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Topical Review

HCN-Encoded Pacemaker Channels: From Physiology and Biophysics to Bioengineering

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Abstract. The depolarizing membrane ionic current I_h (also known as I_f, "f" for funny), encoded by the hyperpolarization-activated cyclic-nucleotide-modulated (HCN1-4) channel gene family, was first discovered in the heart over 25 years ago. Later, I_h was also found in neurons, retina, and taste buds. HCN channels structurally resemble voltage-gated K^+ (Kv) channels but the molecular features underlying their opposite gating behaviors (activation by hyperpolarization rather than depolarization) and non-selective permeation profiles (≥25 times less selective for K^+ than Kv channels) remain largely unknown. Although I_h has been functionally linked to biological processes from the autonomous beating of the heart to pain transmission, the underlying mechanistic actions remain largely inferential and, indeed, somewhat controversial due to the slow kinetics and negative operating voltage range relative to those of the bioelectrical events involved (e.g., cardiac pacing). This article reviews the current state of our knowledge in the structure-function properties of HCN channels in the context of their physiological functions and potential HCN-based therapies via bioengineering.

Key words: HCN channel — Physiology — Bioengineering — Gene transfer — Stem cells

Introduction

Pacemaker activity, the generation of spontaneous cellular electrical rhythms, governs numerous biological processes from the autonomous beating of the heart to respiratory rhythms and sleep cycles. In the heart, abnormal pacing leads to various forms of arrhythmias (e.g., sick sinus syndrome). I_f (or I_h), a diastolic depolarizing current activated by hyperpolarization, is a key player in cardiac pacing. Despite the fact that I_f has been recognized for over 25 years, the encoding genes, collectively known as the hyperpolarization-activated cyclic-nucleotide-gated (HCN) or the so-called pacemaker channel gene family, were only cloned in 1998. To date, four isoforms, namely HCN1 to 4, have been identified. These isoforms exhibit different patterns of gene expression and tissue distribution (Ludwig et al., 1998; Santoro et al., 1998; Santoro & Tibbs, 1999; Santoro et al., 2000; Moosmang et al., 2001), and co-assemble to form heteromeric complexes (except between HCN2 and HCN3) that underlie the native I_f (Chen, Wang & Siegelbaum, 2001; Ulens & Tytgat, 2001; Xue, Marban & Li, 2002; Er et al., 2003). HCN1 is the most abundant isoform in the brain, and is substantially expressed in the sino-atrial (SA) node (but not in the ventricles or atria) of the heart. While HCN3 is present in the central nervous system (CNS) but absent in the heart, HCN2 and HCN4 are found in both. When heterologously expressed, HCN1-4 channels have distinct cAMP sensitivities and gating properties. Of the two predominant isoforms found in the SA node, time-dependent HCN1 currents are ~40 times faster than those of HCN4 channels (Ludwig et al., 1999b; Seifert et al., 1999; Ishii, Takano & Ohmori, 2001; Altomare et al., 2003; Stieber et al., 2003a).

Membrane Topology of HCN Channels

Structurally, HCN channels belong to the super-family of ion channels consisting of four homologous domains pseudo-symmetrically arranged around a central pore. K^+ channels are known to consist of four monomeric subunits (Doyle et al., 1998). For voltage-gated channels, each of the four internal

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repeats is made up of six transmembrane segments, namely, S1-S6 (Fig. 1). The S4 segment carries a ribbon of positively-charged amino acids regularly spaced from one another by two hydrophobic residues, and is the major component of the voltage-sensing apparatus (Yellen, 1998b; Bezanilla, 2000;). S4 moves in response to changes in the transmembrane potential to induce channel opening or closing (Yellen, 1998b; Bezanilla, 2000;). The region between S5 and S6, or the so-called P-loop, inserts back into the membrane to form part of the pore. The P-loops exhibit striking sequence conservation within a given family of channels with similar ionic selectivity, but not among families of different selectivities. For example, Na^+ channels from the electric eel and human have similar P-loops. In fact, all known K^+-selective channels contain the threonine-valine-glycine-tyrosine-glycine (TVGYG) signature motif, except in rare occasions such as the HERG K^+ channels whose middle position is occupied by the conservative aromatic variant phenylalanine. Indeed, the pore is analogous to the active site of an enzyme where major functional (e.g., ionic selectivity, conductance and gating) and pharmacological properties are determined (Li & Tomaselli, 2004). Although HCN channels have adopted the same basic structural design motif and share substantial homology with depolarization-activated voltage-gated K^+ (K_v) channels of the erg, eag, elk and plant KAT1 families in particular, all with fundamental building blocks of 6 transmembrane monomers, two distinct features set them apart: 1) HCN channels are non-selectively permeate by Na^+ and K^+ (with a ratio of 1:4 vs. $\leq$ 1:100 of K^+channels), despite the fact that their pore contains the GYG motif; 2) HCN channels are activated by hyperpolarization rather than depolarization even though they also contain a positively-charged S4 like their depolarization-activated counterparts. The molecular features underlying these vast phenotypic differences have been subjects of much speculation (Ludwig et al., 1999a; Santoro & Tibbs, 1999; Stevens et al., 2001; Chen, Piper & Sanguinetti, 2002). In addition, HCN channels also contain in their C-termini a cyclic-nucleotide binding domain (CNBD) homologous to those found in cyclic-nucleotide gated channels (Zagotta, 1996). CNBD is responsible for accelerating HCN current kinetics by positively shifting steady-state activation upon cAMP binding to increase channel activity, and subsequently the rhythmic firing rate that it modulates (Wainger et al., 2001)

