The funny current was first described in the late 70s (Brown et al. 1979) and in the nearly three decades following this early description, its functional role in the generation of spontaneous activity and in the control of cardiac rate has been intensely investigated and firmly established on the basis of a wealth of experimental evidence (DiFrancesco, 1993; Barbuti et al. 2007).

In SAN cells, the main determinant of pacemaker rate is the duration of diastolic depolarization. \( I_f \) activation at the end of an action potential controls the slope of early diastolic depolarization, and thus determines the duration of diastole by selecting the time after which threshold for a new action potential is reached (Bucchi et al. 2007).

\( I_f \) is also finely modulated by changes of the intracellular CAMP concentration, a mechanism mediating rate modulation by the autonomic nervous system. \( I_f \) activation therefore represents the main physiological mechanism responsible for control of cardiac rate. Not surprisingly, \( I_f \) has been the target of active pharmacological research aimed to develop drugs able to control heart rate. Research in this field has led to development of several funny channel-specific drugs, the “heart rate reducing” agents, which work by selective block of funny channels. Ivabradine, the only such compound having passed the required clinical tests, has been shown to slow heart rate without adverse side effects (such as negative inotropic effects).

Ivabradine block of funny channels occurs at the cytoplasmic channel side and is “current dependent”, i.e. occurs preferentially when ions flow in the outward direction, while inward current “kicks off” drug molecules from their binding site and relieves block (Bucchi et al., 2002). Since ivabradine is also an “open channel blocker” and thus requires hyperpolarization to gain access to the blocking site, its block is strongly “use-dependent” and becomes more efficient when channels cycle frequently between open and closed states (i.e., during tachycardia). Ivabradine is now marketed as a therapeutical tool against stable chronic angina and is being investigated as a potential tool to reduce morbidity-mortality in CAD patients with/without heart failure.

More recently, other clinically relevant applications of the concept of the funny current-based pacemaking have become apparent.

In one example, an inheritable point mutation in the cyclic nucleotide-binding domain of the HCN4 isoform (the main HCN isoform contributing to native funny channels of the sinoatrial node) was shown to cause sinus bradycardia in a large Italian family (Milanesi et al. 2006). The mutation (S672R) behaves as an autosomic dominant mutation and causes the probability curve of heterozygous mutant/wild type HCN4 channels to shift by about 5 mV to the negative direction, thus reducing inward current flow during diastole, which causes a reduced diastolic slope and bradycardia. The more negative position of the activation curve is a constitutive new property of mutated channels and does not alter cAMP-dependent activation. Since acetylcholine has the same effect of shifting the \( I_f \) activation curve to the negative direction, the mutation mimics the effects of a mild vagal stimulation. Other mutations affecting funny channel function, and consequently heart rate, may exist, and the one identified may represent just a special case of a more general mechanism for rhythm disorders based on HCN altered function.

In another example, the concept of \( I_f \)-dependent pacing provides the basic background for the development of newly engineered “biological” pacemakers, whose aim is to eventually replace electronic ones. Techniques used so far for generating biological pacemakers have involved gene-based and, more recently, cell-based approaches, including in situ transfer of HCN2-overexpressing mesenchymal stem cells or transfer of embryonic-stem cell derived spontaneously beating agglomerates. The rationale of these approaches is that funny-channel based pacemaking can be exported to silent cardiac tissue by in situ transfer of \( I_f \)-expressing cells.

Interest in these recent findings is not simply due to the confirmation of the relevance of funny channels to the control of heart rate, but also to the fact that they provide a background for development of tools useful in the clinical setting. More clinically-relevant tools exploiting funny channel-based pacemaking will likely become available in the near future, in connection with a more detailed knowledge of the basic molecular properties of funny channels function and control.


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I$_f$ modulation in Clinical Perspective

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Normal cardiac impulse initiation occurs in the sinus node, in which gradual depolarization during phase 4 of the transmembrane potential results in attainment of threshold and initiation of action potentials that propagate to the rest of the heart. A current referred to as I$_f$ initiates phase 4 depolarization. This current activates on hyperpolarization following termination of a preceding action potential. Although I$_f$ initiates phase 4 depolarization, it is not the only current contributing to this: inward currents carried by Ca also play a role as does the Na/Ca exchanger. Counteracting these depolarizing influences are hyperpolarizing, outward currents carried by K. It is generally accepted that any net increase in inward current or decrease in outward current will increase phase 4 depolarization and cardiac rate.

A key property of the pacemaker current is its modulation by autonomic influences. Catecholamines increase sinus rate by pathways initiated via their binding to beta-1 adrenergic receptors. This binding activates a G-protein coupled pathway in which adenylyl cyclase metabolizes ATP to cyclic AMP and P. The cyclic AMP binds to specific sites near the carboxy terminus of the channel, resulting in increased channel activation and an increased pacemaker potential. The effect of catecholamines to activate the channel is counteracted by muscarinic agonists, such as acetylcholine. The pathway here, too, is mediated via a G protein, with the net result being a braking of catecholamine-induced actions to accelerate rate.

The property of the channel to be activated on hyperpolarization and to bind cyclic AMP has led to the nomenclature: hyperpolarization-activated, cyclic nucleotide-gated (or HCN) channel. There are four HCN channel isoforms; three, HCN1, HCN2 and HCN4 are in heart. HCN3 is in neural tissues. HCN4 and HCN1 contribute to impulse initiation in the sinus node. It has long been appreciated that in a variety of settings it might be desirable to either slow or speed heart rate. Major areas in which slowing of heart rate is often desirable include ischemic heart disease and a variety of post-surgical settings. A primary means for slowing rate has been beta-adrenergic blockade: yet this carries a potential for deleterious effects such as a loss of inotropy. For this reason, agents that act primarily on the HCN channel as a target to reduce I$_f$ and slow rate on that basis have been viewed as desirable. In contrast to many other pharmacological agents, ivabradine has high selectivity and specificity for its target, the HCN channel. Importantly, in blocking the HCN channels that carry I$_p$, ivabradine suppresses but does not stop the sinus node pacemaker’s rate of firing. This reflects the contribution of the other ion channels mentioned above to the pacemaker potential, and also provides an important safety factor for ivabradine. Ivabradine’s clinical success to date provides an example of how highly targeted therapy can result in a risk/benefit advantage over other effective therapies.

Another area of interest with regard to modulating I$_f$ is in settings in which one might want to increase heart rate. The classic example here is the bradycardias that accompany sinoatrial node dysfunction or high degree atrioventricular block. Whereas the standard therapy over the past 60 years has become electronic pacemakers, over the last 5-10 years interest in biological pacemaking as an adjunct or alternative has increased. Although a variety of approaches were tried initially, current attempts focus largely on the use of viral and/or stem cell-based gene and cell therapies to deliver I$_f$ current. The obstacles relating to the use of viral vectors or stem cells in humans constitute formidable challenges, yet preclinical research has proceeded briskly. This has demonstrated that the biological pacemaker is not only capable of providing baseline pacemaker function, but is autonomically responsive and can interact well with electronic pacemakers in a tandem mode of therapy.

