Pharmacology of Hyperpolarization-Activated Cyclic Nucleotide-Gated (HCN) Channels

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Abstract The current produced by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels (termed I_f, cardiac pacemaker "funny" current, and I_h in neurons) is also considered a "pacemaker current" because it plays a key role in controlling the rhythmic activity of cardiac pacemaker cells and spontaneously firing neurons. The pacemaker current is an inward current activated by voltage hyperpolarization and modulated by intracellular cAMP. Voltage-dependent opening of these pacemaker channels is directly regulated by the binding of cAMP. The f-channels are encoded by four genes (HCN1–4) and are widely expressed throughout the heart and central nervous system. This article summarizes the structure, function, and regulation of these channels. Because of their relevance to cardiac pacemaker activity, f-channels are a natural target of drugs aimed at the pharmacological control of heart rate. In this regard, several agents developed for their

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capacity to selectively reduce heart rate act by specifically inhibiting f-channel function. Related compounds that could potentially be used for the treatment of diseases such as angina and heart failure are also discussed.

Keywords Brain • HCN • Heart • I_f • I_h • Ivabradine • Pacemaker

Abbreviations

CAD	Coronary artery disease
cAMP	Cyclic adenosine monophosphate
cGMP	Cyclic ganosine monophosphate
CNBD	Cyclic nucleotide binding domain
HCN	Hyperpolarization-activated cyclic nucleotide-gated I_h
HEK293	Human Embryonic Kidney 293 cells
I_f	Funny current
I_h	Hyperpolarization-activated current
Kv	Voltage-gated potassium channel
LTP	Long-term synaptic plasticity
MI	Myocardial Infarction
PKA	Protein Kinase A
PKC	Protein Kinase C
pS	Pico-Siemens
SAN	Sinoatrial node

1 Introduction

Noma and Irisawa [1] first reported the existence in sinoatrial node (SAN) tissue of a slow, time-dependent inward current that was activated by membrane hyperpolarization. This current has perplexed physiologists since it was first discovered. Initially, its properties were deemed exclusive, for which it has invariably been named I_h (h for hyperpolarization-activated), I_f (f for funny), or I_q (q for queer). Similar currents were later revealed in a diverse range of neuronal and nonneuronal cells, and today these currents are recognized as ubiquitous components of the nervous system. The current contributes to normal pacemaking activity in the sinoatrial and atrioventricular nodes in the atria, and Purkinje fibers in the ventricle [2, 3]. It also plays a role in abnormal spontaneous activity of cardiac myocytes under pathological conditions [4]. The pacemaker current also mediates repetitive firing in neurons and oscillatory activities in neuronal networks. In addition, this current acts to set the resting membrane potential of certain excitatory cells, and

may function in synaptic plasticity and the activation of sperm [5]. A notable characteristic feature of pacemaker channels is their modulation by cyclic nucleotides, i.e., cAMP and cGMP, independently of a phosphorylation process. In the 1990s, several cDNAs encoding pacemaker channels were isolated by molecular cloning [6–9]. On the basis of amino acid sequencing, four mammalian genes were revealed to code for members of the voltage-gated K (Kv) channel superfamily, and cyclic nucleotide-gated channels (see [10] for review). With regard to their dual properties, the channels were termed hyperpolarization-activated cyclic nucleotide-gated cation (HCN) channels. The four isoforms have diverse properties and variable expression patterns and can form functional heteromultimers with particular biophysical and regulatory properties.

Because of their fundamental role in cellular pacemaking, HCN channels are considered as relevant pharmacological targets, in particular with respect to the control of heart beat. A number of organic compounds have been described that block the pacemaker current in a relatively specific manner. Drug interactions and potential applications of their therapeutic use on the pacemaker current will be discussed below.

2 Molecular Structure

As described above, HCN channels belong to the family of cyclic-nucleotide gated channels and may be structurally related to voltage-sensitive potassium channels [11, 12]. Four main isoforms (see Fig. 1a), named HCN1 to HCN4, have been identified so far. The tissue distribution of HCN1, HCN2, HCN3 and HCN4 is heterogeneous, and specific expression of the different isoforms can be summarized briefly as follows: HCN1 is expressed in different regions of the central nervous system (olfactory bulb, cerebral cortex, hippocampus, superior colliculus, and cerebellum) and peripheral nervous system (dorsal root ganglion) [14]. HCN2 is present in most brain regions, with highest expression levels in the olfactory bulb, hippocampus, thalamus, and brain stem. HCN3 is widely expressed in the brain, but at low levels. HCN4 transcripts are selectively expressed in the thalamus and olfactory bulb. Besides expression in the peripheral and central nervous systems, HCN1, HCN2, and HCN4 are expressed in the heart with specific differences according to cardiac regions and species [15, 16].

