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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.

References

Biel M, Ludwig A, Zong X, and Hofmann F (1999a) Hyperpolarization-activated cation channels: a multigene family. *Rev Physiol Biochem Pharmacol* **136:**165–181.

Biel M, Zong X, Ludwig A, Sautter A, and Hofmann F (1999b) Structure and function of cyclic nucleotide-gated channels. *Rev Physiol Biochem Pharmacol* **135:**151–171.

Bradley J, Frings S, Yau KW, and Reed R (2001) Nomenclature for ion channel subunits. *Science (Wash DC)* **294:**2095–2096.

Bradley J, Li J, Davidson N, Lester HA, and Zinn K (1994) Heteromeric olfactory cyclic nucleotide-gated channels: a subunit that confers increased sensitivity to cAMP. *Proc Natl Acad Sci USA* **91:**8890–8894.

Bois P, Bescond J, Renaudon B, and Lenfant J (1996) Mode of action of bradycardic agent, S 16257, on ionic currents of rabbit sinoatrial node cells. *Br J Pharmacol* **118:**1051–1057.

Bönigk W, Altenhofen W, Müller F, Dosé A, Illing M, Molday RS, and Kaupp UB (1993) Rod and cone photoreceptor cells express distinct genes for cGMP-gated channels. *Neuron* **10:**865–877.

Bönigk W, Bradley J, Müller F, Sesti F, Boekhoff I, Ronnett GV, Kaupp UB, and Frings S (1999) The native rat olfactory cyclic nucleotide-gated channel is composed of three distinct subunits. *J Neurosci* **19:**5332–5347.

BoSmith RE, Briggs I, and Sturgess NC (1993) Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (If) in guinea-pig dissociated sinoatrial node cells. *Br J Pharmacol* **110:**343–349.

Chen TY, Peng YW, Dhallan RS, Ahamed B, Reed RR, and Yau KW (1993) A new subunit of the cyclic nucleotide-gated cation channel in retinal rods. *Nature (Lond)* **362:**764–767.

Dhallan RS, Yau KW, Schrader KA, and Reed RR (1990) Primary structure and functional expression of a cyclic nucleotide-activated channel from olfactory neurons. *Nature (Lond)* **347:**184–187.

DiFrancesco D (1993) Pacemaker mechanisms in cardiac tissue. *Annu Rev Physiol* **55:**455–472.

Dzeja C, Hagen V, Kaupp UB, and Frings S (1999) Ca^{2+} permeation in cyclic nucleotide-gated channels. *EMBO (Eur Mol Biol Organ) J* **18:**131–144.

Finn JT, Grunwald ME, and Yau KW (1996) Cyclic nucleotide-gated ion channels: an extended family with diverse functions. *Annu Rev Physiol* **58:**395–426.

Flynn GE, Johnson JP, and Zagotta WN (2001) Cyclic nucleotide-gated channels: shedding light on the opening of a channel pore. *Nature Rev* **2:**643–651.

Frings S, Seifert R, Godde M, and Kaupp UB (1995) Profoundly different calcium permeation and blockage determine the specific function of distinct cyclic nucleotide-gated channels. *Neuron* **15:**169–179.

Gauss R, Seifert R, and Kaupp UB (1998) Molecular identification of a hyperpolarization-activated channel in sea urchin sperm. *Nature (Lond)* **393:**583–587.

Gerstner A, Zong X, Hofmann F, and Biel M (2000) Molecular cloning and functional characterization of a new modulatory cyclic nucleotide-gated channel subunit from mouse retina. *J Neurosci* **20:**1324–1332.

Haynes LW (1992) Block of the cyclic GMP-gated channel of vertebrate rod and cone photoreceptors by l-cis-diltiazem. *J Gen Physiol* **100:**783–780.

Ishii TM, Takano M, Xie LH, Noma A, and Ohmori H (1999) Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node. *J Biol Chem* **274:**12835–12839.

Kaupp UB, Niidome T, Tanabe T, Terada S, Bönigk W, Stühmer W, Cook NJ, Kangawa K, Matsuo H, Hirose T, et al. (1989) Primary structure and functional expression from complementary DNA of the rod photoreceptor cyclic GMP-gated channel. *Nature (Lond)* **342:**762–766.

Kaupp UB and Seifert R (2001) Molecular diversity of pacemaker ion channels. *Annu Rev Physiol* **63:**235–257.

