Commentary

Tuning Ca^{2+} Permeation in Cyclic Nucleotide–gated Channels

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Most ion channels consist of several different subunits, and figuring out the exact role that each subunit polypeptide plays in channel regulation is a daunting and rewarding task. The challenge is to find out (a) which subunits coassemble to form the native channel protein, (b) how many copies of each individual polypeptide are contained in the channel, (c) which subunits contribute to the lining of the conducting pore and which just stick to the internal or external face of the channel, and (d) which of the subunits are neighbors in the native channel. The reward has two equally exciting aspects: the studies can yield glimpses into the mechanisms for fine tuning nearly all functional parameters, including ion selectivity, regulation of open probability (gating), and channel expression. In addition, a modular system of channel assembly may become apparent in which a cell chooses from a repertoire of subunits to build the channel it needs. The report by Hackos and Korenbrot (1999) in this issue is an excellent case in point: cyclic nucleotide–gated (CNG) channels display a fascinating dynamic fine tuning of Ca^{2+} selectivity and this phenomenon depends on the presence of a modulatory β subunit.

CNG channels work as transducer channels in photoreceptors of the vertebrate retina and in olfactory sensory neurons (OSNs) of the nose. In the light-sensitive outer segment of rod and cone photoreceptors, CNG channels conduct a steady inward current in the dark, the "dark current." Activated by cGMP, the second messenger of visual transduction, the channels have an open probability in the dark of just 1–5%, and they close when cGMP is hydrolyzed upon illumination. Thus, photoreceptor channels always work at very low activation levels, prompting Hackos and Korenbrot to study their conducting properties during low activation. The closing of CNG channels in light not only hyperpolarizes the membrane, it also induces a Ca^{2+} signal that plays a pivotal role in phototransduction. The dark current is a mixed cation current with a Ca^{2+} fraction between 12 and 21% (Nakatani and Yau, 1988; Perry and McNaughton, 1991). Steady Ca^{2+} influx in the dark is balanced by Ca^{2+} extrusion through $Na^+/Ca^{2+},K^+$ exchangers, resulting in a stable free Ca^{2+} concentration of ~500 nM. When CNG channels close in light, $[Ca^{2+}]$ drops to ~50 nM due to continuous extrusion by the exchangers, a signal that is sensed by a set of Ca^{2+}-regulated proteins that help the photoreceptor recover after the stimulus (Gray-Keller and Detwiler, 1994). Letting Ca^{2+} into the outer segment is thus an essential part of CNG channel function in photoreceptors.

In OSNs, CNG channels are activated by cAMP, which acts as second messenger during odor stimulation in the sensory cilia. Although our understanding of olfactory signal transduction is by far not as detailed as our concept of phototransduction, it is becoming clear that the ability of CNG channels to conduct Ca^{2+} determines both the rise time and the amplitude of the olfactory receptor current, as well as its termination after the stimulus. Ca^{2+}-gated Cl^- channels are triggered by odor-induced Ca^{2+} influx through CNG channels and cause a depolarizing Cl^- efflux that amplifies the receptor current (Lowe and Gold, 1993). And among the various processes that terminate the receptor current, probably the most rapid is the negative feedback inhibition of CNG channels by Ca^{2+}/calmodulin (Chen and Yau, 1994; Kurahashi and Menini, 1997). Thus, Ca^{2+} signals generated by CNG channels are at the heart of sensory transduction in vision and olfaction.

How the channels interact with Ca^{2+} depends on the set of subunits that coassemble to form the channel protein. CNG channels can form heteromeric proteins containing at least two types of subunits: principal α subunits and modulatory β subunits. Three homologous genes encode distinct α subunits in rods, cones, and OSNs, and a fourth gene supplies two different splice forms of β subunits in rods and OSNs (Chen et al., 1993, 1994; Körschen et al., 1995; Sautter et al., 1998; Bönigk et al., 1999). In addition, a second type of modulatory subunit is part of the olfactory channels (Bradley et al., 1994; Liman and Buck, 1994; Shapiro and Zagotta, 1998). Consequently, three different subunits form the transduction channels of OSNs, and the rod photoreceptor channels have at least two different subunits. It is not clear whether α and β subunits are coassembled in the channels of cone photoreceptors.

All known subunits of CNG channels are integral membrane proteins and appear to contribute to the formation of the channel pore. This is particularly important for cation permeation because the α subunits contribute negatively charged amino-acid resi-

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dues (glutamate or aspartate) to an intrapore cation-binding site. β subunits, on the other hand, have an uncharged glycine in the respective position and attenuate cation binding. The report by Hackos and Korenbrot (1999) now reveals a link between ion selectivity and open probability conferred on the photoreceptor channel by the β subunit. The authors show that the relative Ca^{2+} permeability of heteromeric channels displays a pronounced dependence on the cGMP concentration, with unexpectedly small values at low (physiological) activation levels. This is a surprising result because selectivity and gating are traditionally thought of as independent and associated with different parts of the channel protein. The selectivity filter is determined by geometry and charge density of the intrapore ion-binding site, which is regarded as a fixed feature of the channel (with the notable exception of purinergic receptor channels, which change selectivity with time after activation; Khakh et al., 1999). But this view, as well as the textbook notion that sees the channel gate simply as a plug in the pore, controlled in an all-or-nothing fashion by a voltage sensor or a ligand-binding site, is obviously inappropriate for CNG channels. Apparently, changes of ion selectivity with open probability reflect the ability of photoreceptor CNG channels to adopt more than a single conducting state: at low cGMP concentration, partially liganded channels may open into a subconductance state with relatively low Ca^{2+} permeability. Fully liganded channels switch at elevated cGMP into a different state, which is characterized by higher conductance and increased Ca^{2+} permeability. A similar dependence of ion selectivity on distinct conductance states recently was demonstrated for mutant *Shaker* K^+ channels (Zheng and Sigworth, 1997) and for an NMDA-receptor channel mutant (Schneggenburger and Ascher, 1997). To my knowledge, the report by Hackos and Korenbrot (1999) is the first evidence for such a phenomenon in a native channel, and it has immediate significance for CNG-channel research: physiologically meaningful studies of Ca^{2+} permeation have to be done at the right activation level!

