VIEWPOINT

Funny channel-based pacemaking

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Introduction
What is the origin of normal cardiac pacemaking is an obviously intriguing question, given the fundamental role of pacemaker activity in cardiac function. According to early studies based on experiments in Purkinje fibers, pacemaking was first attributed to the decay of an outward current during diastolic depolarization. The process leading to generation of diastolic depolarization, hence pacemaker activity, was then re-evaluated in the late 1970s with the discovery of the “pacemaker” (“funny”, If) current, which introduced a novel concept of pacemaker generated by activation during diastole of a hyperpolarization-gated inward current.

Two important sets of events have recently brought new elements of interest to the discussion on pacemaking. First, basic concepts in pacemaking have developed into practical applications with clinical relevance. For example, a pharmacological approach to heart rate control is possible today thanks to specific heart rate–reducing drugs. Second, detailed experimentation has led to a deeper knowledge of the complex set of interacting cellular processes that operate during pacemaking. The unraveling of several interacting cellular processes obviously raises questions about the specific roles of individual mechanisms contributing to pacemaking. Here I briefly discuss some of the evidence supporting the view that If is an essential player in pacemaker initiation and control of heart rate.

The funny channel is a marker of cardiac pacemaker tissue
In the heart, functional expression of funny channels under physiological conditions is limited to pacing regions (sinoatrial node [SAN], atrioventricular node [AVN], and conduction tissue). Recent immunofluorescence data have shown that in the rabbit SAN, the region in which hyperpolarization-activated, cyclic nucleotide-gated (HCN4) channels (the main pore-forming subunits of native funny channels in the SAN) are expressed, essentially overlaps the leading pacemaker region of the node, as defined according to standard electrophysiological and morphological criteria. Also in the human heart, HCN4 proteins are strongly expressed in the sinus node and not in the surrounding atrial tissue.

If and pacemaking are in fact linked not only in the adult heart, but throughout development, as apparent for example by the correlation between funny channel expression and the presence of pacemaker activity in developing embryonic ventricular myocytes. Also, developmental studies of the SAN formation by segregation from surrounding atrial tissue demonstrate the existence of a correspondence between the region where pacemaker activity originates and the region of HCN4 expression. For example, pacemaker activity in the mouse embryonic heart initiates from the sinus venosus, the prospective SAN, where in situ hybridization analysis has revealed expression of HCN4 at embryonic day (ED) 8; in fact HCN4 is expressed already at ED7.5, before formation of the sinus venosus in the cardiac mesoderm (cardiac crescent).

Recent investigation has clarified some crucial steps in the gene program for SAN development, highlighting the role of transcription factors that specifically delineate the cardiac conduction system, such as Shox2, Tbx3, and Tbx18. At ED9.5 the mouse SAN begins to form from Tbx18-positive progenitor mesenchymal cells, which differentiate into SAN myocardium; while Tbx18 is necessary for the formation of the SAN, Tbx3 ensures proper pacemaker gene program regulation within the SAN domain. Tbx3 represses atrial differentiation of pacemaker myocytes and prevents the down-regulation of the HCN4 gene, whose expression remains therefore restricted to SAN tissue through different stages of development. These data show that pacemaker activity, development of the SAN, and expression of HCN4 are strictly correlated.

As mentioned, funny channels are also expressed in the AVN and His–Purkinje system. An interesting consideration can be made in this respect: strictly speaking, physiological pacing should only require a single central pacemaker region (i.e., the SAN); why do we then need...
pacemaking capability in the AVN and in the Purkinje fibers, the most peripheral branches of the conduction tissue? This is an example of how basic cellular properties translate into clinically relevant features. It is indeed clinically relevant that atrial fibrillation (AF) and atrioventricular (AV) block are nowhere nearly as life threatening as ventricular fibrillation (VF). This is thanks to the ability of the AVN and/or the ventricular part of the conduction system to pace the ventricles when required. The basic cardiac physiologist’s viewpoint here is that nature has provided the heart with important failsafe mechanisms able to take up pacemaking when SAN activity is impaired. This highlights the importance of having funny channels expressed also in the secondary pacemaker regions, where they can contribute to ventricular pacing in pathological conditions.