Pacemaker channels play a major role in the generation of sinoatrial rhythmic activity. However, their expression is not confined to specialized myocardial cells, such as primary and subsidiary pacemakers. Electrophysiological and molecular data collected over the last ten years (Cerbai & Mugelli, 2006), demonstrated that f-channels are present also in non-pacemaker cardiomyocytes. These channels are highly expressed in fetal and neonatal cardiomyocytes (Cerbai et al, 1999) and even in embryonic stem (ES) cells and ES-derived cardiomyocytes (Sartiani et al, 2007). In the adult heart, I$_f$ current densities and/or mRNA levels of its molecular correlate (i.e. hyperpolarization-activated cyclic nucleotide-gated (HCN) channels) are increased during the development of cardiac hypertrophy and failure in human and rat cardiomyocytes. Figure 1 plots the ratio between current density measured in ventricular cardiomyocytes from rat or human diseased hearts, and respective controls, bars representing confidence intervals (95%). In panel A, points represent the relative increase of I$_f$ in rats with mild or severe left ventricular hypertrophy caused by aortic banding (Mild-LVH) or long-lasting pressure overload (Severe-LVH); in rats with overt heart failure, resulting from uncompensated hypertrophy due to pressure overload (PO-HF) or following a myocardial infarction due to coronary ligation (PMI-HF; performed under ketamine and chlorpromazine (150 and 15 mg/kg, respectively) anaesthesia), and the relative increase of current density in patients undergoing cardiac transplantation for terminal dilated (DCM) or ischemic heart disease and a variety of post-surgical settings.
ischemic cardiomyopathy (ICM). Panel B shows two examples of current recorded in normal and diseased rat myocytes. For all conditions, the relative increase in I_f density was statistically significant versus controls, that is, normotensive rats, sham-operated rats, or undiseased donor hearts not transplanted for technical reasons, with the exception of DCM patients (n.s.: not significant). Over-expression of f-channels in non-pacemaker cells are one of the consequence of the process of cardiac remodeling (Cerbai et al, 1997; Cerbai et al, 1996) and it has been suggested that this phenomenon may represent an arrhythmogenic mechanism in heart failure, a condition associated with high risk for sudden cardiac death. However, it remains controversial whether I_f over-expression might play a role for the increased propensity of arrhythmias in diseased states because I_f current activation was obtained at more negative potentials in working myocardium than in pacemaker cells. The availability of selective f-channel blockers such as ivabradine may help to assess the potential arrhythmogenic function of I_f in heart disease and the role of heart rate in cardiac remodelling.

Figure 1. Ventricular I_f expression is increased in cardiomyopathies.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.
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Multimodal biophotonic imaging of supraventricular pacemaker and conduction system

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Sino-atrial (SA) and AV nodes are integral parts of an anatomically extended atrial pacemaker and conduction system (APCS) that encompasses the entire region between superior and inferior venae cavae and extends into the triangle of Koch. Heart rate and atrio-ventricular (AV) delay are orchestrated by autonomic nervous system within the APCS, which cannot be fully understood without combining both reductionist and integrative approaches that focus on investigation of ionic and structural mechanisms, respectively. We have developed a methodology of multimodal biophotonic imaging that allows structure/function investigation of the rabbit, canine, and human APCS. We applied high-resolution fast fluorescent imaging with voltage sensitive dye di-4-ANEPPS to optically map (OM) action potentials in APCS in the human, canine, and rabbit perfused preparations during normal heart rate, electrical stimulation, and during adrenergic and cholinergic stimulation. Optical coherence tomography (OCT) was applied to map 3D intact fiber structure of the APCS with spatial resolution of 10µm and depth of penetration of 1-2 mm. Immunohistochemistry was used for mapping protein expression in functionally and structurally characterized preparations.

Optical signals integrate electrical activity from 1-2mm of depth into tissue and carry 3D information about the genesis and conduction of the action potential within APCS. In both SA and AV nodal regions we have identified optical signatures from different layers of APCS structure and reconstructed the 3D pattern of excitation in multiple layers. In the canine SA node we have confirmed our earlier finding in the rabbit that a functional block zone (FBZ) exists that electrically uncouples SA node from the septum and thus facilitates source-sink matching. Adrenergic and cholinergic stimulation results in significant anatomic migration of the action potential within APCS. In both SA and AV node has rate-sensitive dual pathway properties that not only generate echo beats and reentrant rhythms but also contribute to normal conduction. The anatomical and functional substrate underlying AV nodal rate-dependent and dual pathway function remains ill-defined. While the compact node (Figure 1A) is considered a pivotal structure in rate-dependent function, nodal inputs are often considered to play a key role in dual pathways. Several hypotheses have been put forward to account for dual pathways. Alternately, the compact node may act as the slow pathway whereas a bypass track made of transitional tissues acts as the fast pathway. Our own histological and functional data indicate that the posterior extension and compact node provide the substrate for slow and fast pathway conduction, respectively. Our current goal is to provide a unified hypothesis for AV nodal rate-dependent and dual pathway function. More specifically, we hypothesize that overall rate-dependent AV nodal properties reflect the lumped expression of the rate-dependent properties displayed by the posterior extension (slow pathway) and compact node (fast pathway). According to this scheme, nodal

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AVN function: one node two pathways

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The rate-dependent properties of the AV node account for the filtering of atrial impulses during supraventricular tachyarrhythmias. These properties may generate important beats-to-beat changes in nodal refractory period and conduction time even in the absence of autonomic modulation. In addition, the AV node has rate-sensitive dual pathway properties that not only generate echo beats and reentrant rhythms but also contribute to normal conduction. The anatomical and functional substrate underlying AV nodal rate-dependent and dual pathway function remains ill-defined. While the compact node (Figure 1A) is considered a pivotal structure in rate-dependent function, nodal inputs are often considered to play a key role in dual pathways. Several hypotheses have been put forward to account for dual pathway function. Initially, a functional asymmetry between the crista terminalis and interatrial septum inputs extending to an unspecified portion of the compact node has been postulated. Some investigators have suggested that inputs alone could provide the substrate for two or more pathways. In contrast, others have concluded that the compact node is the primary site of dual pathways. Alternately, the compact node may act as the slow pathway whereas a bypass track made of transitional tissues acts as the fast pathway. Our own histological and functional data indicate that the posterior extension and compact node provide the substrate for slow and fast pathway conduction, respectively. Our current goal is to provide a unified hypothesis for AV nodal rate-dependent and dual pathway function. More specifically, we hypothesize that overall rate-dependent AV nodal properties reflect the lumped expression of the rate-dependent properties displayed by the posterior extension (slow pathway) and compact node (fast pathway). According to this scheme, nodal
inputs serve as a common proximal pathway whereas the lower nodal bundle serves as a common distal pathway. A common proximal pathway implies that fast or slow pathway conduction can result from any input activation pattern. A common distal pathway implies that its activation can result from either slow or fast pathway conduction. Our data also imply that a slow pathway conduction proceeds directly from the posterior extension to the lower nodal bundle i.e., bypasses the compact node. Conversely, fast pathway conduction from the compact node to the lower nodal bundle bypasses the posterior extension.

