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The Role of Cardiac Pacemaker Currents in Antiarrhythmic Drug Discovery

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CONTENTS

1. THE PACEMAKER CURRENT AS A TARGET FOR THERAPY

From even the most basic physiological observations, it is obvious that parts of the heart can generate their own intrinsic contractile rhythm. With the development of techniques to record electrical activity from tissues, it became clear that contraction of the heart is triggered by an action potential and that most areas of the heart can, under some circumstances, generate rhythmic action potentials, although it is cells in the sinoatrial node (SAN) that have the highest intrinsic frequency, and hence it is these cells that normally provide the drive for the rest of the heart. Even a single, isolated SAN cell will generate rhythmic action potentials separated by a period of slow diastolic depolarization. It is the diastolic depolarization that repeatedly drives the membrane potential towards threshold for action potential firing. The mechanism underlying this diastolic depolarization has been a subject of much puzzlement. By the 1970s, it was clear that to provide a slow depolarization, there must be a slow increase in net inward current, although it was not clear whether this came about as a consequence of the slow inactivation of an outward current (presumed to be a carried by potassium) or the slow activation of an inward current *(1)*. Using the intracellular recording and voltage clamp techniques available at the time, it was difficult to resolve the different components of the current flowing during the diastolic depolarization *(2–4)*. With the advent of patch-clamp techniques, coupled with techniques for preparation of isolated cells

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from various parts of the heart, resolution of individual currents became possible. The mechanism of the diastolic depolarization was greatly clarified by the discovery in pacemaker cells of an inward current that was activated by hyperpolarization *(5–8)*. The current was shown to activate relatively slowly on hyperpolarization and to have low selectivity among cations. These properties are just what one would expect for a pacemaking current

Around the same time as it was discovered in cardiac myocytes, a similar current was identified in photoreceptors *(9)*. Subsequently, similar currents were discovered in other tissues, particularly in parts of the central nervous system (CNS). The current in cardiac tissue was christened I(f) (f for funny, because of its funny properties) and in most other tissues it was called I(h) (h, because of the activation on hyperpolarization). The terms are often used interchangeably.

I(f) or I(h) has been implicated in cardiac *(10)* and neuronal *(11–13)* rhythmogenesis, sensory adaptation *(14,15)*, shaping of synaptic potentials *(16)*, and control of synaptic transmitter release *(17,18)*.

2. THE CHARACTERISTICS OF I(F)

As noted above, the critical and unusual feature of I(f) is its voltage dependence. I(f) is activated by hyperpolarizations with a threshold of approx –40 to –50 mV in the SAN. Figure 1 shows a typical activation curve, which depicts the relative fraction of channels open at steady state as a function of membrane voltage. This relation indicates that the current is activated at voltages near the range of the diastolic depolarization in SAN cells. The fully activated current/voltage (I–V) relation reverses near +10 to +20 mV in physiological solutions as a consequence of the channel having a mixed permeability to Na^+ and K^+. The activation by hyperpolarization and permeability to Na^+ and K^+ are critical properties with respect to the role of I(f) in the generation of diastolic depolarization and hence of spontaneous activity. Hence, membrane potentials around the maximum diastolic potential in SAN cells activate the current, which, because it is a nonspecific cation current, produces an inward, depolarizing current at these potentials. To a large extent, it is this slow activation of I(f) that tends to drive the slow diastolic depolarization until the membrane potential reaches threshold for the triggering of a new action potential.

However, when it was first described, there was initially some skepticism regarding the role of I(f) in pacemaking and, indeed, there is still some degree of controversy as to just how important I(f) is to pacemaking *(19,20)*. Although I(f) seems to be a good candidate for the role of a pacemaker current, the real situation is somewhat more complex. An important caveat is that, rather than being driven by one current, the pacemaking activity of SAN cells is a product of the interplay of many currents *(21)*. This assertion is reinforced by the observation that pacemaking continues (albeit at a slowed rate) when I(f) is blocked *(22)* and from the observation that the *smo* mutant of zebra fish (which has a mutation in pacemaking channels carrying the fast kinetic component of the current which renders them nonfunctional) still has an operational pacemaker, albeit once again at a greatly slowed rate *(23)*. Hence, it seems that the autorhythmicity of SAN cells is driven and stabilized by an interplay of several currents, none of which is crucial, but all of which influence rhythm. This situation provides redundancy and pleiotropism in the regulation of rhythm *(21)*. Nevertheless, it is certainly true that one

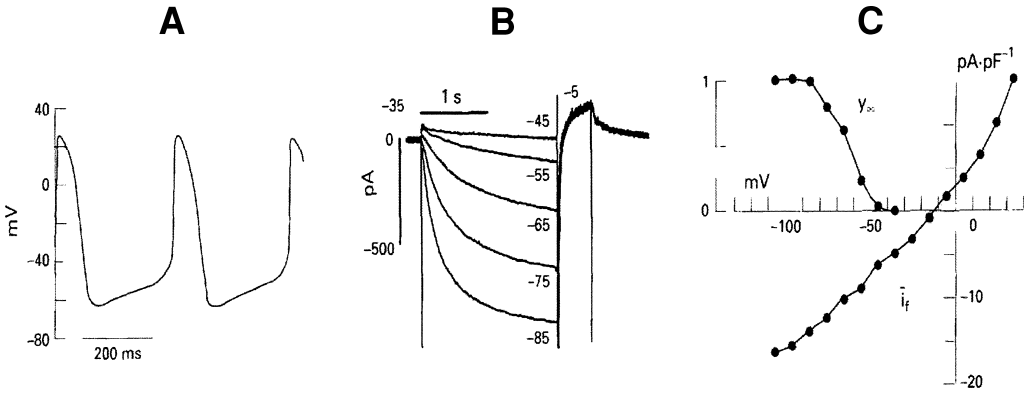

Fig. 1. The biophysical properties of I(f). (**A**) Spontaneous activity of a single SAN cell. Note that the maximum diastolic potential is about –65 mV. (**B**) I(f) current induced in a single SAN cell by a hyperpolarising pulse to the voltages indicated from a holding potential of –35 mV. (**C**) Voltage dependence of activation of I(f), (Y_∞) and fully activated I/V relationship. Reprinted from DiFrancesco, D. (1995), The onset and autonomic regulation of cardiac pacemaker activity: relevance of the f current. *Cardiovasc. Res.* **29,** 4493–4556, with permission from Elsevier Science.

of the major influences on auto rhythmicity is I(f) and, hence, it is sensible to use I(f) as a target in attempts to modulate rhythmicity.

If I(f) is indeed responsible for driving the diastolic depolarization, one would imagine that it should be modulated by sympathetic and parasympathetic stimulation in a way consistent with their chronotropic effects. I(f) is indeed modulated by adrenergic agonists in a way consistent with an increase in heart rate *(24)*.

In 1986, DiFrancesco *(25)* showed that I(f) was carried by channels having a single channel conductance of about 1 pS, and that modulation of I(f) by adrenaline at the single channel level consists of an increase in the open probability without a change in single channel conductance. This effect of adrenaline is mediated via the G-protein/adenyl cyclase/cAMP pathway. Using patch clamp techniques, it has been shown that I(f) channels are modulated by a direct effect of intracellular cAMP at the intracellular side of the channel, independent of any phosphorylation effects *(26)*. The effect of cAMP consists of a facilitation of the opening of single I(f) channels in response to hyperpolarization. Measurement of the voltage dependence of the channel open probability shows that cAMP shifts the probability curve to more positive voltages without modifying its shape *(27)*.

Interestingly, the modulation itself seems to be subject to other influences, notably the action of phosphorylation. Hence, the phosphatase inhibitor calyculin A increases I(f) and potentates the beta-adrenergic mediated response *(28)*, suggesting that there is a so-called tonic level of phosphorylation of the channels that regulates their activity *(29)*. The channels carrying I(f) have also been reported to be modulated by various hormones and growth factors. Epidermal growth factor increases maximal I(f) conductance, apparently acting through tyrosine kinase *(30)*. Vasoactive intestinal peptide produces a slight shift in the voltage dependence of activation of I(f) *(28)*, an effect that may be related to the role of vasoactive intestinal peptide as a cotransmitter with ace-

tylcholine released from vagal nerve endings. The thyroid hormone T3 increases the current density of I(f) in rabbit SAN cells without changing the voltage dependence, an effect that may underlie the sinus tachycardia commonly seen in hyperthyroidism *(31)*. Similar results have been reported with parathyroid hormones *(32)*. In addition, I(f) is modulated by muscarinic agonists in a way consistent with the decrease in heart rate seen with these agents *(33)*.

