Heart rate reduction via selective ‘funny’ channel blockers
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The ‘funny’ current, first described in cardiac pacemaker cells almost 30 years ago, is a key player in the generation of pacemaker activity and the autonomic modulation of heart rate. Because of these specific functions, a search for molecules able to interfere selectively with the ‘funny’ current was undertaken soon after its discovery, with the aim of developing tools for the pharmacological control of heart rate. This search has succeeded in generating a new class of drugs, the heart rate-reducing agents, which act through specific blockade of f-channels; one of these drugs, ivabradine, is presently marketed against stable angina. Because of their many functions in heart and other tissues, pharmacological utilization of “funny” channel properties is an exciting new frontier open to further developments.

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Introduction
Pharmacological reduction of heart rate is a crucial target in the treatment of several pathological conditions characterized by a deficiency of oxygen supply to the working myocardium, such as angina pectoris, a common manifestation of coronary artery disease, ischaemic heart disease and cardiac failure.

Ideally, pharmacological interventions aimed at reducing heart rate selectively should be free of consequences on other cardiovascular parameters; unfortunately, however, conventional drug therapies based on β-blockers and Ca2+ antagonists are often associated with mild to severe decreases in ventricular contractility and modification of vascular tone. This explains why the search for specific heart rate-reducing agents has long been, and still is, a major goal of cardiac pharmacologists.

Cardiac pacemaker rate is controlled by the duration of the diastolic depolarization, and the main source of depolarizing current responsible for this phase is the so-called pacemaker or ‘funny’ (I_f) current. In the past several years, various f-channel inhibitors such as alnidine (ST567), falipamil (AQ-A39), ZD7288 and zatebradine (UL-FS 49) have been developed and shown to reduce heart rate but, because of lack of selectivity and/or presence of undesired side effects, further development aimed at their clinical use has not been pursued. More recently, ivabradine (Servier), a new molecule selected for higher f-channel selectivity and reduced side effects, has been developed and is now being marketed for pharmacological treatment of chronic stable angina.

In this article, we briefly review the properties of the pacemaker current, highlighting those features that are relevant to the block induced by f-channel-inhibiting agents and the related clinical applications.

The ‘funny’ current and cardiac pacemaking
The pacemaker ‘funny’ I_f current is a mixed potassium–sodium inward current that in cardiac pacemaker cells of the sinoatrial (SA) node activates upon membrane hyperpolarization below a threshold of approximately −45 mV, and whose activation range encompasses the range of diastolic depolarization (approximately −65 to −40 mV). In addition to voltage, f-channels are also directly activated by cAMP, which acts as a second messenger in channel modulation; this mechanism is responsible for the control of cardiac rate by autonomic sympathetic and parasympathetic neurotransmitters [1–3].

The role of native f-channels of the SA node in the generation of pacemaker activity and rate regulation is well established [2–6]. Basic evidence for this role relates to the main properties of the I_f current (i.e. activation range overlapping with that of diastolic depolarization; I_f-mediated control of cardiac rate by autonomic transmitters) and the observation that I_f and spontaneous activity correlate in several circumstances (see [7]). Further evidence, discussed below, concerns the ability of substances affecting I_f (e.g. I_f inhibitors) to modify the slope of diastolic depolarization and heart rate.

Cloning in the late 1990s of the molecular correlates of f-channels — the hyperpolarization-activated cyclic nucleotide-gated (HCN) channels — further highlighted
the physiological role of If. The four known isoforms of mammalian HCN channels (HCN1–4) generate ion currents qualitatively similar to native currents, but with quantitative differences in their kinetic properties [2]. The relative abundance of mRNA for HCN subunits varies among tissues, species, age and disease states [8–17]. In heart, HCN channels are highly expressed in the SA node (HCN4 being the major subunit) and to a lesser degree in the conduction tissue, where HCN2 is also expressed [12]; weak HCN expression, mainly HCN2, is also detected in the adult working myocardium [11–13].

