Review

Funny channels in the control of cardiac rhythm and mode of action of selective blockers

Dario DiFrancesco*

University of Milano, Department of Biomolecular Sciences and Biotechnology, Laboratory of Molecular Physiology and Neurobiology, via Celoria 26, 20133 Milano, Italy

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Abstract

“Funny” (f) channels underlie the cardiac “pacemaker” If current, originally described as an inward current activated on hyperpolarization to the diastolic range of voltages in sino-atrial node myocytes [Brown, HF; DiFrancesco, D; Noble, SJ. How does adrenaline accelerate the heart? Nature 1979;280:235–236]. The involvement of funny channels in the generation and modulation of cardiac pacemaker activity has been amply demonstrated by thorough analysis since its discovery. The degree of funny current activation upon termination of an action potential determines the slope of diastolic depolarization, and hence pacemaker frequency; furthermore, If is under cAMP-mediated control by β-adrenergic and muscarinic stimulation and underlies the modulation of cardiac rate by the autonomous nervous system: it therefore represents a mechanism of fundamental physiological relevance.

Their function in pacemaking makes funny channels an obvious target for drugs aiming at regulation of spontaneous activity and cardiac rate. This explains the recent development of “heart rate-reducing” drugs which act as selective f-channel inhibitors, and as such are capable of specifically slow cardiac frequency by decreasing the rate of diastolic depolarization. These substances will be useful in treating diseases such as chronic angina and heart failure. Furthermore, in situ delivery of funny channels, or of a cellular source of funny channels, is a promising new technique for the development of biological pacemakers which may in a near future replace electronic devices. Finally, a channel mutation responsible for one type of a relatively common rhythm disturbance, sinus bradycardia, has been recently identified, highlighting the clinical relevance of funny channels in the pacemaker function.

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Keywords: Cardiac pacemaker, If current, Funny channels, Heart rate; Autonomic control

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* Tel.: +39 02 5031 4931; fax: +39 02 5031 4932.
E-mail address: dario.difrancesco@unimi.it.
1. Introduction

Cardiac pacemaking is an electrical phenomenon, based on the function of ion channel proteins expressed on the membrane of specialized cardiac myocytes, the sino-atrial node (SAN) cells of mammalian heart. “Pacemaker” cells are endowed with the property of spontaneous activity, and generate repetitive action potentials at a constantly controlled rate, thus determining the cardiac frequency and consequently the overall cardiac performance. What gives pacemaker cells this ability? Several mechanisms contribute to provide the cellular and molecular elements necessary for pacemaking to occur, but among them, the $I_f$ current has a major role in providing pacemaking competence.

SAN myocytes are characterized by the presence of a “slow diastolic” phase, which at the termination of an action potential slowly depolarizes the membrane until threshold is reached for a subsequent action potential, thus generating spontaneous, repetitive activity [2]. The origin of this phase has been thoroughly investigated [3,4], and it is now generally recognized that activation of the funny current at the termination of an action potential is the process responsible for generation of the diastolic depolarization.

Originally described in the SAN [1], the funny current has been the object of intense investigation and its properties and function in cardiac pacemaker cells (and, in fact, in several other types of cells where funny channels are expressed) have been described in detail [2,5–8].

In short, the funny current in cardiac cells and discuss therapeutic applications of the concept of pacemaker channels, specifically their potential use in the pharmacological control of heart rate. Review articles addressing more specifically the molecular correlates of native f-channels, the hyperpolarization-activated, cyclic-nucleotide gated (HCN) channels can be found elsewhere [7–9].

2. The funny current generates the diastolic depolarization phase of pacemaker potential

Diastolic depolarization, first recorded in Purkinje fibres, was originally proposed to originate from the decay of a $K^+$ conductance, based on conductance measurements during an action potential [10] or during voltage-clamp [11]. The mechanism proposed was analogous to that predicted by the squid axon Hodgkin-Huxley [12] model of electrical activity, where after termination of an action potential the membrane hyperpolarizes beyond the resting level, and then slowly depolarizes up to the resting membrane potential due to the decay of the previously activated delayed $K^+$ conductance.