3. VARIABILITY OF CHARACTERISTICS OF I(F) IN DIFFERENT TISSUES

Reflecting this wide range of tissues in which it is found and the differing physiological functions with which it is therefore involved, the properties of I(f) (or I[h]) differ significantly in their voltage dependence, activation kinetics, and sensitivity to cAMP in different tissues *(12,34)*. This heterogeneity of properties of I(f) is found not only between tissues but also within organs or tissue types themselves. Hence, biophysical studies have demonstrated that I(f) has a dramatically different voltage dependence of activation in different cardiac regions, with the threshold for activation being –50 mV in SA node, –85 mV in Purkinje myocytes, and –120 mV in adult ventricular myocytes *(35)*. This difference in the voltage-dependent properties of I(f) is highly correlated with the intrinsic pacemaker activity of these tissues; the I(f) current having the most positive activation is found in the tissue with the highest pacing rate (in SA node), whereas the current having the most negative activation is found in tissue that normally exhibits no diastolic depolarization at all (ventricular myocytes). Although some of these differences in current properties may be the result of post-translational modification of the channels or functional modulation by phosphorylation, the most likely explanation is that I(f) currents are carried by different isoforms of the channel in different tissues. Current density is also an important factor, presumably controlled by the level of gene expression; as well as different voltage dependence, I(f) in ventricular cells is present at a very low current density (Fig. 2). With the recent cloning of the channels responsible for I(f), the factors controlling the level of gene expression, and which isoform of the channel is expressed, have become amenable to investigation.

4. MOLECULAR BIOLOGY OF I(F) AND I(H)

Although the properties of I(f) and the single channels underlying the current have been investigated for nearly three decades, particularly because of its importance to cardiac pacemaking *(10)*, cloning of the gene coding for I(f) was only achieved nearly three decades after the original description of I(f), and even then the clone was discovered serendipitously. While searching for proteins interacting with the SH3 binding domain of neural Src, Santoro et al. *(36)* identified a putative member of a new family of channels cloned from mouse brain. Using BLAST searches of expressed sequence tag databases, along with reverse-transcription polymerase chain reaction and screening of cDNA libraries, four isoforms of the channel were subsequently cloned in mammals *(37–40)*. Functional expression of these channels resulted in currents with the hallmarks of the cardiac I(f) or its neuronal equivalent I(h). Although the properties of different isoforms differ quantitatively, all isoforms except one yield currents that are activated by hyperpolarization, are permeable to both K^+ and Na^+, are blocked by

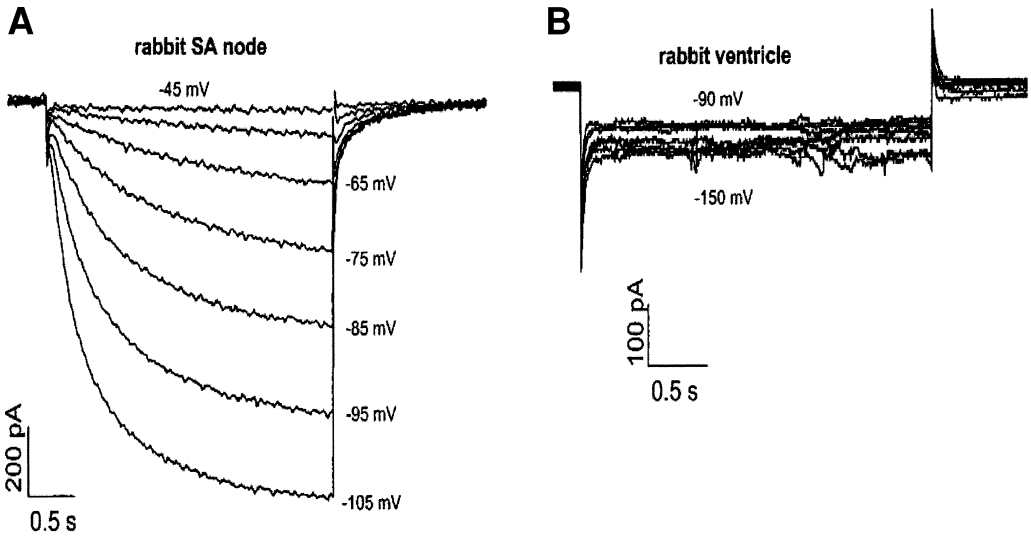

Fig. 2. The relative magnitudes of I(f) in rabbit ventricle and SAN cells. I(f) was evoked by hyperpolarizing voltage steps to the voltages indicated from a holding potential of –35 mV in either a single rabbit SAN cell (**A**) or ventricle cell (**B**). Note the different current scales. Reprinted from Shi, W., Wymore, R., Yu, H., Wu, J., Wymore, R. T., Pan, Z., Robinsin, R., Dixon, J. E., McKinnon, D., and Cohen, I. S. (1999), Distribution and prevalence of hyperpolarization-activated cation channel (HCN) mRNA expression in cardiac tissues. *Circ. Res.* **85,** e1–e6, with permission from Lippincott, Williams & Wilkins Publishers.

cesium (Cs^+) in a voltage-dependent way, and are modulated by a direct action of cAMP on the cytoplasmic side of the channel *(38,39,41)*.

Because of the serendipitous nature of their discovery, the early nomenclature of the gene and its isoforms for pacemaker channels is somewhat inconsistent. Some investigators referred to the isoforms as *HAC1* or *HAC2*, denoting hyperpolarizing activated channel. However, because of the direct action of cAMP and gating by hyperpolarization, the nomenclature suggested by Clapham *(42)* and Biel et al. *(43)* has been generally adopted; that is, of referring to the clones as *HCN* (hyperpolarization-activated, cyclic nucleotide-gated) channels. Compared with the previous nomenclature, *HCN1* corresponds to *HAC2* (*mBCNG-1*); *HCN2* corresponds to *HAC1* (*mBCNG-2*); and *HCN3* corresponds to *HAC3* (*mBCHG-4*).

Cloning of the gene for HCN channels and the consequent ability to perform site-directed mutagenesis and functional studies of channels expressed in heterologous systems has enabled rapid progress in defining the structure and function of the channel protein. The gene transcripts code for a protein of between 800 and 1200 amino acids, with the different protein isoforms having an overall sequence identity of 60%. They are members of the voltage-gated cation-channel superfamily, members of which are characterized by six membrane-spanning segments (S1–S6), including a voltage-sensing S4 segment, and an ion-conducting pore between S5 and S6. It seems safe to assume, by analogy with the voltage-gated family of potassium channels, that functional HCN channels are formed as tetramers of subunits, with each of the four subunits contributing to the pore region. In a structure analogous to the S4 voltage-sensing

region of voltage-gated K^+ channels, the S4 region of HCN channels has two regions each containing five positively charged amino acids (10 positive charges compared with 5–7 in potassium channels). This raises some intriguing questions because the two families of channels are gated in opposite ways, the Kv family of potassium channels being opened by depolarization and HCN channels by hyperpolarization. This conundrum of the biophysics of gating of HCN channels has yet to be resolved *(44)*.

The pore region of HCN channels is also related to that of K^+ channels, having a signature GYG triplet *(45)*. However, the aspartate that follows the GYG signature sequence in most K^+ channels is replaced in HCN channels by arginine, alanine, or glutamine. This presumably underlies the high relative permeability to Na^+ of HCN channels compared to classic K^+ channels *(46)*; values of P_{Na}/P_K for cloned HCN channels are between 0.25 to 0.41 *(37,38,41)*.

In the carboxy terminus, HCN channels contain a sequence with a high degree of homology to the cyclic-nucleotide-binding proteins, such as cyclic nucleotide gated (CNG) channels of photoreceptors and olfactory neurons *(47)*, cAMP-dependent protein kinases, and the catabolic-activator protein of *Escherichia coli*. The mechanism of modulation of the channel by cAMP is thought to consist of a COOH-terminus mediated inhibition of opening, which is partially relieved by cAMP binding, as revealed by site directed mutagenesis of the COOH linker in *HCN1* and *HCN2* isoforms *(48)*.

When expressed in heterologous systems, three of the four *HCN* genes have been shown to generate hyperpolarization-activated currents with distinct biophysical properties. *HCN1* channels activate most quickly and require the least amount of hyperpolarization to open, with a half-maximal voltage, $V_{1/2}$, of about –73 mV *(40)*. *HCN2* channels activate more slowly, require stronger hyperpolarizations ($V_{1/2}$ of –92 mV), but are strongly modulated by cAMP *(38,49,50)*. *HCN4* may activate at even more negative potentials and with the slowest kinetics *(37,49,51)*, although $V_{1/2}$ values reported in the literature are variable. To date, *HCN3* channels have not been found to form functional homomultimers. It is possible that this is caused by the lack of a subunit necessary for full functionality because HCN channels have been shown to be modulated by co-expression of a MinK-related peptide *(52)*.

The existence of different isoforms of the HCN channel provides a convincing molecular basis for the heterogeneity in I(f) among different cells, with SAN cells expressing one isoform and ventricular cells another, consistent with the macroscopic properties of I(f), and hence meeting the physiological requirements of the cell. However, more complicated scenarios are possible. A given cell, for example, could express a mixture of HCN channel isoforms with different properties, a supposition reinforced by the finding that the I(f) current displays fast and slow components in heart *(24,53)* and in neurons *(54)*. Consistent with idea of several different components of I(f) being present simultaneously, a recent zebra fish mutant that displays a slow heart rate was found to be deficient in the fast kinetic component of I(f), whereas the slow component was unchanged *(23)*. As a further intriguing possibility because functional HCN channels are likely tetrameric assemblies of subunits, hybrid channels, or heterotetrameric assemblies of subunits, may also be synthesized in some cells. Although most investigations have been performed to date on homomeric channels, there is evidence that channels can be formed as heteromeric assemblies, which display properties intermediate between the two isoforms *(55)*. This additional complexity may be important in the fine control of pacemaking and may underlie the heterogeneity of cardiac tissue *(56)*.

