

The Pacemaker Current: From Basics to the Clinics

ANDREA BARBUTI, PH.D., MIRKO BARUSCOTTI, PH.D., and DARIO DIFRANCESCO, PH.D.

From the Laboratory of Molecular Physiology and Neurobiology, Department of Biomolecular Sciences and Biotechnology, University of Milan, Milano, Italy

Pacemaker Current. Activation of the pacemaker (“funny,” I_f) current during diastole is the main process underlying generation of the diastolic depolarization and spontaneous activity of cardiac pacemaker cells. I_f modulation by autonomic transmitters is responsible for the chronotropic regulation of heart rate. Given its role in pacemaking, I_f has been a major target of investigation aimed to exploit its rate-controlling function in a clinical perspective. In this short review, we describe some of the most recent clinically relevant applications of the concept of I_f -based pacemaking. (*J Cardiovasc Electrophysiol*, Vol. 18, pp. 342-347, March 2007)

pacemaker current, heart-rate lowering agents, bradycardia, biological pacemaker

The Pacemaker “Funny” Current of Cardiac Myocytes: Function and Modulation

Basic Properties

Heart rhythm initiation and regulation take place in the sinoatrial node (SAN), the pacemaker region of the heart. Cells from this region generate spontaneous action potentials that propagate to the whole cardiac muscle through specific pathways and drive rhythmic cardiac contraction.

Several cellular mechanisms concur to determine the pacemaking process. While the issue of relative contribution of individual mechanisms to pacemaker generation is still debated,¹⁻⁵ evidence collected in the almost three decades since I_f was first described in the SAN⁶ indicates that a major role in the initiation of the diastolic depolarization is played by the I_f (“funny”) current, so called because of its unusual properties.⁷⁻¹¹ These properties include channel opening upon hyperpolarization and a mixed sodium and potassium conductance yielding a reversal potential of about $-10/-20$ mV; in the SAN, I_f has a threshold of activation around $-40/-45$ mV and a half-activation voltage around $-65/-70$ mV,^{12,8} while in Purkinje fibres, the current is activated at more negative voltages in accordance with a more negative voltage range of diastolic depolarization (Fig. 1).

As well as generating spontaneous activity, I_f has also a role in the modulation of cardiac rhythm by autonomic neurotransmitters. Adrenergic agonists increase I_f in the pacemaker range of voltages by a shift of the current activation range to more positive voltages, which thus steepens diastolic (phase 4) depolarization rate and accelerates pace-

maker rhythm; muscarinic agonists slow rhythm by causing the opposite set of changes.¹²⁻¹⁴ Neurotransmitters act on I_f by modulating the cytoplasmic concentration of the second messenger cAMP, which increases upon β -adrenergic and decreases upon muscarinic stimulation; cAMP binds directly to the f-channel and increases the probability of channel opening.¹⁵

The molecular components of f-channels are the so-called Hyperpolarization-activated Cyclic Nucleotide-gated channels, coded for by a family of four genes (*HCN1-4*).^{9,10,16,17} HCN subunits are composed of six transmembrane domains (S1–S6), with the positively charged S4 domain acting as a voltage sensor and the domain between S5 and S6 acting as the conduction pathway and selectivity filter. cAMP binds to channels in the cyclic nucleotide binding domain (CNBD) located within the C-terminus. When expressed in heterologous systems, different isoforms can assemble to form either homotetramers or heterotetramers with voltage dependence and cAMP sensitivity qualitatively similar to, but quantitatively different from, those of native f-channels of the SAN.¹⁸⁻²⁰

Tissue Distribution of I_f in the Heart

The funny current was first described in the SAN⁶ and was extensively characterized originally in this tissue and in Purkinje fibres.^{12,21-25} However, f-channel expression in heart tissues is more widespread. In general, f-channels are densely expressed throughout the cardiac conduction system (SAN, atrioventricular node, Purkinje fibres), where they play an essential role in the generation of the diastolic depolarization responsible for spontaneous activity (Fig. 1).

During embryonic and neonatal cardiac development, f-channels are functionally expressed also in myocytes programmed to develop into working myocardial cells (atrial and ventricular myocytes). However, in adult ventricular and atrial myocytes, a clear-cut physiological role of I_f is not apparent: this is because either the channel density is very low, or the voltage range of channel activation is too negative, or both.^{26,27} Nevertheless, expression of funny channels in working myocardium may become important under cer-

This work was partly supported by MIUR-FIRB and CARIPO grants to Dario Difrancesco.

