Journal Club

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Kinases Bicker Over an Ion Channel

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Review of Magoski and Kaczmarek (<http://www.jneurosci.org/cgi/content/full/25/35/8037>)

Ion channels exist in multiprotein complexes with a variety of signaling proteins that modulate channel activity. Modulators of ion channels may associate with the channel directly or via scaffolding protein(s). Protein kinases constitute one of the most commonly encountered family of proteins in ion channel complexes. Phosphorylation by a kinase may increase or decrease channel activity, depending on the particular kinase that is involved and the specific sites(s) that are phosphorylated (Levitan, 1999). Although many ion channels are regulated by phosphorylation, much less is known about the physiological context of such modulation.

Bag cell neurons of *Aplysia* serve as a model system for phosphorylation-mediated regulation of neuronal excitability. In response to brief stimulation, these neurons respond with an ~30 min barrage of repetitive action potentials called the afterdischarge. This is followed by a refractory period of ~18 h, during which electrical stimulation fails to trigger afterdischarges. The prolonged depolarization accompanying the afterdischarge is driven in part by a voltage-gated nonselective cation channel (Wilson and Kaczmarek, 1993). This channel is regulated by a variety of kinases and phosphatases including protein kinase C (PKC). PKC is colocalized with the cation channel in excised patches and increases channel activity via phosphorylation (Wilson et al., 1998; Magoski et al., 2002). In their recent article in *The Journal of Neuroscience*, Ma-

goski and Kaczmarek (2005) showed that the state of PKC–channel interaction reflects the state of the bag cell neuron: PKC is coupled to the cation channel during the afterdischarge and uncoupled during the refractory state.

Using cultured bag cell neurons, the authors pulled excised inside-out patches and identified nonselective cation channels based on their distinctive electrical properties. Previous studies had shown that application of ATP alone to the cytoplasmic side of these patches causes an increase in open probability (P_O), suggesting that a kinase might be intimately associated with the channel. PKC inhibitor peptides blocked ATP-induced increase in P_O , consistent with a PKC-like kinase (Wilson et al., 1998). Magoski and Kaczmarek (2005) reported that less than one-half of the patches containing these cation channels showed an increased channel P_O in the presence of ATP, whereas the rest showed no change or a decrease in P_O [Magoski and Kaczmarek (2005), their Fig. 1 (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG1>)]. The authors investigated the molecular mechanism underlying this variable response.

Most channels from a given animal responded in the same way. Thus, the authors used batches of neurons from animals that showed no ATP response and asked whether the absence of a response was attributable to a lack of PKC association with the channel or inactive PKC associated with the channel. Application of PKC-activating phorbol ester PMA to the cytoplasmic side of the patch had no effect [Magoski and Kaczmarek (2005), their Fig. 2D (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG2>)]. How-

ever, when cultures were pretreated with PMA, which causes PKC to translocate to the plasma membrane, PKC-dependent modulation was restored [Magoski and Kaczmarek (2005), their Fig. 3 (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG3>)], suggesting that nonresponding patches lacked associated PKC.

Previous studies demonstrated that the Src homology 3 (SH3) domain specifically from Src is critical for the channel–PKC interaction (Magoski et al., 2002). Surprisingly, however, direct application of Src tyrosine kinase to nonresponding patches decreased channel P_O , an effect that could be inhibited by a Src-family tyrosine kinase inhibitor [Magoski and Kaczmarek (2005), their Fig. 4 (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG4>)]. This suggests that Src, via phosphorylation of the channel itself or an associated protein, may inhibit channel activity. Intriguingly, when nonresponders were pretreated with Src kinase inhibitor before patch excision, the ATP response was rescued, and the kinetic profile of the single-channel records resembled a typical PKC-dependent P_O increase [Magoski and Kaczmarek (2005), their Fig. 5 (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG5>)]. Together, these results indicate dual roles for Src. Src inhibits channel activity via tyrosine phosphorylation. However, when Src activity is inhibited, PKC couples with the channel, perhaps by using SH3 domain as a scaffold. However, what causes Src to switch from a kinase to a scaffold, and vice versa, remains unanswered. The authors then asked whether the lack of response in some patches was attributable to an association of the channel with Src? If

Received Sept. 28, 2005; accepted Jan. 5, 2006.

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DOI:10.1523/JNEUROSCI.4133-05.2006

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Figure 1. Schematic summarizing the results presented by Magoski and Kaczmarek (2005). Bag cell neurons of *Aplysia*, when stimulated, undergo prolonged depolarization (afterdischarge), followed by a refractory period. In resting neurons, PKC associates with the cation channel, perhaps via Src, and contributes to the depolarization underlying the afterdischarge. In contrast, when these neurons enter the refractory state, PKC dissociates from the channel, and Src may inhibit the channel via tyrosine phosphorylation of the channel or an associated protein.

this were true, Src inhibition should rescue the ATP response in nonresponding patches. Application of Src inhibitor alone or Src inhibitor followed by PMA were ineffective, suggesting that neither Src nor PKC are associated with the channel in nonresponding patches [Magoski and Kaczmarek (2005), their Fig. 6 (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG6>)].

The most compelling evidence that justifies the title of the paper comes from their last two figures. Resting neurons had less phosphotyrosine staining and more ATP responding patches than refractory

neurons [Magoski and Kaczmarek (2005), their Figs. 7 (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG7>) and 8 (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG8>)]. Furthermore, the kinetic profile of the enhanced ATP response in resting neurons followed the pattern that is observed for a typical PKC-dependent P_O increase [Magoski and Kaczmarek (2005), their Fig. 8 (<http://www.jneurosci.org/cgi/content/full/25/35/8037/FIG8>)]. In conclusion, these results support the hypothesis that the cation channels excised from resting neurons are coupled to PKC, but those from refractory neurons are not (Fig. 1).

Therefore, what is the real molecular trigger for transition from afterdischarge to refractoriness: is it PKC dissociation, Src association, or some other event coupled to these? We know that PKC activity and cation channel activity are elevated at the onset of the afterdischarge; thus, it is likely that PKC and the channel associate to initiate the afterdischarge. At some point during the afterdischarge, Src may phosphorylate the channel or an associated protein and initiate the switch to refractoriness. It would be interesting to know whether Src inhibition during the afterdischarge blocks the transition to refractoriness. Conversely, does inhibition of Src in refractory neurons reverse the increased P_O ATP response? As with many good papers, this paper leaves us with more questions than answers.

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