5. DISTRIBUTION OF HCN CHANNELS

As a logical development of earlier studies demonstrating the presence and properties of I(f) in a variety of tissues, efforts have now been directed to studies defining the relative expression levels of the different isoforms of the *HCN* gene.

The relative expression levels of the different isoforms varies considerably throughout the heart, consistent with the different properties of I(f) and the different intrinsic pacemaker activity of the tissues. Hence, in SAN, the dominant isoform of the channel appears to be *HCN4*, with lesser amounts of *HCN1*, whereas in Purkinje fibers, there are equal amounts of *HCN1* and *HCN4* with a smaller amount of *HCN2*. In ventricle, the dominant isoform appears to be primarily *HCN2 (57)*. It should be noted that heterogeneity of cellular properties in the heart is found not only at the "gross" level (i.e., between SAN, atria, and ventricles), but that this heterogeneity can be traced down almost to a single cell level. For instance, the SAN shows a gradation of properties throughout its structure, in terms of the electrophysiology of the tissue and the ion channels expressed *(58,59)*. It may be that this phenotypic heterogeneity is a reflection of the different mix of *HCN4* and *HCN1* expressed in these cells, giving central pacemaker cells a higher intrinsic rate than peripheral cells.

As well as a high level of expression in heart, *HCN* isoforms are also expressed in various parts of the CNS, where *HCN1-4* show distinct but overlapping patterns of mRNA expression *(14,39,50,60,61)*. *HCN1* is expressed selectively in specific brain regions, including the hippocampus, layer 5 cells of the neocortex, and Purkinje cells of the cerebellum. *HCN2* is widely expressed throughout brain, including neocortex, hippocampus, and thalamus. Finally, *HCN4* is expressed in a restricted manner in subcortical and lower brain regions.

HCN expression can also be found in the periphery, notably in smooth muscle *(62)*. Indeed, it seems likely that HCN channels are expressed in any tissue that displays intrinsic rhythmic activity. Note also that HCN may be involved in some unexpected functions, for example, sour taste perception *(63)*. Importantly, in the context of side effects of specific bradycardic agents, *HCN1* channels are highly expressed in the retina *(60)*.

6. RELEVANCE OF I(F) TO CLINICAL CONDITIONS

An increased heart rate increases myocardial oxygen demand and decreases time for myocardial relaxation and diastolic ventricular filling. Because of the increased transmural pressure on the coronary perfusion vessels during systole, perfusion is greatly limited, or prevented entirely, during much of the cardiac cycle and perfusion occurs mostly during diastole. Hence, for example, in the presence of a compromised coronary flow because of coronary artery stenosis, a decrease in diastolic perfusion time secondary to an increase in heart rate may further reduce overall coronary perfusion, to the point where the myocardium can become ischemic. Generally, the subendocardium is most vulnerable to ischemia, and tachycardia has indeed been shown to exacerbate this vulnerability *(64,65)*. Under these circumstances, drugs that block sinus tachycardia, reduce heart rate at rest, or both could be expected to increase the diastolic coronary perfusion time and hence improve overall perfusion and function of the ischemic subendocardium *(66,67)*. This, at least partly, is the rationale behind the use of beta-blocking agents and the rate-lowering Ca^{2+} channel-blocking agents, such as diltiazem

and verapamil. However, interpretation of the action of these agents, particularly the calcium channel blockers, is complicated by their tendency to reduce blood pressure and myocardial contractility, both of which reduce myocardial oxygen demand and, indirectly, affect coronary perfusion. One would nevertheless predict that a pure bradycardic agent without these attendant pressor effects should be beneficial in improving coronary perfusion and resistance of the myocardium to ischemia. This prediction is born out in practice: slowing heart rate with ZD 7288 in dogs reduces the severity of myocardial ischemia produced by left anterior descending coronary artery (LAD) occlusion *(68)*, with similar results having been shown in pigs *(69)*. Because I(f) is the primary determinant of heart rate, I(f) blockers therefore have a promising role as anti-ischemic agents.

In addition, blockers of I(f) may be useful in contexts other than when the myocardium is vulnerable to ischemia. I(f) appears to be upregulated in the ventricle during cardiac failure, leading to enhanced autorhythmicity *(70)*; however, note the results from a previous study *(71)*. It seems possible that this enhanced autorhythmicity may be responsible for the greater propensity to arrhythmia in cardiac failure, and hence blockers of I(f) may also be useful as antiarrhythmic agents in this situation. This would constitute an action separate from their bradycardic effects, although the bradycardic effect would still be present, and perhaps provide an additional benefit.

7. TYPES OF DRUGS UNDER CURRENT DEVELOPMENT

One of the earliest blockers of I(f) described was Cs^+ *(72)*, which is moderately specific for I(f), although it does block other ionic currents in addition, especially at higher doses *(73)*. Difficulties with using Cs^+ in physiological solutions and its lack of specificity sparked the search for organic blockers of I(f) in the hope of generating a more specific and more potent compounds. The following two broad lineages of compounds have emerged from these efforts:

1. A series of compounds related to phentolamine and clonidine, the prototype being alinidine (*N*-allyl-clonidine), with the latest compound being ZD-7288 *(74)*.
2. A series of compounds based on modifications of verapamil, the prototype being falipamil (or AQ-A 39), from which zatebradine *(75)* and ivabradine (S-16257-2) have been developed.

7.1. Alinidine and Congeners

Alinidine (or ST567) was shown to be bradycardic as long as 20 yr ago in dogs *(76)* and in humans *(77)*. Although it blocks I(f), alinidine is not very specific in its action, also blocking the slow inward current and outward currents in rabbit SAN cells *(78)*. There also have been suggestions that alinidine can block anion channels *(79)*, an action that may underlie its sometimes reported negative inotropic effect *(80)*. In addition, alinidine displays a variety of pharmacological properties, for example, it antagonizes cromakalim and hence inhibits K_{ATP} channel opening *(81)*. It has been shown that alinidine exhibits rate-independent cardioprotective effects, perhaps as a consequence of adenosine antagonist properties *(82,83)*. Alinidine has also been shown to be antimuscarinic—in paced left rat atria, alinidine acted as competitive antagonist against oxotremorine with a pA_2 of 5.82, and in guinea pig papillary muscle it antagonized carbachol with a pA_2 value of 5.58 *(84)*. Early reports that alinidine slows conduction through the atrioventricular (AV) node *(76)* have not been confirmed in later studies

(85). Although alinidine has been shown to prolong the effective refractory period in the AV node of dogs subject to coronary artery occlusion *(86,87)*, an effect on refractory period was not noted in humans at a dose of 40 mg *(88)*.

Despite the potential difficulties with nonspecific actions, alinidine has been shown to have very few acute side effects in humans *(89,90)*. Note, however, that the latter study (albeit with a limited numbers of subjects) reported that alinidine did not appear to enhance myocardial salvage or preservation of left ventricular function or to reduce the incidence of major arrhythmias in the early phase of myocardial infarction. Nevertheless, interest in alinidine congeners continues with a number of compounds still under investigation *(91)*. Many of these exhibit most of the nonspecific effects of alinidine and so are unlikely to be any more useful than alinidine as bradycardic agents. However, one compound, ZD7288, has been developed that shows some promise as a therapeutic agent.

7.1.1. ZD 7288

ZD 7288 (Fig.3A) has been shown to be bradycardic in rabbits and guinea pig *(92)* and to block I(f) in dissociated guinea pig SAN cells *(93)*. The block is not use dependent (in contrast to that produced by zatebradine) and has been suggested to be caused by an action of the very hydrophobic ZD 7288 molecule at an intracellular site on the HCN channel *(94)*. In sheep Purkinje fibers, the blocking of I(f) by ZD 7288 appears to show so-called reverse use-dependence, and it has been suggested that this may limit its efficacy under physiological conditions *(95)*. ZD 7288 is more selective than alinidine or UL-FS 49, producing less prolongation of the action potential in SAN cells at bradycardic concentrations *(96)*.

As noted previously, HCN channels are widely expressed in CNS and some peripheral tissues, and one could expect that blockers of HCN channels will have effects in these tissues as well as in the heart. In this context, most interest in ZD 7288 at present seems to be centered on its actions in neurons *(97,98)*. ZD 7288 inhibits I(h) in rod photoreceptors *(99)*, a finding that has relevance to one of the side effects of HCN channel blockers, which is that they produce visual disturbances.

As well as side effects produced by blockade of HCN channels in extracardiac tissues, which will be a common effect to all specific bradycardic agents, a difficulty with the clinical use of ZD 7288 may arise as a consequence of its kinetics; in in vitro preparations, the action appears to be essentially irreversible *(95)*.