All isoforms are composed of six transmembrane segments (see Fig. 1a, b) organized in a similar manner to other voltage-sensitive ion channels, i.e., voltage sensing located in the fourth segment and pore region between the fifth and the sixth segments. Voltage sensing is attributed to repetitive, positively charged amino acids (Lys and Arg) in the fourth segment, with activation or deactivation of the channel taking place according to changes in the transmembrane electrical field. While HCN channels are permeable to sodium and potassium, the pore region is characterized by a GYG sequence, which is generally considered to be a specific requirement for K channel selectivity. No clear explanation for this apparent contradiction has yet been provided. In addition to this classical transmembrane

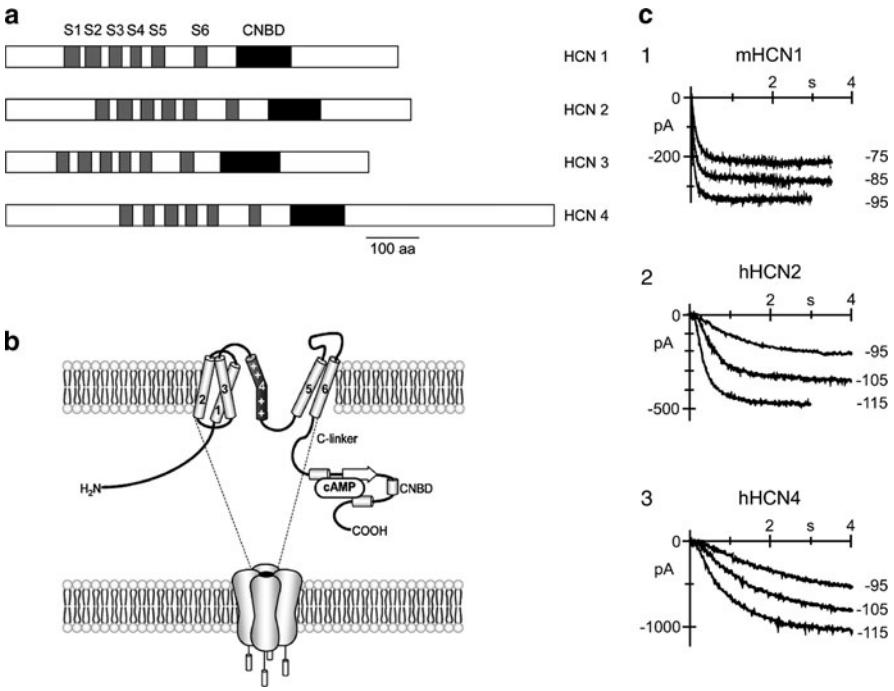

Fig. 1 (**a**) The four isoforms of the HCN family. The six transmembrane segments S1–S6 are numbered 1–6, CNBD indicates the cyclic nucleotide-binding domain. (**b**) HCN channels are tetramers. One monomer is composed of six transmembrane segments including the voltage sensor (S4) and the pore region between S5 and S6. The pore region contains the selectivity filter carrying the GYG motif. The COOH terminal channel domain is composed of the C-linker and the cyclic nucleotide-binding domain (CNBD). (**c**) Kinetic properties of the different HCN isoforms. Activation traces recorded on hyperpolarization to the indicated voltages of HEK-293 cells expressing mouse HCN1 (1), human HCN2 (2), and human HCN4 (3) channels (modified from [13])

voltage-dependent machinery, HCN proteins are characterized by a C-terminus domain, which contains consensus sequences able to bind with cyclic nucleotides (a 120 amino acid-long cyclic nucleotide binding domain, CNBD). From a functional point of view, as detailed below, channel activation is triggered by membrane hyperpolarization. The latter is facilitated by the prior binding of a cyclic nucleotide to the CNBD region. A high degree of conservation between the four HCN isoforms is observed in the transmembrane region, in the C-terminus CNBD, and in the peptide located between the sixth transmembrane domain and the CNBD (an 80 amino acid-long C-linker). In contrast, a high diversity is found in the N-terminal region and the C-terminal region downstream of the CNBD.

HCN subunits must assemble as tetramers, organized around the central pore region, to constitute functional channels. Biophysical and pharmacological properties depend on subunit composition and heterotetramerization may be necessary to constitute channels with current properties identical to native currents [17, 18].

3 Basic Biophysical Properties of HCN Channel Subtypes

An important feature of HCN channels is their activation by hyperpolarization. Generally, h-currents activate with hyperpolarizing steps to potentials negative to −50 to −70 mV. Unlike most other voltage-gated currents, I_h does not inactivate. Activation of I_h is preceded by a delay, resulting in a typical sigmoidal time course of onset. Several mechanisms have been described in the literature to explain the processes of I_h activation and deactivation that follow this delay [19]. The kinetic features of I_h require complex multi-state kinetic modeling based on the existence of distinct "delaying" and proper "gating" processes [20, 21]. Depending on the cell type, the kinetics of activation of the current are quite variable; these differences could reflect the diverse intrinsic activation properties of distinct HCN channel isoforms underlying the current and/or the experimental conditions, or even the cellular microenvironment of the HCN-channel [22].