This idea was subsequently strongly supported by the description in Purkinje fibres of the so-called $I_{K2}$ current, reported as a pure $K^+$ current activated upon depolarization in the diastolic range of voltages [13,14]. According to this description, the $I_{K2}$ decay was the process underlying diastolic depolarization, and $I_{K2}$ had the properties expected for the current predicted by Weidmann’s and Vassalle’s experiments. The relevance of $I_{K2}$ to pacemaking was strengthened by evidence of the involvement of this component in rate acceleration caused by sympathetic stimulation [15]. The experimental evidence for a $K^+$ conductance decay hypothesis as the mechanism driving diastolic depolarization in Purkinje fibres was therefore firmly established to all accounts, and the mechanism was regarded as indisputable for over a decade. The $I_{K2}$ current was considered as one of the best described cardiac components. Yet, the $I_{K2}$ interpretation and consequently the $K^+$-conductance decay hypothesis, were deeply incorrect. In the late 1970s and early 1980s, a set of new experimental data appeared which paved the way to the demonstration that the Purkinje fibre pacemaker current was not an outward current activated on depolarization, but was no less than just the opposite, i.e., an inward current activated on hyperpolarization.

Among the findings that contributed to the re-interpretation of the Purkinje fibre’s $I_{K2}$, an important one was the discovery of the funny ($I_f$) current in the sino-atrial node. The first detailed report of this current describing its elementary properties and role in the generation of spontaneous activity in the SAN, as well as the involvement in catecholamine-induced control of rate appeared in 1979 [1]. Records of the same current had appeared in previous publications in both mammalian and amphibian heart, but the component had not been considered physiologically relevant [16,17].

The “funny” current had atypical features, which justified its name: it was inward and activated on hyperpolarization within a voltage range comprising the range of diastolic depolarization and had unusually slow kinetics. These properties made $I_f$ the most obvious candidate in the search for components involved in the initiation and control of pacemaking. Several features of the funny current in SAN cells were surprisingly similar to those of the $I_{K2}$ current in Purkinje fibres [18]. The puzzle of having two nearly identical components of a totally different ionic nature was solved two years after the finding of $I_f$ by the demonstration that $I_{K2}$ was in fact, like $I_f$, an inward current activated on hyperpolarization and carried by Na⁺ and K⁺, rather than a pure $K^+$ current activated on depolarization [19,20]. How could an inward current, reversing close to $-10$ to $-20$ mV, look like a pure $K^+$ current? The illusion had been caused by the presence, in Purkinje fibres, of a large $K^+$ inwardly-rectifying component, called $I_{K1}$, which decreases during the strong hyperpolarizing steps used to study $I_{K2}$: the superimposition of this component with $I_f$ generates a “fake” reversal potential close to the $K^+$ equilibrium potential ($E_{K^+}$). Removal of $I_{K1}$ (by Ba²⁺-induced block) abolished reversal near $E_{K^+}$ [19]: this latter result was particularly significant since it “unmasked” the real inward nature of the Purkinje fibre’s pacemaker current and allowed for the first time to visualise the “conversion” of $I_{K2}$ into an inward, hyperpolarization-activated current. These results established that $I_{K2}$ was a “camouflaged” $I_f$, the two “pacemaker” currents in the two cardiac tissues being indeed of identical nature, and led to a rational, integrated interpretation of the origin of cardiac pacemaking in all different pacing regions of the heart.

Following the re-interpretation of $I_{K2}$ and its identification with the nodal $I_f$, the funny current was systematically characterized in the SAN [5]. Importantly, the findings in cardiac pacemaker cells set the pace for the identification and description of ionic currents with similar properties in a large variety of neurons and other cell types, such as smooth muscle cells
The funny current mediates autonomic control of cardiac rate

The relevance of $I_f$ to pacemaking does not only derive from its role in the generation of diastolic depolarization but also from its involvement in neurotransmitter-induced control of cardiac rate. Since it was first described in the SAN, $I_f$ was shown to mediate the acceleratory effect of adrenaline on pacemaker rate [1]. This is caused by a shift of the voltage dependence of the current activation curve (i.e., the f-channel open probability curve) to more positive voltages induced by $\beta$AR stimulation and associated to an increased activity of membrane adenylate-cyclase and a higher intracellular cAMP, the second messenger in $I_f$ regulation and modulating its rate is established, cardiac (and