Address for correspondence: Dario DiFrancesco, Ph.D., Laboratory of Molecular Physiology and Neurobiology, Department of Biomolecular Sciences and Biotechnology, University of Milano, via Celoria 26, 20133 Milano, Italy. Fax: 39-02-5031-4932; E-mail: dario.difrancesco@unimi.it

doi: 10.1111/j.1540-8167.2006.00736.x

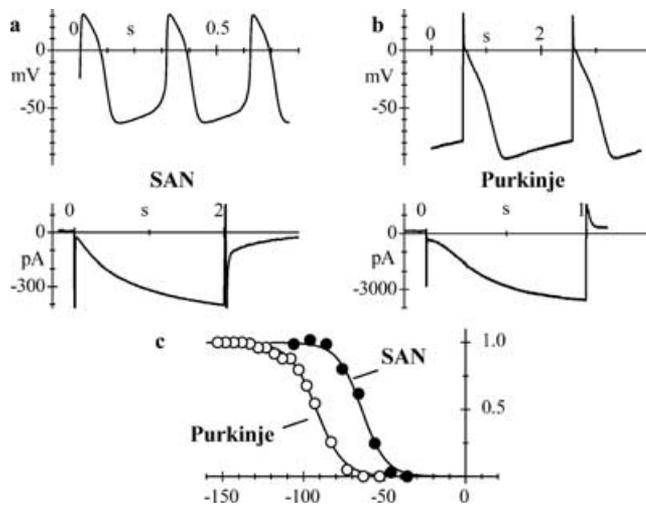


Figure 1. A, B: Sample action potentials (upper) and I_f current records (lower) in a SAN cell (A) and in a Purkinje fibre (B). C: Activation curves of I_f in a SAN cell and in a Purkinje fibre, as indicated. Note that the current is activated at more negative voltages in the Purkinje fibre than in the SAN.

tain pathological conditions. For example, I_f is upregulated in ventricular myocytes of hypertrophic hearts as a consequence of remodeling, leading to the hypothesis that it may contribute to arrhythmias in chronic hypertension and cardiac failure, a condition associated with increased risk for sudden cardiac death.²⁸

It is long known that cardiac fibers surrounding atrioventricular valves may display rhythmic activity.^{29,30} Beating cells from the region surrounding the mitral valve in the rabbit express a large I_f , indicating a correlation between spontaneous activity and the pacemaker current.³¹ More recently, the tissue surrounding canine and human pulmonary veins was shown to contain pacing cells expressing I_f .³²⁻³⁵ Fast atrial pacing appears to upregulate I_f and increase the potential of pulmonary vein cardiomyocytes to develop arrhythmias.³³ The evidence that atrial fibrillation is often triggered by electrical activity of the regions surrounding pulmonary veins thus suggests a possible arrhythmogenic contribution of I_f of pulmonary vein myocytes to atrial fibrillation.

Funny Channel Subunit Composition and Protein-Protein Interactions

The finding that different HCN isoforms have variable kinetics and cAMP sensitivity and that heteromeric assembly of two types of subunits generates channels with different properties^{18,20} supports the view that a variable isoform distribution and/or subunit composition might explain the changing properties of I_f in different cardiac regions (see SAN vs Purkinje fibre in Fig. 1). HCN isoforms are indeed differently distributed throughout the heart.³⁶

However, heterologous expression of various HCN isoforms, either alone or in combination, failed to reproduce native pacemaker current properties. For example, rabbit SAN myocytes express ~80% HCN4 and ~20% HCN1 mRNAs,³⁶ but neither the coexpression of these two isoforms nor the expression of covalently linked tandem constructs can reproduce native f-channel properties.²⁰ Also, HCN4 activates more slowly than HCN2 and with a half-maximum activation

voltage range about 10 mV less negative when expressed in either homologous (newborn ventricular myocytes) or heterologous (HEK293 cells) expression systems, but both isoforms activate some 16–19 mV more positively in myocytes than in HEK293 cells.³⁷

These data indicate that the HCN subunit composition cannot be the only determinant of the properties of native I_f , and that “context dependence” must play an important role in defining f-channel characteristics. “Context” dependence could, for example, arise from a specific cellular environment in which channels are preferentially located for more efficient interaction with accessory proteins and signaling molecules.

HCN channels do indeed interact with several proteins. Coexpression of KCNE2 with HCN1 and HCN2, for example, increases channel conductance and accelerates kinetics with no effect on voltage dependence, suggesting that KCNE2 acts as a β -subunit.^{38,39} Expression of KCNE2 with HCN4 causes a similar increase in conductance, but slows channel kinetics and shifts the activation curve to more negative voltages.⁴⁰

It has also been shown that HCN4 channels membrane localization involves interaction with caveolin-rich membrane microdomains (caveolae), specialized membrane compartments normally enclosing proteins involved in signal transduction pathways, and that disrupting this interaction deeply affects channel properties.⁴¹ More recently, we found that the confinement of pacemaker channels within membrane caveolae has a role in adrenergic modulation. Experimental evidence indeed indicates that adrenergic modulation of I_f and rate is better performed by β_2 than β_1 receptor stimulation, due to the colocalization of β_2 receptors and f-channels within caveolar spaces.⁴²

Clinical Relevance of I_f

Funny Channel Mutations Can Lead to Rhythm Disorders

Among the recent achievements of ion channel studies, a most important one is the finding of a continuously growing number of inherited diseases that are caused by defects of ion channel function.⁴³

Several channel mutations are responsible for cardiac diseases. This is not surprising because the contractile activity depends so strictly upon the proper working state of the many ion channels and receptors involved in action potential generation and excitation-contraction coupling. Cardiac channelopathies include several types of the Long QT syndrome, the Brugada syndrome, and the catecholaminergic polymorphic ventricular tachycardia to name a few, and involves K^+ channels α and β subunits, Na^+ channels, L-type Ca^{2+} channels, and the ryanodine receptor.⁴⁴⁻⁴⁶ Progress in the field of channelopathies is important since it provides basic knowledge for development of pharmacological therapies specifically targeted to an identified channel type.