7.2. Falipamil (AQ-A 39) and Congeners

An alternative development track for blockers of I(f) has been made through modifications to the benzenacetonitrile Ca^{2+} channel antagonist verapamil. Structural modification by replacement of the lipophilic alpha-isopropylacetonitrile moiety by various heterocyclic ring systems has led to a new range of molecules with specific bradycardic activity, the prototype being falipamil (AQ-A 39). Falipamil has been shown to be bradycardic in many isolated tissue preparations, including guinea pig *(100)*; rabbit *(101)*; dog *(102)*; anesthetized cats, dogs *(103)*, and pigs *(104)*; and in humans *(105)*.

Despite the production of similar effects on heart rate, AQ-A 39 differs from alinidine and mixidine in that it does not depress cardiac contractility in anesthetized dogs with spontaneous heart rates *(106)*. A similar observation has been made in anesthetized pigs, at least for arterial plasma concentrations lower than 1500 ng/mL *(104)*.

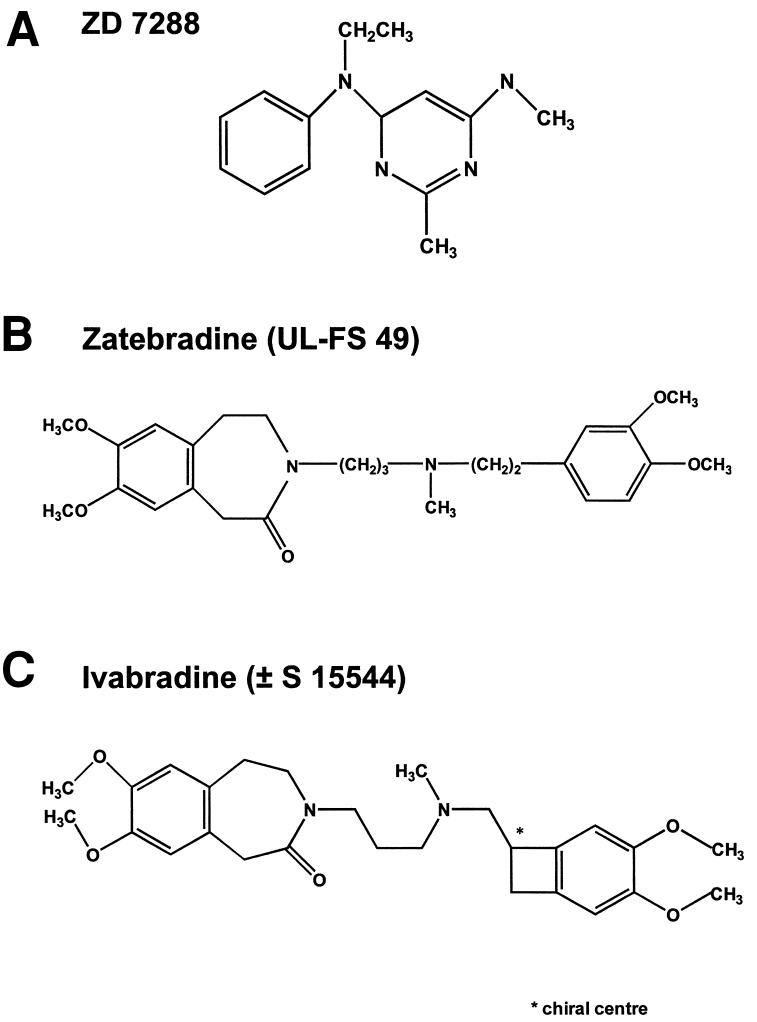

Fig. 3. Depicts the chemical structures of several novel I(f) blockers in development. (**A**) shows ZD 7288 (4-[*N*-ethyl-*N*-phenylamino]-1,2-dimethyl-6-[methylamino] pyridinium chloride), (**B**) shows Zatebradine (UL-FS 49 or 1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-][2-(3,4-dimethoxyphenyl)-ethyl) methylimino]propyl]-2*H*-3-benzazepin-2-on-hydrochloride), and (**C**) shows (±) S 15544 (the racemic parent of Ivabradine; (±) *S* 15544 or 7,8-dimethoxy3-[3-][(4,5-dimethoxybenzocyclobutan-1-yl) methyl] methyl-amino] propyl}1,3,4,5-tetrahydro-2*H*-3-benzazepin-2-one). Note that ivabradine has a chiral carbon center.

Consistent with these bradycardic actions, falipamil shows protective effects against ischemia in anesthetized dogs subjected to 15 min of coronary artery occlusion, followed by 3 h of reperfusion. In this study, both AQ-AH 208 and AQ-A 39 (falipamil) produced similar decreases in heart rate (24%) and increases in the endocardial/epicardial distribution of collateral blood flow. During occlusion and throughout reperfusion, both compounds also produced a significant improvement in the percentage of shortening of the ventricle in the ischemic-reperfused region *(107)*. A similar result has been reported in humans; in a randomized, controlled study 10 male patients with

angiographically confirmed ischemic heart disease received AQ-A 39 (falipamil) in a single intravenous dose (2 mg/kg). After submaximal exercise, heart rate during placebo was 129 ± 3, and during AQ-A 39, it was 113 ± 3 beats min^{-1}. AQ-A 39 did not affect systolic arterial pressure and improved exercise tolerance *(108)*.

Some problems in the clinical use of falipamil may arise because of its pharmacokinetic properties, such as its terminal half-life ($t_{1/2}$). In human plasma, the $t_{1/2}$ was determined to be 1.8 ± 0.6 h *(109)*. In addition, falipamil has been reported to have anticholinergic effects in isolated SAN preparations in addition to its rate-dependent block of I(f) *(110)*. In intact animals, this anticholinergic effect can be manifest in vagolytic effects, and these can result in a paradoxical increase in heart rate in some circumstances *(111)*.

As further development has proceeded, falipamil has been submitted to further optimization mainly by manipulation of the phthalmidine moiety, resulting in a second generation of specific bradycardic agents with increased potency and selectively and a prolonged duration of action, represented by the benzazepinone-derivative UL-FS 49 (zatebradine; ref. *112*).

7.2.1. Zatebradine (UL-FS 49)

Zatebradine (Fig. 3B) blocks sinus tachycardia; it has been shown to markedly attenuate exercise-induced heart rate both in animal models *(113–116)* and in humans *(117–120)* at concentrations that do not affect the inotropic or lusitropic state or vascular tone. Zatebradine exhibits a less anticholinergic effect than falipamil *(121)*. In rabbit SAN cells *(75)* and sheep Purkinje fibers *(122)*, the bradycardic effect of zatebradine has been attributed to a use-dependent inhibition of I(f), caused by interaction with the open state of the channel that reduces open probability *(123)*. This is in contrast to the mode of action of ZD 7288 (and other alinidine-related compounds) that interact with the HCN channel in a way that reduces the single-channel conductance and that do not show the same use dependence. At concentrations that block I(f), zatebradine has minimal effects on the L-type Ca^{2+} current (ICa) or the delayed rectifier K^+ current (IKr) in rabbit sinoatrial cells *(75)*, although in spontaneously beating rabbit SAN cells zatebradine prolonged the duration of the action potential, suggesting a K^+ channel block *(75,124)*. More recent electrophysiological studies demonstrate that zatebradine also prolongs action potential duration in guinea pig papillary muscles and rabbit Purkinje fibers *(125,126)*, an effect that was more prominent in Purkinje fibers than in papillary muscles. Consistent with its bradycardic effects, zatebradine has been shown to confer protection against exercise-induced regional contractile dysfunction in dogs *(66,67,127)* and in anesthetized pigs *(69)*.

In 1999, zatebradine was in Phase III clinical trials. However, the Zatebradine Study Group, from a randomized double-blind, placebo-controlled, multicenter study in patients with chronic stable angina pectoris taking extended-release nifedipine, concluded that zatebradine seemed to provide no additional antianginal benefit. This group raised questions regarding the benefit of heart rate reduction alone as an antianginal approach to patients with chronic stable angina *(128)*. This conclusion was later reinforced by Glasser et al. *(129)*, who noted that "despite significant reductions in resting and exercise heart rate, there were no clinically significant effects on myocardial ischemia, suggesting that the anti-ischemic effect of heart rate reduction should be re-evaluated."

These studies raise some questions about the likely usefulness of specific bradycardic agents in cardiac disease, although it may be that their conclusions can

only be interpreted as being relevant to zatebradine. In addition to these doubts about efficacy, the class III actions of zatebradine *(130)* have spurred efforts to improve the specificity of its I(f)-blocking actions. This has resulted in development of the compound ivabradine.

7.2.2. Ivabradine (S-16257-2)

Ivabradine is the (+)-enantiomer of the racemic compound S15544 (note that zatebradine is nonchiral). It is similarly bradycardic as zatebradine in isolated tissues *(125)*, by the same mechanism, that is, a block of I(f) *(131)*. The major improvement of ivabradine over zatebradine is that it produces much less prolongation of the action potential in the bradycardic concentration range *(125)*. Because the prototype compound for ivabradine, S15544, is a racemate, it is possible to compare the isomers and to "dissect out" the action potential prolonging action (*see* Fig. 3B for chemical structure). Most of the action potential prolonging effect resides in the (–)-enantiomer (S16260), with the (+)-enantiomer (S16257, ivabradine) having a much smaller effect *(132)*.