Hyperpolarization-activated Opening of HCN Channels

For classical depolarization-activated channels such as voltage-gated Na^+, Ca^{2+} and K^+ channels, previous functional studies have established that the positively charged S4 moves outward upon membrane depolarization to induce the transition of channels from a closed conformation to an ion-conducting open state by opening the activation gate (*see*, Yellen, 1998a, and Bezanilla, 2000, for review). Hyperpolarization causes S4 to return to its inward position, thereby shutting the gate (deactivation). Crystal structures of the voltage-dependent K^+ channel Kv1.2 further show that the voltage sensors are essentially independent domains inside the membrane (Long, Campbell & Mackinnon, 2005). During the process of voltage-sensing, they exert mechanical effects on the pore by constricting or dilating the inner pore-lining S6 inner helices via the S4-S5 linker helices. Opposite to the depolarization-activated counterparts, HCN channels are activated by hyperpolarization but deactivated upon depolarization. The mechanism underlying this fundamental gating difference is unknown. Using cysteine scanning mutagenesis, Larsson and colleagues (Mannikko, Elinder & Larsson, 2002) provided the first evidence that the voltage-sensing mechanisms of the sea urchin sperm HCN (spHCN) and classical Kv channels are conserved (i.e., the spHCN S4 moves outward and inward during depolarization and hyperpolarization, respectively). Similar S4 movements have also been demonstrated in mammalian HCN (Bell et al., 2004) and KAT1 (Latorre et al., 2003) channels. Various lines of evidence suggest that the S4-S5 linker and the C-linker come into close proximity during gating (Prole & Yellen, 2006). More recently, cooperativity between single HCN channels has also been reported (Dekker & Yellen, 2006). However, the molecular coupling between such conserved S4 movements and the opposite gating behaviors of HCN channels remains unresolved although a moving hinge mechanism has been proposed (Henrikson et al., 2002).

Not only does the S4 voltage-sensing domain of HCN channels prominently influence gating (Chen et al., 2000; Vaca et al., 2000; Mannikko et al., 2002;), the HCN pore is also coupled to activation and deactivation in a manner analogous to those observed in K_v and Na_v channels (Xue & Li, 2002; Azene, Xue & Li, 2003; Azene et al., 2005a;). The external S1-S2, S3-S4 linkers also influence gating (Ishii et al., 2001; Henrikson et al., 2002; Lesso & Li, 2003; Stieber et al., 2003b; Tsang, Lesso & Li, 2004a, 2004b), and may underlie isoform-specific gating properties.

Permeation and Conduction Characteristics of HCN Channels

Although native I_f recorded from different tissues and heterologously expressed HCN1-4 channels exhibit considerable differences in gating properties, their permeation and conduction properties are much less

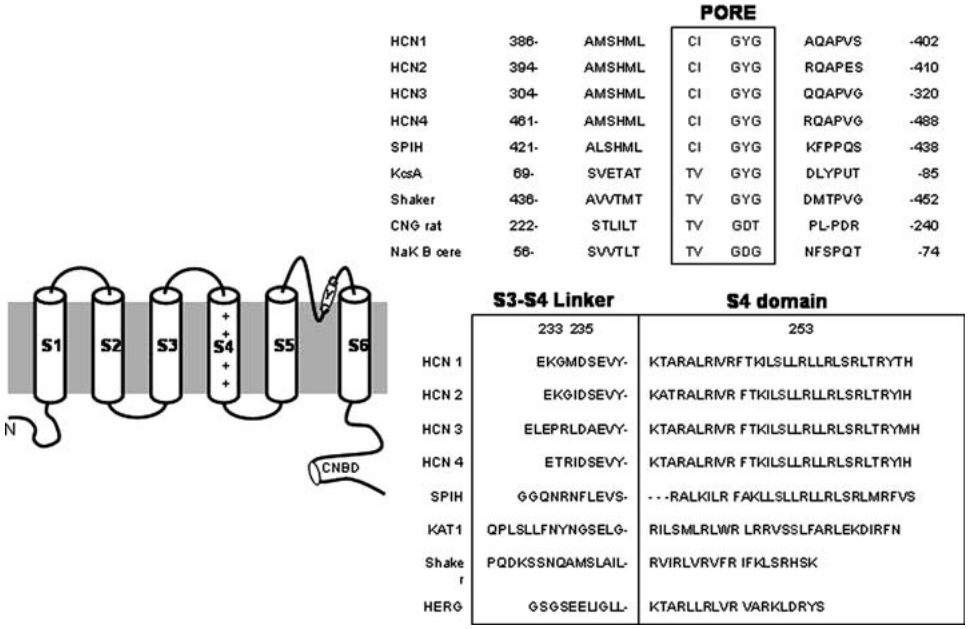

Fig. 1. An HCN monomeric subunit. The approximate locations of the TVGYG motif and the cyclic nucleotide-binding domain (CNBD) are highlighted. Sequence comparison (*middle* and *right*) of the S5-S6, the P-loops and the S4 of various HCN and K^+ channels. The HCN S4 is larger than those of depolarization-activated voltage-gated channels.