HCN channels, the molecular components of native funny channel, were cloned later than K^+ , Na^+ , and Ca^{2+} channels, and “funny” channelopathies have therefore been investigated only recently. What might we expect from a defective funny channel? If funny channels are indeed important in the initiation of spontaneous activity and rate regulation, modifications of their functional properties should lead to disturbances of cardiac rhythm generation and/or

regulation. Animal studies had already indicated the possibility that f-channel mutations could be associated with defects of rhythm. For example, a recessive mutation in the zebrafish *Danio rerio* (slow mo) causing a reduced heart rate in the embryo was found to be associated with a strongly reduced I_f current.⁴⁷

Thus far, only few reports in humans have investigated rhythm disturbances related to mutations in HCN4, the HCN isoform of highest expression in SAN tissue.⁴⁸⁻⁵⁰

In the most recent study performed on a large (27-member) Italian family, a missense mutation in exon 7 of the *HCN4* gene was associated with a hereditary form of asymptomatic sinus bradycardia⁵⁰ (Fig. 2).

In this study, 15 individuals carrying the mutation had heart rates in the range 43–60 bpm, with a mean of 52 bpm, while 12 wild-type individuals had rates in the range 64–81 bpm, with a mean of 73 bpm. Bradycardia and the *HCN4* gene cosegregated tightly (two-point LOD score of 5.07–5.66 according to penetrance values ranging from 0.7 to 1). The mutated *HCN4* gene codes for a protein with a single aminoacid substitution in the CNBD (S672R), near the cAMP binding site (Fig. 2B). The effect of the mutation was to shift the channel activation curve to more negative voltages (by about 5 mV for heteromeric wild-type/mutant channels, Fig. 2C) without affecting cAMP-induced activation. Thus, a single-point mutation in HCN4 is responsible for a constitutive new biophysical channel property. A leftward shift of the activation curve mimics the effects of a mild vagal stimulation¹⁴: with a reduced inward current, the diastolic depolarization is less steep, leading to a prolonged beat-to-beat interval and, thus, to bradycardia. Since several other mutations can modify HCN4 kinetics,⁵¹ the S672R mutation may represent a special case of a broader mechanism for bradycardia/tachycardia based on HCN4 kinetic alterations.

In general, these results illustrate that the correlation between the funny current and heart rate is not only relevant to normal physiological conditions, but also has important consequences in the framework of cardiac pathologies involving rhythm disturbances. Below, we discuss another important aspect of this correlation, one where pathological expression of HCN channels in ventricular muscle may have arrhythmogenic potential.

I_f and Hypertrophy

Funny channels are abundantly expressed in all cardiac regions during fetal and neonatal stages of heart development, but soon after birth, their expression in the working myocardium decreases and the voltage range of activation shifts to voltages more negative than the resting potential; under these conditions, the I_f current does not contribute to normal electrical activity.^{26,27}

However, under specific pathological conditions such as cardiac hypertrophy and failure, the membrane expression of f-channels can again increase. This has been shown, for example, in animal studies of spontaneously hypertensive rats (SHR), a well-characterized model of cardiac hypertrophy caused by pressure overload where animals develop a severe left ventricular hypertrophy over the course of their life. Ventricular cells from old SHRs have action potentials with a clear diastolic depolarization and a much higher I_f current density than aged-matched cells from normotensive

animals^{52,53}; the larger I_f was found to correlate with an increased expression of the HCN2 and HCN4 subunits of the pacemaker channel.⁵⁴

Pathological alterations of cardiac function may lead to modification of I_f also in humans. The properties of I_f in myocytes isolated from explanted human hearts with dilated or ischemic cardiomyopathy were similar to those of I_f in hypertrophied rats.⁵⁵⁻⁵⁷ In patch-clamp experiments, I_f was recorded more frequently in myocytes from dilated or ischemic hearts than in control myocytes, and the current density was higher (even though the increment was not always significant)⁵⁶; also, the I_f activation range was more depolarized in diseased than in control myocytes.⁵⁷

Along with a reduced expression of inwardly rectifying K^+ current shown to occur in human failing hearts,^{56,58} the higher expression of functional f-channels is bound to increase electrical instability, which, especially under the elevated β -adrenergic signaling typical of these pathological conditions, might contribute to trigger fatal arrhythmias.