**Funny channel mutations altering functional behavior generate arrhythmias**

Channelopathies are diseases caused by dysfunctional ion channels. Several cardiac pathologies are known that depend on defective Na⁺, K⁺, and Ca²⁺ channels, and recent evidence for “funny” channelopathies has also appeared. If the main role of funny channels in the heart is generation of spontaneous activity and rate control, it should be expected that dysfunctional funny channels cause arrhythmic behavior. Indeed several reports now indicate a correlation between mutations of HCN4 channels and rhythm disturbances. For example, a single-point mutation (S672R) of the HCN4 protein was associated with asymptomatic sinus bradycardia in a large Italian family. Functional studies revealed that S672R is a loss-of-function type of mutation, which in expression experiments mimicking heterozygosity, caused a negative shift of about 5 mV of the If activation curve relative to wild-type proteins. This is a cholinergic-type of effect, due to a constitutive change of funny channel properties rather than to increased muscarinic stimulation, and is fully consistent with the bradycardia associated with the HCN4 mutation. Other HCN4 mutations reported to be between mutations of HCN4 channels and rhythm disturbances. For example, a single-point mutation (S672R) of the HCN4 protein was associated with asymptomatic sinus bradycardia in a large Italian family. Functional studies revealed that S672R is a loss-of-function type of mutation, which in expression experiments mimicking heterozygosity, caused a negative shift of about 5 mV of the If activation curve relative to wild-type proteins. This is a cholinergic-type of effect, due to a constitutive change of funny channel properties rather than to increased muscarinic stimulation, and is fully consistent with the bradycardia associated with the HCN4 mutation. Other HCN4 mutations reported to be associated with arrhythmias include L573X, a truncated protein lacking the CNBD at the C-terminal, and the single-point mutation D533N (see Milanesi, et al). Another arrhythmia-related HCN4 mutation is G480R; in contrast with other mutations, this is located in the pore region and modifies the GYG selectivity sequence typical of K⁺-permeable channels. Functional studies have associated the G480R mutation with a reduced If, which justifies the asymptomatic bradycardia of affected family members. These data suggest the existence of a general mechanism for sinus arrhythmias caused by defective funny channels, and highlight the role of funny channels in normal pacemaker activity.

**Specific block of funny channels causes specific rate slowing**

Heart rate reduction is a recognized therapeutic target in several cardiac conditions, such as ischemic heart disease and heart failure. Slowing is associated with a decreased oxygen demand and an increased diastolic time of myocardial perfusion, conditions ameliorating both aspects of myocardial oxygen balance. Indeed in both ischemic heart disease and heart failure, morbidity/mortality reduction associated with the use of β-blockers and Ca²⁺ antagonists are at least partly attributable to their rate reducing action. However, the use of β-blockers and Ca²⁺ antagonists have limitations because these substances not only slow rate, but exert several other effects on the cardiovascular (typically negative inotropism) and other systems, which sometimes represent contraindications. Thus, “pure” heart rate reducing agents might be useful in ischemic heart disease and other cardiovascular conditions, which accounts for the interest of drug companies in their development.

The role of funny channels in the generation of spontaneous activity and rate control makes them ideal targets in the search for pure heart rate–reducing agents. Several substances able to slow rate by block of funny channels are known today. Of these substances, ivabradine is the only one commercially available for therapeutic use in chronic stable angina. Clinical studies have shown that ivabradine reduces heart rate without other significant cardiovascular side effects. In these studies ivabradine did not modify cardiovascular parameters such as the corrected QT interval (QTc), the PR and QRS intervals, conductivity and refractoriness measured in atrium, AVN, His–Purkinje system and ventricles, left ventricular ejection fraction, and stroke volume. Studies of patients with stable angina further showed the efficacy of ivabradine as an antianginal and anti-ischemic agent.

In vitro studies have shown that ivabradine, in a low-concentration range, is a specific blocker of funny channels. Detailed investigation of native funny channels has revealed that ivabradine blocks by entering channel pores from the intracellular side and is an open-channel blocker (i.e., requires open channels to reach its binding site within the pore). At the same time, block is current-dependent, being favored by depolarization when drug molecules are “kicked in” their blocking site by the outward flow of permeating Na⁺/K⁺ ions. This latter property is peculiar of ivabradine and is lacking in other If blocking drugs. The above properties result in a marked “use dependence” of funny channel block by ivabradine; in other words, block is especially efficient when funny channels are cycled repetitively through open/closed states, suggesting that the drug efficiency as a rate-reducing agent might increase at high rates (tachycardia). The specificity of the action of ivabradine on heart rate and the lack of cardiovascular side effects have important consequences on the clinical use of the drug, but also importantly represent a direct demonstration of the role of funny channels in pacemaking.