variable. All currents reported are cation-selective but only weakly selective for K^+ over Na^+, with permeability ratios of about 4:1 in spite of the presence of the signature GYG sequence in the pore (Zagotta & Siegelbaum, 1996; Ludwig et al., 1999a; Santoro & Tibbs, 1999). Under physiological conditions, the native currents are mostly carried by Na^+ due a greater electrochemical gradient. Like in K^+ channels, GYG is also a prerequisite for ion conduction of HCN channels (Xue et al., 2002; Er et al., 2003). Sharing the basic architectural plan with voltage-gated K^+ channels and bacterial K^+ channel KcsA, the ion-conducting pore of the HCN channels is lined by the re-entrant P-loop and the transmembrane segment S6. Review of the KcsA channel crystal structure shows that the narrow and highly selective filter is formed by four TVGYG sequences, each from one subunit; the carbonyl oxygen atoms in the peptides from the TVGYG residues together with the hydroxyl oxygen atom from the threonine point towards the center of the conduction pathway to form four ion-binding sites that stabilize the nearly completely dehydrated potassium ions (Doyle et al., 1998). Figure 1 shows the partial sequence alignment comparing the HCN channel with other members in the K^+ channel family. Despite the presence of GYG sequence, the difference in sequence in the immediate vicinity of the GYG signature has been shown to have important implications in the K^+ channel ion selectivity (Heginbotham et al., 1994). A recent crystal structure study on a NaK channel from *Bacillus cereus* (Shi et al., 2006), a non-selective Na^+ and K^+ channel, showed sequence similar to the KcsA channel except for its selectivity filter which resembled the cyclic-nucleotide-gated (CNG) channel, another member of the K^+ channel family that may give insight on the HCN channel ion selectivity. Both the bacterial NaK channel and the CNG channel have the tyrosine residue in the TVGYG sequence of the selectivity filter replaced by aspartate (Figure 1). This results in the change of two K^+ binding sites in the KcsA channel to a vestibule, which may account for the loss of ion selectivity. In the case of the HCN channels, the highly conserved threonine and valine in the TVGYG sequence, whose carbonyl oxygens form the ion stabilizer in most K^+ channels, are replaced consistently by cysteine and isoleucine, respectively, in all 4 isoforms. These changes may alter the backbone structure and indirectly alter the binding sites, contributing to the loss of selectivity for K^+ ions in the HCN channels.

Although relatively non-selective for K^+ and impermeable to anions, permeation of HCN channels, however, depends strongly on both external K^+ and Cl^-. In fact, HCN channels do not conduct inward currents in the complete absence of K^+ or when external Cl^- is replaced by large organic ions. The mechanism that underlies the effects of K^+ and Cl^- on permeation is unknown. Non-conductance could be secondary to changes in gating. Alternatively, it could result from direct high-affinity binding of other permeant ions to the pore in the absence of K^+ and Cl^-, thereby plugging the conduction pathway. Nonetheless, HCN1-4 have similar pharmacological profiles. For instance, the four

isoforms are inhibited by Cs^+ and the organic blocker ZD7288 but completely insensitive to other known K^+ channel blockers such as Ba^{2+} and tetraethylammonium (TEA^+) (Azene et al., 2005a).

Physiology

HCN1, HCN2, and HCN4 are expressed in the heart. Indeed, I_f is one of the key players that prominently modulate the rhythmic firing activity of nodal pacemaker cells (Robinson & Siegelbaum, 2003). Perhaps, it does not come as a surprise that the SA node, a highly specialized cardiac tissue consisting of only a few thousand pacemaker cells that paces the heart, expresses I_f with the highest density and the most positive activation profile. At the mRNA level, HCN4 accounts for over 80% of the total HCN transcripts found in the nodal cells. A familial form of sinus bradycardia associated with a mis-sense HCN mutation has been reported (Milanesi et al., 2006). Although I_f is most abundant in the SA node, it is also found at various levels in the atrio-ventricular node, the Purkinje fibers, the atria and the ventricles (Cerbai, Barbieri & Mugelli, 1994; Cerbai et al., 1997, 2001; Hoppe et al., 1998; Fernandez-Velasco et al., 2003;). Indeed, the expression of I_f in the human atria has been suggested to be a potential contributor to atrial ectopy (Zorn-Pauly et al., 2004), while upregulated ventricular I_f in diseased states, such as heart failure, hypertrophy and hypertension, has been proposed to predispose the heart with associated arrhythmias (Cerbai et al., 1994, 1997, 2001; Hoppe et al., 1998; Fernandez-Velasco et al., 2003).

I_h is also abundantly expressed in central neurons, as well as peripheral neurons such as sensory neurons, mechanosensitive fibers, and dorsal root ganglion (Moosmang et al., 2001; Doan et al., 2004). In HCN2 knock-out mouse (Ludwig et al., 2003), there is a near-complete loss of I_h in the thalamocortical relay neurons associated with spontaneous absence seizure. Pathophysiologically, nerve injury in the dorsal root ganglion markedly increases I_h and results in pacemaker-driven spontaneous action potentials in the ligated nerve (Chaplan et al., 2003).