All four HCN channel types (HCN1–4) display the principal biophysical properties of the native pacemaker current. Nevertheless, the biophysical properties vary according to experimental parameters and also diverge depending on the expression system or cell type. In general, the different isoforms differ from each other with respect to their voltage dependence and their degree of cAMP-dependent modulation. HCN2 has a more negative activation threshold than HCN1 and HCN4 [17, 20, 23]. The kinetics for voltage-dependent activation vary between the HCN channel subtypes (see Fig. 1c).

HCN1 is the fastest channel (time constant of 25–300 ms) depending on the voltage values employed [9, 24], while HCN4 is the slowest channel [25–27], displaying time constant values between a few hundred milliseconds at −140 mV up to several seconds at −70 mV. HCN2 and HCN3 activate with kinetics that range between those for HCN1 and HCN4 [9, 20, 23].

Evidence for the ion permeation properties of pacemaker channels is derived from experiments on Purkinje fibers and on isolated rabbit SA node myocytes [28, 29]. Ionic substitution experiments identified Na^+ and K^+ ions as carriers of the cardiac pacemaker f-current, with an Na^+/K^+ permeability ratio of 0.27 [28, 30]. Accordingly, HCN channels are more permeable to K^+ than to Na^+ (with permeability ratios of about 4:1 (see [16] for review). Despite this preference for K^+ conductance, h-channels carry an inward Na^+ current under physiological conditions. Moreover, the global conductance of the pacemaker current increases with external K^+ concentration [28]. It has been reported that HCN channels also display a very low permeability to Ca^{2+} [31, 32].

An ongoing discussion concerns the value of the single channel conductance of pacemaker channels [10]. Native channels recorded in isolated rabbit SAN myocytes have a very small single channel conductance estimated to be only 1 pS (see Fig. 2a) [29]. However, single channel currents recorded by Michels and coworkers [33] do not appear to be the same as those reported previously by DiFrancesco [29]. One difference is in the conductance, which for native pacemaker channels is nearly 20-fold higher than that previously reported. The

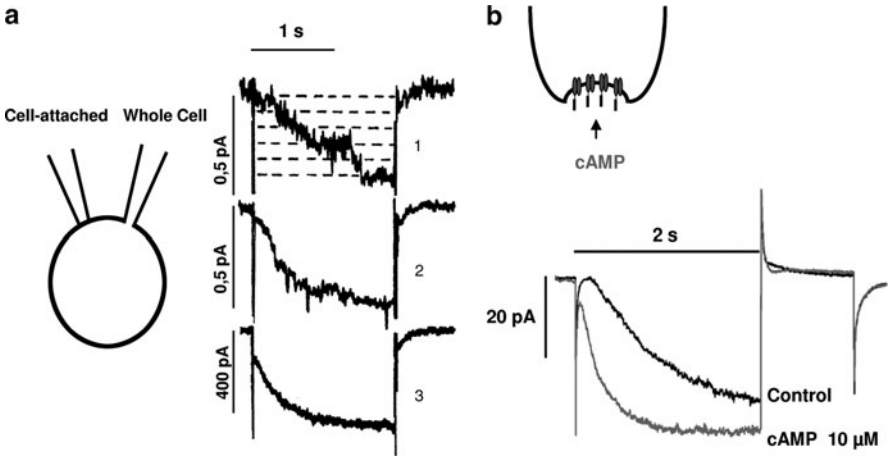

Fig. 2 (**a**) Representative recordings of pacemaker currents from whole-cell and cell-attached configurations. *1* – Single channel traces recorded from a cell-attached patch during hyperpolarization to −102 mV from a holding potential of −32 mV. *2* – Average of nine cell-attached traces. *3* – Whole-cell pacemaker current recorded with a second pipette during the same pulses (modified from [29]). (**b**) Action of cAMP on I_f activation in an inside-out macropatch. The I_f current was activated on hyperpolarization to −95 mV in a macropatch exposed to cAMP (10 µM) on the inside as represented in the *inset*

conductances reported for HCN isoforms are also very elevated (13- to 35-fold), and while the reason for this major discrepancy was not identified, it could be explained by the different cell preparations or experimental conditions (i.e., patch configurations) used. However, ensemble records shown by Michels and collaborators [33] for HCN isoforms and native h-current seem flat and do not divulge any time dependence, reflecting an instantaneous rather than a time-dependent behavior. Thus, it remains unclear whether pacemaker channels could exhibit two distinct conductances and/or different kinetics.

4 Role

As mentioned above, HCN channels generate and/or regulate neuronal and cardiac excitability. Several physiological roles have been ascribed to HCN channels, which are the consequences of their particular biophysical properties (see [16] for review).