I_f as a Preferential Pharmacological Target for Chronic Stable Angina

Common pharmacological interventions aimed to reduce heart rate are based on drugs targeted to block the β -adrenergic cascade and/or reduce the L-type Ca^{2+} current. Unfortunately, in addition to effectively reducing cardiac chronotropism, these drugs also induce unwanted side effects, typically a substantial reduction of the contractile force of working myocardium (negative inotropism) due to inhibition of Ca^{2+} entry. In the absence of alternative pharmacological interventions and despite the adverse side effects, β -blockers and Ca^{2+} antagonists are of large use in therapies requiring heart rate reduction. It is therefore not surprising that researchers working in the field of experimental cardiac pharmacology have long sought to find more specific heart-rate lowering agents, that is, molecules whose spectrum of action target more specifically the molecular mechanisms that control pacemaker rate. Given its primary role in pacemaking, drugs able to interfere selectively with the funny current have therefore been actively investigated since the current was first described in 1979.⁶

Several heart rate-reducing drugs with f-channel blocking properties have been developed. Early such substances include alnidine (the N-allyl-derivative of clonidine), zatebradine (UL-FS49), cilobradine (DK-AH26) and falipamil (AQ-A39) (compounds originally derived from the Ca^{2+} channel blocker verapamil), and ZD7288. These substances cause a reduction of the diastolic depolarization slope in pacemaker cells, hence a slowing of cardiac rate, by blocking I_f .⁵⁹

Ivabradine (S16257), a more recently developed compound, is at present the only member of the family of heart-rate-reducing agents having completed clinical development for chronic stable angina,⁶⁰ and is now commercially available in several European countries. Efficacy against angina was investigated in a double-blind, placebo-controlled study of patients with stable angina, showing improvements in exercise tolerance and time of onset of exercise-induced ischemia⁶¹ and in noninferiority tests of ivabradine versus atenolol.⁶² The action of ivabradine is characterized by a specific block of I_f , with no appreciable effects on Ca^{2+} or K^+ currents at concentrations affecting I_f . This results in a selective reduction of the steepness of diastolic depo-

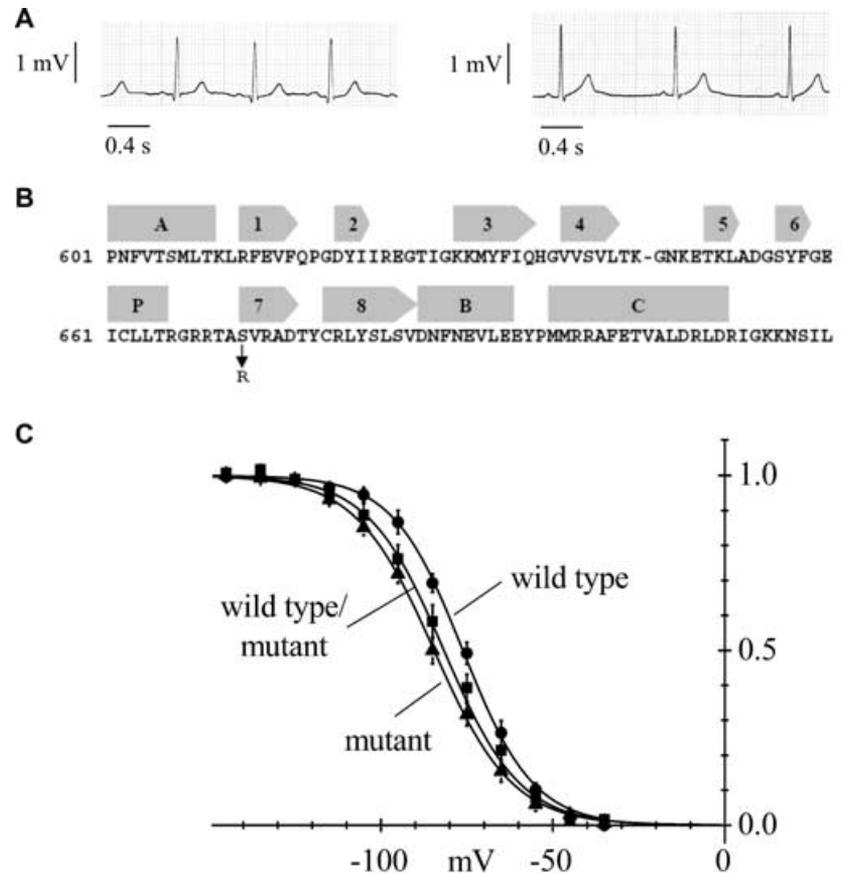


Figure 2. A: ECGs from a wild-type individual (left) and a bradycardic individual from the same family carrying the heterozygous mutation S672R (right). B: Sequence of the CNBD of hHCN4 indicating the site of the point mutation S672R (arrow). C: Mean activation curves of wild-type hHCN4 channels, homozygous mutant S672R channels and heterozygous wild-type-mutant channels expressed in HEK293 cells. The wild-type-mutant curve is shifted by about 5 mV to the left relative to the wild-type curve. Panels a and c are modified from Ref. 50 (with permission).

larization, hence of rate slowing without changes of other action potential parameters,^{63,64} which, in turn, is reflected by the lack of side effects on cardiovascular performance, and in particular lack of effects on myocardial contractility and corrected QT interval.⁶⁰ Also, the efficacy of specific heart-rate lowering by ivabradine to reduce morbidity–mortality in CAD patients with impaired left ventricular function is now under investigation in a large-scale multicenter study.⁶⁵

HCN-Based Biological Pacemakers

The funny channel blocking drugs discussed above are useful for inducing a controlled inhibition of cardiac pacemaking and slowing of heart rate. An obvious question arising is whether the f-channel properties can also be exploited for the opposite function, that is, to accelerate cardiac rhythm, or in fact even to induce *de novo* pacemaker activity in normally quiescent cells.