In support of our hypothesis is the finding that nodal inputs are not functionally asymmetric enough to account for dual pathways. In addition, rate-dependence of nodal inputs is trivial. Our data also provide direct evidence for the involvement of the posterior extension and compact node in slow and fast pathway, respectively. Fast pathway conduction prevails in long and intermediate cycle length ranges whereas slow pathway conduction takes place in short cycle length range because of the long refractory period of the fast pathway (Figure 1B). When fast pathway conduction prevails, slow pathway conduction is concealed. Ablation microlesions targeting the posterior extension amputate the left steep portion of the nodal recovery curve but leave intact the flat portion of the curve corresponding to the fast pathway (Figure 1C). When microlesions are applied at the junction between transitional and compact node tissues, nodal conduction time is not altered over short cycle length range (Figure 1D). That ablation reveals otherwise concealed slow pathway conduction as prolonged nodal conduction times over intermediate and long cycle lengths. Microlesions applied at both sites cause a complete AV block. Taken together, the above data indicate that the overall nodal recovery curve is a composite of recovery properties of slow and fast pathway, each accounting for a specific portion of the curve. Preliminary data show that, except for an upward shift, the recovery curves of slow and fast pathways were not otherwise altered by fatigue. Therefore, overall fatigue may also be explained by individual effects of rate on slow and fast pathway.

In conclusion, overall rate-dependent AV nodal function arises from the individual properties of the slow and fast pathway. Consequently, AV nodal rate-dependent and dual pathway properties can be reconciled and lumped into a single functional scheme.
node’), although NF-positive, expresses some Cx43 and Na\textsubscript{a,1.5} and less HCN4 and we suggest that it is made up of NH cells (transitional Nodal-His cells as identified using electrophysiology). The fast pathway is most likely made up of transitional cells (NF-negative like atrial cells, but small and dispersed like nodal cells). Although it expresses Cx43 like the working myocardium, it may express Na\textsubscript{a,1.5} at a reduced level compared to the working myocardium. It is likely, therefore, that the fast pathway is made up of AN cells (transitional Atrial-Nodal cells as identified using electrophysiology). We have tested this `model’ of the atrioventricular node using computer modelling and we are able to simulate many of the physiological and pathophysiological behaviours of the atrioventricular node. For example, a model of a string of atrial cells connected via parallel strings of N cells (slow pathway) and AN cells (fast pathway) to a string of NH cells (penetrating bundle) – each cell type has its own biophysically detailed action potential – is able to simulate atrioventricular nodal reentrant tachycardia.

Fig. 1. Diagram of heart incorporating detailed anatomical models of the sinoatrial and atrioventricular nodes.

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Calcium channels and cardiac pacemaker activity: from ionic currents to genes

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The spontaneous activity of pacemaker cells in the sino-atrial node (SAN) controls the heart rhythm and rate (HR) under physiological conditions. Automaticity is due to the diastolic depolarization (DD), a slow depolarization phase which drives the membrane voltage from the end of an action potential to the threshold of a new action potential. SAN cells express a wide array of ionic channels, but we have limited knowledge about their functional role in the genesis and regulation of heart automaticity. Particularly, the role of L- and T-type calcium channels in the generation of the DD has been matter of debate. Indeed, even if L- and T-type channels have been proposed to contribute to pacemaking (1), we lack genetic evidence linking the activity of specific voltage-dependent calcium channels genes to dysfunction of cardiac automaticity. We have thus developed a technique to isolate pacemaker cells from mouse SAN (2) and AVN (3). During the last few years, we have studied pacemaker activity in mice lacking L-type Cav1.3 (Cav1.3-/-) and T-type Cav3.1 (Cav3.1-/-) calcium channels (3). We found severely slowed and erratic pacemaking in SAN and AVN cells from Cav1.3-/- mice and demonstrated that this phenomenon is due to abolition of ICa,L in the diastolic depolarization range. Cav1.3-/- mice have pronounced bradycardia, strong SAN dysfunction and display sporadic II-degree AV blocks (4). Atrial fibrillation and flutter were also observed in Cav1.3-/- mice. SAN dysfunction, but not bradycardia could be compensated by crossing Cav1.3-/- animals with mice lacking the cardiac IK\textsubscript{Ach} (5).

This observation indicates that Cav1.3 channels are essential for stabilizing SAN rate under vagal activation.

On the other hand, disruption of the gene coding for Cav3.1 channels abolished ICa,T in SAN and AVN cells. Cav3.1-/- mice had moderately reduced HR (10%) and slowed AVN conduction (3). The lack of Cav3.1 channels prolonged the SAN recovery time and slowed pacemaker activity of individual SAN cells through a pure reduction of the slope of the diastolic depolarisation. However, the heart rate variability (HRV) of Cav3.1-/- mice was not significantly different from that of wild-type animals, indicating that even if Cav3.1 channels contribute to the setting of the basal HR they have reduced impact on the autonomic regulation of HR. In conclusion, our studies indicate that L-type Cav1.3 and T-type Cav3.1 channels play specific and differential roles in the genesis and pacemaker activity. A model of activation of these channels during mouse SAN pacemaking will be discussed.


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Sick Sinus Syndrome - what is the problem: action, connection or remodelling?

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Sinus node dysfunction is commonly known as sick sinus syndrome (SSS). It is a frequent cause of arrhythmias, especially in elderly patients but it can also occur at all ages, including in the newborn. Sinus node dysfunction is the major cause necessitating pacemaker implantation and accounts for approximately half of all patients requiring a permanent pacemaker. SSS may be due to different causes e.g. arterial hypertension, coronary artery disease, trauma, infectious diseases, drugs, electrolyte imbalances or familiar diseases. Manifestations include severe sinus bradycardia, sinus pauses or arrest, sinus node exit block, chronic atrial tachyarrhythmias, alternating periods of atrial bradycarrhythmias and tachyarrhythmias, and inappropriate responses of heart rate during exercise or stress.

Besides disease-associated reasons, one reason for developing idio-patric sinus syndrome are mutations in genes coding for ion channels involved in pacemaking. The role of the cardiac sodium channel encoded mainly by SCN5A (Nav1.5) in sino-atrial node depolarisation is unclear. Mutations have been identified in patients with sinus node dysfunction. Consistent with the electrophysiology, Nav1.5 is present in atrial muscle and the periphery of the node but absent from the center of the sino-atrial node. Despite this, knockout of Nav1.5 in a mouse model results in bradycardia, a delay in sinus node conduction time, and block of sinus node conduction. Knockout of the Na-channel β2 subunit also results in bradycardia and sinus node dysfunction. Recently, various brain-type Na channels, including Nav1.1, have also been shown to be expressed in cardiac myocytes. Nav1.1, in contrast to Nav1.5, is present in the node as well as the atrial muscle and seems to play a functional role in proper sinus node function. In the working myocardium the principal calcium channel isoform is Cav1.2 responsible for ICa,L. But, in the node, Cav1.3 seems to be the major player. Knocking out Cav1.3 causes bradycardia and sinus dysfunction, linked to abolition of the major component of ICa,L. In addition, knockout of Cav3.1, that is carrying ICa,T, causes bradycardia, slows the intrinsic heart rate, and prolongs sinus node recovery time. HCN channels (HCN1, 2, and 4) that are highly expressed in the node are responsible for If. The principal channel in the node is HCN4. HCN4 knockout is lethal at embryonic stages, but the embryos exhibit bradycardia accompanied by dramatic decrease in cardiac If. Knockout of the HCN2 gene results also in a reduction of If in vitro but in vivo there is no significant bradycardia, but sinus dysfunction is observed. Gap junction channels, composed of connexins, are responsible for electric coupling between cells. However, in contrast to atrial muscle, electric coupling within the node is poor and conduction is slow. This protects the pacemaker from the hyperpolarizing influence of the surrounding atrial muscle. There is evidence that electric coupling is stronger in nodal periphery because of interdigitations of atrial and nodal cells in the periphery and it has been suggested that this permits the node to drive the surrounding atrial muscle and at the same time to be protected from the hyperpolarizing influence of the atrial muscle. In Cx40 knockout mice, there is evidence of bradycardia, sinus node exit and entry block, and a prolongation of the sinus node conduction time.

