inhibitory synaptic vesicles from *Clcn-3<sup>-/-</sup>* mice are significantly less acidic than those from *Clcn-3<sup>+/+</sup>* mice.

Why might GABAergic synaptic vesicles need to be acidified in order to refill properly? In vitro studies had shown that VGAT in GABAergic synaptic terminals acts as a cotransporter or symporter for chloride ions (Cl<sup>-</sup>) with GABA. During transport, VGAT acts as a proton-GABA exchanger. High rates of exchange strongly depend on the size of the pH gradient in the vesicle lumen versus cytoplasm. Riazanski and colleagues' view is that CLC-3 helps GABAergic interneurons maintain rapid firing by ensuring optimal pH differences between vesicle lumen and cytoplasm, and the availability of protons for rapid GABA loading into vesicles.

An alternative theory is that CLC-3's role is to act as a chloride transporter, providing a pathway to clear the vesicle of high Cl<sup>-</sup> concentrations. During refilling, two Cl<sup>-</sup> ions accompany GABA as it enters the synaptic vesicle. During periods of high GABA reuptake, inhibitory vesicles would begin to acquire high concentrations of Cl<sup>-</sup> engulfed from the synaptic cleft. The equilibrium potential for Cl<sup>-</sup> would be expected to drive its movement out of the vesicle, eventually inhibiting GABA uptake. In this alternative view, CLC-3 might allow the VGAT transporter to work more efficiently at rapidly refilling

vesicles during recycling and help to maintain rapid firing at inhibitory synapses. Regardless of which theory is correct, this new study places CLC-3 and Cl<sup>-</sup> in a pivotal role at the presynaptic ending, where they appear to regulate inhibitory synaptic function by optimizing GABA loading into presynaptic vesicles.

The curious patterns of neurodegeneration in *Clcn-3<sup>-/-</sup>* mice can now be explained as well. One type of GABAergic interneuron called HIPP (hilar neuron with its axon distributed in the perforant path termination zone) is found in the hilus of the dentate gyrus. HIPP cells provide recurrent inhibitory feedback onto granule neurons, thereby regulating levels of excitation and rapidly undergo degeneration after status epilepticus in some rodent models of MTLE. In *Clcn-3<sup>-/-</sup>* mice, selectively eliminating this type of feedback inhibition might be the cause of limbic seizures and the demise of hilar interneurons. With prolonged seizures, principal neurons become excitotoxic, explaining the characteristic spatiotemporal patterns of widespread degeneration.

The findings also have broader implications for understanding how interneuron dysfunction may contribute to other forms of epilepsy or neuropsychiatric disorders. Cortical and hippocampal GABAergic interneurons exhibit considerable diversity. Determining whether CLC-3 expression is confined to particular functional subtypes could add to our understanding of their molecular and functional differences. The finding that CLC-3 is within perisomatic inhibitory endings on CA1 pyramidal neurons is consistent with expression in basket cells. As this interneuron type helps regulate rhythmic oscillations of ensembles of cortical and hippocampal neurons, defects in reduced quantal release of GABA at basket cell synapses might lead to an early phenotype characterized by loss of oscillations in the gamma frequency range. It may now become possible to selectively knock down CLC-3 in subclasses of interneurons to test their roles.

by Janice R. Naegele, PhD

## References

1. Stobrawa SM, Breiderhoff T, Takamori S, Engel D, Schweizer M, Zdebik AA, Bosl MR, Ruether K, Jahn H, Draguhn A, Jahn R, Jentsch TJ. Disruption of CLC-3, a chloride channel expressed on synaptic vesicles, leads to a loss of the hippocampus. *Neuron* 2001;29:185–196.
2. Dickerson LW, Bonthuis DJ, Schutte BC, Yang B, Barna TJ, Bailey MC, Nehrke K, Williamson RA, Lamb FS. Altered GABAergic function accompanies hippocampal degeneration in mice lacking CLC-3 voltage-gated chloride channels. *Brain Res* 2002;958:227–250.
3. Riazanski V, Deriy LV, Shevchenko PD, Le B, Gomez EA, Nelson DJ. Presynaptic CLC-3 determines quantal size of inhibitory transmission in the hippocampus. *Nat Neurosci* 2011;14:487–494.



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