In general, HCN channels engender and regulate neuronal and cardiac firing rates. Besides acting as a pacemaker, the HCN current also functions as a regulator of resting potential and membrane resistance. The current stabilizes the resting membrane potential because small hyperpolarizations activate the pacemaker channels, whose inward currents depolarize the cell. This depolarization, as a

consequence, deactivates the HCN channels, preventing continued departure from the resting potential. The h-channels possess an inherent negative-feedback property. On the contrary, neurotransmitters can influence rhythmic activity in both the heart and the nervous system by either increasing or decreasing the level of cAMP, which in turn directly modulates the activation kinetics and maximal current of HCN channels.

The pacemaker current is not only involved in principal rhythm generation but it also plays a key role in the regulation of heart rate by the autonomic nervous system. HCN channels are also implicated in several essential neuronal functions, which were described elegantly and in great detail by Biel et al. [16]. At least seven physiological roles have been ascribed to the h-current (1) in dendritic integration, (2) in the control of working memory, (3) in constraining hippocampal LTP (long-term synaptic plasticity), (4) in motor learning, (5) in synaptic transmission, (6) in resonance and oscillations, and (7) in the generation of thalamic rhythms.

5 Regulation

One of the most interesting characteristics of the pacemaker current is its regulation by cyclic nucleotides independently of a phosphorylation process. This mode of action of cAMP was first demonstrated by inside-out macro-patch f-current studies in rabbit SAN cells (see Fig. 2b) [34]. It was shown that cAMP directly binds to the inner face of the channel and facilitates the activation of I_h by shifting its voltage dependence of gating to more positive potentials. Further evidence of this direct regulation has been demonstrated using cAMP analogues. This study indicates that the channel's binding site may be structurally similar to the cyclic nucleotide binding site of olfactory receptor channels [35, 36]. The region implicated in ligand binding and the functional transfer of cAMP-mediated channel gating is located in the C-terminus [37, 38]. Wainger et al. [39] reported that deletion of the CNBD shifted the activation curves of HCN channels to more positive voltages by an amount similar to the maximal shift seen with saturating concentrations of cAMP. This indicates that cAMP binding enhances gating by removing a basal inhibitory action operated by the C-terminus. This mechanism had been previously suggested by Barbuti et al. [40]. cAMP shifts the $V_{1/2}$ value of human HCN2 and HCN4 channels to more positive voltages. In contrast, HCN1 and HCN3 are only weakly affected by cAMP [23, 37, 39]. All pharmacological agents or neurotransmitters, which are able to induce a change in the intracellular cAMP level modulate the h-current and consequently influence cellular pacemaking activity. For example, the adrenergic and cholinergic modulation of heart rate are directly related to the intracellular cAMP level, with cAMP acting as a major second messenger in f-channel regulation [20]. In the nervous system, serotonergic receptors in mammalian and crustacean motoneurons and in mammalian substantia nigra pars compacta neurons, as well as noradrenergic beta-receptors in neurons of the medial nucleus of the trapezoid body, and histaminergic H2 receptors in thalamic neurons

have been shown to modulate I_f via their modulation of intracellular cAMP levels (see [5, 16] for reviews). It has been reported that adenosine A1 receptors in thalamic and mesopontine neurons, and μ-opioïd receptors in nodose ganglion cells, are negatively coupled to adenylate cyclase, inducing a shift in the I_h activation curve [41, 42]. As is the case in neurons, I_f in the heart is enhanced by the stimulation of histamine H2 receptors and decreased by the action of adenosine [43, 44]. The current can also be regulated in both brain [45] and heart [46] by nitric oxide. This gas elevates cGMP levels by stimulation of soluble guanylate cyclase. Subsequent studies have suggested that I_h activity may also be regulated by protein phosphorylation and dephosphorylation [47]. A number of protein kinases have been implicated, including PKA [48, 49], PKC [48], and tyrosine kinases [50, 51]. It has also been shown that triiodothyronine (T3) enhances the pacemaker current in SA node cells isolated from rabbit heart by increasing maximal conductance without inducing a shift of the activation curve, signifying an overexpression of f-channels that possibly leads to the acceleration of the resting heart rate observed in hyperthyroïdism [52]. This overexpression has been corroborated by Pachuki et al. [53].

6 Pharmacology

6.1 Alinidine (ST567) and Clonidine

Alinidine (see Fig. 3 for chemical structure) is an antiarrhythmic drug that acts primarily on the sinus node. This *N*-allyl derivative of clonidine, a well-known α2 adrenoreceptor agonist, is a specific bradycardic agent. In the rabbit SAN, alinidine blocks I_f in the micromolar range [54]. This inhibition is associated with a reduced slope of slow diastolic depolarization and slightly prolonged action potential duration [55]. Experiments performed on sheep Purkinje fibers revealed that I_f inhibition by alinidine is a consequence of a decreased maximal conductance associated with a shift in the activation curve toward more negative potentials. These effects do not appear to be use- or frequency-dependent [56]. At present, despite its bradycardic action, alinidine is not used in therapy since it is not specific enough for I_f. Indeed, this drug also has inhibitory properties on other ionic currents as in the case, for example, of potassium and calcium conductances [54].