Ivabradine is bradycardic and alters neither myocardial contractility nor coronary vasomotion at rest and during exercise in normal dogs *(133)*. It has been shown to be effective against exercise-induced myocardial ischemia in dogs *(134)*. In humans, iv ivabradine in the first Phase I study was shown to produce a decrease of maximal heart rate during exercise *(135)*. In later Phase I trials, pharmacokinetic studies have been conducted in humans *(136)*. Ivabradine is currently in later stage clinical trials. It remains to be seen whether it will prove to be a more effective anti-ischemic agent than zatebradine, thus proving the concept that pure bradycardic agents can be useful as therapeutic agents for angina and other conditions producing myocardial ischemia.

8. CONCLUSIONS

There are a range of side effects typical for specific bradycardic agents, such as effects on the lungs. Animal studies show that relatively high iv doses of zatebradine contract guinea pig airways by a histamine-like mechanism, and zatebradine can reduce FEV1 in humans *(137)*. Besides these generalized extraneous effects of specific bradycardic agents and the usual problems of pharmacokinetics and metabolism that must be overcome, a range of side effects is possible that are related to the fundamental pharmacodynamics of these agents. Because HCN channels are found in a variety of extracardiac tissues, notably the CNS, it may be unavoidable that these agents will produce CNS actions and these may limit their usefulness as antianginal agents. Indeed, one of the common side effects already documented for these agents is a disturbance of vision, a consequence of the presence of HCN channels in the photoreceptors. However, this problem of a diffuse target for therapeutic intervention has not inhibited the development of other drugs, such as class I or class III antiarrhythmic agents. The target for these agents, sodium channels, or potassium channels, respectively, are widespread in extracardiac tissues, but this does not necessarily impede the clinical use of these agents. There is no reason to suppose that the presence of HCN channels in extracardiac tissues will be more of an impediment to the usefulness of specific bradycardic agents, although side effects may inevitably occur. In fact, the situation with specific bradycardic agents may offer more scope for drug development than has currently been exploited. Because HCN channels exist in at least four isoforms, one

can speculate that it may be possible to develop blockers specific for a given isoform, in the same way that blockers of specific voltage-dependent potassium channels have been developed. If this can be done, tissue-specific blockers of HCN channels would enable the development of agents that specifically target the SAN or CNS to tailor their therapeutic actions.

REFERENCES

1. DiFrancesco, D. (1995) The onset and autonomic regulation of cardiac pacemaker activity: Relevance of the f current. *Cardiovasc Res.* **29,** 449–456.
2. Yanagihara, K. and Irisawa, H. (1980) Inward current activated during hyperpolarization in the rabbit sinoatrial node cell. *Pflugers Arch.* **385,** 11–19.
3. Brown, H. F., Giles, W., and Noble, S. J. (1977) Membrane currents underlying activity in frog sinus venosus. *J. Physiol.* **271,** 783–816.
4. DiFrancesco, D. (1981) A new interpretation of the pace-maker current in calf Purkinje fibres. *J. Physiol.* **314,** 359–376.
5. Brown, H. and DiFrancesco, D. (1980) Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node. *J. Physiol.* **308,** 331–351.
6. DiFrancesco, D. and Ojeda, C. (1980) Properties of the current if in the sino-atrial node of the rabbit compared with those of the current iK, in Purkinje fibres. *J. Physiol.* **308,** 353–367.
7. Irisawa, H. and Noma, A. (1984) Pacemaker currents in mammalian nodal cells. *J. Mol. Cell Cardiol.* **16,** 777–781.
8. Maylie, J. and Morad, M. (1984) Ionic currents responsible for the generation of pacemaker current in the rabbit sino-atrial node. *J. Physiol.* **355,** 215–235.
9. Bader, C. R., Macleish, P. R., and Schwartz, E. A. (1979) A voltage-clamp study of the light response in solitary rods of the tiger salamander. *J. Physiol.* **296,** 1–26.
10. DiFrancesco, D. (1993). Pacemaker mechanisms in cardiac tissue. *Annu. Rev. Physiol.* **55,** 455–472.
11. McCormick, D. A. and Pape, H. C. (1990) Properties of a hyperpolarization-activated cation current and its role in rhythmic oscillation in thalamic relay neurones. *J. Physiol.* **431,** 291–318.
12. Pape, H. C. (1996) Queer current and pacemaker: The hyperpolarization-activated cation current in neurons. *Annu. Rev. Physiol.* **58,** 299–327.
13. Maccaferri, G. and McBain, C. J. (1996) The hyperpolarization-activated current (Ih) and its contribution to pacemaker activity in rat CA1 hippocampal stratum oriens-alveus interneurones. *J. Physiol.* **497,** 119–130.
14. Luthi, A. and McCormick, D.A. (1998) H-current: Properties of a neuronal and network pacemaker. *Neuron* **21,** 9–12.
15. Demontis, G. C., Longoni, B., Barcaro, U., and Cervetto, L. (1999) Properties and functional roles of hyperpolarization-gated currents in guinea-pig retinal rods. *J. Physiol.* **515,** 813–828.
16. Magee, J. C. (1999) Dendritic Ih normalizes temporal summation in hippocampal CA1 neurons. *Nat. Neurosci.* **2,** 848.
17. Beaumont, V. and Zucker, R. S. (2000) Enhancement of synaptic transmission by cyclic AMP modulation of presynaptic Ih channels. *Nat. Neurosci.* **3,** 133–141.
18. Southan, A. P., Morris, N. P., Stephens, G. J., and Robertson, B. (2000) Hyperpolarization activated currents in presynaptic terminals of mouse cerebellar basket cells. *J. Physiol.* **526,** 91–97.
19. DiFrancesco, D. (1995) The pacemaker current (I(f)) plays an important role in regulating SA node pacemaker activity. *Cardiovasc. Res.* **30,** 307–308.

20. Vassalle, M. (1995) The pacemaker current (I(f)) does not play an important role in regulating SA node pacemaker activity. *Cardiovasc. Res.* **30,** 309–310.
21. Noble, D., Denyer, J. C., Brown, H. F., and DiFrancesco, D. (1992) Reciprocal role of the inward currents ib, Na and i(f) in controlling and stabilizing pacemaker frequency of rabbit sino-atrial node cells. *Proc. R Soc. Lond. B Biol. Sci.* **250,** 199–207.
22. Liu, Y. M., Yu, H., Li, C. Z., Cohen, I. S., and Vassalle, M. (1998) Cesium effects on If and IK in rabbit sinoatrial node myocytes. Implications for SA node automaticity. *J. Cardiovasc. Pharmacol.* **32,** 783–790.
23. Baker, K., Warren, K. S., Yellen, G., and Fishman, M. C. (1997) Defective "pacemaker" current (Ih) in a zebrafish mutant with a slow heart rate. *Proc. Natl. Acad. Sci. USA* **94,** 4554–4559.
24. DiFrancesco, D., Ferroni, A., Mazzanti, M., and Tromba, C. (1986) Properties of the hyperpolarizing-activated current (if) in cells isolated from the rabbit sino-atrial node. *J. Physiol.* **377,** 61–68.
25. DiFrancesco, D. (1986) Characterization of single pacemaker channels in cardiac sino-atrial node cells. *Nature* **324,** 470–473.
26. DiFrancesco, D. and Tortora, P. (1991) Direct activation of cardiac pacemaker channels by intracellular cyclic AMP. *Nature* **351,** 145–147.
27. DiFrancesco, D. and Mangoni, M. (1994) Modulation of single hyperpolarization-activated channels (i(f)) by cAMP in the rabbit sino-atrial node. *J. Physiol.* **474,** 473–482.
28. Accili, E. A., Redaelli, G., and DiFrancesco, D. (1997) Differential control of the hyperpolarization-activated current (i(f)) by cAMP gating and phosphatase inhibition in rabbit sino-atrial node myocytes. *J. Physiol.* **500,** 643–651.
29. Chang, F., Cohen, I. S., DiFrancesco, D., Rosen, M. R., and Tromba, C. (1991) Effects of protein kinase inhibitors on canine Purkinje fibre pacemaker depolarization and the pacemaker current i(f). *J. Physiol.* **440,** 367–384.
30. Wu, J. Y., Yu, H., and Cohen, I. S. (2000) Epidermal growth factor increases i(f) in rabbit SA node cells by activating a tyrosine kinase. *Biochim. Biophys. Acta.* **1463,** 1–19.
31. Renaudon, B., Lenfant, J., Decressac, S., and Bois, P. (2000) Thyroid hormone increases the conductance density of f-channels in rabbit sino-atrial node cells. *Receptors Channels* **7,** 1–8.
32. Hara, M., Liu, Y. M., Zhen, L., Cohen, I. S., Yu, H., Danilo P, Jr., et al. (1997) Positive chronotropic actions of parathyroid hormone and parathyroid hormone-related peptide are associated with increases in the current, I(f), and the slope of the pacemaker potential. *Circulation* **96,** 3704–3709.
33. DiFrancesco, D. and Tromba, C. (1988) Inhibition of the hyperpolarization-activated current (if) induced by acetylcholine in rabbit sino-atrial node myocytes. *J. Physiol.* **405,** 477–491.
34. Santoro, B. and Tibbs, G. R. (1999) The HCN gene family: Molecular basis of the hyperpolarization-activated pacemaker channels. *Ann. NY Acad. Sci.* **868,** 741–764.
35. Yu, H., Chang, F., and Cohen, I. S. (1995) Pacemaker current If in adult canine cardiac ventricular myocytes. *J. Physiol.* **485,** 469–483.
36. Santoro, B., Grant, S. G., Bartsch, D., and Kandel, E. R. (1997) Interactive cloning with the SH3 domain of N-src identifies a new brain specific ion channel protein, with homology to eag and cyclic nucleotide-gated channels. *Proc. Natl. Acad. Sci. USA* **94,** 14,815–14,820.
37. Ishii, T. M., Takano, M., Xie, L. H., Noma, A., and Ohmori, H. (1999) Molecular characterization of the hyperpolarization-activated cation channel in rabbit heart sinoatrial node. *J. Biol. Chem.* **274,** 12,835–12,839.
38. Ludwig, A., Zong, X., Jeglitsch, M., Hofmann, F., and Biel, M. (1998) A family of hyperpolarization-activated mammalian cation channels. *Nature* **393,** 587–591.
39. Santoro, B., Liu, D. T., Yao, H., Bartsch, D., Kandel, E. R., Siegelbaum, S. A., and Tibbs, G. R. (1998). Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain. *Cell* **93,** 717–729.