HCN channels have also been suggested to serve as proton receptors that are responsible for mediating the detection of sour taste by taste buds (Stevens et al., 2001). Lowering the extracellular pH causes a dose-dependent flattening of the activation curve and a positive voltage shift in the half-maximal activation voltage. Most recently, we have also described the presence of I_h in pancreatic β cells that mediates glucose-stimulated insulin secretion (El-kholy et al., 2007). Collectively, it is apparent that I_h is central to rhythmic electrical events, physiologically or pathophysiologically.

Mechanistic Role of I_f

Although I_f is known to be functionally important in pacing, its mechanistic action is largely inferential. For instance, I_f has been presumed to exert its effect on cardiac pacing by providing a time-dependent inward current to drive diastolic depolarization to the action potential threshold after each excitation cycle. However, HCN channels exhibit intrinsically slow kinetics and negative activation profiles relative to the time scale and the voltage range of cardiac pacing. In addition, neither overexpression of wild-type HCN1, HCN2 or HCN4 alone suffices to cause automaticity in normally quiescent adult left ventricular cardiomyocytes that lack I_f (Qu et al., 2001; Er et al., 2003; Xue et al., 2007). Albeit with a slow pacing rate, ventricular automaticity, on the other hand, could be achieved from a different approach by genetic suppression of inward rectifier K^+ current (I_{K1}), an antagonistic current of I_f (Miake, Marban & Nuss, 2003), implicating that I_f merely plays a secondary rather than an active role in generating electrical rhythms. Collectively, these observations make the physiological relevance of I_f to cardiac pacing somewhat controversial. Recently, we successfully induced automaticity in normally quiescent left ventricular cardiomyocytes (LVCM) by overexpressing I_f via somatic gene transfer of an engineered HCN1 construct so as to obtain a better understanding of the mechanistic role of I_f (Xue et al., 2007). Isolated LVCM from adult guinea pig injected with engineered HCN1-ΔΔΔ exhibit I_f-like current after subtraction of I_{K1} (Fig. 2*A* bottom), compared to control cells (Fig. 2*A* top). Automaticity is evident only in the HCN1-ΔΔΔ-transduced LVCM (Fig. 2*B* bottom) and not in the control (Fig. 2*B* top). More importantly, the induced pacing rate and the phase-4 slope of action potentials are proportional to the magnitude of I_f, suggesting that I_f plays a *primary* role in cardiac pacing. Although I_f and I_{K1} counterbalance each other within a certain voltage, I_{K1} hyperpolarizes the resting membrane potential, which in turn can result in higher opening probability of hyperpolarization-activated I_f. Thus, a fine balance between these membrane potential-determining currents is the key to automaticity induction and modulation.

Potential HCN-Based Therapies via Bioengineering

Normal heartbeats originate in the SA node and as a result, malfunctions of pacemaker cells due to disease or aging lead to various forms of arrhythmias (e.g., bradycardias or abnormally low heart rates). While conventional treatments of pharmacological intervention and/or implantation of electronic pacemakers are effective, they are also associated with