Recently, the α2 adrenoreceptor agonist clonidine was shown to have a direct inhibitory effect on I_f [57]. In that study, using knockout mice for the three α2 adrenoreceptors, the authors showed that clonidine induced bradycardia. Electrophysiological measurements in SAN cells confirmed that clonidine lowered the frequency of pacemaker potentials through I_f inhibition with an IC_{50} around 3 μM. HCN2 and HCN4 heterologously expressed in HEH293 cells present a similar sensitivity to clonidine, with an IC_{50} of 10 μM. This inhibition was associated with a shift in the activation curve toward more negative potentials.

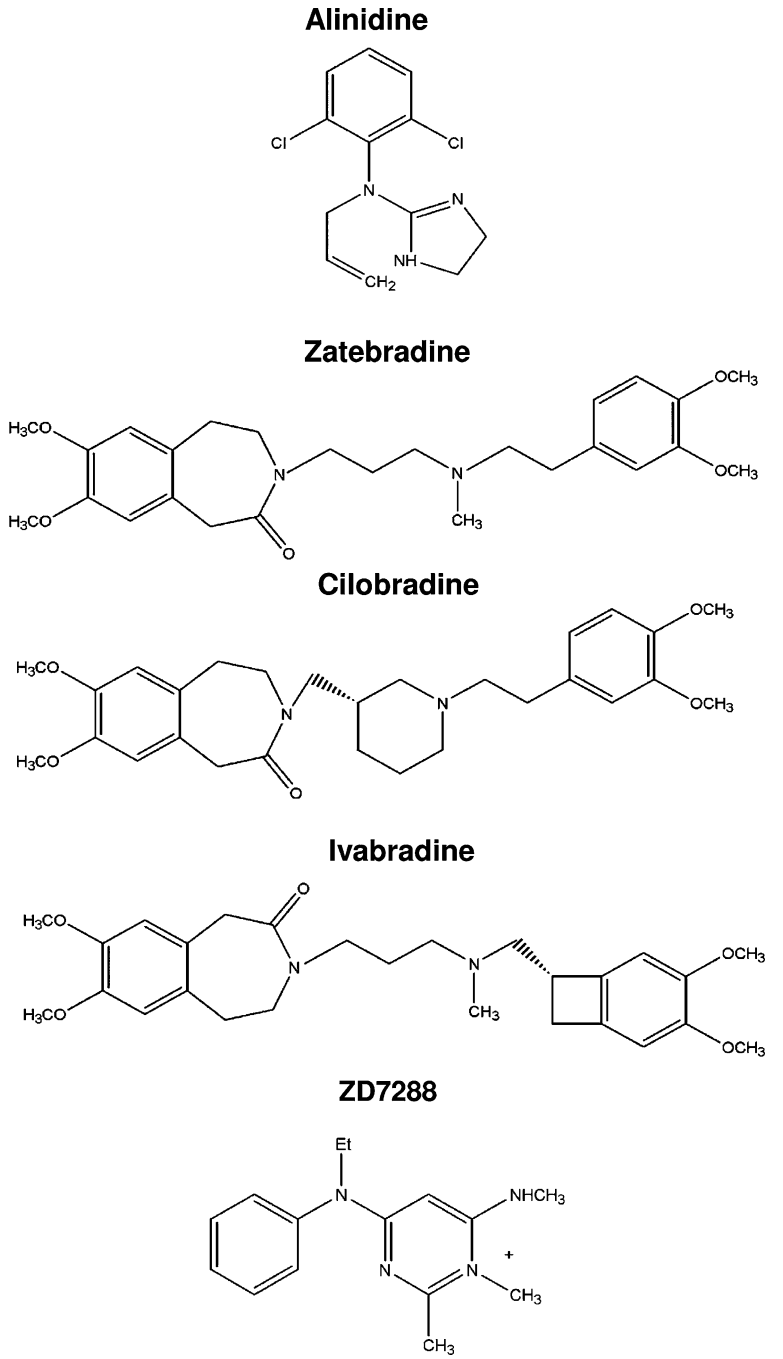

Fig. 3 Chemical structures of alinidine, zatebradine, cilobradine, ivabradine and ZD 7288

This study raises questions concerning the level of contribution of HCN channel inhibition in the bradycardic actions of clonidine observed in mouse. However, the IC_{50} for HCN2 and HCN4 seems very high to explain a major contribution of I_f inhibition in the therapeutic effects of clonidine in humans.