Fig. 1. Rate modulation by the autonomic nervous system is mediated by changes in $I_f$. (a) Isoprenaline accelerates and acetylcholine slows spontaneous rate in isolated SAN cells. Note that the action potential shape and duration are unaltered, and that rate is modified by changing the steepness of the diastolic depolarization phase of the action potential. (b) Isoprenaline increases and acetylcholine decreases $I_f$ during voltage-clamp steps from $-35$ to $-85$ mV in a SAN cell; these changes are responsible for the changes in diastolic depolarization slope in panel a and are caused by a rightward and a leftward shift of the activation curve, respectively. (c) Modified from [21] and from [57] (with permission).
non-cardiac) pacemaking is clearly a complex cellular process whose accomplishment requires the contribution of several mechanisms. In particular, there is now substantial evidence that sarcoplasmic reticulum (SR) Ca\(^{2+}\) transients affect heart rate via a process involving the Na–Ca exchanger\[^{27}\]. For example, inhibition of Ca\(^{2+}\) release from the SR slows spontaneous rate and impairs rate acceleration induced by \(\beta\)-adrenergic receptor (\(\beta\AR\)) stimulation, leading to the proposal that SR Ca\(^{2+}\) transients mediate \(\beta\AR\) modulation of rate\[^{28}\]. However, it can be shown that disruption of Ca\(^{2+}\) release from the SR does not inhibit the cAMP-\(I_f\)-rate modulation process\[^{29}\], since the same conditions impair \(f\)-channel modulation by \(\beta\AR\), but not by cAMP; these data suggest that inhibition of \(\beta\AR\) modulation of rate is due to uncoupling between \(\beta\ARs\) and \(f\)-channels, and do not substantiate evidence in favour of the view that Ca\(^{2+}\) homeostasis has an independent role in driving pacemaker generation\[^{30}\].

4. The dual voltage- and cAMP-dependent regulation of \(f\)-channels

Autonomic \(\beta\)-adrenergic and cholinergic stimuli modify the degree of activation of \(f\)-channels, hence heart rate, by increasing and decreasing, respectively, the activity of adenylate-cyclase and intracellular cAMP, which is the second messenger of \(I_f\) regulation\[^{21–24}\].

How does cAMP activate \(f\)-channels? When this was first investigated in inside-out patches of SAN cell membranes, it led to the surprising finding that cAMP activates \(f\)-channels by direct binding, rather than by cAMP-mediated phosphorylation\[^{31}\]. This was the first demonstration of a kinship, later confirmed by the cloning of HCN channels, between \(f\)-channels and the cyclic-nucleotide-gated (CNG) channels of sensory neurons.

An important, if still unusual, property of \(f\)-channels is their dual dependence upon voltage hyperpolarization and intracellular cAMP. While the hyperpolarization-dependent activation is functional to the generation of diastolic depolarization and spontaneous activity, the cAMP-dependent activation endows funny channels with the ability to mediate neurotransmitter-induced control of heart rate. The action of cAMP on the current activation curve is equivalent to a depolarizing voltage shift (of some 11 mV at saturating concentrations\[^{31}\]). This observation raised the obvious question whether voltage hyperpolarization and cAMP share a common mechanistic action on \(f\)-channel gating, favouring a channel configuration which is more likely to open.

Experimental data from inside-out patches exposed to pronase, which by cleavage of internal portions of \(f\)-channels causes a large depolarizing shift of the channel open probability curve (\(+56\,\text{mV}\)) and abolishes cAMP dependence, led to the hypothesis of the existence of a basal inhibitory action exerted by a proteolysis-sensitive internal domain on channel gating which could be removed by either hyperpolarization or cAMP binding\[^{32}\]. Later confirmation of this hypothesis, and the identification of the C-terminus, the channel region binding cAMP (at the cyclic-nucleotide binding domain, CNBD), as a key element in the basal channel inhibitory action, was achieved by work on HCN isoforms\[^{33–35}\].

How does cAMP act to remove a basal inhibitory channel inhibition, and cause the open probability curve to shift positive? This was investigated theoretically, and an allosteric model of channel activation was developed which is able to explain the cAMP-induced shift of the open probability curve, as well as the sigmoidal dose-dependence of the shift against cAMP concentration\[^{36}\]. According to this model, pacemaker channels are viewed as tetramers and each of the four subunits carries a voltage sensor which is independently gated by voltage; however, opening/closing reactions occur allosterically and involve concerted transitions of all four subunits\[^{36,37}\] (Fig. 2).

