SA1

Setting the Pace

O.F. Hutter

Institute of Physiology, University of Glasgow, Glasgow, UK

For much of the 19th century "enquiry into the function of the different tissues of the animal body has been marked by a general tendency to exalt the importance of Nerve and correspondingly to deprecate the powers of Muscle"[1]. In the case of the frog heart, the demonstrably most rhythmic sinus venosus had been recognized as the origin of each beat[2], but the spring of automaticity was long considered to reside not in the muscular component of that chamber but in ganglion cells buried within it. Against this background, the myogenic theory of cardiac automaticity advocated by Foster[3,4], Gaskell[5,6], and McWilliam[7,8] was slow to gain acceptance over the neurogenic theory. The socio-economic factors that arguably played a part in stoking this prolonged controversy will be recalled. And some lessons for the Body Politic that may be drawn from modern discoveries on the mechanism of pacemaking will be explored.

Ranson, W.B. J Physiol. 5, 261- 372, 1885

Stanius H.F. Arch Anat Physiol. 85-100, 1852

Foster, M. Rep Br Assoc Adv Sci 29, 160, 1859

Foster, M. A Textbook of Physiology, ed 2. London: Macmillan & Co., 1887.

Gaskell, W.H. J Physiol. 4, 43-127, 1883

Gaskell W.H. in Schaefer E. A. ed. Textbook of Physiology, vol 2, Edinburgh: Young J Pentland, 1900

McWilliam, J.A. J Physiol. 6, 192-292, 1885

McWilliam, J.A. J Physiol. 9, 167-212, 1888

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SA2

The evolution of knowledge concerning the morphology of the conduction tissues

R.H. Anderson

Cardiac Unit, Institute of Child, London, UK

In the years leading up to the turn of the nineteenth century, most debates relating to conduction of the cardiac impulse revolved around whether the process was myogenic or neurogenic. Stannius then showed unequivocally that conduction was myogenic, but his studies failed to clarify the anatomic substrates for initiation and dissemination of the cardiac impulse. Indeed, anatomical studies in the final decade of the 19th century had served only to confuse the issue. In 1893, Wilhelm His the younger, working in Leipzig, proposed that a solitary muscular fascicle crossed the plane of atrioventricular insulation. Stanley Kent, a physiologist working in London, suggested that multiple pathways permitted atrioventricular conduction in the normal heart. The confusion was sufficiently great for Arthur Keith, one of the foremost anatomists of the time, to express scepticism at the existence of the purported atrioventricular bundle. He was then sent, by James Mackenzie, the account of the atrioventricular conduction axis produced by Sunao Tawara, a Japanese pathologist working in the laboratory of Ludwig Aschoff, at Marburg in Germany. As Keith explained, with Tawara's account to guide him, he was able to demonstrate unequivocally the atrioventricular bundle, and to trace it from the atrioventricular node to the ventricular conducting fascicles. It was no exaggeration for Keith to state that the discovery of the atrioventricular conduction axis by Tawara ushered in a new epoch for cardiac research.

Keith had not only been asked by Mackenzie to verify the findings of Tawara, but also by Wenckebach to explore the structure of the junction between the superior caval vein and the right atrium. Stimulated by the findings of Tawara, and helped by Martin Flack, a medical student at the time, he re-examined the cavoatrial junctions of several mammalian hearts. It was Flack who made the crucial breakthrough, finding a wonderful structure within the terminal groove, a structure that was found in all animals studied, including man. Thus was found the sinus node. Controversy then shifted to the internodal atrial myocardium, but Aschoff and Monckeberg, at an important meeting held in Erlangen, Germany, in 1910, established the criteria for anatomic recognition of histologically specialised tissues that retain their relevance even today. They pointed out that the nodes were histologically discrete, and could be traced through serial sections, whilst tracts shared both these features, also being insulated from the adjacent areas of working myocardium. Using these criteria, we can now recognise the structures identified by Kent as remnants of an extensive array of specialised tissue in the developing heart which become sequestrated within the atrial vestibules in the postnatal heart. All the works of these giants of the past, therefore, retain their relevance for our current understanding of the anatomic disposition of the conduction tissues of the heart.

His Jr W (1893). Die Tätigkeit des embryonalen Herzens und deren Bedeutung für die Lehre von Herzbewegung beim Erwachsenen. Arbeit aus der Medizin Klinik zu Leipzig 14.

Tawara S (2000). The conduction system of the mammalian heart. An anatomico-histological study of the atrioventricular bundle and the Purkinje fibers. Imperial College Press, London.

Kent A.F.S. (1913). The structure of the cardiac tissues at the auricular-ventricular junction. J Physiol 47, xvii-xviii.

Keith A, Flack M (1907). The form and nature of the muscular connections between the primary divisions of the vertebrate heart. J Anat Physiol 41:172-189.