6.2 Zatebradine (UL-FS 49) and Cilobradine (DK-AH 269)

Zatebradine (UL-FS 49) and Cilobradine (DK-AH 269) (see chemical structures in Fig. 3) are bradycardic agents derived from structural modification of the calcium-antagonist verapamil. Both zatebradine and cilobradine cause a use-dependent block of I_f in cardiac Purkinje fibers, isolated SAN cells and the I_h current in nerve cells. More specifically, these compounds induce a concentration- and voltage-dependent inhibition of I_f, which slows diastolic depolarisation and decreases the spontaneous firing rate [58–63].

Cilobradine and zatebradine have a similar IC_{50} for the different HCN subtypes. However, use-dependent block kinetics depend on the isoform of the channel under consideration. Indeed, a study using the heterologous expression of HCN in HEK293 cells revealed that 5 μM cilobradine induces a tenfold faster use-dependent block for HCN3 and HCN4 than for HCN2 and HCN1 [63]. However, it is important to delve further into this apparent HCN isoform selectivity since the kinetics of block depend on protocol parameters such as the frequency and duration of hyperpolarizing pulses.

Interestingly, I_f recorded in murine SAN cells presents a similar use-dependent block compared to HCN4 [63]. Although cilobradine and zatebradine have similar use-dependent blocking properties, these drugs present a marked difference in their potency. Indeed, cilobradine blocks I_f and heterologously expressed HCN channels more effectively and faster than zatebradine [62, 63]. This is essentially a consequence of a slower dissociation rate associated presumably with a higher association rate.

To date, the binding site of zatebradine and cilobradine has not been fully elucidated. An investigation of the blocking mechanism of I_f by zatebradine in rabbit SAN cells indicates that this molecule blocks the channel by entering the open channel pore from the intracellular side for a distance of 39% of the membrane thickness [64]. Furthermore, a recent study using an alanine scanning mutagenesis approach revealed that mutations A425G or I432A in the S6 segment of HCN2 attenuated the block by cilobradine [65]. This block was even less effective in the double mutant I432A/A425G. These results indicate that cilobradine probably interacts with these specific residues of the S6 segment.

Besides its inhibitory action on I_f and I_h, zatebradine has been shown to block potassium currents [58, 66]. As a consequence, blocking the repolarizing current I_k in the myocardium would prolong the action potential in this tissue [67–69]. Furthermore, patients treated with zatebradine developed symptoms of visual disorders, which could be explained by the inhibitory effect of zatebradine on

retinal HCN channels [70–72]. These blocking characteristics on myocardial I_k and retinal I_h limit the possible clinical applications of zatebradine. Cilobradine appeared to be more specific than zatebradine without having any apparent effect on action potential shape when tested at low concentration on guinea-pig Purkinje fibers [62]. Experiments showed that cilobradine reduced heart rate in the rabbit without producing any negative inotropic effect, and reduced angina pectoris [73]. On that account, cilobradine might be an interesting candidate molecule for therapeutic approaches to combat cardiac diseases.

6.3 Ivabradine (S 16257)

Given that an elevated heart rate correlates with increased mortality in some cardiac diseases such as angina and heart failure, lowering heart rate is beneficial because of the reduced associated demand for oxygen and improvement in diastolic myocardial perfusion. To this extent, specific heart rate-reducing agents targeting f-channels were developed for their ability to slow heart rate by suppressing the rate of diastolic depolarization with limited side effects on action potential duration and inotropic state [74]. Among these "pure bradycardic agents", ivabradine (S 16257) [75], a compound with highly specific f-channel binding properties and typical reduction of I_f conductance, was the first I_f blocker used in clinical development and therapeutic application (see Fig. 3 for its chemical structure).

Patch-clamp studies on rabbit sinus node cells showed a selective use-dependent block of I_f in the same concentration range as that reducing the slope of spontaneous diastolic depolarisation [76, 77] (see Fig. 4a, b). Also, for concentrations close to the IC_{50} of I_f inhibition, no effect was observed on $I_{Ca,L}$, $I_{Ca,T}$, and I_{Kr}, suggesting a specific action of ivabradine on I_f in sinoatrial cells. In general, the ivabradine effect is comparable to that of zatebradine and cilobradine, since all of these drugs alter I_f by decreasing maximal conductance without changing the voltage dependence of current activation. This heart rate-reducing agent interacts with f-channels from the intracellular side [76]. Elegant studies performed on f-channels in SA node cells have provided further evidence for the precise blocking mechanism of ivabradine (for details see [77]). Ivabradine is an open-channel blocker with a block exerted when channels deactivate on depolarization (see Fig. 4c). This use-dependent property of ivabradine corresponding to drug accumulation during repetitive activity is clinically useful with respect to the better efficiency obtained at higher heart rates, when a bradycardic action is expected. The voltage-dependence of the block is a major property of ivabradine block facilitation by channel open/close cycling, with the block being stronger at depolarized voltages. The block of f-channels from the intracellular side and a better efficiency obtained at depolarized than at hyperpolarized voltages result from the chemical nature of ivabradine (positively charged at physiological pH by the presence of a quaternary ammonium ion). The action of ivabradine block can be described as "current"-dependent in that ivabradine blocks current flow by entering the pore and competing with permeating