SSS is caused by different conditions with multiple causes. Fibrosis, ion channels, variants related to heart failure, ageing and AF are determinants. Assuming that these conditions cause ‘electronic remodelling’, manipulation of ion channel gene expression in the node could be a powerful therapeutic tool protect and restore proper nodal function. Therefore research has put much interest in generating ‘biopacemakers’ by manipulation of ion channel genes and gene expression.


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Tandem biological and electronic pacemaking

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Many laboratories are pursuing the use of gene and cell therapies to treat bradycarrhythmias, an approach that has been termed creating a biological pacemaker. While divergent approaches have been explored, recent efforts have focused on exogenous expression of genes from the HCN family, or on the use of cells that endogenously express members of this gene family. The HCN gene family is responsible for the pacemaker current, If, which contributes to normal automaticity in the sinoatrial node. The members of this family differ in their gating characteristics and cAMP responsiveness. Thus, one question being asked is which HCN gene is optimal for creating a biological pacemaker, or whether a mutated form of HCN would function better than any of the individual native isoforms.

To fully assess functionality of any biological pacemaker, it must be studied in an in vivo setting where the normal pacemaking activity is suppressed. To do this safely requires the simultaneous implantation of an electronic pacemaker in a tandem configuration, where the electronic device operates as a demand pacemaker set at a low rate. This approach has several advantages: 1) it provides an increased level of safety, and mirrors what is the likely configuration of any clinical trial; 2) the electronic pacemaker provides a continuous record of performance of both the biological and electronic pacemakers.

We report results with an HCN2 based biological pacemaker studied in tandem mode and delivered both as a gene therapy delivered in an adenoviral vector and as a cell therapy delivered via an adult human mesenchymal stem cell. In both cases, the electronic pacemaker accounts for less than 1/3 of the heartbeats under optimal implantation conditions. In both forms of therapy, the HCN2 based biological pacemaker is able to maintain
an average heart rate on the order of 55 beats per minute. The tandem pacemaker also exhibited greater responsiveness to autonomic agonists than is observed when the electronic pacemaker is operating alone.

We also have investigated whether variations on the HCN2 gene result in better performance when studied in a tandem configuration. We studied a point mutation in the murine HCN2 gene (E324A) which results in a current that activates more quickly and at less negative voltages. However, these benefits were opposed by poorer expression of the exogenous gene. We also studied a chimeric channel, in which the transmembrane portion of HCN2 has been replaced by HCN1. This results in a current which activates faster than HCN2 but with similar voltage dependence when expressed in myocytes using a gene therapy approach. Unlike HCN2 based gene therapy, the chimeric channel was associated with periods of ventricular tachycardia in vivo.

In summary, an HCN2 based biological pacemaker operating in tandem with an electronic pacemaker provides a level of performance in terms of basal heart rate, autonomic responsiveness, and reduced battery drain that is potentially superior to that of an electronic pacemaker alone. It remains to be determined if variations on the native HCN genes can provide improved functionality than HCN2 in this configuration.

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Diversity in the mechanisms underlying the roles of cytosolic Ca\(^{2+}\) in initiation and regulation of the pacemaker activity in the sino-atrial node

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Over slightly more than a decade, particular attention has been focussed on whether spontaneous pacemaker activity in the sino-atrial (SA) node arises primarily from the voltage-dependent properties of ion channels in the cell membrane or whether cytosolic Ca\(^{2+}\) plays a significant role in regulating this activity. The purpose of this presentation is to briefly review progress over the last decade and to present recent observations from our laboratory that indicate substantial complexity in the many roles for cytosolic Ca\(^{2+}\) in regulating pacemaker activity.

Initial work focussed on Ca\(^{2+}\) release from the sarcoplasmic reticulum, but it is clear that even in the absence of a functional SR cytosolic Ca\(^{2+}\) may still regulate pacemaker activity. Our observations continue to support a role for Ca\(^{2+}\) release from the SR in the absence of neurotransmitters and hormones, and the importance of this SR-released Ca\(^{2+}\) is heightened, for example, following \(\beta\)-adrenoceptor stimulation. Under the conditions of our experiments, application of ryanodine to block Ca\(^{2+}\) release and/or cyclopiazonic acid or thapsigargin to block Ca\(^{2+}\) uptake into the SR slows but does not stop spontaneous activity. Our observations support a continuing importance of cytosolic Ca\(^{2+}\) in regulating pacemaker activity when SR function is suppressed (see below). At least part of the role of the SR-released Ca\(^{2+}\) is to provoke electrogenic Na\(^+\)/Ca\(^{2+}\) exchange (NCX) currents at the foot of the action potential, perhaps associated with Ca\(^{2+}\) sparks. However, under the conditions of our experiments, we detect sparks as separable events in only about 30% of guinea-pig SA-node cells, and in any case there may be additional roles of the subsarcolemmal Ca\(^{2+}\).

Cytosolic Ca\(^{2+}\) (derived from the SR or from entry through the surface membrane) may play a role by directly influencing membrane transporters (e.g. NCX), via Ca\(^{2+}\)-calmodulin-dependent protein kinase (CaM kinase II) or via other actions mediated by calmodulin. Our recent observations support the fundamental importance of NCX in maintaining pacemaker activity, since suppression of this pathway by rapid switch to low Na\(^+\) solutions (with Li\(^+\) as replacement) caused immediate cessation of beating before there was time for the secondary effects that are expected to occur following changes in cytosolic Ca\(^{2+}\). When this essential pathway for Ca\(^{2+}\) balance is inhibited. Depolarizing ionic currents via NCX could arise from the conventional electrogenic 3Na\(^+:1Ca^{2+}\) mode of NCX or from a recently proposed Na\(^+\)-leak pathway through the same protein. Another potentially important player in pacemaker activity is the \(I_f\) current that is activated by hyperpolarization and regulated by cytosolic cAMP. Our recent observations shed light on regulation of this pathway by cytosolic Ca\(^{2+}\) and provide a possible resolution of the controversy about the role of this pathway in the absence of \(\beta\)-adrenoceptor stimulation. Evidence for the presence of Ca\(^{2+}\)-stimulated adenyl cyclase, AC1, in SA node but not ventricular muscle was found both using PCR to detect mRNA and using Western blotting to detect a protein of the expected molecular mass. Immunocytochemistry showed localisation of AC1 at the surface membrane. Functional electrophysiological studies supported the hypothesis that cytosolic Ca\(^{2+}\) activates AC1 to catalyse the formation of cAMP which in turn shifts activation of \(I_f\) in the depolarizing direction so that there is more \(I_f\) current at the pacemaker range of potentials. A further point is that conventional voltage-clamp protocols may lead to lower cytosolic Ca\(^{2+}\) than would be the case in a beating SA node cell and therefore to underestimation of the \(I_f\) current. In addition, the significance of the presence of AC1 at the surface membrane may be much wider than its importance for \(I_f\). Stimulation of AC1 by subsarcolemmal Ca\(^{2+}\) may also lead to activation of PKA and phosphorylation of other channels including Ca\(^{2+}\) and K\(^+\) channels. The effects of cytosolic Ca\(^{2+}\) on enzymes are not expected to be confined to AC1 since there will be activation of CaM kinase II and there may also be additional effects on yet more Ca\(^{2+}\)-regulated enzymes. For example, NO shows complex biphasic effects on pacemaker activity and some enzymes in the proposed pathways are Ca\(^{2+}\) sensitive. There is considerable potential for interaction and feedback in all these pathways which will also depend on the balance between phosphorylating enzymes and phosphatases (that can also be Ca\(^{2+}\) sensitive).