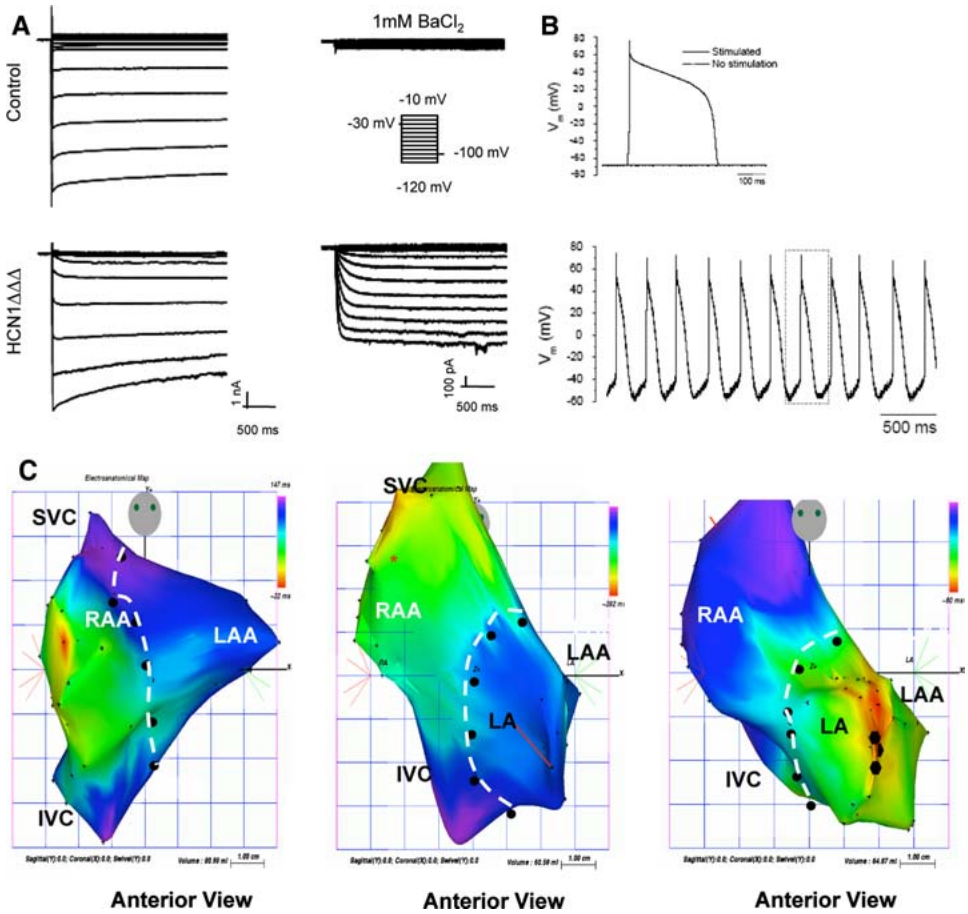

Fig. 2. HCN1-ΔΔΔ overexpression converted silent ventricular cardiomyocytes into electrically active pacemaker-like cells. (*A*) I_{K1} currents of control LVCM (*top left*) are comparable to HCN1-ΔΔΔ-transduced LVCM (*bottom left*), but I_f-like currents are evident only in the HCN1-ΔΔΔ-transduced LVCM (*bottom right*) with elimination of I_{K1} by Ba^{2+} compared to control (*top right*). (*B*) Action potential must be triggered by external stimulus in control LVCM (*top*), but automaticity uniquely exists in LVCM transduced with HCN1-ΔΔΔ (*bottom*). (*C*) Electroanatomic maps of control porcine heart with SA node pacing (*left*) and sick sinus syndrome (SSS) porcine heart with electronic right atrial pacing (*middle*) and SSS porcine heart with pacing by left atrium focally transduced with a recombinant adenovirus which overexpresses HCN1-ΔΔΔ (*right*) (Figures adapted from Tse et al., 2006).

significant risks (e.g., infection, hemorrhage, lung collapse and death) and expenses. Other disadvantages include limited battery life (replaced every 5-10 years), permanent implantation of catheters into the heart, and lack of autonomic neurohumoral responses, etc. Therefore, there is a great need to develop more effective, economical, and perhaps, biological alternatives. With an improved understanding of the molecular properties of HCN-encoded I_f, bioengineered pacemakers have become possible.

Several gene-based approaches have been explored to confer upon non-pacing cardiac muscle cells the ability to intrinsically generate rhythmic action potentials like the genuine electrically active nodal pacemaker cells. Since the four HCN isoforms co-assemble to form both homomeric and heteromeric channels, the molecular identity of endogenous I_f is complex. Furthermore, different interacting subunits and modulatory factors are known to present in different regions of the heart, but the accessory proteins of HCN have not been identified. Thus, the native currents are difficult to reproduce by genetic expression of a single HCN isoform. Indeed, overexpression of wild-type (WT) HCN1 (Xue et al., 2007) or HCN2 (Qu et al., 2001) channels alone in normally quiescent cardiac ventricular muscle cells are insufficient to cause cellular rhythmic oscillations, as measured by patch-clamp recordings. The hurdle is surmountable, however, by protein engineering. We first approached the problem mathematically by simulating the action potential, using a computational model to understand the contribution of I_f to pacing (Azene et al., 2005b). The numerical results yielded a number of biophysical criteria that must be satisfied for proper pacing to occur. With the necessary criteria in mind, we engineered pacemakers from non-pacing cardiomyocytes (Tse et al., 2006; Xue et al.,

2007) by transduction of modified HCN1 construct with deleted residues EVY at position 235-7 in the S3-S4 linker (HCN1-ΔΔΔ) to favor channel activation (Henrikson et al., 2003; Lesso & Li, 2003; Tsang et al., 2004a, b). This engineered-construct has been proven to produce pacing in vitro but more importantly in vivo. In a sick sinus syndrome porcine model, pacing of the heart was restored and originated from the site of focal transduction in the left atrium with HCN1-ΔΔΔ injection (Fig. 2*C*) (as opposed to the right atrium where the native SA node is anatomically located). The left atrium was strategically chosen to create a reverse conduction pattern. In other words, we were able to anatomically translocate the SA node from the right to the left atrium. A similar strategy of genetically engineering pacing cells with engineered HCN2 channels that favored activation has also been independently employed (Qu et al., 2003; Plotnikov et al., 2004; Bucchi et al., 2006). Collectively, these results demonstrate great potential for bioengineering pacemaker cells through genetic modification of HCN expression in non-pacing cells. Another potential platform for pacemaker gene delivery is the use of genetically engineered human embryonic stem cells or other stem cell types (e.g., mesenchymal stem cells). These cell-based strategies have been described and reviewed elsewhere (Gepstein, 2005; Xue et al., 2005; Li et al., 2006; Robinson et al., 2006).

Conclusion

Pacemaker activity, the generation of spontaneous cellular electrical rhythms, governs numerous biological processes from the autonomous beating of the heart to respiratory rhythms and sleep cycles. Not only is the biology of HCN channels intriguing but an improved understanding of this unique class of signaling proteins may also lead to the development of novel therapies for diseases that involve disrupted electrical rhythms (e.g., sinus node dysfunction).