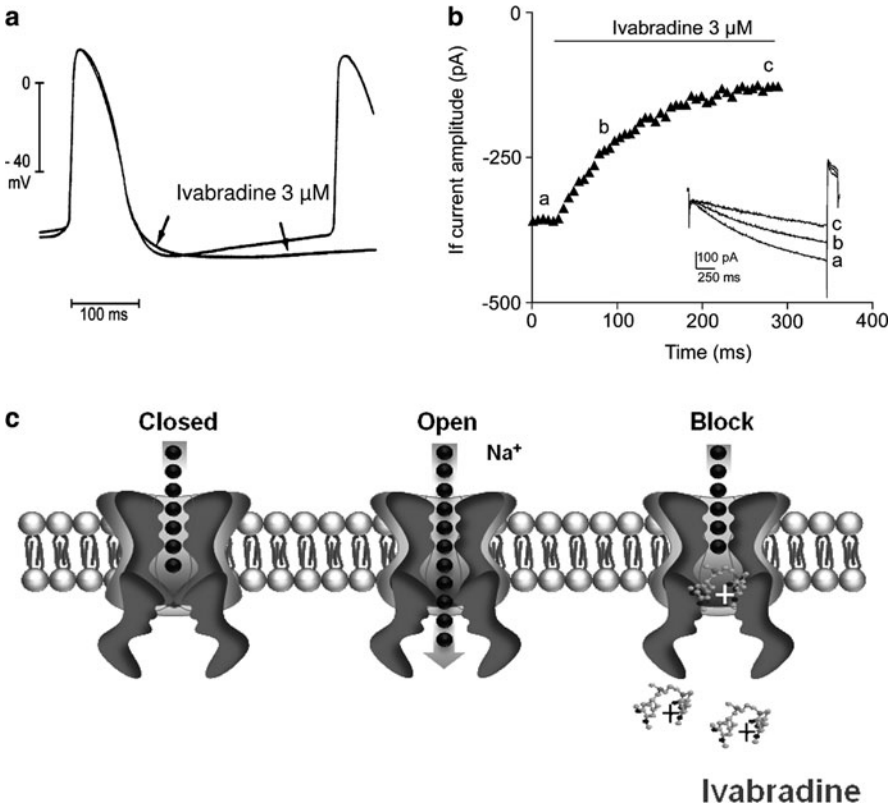

Fig. 4 (**a**) Recordings of spontaneous action potentials of rabbit sinus node preparation before and during ivabradine (3 μM) application (modified from [68]). (**b**) Use-dependent block of I_f by ivabradine (3 μM). Current was elicited by hyperpolarizing steps to −100 at 1/6 Hz. The graph plots the current–amplitude before and during ivabradine application. *Inset figures* show a set of three I_f traces specified on the graph (*a*, *b*, *c*). (**c**) Schematic representation of the specific mechanism of I_f channel blockade by ivabradine. Ivabradine enters the channel pore from the intracellular side of the channel and binds to a site in the ion permeation pathway

ions for a binding site along the permeation pathway when ions flow in the outward direction. Unblocking takes place when the current is inward during hyperpolarization. The dependence of block upon current flow was limited to HCN4, the predominant subtype present in the mammalian SAN, and is not significant for HCN1 [63, 78, 79]. This feature distinguishes the action of ivabradine from the other heart rate-reducing agents that reduce I_f in a voltage-dependent manner independently of the electrochemical gradient.

The anti-ischemic efficacy of ivabradine was initially established in pig and dog models mimicking exercise-induced angina pectoris [80–82], and was later confirmed in patients with stable angina [83, 84]. Ivabradine is, at present, the only member of the family of specific heart rate-reducing agents to have completed clinical assessment for the treatment of stable angina. Ivabradine has no side effects

on cardiac contractility and has potential for therapeutic use in patients with coronary artery disease (CAD). A large-scale, multicenter clinical trial (BEAUTI$_F$UL: the morBidity-mortality EvAlUaTion of the I_f inhibitor ivabradine in patients with coronary disease and left ventricular dysfunction) has evaluated the efficacy of ivabradine at reducing morbidity/mortality in patients with impaired left ventricular function [85, 86]. In these patients with a heart rate $\geq$70 bpm, ivabradine significantly reduces important coronary events such as myocardial infarction (MI) (by 36%) and coronary revascularisation (by 30%). Moreover, in a subgroup of patients with limited angina, the BEAUTI$_F$UL study showed that ivabradine (Procoralan) reduced the risk of the combination of primary endpoint-cardiovascular death, hospitalization for acute MI, or new or worsening heart failure by 24% in all angina patients, and by 31% in those with a heart rate $\geq$70 bpm. More recently, the Systolic Heart Failure Treatment with the If inhibitor ivabradine Trial (SHIFT) study has confirmed that high heart rate is a risk factor in heart failure, and the results support the importance of heart rate reduction with ivabradine for improvement of clinical outcomes in heart failure [87, 88]. Other ongoing clinical trials (SIGNI$_F$Y and VIVI$_F$Y) will allow a more precise analysis of the therapeutic action of ivabradine in CAD and acute coronary syndrome.

