Biophysical *Journal*
New and Notable

Biophysical Society

New Insights into the Mechanism of Ca^{2+}-Dependent Inactivation of NMDA Receptors

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Calcium-dependent inactivation (CDI) is an important mechanism that is common to a variety of ion channels, and is particularly important to the function of the N-methyl-D-aspartic acid (NMDA) receptor (1,2). The NMDA receptor is a class of glutamate receptor that is a mediator of synaptic plasticity and has been a target for neurological drug development. In particular, spike timing-dependent plasticity is thought to be influenced by CDI (3). CDI is mediated by the interaction of calmodulin with a sequence on the intracellular C-terminal domain (C0) of the NMDA receptor 1 (NR1) subunit of the NMDA receptor (4) and by the binding of calcium to calmodulin. Although the general outlines of the mechanism of CDI have been well known for over 20 years, the important details of the process have been lacking, in particular the time course of CDI after NMDA receptor activation. Reported in this issue of *Biophysical Journal*, Iacobucci and Popescu (5) have designed a series of clever experiments that shed new light on this important process.

The goals of their study were to define precisely the kinetics of CDI, to localize the calcium sensor (calmodulin) relative to the ion channel pore, and to determine if calmodulin binds to NR1 in the apo or holo form. The key to these measurements was a robust definition of CDI. This involved characterizing and excluding Ca^{2+}-independent sources of inhibition. The other major Ca^{2+}-dependent inhibition at the external DRPEER motif did not contribute to the results because the time course of inhibition at this site is rapid, present at time zero in the CDI measurements, and, thus, subtracted from the time course. In addition, a heterologous human embryonic kidney cell (HEK293) expression system for NR1-2a/NR2A receptors and calmodulin was used to facilitate independent transfection of various forms of calmodulin and NMDA receptors. By using this system, it became clear that endogenous calmodulin is generally insufficient to produce high levels of CDI. The greatest extent of CDI (overexpression of calmodulin) is ~80%, with a residual activity of ~20%. This suggests that CDI can be cell- and state-dependent and potentially affected by calmodulinopathies.

Using these metrics, the time constant of the onset of CDI was 500 ms and recovery was 9.3 s. This is an important finding because, although the equilibrium level of CDI would not be reached during a single synaptic event, it would be significant during that time and multiple closely timed events would add to the degree of CDI. This provides a more dynamic, activity-dependent tuning of the postsynaptic signal.

The next question was to determine the location of calmodulin relative to the mouth of the channel. This involved estimating an average unitary Ca^{2+} flux for a given external Ca^{2+} concentration and then using that to produce a relationship between CDI and steady-state Ca^{2+} flux (half the greatest level of CDI at 0.024 pA of Ca^{2+} current). Using this finding and employing Fick's law, the authors were able to calculate the distance (~9 nm) from the mouth of the channel to the site of action, presumably calmodulin.

It is well known that calmodulin can interact with other proteins in either its apo or holo (calcium-bound) form. If bound to NR1 in the apo form, calmodulin would be primed to sense calcium influx through the channel. Although in vitro measurements clearly show that both apo and holo calmodulin can interact with the C0 region of NR1, with a higher affinity for the holo than the apo form (6), it was not clear whether the receptor was primed for CDI by prebound apo-calmodulin or if Ca^{2+} was bound to calmodulin that then bound to C0, thus producing CDI. The authors derived predictions for the relationship between CDI and the p_o (probability of being in the open state) for each of these two models. Unfortunately, with native affinity of calmodulin for the NR1 C0 and the maximum p_o of wild-type

Submitted September 25, 2017, and accepted for publication October 3, 2017.

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Editor: Vasanthi Jayaraman.
https://doi.org/10.1016/j.bpj.2017.10.008

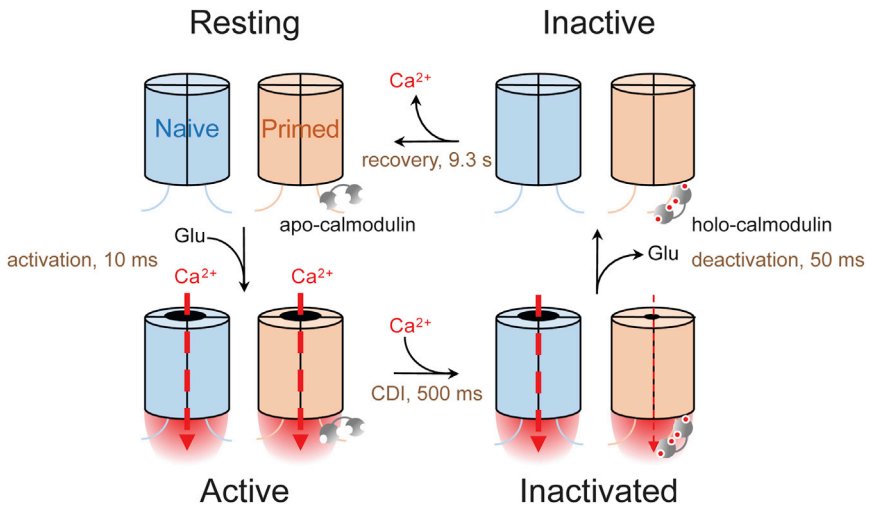

FIGURE 1 Schematic summarizing the proposed mechanism of CDI on NMDA receptors. Apo calmodulin binds to C0 of NR1 and primes the channel for inactivation (*Resting*). Glutamate binds to NR2 (glycine is prebound to NR1), and the channel is activated (*Active*). Ca^{2+} transits the channel and binds to calmodulin, triggering CDI (*Inactivated*). During inactivation, channels with holo calmodulin show decreased conductance. Glutamate dissociates, closing the channel (*Inactive*). Ca^{2+} dissociates, recovering the initial state. To see this figure in color, go online.

NR1a/N2A receptors (0.62), either model could explain the data; however, the predicted holo calmodulin affinity for NR1 was lower than expected. By using a double mutation that lowered the calmodulin affinity and increased channel p_o, the data strongly supported the model favoring prebound apo-calmodulin.

Although many pieces of this puzzle were known before this study, this whole-cell model of calmodulin action provides a comprehensive picture of CDI. As shown in Fig. 1, calmodulin is prebound to the C-terminal C0 region of NR1 ~9 nm from the mouth of the channel. Given the considerable intracellular buffering of Ca^{2+}, it is the influx of calcium through the channel after activation (binding of glutamate to NR2 and glycine to NR1) that increases the local concentration sufficiently to bind calmodulin and trigger CDI. This occurs with a time course of 500 ms. Glutamate unbinds (deactivation) and then Ca^{2+} dissociates from calmodulin with a time constant of 9.3 s.

Although this study represents a notable increase in our understanding of CDI, this is not the end of the story. Previous work (7) has suggested that the activation of bound calmodulin by Ca^{2+} displaces α-actinin, which in turn allows the binding of Ca^{2+}/calmodulin-dependent kinase II (CaMKII). The subsequent molecular events leading to CDI remain open questions. Various other questions remain as well. For example, is there any cross talk between NMDA receptors or is each receptor sensitive to only Ca^{2+} flux through its own channel? Are the two Ca^{2+} binding sites on calmodulin equivalent with respect to CDI? Why is the greatest extent of CDI 80%; are some receptors insensitive or is gating impaired by CDI but still present? What is the exact physiological role of CDI and is it neuroprotective in some neurological diseases? Future single molecule experiments using calmodulin mutations in combination with structure-dynamic investigations may shed light on some of these questions.

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