Stannius HF (1852). Zwei Reihen physiologischer Versuche. Arch Anat Physiol Med 85-100.

His Jr W (1893). Die Tätigkeit des embryonalen Herzens und deren Bedeutung für die Lehre von Herzbewegung beim Erwachsenen. Arbeit aus der Medizin Klinik zu Leipzig 14.

Aschoff L (1910). Referat uber die Herzstorungen in ihren Beziehungen zu den Spezifischen Muskelsystem des Herzens. Verh Dtsch Pathol Ges 14: 3-35.

Mönckeberg JG (1910). Beitrage zur normalen und pathologischen Anatomie des Herzens. Verh Dtsch Pathol Ges 14: 64-71.

Janse MJ, Anderson RH (1974). Specialized internodal atrial pathways - fact or fiction? Eur J Cardiol 2: 117-136.

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SA3

Early recordings of ionic currents underlying pacemaking

H.F. Brown

University of Oxford (formerly), Gairloch, UK

A brief account will be given of investigations into pacemaking carried out in Oxford 30 years ago. At this time voltage clamp studies had to use multicellular preparations. These presented challenges and gave some unexpected results.

Brown HF, Giles W & Noble SJ (1977) J Physiol **271** 783-816

Brown H, DiFrancesco D & Noble S (1979) J Exp Biol **81** 175-204

Brown H & DiFrancesco D (1980) J Physiol **308** 331-351

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SA4

The sinus node revisited

T. Opthof

Department of Medical Physiology, University Medical Center, Utrecht, Center for Heart Failure Research, Experimental Cardiology Group, Amsterdam, Netherlands

History

The sinus node was discovered by Arthur Keith and his student Martin Flack in 1906, when they were working on a series of cardiac preparations from several species[1]. In fact the sinus node was first observed in the mole's heart and the famous Keith and Flack paper was published one year later in a journal that no longer exists[2]. Because the sinus node is the '*primum movens*', but also the '*ultimum moriens*', its discovery did not definitely settle the debate on the question whether or not automaticity is an intrinsic feature of the heart, as appears from the famous review of Eyster and Meek, published as the first paper of the first issue of *Physiological Reviews* in 1921[3].

Automaticity yes, oscillation no

Today we know that the sinus node displays automaticity, although none of its components has an oscillatory behaviour. In his monograph *The Music of Life* Denis Noble[4] describes how difficult it was for him as a student to get access to what was a powerful computer at that time for modelling work on heart rhythm. "Mr. Noble, where is the oscillator in your equations. What is it that you expect to drive the rhythm?"[4] was the question that remained unanswered. The answer is that the sinus node is not an oscillator. It is primarily the absence of the powerful inward rectifier current (I_{K1}) that allows the sinus node to be spontaneously active. In working cardiac muscle the resting membrane potential is almost identical to the equilibrium potential for K^+, because the conductance of I_{K1} is predominant during diastole.

Automaticity and membrane currents

The sinus node has no resting membrane potential. Still, full blockade of the L-type Ca^{2+} current ($I_{Ca\text{-}L}$) or the rapid delayed rectifier current (I_{Kr}) causes quiescence at about -30 mV at least in single sinus nodal myocytes[5,6]. The large difference between the maximum diastolic potential (-65 mV) and this 'resting potential' creates a physiological condition in which membrane currents, which as stated above lack oscillatory behaviour themselves, are - and remain - out of phase and keep 'each other going'. In the absence of I_{Kr}, the membrane cannot reach the negative potential required for activation of $I_{Ca\text{-}L}$ and I_f ("the pacemaker current"). In the absence of $I_{Ca\text{-}L}$, there is no action potential and no activation of I_{Kr}. Ironically, absence of I_f does not abolish pacemaking, but the current is still important for tuning of heart rate by the autonomic nervous system.

Reductionism: sinus node cells are no *pars pro toto*

In an era of DNA-mania[4] and preponderance of reductionism it is important to emphasize that isolated sinus node myocytes lack the feature of regularity[7]. An isolated sinus node or right atrium beats at almost perfectly constant cycle length. *In situ*, physiological influences create heart rate variability which is, however, not an intrinsic feature of the sinus node. The fact that an inhibitor of I_{Kr} will silence isolated sinus node cells and will also silence strips of sinus node tissue, if detached from the right atrium[8], exemplifies that sinus node cells behave differently when isolated, when connected to each other (like in an isolated sinus node) or in the intact heart (when the sinus node is connected with the right atrium). This is not restricted to sinus node cells. It is caused by electrotonic interaction and it is observed in the ventricles as well, where action potentials are substantially shorter in intact tissue than in isolated myocytes[9]. It should be realized that computer models are based on data obtained in single cells and that the membrane currents involved are *not* necessarily identical in the intact organ.

