International Union of Pharmacology. XLII. Compendium of Voltage-Gated Ion Channels: Cyclic Nucleotide-Modulated Channels

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Abstract——This summary article presents an overview of the molecular relationships among the voltage-gated cyclic nucleotide-modulated channels and a standard nomenclature for them, which is derived from the *IUPHAR Compendium of Voltage-Gated Ion Channels.*[1] The complete Compendium, including data tables for each member of the cyclic nucleotide-modulated channel family can be found at <http://www.iuphar-db.org/iuphar-ic/>.

Cyclic Nucleotide-Gated Channels

The family of cyclic nucleotide-modulated channels comprises two groups: the cyclic nucleotide-gated (CNG) channels, and the hyperpolarization-activated, cyclic nucleotide-gated (HCN) channels. Cyclic nucleotide-gated (CNG) cation channels are ion channels whose activation is mediated by the direct binding of cGMP or cAMP to the channel protein (Finn et al., 1996; Biel et al., 1999; Flynn et al., 2001). CNG channels are expressed in the cilia of olfactory neurons and in outer segments of rod and cone photoreceptor neurons, where they play key roles in sensory transduction. Low levels of CNG channel transcripts have also been found in a variety of other tissues including brain, testis, kidney, and heart. Despite the fact that their gating is only slightly voltage-dependent, CNG channels are members of the superfamily of voltage-gated cation channels. Like other members of this large gene family, CNG channel subunits contain six transmembrane segments (S1–S6) including a positively charged S4 segment and an ion-conducting pore loop between S5 and S6. CNG channels pass monovalent cations, such as Na^+ and K^+ but do not discriminate between them. Calcium is also permeable but at the same time acts as a voltage-dependent blocker of monovalent cation permeability (Frings et al., 1995; Dzeja et al., 1999). The C terminus of all CNG channels contains a cyclic nucleotide-binding domain (CNBD) that has significant sequence similarity to the CNBDs of other cyclic nucleotide receptors (Kaupp et al., 1989). CNG channels reveal a higher sensitivity for cGMP than for cAMP. The extent of ligand discrimination varies significantly between the individual CNG channel types. Photoreceptor channels strongly discriminate between cGMP and cAMP whereas the olfactory channel is almost equally sensitive to both ligands.

Based on phylogenetic relationship, the six CNG channel subunits identified in mammals are divided in two subfamilies, the α subunits (CNGA1–CNGA4) and the β subunits (CNGB1 and CNGB3) (Bradley et al., 2001). When expressed in heterologous expression systems, α subunits—with the exception of CNGA4—form functional homomeric channels. By contrast, β subunits and CNGA4 do not yield functional channels. However, when co-expressed with CNGA1–CNGA3 these subunits confer novel properties (e.g., single channel flickering, increased cAMP sensitivity) that are characteristic of native CNG channels. Native CNG channels are believed to be tetramers composed of α and β subunits. Although the exact stoichiometry of native channels has not yet been determined, the subunit composition is known for the rod photoreceptor channel CNGA1 (Kaupp et al., 1989), CNGB1a (Körschen et al., 1995), for the cone photoreceptor channel CNGA3 (Bönigk et al., 1993), CNGB3 (Gerstner et al., 2000), and for the olfactory channel CNGA2 (Dhallan et al., 1990; Ludwig et al., 1990), CNGA4 (Bradley et al., 1994; Liman and Buck, 1994), CNGB1b (Sautter et al., 1998; Bönigk et al., 1999).

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Drugs That Act on CNG Channels

Several drugs have been reported to block CNG channels, although not with very high affinity. The most specific among these drugs is L-*cis* diltiazem which blocks CNG channels in a voltage-dependent manner at micromolar concentration (Haynes, 1992). The D-*cis* enantiomer of diltiazem that is used therapeutically as a blocker of the L-type calcium channel, is much less effective than the L-*cis* enantiomer in blocking CNG channels. High affinity binding of L-*cis* diltiazem is only seen in heteromeric CNG channels containing the CNGB1 subunit (Leinders-Zufall and Zufall, 1995). CNG channels are also moderately sensitive to block by some other inhibitors of the L-type calcium channel (e.g., nifedipine), the local anesthetic tetracaine and calmodulin antagonists (Finn et al., 1996). Interestingly, LY83583 [6-(phenylamino)-5,8-quinolinedione] blocks both the soluble guanylate cyclase and some CNG channels at similar concentrations (Leinders-Zufall and Zufall, 1995). H-8 [*N*-2-(methylamino)ethyl-5-isoquinoline-sulfonamide], which has been widely used as a nonspecific cyclic nucleotide-dependent protein kinase inhibitor, blocks CNG channels, though at significantly higher concentrations than needed to inhibit protein kinases (Wei et al., 1997).