Since no pharmacological tools able to directly activate funny channels or increase their expression are presently available, a simple pharmacological approach is not applicable. However, genetic or cellular approaches based on *in situ* delivery of HCN channels can indeed be adopted to produce cell substrates able to pace an otherwise quiescent tissue, in the pursuit of strategies to develop “biological pacemakers” eventually able to replace the electronic devices used today.

Different strategies have, in fact, been used so far to generate an HCN-based “biological pacemaker:” (1) adenoviral-mediated HCN2 infection,^{66,67} (2) implant of mesenchymal stem cell (MSC) overexpressing HCN2 channels,⁶⁸ and (3)

implant of spontaneously beating human embryonic stem cells (hES)-derived cardiomyocytes.^{69,70}

In vitro pilot experiments⁷¹ have shown that adenoviral-mediated overexpression of the HCN2 isoform in primary neonatal ventricular cultures increases significantly the slow diastolic depolarization rate and the rate of spontaneous activity (from 48 to 88 bpm). Further *in vivo* studies confirmed that direct injection of HCN2-adenovirus into the left atrium⁶⁶ or into the left bundle-branch⁶⁷ of dogs could induce spontaneous activity. This was supported by evidence that following sinus rhythm suppression by vagal stimulation, spontaneous beating persisted and the heart rhythm was driven by activity originating from the site of injection. Patch-clamp analysis also showed a highly increased expression of I_f in cells isolated from left atrium or left bundle branch of dogs infected with HCN2 relative to control dogs.

Since adenoviral infection does not ensure stable expression of HCN channels and the use of viruses is not suitable in a clinical perspective, a second approach was attempted by use of human MSCs engineered to express high levels of HCN2 channels.⁶⁸ Due to expression of HCN2 channels, engineered MSCs behave as a source of pacemaker current able to elicit spontaneous activity in otherwise resting ventricular cells, as shown both in ventricular cultures and in whole hearts.⁷²

A third approach not requiring direct manipulation of HCN genes employs hES cells previously differentiated into spontaneously active cells.^{69,70} The differentiated hES cells are inherently more similar to native pacemaker cells of the SAN than engineered mesenchymal cells, since they are able

to beat spontaneously. Even though a direct involvement of the I_f current has not been investigated in these latter studies, it has been previously shown that hES-derived cardiomyocytes do not express inward rectifying currents, normally involved in setting a stable resting potentials, while they do express a substantial I_f current⁷³ that may contribute to the pacemaking mechanism.

Conclusions

A wealth of experimental evidence obtained in the nearly three decades following its discovery has firmly established the role of the funny current in the generation and control of pacemaker activity. Recently, the concept of the funny current-based pacemaking has developed into clinically relevant applications. First, specific reduction of heart rate by pharmacological block of f-channels represents an efficient therapeutical tool against chronic angina and is being investigated as a potential tool to reduce morbidity–mortality in CAD patients with/without heart failure. Second, f-channel mutations affecting channel function can lead to inherited arrhythmias, as shown, for example, by the finding that a specific point-mutation in HCN4 reduces inward current flow during diastole and causes sinus bradycardia⁵⁰; the search is on for other mutations affecting funny channel kinetics, since the one identified likely represents only a special case of a more general mechanism for rhythm disorders based on HCN altered function. Third, the concept of I_f -dependent spontaneous activity is at the basis of the development of newly engineered “biological” pacemakers.

Clearly, in the near future, understanding of finer molecular details will further help the acquisition of clinically relevant tools based on the f-channel-mediated control of cardiac chronotropism.