This model introduces an interesting concept: to account for the activating action of cAMP, it is not necessary to assume that cAMP molecules have an “active” function, i.e., it is not neces-

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**Fig. 2.** cAMP-induced \(f\)-channel activation can be accounted for by an allosteric model of channel gating. (a) The \(f\)-channel activation curve, as measured in an inside-out macro-patch, shifts to more positive voltages upon perfusion of 10 \(\mu\text{M}\) cAMP, reproducing the action of \(\beta\AR\)-stimulation (Fig. 1). (b) Shifts of the activation curve are plotted against cAMP concentration in a dose–response relationship. Experimental datapoints are fitted by an equation (full line) derived from an allosteric model where cAMP binds to open channels more favorably than to closed channels (c). Modified from\[^{36}\] (with permission).
sary to assume that cAMP binding to closed channels causes an increased probability of opening. Indeed, the action of cAMP is accounted for by the simple assumption that cAMP has a higher binding affinity to open than to closed channels (Fig. 2c).

5. Funny channels as tools for gene/cell therapy and pharmacological control of heart rate

The generation and modulation of heart rate by the funny current are mechanisms of basic physiological significance, but they may also represent tools for interventions aimed to the control of cardiac chronotropism by gene/cell or pharmacological approaches. Molecular/cellular approaches today allow the pacemaker function of f-channels to be transferred to resting or defective spontaneously active cardiac cells, in both in vitro cultures and in vivo conditions, as a basis for the development of “biological” pacemakers.

Several cardiac rhythm disturbances such as severe sinus bradycardia, sinus arrest or atrio-ventricular block are normally “biological” pacemakers. Transfer of the “pacemaker” functions and in vivo conditions, as a basis for the development of defective spontaneously active cardiac cells, in both in vitro cultures which either express native pacemaker channels or are engineered to this purpose [41,42]. This aspect of the exploitation of funny channel properties is treated in detail in another chapter of this issue [43].

The existence of molecules interacting with ion flow through funny channels is known since early studies of f-channels, such as alteration of the duration of action potentials or cardiac inotropism. A pharmacological approach to heart rate control is therefore based on the development of molecules able to interact specifically with funny channels.

6. Heart-rate-reducing agents and the selective block of funny channels

The existence of molecules interacting with ion flow through funny channels is known since early studies of f in the Purkinje fibres and SAN cells. Cs+ and Rb+ ions, for example, reduce inward f when applied externally [44]. These ions however block other types of channels and are not specific f-channel blockers. In the 1980’s, drugs originally termed “Pure Bradycardic Agents” (PBAs) were developed based on their ability to slow heart rate specifically by depressing diastolic depolarization rate, with limited side effects on action potential duration and inotropic state. These substances are clearly potentially important for therapeutic use in diseases where heart rate reduction is beneficial, such as angina and heart failure, since lowering heart rate decreases oxygen demand and improves diastolic myocardial perfusion. Also, several studies show a link between elevated heart rate and mortality, which corroborates the concept of heart rate reduction as a convenient therapeutic approach [45].

Although believed for some time to be Ca2+-antagonists [46], PBAs were in fact shown to be f-channel blockers [47,48]. The first such drug to be developed was alinidine, an N-allyl derivative of clonidine [49]. This was followed by other molecules developed with the aim of improving specificity of the rate-slowing action against side effects, such as falipamil (AQ-A39) and its congener UL-FS49, and ZD7288 [48,50]. A more recently developed compound with highly specific f-channel binding and heart rate-reducing action is ivabradine (Fig. 3, [51]).

The specificity of pure bradycardic action results from the selectivity of f-channel block, since pure f inhibition causes changes of pacemaker activity that only involve a reduced slope of diastolic depolarization rate, without significantly affecting other action potential parameters (Fig. 3a). It is interesting to observe that although the overall slowing action of rate-reducing agents is similar to that exerted by parasympathetic stimulation, the mode of action of these drugs and ACh on f-channel differs. Indeed, while ACh inhibits f by shifting the current activation curve to more negative voltages (Fig. 1c), rate-reducing agents simply reduce the overall f conductance (Fig. 3) as a typical effect of ion channel block.

