Commentary

Cooperativity in Cyclic Nucleotide-Gated Ion Channels

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Cooperativity is one of the marvelous "emergent" behaviors of macro-molecules undergoing multimolecular reactions (Perutz, 1990). Cooperativity sculpts the potential energy landscape of such a reaction so as to create a seemingly teleological pathway for reaching and stabilizing a particular state—for example, a multimeric protein with multiple ligand sites occupied. Ligand-gated ion channels provide excellent opportunities for examination of the function and the mechanistic nature of cooperative behavior (Jackson, 1989, 1994; Changeux and Edelstein, 1994). Cooperative ligand activation of ion channels can serve to create exquisitely sensitive molecular switches at chemical synapses. And single-channel recording can provide unique possibilities for investigating the mechanism(s) underlying cooperativity in single molecules (e.g., Jackson, 1994). While full understanding of the physical mechanisms underlying cooperative gating will require tertiary structure information of multiple structural forms of a channel—information unlikely to be generally available in the near term—partial insights into the phenomenon obtained with functional assays are greatly to be welcomed.

In this issue of *The Journal of General Physiology*, Karpen and Brown (1996) refine their application of the technique of covalent activation of the cyclic nucleotide-gated channel of retinal rods with the photoaffinity analogue 8-*p*-azidophenacylthio-cGMP, and in doing so advance the investigation of cooperative channel gating. The novel technical feat of the effort of Brown and Karpen (1996) is the production of membrane patches reasonably argued to contain only fully liganded channels, or channels having one unliganded nucleotide binding site.

Although cyclic nucleotide-gated ion channels (CNCs) took their seat in the congress of known ligand-gated channels just a little over a decade ago (Fesenko et al., 1985), work on them has proceeded very rapidly. They are well characterized functionally as nondesensitizing, cooperatively gated, and weakly specific cation channels with rectifying properties dependent on divalent cation blocking (Yau and Baylor, 1989; Kaupp, 1991). Beginning with the bovine retinal rod channel (Kaupp et al., 1989) more than 10 distinct CNCs have been cloned and sequenced; of these most have been characterized functionally after re-expression (Yau and Chen, 1995). The generic native CNC is likely a heteromultimer comprising four (or possibly five) subunits, each having six transmembrane segments, with a ligand binding domain on the cytoplasmic side (Fig. 1; Kaupp, 1991; Molday and Hsu, 1994; Yau and Chen, 1995). CNCs constitute a distinct and archetypal family of ligand-gated channels, likely formed by the combination of a general nucleotide binding domain with an ur-channel subunit having a domain closely resembling the S4 voltage-sensing domain of some classes of K channels (Jan and Jan, 1990; Yau and Chen, 1995). This view is now strongly supported by recent work on mutant and chimeric CNCs (Heginbotham et al., 1992; Goulding et al., 1994), and a growing body of sequence homology data. CNCs have two known subunits: the α-subunit, originally cloned by Kaupp et al. (1989), can form homomultimeric channels; the β-subunit, discovered later (Chen et al., 1993) forms functional channels only in conjunction with the α-subunit. (Excellent reviews of CNC structure and functional properties can be found in Kaupp, 1991; Molday and Tsu, 1994; Yau and Chen, 1995; Menini, 1995.)

The nucleotide dependence of steady state currents through CNCs is usually well described by the Hill equation

$$J/J_{max} = \frac{cG^n}{cG^n + K_{1/2}^n} \quad (1)$$

where J is the average current through a patch of membrane exposed at some particular transmembrane potential to a concentration cG of cGMP, J_{max} is the maximum average current at high cG, $K_{1/2}$ is the value of cG yielding 50% activation, and n, the Hill constant. Accurate estimation of the parameters of Eq. 1 requires very stable recordings. Thus, the absence of desensitization is both a fundamental property of CNC gating, and of considerable practical importance in experiments such as those of Brown and Karpen (1996), in which individual patches may be investigated for hours, and the resultant data subsequently analyzed with Eq. 1 or related