References

- Vinogradova TM, Bogdanov KY, Lakatta EG: beta-Adrenergic stimulation modulates ryanodine receptor Ca^{2+} release during diastolic depolarization to accelerate pacemaker activity in rabbit sinoatrial nodal cells. *Circ Res* 2002;90:73-79.
- DiFrancesco D, Robinson RB: Beta-modulation of pacemaker rate: Novel mechanism or novel mechanics of an old one? *Circ Res* 2002;90:E69-E69.
- Bucchi A, Baruscotti M, Robinson RB, DiFrancesco D: $I(f)$ -dependent modulation of pacemaker rate mediated by cAMP in the presence of ryanodine in rabbit sino-atrial node cells. *J Mol Cell Cardiol* 2003;35:905-913.
- Lipsius SL, Huser J, Blatter LA: Intracellular Ca^{2+} release sparks atrial pacemaker activity. *New Physiol Sci* 2001;16:101-106.
- Honjo H, Inada S, Lancaster MK, Yamamoto M, Niwa R, Jones SA, Shibata N, Mitsui K, Horiuchi T, Kamiya K, Kodama I, Boyett MR: Sarcoplasmic reticulum Ca^{2+} release is not a dominating factor in sinoatrial node pacemaker activity. *Circ Res* 2003;92:e41-e44.
- Brown HF, DiFrancesco D, Noble SJ: How does adrenaline accelerate the heart? *Nature* 1979;280:235-236.
- DiFrancesco D: The cardiac hyperpolarizing-activated current, if . Origins and developments. *Prog Biophys Mol Biol* 1985;46:163-183.
- DiFrancesco D: Pacemaker mechanisms in cardiac tissue. *Annu Rev Physiol* 1993;55:455-472.
- Accili EA, Proenza C, Baruscotti M, DiFrancesco D: From funny current to HCN channels: 20 years of excitement. *New Physiol Sci* 2002;17:32-37.
- Robinson RB, Siegelbaum SA: Hyperpolarization-activated cation currents: From molecules to physiological function. *Annu Rev Physiol* 2003;65:453-480.
- DiFrancesco D: Serious workings of the funny current. *Prog Biophys Mol Biol* 2006;90:13-25.
- DiFrancesco D, Ferroni A, Mazzanti M, Tromba C: Properties of the hyperpolarizing-activated current (if) in cells isolated from the rabbit sino-atrial node. *J Physiol (Lond)* 1986;377:61-88.
- DiFrancesco D, Tromba C: Muscarinic control of the hyperpolarization-activated current (if) in rabbit sino-atrial node myocytes. *J Physiol (Lond)* 1988;405:493-510.
- DiFrancesco D, Ducouret P, Robinson RB: Muscarinic modulation of cardiac rate at low acetylcholine concentrations. *Science* 1989;243:669-671.
- DiFrancesco D, Tortora P: Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. *Nature* 1991;351:145-147.
- Santoro B, Tibbs GR: The HCN gene family: Molecular basis of the hyperpolarization-activated pacemaker channels. *Ann N Y Acad Sci* 1999;868:741-764.
- Biel M, Ludwig A, Zong X, Hofmann F: Hyperpolarization-activated cation channels: A multi-gene family. *Rev Physiol Biochem Pharmacol* 1999;136:165-181.
- Viscomi C, Altomare C, Bucchi A, Camatini E, Baruscotti M, Moroni A, DiFrancesco D: C terminus-mediated control of voltage and cAMP gating of hyperpolarization-activated cyclic nucleotide-gated channels. *J Biol Chem* 2001;276:29930-29934.
- Ulens C, Tytgat J: Functional heteromerization of HCN1 and HCN2 pacemaker channels. *J Biol Chem* 2001;276:6069-6072.
- Altomare C, Terragni B, Brioschi C, Milanese R, Pagliuca C, Viscomi C, Moroni A, Baruscotti M, DiFrancesco D: Heteromeric HCN1-HCN4 channels: A comparison with native pacemaker channels from the rabbit sinoatrial node. *J Physiol (Lond)* 2003;549:347-359.
- Brown HF, DiFrancesco D: Voltage clamp investigations of membrane currents underlying pacemaker activity in rabbit sino-atrial node. *J Physiol (Lond)* 1980;308:331-351.
- DiFrancesco D, Ojeda C: Properties of the current if in the sino atrial node of the rabbit compared with those of the current $iK2$ in Purkinje fibres. *J Physiol (Lond)* 1980;308:353-367.
- DiFrancesco D: A new interpretation of the pace-maker current in calf Purkinje fibres. *J Physiol (Lond)* 1981;314:359-376.
- DiFrancesco D: A study of the ionic nature of the pace-maker current in calf Purkinje fibres. *J Physiol (Lond)* 1981;314:377-393.
- DiFrancesco D: Characterization of single pacemaker channels in cardiac sino-atrial node cells. *Nature* 1986;324:470-473.
- Robinson RB, Yu H, Chang F, Cohen IS: Developmental change in the voltage-dependence of the pacemaker current, if , in rat ventricle cells. *Pflug Arch Eur J Phy* 1997;433:533-535.
- Cerbai E, Pino R, Sartiani L, Mugelli A: Influence of postnatal-development on $I(f)$ occurrence and properties in neonatal rat ventricular myocytes. *Cardiovasc Res* 1999;42:416-423.
- Cerbai E, Mugelli A: $I(f)$ in non-pacemaker cells: Role and pharmacological implications. *Pharmacol Res* 2006;53:416-423.
- Wit AL, Fenoglio JJ Jr, Wagner BM, Bassett AL: Electrophysiological properties of cardiac muscle in the anterior mitral valve leaflet and the adjacent atrium in the dog. Possible implications for the genesis of atrial dysrhythmias. *Circ Res* 1973;32:731-745.
- Wit AL, Fenoglio JJ Jr, Hordof AJ, Reemtsma K: Ultrastructure and transmembrane potentials of cardiac muscle in the human anterior mitral valve leaflet. *Circulation* 1979;59:1284-1292.
- Anumonwo JM, Delmar M, Jalife J: Electrophysiology of single heart cells from the rabbit tricuspid valve. *J Physiol (Lond)* 1990;425:145-167.
- Chen YJ, Chen SA, Chang MS, Lin CI: Arrhythmogenic activity of cardiac muscle in pulmonary veins of the dog: Implication for the genesis of atrial fibrillation. *Cardiovasc Res* 2000;48:265-273.
- Chen YJ, Chen SA, Chen YC, Yeh HI, Chan P, Chang MS, Lin CI: Effects of rapid atrial pacing on the arrhythmogenic activity of single cardiomyocytes from pulmonary veins: Implication in initiation of atrial fibrillation. *Circulation* 2001;104:2849-2854.
- Greenwood IA, Prestwich SA: Characteristics of hyperpolarization-activated cation currents in portal vein smooth muscle cells. *Am J Physiol-Cell Ph* 2002;282:C744-C753.
- Perez-Lugones A, McMahon JT, Ratliff NB, Saliba WI, Schweikert RA, Marrouche NF, Saad EB, Navia JL, McCarthy PM, Tchou P, Gillinov AM, Natale A: Evidence of specialized conduction cells in human pulmonary veins of patients with atrial fibrillation. *J Cardiovasc Electrophysiol* 2003;14:803-809.
- Shi W, Wymore R, Yu H, Wu J, Wymore RT, Pan Z, Robinson RB, Dixon JE, McKinnon D, Cohen IS: Distribution and prevalence of hyperpolarization-activated cation channel (HCN) mRNA expression in cardiac tissues. *Circ Res* 1999;85:e1-e6.