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References

Altomare, C., Terragni, B., Brioschi, C., Milanesi, R., Pagliuca, C., Viscomi, C., Moroni, A., Baruscotti, M., DiFrancesco, D. 2003. Heteromeric HCN1-HCN4 channels: a comparison with native pacemaker channels from the rabbit sinoatrial node. *J. Physiol.* **549**:347–359

Azene, E., Xue, T., Li, R.A. 2003. Molecular basis of the effects of potassium of heterologously-expressed pacemaker (HCN) channels. *J. Physiol.* **547**:349–356

Azene, E.M., Sang, D., Tsang, S.Y., Li, R.A. 2005a. Pore-to-gate coupling of HCN channels revealed by a pore variant that contributes to gating but not permeation. *Biochem. Biophys. Res. Commun.* **327**:1131–1142

Azene, E.M., Xue, T., Marban, E., Tomaselli, G.F., Li, R.A. 2005b. Non-equilibrium behavior of HCN channels: insights into the role of HCN channels in native and engineered pacemakers. *Cardiovasc. Res.* **67**:263–273

Bell, D.C., Yao, H., Saenger, R.C., Riley, J.H., Siegelbaum, S.A. 2004. Changes in local S4 environment provide a voltage-sensing mechanism for mammalian hyperpolarization-activated HCN channels. *J. Gen. Physiol.* **123**:5–19

Bezanilla, F. 2000. The voltage sensor in voltage-dependent ion channels. *Physiol. Rev.* **80**:555–592

Bucchi A., Plotnikov A.N., Shlapakova I., Danilo P. Jr., Kryukova Y., Qu J., Lu Z., Liu H., Pan Z., Potapova I., Knight B.K., Girouard S., Cohen I.S., Brink P.R., Robinson R.B., Rosen M.R. 2006. Wild-type and mutant HCN channels in a tandem biological-electronic cardiac pacemaker. *Circulation.* **114**:992–999

Cerbai, E., Barbieri, M., Mugelli, A. 1994. Characterization of the hyperpolarization-activated current, I(f), in ventricular myocytes isolated from hypertensive rats. *J. Physiol.* **481**:585–591

Cerbai, E., Pino, R., Porciatti, F., Sani, G., Toscano, M., Maccherini, M., Giunti, G., Mugelli, A. 1997. Characterization of the hyperpolarization-activated current, I(f), in ventricular myocytes from human failing heart. *Circulation* **95**:568–571

Cerbai, E., Sartiani, L., DePaoli, P., Pino, R., Maccherini, M., Bizzarri, F., DiCiolla, F., Davoli, G., Sani, G., Mugelli, A. 2001. The properties of the pacemaker current I(F)in human ventricular myocytes are modulated by cardiac disease. *J. Mol. Cell Cardiol.* **33**:441–448

Chaplan, S.R., Guo, H.Q., Lee, D.H., Luo, L., Liu, C., Kuei, C., Velumian, A.A., Butler, M.P., Brown, S.M., Dubin, A.E. 2003. Neuronal hyperpolarization-activated pacemaker channels drive neuropathic pain. *J. Neurosci.* **23**:1169–1178

Chen, J., Mitcheson, J.S., Lin, M., Sanguinetti, M.C. 2000. Functional roles of charged residues in the putative voltage sensor of the HCN2 pacemaker channel. *J. Biol. Chem.* **275**:36465–36471

Chen, J., Piper, D.R., Sanguinetti, M.C. 2002. Voltage sensing and activation gating of HCN pacemaker channels. *Trends Cardiovasc. Med.* **12**:42–45

Chen, S., Wang, J., Siegelbaum, S.A. 2001. Properties of hyperpolarization-activated pacemaker current defined by coassembly of HCN1 and HCN2 subunits and basal modulation by cyclic nucleotide. *J. Gen. Physiol.* **117**:491–504

Dekker J.P., Yellen G. 2006. Cooperative gating between single HCN pacemaker channels. *J. Gen. Physiol.* **128**:561–567

Doan, T.N., Stephans, K., Ramirez, A.N., Glazebrook, P.A., Andresen, M.C., Kunze, D.L. 2004. Differential distribution and function of hyperpolarization-activated channels in sensory neurons and mechanosensitive fibers. *J. Neurosci.* **24**:3335–3343

Doyle, D.A., Morais Cabral, J., Pfuetzner, R.A., Kuo, A., Gulbis, J.M., Cohen, S.L., Chait, B.T., MacKinnon, R. 1998. The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* **280**:69–77

El-kholy, W., Xue, T., Diao, J., Chan, C., Cho, H., Wang, X., MacDonald, P., Tsushima, R., Wheeler, M., Li, R. 2004. Hyperpolarization-activated currents in pancreatic β cells modulate insulin secretion. *Biophys. J.* **86(Suppl.)**:293a

El-kholy W., MacDonald, P.E., Fox, J.M., Bhattacharjee, A., Xue T., Gao, X., Zhang, Y., Sticher, J., Li, R.A., Tsushima, R.G., wheeler, M.B. 2007. Hyperpolarization-activated cyclic nucleotide-gated channels in Pancreatic beta cells. *Mol. Endocrinol.* **21**(3):753–761

Er, F., Larbig, R., Ludwig, A., Biel, M., Hofmann, F., Beuckelmann, D.J., Hoppe, U.C. 2003. Dominant-negative suppression of HCN channels markedly reduces the native pacemaker current I(f) and undermines spontaneous beating of neonatal cardiomyocytes. *Circulation* **107**:485–489

Fernandez-Velasco, M., Goren, N., Benito, G., Blanco-Rivero, J., Bosca, L., Delgado, C. 2003. Regional distribution of hyperpolarization-activated current (If) and hyperpolarization-activated cyclic nucleotide-gated channel mRNA expression in ventricular cells from control and hypertrophied rat hearts. *J. Physiol.* **553**:395–405