Hyperpolarization-Activated, Cyclic Nucleotide-Gated Channels

The hyperpolarization-activated, cyclic nucleotide-gated (HCN) cation channels are members of the superfamily of voltage-gated cation channels (Biel et al., 1999; Santoro and Tibbs, 1999; Kaupp and Seifert, 2001). In contrast to most other voltage-gated channels, HCN channels open upon hyperpolarization and close at positive potential. The cyclic nucleotides, cAMP and cGMP, enhance HCN channel activity by shifting the activation curve of the channels to more positive voltages. The stimulatory effect of cyclic nucleotides is not dependent on protein phosphorylation but is due to a direct interaction with the HCN channel protein. The current produced by HCN channels, termed I_h, I_f, or I_q, is found in a variety of excitable cells including neurons, cardiac pacemaker cells, and photoreceptors (Pape, 1996). The best understood function of I_h is to control heart rate and rhythm by acting as "pacemaker current" in the sinoatrial (SA) node (DiFrancesco, 1993). I_h is activated during the membrane hyperpolarization following the termination of an action potential and provides an inward Na^+ current that slowly depolarizes the plasma membrane. Sympathetic stimulation of SA node cells raises cAMP levels and increases I_h, thus accelerating diastolic depolarization and heart rate. Stimulation of muscarinic acetylcholine receptors slows down heart rate by the opposite action. In neurons, I_h fulfills diverse functions, including generation of pacemaker potentials, "neuronal pacemaking" (Pape, 1996), determination or resting potential (Pape, 1996), transduction of sour taste (Stevens et al., 2001), and control of synaptic plasticity (Mellor et al., 2002).

In mammals, the HCN channel family comprises four members (HCN1–HCN4) that share about 60% sequence identity to each other (Gauss et al., 1998; Ludwig et al., 1998, 1999; Santoro et al., 1998). HCN channels contain six transmembrane helices (S1–S6) and are believed to assemble in tetramers. The S4 segment of the channels is positively charged and serves as voltage sensor. The C terminus of all HCN channels contains a cyclic nucleotide-binding domain that confers regulation by cyclic nucleotides. When expressed in heterologous systems, all four HCN channels generate currents displaying the typical features of native I_h: (*i*) activation by membrane hyperpolarization; (*ii*) permeation of Na^+ and K^+ with a permeability ratio P_{Na}/P_K of about 0.2; (*iii*) positive shift of voltage dependence of channel activation by direct binding of cAMP; (*iv*) channel block by extracellular Cs^+. The channels HCN1–HCN4 mainly differ from each other with regard to their speed of activation and the extent by which they are modulated by cAMP. HCN1 is the fastest channel, followed by HCN2, HCN3, and HCN4. Unlike HCN2 and HCN4, whose activation curves are profoundly shifted by cAMP (Ludwig et al., 1998, 1999; Ishii et al., 1999; Seifert et al., 1999), HCN1 is only weakly affected by cAMP (Wainger et al., 2001).

HCN channels are found in neurons and heart cells. In SA node cells, HCN4 represents the predominantly expressed HCN channels isoform (Ishii et al., 1999; Moosmang et al., 2001). In mouse brain, all four HCN subunits have been detected (Moosmang et al., 1999; Santoro et al., 2000). The expression levels and the regional distribution of the HCN channel mRNAs vary profoundly between the respective channel types. HCN2 is the most abundant neuronal channel and is found almost ubiquitously in the brain. By contrast, HCN1 and HCN4 are enriched in specific regions of the brain such as thalamus (HCN4) or hippocampus (HCN1). HCN3 mRNA is uniformly expressed throughout the brain at very low levels. HCN channels have also been detected in the retina and some peripheral neurons such as dorsal root ganglion neurons (Moosmang et al., 2001).

Drugs That Act on HCN Channels

Given the key role of HCN channels in cardiac pacemaking, these channels are promising pharmacological targets for the development of drugs used in the treatment of cardiac arrhythmias and ischemic heart disease. Several blockers of native I_h channels are known. The most extensively studied blocker is ZD7288 [4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino)pyrimidinium chloride] (BoSmith et al., 1993). Low micromolar concentrations of this agent specifically block both native I_h and cloned HCN channels in a voltage-dependent

manner. The bradycardic agent ivabradine, which is chemically unrelated to ZD7288, reveals a similar affinity and specificity for I_h as ZD7288 (Bois et al., 1996). Other blockers of I_h are zatebradine (Raes et al., 1998), a derivative of verapamil, and alinidine (Van Bogaert and Goethals, 1987), a derivative of clonidine. These agents block I_h at comparable concentrations as ZD7288. However, they are less selective for I_h because they can also inhibit the current mediated by some K_{ir} channels at concentrations that reduce I_h.

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