To most people, relative Ca^{2+} permeability is a somewhat cryptic parameter. It is usually interpreted as the relative ease with which two ion species (here Ca^{2+} and Na^+) can enter a channel, but it doesn't tell you how efficiently a channel conducts Ca^{2+} into the cell. Recent studies of Ca^{2+} interaction with CNG channels have yielded a concept for Ca^{2+} permeation that may help us appreciate the results presented by Hackos and Korenbrot (1999). Interaction of CNG channels with extracellular Ca^{2+} is determined by the Ca^{2+} affinity of the intrapore binding site. This site is formed by a set of four negatively charged residues in channels consisting of only α subunits or by a combination of charged and uncharged residues in channels containing α and β subunits. The α subunits of rods, cones, and OSNs show marked intrinsic differences in Ca^{2+} affinity, and coassembly with β subunits reduces Ca^{2+} affinity (Dzeja et al., 1999; Seifert et al., 1999). Consequently, a variety of CNG channels with quite diverse affinities for extracellular Ca^{2+} results from the combinations of the various α and β subunits. When Ca^{2+} enters a high-affinity CNG channel, it is tightly bound, blocks the passage of monovalent cations, and stays in the pore for a relatively long time. Therefore, Ca^{2+} blockage of monovalent currents is very effective in high-affinity channels, but the rate of Ca^{2+} permeation is low. In contrast, low-affinity CNG channels show a less efficient Ca^{2+} block of monovalent current but allow higher rates of Ca^{2+} permeation (a larger Ca^{2+} influx) because Ca^{2+} ions move through the pore more easily.

Thus, Ca^{2+} influx is inversely related to Ca^{2+} affinity in these channels. But how is the relative Ca^{2+} permeability (as determined by Hackos and Korenbrot from reversal potentials with intracellular Ca^{2+}) related to Ca^{2+} affinity (as determined from the blockage of monovalent currents by extracellular Ca^{2+})? Earlier studies have shown that the higher the Ca^{2+} affinity of a CNG channel, the lower is its relative Ca^{2+} permeability (Frings et al., 1995). Consistent with this result, Hackos and Korenbrot (1999) show that recombinant rod photoreceptor channels containing both α and β subunits have a higher relative Ca^{2+} permeability than α homomers; and the Ca^{2+} affinity in rod αβ channels is lower than in α homomers (Körschen et al., 1995). Furthermore, the reduced relative Ca^{2+} permeability at low activation levels found by Hackos and Korenbrot (1999) is associated with an increase of Ca^{2+} affinity, as reported by Colamartino et al. (1991).

Taken together, high values of relative Ca^{2+} permeability suggest high levels of Ca^{2+} influx and low Ca^{2+} affinity in CNG channels. As permeability and flux rates are not necessarily linked (one reflecting the access to the pore, the other the binding strength), this phenomenological correlation is food for thought and may stimulate further investigations into Ca^{2+} permeation in these channels. But it already gives some insight into how the dark current is shaped in such a way that current amplitude and Ca^{2+} influx maintain just the right balance necessary for phototransduction: at the low cGMP concentrations in photoreceptors, relative Ca^{2+} permeability of CNG channels is low, implying that Ca^{2+} affinity is high. This means that Ca^{2+} efficiently suppresses Na^+ influx and that Ca^{2+} influx is retarded by strong binding. The result is a small dark current (approximately −40 pA) with a relatively high Ca^{2+} fraction (12–21%). At higher cGMP levels (which apparently don't occur in photoreceptors), CNG channels would decrease their Ca^{2+} affinity. This would lead to larger currents (exceeding the increment caused by

increased open probability) with a relatively smaller Ca^{2+} component, but a larger overall Ca^{2+} influx. These relations between Ca^{2+} affinity, fractional Ca^{2+} current, and Ca^{2+} influx govern the physiological functions of CNG channels and should be kept in mind when predicting effects of the fine-tuning of Ca^{2+} permeation described Hackos and Korenbrot (1999).

Interestingly, the authors demonstrate that both rod and cone CNG channels show cGMP dependence of relative Ca^{2+} permeability. Since this property is conferred to the rod channel by its β subunit, maybe cone channels also possess a β subunit. This is particularly interesting because the β subunit contains a calmodulin-binding site (Weitz et al., 1998), which may mediate regulatory effects by Ca^{2+}/calmodulin in rods and cones. The relative Ca^{2+} permeability at low activation levels is much higher in cones than in rods. Such a pronounced difference in Ca^{2+} permeation is expected to cause differences in the dynamics of Ca^{2+} handling between the two photoreceptor types and may be one of the reasons why cones show faster recovery after a light stimulus. Finally, in cells where CNG channels can reach high levels of activity, the dynamic tuning of Ca^{2+} permeation may constitute a regulatory mechanism that becomes effective as Ca^{2+} affinity changes with open probability.

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