Gepstein, L. 2005. Stem cells as biological heart pacemakers. *Expert Opin. Biol. Ther.* **5**:1531–1537

Heginbotham, L., Lu, Z., Abramson, T., MacKinnon, R. 1994. Mutations in the K^+ channel signature sequence. *Biophys. J.* **66**:1061–1067

Henrikson, C.A., Dong, P.H., Marban, E., Li, R.A., 2002 Molecular Basis of hyperpolarization-activated gating of pacemaker (HCN) channels. *Circulation.* **106**:226, 1137 Suppl. S

Henrikson C., Xue T., Dong P., Sang D., Marban E., Li R.A. 2002. Identification of a surface charged residue in the S3-S4 linker of pacemaker (HCN) channel that influences activation gating. *J. Biol. Chem.* **78**(16):13647–13654

Hoppe, U.C., Jansen, E., Sudkamp, M., Beuckelmann, D.J. 1998. Hyperpolarization-activated inward current in ventricular myocytes from normal and failing human hearts. *Circulation* **97**:55–65

Ishii, T.M., Takano, M., Ohmori, H. 2001. Determinants of activation kinetics in mammalian hyperpolarization-activated cation channels. *J. Physiol.* **537**:93–100

Latorre, R., Olcese, R., Basso, C., Gonzalez, C., Munoz, F., Cosmelli, D., Alvarez, O. 2003. Molecular coupling between voltage sensor and pore opening in the Arabidopsis inward rectifier K^+ channel KAT1. *J. Gen. Physiol.* **122**:459–469

Lesso, H., Li, R.A. 2003. Helical secondary structure of the external S3-S4 linker of pacemaker (HCN) channels revealed by site-dependent perturbations of activation phenotype. *J. Biol. Chem.* **278**:22290–22297

Li, R.A., Moore, J.C., Tarasova, Y.S., Boheler, K.R. 2006. Human embryonic stem cell-derived cardiomyocytes: therapeutic potentials and limitations. *J. Stem Cell* **1**:109–124

Li, R.A., Tomaselli, G.F. 2004. Using the deadly mu-conotoxins as probes of voltage-gated sodium channels. *Toxicon* **44**:117–122

Long, S.B., Campbell, E.B., Mackinnon, R. 2005. Voltage sensor of Kv1.2: structural basis of electromechanical coupling. *Science* **309**:903–908

Ludwig, A., Budde, T., Stieber, J., Moosmang, S., Wahl, C., Holthoff, K., Langebartels, A., Wotjak, C., Munsch, T., Zong, X., Feil, S., Feil, R., Lancel, M., Chien, K.R., Konnerth, A., Pape, H.C., Biel, M., Hofmann, F. 2003. Absence epilepsy and sinus dysrhythmia in mice lacking the pacemaker channel HCN2. *Embo J.* **22**:216–224

Ludwig, A., Zong, X., Hofmann, F., Biel, M. 1999a. Structure and function of cardiac pacemaker channels. *Cell Physiol. Biochem.* **9**:179–186

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

Ludwig, A., Zong, X., Stieber, J., Hullin, R., Hofmann, F., Biel, M. 1999b. Two pacemaker channels from human heart with profoundly different activation kinetics. *Embo J.* **18**:2323–2329

Mannikko, R., Elinder, F., Larsson, H.P. 2002. Voltage-sensing mechanism is conserved among ion channels gated by opposite voltages. *Nature* **419**:837–841

Miake, J., Marban, E., Nuss, H.B. 2003. Functional role of inward rectifier current in heart probed by Kir2.1 overexpression and dominant-negative suppression. *J. Clin. Invest.* **111**:1529–1536

Milanesi, R., Baruscotti, M., Gnecchi-Ruscone, T., DiFrancesco, D. 2006. Familial sinus bradycardia associated with a mutation in the cardiac pacemaker channel. *N. Engl. J. Med.* **354**:151–157

Moosmang, S., Stieber, J., Zong, X., Biel, M., Hofmann, F., 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

Plotnikov, A.N., Sosunov, E.A., Qu, J., Shlapakova, I.N., Anyukhovsky, E.P., Liu, L., Janse, M.J., Brink, P.R., Cohen, I.S., Robinson, R.B., Danilo, P., , Jr., Rosen, M.R. 2004. Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. *Circulation* **109**:506–512

Prole D.L., Yellen G. 2006. Reversal of HCN Channel Voltage Dependence via Bridging of the S4-S5 Linker and Post-S6. *J. Gen. Physiol.* **128**:273–282

Qu, J., Barbuti, A., Protas, L., Santoro, B., Cohen, I.S., Robinson, R.B. 2001. HCN2 overexpression in newborn and adult ventricular myocytes: distinct effects on gating and excitability. *Circ. Res.* **89**:E8–14

Qu, J., Plotnikov, A.N., Danilo, P., , Jr., Shlapakova, I., Cohen, I.S., Robinson, R.B., Rosen, M.R. 2003. Expression and function of a biological pacemaker in canine heart. *Circulation* **107**:1106–1109

Robinson, R.B., Brink, P.R., Cohen, I.S., Rosen, M.R. 2006. I(f) and the biological pacemaker. *Pharmacol. Res.* **53**:407–415

Robinson, R.B., Siegelbaum, S.A. 2003. Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu. Rev. Physiol.* **65**:453–480