7. Mode of f-channel block by ivabradine

A typical feature of heart rate-reducing agents is their use-dependence, according to which the effect of drug application accumulates during repetitive activity [47]. This feature results from an accumulation of f current inhibition during repetitive activation/deactivation protocols [51] and is therapeutically use-
f-channels is "current"-dependent [51]. The "current-dependence" of block can be interpreted biophysically with the assumption that ivabradine molecules are "kicked into" the pore from the intracellular side of the channel (and reach the blocking site) when ions flow in the outward direction during a depolarization, and are "kicked out" when ions flow in the inward direction during a hyperpolarization. A current-dependent block is characteristic of inwardly rectifying K+ channels, through which permeation occurs according to a multi-ion, single-file mechanism [52]. This hypothetical mechanism of f-channel block by ivabradine is illustrated in the 3D-reconstruction of Fig. 5.

The "current-dependence" of block can therefore be defined as "current-dependent".

The theoretical structure of the human HCN4, the major cardiac HCN isoform, was reconstructed by the Swiss Model program, based on homology with the known X-ray crystal structure of the 2-transmembrane-domain potassium MthK channels [53]. According to this model reconstruction, HCN4 channels have a "pore cavity" just below the intracellular side of the selectivity filter where K+ and Na+ ions, represented by yellow spheres, may bind along their pathway across the channels. Ivabradine molecules are positively charged at physiological pH since they include a tertiary ammonium ion, and could therefore in theory bind to the channel within the pore cavity in such a way as to affect the binding of permeating ions to the pore sites. The reconstruction in Fig. 5 is fully speculative, but would be able to explain the current-dependence of f-channel block by ivabradine.

![Fig. 4. Inward current flow is required for ivabradine-induced I_h block relief by hyperpolarization. (a) I_h block by ivabradine induced with repetitive stimulation (−100 mV/5 mV) (trace 1) is partially relieved by a long step to −100 mV (trace 2). (b) If the same protocol is repeated in the presence of Cs+, an external blocker of f-channels which stops current flow, no block is relieved (compare traces 1 and 2). Modified from [51] with permission.](image-url)
debate can be found elsewhere (see for example [56]). Today, a bulk of evidence has accumulated leaving little doubt concerning bradycardia[54]. The mutation affects the f-channel function by the HCN4 in the CNBD is responsible for a familial form of sinus diac rhythm. Specifically, by investigating a large Italian family by evidence that channel mutations may affect normal car-
ation and rate control by f-channels has received recent support in situ transfection of HCN channels or by stem-cell approaches. Finally, the recent finding of a point mutation of HCN4 leading to inherited sinus bradycardia in man [54] has for the first time shown a direct involvement of altered f-channel function in a common form of rhythm disturbance.

8. HCN channelopathies

The clinical relevance of the mechanism of pacemaker gener-
ation and rate control by f-channels has received recent support by evidence that channel mutations may affect normal cardiac rhythm. Specifically, by investigating a large Italian family we have shown that an autosomal dominant point-mutation of HCN4 in the CNBD is responsible for a familial form of sinus bradycardia [54]. The mutation affects the f-channel function by shifting the f current activation curve to more negative voltages (by about 5 mV), an effect which mimics that of a low dose of ACh [26], thus explaining the rate slowing associated with the mutation.

9. Conclusions

The role of f in pacemaking, and specifically the extent to which f can be considered as the main determinant of pacemaker activity, has long been debated since the original description of the funny current [2-4,55], and more detailed accounts of this debate can be found elsewhere (see for example [56]). Today, a bulk of evidence has accumulated leaving little doubt concerning the role played by f in the generation and control of pacemaker activity. Since the cloning of HCN channels in the late 1990s, some of the molecular aspects underlying the function of the funny current have been appreciated. More molecular details are likely to become available in the next few years while structurally relevant parts of HCN channels are crystallized.

Their role in cardiac pacemaking naturally make funny channels a target for the development of substances specifically developed for pharmacological control of heart rate, such as the heart rate-reducing agents, potentially useful in the treatment of several cardiac diseases. Furthermore, “biological” pacemakers can be devised based on the novel concept of delivering the pacemaker function to recipient cardiac tissue by either stable in situ transfection of HCN channels or by stem-cell approaches.