Körschen HG, Illing M, Seifert R, Sesti F, Williams A, Gotzes S, Colville C, Müller F, Dosé A, Godde M, et al. (1995) A 240 kDa protein represents the complete beta subunit of the cyclic nucleotide-gated channel from rod photoreceptor. *Neuron* **15:**627–636.

Leinders-Zufall T and Zufall F (1995) Block of cyclic nucleotide-gated channels in salamander olfactory receptor neurons by the guanylyl cyclase inhibitor LY83583. *J Neurophysiol* **74:**2759–2762.

Liman ER and Buck LB (1994) A second subunit of the olfactory cyclic nucleotide-gated channel confers high sensitivity to cAMP. *Neuron* **13:**611–621.

Ludwig A, Zong X, Jeglitsch M, Hofmann F, and Biel M (1998) A family of hyperpolarization-activated mammalian cation channels. *Nature (Lond)* **393:**587–591.

Ludwig A, Zong X, Stieber J, Hullin R, Hofmann F, and Biel M (1999) Two pacemaker channels from human heart with profoundly different activation kinetics. *EMBO (Eur Mol Biol Organ) J* **18:**2323–2329.

Ludwig J, Margalit T, Eismann E, Lancet D, and Kaupp UB (1990) Primary structure of cAMP-gated channel from bovine olfactory epithelium. *FEBS Lett* **270:**24–29.

Mellor J, Nicoll RA, and Schmitz D (2002) Mediation of hippocampal mossy fiber long-term potentiation by presynaptic ih channels. *Science (Wash DC)* **295:**143–147.

Moosmang S, Biel M, Hofmann F, and Ludwig A (1999) Differential distribution of four hyperpolarization-activated cation channels in mouse brain. *Biol Chem* **380:**975–980.

Moosmang S, Stieber J, Zong X, Biel M, Hofmann F, and Ludwig A (2001) Cellular expression and functional characterization of four hyperpolarization-activated pacemaker channels in cardiac and neuronal tissues. *Eur J Biochem* **268:**1646–1652.

Pape HC (1996) Queer current and pacemaker: the hyperpolarization-activated cation current in neurons. *Annu Rev Physiol* **58:**299–327.

Raes A, Van de Vijver G, Goethals M, and van Bogaert PP (1998) Use-dependent block of Ih in mouse dorsal root ganglion neurons by sinus node inhibitors. *Br J Pharmacol* **125:**741–750.

Santoro B, Chen S, Lüthi A, Pavlidis P, Shumyatsky GP, Tibbs GR, and Siegelbaum SA (2000) Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. *J Neurosci* **20:**5264–5275.

Santoro B, Liu DT, Yao H, Bartsch D, Kandel ER, Siegelbaum SA, and Tibbs GR (1998) Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. *Cell* **93:**717–729.

Santoro B and Tibbs GR (1999) The HCN gene family: molecular basis of the hyperpolarization-activated pacemaker channels. *Ann NY Acad Sci* **868:**741–764.

Sautter A, Zong X, Hofmann F, and Biel M (1998) An isoform of the rod photoreceptor cyclic nucleotide-gated channel beta subunit expressed in olfactory neurons. *Proc Natl Acad Sci USA* **95:**4696–4701.

Seifert R, Scholten A, Gauss R, Mincheva A, Lichter P, and Kaupp UB (1999) Molecular characterization of a slowly gating human hyperpolarization-activated channel predominantly expressed in thalamus, heart, and testis. *Proc Natl Acad Sci USA* **96:**9391–9396.

Stevens DR, Seifert R, Bufe B, Muller F, Kremmer E, Gauss R, Meyerhof W, Kaupp UB, and Lindemann B (2001) Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. *Nature (Lond)* **413:**631–635.

Van Bogaert PP and Goethals M (1987) Pharmacological influence of specific bradycardic agents on the pacemaker current of sheep cardiac Purkinje fibers. A comparison between three different molecules. *Eur Heart J* **(Suppl L):**35–42.

Wainger BJ, DeGennaro M, Santoro B, Siegelbaum SA, and Tibbs GR (2001) Molecular mechanism of cAMP modulation of HCN pacemaker channels. *Nature (Lond)* **411:**805–810.

Wei JY, Cohen ED, and Barnstable CJ (1997) Direct blockade of both cloned rat rod photoreceptor cyclic nucleotide-gated ion-selective cation (CNG) channel alpha-subunit and native CNG channels from Xenopus rod outer segments by H-8, a nonspecific cyclis nucleotide-dependent protein kinase inhibitor. *Neurosci Lett* **233:**37–40.