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Review

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

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Abstract

"Funny" (f) channels underlie the cardiac "pacemaker" I_f 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, I_f 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.

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

Contents

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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 I_f 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 expresssed) have been described in detail [2,5–8].

In this short review I will summarize the properties of 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/−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 Ba^{2+}-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

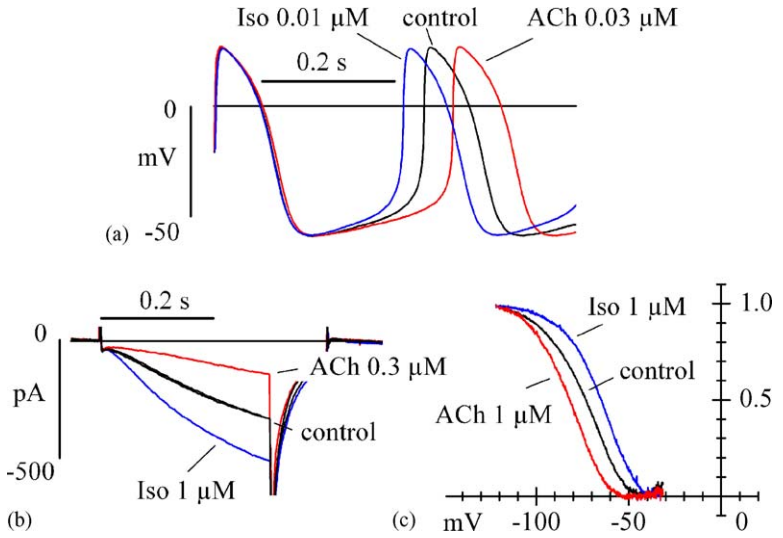

Fig. 1. Rate modulation by the autonomic nervous system is mediated by changes in I_f. (a) Isoprenaline accelerates and acetylcholine slows spontaneous rate in isolated SAN cells. Note that the action potential shape and duration are unaltered, and that rate is modified by changing the steepness of the 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 [2] and from [57] (with permission).

[2,6]. The properties of the funny current are apt to generate a slowly developing diastolic depolarization (Fig. 1). It is carried by both Na^+ and K^+ ions, in itself an unusual feature, with a reversal potential of about −10/−20 mV in normal Tyrode solution; it is activated on hyperpolarization with a threshold potential of about −40/−50 mV, and a saturation potential of about −100 mV; it activates slowly on hyperpolarization to the range of current activation (for example, at −84 mV the time constant of activation is in the range of 500 ms [21]), and deactivates fast on depolarization to positive voltages.

The pacemaker mechanism, i.e., the process leading to generation of the diastolic depolarization phase of the action potential in pacemaker cells, can therefore be explained as follows (see Fig. 1): I_f is deactivated during the upstroke of an action potential, but turns on during repolarization, when the voltage enters the current activation range (below −40 mV); slow activation of the inward I_f antagonizes the hyperpolarizing effect of the outward decaying I_K (the delayed K^+ current responsible for repolarization) until maximum diastolic potential (MDP) is reached, and then causes the membrane voltage to slowly depolarize until threshold is reached for fast inward current activation (Ca^{2+}-current) and a new action potential.

3. 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 βAR stimulation and associated to an increased activity of membrane adenylate-cyclase and a higher intracellular cAMP, the second messenger in I_f modulation [21] (Fig. 1).

The depolarizing shift increases the I_f current availability at diastolic voltages, so that there is more inward current flowing during diastole and a consequent acceleration of diastolic depolarization slope and rate (Fig. 1, blue traces). This is the way the sympathetic stimulus causes a positive chronotropic effect.

Later studies showed that the pacemaker current is also strongly modulated by acetylcholine [22–24]. The action of ACh is opposite to that of catecholamines, i.e. ACh inhibits I_f by shifting its activation curve to more negative voltages, which decreases the current availability during diastolic depolarization and causes the heart rate to slow down. As with sympathetic stimulation, cAMP is the second messenger in ACh-induced I_f inhibition: by stimulating muscarinic receptors, ACh inhibits adenylate-cyclase and cAMP production, and thus induces a negative shift of the I_f activation curve.

The demonstration of the ACh-dependent I_f inhibition had an impact on the physiological regulation of rate by parasympathetic innervation, and modified the generally accepted view that the vagal control of cardiac slowing results from the opening of ACh-activated K^+ channels ($I_{K,\,ACh}$ current [25]). Indeed, investigation of the ACh action on the two currents in SAN cells revealed that much lower concentrations of ACh are required to inhibit I_f than to activate $I_{K,\,ACh}$, and that concentrations as low as 3–30 nM, which do not activate $I_{K,ACh}$ but do inhibit I_f, are able to induce slowing of pacemaker activity [26]. These data showed therefore for the first time that ACh-induced I_f inhibition, and not K^+-current activation, is the process underlying slowing of cardiac rate by low ACh doses, corresponding to moderate vagal activity.