40. Vaccari, T., Moroni, A., Rocchi, M., Gorza, L., Bianchi, M. E., Beltrame, M., and DiFrancesco, D. (1999) The human gene coding for HCN2, the pacemaker channel of the heart. *Biochim. Biophys. Acta.* **1446,** 419–425.
41. Moroni, A., Barbuti, A., Altomare, C., Viscomi, C., Morgan, J., Baruscotti, M., and DiFrancesco, D. (2000) Kinetic and ionic properties of the human HCN2 pacemaker channel. *Pflügers Arch.* **439,** 618–626.
42. Clapham, D. E. (1998) Not so funny anymore: Pacing channels are cloned. *Neuron* **21,** 5–7.
43. Biel, M., Ludwig, A., Zong, X., and Hofmann, F. (1999) Hyperpolarization-activated cation channels: A multi-gene family. *Rev. Physiol. Biochem. Pharmacol.* **136,** 165–181.
44. Vaca, L., Stieber, J., Zong, X., Ludwig, A., Hofmann, F., and Biel, M. (2000) Mutations in the S4 domain of a pacemaker channel alter its voltage dependence. *FEBS Lett.* **479,** 35–40.
45. Doyle, D. A., Morais Cabral, J., Pfuetzner, R. A., Kuo, A., Gulbis, J. M., Cohen, S. L., et al. (1998) The structure of the potassium channel: molecular basis of K^+ conduction and selectivity. *Science* **280,** 69–77.
46. Heginbotham, L., Lu, Z., Abramson, T., and MacKinnon, R. (1994) Mutations in the K^+ channel signature sequence. *Biophys. J.* **66,** 1061–1067.
47. Zagotta, W. N. and Siegelbaum, S. A. (1996) Structure and function of cyclic nucleotide-gated channels. *Annu. Rev. Neurosci.* **19,** 235–263.
48. Wainger, B. J., DeGennaro, M., Santoro, B., Siegelbaum, S. A., and Tibbs, G. R. (2001) Molecular mechanism of cAMP modulation of HCN pacemaker channels. *Nature* **411,** 805–810.
49. Ludwig, A., Zong, X., Stieber, J., Hullin, R., Hofmann, F., and Biel, M. (1999) Two pacemaker channels from human heart with profoundly different activation kinetics. *EMBO J.* **18,** 2323–2329.
50. Santoro, B., Chen, S., Luthi, A., Pavlidis, P., Shumyatsky, G. P., Tibbs, G. R., et al. (2000) Molecular and functional heterogeneity of hyperpolarization-activated pacemaker channels in the mouse CNS. *J. Neurosci.* **20,** 5264–5275.
51. Seifert, R., Scholten, A., Gauss, R., Mincheva, A., Lichter, P., and Kaupp, U. B. (1999) Molecular characterization of a slowly gating human hyperpolarization-activated channel predominantly expressed in thalamus, heart, and testis. *Proc. Natl. Acad. Sci. USA* **96,** 9391–9396.
52. Yu, H., Wu, J., Potapova, I., Wymore, R. T., Holmes, B., Zuckerman, J., et al. (2001) MinK-related peptide 1: A beta subunit for the HCN ion channel subunit family enhances expression and speeds activation. *Circ. Res.* **88,** E847–E87.
53. Maruoka, F., Nakashima, Y., Takano, M., Ono, K., and Noma, A. (1994) Cation-dependent gating of the hyperpolarization-activated cation current in the rabbit sino-atrial node cells. *J. Physiol.* **477,** 423–435.
54. Solomon, J. S. and Nerbonne, J. M. (1993) Two kinetically distinct components of hyperpolarization-activated current in rat superior colliculus-projecting neurons. *J. Physiol.* **469,** 291–313.
55. Chen, S., Wang, J., and Siegelbaum, S. A. (2001) Properties of hyperpolarization-activated pacemaker current defined by coassembly of HCN1 and HCN2 subunits and basal modulation by cyclic nucleotide. *J. Gen. Physiol.* **117,** 491–504.
56. Boyett, M. R., Honjo, H., and Kodama, I. (2000) The sinoatrial node, a heterogeneous pacemaker structure. *Cardiovasc. Res.* **47,** 658–687.
57. Shi, W., Wymore, R., Yu, H., Wu, J., Wymore, R. T., Pan, Z., et al. (1999) Distribution and prevalence of hyperpolarization-activated cation channel (HCN) mRNA expression in cardiac tissues. *Circ. Res.* **85,** e1–e6.
58. Zhang, H., Holden, A. V., and Boyett, M. R. (2000) Gradient model versus mosaic model of the sinoatrial node. *Circulation* **103,** 584–588.

59. Nikmaram, M. R., Boyett, M. R., Kodama, I., Suzuki, R., and Honjo, H. (1997) Variation in effects of Cs^+, UL-FS-49, and ZD-7288 within sinoatrial node. *Am. J. Physiol.* **272,** H2782–H2792.
60. Moosmang, S., Stieber, J., Zong, X., Biel, M., Hofmann, F., and Ludwig, A. (2001) Cellular expression and functional characterization of four hyperpolarization-activated pacemaker channels in cardiac and neuronal tissues. *Eur. J. Biochem.* **268,** 1646–1652.
61. Monteggia, L. M., Eisch, A. J., Tang, M. D., Kaczmarek, L. K., and Nestler, E. J. (2000) Cloning and localization of the hyperpolarization-activated cyclic nucleotide-gated channel family in rat brain. *Brain Res. Mol. Brain Res.* **81,** 129–139.
62. Greenwood, I. A. and Prestwich, S. A. (2002) Characteristics of hyperpolarization-activated cation currents in portal vein smooth muscle cells. *Am. J. Physiol. Cell Physiol.* **282,** C744–C753.
63. Stevens, D. R., Seifert, R., Bufe, B., Muller, F., Kremmer, E., Gauss, R., et al. (2001) Hyperpolarization-activated channels HCN1 and HCN4 mediate responses to sour stimuli. *Nature* **413,** 631–635.
64. Boudoulas, H., Rittgers, S., Lewis, R., Leier, C., and Weissler, A. (1979) Changes in diastolic time with various pharmacologic agents: Implications for myocardial perfusion. *Circulation* **60,** 164–169.
65. Hoffman, J. (1990) Autoregulation and heart rate. *Circulation* **82,** 1880–1881.
66. Guth, B., Heusch, G., Seitelberger, R., and Ross, J. (1987) Elimination of exercise-induced regional myocardial dysfunction by a bradycardic agent in dogs with chronic coronary stenosis. *Circulation* **75,** 661–669.
67. O'Brien, P., Drage, D., Saeian, K., Brooks, H., and Warltier, D. (1992) Regional redistribution of myocardial perfusion by UL-FS49, a selective bradycardic agent. *Am. Heart J.* **123,** 5665–5674.
68. Schlack, W., Ebel, D., Grunert, S., Halilovic, S., Meyer, O., and Thamer, V. (1998) Effect of heart rate reduction by 4-(N-ethyl-N-phenyl-amino)-1,2-dimethyl-6-(methylamino)pyrimidinium chloride on infarct size in dog. *Arzneimittelforschung* **48,** 26–33.
69. Indolfi, C., Guth, B. D., Miura, T., Miyazaki, S., Schulz, R., and Ross J. Jr. (1989) Mechanisms of improved ischemic regional dysfunction by bradycardia. Studies on UL-FS 49 in swine. *Circulation* **80,** 983–993.
70. Cerbai, E., Sartiani, L., DePaoli, P., Pino, R., Maccherini, M., Bizzarri, F., et al. (2001) The properties of the pacemaker current I(F)in human ventricular myocytes are modulated by cardiac disease. *J. Mol. Cell Cardiol.* **33,** 441–448.
71. Hoppe, U. C., Jansen, E., Sudkamp, M., and Beuckelmann, D. J. (1998) Hyperpolarization-activated inward current in ventricular myocytes from normal and failing human hearts. *Circulation* **97,** 55–65.
72. DiFrancesco, D. (1982) Block and activation of the pace-maker channel in calf purkinje fibres: effects of potassium, caesium and rubidium. *J. Physiol.* **329,** 485–507.
73. Zhou, Z. and Lipsius, S. L. (1992) Properties of the pacemaker current (If) in latent pacemaker cells isolated from cat right atrium. *J. Physiol.* **453,** 503–523.
74. BoSmith, R. E., Briggs, I., and Sturgess, N. C. (1993) Inhibitory actions of ZENECA ZD7288 on whole-cell hyperpolarization activated inward current (If) in guinea-pig dissociated sinoatrial node cells. *Br. J. Pharmacol.* **110,** 343–349.
75. Goethals, M., Raes A., and Van Bogaert, P. P. (1993) Use-dependent block of the pacemaker current I(f) in rabbit sinoatrial node cells by zatebradine (UL-FS 49). On the mode of action of sinus node inhibitors. *Circulation* **88,** 2389–2401.
76. Traunecker, W. and Walland, A. (1980) Haemodynamic and electrophysiologic actions of alinidine in the dog. *Arch. Int. Pharmacodyn. Ther.* **244,** 58–72.
77. Harron, D. W., Jady, K., Riddell, J. G., and Shanks, R. G. (1982) Effects of alinidine, a novel bradycardic agent, on heart rate and blood pressure in man. *J. Cardiovasc. Pharmacol.* **4,** 213–220.