Santoro, B., Chen, S., Luthi, A., Pavlidis, P., Shumyatsky, G.P., Tibbs, G.R., Siegelbaum, S.A. 2000. Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. *J. Neurosci.* **20**:5264–5275

Santoro, B., Liu, D.T., Yao, H., Bartsch, D., Kandel, E.R., Siegelbaum, S.A., Tibbs, G.R. 1998. Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. *Cell* **93**:717–729

Santoro, B., Tibbs, G.R. 1999. The HCN gene family: molecular basis of the hyperpolarization-activated pacemaker channels. *Ann. N. Y. Acad. Sci.* **868**:741–64

Seifert, R., Scholten, A., Gauss, R., Mincheva, A., Lichter, P., Kaupp, U.B. 1999. Molecular characterization of a slowly gating human hyperpolarization-activated channel predominantly expressed in thalamus, heart, and testis. *Proc. Natl. Acad. Sci. U S A* **96**:939193–6

Shi, N., Ye, S., Alam, A., Chen, L., Jiang, Y. 2006. Atomic structure of a Na^+- and K^+-conducting channel. *Nature* **440**:570–574

Stevens, D.R., Seifert, R., Bufe, B., Muller, F., Kremmer, E., Gauss, R., Meyerhof, W., Kaupp, U.B., Lindemann, B. 2001. Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. *Nature* **413**:631–5

Stieber, J., Herrmann, S., Feil, S., Loster, J., Feil, R., Biel, M., Hofmann, F., Ludwig, A. 2003a. The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart. *Proc. Natl. Acad. Sci. U S A* **100**:15235–15240

Stieber J., Thomer A., Much B., Schneider A., Biel M., Hofmann F. 2003b. Molecular basis for the different activation kinetics of the pacemaker channels HCN2 and HCN4. *J. Biol. Chem.* **278:**33672–33680

Tsang, S.Y., Lesso, H., Li, R.A. 2004a. Critical intra-linker interactions of HCN1-encoded pacemaker channels revealed by interchange of S3-S4 determinants. *Biochem. Biophys. Res. Commun.* **322**:652–658

Tsang, S.Y., Lesso, H., Li, R.A. 2004b. Dissecting the structural and functional roles of the S3-S4 linker of pacemaker (hyperpolarization-activated cyclic nucleotide-modulated) channels by systematic length alterations. *J. Biol. Chem.* **279**:43752–3759

Tse H.F., Xue T., Lau C.P., Siu C.W., Wang K., Zhang Q.Y., Tomaselli G.F., Akar F.G., Li R.A. 2006. Bioartificial Sinus Node Constructed via In Vivo Gene Transfer of an Engineered Pacemaker HCN Channel Reduces the Dependence on Electronic Pacemaker in a Sick-Sinus Syndrome Model. *Circulation*

Ulens, C., Tytgat, J. 2001. Functional heteromerization of HCN1 and HCN2 pacemaker channels. *J Biol Chem* **276**:6069–72

Vaca, L., Stieber, J., Zong, X., Ludwig, A., Hofmann, F., Biel, M. 2000. Mutations in the S4 domain of a pacemaker channel alter its voltage dependence. *FEBS Lett* **479**:35–40

Wainger, B.J., DeGennaro, M., Santoro, B., Siegelbaum, S.A., Tibbs, G.R. 2001. Molecular mechanism of cAMP modulation of HCN pacemaker channels. *Nature* **411**:805–810

Xue, T., Cho, H.C., Akar, F.G., Tsang, S.Y., Jones, S.P., Marban, E., Tomaselli, G.F., Li, R.A. 2005. Functional integration of electrically active cardiac derivatives from genetically engineered human embryonic stem cells with quiescent recipient ventricular cardiomyocytes: insights into the development of cell-based pacemakers. *Circulation* **111**:11–20

Xue, T., Li, R.A. 2002. An external determinant in the S5-P linker of the pacemaker (HCN) channel identified by sulfhydryl modification. *J. Biol. Chem.* **277**:46233–46242

Xue, T., Marban, E., Li, R.A. 2002. Dominant-negative suppression of HCN1- and HCN2-encoded pacemaker currents by an engineered HCN1 construct: insights into structure-function relationships and multimerization. *Circ. Res.* **90**: 1267–1273

Xue T., Siu C.W., Lieu D.K., Lau C.P., Tse H.F., Li R.A. 2007. Mechanistic role of If revealed by induction of ventricular automaticity via somatic gene transfer of gating-engineered pacemaker (HCN) channels. *Circulation* In press

Yellen, G. 1998a. The moving parts of voltage-gated ion channels. *Q. Rev. Biophys.* **31**:239–295

Yellen G. 1998b. Premonitions of ion channel gating. *Nat. Struct. Biol.* **5**:421

Zagotta, W.N. 1996. Molecular mechanisms of cyclic nucleotide-gated channels. *J. Bioenerg. Biomembr.* **28**:269–278

Zagotta, W.N., Siegelbaum, S.A. 1996. Structure and function of cyclic nucleotide-gated channels. *Annu. Rev. Neurosci.* **19**:235–263

Zorn-Pauly, K., Schaffer, P., Pelzmann, B., Lang, P., Machler, H., Rigler, B., Koidl, B. 2004. If in left human atrium: a potential contributor to atrial ectopy. *Cardiovasc. Res.* **64**:250–259