The role of HCN channels in pacemaking has been confirmed in several ways. For example, overexpression of the HCN2 isoform strongly increased the spontaneous rate by inducing a diastolic depolarization phase in neonatal rat ventricular myocytes [15], whereas dominant-negative suppression of HCN2 and HCN4 isoforms blocked spontaneous pacing [18]. Recently, we found in a large Italian family that a specific point mutation in the human gene coding for HCN4 (R672S) is associated with inherited sinus bradycardia [19**]; the mutation shifts the current activation curve to more negative potentials and, acting much like a mild vagal stimulation, reduces the depolarizing current during diastole and thus slows heart rate. This is the first reported HCN channel mutation specifically affecting cardiac rate and provides further direct evidence for the role of If in cardiac rate control.

I<sub>f</sub> inhibitors: heart rate-reducing agents

The If current is active at diastolic potentials and its functional contribution is therefore particularly suited to controlling the rate of diastolic depolarization, which determines the time interval between consecutive action potentials. Owing to this specific involvement, the pacemaker current represents an ideal target for the development of pure heart rate-modulating agents that lack additional undesired effects on cardiovascular performance.

In the past three decades, several molecules with heart rate-reducing properties have been developed and identified as f-channel blockers. Early drugs identified as pure bradycardic agents include alinidine (ST567), ZD7288 and zatebradine (UL-FS49) and its derivatives; a more recent drug is ivabradine (S16257). The main action of these substances is to induce a reduction of the diastolic depolarization slope by blocking If [3], although high concentrations might affect other channels and lead to arrhythmias [20,21**].

Alinidine

Alinidine was one of the first compounds developed as a bradycardic agent. It was found to slow heart rate by prolonging the diastolic depolarization phase, an effect caused by a twofold inhibitory action on the pacemaker current (i.e. a reduction of the maximal channel conductance and a shift of the activation curve of the current to more negative voltages); the action of alinidine is not use- or frequency-dependent [22]. Despite its efficacy in reducing heart rate, development of alinidine was discontinued owing to the lack of specificity of its action. Indeed, alinidine also blocks calcium and potassium currents and prolongs the action potential repolarization process, which makes the compound less attractive for therapeutic use [22,23].

Zatebradine and related compounds

Zatebradine (UL-FS49), cilobradine (DK-AH26) and falipamil (AQ-A39) are bradycardic agents derived from the Ca<sup>2+</sup> channel blocker verapamil. The bradycardic effect induced by these drugs is mediated by a use-dependent block of the pacemaker current, without modification of the voltage dependence [24–26]. A detailed investigation of the If blocking mechanism by zatebradine showed that block results from drug molecules entering the channel pore from the intracellular side for a distance of ~39% across the electrical field only when channels are in the open state [27]. Although zatebradine is more specific than alinidine in its ‘pure bradycardic’ action, patients treated with zatebradine developed undesired symptoms of visual disorders, which limited possible clinical applications. Expectedly, the visual effects were found to be caused by zatebradine-induced block of the If current, the neuronal counterpart of If, which plays a major functional role in the transduction of light signal in photoreceptors [28,29]. Reduced infarct size and reduced mortality following zatebradine administration have been reported in rats with myocardial infarction, although adverse effects were observed on left ventricular remodeling [30].

Cilobradine is a new compound that is similar to zatebradine, but with improved selectivity for If. When tested in guinea-pig Purkinje fibres, cilobradine (1 μM) reduced the If current by 80% and, at a concentration of 0.3 μM, strongly slowed the rate of diastolic depolarization without apparent changes in action potential shape and duration [26]. When cilobradine was tested in rabbit hearts, data confirmed its heart rate-inhibiting effect and a lack of substantial inotropic or vascular alterations [31].