78. Satoh, H. and Hashimoto, K. (1986) Electrophysiological study of alinidine in voltage clamped rabbit sino-atrial node cells. *Eur. J. Pharmacol.* **121,** 211–219.
79. Millar, J. S. and Williams, E. M. (1981) Pacemaker selectivity: Influence on rabbit atria of ionic environment and of alinidine, a possible anion antagonist. *Cardiovasc. Res.* **15,** 335–350.
80. Jaski, B. E. and Serruys, P. W. (1985) Anion-channel blockade with alinidine: A specific bradycardic drug for coronary heart disease without negative inotropic activity? *Am. J. Cardiol.* **56,** 270–275.
81. McPherson, G. A. and Angus, J. A. (1989) Phentolamine and structurally related compounds selectively antagonize the vascular actions of the K^+ channel opener, cromromakalim. *Br. J. Pharmacol.* **97,** 941–949.
82. Streller, I. and Walland, A. (1990) Antiischemic effects of alinidine in paced isolated rat hearts. *Basic Res Cardiol.* **85,** 71–77.
83. Lang, U., Streller, I., and Walland, A. (1989) Alinidine antagonizes the myocardial effects of adenosine. *Eur. J. Pharmacol.* **164,** 13–22.
84. Lang, U. and Walland, A. (1989) Alinidine reverses the descending staircase of isolated rat atria by an antimuscarinic action. *Naunyn Schmiedebergs Arch. Pharmacol.* **339,** 456–463.
85. Takeda, M., Furukawa, Y., Ogiwara, Y., Saegusa, K., Haniuda, M., Akahane, K., et al. (1989) Effects on atrio-ventricular conduction of alinidine and falipamil injected into the AV node artery of the anesthetized dog. *Arch. Int. Pharmacodyn Ther.* **297,** 39–48.
86. Aidonidis, I., Brachmann, J., Rizos, I., Zacharoulis, A., Stavridis, I., Toutouzas, P., et al. (1995) Electropharmacology of the bradycardic agents alinidine and zatebradine (UL-FS 49) in a conscious canine ventricular arrhythmia model of permanent coronary artery occlusion. *Cardiovasc. Drugs Ther.* **9,** 555–563.
87. Boucher, M., Chassaing, C., and Chapuy, E. (1995) Cardiac electrophysiologic effects of alinidine, a specific bradycardic agent, in the conscious dog: Plasma concentration-response relations. *J. Cardiovasc. Pharmacol.* **25,** 229–233.
88. Meinertz, T., Kasper, W., and Jahnchen, E. (1987) Alinidine in heart patients: Electrophysiologic and antianginal actions. *Eur. Heart J.* **8 ,** 109–114.
89. Koenig, W., Stauch, M., Sund, M., Wanjura, D., and Henze, E. (1990) Hemodynamic effects of alinidine (ST 567) at rest and during exercise in patients with chronic congestive heart failure. *Am. Heart J.* **119,** 1348–1354.
90. Van de Werf, F., Janssens, L., Brzostek, T., Mortelmans, L., Wackers, F. J., Willems, G. M., et al. (1993) Short-term effects of early intravenous treatment with a beta-adrenergic blocking agent or a specific bradycardiac agent in patients with acute myocardial infarction receiving thrombolytic therapy. *J. Am. Coll. Cardiol.* **22,** 407–416.
91. C hallinor-Rogers, J. L., Rosenfeldt, F. L., Du, X. J., and McPherson, G. A. (1997) Antiischemic and antiarrhythmic activities of some novel alinidine analogs in the rat heart. *J. Cardiovasc. Pharmacol.* **29,** 499–507.
92. Leitch, S. P., Sears, C. E., Brown, H. F., and Paterson, D. J. (1995) Effects of high potassium and the bradycardic agents ZD7288 and cesium on heart rate of rabbits and guinea pigs. *J. Cardiovasc. Pharmacol.* **25,** 300–306.
93. BoSmith, R. E., Briggs, I., and Sturgess, N. C. (1993) Inhibitory actions of Zeneca ZD7288 on whole-cell hyperpolarization activated inward current (If) in guinea-pig dissociated sinoatrial node cells. *Br. J. Pharmacol.* **110,** 343–349.
94. Rothberg, B. S., Shin, K. S., Phale, P. S., and Yellen, G. (2002) Voltage-controlled gating at the intracellular entrance to a hyperpolarization-activated cation channel. *J. Gen. Physiol.* **119,** 83–91.
95. Berger, F., Borchard, U., Gelhaar, R., Hafner, D., and Weis, T. (1994) Effects of the bradycardic agent ZD 7288 on membrane voltage and pacemaker current in sheep cardiac Purkinje fibres. *Naunyn Schmiedebergs Arch Pharmacol.* **350,** 677–684.

96. Briggs, I., BoSmith, R. E., and Heapy, C. G. (1994) Effects of Zeneca ZD7288 in comparison with alinidine and UL-FS 49 on guinea pig sinoatrial node and ventricular action potentials. *J. Cardiovasc. Pharmacol.* **24,** 380–387.
97. Harris, N. C. and Constanti, A. (1995) Mechanism of block by ZD 7288 of the hyperpolarization-activated inward rectifying current in guinea pig substantia nigra neurons in vitro. *J. Neurophysiol.* **7,** 2366–2378.
98. Gasparini, S.and DiFrancesco, D. (1997) Action of the hyperpolarization-activated current (Ih) blocker ZD 7288 in hippocampal CA1 neurons. *Pflugers Arch.* **435,** 99–106.
99. Satoh, T. O. and Yamada, M. (2000) A bradycardiac agent ZD7288 blocks the hyperpolarization-activated current (I(h)) in retinal rod photoreceptors. *Neuropharmacology* **39,** 1289–1291.
100. Hohnloser, S., Weirich, J., Homburger, H., and Antoni, H. (1982) Electrophysiological studies on effects of AQ-A 39 in the isolated guinea pig heart and myocardial preparations. *Arzneimittelforschung* **32,** 730–734.
101. Senges, J., Rizos, I., Brachmann, J., Anders, G., Jauernig, R., Hamman, H. D., et al. (1983) Effect of nifedipine and AQ-A 39 on the sinoatrial and atrioventricular nodes of the rabbit and their antiarrhythmic action on atrioventricular nodal reentrant tachycardia. *Cardiovasc. Res.* **17,** 132–134.
102. Kawada, M., Satoh, K., and Taira, N. (1984) Analyses of the cardiac action of the bradycardic agent, AQ-A 39, by use of isolated, blood-perfused dog-heart preparations. *J. Pharmacol. Exp. Ther.* **228,** 484–490.
103. Dammgen, J., Kadatz, R., and Diederen, W. (1981) Cardiovascular actions of 5,6-dimethoxy-2-(3-[(alpha-(3,4-dimethoxy) phenylethyl)-methylamino] propyl) phthalimidine (AQ-A 39), a specific bradycardic agent. *Arzneimittelforschung* **31,** 666–670.
104. Verdouw, P. D., Bom, H. P., and Bijleveld, R. E. (1983) Cardiovascular responses to increasing plasma concentrations of AQ-A 39 Cl, a new compound with negative chronotropic effects. *Arzneimittelforschung* **33,** 702–706.
105. Hilaire, J., Broustet, J. P., Colle, J. P., and Theron, M. (1983) Cardiovascular effects of AQ-A 39 in healthy volunteers. *Br. J. Clin. Pharmacol.* **16,** 627–631.
106. Siegl, P. K., Wenger, H. C., and Sweet, C. S. (1984) Comparison of cardiovascular responses to the bradycardic drugs, alinidine, AQ-A 39, and mixidine, in the anesthetized dog. *J. Cardiovasc. Pharmacol.* **6,** 565–574.
107. Gross, G. J., Daemmgen, J. W. (1986) Beneficial effects of two specific bradycardic agents AQ-A39 (falipamil) and AQ-AH 208 on reversible myocardial reperfusion damage in anesthetized dogs. *J. Pharmacol. Exp. Ther.* **238,** 422–428.
108. Gilfrich, H. J., Oberhoffer, M., and Witzke, J. (1987) Comparison of AQ-A 39 with propanolol and placebo in ischaemic heart disease. *Eur. Heart J.* **8(Suppl L),** 147–151.
109. Roth, W., Koss, F. W., Hallinan, D., Lambe, R., and Darragh, A. (1990) Pharmacokinetics of falipamil after intravenous administration to humans. *J. Pharm. Sci.* **79,** 415–419.
110. Osterrieder, W., Pelzer, D., Yang, Q. F., and Trautwein, W. (1981) The electrophysiological basis of the bradycardic action of AQA 39 on the sinoatrial node. *Naunyn Schmiedebergs Arch Pharmacol.* **317,** 233–237.
111. Boucher, M., Chassaing, C., Chapuy, E., and Duchene-Marullaz, P. (1994) Chronotropic cardiac effects of falipamil in conscious dogs: Interactions with the autonomic nervous system and various ionic conductances. *J. Cardiovasc. Pharmacol.* **23,** 569–575.
112. Lillie, C. and Kobinger, W. (1986) Investigations into the bradycardic effects of UL-FS 49 (1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-[[2-(3,4-dimethoxy-phenyl)ethyl]methylimino]propyl]-2H-3-benzazepin-2-on hydrochloride) in isolated guinea pig atria. *J. Cardiovasc. Pharmacol.* **8,** 791–797.
113. Johnston, W., Vinten-Johansen, J., Tommasi, E., and Little, W. (1991) ULFS-49 causes bradycardia without decreasing right ventricular systolic and diastolic performance. *J. Cardiovasc. Pharmacol.* **18,** 528–534.