The above arguments show the complex roles so far identified for cytosolic Ca\(^{2+}\) in the pacemaker activity of SA node. However, even greater complexity may be revealed if endogenous substances such as IP\(_3\) in turn modulate this Ca\(^{2+}\).

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High constitutive activities of both adenylyl cyclases and phosphodiesterases regulate local subsarcolemmal RyR Ca\(^{2+}\) release to control normal sinoatrial node cell automaticity

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Rabbit sinoatrial node cells (SANC) possess localized, sub-membrane Ca\(^{2+}\) releases (LCR) via ryanodine receptors (RyR) which occur during a late part of the diastolic depolarization (DD) (1). Regardless of size, SANC exhibit intense RyR, Na\(^{+}\)-Ca\(^{2+}\) exchanger (NXC) and submembrane NCX/RyR colocalization (2). Spontaneous, roughly periodic LCR activate inward NCX current, accelerate the nonlinear DD rate and thus control the SANC spontaneous beating rate (1,3). The basal cAMP content of isolated SANC homogenates is markedly increased compared to that in ventricular myocytes, and LCR periodicity in SANC is precisely controlled by cAMP PKA-dependent phosphorylation of Ca\(^{2+}\) cycling proteins (4). This high basal level of cAMP is required for spontaneous beating and is due to constitutively activated adenylate cyclases (ACs) in SANC, since specific AC inhibitor, MDL-12,330A (400 \(\mu\)mol/L) markedly reduces cAMP content and the same concentration of MDL stops spontaneous beating (4). We hypothesized that a high level of cAMP in SANC might be a result of low cAMP degradation by phosphodiesterases (PDEs).

Spontaneously beating SANC were isolated from adult rabbit hearts, according to the NIH ethical requirements. To study Ca\(^{2+}\) cycling in SANC we employed confocal microscopy with Fluor-3 as a Ca\(^{2+}\) indicator. We simultaneously recorded spontaneous SANC action potentials (perforated patch-clamp) and line-scan images beneath sarcolemma. The cAMP content of isolated SANC homogenates was assessed by radioisotope assay. Phospholamban (PLB) phosphorylation was measured by Western blotting of SANC homogenates with antibodies to total PLB and phosphorylated PLB at Serine 16.

To test our hypothesis we employed a broad-spectrum PDE inhibitor, IBMX. Contrary to our expectations, IBMX (100 \(\mu\)mol/L), induced 9-fold increase in the cAMP level and ~55% increase in the SANC firing rate (from 145 ± 8 to 221 ± 6 beat/min, n=10) demonstrating a high basal activity of PDEs in SANC. The PDE superfamily consists of 11 families and PDEs 1-5 are present in the heart (5). Using specific inhibitors against different PDE subtypes we determined which PDE subtype relate to an increase in the spontaneous SANC beating rate. A suppression of PDE3 by milrinone (50 \(\mu\)mol/L) produced 47% increase in the beating rate (from 145 ± 13 to 207 ± 10 beat/min, n=9), i.e., similar to the effect of IBMX, and exceeding effects of a saturating concentration of \(\beta\)-AR agonist isoproterenol (35%, n=9). The effects of other PDE inhibitors on the spontaneous beating rate were relatively small. Suppression of either total PDE activity by IBMX or PDE3 activity by milrinone markedly increased cAMP PKA-dependent PLB phosphorylation (Fig. 1), and this was reversed by a specific PKA inhibitor peptide, PKI. Additionally, milrinone produced ~46% increase in the amplitude of L-type Ca\(^{2+}\) current from 13.3 ± 1.6 in control, to 19.0 ± 2.2 pA/pF with milrinone (n=8, P<0.05). The positive chronotropic effect of PDE inhibition is critically dependent upon subsarcolemmal RyR Ca\(^{2+}\) release during the late DD. Specifically, either IBMX or milrinone decreased the LCR period (the interval from a prior AP induced SR Ca\(^{2+}\) transient to subsequent LCR occurrence), increased the LCR amplitude (P<0.01) and size (P<0.01) leading to earlier and augmented LCR Ca\(^{2+}\) release. This PDE inhibition-induced reduction in the LCR period was greater than that produced by isoproterenol. The LCR period following IBMX, milrinone or ISO was highly correlated with the concomitant decrease in the SANC spontaneous cycle length (Fig 2). When RyRs were disabled by ryanodine, both IBMX and milrinone failed to amplify LCRs, to accelerate the DD rate and to increase the SANC firing rate, despite preserved PDE inhibition induced augmentation of L-type Ca\(^{2+}\) current. Thus, high basal constitutive PDE activity in SANC coexists with constitutively active AC, providing a negative feedback on the latter to control cAMP level. Constitutively active PDEs, via reduction in cAMP-mediated, PKA-dependent protein phosphorylation prolong the local RyR Ca\(^{2+}\) release period to control the basal spontaneous SANC beating rate.

Figure 1. A, representative Western blot of the basal level of PLB phosphorylation at serine16, and total PLB in SANC prior to and following milrinone (50 \(\mu\)mol/L), IBMX (100 \(\mu\)mol/L) or \(\beta\)-AR stimulation (1 \(\mu\)mol/L, ISO). B, relative values of phosphorylated PLB normalized to total PLB (n=8).
Heart failure in the sinoatrial node

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Heart failure (HF) is increasingly prevalent in Western countries. It is a major risk factor of life-threatening ventricular arrhythmias and sudden death, and an important risk factor for atrial fibrillation (AF)[1]. Poorly treated HF has a mortality rate at five years of about 60%[2]. Abnormalities in sinoatrial node (SAN) function are common in HF and may contribute to bradyarrhythmic death[1]. However, detailed data concerning SAN remodelling in HF is limited. Patients with HF show electrophysiological remodelling of sinoatrial node (SAN) function and regulation. They show decreased heart rate and heart rate variability[3]. Severe HF patients also show intermodal conduction slowing, dispersion in excitability and prolongation of the SAN recovery time[4].