ZD7288

Early investigation of ZD7288 showed that this compound reduces the spontaneous rate of intact right atria, but does not modify ventricular contractility [32]. When tested in intact SA node, ZD7288 (0.3 μM) induced a slowing of rate (~61%), but also caused a modest prolongation (+10%) of action potential duration [33]. Experiments in single SA node myocytes isolated from guinea-pig hearts showed that the primary effect of ZD7288 is to
block the If current, with minor effects on Ca\textsuperscript{2+} and K\textsuperscript{+} currents. For example, 1 \mu M ZD7288 reduced the pacemaker and Ca\textsuperscript{2+} currents by 82% and 18%, respectively; when a concentration of 0.1 \mu M was tested on the delayed K\textsuperscript{+} current, a decrease of \sim 1% was observed [34]. The moderate action potential prolongation observed in intact SA node experiments is therefore attributable to the action of ZD7288 on currents other than If. Analysis of the action of the drug on the If current revealed a use-independent block associated with a shift of the channel activation curve to more negative potentials (\sim 16.2 mV) and a decrease of maximal channel conductance (\sim 52% at 0.3 \mu M) [34]. A limitation of the potential clinical use of ZD7288 was again reduced channel specificity. Indeed, in addition to its heart rate-inhibiting effect, ZD7288 also affects the neuronal Ih current in several regions of the central nervous system, such as the substantia nigra, the hippocampal CA1 region, thalamocortical neurons and photoreceptors [35,36].

Ivabradine
Ivabradine (S16257) represents a new compound in the coronary artery disease pharmacopeia, and has recently been approved by the European Medicines Evaluation Agency as a new symptomatic treatment for patients with chronic stable angina pectoris. The efficacy of this drug and its mechanism of action have been extensively investigated both in preliminary studies in vitro and in animal models, as well as in clinical trials. At a concentration of 3 \mu M, ivabradine reduces the rate of spontaneous firing in the isolated rabbit SA node by 24%, and its bradycardic action is associated with a decrease in the slope of the slow diastolic depolarization, with no alteration of action potential amplitude and only a minor increase of its duration (+9%) [37]. In agreement with its selective effect on diastolic depolarization, ivabradine is highly specific for f-channels at the same concentration, and strongly reduces the If current (\sim 60%) without affecting T-type Ca\textsuperscript{2+}, L-type Ca\textsuperscript{2+} and delayed outward K\textsuperscript{+} currents (Figure 1) [20].

Investigation of the detailed blocking mechanism of native f-channels [38] has shown that ivabradine acts by accessing the channels from their intracellular side and by exerting use- and current-dependent block. Ivabradine can access the blocking site only when channels are in the open state (state-dependence) and its block is favoured by depolarization; however, block is not dependent upon voltage per se, but depends upon the presence of an outward flow of current through the channel (current-dependence). This latter property is unique and is not found in other f-channel inhibitors such as zatebradine and ZD7288, whose block is purely voltage dependent [38]. Because channels open on hyperpolarization, and block is favoured by depolarization, block develops quickly and strongly when channels cycle through open/

Figure 1

Heart rate-reducing action of If inhibitors. (a) Zatebradine (3 \mu M, left) and ivabradine (0.3 \mu M, right) slow the rate of spontaneous activity of isolated SA node myocytes by reducing the steepness of diastolic depolarization; this action is attributable to drug-induced If inhibition [18]: left, zatebradine 10 \mu M; right, ivabradine 3 \mu M. Note that action potential prolongation in (a) reflects partial block of K\textsuperscript{+} components by zatebradine (3 \mu M).
(c) Prolongation of time to limiting angina (measured during double-blind dose-ranging, standardized bicycle exercise tolerance tests, for protocols see [39]) plotted for various doses of ivabradine at peak of drug activity (mean \pm SEM). Asterisks indicate values significantly different from placebo (P < 0.05). Data in (a) reproduced with permission from [37]; data in (c) are replotted from [39].
closed states at high rates (use-dependence), a useful property for a drug targeted to be more effective during tachycardia (accelerated heart rate).