114. Chen, Z. and Slinker, B. (1992) The sinus node inhibitor UL-FS 49 lacks significant inotropic effect. *J. Cardiovasc. Pharmacol.* **19,** 264–271.
115. Van Woerkens, L., van der Giessen, W., and Verdouw, P. (1992) The selective bradycardic effects of zatebradine (UL-FS 49) do not adversely affect left ventricular function in conscious pigs with chronic coronary artery occlusion. *Cardiovasc. Drugs Ther.* **6,** 59–65.
116. Breall, J., Watanabe, J., and Grossman, W. (1993) Effect of zatebradine on contractility, relaxation and coronary blood flow. *J. Am. Coll. Cardiol.* **21,** 471–477.
117. Pistchner, H., Muno, E., Vens-Cappel, F., Schulte, B., Schlepper, M., de Moura-Sieber, V., et al. Antiischemic, antianginal, and hemodynamic effects of ULFS 49 Cl (a new heart-rate-reducing agent) in patients with angiographically proven CAD, in *Sinus Node Inhibitors: A New Concept in Angina Pectoris* (Hjalmarson, Å., Remme, W., eds.), Springer, New York, 1991, pp. 45–53.
118. Baiker, W., Czako, E., Keck, M., and Nehmiz, G. Efficacy and duration of action of three doses of zatebradine (ULFS 49 Cl) in patients with chronic angina pectoris compared to placebo, in *Sinus Node Inhibitors: A New Concept in Angina Pectoris* (Hjalmarson, Å., Remme, W., eds.), Springer, New York, 1991, pp. 55–63.
119. Franke, H., Su CAPF, Schumacher, K., and Seiberling, M. (1987) Clinical pharmacology of two specific bradycardic agents. *Eur. Heart J.* **8(Suppl L),** 91–98.
120. Roth, W., Bauer, E., Heinzel, G., Cornelissen, P., van Tol, R., Jonkman, J., and Zuiderwijk, P. (1993) Zatebradine: pharmacokinetics of a novel heart-rate-lowering agent after intravenous infusion and oral administration to healthy subjects. *J. Pharm. Sci.* **82,** 99–106.
121. Kobinger, W. and Lillie, C. (1984) Cardiovascular characterization of UL-FS 49, 1,3,4,5-tetrahydro-7,8-dimethoxy-3-[3-][2-(3,4-dimethoxyphenyl)ethyl] methylimino]propyl]-2H-3-benzazepin-2-on hydrochloride, a new "specific bradycardic agent." *Eur. J. Pharmacol.* **104,** 9–18.
122. Van Bogaert, P. P., Goethals, M., and Simoens, C. (1990) Use- and frequency-dependent blockade by UL-FS 49 of the if pacemaker current in sheep cardiac Purkinje fibres. *Eur. J. Pharmacol.* **187,** 241–256.
123. DiFrancesco, D. (1994) Some properties of the UL-FS 49 block of the hyperpolarization-activated current (i(f)) in sino-atrial node myocytes. *Pflugers Arch.* **427,** 64–70.
124. Doerr, T. and Trautwein, W. (1990) On the mechanism of the "specific bradycardic action" of the verapamil derivative UL-FS 49. *Naunyn Schmiedebergs Arch Pharmacol.* **341,** 331–340.
125. Thollon, C., Cambarrat, C., Vian, J., Prost, J. F., Peglion, J. L., and Vilaine, J. P. (1994) Electrophysiological effects of S 16257, a novel sino-atrial node modulator, on rabbit and guinea-pig cardiac preparations: Comparison with UL-FS 49. *Br. J. Pharmacol.* **112,** 37–42.
126. Perez, O., Gay, P., Franqueza, L., Carron, R., Valenzuela, C., Delpon, E., et al. (1995) Electromechanical effects of zatebradine on isolated guinea pig cardiac preparations. *J. Cardiovasc. Pharmacol.* **26,** 46–54.
127. Raberger, G., Krumpl, G., and Schneider, W. (1987) Effects of the bradycardic agent UL-FS 49 on exercise-induced regional contractile dysfunction in dogs. *Int. J. Cardiol.* **14,** 343–354.
128. Frishman, W. H., Pepine, C. J., Weiss, R. J., and Baiker, W. M. (1995) Addition of zatebradine, a direct sinus node inhibitor, provides no greater exercise tolerance benefit in patients with angina taking extended-release nifedipine: Results of a multicenter, randomized, double-blind, placebo-controlled, parallel-group study. The Zatebradine Study Group. *J. Am. Coll. Cardiol.* **26,** 305–312.
129. Glasser, S. P., Michie, D. D., Thadani, U., and Baiker, W. M. (1997) Effects of zatebradine (ULFS 49 CL), a sinus node inhibitor, on heart rate and exercise duration in chronic stable angina pectoris. Zatebradine Investigators. *Am. J. Cardiol.* **79,** 1401–1405.

130. Valenzuela, C., Delpon, E., Franqueza, L., Gay, P., Perez, O., Tamargo, J., and Snyders, D. J. (1996) Class III antiarrhythmic effects of zatebradine. Time-, state-, use-, and voltage-dependent block of hKv1.5 channels. *Circulation* **94,** 562–570.
131. Bois, P., Bescond, J., Renaudon, B., and Lenfant, J. (1996) Mode of action of bradycardic agent, S 16257, on ionic currents of rabbit sinoatrial node cells. *Br. J. Pharmacol.* **118,** 1051–1057.
132. Thollon, C., Bidouard, J. P., Cambarrat, C., Lesage, L., Reure, H., Delescluse, I., et al. (1997) Stereospecific in vitro and in vivo effects of the new sinus node inhibitor (+)-S 16257. *Eur. J. Pharmacol.* **339,** 43–51.
133. Simon, L., Ghaleh, B., Puybasset, L., Giudicelli, J. F., and Berdeaux, A. (1995) Coronary and hemodynamic effects of S 16257, a new bradycardic agent, in resting and exercising conscious dogs. *J. Pharmacol. Exp. Ther.* **275,** 659–666.
134. Monnet, X., Ghaleh, B., Colin, P., de Curzon, O. P., Giudicelli, J. F., and Berdeaux, A. (2001) Effects of heart rate reduction with ivabradine on exercise-induced myocardial ischemia and stunning. *J. Pharmacol. Exp. Ther.* **299,** 1133–1139.
135. Carre, F., Denolle, T., Lecoz, F., Violet, I., Lerebours, G., and Gandon, J. M. (1995). First intravenous phase I of S 16257, a new bradycardic agent: Effects on the maximal exercise parameters. *Thérapie* **50,** 377.
136. Duffull, S. B., Chabaud, S., Nony, P., Laveille, C., Girard, P., and Aarons, L. (2000) A pharmacokinetic simulation model for ivabradine in healthy volunteers. *Eur. J. Pharm. Sci.* **10,** 285–294.
137. Maesen, F. P., Smeets, J. J., van Noord, J. A., Nehmiz, G., Wald, F. D., and Cornelissen, P. J. (1994) Effect of zatebradine, a novel 'sinus node inhibitor,' on pulmonary function compared to placebo. *Pulm. Pharmacol.* **7,** 349–355.

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