While the role played by I_f in generating the diastolic depolarization and modulating its rate is established, cardiac (and

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 β-adrenergic receptor (βAR) stimulation, leading to the proposal that SR Ca^{2+} transients mediate βAR 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 βAR, but not by cAMP, these data suggest that inhibition of βAR modulation of rate is due to uncoupling between βARs 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 β-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 mV) and abolishes cAMP dependence, led to the hypothesis of the existance 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-

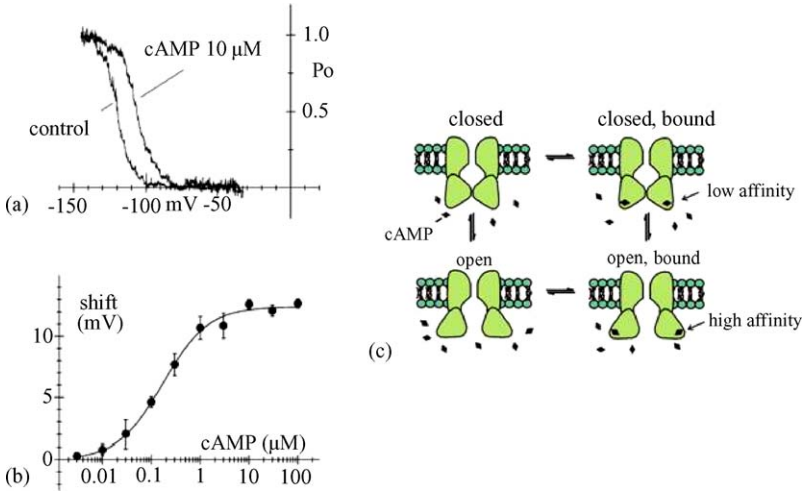

Fig. 2. cAMP-induced f-channel activation can be accounted for by an allosteric model of channel gating. (a) The f-channel activation curve, as measured in an inside-out macro-patch, shifts to more positive voltages upon perfusion of 10 µM cAMP, reproducing the action of βR-stimulation (Fig. 1). (b) 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 favourably 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 coltures 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 treated by the implantation of electronic pacemakers. Gene and cell therapy methods are being developed today to create biological pacemakers, which are expected to have several advantages over electronic ones [38]. Transfer of the "pacemaker" function is a feasible approach, as shown for example by evidence that injecting an adenovirus expressing human HCN2 gene in the right atrium [39] or in the left bundle branch [40] of anesthetized dogs results in idioventricular rhythm that is faster than control rhythm and is linked to local overexpression of HCN2 channels. An alternative to in situ HCN transfection is the use of stem cells 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 relevance of I_f to pacemaker generation makes f-channels natural targets in the search for drugs able to specifically modify the diastolic depolarization phase of the action potential without unwanted side-effects due to interference with K^+ or Ca^{2+} 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 existance of molecules interacting with ion flow through funny channels is known since early studies of I_f in the Purkinje fibres and SAN cells. Cs^+ and Rb^+ ions, for example, reduce inward I_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" (PBA's) 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

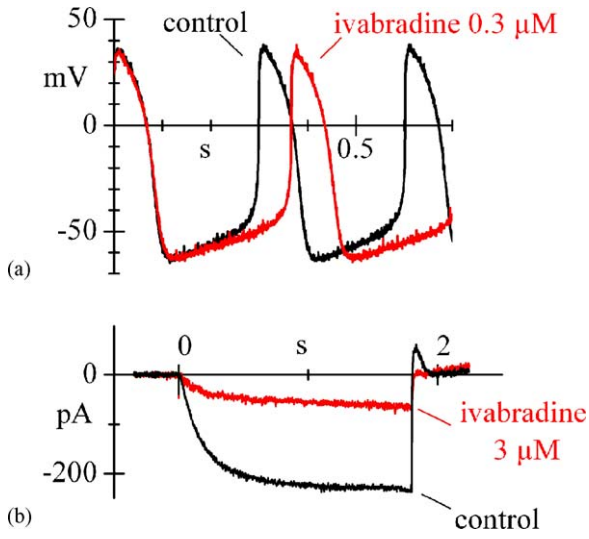

Fig. 3. Block of f-channels by ivabradine. (a) Ivabradine slows spontaneous activity by reducing specifically the rate of diastolic depolarization. (b) Steady-state inhibition of I_f by ivabradine 3 µM during repetitive voltage-clamp steps to −100/+5 mV (1/6 Hz). Ivabradine inhibits I_f by reducing the I_f conductance, rather than by altering the activation curve.

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 Ca^{2+}-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 I_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 I_f by shifting the current activation curve to more negative voltages (Fig. 1c), rate-reducing agents simply reduce the overall I_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 I_f current inhibition during repetitive activation/deactivation protocols [51] and is therapeutically use-

ful since it implies that the slowing action of these drugs will be stronger at higher heart rates, when the bradycardic effect is most valuable.

Use-dependence derives from some of the blocking properties of f-channels by rate-reducing agents, such as the dependence of block upon channel opening ("open-channel" block) and the voltage-dependence of block, according to which block is stronger at depolarized voltages. These two properties are apparently in contrast, since the former requires voltage hyperpolarization for channel opening, and the latter requires voltage depolarization for an increased block efficiency, but in fact they are the basis for block facilitation by repetitive channel open/close cycling.

