Commentary

Modulating Modulation

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Reuter (1967) reported that a voltage-dependent conductance, the calcium conductance of cardiac muscle, could be enhanced by stimulation of receptors for norepinephrine. Besides explaining a crucial element in the control of the heart by the sympathetic nervous system, Reuter's observation began a major branch of electrophysiology. Transmitter modulation of voltage-dependent ion channels by hormones and transmitters is now appreciated as an important control mechanism in heart, smooth muscle, neuroendocrine cells, and neurons. Virtually every type of voltage-dependent ion channel is subject to some kind of modulation.

Of all the examples of transmitter regulation of voltage-dependent channels, probably none has been more thoroughly studied than inhibition of calcium current in neurons, originally described by Dunlap and Fischbach (1981) in sensory neurons. A few years after their paper, the patch clamp technique made voltage-clamp recordings from neurons an everyday technique, and the phenomenon they described proved to be present in virtually every kind of neuron and to be elicited by a wide variety of transmitters. By now, the subject has been grist for the mills of at least three scientific generations of investigators. Their efforts have revealed an unexpected variety of second messenger pathways involved in calcium channel modulation (reviewed by Hille, 1994; Dolphin, 1998; Ikeda and Dunlap, 1999). In rat sympathetic neurons alone, Hille and colleagues have elucidated five different pathways used by nearly a dozen transmitters that inhibit calcium current. Less detailed studies in other neurons suggest that many of these pathways are widely used.

Now, just when many might have thought that the subject was nearly exhausted, Barrett and Rittenhouse (2000) show that there is more to be found. Their paper follows up an observation by Swartz and colleagues that the most common mechanism of transmitter inhibition of neuronal calcium channels, which involves a rapid, membrane-delimited pathway, can itself be modulated: the pathway is inhibited by phorbol esters, most likely by stimulation of protein kinase C (Swartz, 1993; Swartz et al., 1993). Subsequent investigations using rat sympathetic neurons showed that protein kinase C disrupts multiple membrane-delimited pathways of calcium channel inhibition (which use at least three different kinds of G proteins), but not another pathway that uses a cytoplasmic messenger (Shapiro et al., 1996). The effect is downstream of transmitter receptors, since protein kinase C is still effective when receptors are bypassed by using the nonhydrolyzable GTP analog GTP-γ-S to activate G proteins (Swartz, 1993; Zhu and Ikeda, 1994). Barrett and Rittenhouse (2000) have now found that the effect of protein kinase C is prevented if the calcium channels are already inhibited by maximal G protein stimulation. This finding is of interest for understanding both the molecular events involved and how they are used in a physiological context.

The beauty of experiments on G-protein modulation of neuronal calcium channels is the directness with which mental pictures of molecular mechanisms can be built from traces on the oscilloscope. As a result of much work, there is now good evidence that the rapid, membrane-delimited G-protein inhibition of calcium channels is mediated by the βγ subunits of G-proteins binding directly to the calcium channel (Ikeda 1996; Herlitze et al., 1996; De Waard et al., 1997; Zamponi et al., 1997). In relating oscilloscope traces to molecular events, the crucial experimental protocol is one introduced by Elmslie et al. (1990) to reveal a phenomenon called facilitation: the inhibition of calcium channels can be temporarily reversed by a long, strong depolarizing pulse. The mental picture is that during the depolarization, the affinity of the channel for G-proteins is dramatically reduced (as a result of the conformational change associated with channel activation), and the G-protein comes off the channel. This simple picture, though still unproven, offers a satisfying explanation for a variety of complex changes in the kinetics and voltage dependence of channel currents, including the time dependence of reinhibition after the facilitating depolarization (reviewed by Dolphin, 1998; Zamponi and Snutch, 1998; Ikeda and Dunlap, 1999). The facilitation protocol has been the basic tool for investigating G-protein control of calcium channels ever since Elmslie and colleagues described it. (In fact, it is a measure of how familiar the protocol has become that the original paper is not always cited.)

The molecular picture inferred from the facilitation

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protocol has turned out to fit extremely well with direct studies of the molecules involved. Binding of G-protein βγ subunits to fusion proteins from the calcium channel has been observed in direct biochemical studies (De Waard et al., 1997; Zamponi et al., 1997). In addition, this interaction can be disrupted by protein kinase C–mediated phosphorylation of the calcium channel peptides (Zamponi et al., 1997), and when mutated channels lacking two particular phosphorylation sites were expressed, protein kinase C no longer blocked G-protein inhibition (Hamid et al., 1999). In this context, the results of Barrett and Rittenhouse predict that the phosphorylation of these sites can be prevented (or at least greatly slowed) if G-protein βγ subunits are bound to the channel to start with. An additional point of close correspondence between studies on cloned and native channels concerns a likely interaction between G-proteins and the β subunit of the calcium channel in regulating gating of the channel. The β subunit of the calcium channel is a cytoplasmic protein that alters the voltage dependence and kinetics of gating and binds to a region of the main calcium channel subunit (the cytoplasmic loop between the first and second pseudosubunit domains) at which G-protein βγ subunits also interact. In both native neurons and cloned channels, depleting the channel β subunit enhances G-protein inhibition, suggesting functional competition between the two proteins (Campbell et al., 1995; Roche et al., 1995; Bourinet et al., 1996). In heterologously expressed channels, one effect of the calcium channel β subunit is to speed inactivation, and in Barrett's and Rittenhouse's experiments on sympathetic neurons, they note that inactivation is fastest under conditions where G-protein binding is expected to be minimized (internal dialysis with GDP-β-S, after stimulation of protein kinase C, or after facilitating depolarizations).

What is the physiological significance of all of this? So far, the clearest physiological role for G-protein inhibition of calcium channels is in mediating neurotransmitter inhibition of synaptic transmission, by reducing calcium entry into the presynaptic terminal. There are many parallels between the actions of transmitters on calcium channels (usually studied in cell bodies) and on synaptic transmission (although G-proteins also can act more directly on transmitter release by inhibiting still-unknown steps in exocytotosis). In fact, it has been observed that phorbol esters can reduce or eliminate transmitter inhibition of synaptic transmission at some synapses (Swartz et al., 1993). The new results suggest that this effect will itself depend on whether or not the presynaptic calcium channels are already inhibited by G-proteins when kinase C is activated. In Barrett's and Rittenhouse's experiments, the dramatic effects of G-protein inhibition in preventing kinase C actions were seen after maximal activation of G-proteins with intracellular GTP-γ-S, and it remains to be seen whether physiological activation of G-proteins by transmitters can be equally effective. Perhaps the effect of more physiological activation of G-proteins will be to slow the development of kinase C effects.

An even more fundamental unanswered question is how and when protein kinase C is activated at particular cells or synapses during normal functioning of the nervous system. In studies on isolated cells, phorbol esters were very effective in blocking G-protein inhibition of channels, but it has proven difficult to find equivalent effects of natural transmitters like acetylcholine and glutamate that would be expected to activate protein kinase C (K.J. Swartz, personal communication). As for many other aspects of ion channel function, our increasing understanding of complex molecular mechanisms has surpassed our knowledge of how those mechanisms are actually used.

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