**Viewing contributions to activity of If and Ca²⁺ oscillations in the same cell**

The properties of funny channels discussed earlier are unique in correlating the If function to rhythm generation and control of rate. Such a tight correlation is not apparent
in other mechanisms also active and essential to proper rhythmic function, such as Ca\textsuperscript{2+} transients. Thus, even if spontaneous local oscillations of subsarcolemmal Ca\textsuperscript{2+} seem to be normally functional in SAN, but not in ventricular cells,\textsuperscript{18} they are a manifestation of a general mechanism involving several Ca\textsuperscript{2+}-dependent processes that occur in all myocytes. Accordingly, impairment of Ca\textsuperscript{2+} cycling is likely to have the heaviest impact on ventricular function, rather than to simply upset rhythm generation and/or heart rate; for example, specific RyR2 mutations typically lead to catecholaminergic polymorphic ventricular tachycardia. Finally, also because of its more ubiquitous diffusion within all cardiac regions, Ca\textsuperscript{2+} cycling is an unlikely target for pharmacological interventions aiming to specifically control pacemaker activity and rate.

In my view, I\textsubscript{f} and SR Ca\textsuperscript{2+} transients cooperate in an integrated system whose aim is to maximize the safety and reliability of pacemaking, but their specific functional roles are different: I\textsubscript{f} is in charge of initiating diastolic depolarization and controlling its steepness, hence heart rate, in the pacemaker range of voltages. Even if modulated by intracellular agents and pathways (cyclic adenosine monophosphate, ancillary subunits, modulatory proteins, phosphorylation, PIP2, etc.) as well as by voltage, its function is electrical in essence. Ca\textsuperscript{2+} cycling, on the other hand, controls contraction, and its essential role is linked to the mechanical function of the cell.

Even if serving different cellular functions, I\textsubscript{f}-generated voltage changes and Ca\textsuperscript{2+} fluctuations must be in phase and need therefore to be tuned in any single cell. A way to visualize the contribution to membrane voltage of the 2 processes in the same cell is proposed in Figure 1. Experimenters working on pacing myocytes know by experience that microelectrode impaling (in multicellular preparations) or patch-clamp pipette sealing (in single cells) may often slightly damage cells and induce a depolarizing leak. Partially depolarized pacemaker cells do not rest at a stable voltage level; they undergo oscillations of a few millivolts in amplitude, which sometimes get larger with time, while the seal is recovering, until full-blown action potentials are restored.

Voltage oscillations recorded at a voltage level around -25 mV (at which no I\textsubscript{f} is activated) in a single, depolarized SAN cell are shown in Figure 1B. In this experiment hyperpolarizing (outward) current steps of various amplitudes (20, 40, 60 pA) (Figure 1A) were applied to push the membrane voltage down to levels in the pacemaker range. As is clearly apparent in Figure 1C, where the trace during the 60 pA step is plotted on an expanded time scale, outward current injection led to an apparently bizarre result, i.e., to
resumption of pacing. Normally in fact, excitable cells may become spontaneously active by injection of depolarizing current. This result has been reported, and demonstrates that bringing the frequency of spontaneous activity into the proper range of If activation (close to −50 mV in Figure 1) is essential to restore pacemaker activity. The experiment in Figure 1 also allows comparison of the frequency of spontaneous activity with the frequency of membrane potential oscillations at a depolarized level. This is interesting in that membrane voltage oscillations at depolarized levels likely reflect intracellular Ca^{2+} fluctuations acting through the Na^+ /Ca^{2+} exchanger. In agreement with this view, a rough estimation of the rate of membrane oscillations in the absence of current injection (Figure 1B) yields a value of 3.3 Hz; this is not too dissimilar to the frequency of about 4 Hz reported for local intracellular Ca^{2+} releases and related current fluctuations in voltage-clamp conditions in rabbit SAN cells.

The rate of spontaneous activity during the 60 pA step in Figure 1C was 2.9 Hz; this confirms the similarity between the frequency of action potentials and that of spontaneous Ca^{2+} oscillations, which I interpret as a necessary condition for proper tuning of the 2 repetitive processes, rather than an indication that a Ca^{2+} clock drives pacemaking. The data in Figure 1 highlight the stabilizing role of the funny current and indicate that generation of a regular rhythm depends crucially on activation of If in the proper range of membrane voltages.

Conclusions

Funny channels have properties suitable to generate a slow depolarization after repolarization of an action potential and to control its steepness by means of a cyclic adenosine monophosphate–dependent mechanism able to mediate the autonomic modulation of heart rate. Their functional expression is restricted, in physiological conditions, to pacemaker regions throughout cardiac development, which justifies the classification of the HCN4 gene as a marker of pacemaker tissue. Pharmacological block of the If current leads to specific heart rate reduction and represents a novel therapeutic approach to stable angina with potential applications to a wider range of cardiac conditions. Finally, specific funny channel mutations affecting their normal function can cause rhythm disturbances such as bradycardia. This and other evidence support the notion that funny channels have a major role in the generation of spontaneous activity and rate control.

References