Clinical studies performed to test the ability of ivabradine to prevent angina symptoms and the underlying ischemia [39,40] (for review, see [41]) have shown that, in agreement with data obtained in vitro and in animal models, ivabradine reduces heart rate at rest and during exercise, without modification of parameters unrelated to heart rate, and displays anti-ischemic properties. As with zatebradine, ivabradine treatment caused visual symptoms which, however, were mild and normally well tolerated. These results demonstrated the efficacy of ivabradine for angina prevention and validated the clinical requirements for drug marketing [42] (see also Update).

**Specific effects on HCN channels**

More detailed molecular insight into the mechanisms of f-channel inhibition by heart rate-reducing agents has been achieved by investigation of their action on heterologously expressed individual HCN isoforms. An important aim of this approach is to provide information ultimately useful to help design new isoform-specific molecules.

Zatebradine, cilobradine and ivabradine block currents elicited by homomeric expression of all four HCN isoforms in a use-dependent manner, with half-block values in the micromolar range and Hill coefficients close to 1.0; these values are similar to those reported for native rabbit sinoatrial If [20,21,38,43].

Investigation of the action of ZD7288 on HCN1 channels in inside-out patches from HEK293 cells has revealed a highly voltage-dependent block that requires opening of channels and which is relieved by hyperpolarization [44]. According to this study, specific sites in the S6 domain are important in block reversibility, and drug molecules are trapped by an intracellular activation gate when channels are closed.

Details of ivabradine block of HCN1 and HCN4, two isoforms expressed in SA node tissue [12,14], have also been investigated by heterologous expression in HEK293 cells (Figure 2) [43**]. The analysis has shown that HCN4 block is similar in many aspects to that of native f-channels of the SA node; specifically, ivabradine is an ‘open-channel’ blocker of HCN4, and the concentration-response curves of HCN4 and f-channel block essentially coincide. These data are expected in view of the fact that HCN4 is the most highly expressed HCN isoform in the SA node [12]. Unexpectedly, however, ivabradine was found to exert a ‘closed-channel’ type of block on HCN1 [43**]. Interpretation of these data implies that binding
depends upon drug interactions with specific channel residues, which are either distinct or spatially oriented in a different way in the two isoforms. Structure–function studies of mutated channels are likely to be required to address which residues are involved in state-dependent binding.

Conclusions
Recent advances in our understanding of the molecular basis of cardiac pacemaking and the pharmacology of ion channels have allowed us to identify novel approaches to the specific control of heart rate that do not involve modifications of other cardiovascular parameters and thus lack potentially adverse side effects.

The molecular target of these approaches is the pacemaker ‘funny channel’ of the SA node. To date, several f-channel inhibitory compounds have been tested and, among these, ivabradine has succeeded in gaining clinical approval and is now on the market. Because HCN channel isoforms are unevenly distributed among excitable tissues, the identification of new isoform-specific compounds is expected to allow in the near future the development of therapeutic applications based on cell/tissue-specific pharmacology.

Update
Specific heart rate inhibitors such as ivabradine have no side effects on cardiac inotropism, and have potential for therapeutic use in patients with coronary artery disease. Indeed, a large-scale multicenter clinical trial (BEAUTIFUL: the morbidity-mortality Evaluation of the If) launched to evaluate the efficacy of ivabradine at reducing morbidity/mortality in CAD patients with impaired left ventricular function has been completed.

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References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- of outstanding interest

The authors evaluated the block by zatebradine, cilobradine and ivabradine of all four HCN isoforms, finding no indication of isoform specificity of block at steady-state, whereas IC50 values differed among drugs. Telemetric electrocardiogram recordings in mice revealed that, at high concentrations, all sinus node inhibitors induced arrhythmias, characterized by periodic fluctuations in the interval between T and P waves, which the authors attributed primarily to Ii inhibition, while not discounting possible effects on other currents.