Modulation of the cGMP-gated channel by calcium

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Abstract: Calcium acting through calmodulin has been shown to regulate the affinity of cyclic nucleotide-gated channels expressed in cell lines. But is calmodulin the Ca-sensor that normally regulates these channels?

[MOLDAY & HSU] In the first five sections of their target article, MOLDAY & HSU provide a concise overview of the biochemical and functional properties of the cGMP-gated channel in photoreceptors. In the next two sections they concentrate on the evidence that calcium acts through calmodulin to regulate the affinity of cGMP-gated channel for its ligand. Recent work from their laboratories has demonstrated that this modulatory action cannot be measured in the homo-ologomeric channel formed by the human alpha subunit expressed in a cell line, but it can be measured when the alpha subunit is coexpressed with the longer of the two splice forms of the beta subunit. The modulatory action of calmodulin on the rod cGMP-gated channel is therefore mediated through the beta subunit. In contrast, the effect of calmodulin on the cyclic nucleotide-gated channel from rat olfactory receptors is mediated through the alpha subunit. A second important difference between the two types of receptor is that calmodulin has a stronger action on the olfactory channel: calcium-calmodulin increases the $K_{1/2}$ of the expressed olfactory channel over 10-fold, whereas the $K_{1/2}$ of the rodchannel is increased 1.5-to-2-fold.

When a rod responds to light, calcium levels fall inside the outer segment, and MOLDAY & HSU suggest that this will relieve the effect of calmodulin on the channel, increasing its affinity for cGMP and therefore promoting the re-opening of channels during the process of light adaptation. Unfortunately, we have relatively little information about this proposed action. Gray-Keller and Detwiler (1994) have reported that calmodulin or calmodulin inhibitors introduced into a transducing rod through a patch pipette have no effect on phototransduction. We have made similar observations using an alternative preparation, the truncated salamander rod outer segment. This preparation leaves the outer segment relatively intact and many properties of the phototransduction mechanism are maintained (Lagnado & Baylor 1994), but with the advantage that it is possible to manipulate internal levels of calcium and cGMP. We find that calcium over the range 200nM to 10nM regulates the channels affinity for cGMP. At low cGMP concentrations the current is increased about 2-fold when Ca is removed. A diffusible medi tor is involved, since the ability to modulate the channel affinity for cGMP by changing calcium gradually washes ou However, there does not seem to be any effect of addin calmodulin or calmodulin inhibitors such as mastoparan. A these observations are unpublished. A similar observation found in the original study of Kramer and Siegelbaum (1992) o olfactory receptors, where the ability of calcium to modulate th cAMP-dependent current did not seem to involve calmoduli

Although we are still investigating a possible action of ca modulin, the obvious implication of the results we have so far that some other calcium-binding protein modulates the cha nel. Recently, three new calcium-binding proteins have bee discovered in the rod outer segment: recoverin, GCAP and p2 All of these are "calmodulin-like," in the sense that they a soluble, possess multiple E-F hands, and have molecul weights of 20–24 kD. It does not seem that recoverin binds the channel but it will be interesting to see if GCAP or p24 ca The idea that there may be a second calcium-sensitive modul tor of the channel has also been suggested by Gordon ar Zimmerman (1994), who found evidence for an endogeno modulator of the cGMP-gated channel which was lost at lo calcium.

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