In animal models, HF decreases intrinsic heart rate and circadian rhythmicity [5,6,7]. In rabbit, myocytes from within the SAN region demonstrate that the HF induced increase in cycle length is caused by a reduction in diastolic depolarization rate[8]. HF impairs single sinus-node cell automaticity by downregulating the hyperpolarization-activated “pacemaker” current (If), without changing voltage dependence or kinetics. Other currents involved in pacemaking, the T-type and L-type calcium current, rapid and ultra-rapid delayed rectifier current, transient outward currents, and sodium-calcium exchange current are unaltered[8]. In a canine model in which congestive heart failure was induced by overdrive pacing, the hyperpolarization-activated cyclic nucleotide-gated (HCN) channel subunits HCN2 and HCN4 underlying If, are downregulated. In the same model an upregulation of atrial HCN4 is observed which may help to promote atrial arrhythmia formation[7].

Patients with heart failure produces complex endocrine changes, including alterations in atrial and brain natriuretic peptides, and changed concentrations of arginine vasopressin, angiotensin-II and aldosterone. Many of these changes could affect SAN function and need further investigation. In addition, heart failure is associated with profound changes in the balance of the autonomic nervous system, such as activation of the sympathetic nerve, parasympathetic withdrawal and increased catecholamine levels. Changes in adrenergic receptor number, function and stimulation also occur[9]. In animal models HF decreases intrinsic heart rate with a larger responsiveness to acetylcholine and a decreased circadian rhythmicity. The responsiveness of the SAN to beta-adrenergic stimulation with noradrenaline is not changed[5].

Only throughout the last decade, intracellular calcium (Ca^{2+}) has been recognized as an additional mechanism through which beta-adrenergic stimulation exerts its positive chronotropic action. Beta-adrenergic stimulation increases Ca^{2+} transients and augment spontaneous and triggered Ca^{2+} releases during diastolic depolarisation. This promotes Ca^{2+} transport across the sarcolemmal membrane by the sodium calcium exchanger (NCX), which delivers a depolarising current at diastolic potentials. In this way also INCX, albeit indirectly, is increased by beta-adrenergic stimulation and helps to accelerate pacemaker activity[10]. It is unknown to what extent ACh stimulation modulates Ca^{2+} transients and Ca^{2+} releases during diastolic depolarisation in pacemaker cells. Moreover, the role of Ca^{2+} to the negative chronotropic action of ACh in non-stimulated and beta-adrenergic stimulated nodal myocytes is still unclear. These questions in relation to the occurrence of heart failure will be addressed during the presentation.


Development of the cardiac conduction system and the nodes

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One of the most fascinating aspects in the formation of the heart is the very early development of the electrical patterning as can be registered by the ECG, which is the registration of the rhythmic waves of depolarizing activity over the cardiac muscle. In the mature heart the conduction system is held responsible for the rhythmic excitations and contractions. However, in chicken embryos of less than three days of development, when the formation of the atrial and ventricular working myocardium has just been initiated, an adult type of ECG can be monitored. Generally, the conduction system is defined as the system of specialized myocardial tissues responsible for initiation and propagation of the sinus impulse. If we accept this functional definition, then the early embryonic heart has a conduction system, which is already in place, because there is an adult type of ECG. On the other hand, if we would apply strict anatomical and histological definitions, the embryonic heart undeniably lacks a conduction system. This field of tension concerning the recognition of the conduction system using physiological versus anatomical or histological criteria has led to many controversies in the field of the development of the cardiac specialized tissues. Why certain areas of the heart tube do not develop into the working myocardium of the chambers and contribute to the formation of the cardiac conduction system is one of the key questions of cardiac embryology. By our recent findings that the transcriptional repressors Tbx2 and Tbx3 repress working myocardium formation, we are beginning to understand these morphogenetic processes. Detailed reconstructions of the developmental patterns of expression of Tbx3 during development have revealed that Tbx3 is expressed in those areas of the heart tube that do not become working myocardium, i.e. in the sinus node region, internodal region, atrioventricular junction, atrioventricular bundle and bundle branches. These areas comprise not only the conventional conduction system, but also the highly controversial areas of the internodal region and the entire atrioventricular junction.

Cells are added continuously to the venous pole of the heart. An intriguing question is how the growing embryonic heart tube maintains the leading pacemaker at the inflow of the heart. Polarity along the longitudinal axis of the initial heart tube ensures that in the very early phases of heart development the dominant pacemaker is at the inflow of the heart. With the subsequent growth of the heart, so-called sinus venosus myocardium is added at the intake of the heart. The addition of this venous myocardium is under control of the T-box transcription factor Tbx18. Initially, this myocardium does not express the transcription factor Nkx2-5, which, in turn, permits the gene encoding pacemaker channel Hcn4 and Tbx3 to be expressed here. Upon maturation, the venous myocardium acquires the atrial phenotype, except the sinoatrial region where Tbx3 controls the maintenance of the nodal phenotype. Thus, the most recently added myocardium always has the highest pacemaker activity, which is lost upon maturation of the myocardium toward the atrial lineage. The sinus node escapes this maturation by the action of Tbx3.

Finally, an ever recurring theme is whether the embryonic origin of the pulmonary myocardium would explain the frequent occurrence of arrhythmias taking origin from this region. Some groups suggest a common origin of the systemic myocardium, which includes the sinus node, and pulmonary myocardium. This would explain that arrhythmias can originate from other regions such as the pulmonary vein myocardium. It is common wisdom that fish do not have lungs, and only systemic venous returns where pacemaker activity resides. During evolution the pulmonary venous return developed at the dorsal side of the atrium in the pulmo-pharyngeal region. It is fascinating to observe that all recent molecular data have demonstrated that the pulmonary myocardial lineage is essentially different from the systemic myocardial lineage. These data have fundamentally changed our interpretations of these arrhythmias.

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Ionic mechanisms underlying the pacemaker potential in the sinoatrial node cell

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The time- and voltage-dependent gating of ionic channels plays a primary role in the automaticity of membrane potential changes. The experimental evidence described so far indicates that the following gating mechanisms underlie the slow diastolic depolarization. The delayed rectifier K+ channels are activated during the preceding action potential and are deactivated by the negative potential during diastole. The activation of K+ channels results in the time-dependent depolarization of the membrane, provided that the amplitude of the background inward current is of significant amplitude. The inactivation of the L-type Ca2+ channels is gradually removed during the early diastolic period and this channel gating results in a time-dependent increase in the inward current. Because of its sustained nature, K+ also contributes to the net inward current. The negative membrane potential near the maximum diastolic potential activates If. Finally, the later phase of diastole depolarization activates the L-type Ca2+ channel, triggering the maximum rate of rise of the action potential. These time- and voltage-dependent changes in membrane conductance occur in the presence of a significant background conductance.
Channel gating driving membrane depolarization during diastole. Deactivation of IK
Removal of inactivation of ICaL
Activation of Ist
Activation of ICa,L
Activation of ICa,T
Background conductances
Ib,Na
IK,ACh
INa/K
INa/Ca
IK,ATP