S16257 (ivabradine) is a new compound which recapitulates the properties of heart rate-reducing agents but also possesses some specific features. It is presently the only member of the family having completed clinical development for stable angina, and viability for clinical use in ischaemic heart disease and cardiac failure has been evaluated in detail [45]. Studies in SAN cells have shown that ivabradine – f-channel binding/unbinding reactions are restricted to the open channel state, implying that ivabradine (like UL-FS49) is an open channel blocker [51]. Furthermore, ivabradine block of f-channels occurs at the intracellular channel side, and is more efficient at depolarized than at hyperpolarized voltages. These properties result from the positively charged nature of the blocking molecule (due to the presence of a tertiary ammonium ion) and its tendency to cross channels (from the intracellular to the extracellular side) more easily during a depolarization [51].

A distinctive property of ivabradine, not found in other rate-reducing agents, is that its blocking action is not intrinsically voltage-dependent, but rather depends on the direction of ion flow across the channel pore; in other words, ivabradine block of f-channels is "current"-dependent [51].

An experiment indicating the relevance of ion flow in the f-channel blocking mechanism of ivabradine is shown in Fig. 4 [51]. Both protocols in a and b consisted of a series of repetitive activation/deactivation steps (−100/+5 mV) applied in the presence of 3 μM ivabradine; following full block development, a long hyperpolarizing step to −100 mV was applied in the absence (a) or in the presence of 5 mM Cs^+ (b), after which the activation/deactivation pulsing protocol was resumed. Cs^+ is a known f-channel blocker which acts extracellularly [44], and as expected, no current flow through f-channels was observed during perfusion with Cs^+. As apparent by comparing current records just before and just after the long step (insets), the long hyperpolarization clearly removed part of the block in the absence (a), but not in the presence of Cs^+ (b). The most straightforward interpretation of these data indicates that voltage hyperpolarization is not by itself responsible for block removal, and that an inward ionic flow is required [51]. This and additional evidence shows that the electrochemical gradient, more than absolute voltage across channels, determines the extent of block, which can therefore be defined as "current-dependent".

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.

Here 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.

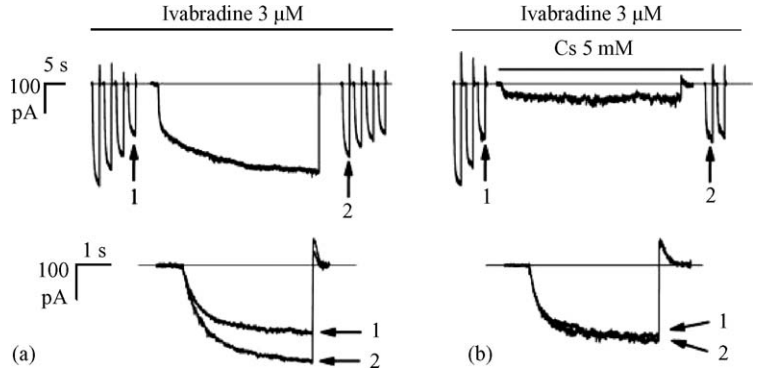

Fig. 4. Inward current flow is required for ivabradine-induced I_f block relief by hyperpolarization. (a) I_f block by ivabradine induced with repetitive stimulation (−100 mV/+5 mV) (trace 1) is partially relieved by a long step to −100 mV (trace 2). (b) If the same protocol is repeated in the presence 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).

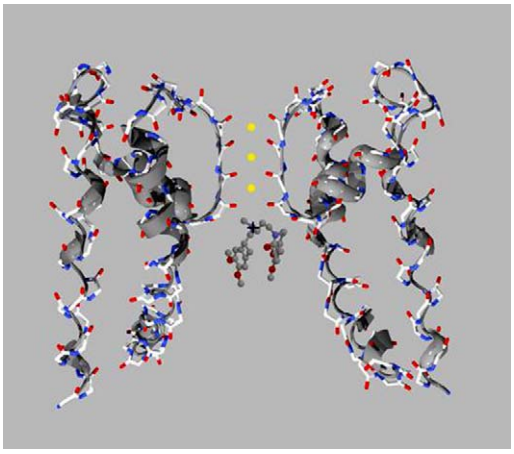

Fig. 5. Hypothetical interaction of ivabradine molecules with ion permeation across f-channels. Alignment of human HCN4 with the K^+ channel MthK [53] by ClustalX alignment procedure and 3D-modelling by DeepView-Swiss-PdbViewer led to the structure shown. Only two of the four subunits (S5–S6 transmembrane domains) are shown. Interaction of the charged ivabradine molecule with the positively charged permeating ions (yellow dots) in the pore could explain the current dependence of block.

8. HCN channelopathies

The clinical relevance of the mechanism of pacemaker generation 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 I_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 I_f in pacemaking, and specifically the extent to which I_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 I_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. 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.

Acknowledgments

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