Although the f-current is the main ionic mechanism involved in the genesis and regulation of the spontaneous activity of SA node cells under physiological conditions, overexpression of pacemaker channels can be observed in pathological situations such as thyrotoxicosis, leading to sinus tachycardia [52, 53]. Overexpression of f-channels is also observed in rat hypertrophied myocytes [89] and in failing human heart [90]. Under these pathological conditions, I_f could be interacting with other mechanisms and thus contribute to the appearance of ventricular arrhythmias. In this way, we have reported that ivabradine inhibits the I_f current in atrial myocytes isolated from human right appendages with characteristics similar to those described previously in rabbit sinus node cells. In this human atrial tissue, the major HCN gene subtype detected was HCN2. The action of ivabradine could be beneficial in limiting the genesis of ectopic atrial arrhythmias, in which I_f may be involved [91].

Ivabradine could thus offer a new therapeutic strategy (or be a good candidate) to reduce these arrhythmias.

6.4 ZD 7288

ZD 7288 [4-(*N*-ethyl-*N*-phenylamino)-1,2-dimethyl-6-(methylamino) pyrimidinium chloride] (see chemical structure in Fig. 3), originally named ICI D7288, has been shown to reduce the I_f current in many different physiological preparations. In the heart, at concentrations lower than 1 μM, ZD 7288 was reported to decrease the spontaneous beating rate of guinea-pig isolated right atria with no effect on the contractile force of paced left atria [92]. This effect was attributed to a strong and specific inhibition of I_f at concentrations less than 1 μM [93]. ZD 7288 was

also shown to block the neuronal I_h current, although with less affinity than in cardiac cells [94, 95]. Precise mechanisms and molecular determinants of the effects of ZD 7288 have been the subject of continued efforts. In 2001, Shin and collaborators [96] characterized I_f blockade mechanisms by specific analysis of the effects of ZD 7288 on HCN1 expressed in HEK293 cells. They found that the blocking effects of ZD 7288 required channel opening and that the drug was trapped by closing of the channel. Interestingly, the ZD 7288 binding site has been located in the pore lining of the channel. A recent study by Cheng et al. mapped the binding site of ZD 7288 on HCN2 expressed in Xenopus oocytes [65]. Using site-directed mutagenesis, these authors reported that two amino acids located in the 6th transmembrane domain of the protein (Ala425 and Ile432) were determinant for the effect of the compound.

7 Conclusions and Future Directions

The hyperpolarization-activated cation current is clearly an important target for the treatment of stable angina in the heart. Modulation of I_h may also be a promising approach for the treatment of disease processes in the central and peripheral nervous systems. Several results suggest that HCN channels also play a prominent role in neuropathic pain (for recent review, see [97]). In this case, I_h blockers could be beneficial for analgesic therapy. Furthermore, the use of I_h blockers has also been implicated in epilepsy therapy [98]. However, given the complexity of the cellular mechanisms leading to these diseases, a clear notion for a rational design of antiepileptic I_h channel modulators has not yet emerged. Finally, HCN channels may contribute to the clinical actions of general anesthetics. Native neuronal I_h as well as heterologously expressed HCN channels are inhibited by clinically relevant concentrations of anesthetics [99–102]. In principle, existing blockers could be plausible molecular candidates. Nevertheless, these drugs inhibit all HCN isoforms with no apparent subtype selectivity and would also exert bradycardic effects. Ideal I_h neuronal blockers should not interfere with the function of sinoatrial HCN4 channels.

HCN isoforms could serve as new genetic targets in the modulation of cellular rhythmicity. For example, it was shown that HCN4 mutations underlie certain congenital cardiac arrhythmias such as the inherited form of the sick sinus syndrome [103]. Furthermore, it is essential to amplify the range of available therapeutic agents specifically targeting cardiac pacemaker channels, for instance. In this way, high affinity, subtype-specific HCN channel blockers must be developed. Moreover, one of the most important challenges in the coming years is the development of biological pacemakers, which may replace electronic devices. Indeed, pacemaker cells derived from stem cells and/or the stable in situ transfection of HCN channels represent a promising novel approach for the development of such pacemakers.

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