The central question is the relative amplitudes of these current components. These would be quantitatively estimated over the entire range of the diastolic depolarization by incorporating the experimentally-derived characteristics of each current system into a mathematical model of the pacemaker action potential. To date various types of SA node models have been proposed. (Yanagihara, Noma, & Irisawa, 1980; Noble, & Noble, 1984; Wilders, Jongmsa, & Van Ginneken, 1991; Demir et al., 1994). Our model (Sarai et al., 2003) successfully reconstructs the experimental action potentials at various concentrations of external Ca2+ and K+. Increasing the amplitude of L-type Ca2+ current (ICaL) prolongs the duration of the action potential and thereby slightly decreases the spontaneous rate. On the other hand, a negative voltage shift of ICaL gating by a few mV markedly increases the spontaneous rate. When the amplitude of sustained inward current (Ist) is increased, the spontaneous rate is increased irrespective of the ICaL amplitude. Increasing [Ca2+]o shortens the action potential and increases the spontaneous rate. When the spontaneous activity is stopped by decreasing ICaL amplitude, the resting potential is around –35 mV over 1-15 mM [K+]o because of the presence of the background non-selective cation current. The unique role of individual voltage- and time-dependent ion channels is clearly demonstrated and distinguished from that of the background current by calculating an instantaneous equilibrium potential during the course of the spontaneous activity.


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SA20

A novel role of P21 activated kinase-1 (Pak1)-mediated signaling in regulation of cardiac pacemaker channels

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Modulation of ion channel activity is a fundamental mechanism for heart function including cardiac pacemaker activity. Pathways regulating the phosphorylation of ion channel in cardiac cells are relatively well understood; however, the counterbalance mechanism by dephosphorylation of these proteins remains unclear.

Here we report a novel role of P21 activated kinase-1 (Pak1)-mediated signaling in blunting isoproterenol (ISO)-induced enhancement of L-type Ca2+ current (ICaL) and delayed rectifier potassium current (IK) in sino-atrial node (SAN) pacemaker cells. (Ke et al., 2007) We demonstrated that there is abundant expression of endogenous Pak1 in cardiac pacemaker cells. Over-expressing Pak1 in these cells by adenovirus expressing constitutively active Pak1 (Ad-Pak1) blunts β-adrenoceptor agonist ISO-induced up-regulation of ICaL and IK. ISO (100 nM) increased the amplitudes of ICaL by ~57 % and IK by ~410 % in Ad-LacZ infected cells, whereas the corresponding increases were only ~13 % and ~70 % in the Ad-Pak1 infected cells. Moreover, the IK decay time constant was reduced (by 39 ± 8 %, Fig. 4G-1). However, the effect of ISO on the rate of IK decay in the Ad-Pak1 group was much less than that of the control group, and indeed not statistically significant (17 ± 9 %). Such a difference in the response to ISO between the two groups indicates that the effect of ISO on ICaL and IK was significantly attenuated in cells infected with Ad-Pak1. This effect of active Pak1 mainly presented when ICaL and IK activity was enhanced by β-stimulation. Such effect can be reversed by inhibition of PP2A. Furthermore, L-type Ca2+ channels (alpha 1C) associate with Pak1 in SAN tissue and PP2A co-immunoprecipitates with endogenous Pak1 in sinoatrial node (SAN) tissue and expression of constitutively active Pak1 blunts the ISO-induced chronotropic action on pacemaker activity of intact SAN preparations. Thus, our data demonstrate that a Pak1 signaling pathway exists in cardiac pacemaker cells and this pathway may play an important role in the regulation of ion channel activity in the heart.


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Ageing to arrhythmias: Conundrums of the ageing heart
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Background: The size of the elderly population is continually increasing and it has been predicted that by 2035 over 25% of the Western population will be elderly (≥ 65 years of age) (Lakatta, 2002). Dysfunction of the sinoatrial (SA) node has been shown to be at its highest incidence within the elderly population. Those who suffer SA node dysfunction experience a range of symptoms including dizziness, fatigue and palpitations, clinically observed as rhythm disturbances, sinus pauses and arrhythmias; the patients are classed as suffering ‘sick sinus syndrome’ and without clinical intervention are at high risk of sudden death (Ross & Kenny, 2000). Effective long-term treatment employs catheter ablation and/or implantation of an artificial pacemaker. However, elderly patients are frequently unfit for surgery, and those over 70 years of age with sick sinus syndrome experience minimal improvement in their cardiac function (Fleischmann et al. 2006). My hypothesis was to prove the existence of an age-dependent decrease in the protein expression of the gap junction connexin43 (Cx43) and the channel Cav1.2, accompanying an associated decline in pacemaker function. Furthermore, I hypothesised that the stress activated c-jun N-terminal kinase (JNK), an intracellular signalling mediator, is implicated in the reduced expression of these proteins.

Methods and Results: The SA node region was dissected from rodents between birth and the end of their lifespan. My data cated in the reduced expression of these proteins.

JNK in the heart implicating JNK in the regulation of Cx43 protein and SA node remodelling during progressive ageing (n=5 [≥0.3484; R2=0.96]).

Conclusion: This is the first evidence that the JNK-mediated pathway is a signalling mechanism implicated in cardiac ageing, associating with the age-dependent decline of Cx43 and Cav1.2 channel with the SA node. By further defining the degenerative changes limiting the cardiac pacemaker function in the elderly and the mechanisms responsible for these it may prove possible to limit the progression of SAN dysfunction in the elderly.

Electrophysiological consequences of myocyte-fibroblast electrotonic interaction have been studied in ‘wet’ [7] and ‘dry’ model systems [8]. They suggest that – in addition to forming obstacles to electrical conduction – fibroblasts could act as i) passive electrotonic loads / current sinks or, ii) if bridging gaps between myocyte groups as conductors of excitation. In situ, this could affect crucial electrophysiological parameters such as excitability, refactoriness and electrical load (which are key determinants of arrhythmogenic tissue properties), and/or provide conduction pathways outside the traditional conceptual framework of myocyte-myocyte only coupling, such as seen in cases of donor-recipient cardiac coupling across transplantation scars (which occurs in about 10% of human heart transplants), or with electrical invasion of post-infarct scar tissue [6]. This possibility has triggered investigations into the potential of genetically modified fibroblasts to act as modulators of cardiac automaticity [9], and it may form an interesting, if unconventional, target for anti-arrhythmic drug actions [10].

Thus, cardiac fibroblasts contribute to cardiac electrophysiology, apparently assuming roles beyond the formation of passive obstacles, by acting as current sinks and/or electrical conduction pathways. Details of in situ myocyte-fibroblast interaction in different regions of the heart, and the mechanisms that govern regulation of heterogeneous cell interaction in health and disease, are still ill-explored, and form a potentially exciting target for further research.


PC holds a Junior Research Fellowship of Christ Church College Oxford, PK is a Senior Research Fellow of the British Heart Foundation.

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SA23

Ionic currents and channels in the human heart

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Use of conventional microelectrodes allows recording of the diverse shapes of human cardiac action potentials, each of which is characteristic for the various locations within the heart. The single cell voltage clamp technique enables analysis of the underlying ionic current and molecular biology and genetics provide tools to study expression and modification of ion conducting channels including their regulatory subunits. Atrial and ventricular cardiac tissue change their structure and function in response to disease (remodeling) Pathological expression and function of ion channels is relevant in clinical medicine since they can lead to life-threatening arrhythmias in particular when the ventricles are involved.