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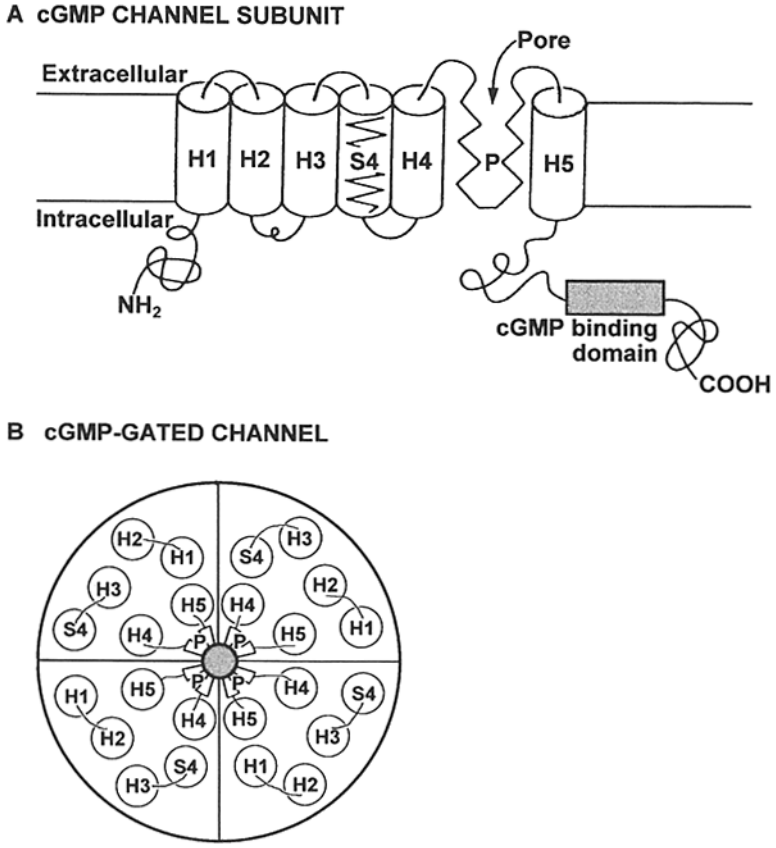

FIGURE 1. (*A*) Hypothesized secondary structure of a subunit of a cyclic nucleotide-gated cation channel. The putative membrane-spanning helices H1-H5 correspond to linear segments of the peptide chain as originally labeled by Kaupp et al. (1991); further modifications of the scheme are as found in Bönigk et al. (1993); see also Molday (1994) and Chen and Yau (1995). After Bönigk et al. (1993). (*B*) Possible arrangement of four subunits to form a channel; the perspective is looking down on the channel from the extracellular face. Modified from Alsobrook and Stevens (1988).

variants. Analysis of kinetic schemes that yield Eq. 1 show that n provides an estimate of the minimum number of ligands needed to produce measureable activation. The general adequacy of the Hill equation with n = 2–3 as a description of CNC currents is the empirical basis for the hypothesis that the gating process is mechanistically cooperative. A general issue in the application of such kinetic schemes, however, is whether the system is molecularly (or functionally) homogeneous. If not, then the current data will sample properties of several related functional systems in parallel, and the estimated parameters are not readily interpretable. It is therefore important to note that for a homogeneous population of channels activated by a single binding site n is expected to be unity.

A basic finding of Karpen and Brown (1996) is that $n = 0.65$ (very reliably < 1) for the residual current activable by cGMP after covalent activation of $\sim 80\%$ of the cGMP-gated current of a patch of native rod membrane. Karpen and Brown (1996) make a strong case that such high covalent activation produces a channel population with only one binding site unliganded (1BUs). Such a value of n technically classifies the system as having a negative cooperativity. Karpen and Brown (1996), however, show the finding that $n < 1$ is best accounted for by heterogeneity in the dissociation constant for cGMP of the 1BU sites, with nearly 30-fold relative affinities of the two sites of the best-fitting two-site model (Karpen and Brown, Fig. 5 *B*). Moreover, they also report that n is almost identically equal to 1 for activation of 1BUs of some presumably homomultimeric CNC channels (obtained by expressing the α-subunit in *Xenopus* oocytes). It is therefore reasonable to infer that the heterogeneity in ligand affinity of 1BUs in native membrane patches is due to the presence of the β-subunit in native channels. The observed heterogeneity in cGMP binding of 1BUs may also result from an intrinsic heterogeneity of the presumed homomultimers, because n is usually found to be < 1 for channels expressed in oocytes. The mechanistic basis for such heterogeneity is obscure, but a similar heterogeneity (over time) has been observed in voltage-dependent sodium channels in oocytes (Zhou et al. 1991) and in planar bilayers (Chabala et al., 1991). In the latter system, at least, one can exclude the possibility that covalent modification of the channel protein is involved.

Future studies of the molecular basis for the heterogeneity in cGMP affinity are likely to depend on single-channel experiments having 1BU channels in the patches. In such a system, the prediction of binding sites with different ligand affinity should be readily

tested, given the large difference in apparent affinity between the inferred bimudal population of 1BU sites. Moreover, single-channel data of 1BU channels should also provide valuable new insights into the kinetics of the gating mechanism. An even more ambitious undertaking would be to attempt to prepare patches with single channels having only a single covalently attached ligand; detection of the number of ligands might be possible with single-photon fluorescence methods. Such channels might provide the grist for tests of CNC gating models based on Monod-Wyman-Changeux allosteric mechanisms (Goulding et al., 1994; Jackson, 1994; Changeux and Edelstein, 1994).

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