Journal Club

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Cereblon Regulates BK Channel Expression at Presynaptic and Postsynaptic Sites in Excitatory Synapses

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Review of Choi et al.

Cereblon is a substrate receptor protein for the CRL4A E3 ubiquitin ligase complex. As such, cereblon binds to different proteins to promote their ubiquitination and degradation (Zheng and Shabek, 2017). Cereblon is highly expressed in brain tissues, including the hippocampus, cortex, cerebellum, and striatum (Higgins et al., 2010; Aizawa et al., 2011). Missense mutations of this protein and deletion or microduplication of the chromosomal segment harboring the *Cereblon* gene have been linked to human intellectual disability (Higgins et al., 2004; Dijkhuizen et al., 2006; Papuc et al., 2015).

To elucidate how cereblon mutations might lead to intellectual disability, Choi et al. (2018) studied behavior and synaptic function in mice lacking or expressing mutated forms of cereblon. Macroscopic brain architecture and dendritic spine morphology were similar in wild-type and

cereblon-null animals. Furthermore, no apparent differences were found in spontaneous synaptic transmission or long-term synaptic plasticity after cereblon deletion. Similar results were found in *Drosophila*. In contrast, lack of cereblon led to a reduction in the amplitude of evoked excitatory synaptic transmission (eEPSC). Furthermore, mice lacking cereblon performed abnormally in passive avoidance and novel object recognition tests. These results suggest that loss of cereblon impairs cognitive function by reducing presynaptic glutamate release probability.

The probability of glutamate release from presynaptic terminals was assessed by computing the paired-pulse ratio: the relative amplitude of postsynaptic responses elicited by two presynaptic spikes (second response/first response). This ratio was increased, suggesting release probability was reduced after cereblon knock-out in both species. This presynaptic impairment was also present in animals expressing mutant forms of cereblon that mimic mutations related to mild intellectual disability in humans, namely, the R419X cereblon mutant in mice and the G552X mutant in *Drosophila*. A similar decrease in glutamate release was suggested by experiments involving a short-term synaptic plasticity protocol consisting of 20-pulse train stimulation: the amplitude of successive eEPSCs in-

creased in cereblon-null mice, suggesting a presynaptic origin of the impairment.

As part of the CRL4A E3 ubiquitin ligase complex, cereblon regulates the trafficking and plasma membrane expression of the large-conductance Ca^{2+} -activated K^{+} channel (BK) α subunit (Jo et al., 2005; Liu et al., 2014). BK channels are thought to be key regulators of neurotransmitter release in many synapses (for review, see Grigoli et al., 2016). Therefore, Choi et al. (2018) studied whether the reduction in neurotransmitter release in cereblon-null animals resulted from modifications in BK function and/or channel expression levels. Total expression levels of BK channels in hippocampal CA1 pyramidal neurons were unaffected by cereblon knock-out, but surface expression and the amplitude of BK-mediated currents were increased. Furthermore, blocking BK currents restored glutamate release probability to wild-type levels and rescued abnormal cognitive behaviors in cereblon-null animals.

Choi et al. (2018) present clear evidence that loss of cereblon impairs cognitive function by increasing surface expression of BK channels and reducing presynaptic glutamate release probability in excitatory synapses. However, how these effects are linked remains unclear. Because BK channels are expressed at both presynaptic and postsynaptic synaptic terminals, as well as in the soma of multiple cellular types in the central nervous system (Latorre et

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al., 2017), mutations disrupting surface expression of BK channels at any of these locations might impair synaptic transmission.

At presynaptic active zones, BK channels coassemble with voltage-gated Ca^{2+} channels (VGCCs) (Grunnet and Kaufmann, 2004; Berkefeld et al., 2006). Opening of VGCCs produces the Ca^{2+} influx necessary for BK activation, which in turn repolarizes the presynaptic terminal, curtailing the opening of VGCCs and thus terminating Ca^{2+} influx and neurotransmitter release. Therefore, the increase in BK function occurring after cereblon deletion would be expected to reduce Ca^{2+} influx at the presynaptic terminal, thus reducing release probability. BK blockers would abolish this “brake” in VGCC-dependent Ca^{2+} influx, increasing glutamate release. However, this model is difficult to reconcile with previous results demonstrating that BK channels do not modulate transmitter release under basal conditions or during high-frequency stimulation in hippocampal CA3–CA1 synapses (Hu et al., 2001). The model is also inconsistent with results demonstrating that mutations disrupting the BK channel orthologue encoded by the *slowpoke* gene have little effect on glutamate release in *Drosophila* neuromuscular junction (Lee et al., 2008). An alternative model to explain the effects of cereblon deletion on glutamate release is that cereblon modulates BK channels at the soma of the presynaptic cell.

Somatic BK channels play a pivotal role in modulating action potential shape and frequency (Sailer et al., 2006), with inhibition of BK channels leading to either an increase or a decrease in action potential firing (Latorre et al., 2017). In hippocampal CA3 pyramidal neurons (the presynaptic cells in the mouse experiments by Choi et al., 2018), a decrease in BK channel activity is associated with a broadening of action potential duration and an increase in glutamate release, a situation that was reversed using a BK channel blocker (Deng et al., 2013). Conversely, the increase in BK channel function described by Choi et al. (2018) would be expected to shorten the action potential duration in CA3 pyramidal neurons, causing a decrease in glutamate release; the subsequent application of a BK channel blocker would then abolish this decrease, as described in the article.

Finally, some results in the work by Choi et al. (2018) cannot be explained without taking into account possible roles of postsynaptic BK channels. Indeed, the

authors measured BK current amplitude only in CA1 pyramidal neurons, the postsynaptic cells in their experiments. In postsynaptic cells, BK channels are often located in close proximity with postsynaptic proteins, such as PSD95 and NMDA receptors (NMDARs). Indeed, a functional coupling between NMDAR and BK channels has been described in olfactory bulb granule cells (Isaacson and Murphy, 2001). In this context, an increase in BK channel expression/function would reduce the influx through NMDAR at the postsynaptic terminals in a similar way to what has been described for VGCC–BK association: Ca^{2+} influx through NMDAR would be enough to produce BK activation, thus repolarizing the postsynaptic terminal and terminating NMDAR-dependent Na^+ and Ca^{2+} influx. Consistent with this mechanism, a decrease in the amplitude of the eEPSCs (due to both AMPA and NMDA receptors) was observed (Choi et al., 2018, their Fig. 2A), without any modification of the spontaneous EPSC (mainly due to AMPA receptors) (Choi et al., 2018, their Fig. 2B). This could also explain why eEPSCs were reduced in mice, whereas *Drosophila* excitatory junction currents were unaffected in cereblon-deficient animals, given that glutamatergic postsynaptic receptors at the *Drosophila* neuromuscular junction (DGluRIIA and DGluRIIB) are homologous to vertebrate AMPA and kainate receptors (Petersen et al., 1997; Davis et al., 1998). An inhibitory effect of BK on NMDAR could also be presumed from the current traces of EPSCs recorded from cereblon knock-out mice because the decay kinetics are slightly accelerated compared with wild-type (e.g., Choi et al., 2018, their Fig. 2C), as they would be if NMDAR-driven currents were inhibited.

In conclusion, the work by Choi et al. (2018) highlights the importance of BK channels on synaptic excitability, specifically related to their location. Undoubtedly, these results open a new and exciting avenue for research. In particular, future work should address the extent to which presynaptic and postsynaptic BK channels contribute to altered synaptic transmission and cognitive defects. More broadly, BK channels might be a promising pharmacological target in the search for new ways of treating intellectual disabilities.

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