Concentrating on repolarising ionic currents I will give a brief overview of the diversity of changes encountered in the diseased heart with particular emphasis on atrial fibrillation and heart failure. During remodeling, not only the expression level of ion channels but also their electrophysiological properties can significantly change. In addition, their responsiveness to drugs can become modified, where gain or loss of function may be similarly deleterious.

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Moderate hypothermia facilitates termination of spiral wave reentry in the ventricle

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Moderate hypothermia (33°C) has been shown to improve defibrillation success by DC shocks compared with normothermia (37°C) and severe hypothermia (30°C) in cardiac arrest due to ventricular fibrillation/tachycardia (VF/VT), but the mechanisms are unknown. We hypothesized that moderate hypothermia may prevent spiral wave functional reentry, and...
we investigated the dynamics of spiral waves induced in the two-dimensional ventricular myocardium of Langendorff-perfused rabbit hearts by means of optical mapping. Moderate and severe hypothermia (33 and 30°C, respectively) caused a significant prolongation of the action potential and a significant decrease in conduction velocity under basic stimulation at 2.5 Hz. VT/VFs induced by DC shocks often self-terminated at moderate hypothermia: the duration of TV/FVs was reduced dramatically at moderate hypothermia compared with nonthermia and severe hypothermia. Spiral waves during VT at normothermia rotated around a functional line of block and were stationary, whereas those at moderate and severe hypothermia were characterized by disorganization with frequent wave breakups. Phase maps during VT/VFs at moderate hypothermia showed collision of counter-rotating phase singularities (PSs), resulting in their mutual annihilation, and exit of PSs from the anatomical boundaries. These results suggest that moderate hypothermia may facilitate self-termination of spiral waves through the decrease of their generation/extinction ratio.

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SA26

Human heart β-adrenoceptors and endothelin receptors: localization and function

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The contractile state of the human heart is modulated by numerous G-protein coupled receptors including β-adrenoceptors and endothelin receptors. Both β1- and β2-adrenoceptors are localized throughout the heart including the sinus node, atrium, atrioventricular conducting system and ventricle. In failing hearts with idiopathic dilated cardiomyopathy or ischemic heart disease, the density of β1- and β2-adrenoceptors in the interatrial and ventricular septa and the atrioventricular conducting system was assessed. Sections (10 μm) were cut on a cryostat and labeled with (-)-[125I]cyanopindolol in the absence and presence of 70 nM ICI 118,551 to block both ETA and ETB receptors or 1 µM (-)-propranolol to block both β1- and β2-adrenoceptors. These studies revealed a lower density of β1-adrenoceptors in the bundle of His than in the atrioventricular node or interatrial and ventricular septa. On the other hand there was a uniform distribution of β2-adrenoceptors throughout these regions. The sympathetic nervous system may have a greater modulatory effect on conduction in the atrioventricular node compared to the bundle of His due to different β1-adrenoceptor densities.

In atrium and ventricle from failing and non-failing human hearts, activation of β1- or β2-adrenoceptors causes increases in contractile force, hastening of relaxation and cyclic AMP dependent protein kinase phosphorylation of proteins implicated in hastening of relaxation, phospholamban, troponin I and C-protein. These observations are consistent with coupling of both β1- and β2-adrenoceptors to stimulatory Gsα-protein.

Human β1-adrenoceptors exist in multiple forms including ‘affinity states’ and polymorphisms. Activation by noradrenaline elicits powerful increases in contractile force and hastening of relaxation. These effects are blocked with high affinity by β-blockers including propranolol, (-)-pindolol, (-)-CGP12177 and carvedilol. Some β-blockers, typified by (-)-pindolol and (-)-CGP12177, not only block the receptor, but also activate it, but at much higher concentrations (~2 log-units) than those required to block the receptor. Abrogation of cardiostimulant effects of (-)-CGP12177 in β1-/β2-adrenoceptor double knockout mice but not β2-adrenoceptor knockout mice revealed an obligatory role of the β1-adrenoceptor. On the basis of these results, the β1-adrenoceptor is thought to exist in two ‘affinity states’, β1H- and β1L-adrenoceptor, where β1H-adrenoceptor is activated by noradrenaline and blocked with high affinity by β-blockers, and β1L-adrenoceptor is activated by drugs such as (-)-CGP12177 and blocked with low affinity by β-blockers such as (-)-propranolol. In human heart (-)-CGP12177 and (-)-pindolol cause increases in contractile force and hastening of relaxation.

There are two common polymorphic locations of the β1-adrenoceptor, at amino acids 49 (Ser/Gly) and 389 (Gly/Arg). In the non-failing heart the cardiostimulant effects of noradrenaline at β1H-adrenoceptors and (-)-CGP12177 at β1L-adrenoceptors are conserved across β1-adrenoceptor polymorphisms. On the other hand, the effect of β-blockers in human heart failure may be determined by β1-adrenoceptor polymorphisms. Idiopathic cardiomyopathy patients receiving a maximal dose of carvedilol >1 year exhibit greater increases in left ventricular ejection fraction if they have Arg389 compared to patients carrying Gly389, P<0.05.

In failing hearts, the density of ETA and ETB receptors in the interatrial and ventricular septa and the atrioventricular conducting system was assessed. Sections were cut on a cryostat and labeled with [125I]ET-1 in the absence and presence of 100 nM BQ123 to block ETA receptors or 200 nM BQ3020 to block ETB receptors or 1 µM ET-1 to block both ETA and ETB receptors. Both ETA and ETB receptors are located in the interatrial and interventricular septum, the atrioventricular node and the penetrating and branching bundles of His. There was a higher proportion of ETB receptors in the atrioventricular conducting system compared with surrounding atrial and ventricular myocardium. While these studies might predict a greater role or ETB receptors in the atrioventricular conducting system compared to surrounding atrial and ventricular myocardium, the role of ETA and ETB receptors in the human atrioventricular conducting system has however not been clarified, particularly in heart failure which is associated with elevated levels of the endogenous agonist, ET-1. On the otherhand it has been shown that ET-1 causes increases in contractile force in human atrium and ventricle.

Parts of this work were carried out in the laboratories of Professor Roger Summers (Melbourne) and Dr Anthony Davenport (Cambridge).

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The Purkinje cell 2007 style

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There is a long history for Purkinje cells. In 1845, Purkinje first described Purkinje fibers strands on the endocardial surface of sheep hearts. In 1906, Tawara described these strands as a dense layer of connective tissue surrounding short cells (Purkinje cells) which have variable shapes and could from bundles that branched within the septum forming a network along the endocardial surface. Structural studies continued with various reports about the presence and absence of t-tubules, thus distinguishing Pcells from their ventricular cell neighbors. Recent data suggest that t-tubules occasionally are present in Purkinje fibers but their frequency depends on the size of the heart.

During this presentation we will first review what is known about the differences in intrinsic ion channel function between Purkinje cells and ventricular cells that underlie their markedly different action potential phenotypes. Second we will discuss what is known about the differences in EC coupling that separate the Purkinje cell from the ventricular cell and their potential for reverse EC coupling. Finally we will discuss what is known about the remodeled Purkinje cell in terms of enhanced pacemaker function.

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