37. Qu J, Altomare C, Bucchi A, DiFrancesco D, Robinson RB: Functional comparison of HCN isoforms expressed in ventricular and HEK 293 cells. *Pflug Arch Eur J Phys* 2002;444:597-601.
38. Yu H, Wu J, Potapova I, Wymore RT, Holmes B, Zuckerman J, Pan Z, Wang H, Shi W, Robinson RB, El Maghrabi MR, Benjamin W, Dixon J, McKinnon D, Cohen IS, Wymore R: MinK-related peptide 1: A beta subunit for the HCN ion channel subunit family enhances expression and speeds activation. *Circ Res* 2001;88:E84-E87.
39. Qu J, Kryukova Y, Potapova IA, Doronin SV, Larsen M, Krishnamurthy G, Cohen IS, Robinson RB: MiRP1 modulates HCN2 channel expression and gating in cardiac myocytes. *J Biol Chem* 2004;279:43497-43502.
40. Decher N, Bundis F, Vajna R, Steinmeyer K: KCNE2 modulates current amplitudes and activation kinetics of HCN4: Influence of KCNE family members on HCN4 currents. *Pflug Arch Eur J Phys* 2003;466:633-640.
41. Barbuti A, Gravante B, Riolfo M, Milanese R, Terragni B, DiFrancesco D: Localization of pacemaker channels in lipid rafts regulates channel kinetics. *Circ Res* 2004;94:1325-1331.
42. Barbuti A, Terragni B, Brioschi C, DiFrancesco D: Localization of f-channels to caveolae mediates specific beta(2)-adrenergic receptor modulation of rate in sinoatrial myocytes. *J Mol Cell Cardiol* 2007;42:71-78.
43. Ashcroft FM: *Ion Channels and Disease*. New York: Academic Press; 2000.
44. Sanguinetti MC, Tristani-Firouzi M: hERG potassium channels and cardiac arrhythmia. *Nature* 2006;440:463-469.
45. Priori SG, Schwartz PJ, Napolitano C, Bloise R, Ronchetti E, Grillo M, Vicentini A, Spazzolini C, Nastoli J, Bottelli G, Folli R, Cappelletti D: Risk stratification in the long-QT syndrome. *N Engl J Med* 2003;348:1866-1874.
46. Priori SG, Napolitano C, Tiso N, Memmi M, Vignati G, Bloise R, Sorrentino V, Danielli GA: Mutations in the cardiac ryanodine receptor gene (hRyR2) underlie catecholaminergic polymorphic ventricular tachycardia. *Circulation* 2001;103:196-200.
47. Baker K, Warren KS, Yellen G, Fishman MC: Defective "pacemaker" current (I_h) in a zebrafish mutant with a slow heart rate. *Proc Natl Acad Sci USA* 1997;94:4554-4559.
48. Schulze-Bahr E, Neu A, Friederich P, Kaupp UB, Breithardt G, Pongs O, Isbrandt D: Pacemaker channel dysfunction in a patient with sinus node disease. *J Clin Invest* 2003;111:1537-1545.
49. Ueda K, Nakamura K, Hayashi T, Inagaki N, Takahashi M, Arimura T, Morita H, Higashiesato Y, Hirano Y, Yasunami M, Takishita S, Yamashina A, Ohe T, Sunamori M, Hiraoka M, Kimura A: Functional characterization of a trafficking-defective HCN4 mutation, D553N, associated with cardiac arrhythmia. *J Biol Chem* 2004;279:27194-27198.
50. Milanese R, Baruscotti M, Gnecci-Ruscone T, DiFrancesco D: Familial sinus bradycardia associated with a mutation in the cardiac pacemaker channel. *N Engl J Med* 2006;354:151-157.
51. Vaca L, Stieber J, Zong X, Ludwig A, Hofmann F, Biel M: Mutations in the S4 domain of a pacemaker channel alter its voltage dependence. *FEBS Lett* 2000;479:35-40.
52. Cerbai E, Barbieri M, Mugelli A: Characterization of the hyperpolarization-activated current, I(f), in ventricular myocytes isolated from hypertensive rats. *J Physiol (Lond)* 1994;481(Pt 3):585-591.
53. Cerbai E, Barbieri M, Mugelli A: Occurrence and properties of the hyperpolarization-activated current I_f in ventricular myocytes from normotensive and hypertensive rats during aging. *Circulation* 1996;94:1674-1681.
54. Fernandez-Velasco M, Goren N, Benito G, Blanco-Rivero J, Bosca L, Delgado C: Regional distribution of hyperpolarization-activated current (I_f) and hyperpolarization-activated cyclic nucleotide-gated channel mRNA expression in ventricular cells from control and hypertrophied rat hearts. *J Physiol (Lond)* 2003;553:395-405.
55. Cerbai E, Pino R, Porciatti F, Sani G, Toscano M, Maccherini M, Giunti G, Mugelli A: Characterization of the hyperpolarization-activated current, I(f), in ventricular myocytes from human failing heart. *Circulation* 1997;95:568-571.
56. Hoppe UC, Jansen E, Sudkamp M, Beuckelmann DJ: Hyperpolarization-activated inward current in ventricular myocytes from normal and failing human hearts. *Circulation* 1998;97:55-65.
57. Cerbai E, Sartiani L, DePaoli P, Pino R, Maccherini M, Bizzarri F, DiCiolla F, Davoli G, Sani G, Mugelli A: The properties of the pacemaker current I(f) in human ventricular myocytes are modulated by cardiac disease. *J Mol Cell Cardiol* 2001;33:441-448.
58. Beuckelmann DJ, Nabauer M, Erdmann E: Alterations of K⁺ currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ Res* 1993;73:379-385.
59. Baruscotti M, Bucchi A, DiFrancesco D: Physiology and pharmacology of the cardiac pacemaker ("funny") current. *Pharmacol Ther* 2005;107:59-79.
60. DiFrancesco D, Camm JA: Heart rate lowering by specific and selective I(f) current inhibition with ivabradine: A new therapeutic perspective in cardiovascular disease. *Drugs* 2004;64:1757-1765.
61. Borer JS, Fox K, Jaillon P, Lerebours G: Antianginal and antiischemic effects of ivabradine, an I(f) inhibitor, in stable angina: A randomized, double-blind, multicentered, placebo-controlled trial. *Circulation* 2003;107:817-823.
62. Tardif JC, Ford I, Tendera M, Bourassa MG, Fox K: Efficacy of ivabradine, a new selective I(f) inhibitor, compared with atenolol in patients with chronic stable angina. *Eur Heart J* 2005;26:2529-2536.
63. Bois P, Bescond J, Renaudon B, Lenfant J: Mode of action of bradycardic agent, S 16257, on ionic currents of rabbit sinoatrial node cells. *Br J Pharmacol* 1996;118:1051-1057.
64. DiFrancesco D: Funny channels in the control of cardiac rhythm and mode of action of selective blockers. *Pharmacol Res* 2006;53:399-406.
65. Fox K, Ferrari R, Tendera M, Steg PG, Ford I: Rationale and design of a randomized, double-blind, placebo-controlled trial of ivabradine in patients with stable coronary artery disease and left ventricular systolic dysfunction: The morbidity-mortality Evaluation of the I(f) inhibitor ivabradine in patients with coronary disease and left ventricular dysfunction (BEAUTIFUL) study. *Am Heart J* 2006;152:860-866.
66. Qu J, Plotnikov AN, Danilo P Jr, Shlapakova I, Cohen IS, Robinson RB, Rosen MR: Expression and function of a biological pacemaker in canine heart. *Circulation* 2003;107:1106-1109.
67. Plotnikov AN, Sosunov EA, Qu J, Shlapakova IN, Anyukhovsky EP, Liu L, Jansse MJ, Brink PR, Cohen IS, Robinson RB, Danilo P Jr, Rosen MR: Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. *Circulation* 2004;109:506-512.
68. Potapova I, Plotnikov A, Lu Z, Danilo P Jr, Valiunas V, Qu J, Doronin S, Zuckerman J, Shlapakova IN, Gao J, Pan Z, Herron AJ, Robinson RB, Brink PR, Rosen MR, Cohen IS: Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers. *Circ Res* 2004;94:952-959.
69. Kehat I, Khimovich L, Caspi O, Gepstein A, Shofti R, Arbel G, Huber I, Satin J, Itskovitz-Eldor J, Gepstein L: Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol* 2004;22:1282-1289.
70. Xue T, Cho HC, Akar FG, Tsang SY, Jones SP, Marban E, Tomaselli GF, Li RA: 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* 2005;111:11-20.
71. Qu J, Barbuti A, Protas L, Santoro B, Cohen IS, Robinson RB: HCN2 overexpression in newborn and adult ventricular myocytes: Distinct effects on gating and excitability. *Circ Res* 2001;89:E8-E14.
72. Rosen MR, Brink PR, Cohen IS, Robinson RB: Genes, stem cells and biological pacemakers. *Cardiovasc Res* 2004;64:12-23.
73. Satin J, Kehat I, Caspi O, Huber I, Arbel G, Itzhaki I, Magyar J, Schroder EA, Perlman I, Gepstein L: Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes. *J Physiol (Lond)* 2004;559:479-496.