Functional inhomogeneity

The sinus node displays "functional inhomogeneity". Its basic elements have each a (different) intrinsic cycle length and a (different) responsiveness to ions, temperature, mechanoelectrical feedback, drugs and last but not least (neuro)hormones. Thus, changes in one of these parameters will induce pacemaker shifts within the nodal area[10]. Because the sinus node cells are connected with each other by connexins, there is one prevailing cycle length in the whole nodal area. Changes in cycle length, e.g. after an increase in norepinephrine, may result from a shortening in cycle length of a pacemaker area that was not determining the cycle length of the whole sinus node before the change in autonomic tone. The chronotropic response of the intact sinus node cannot be deduced from the chronotropic responses of its constituent cells. For changes directed at acceleration of heart rate, nodal areas with high responsiveness to the agent involved determine heart rate. For changes directed at deceleration of heart rate (as with vagal stimulation) areas with low responsiveness determine heart rate. Functional inhomogeneity is an important concept for the understanding of heart rate variability and "accentuated antagonism" of the autonomic nervous system.

Keith A. The sino-auricular node: a historical note. Br Heart J 1942; 4: 77-79.

Keith A, Flack M. The form and nature of the muscular connection between the primary divisions of the vertebrate heart. J Anat Physiol 1907; 41: 172-189.

Eyster JAE, Meek WJ. The origin and conduction of the heart beat. Physiol Rev 1921; 1: 1-43.

Noble D. The Music of Life. Oxford University Press, 2006.

Verheijck EE, van Ginneken ACG, Wilders R, Bouman LN. Contribution of L-type Ca2+ current to electrical activity in sinoatrial nodal myocytes of rabbits. Am J Physiol 1999; 276: H1064-H1077.

Verheijck EE, van Ginneken ACG, Bourier J, Bouman LN. Effects of delayed rectifier current blockade by E-4031 on impulse generation in single sinoatrial nodal myocytes of the rabbit. Circ Res 1995; 76: 607-615.

Opthof T, VanGinneken ACG, Bouman LN, Jongsma HJ. The intrinsic cycle length in small pieces isolated from the rabbit sinoatrial node. J Mol Cell Cardiol 1987; 19: 923-934.

Verheijck EE, Wilders R, Bouman LN. Atrio-sinus interaction demonstrated by blockade of the rapid delayed rectifier current. Circulation 2002; 105: 880-885.

Conrath CE, Opthof T. Ventricular repolarization. An overview of (patho)physiology, sympathetic effects, and genetic aspects. Prog Biophys Mol Biol 2006; 92: 263-307.

Boyett MR, Honjo H, Kodama I. The sinoatrial node, a heterogeneous pacemaker structure. Cardiovasc Res 2000; 47: 658-687.

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SA5

Funny channels: relevance to pacemaking and importance as a new drug target

D. DiFrancesco

Dept Biomolecular Sciences & Biotechnology, University of Milano, Milano, Italy

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 patiens 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.

Brown HF, DiFrancesco D & Noble SJ (1979) Nature 280, 235-236.

DiFrancesco D (1993) Ann. Rev. Physiol. 55, 455-472.

-arbuti A, Baruscotti M & DiFrancesco D (2007) J. Cardiovasc. Electrophysiol. 18, 1-6.

Bucchi et al. (2007) J. Mol. Cell. Cardiol. (in press).

Milanesi R et al. (2006) New Engl. J. Med. 354, 151-157.

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SA6

I_f modulation in Clinical Perspective

M.R. Rosen

Pharmacology, Columbia University, New York, NY, USA

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_f, 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.

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SA7

I_f in heart failure

A. Mugelli and E. Cerbai

Center of Molecular Medicine (C.I.M.M.B.A.), University of Florence, Florence, Italy

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 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.

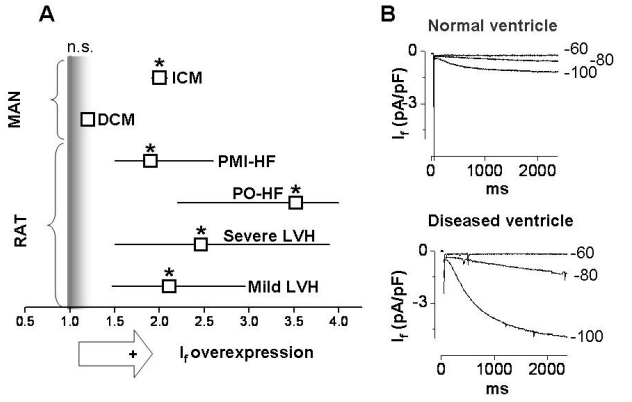

Figure 1. Ventricular I_f expression is increased in cardiomyopathies.

Cerbai,E., Barbieri,M. & Mugelli,A. (1996) Occurrence and properties of the hyperpolarization-activated current I_f in ventricular myocytes from normotensive and hypertensive rats during aging. Circulation, 94, 1674-1681.

Cerbai,E., Pino,R., Porciatti,F., Sani,G., Toscano,M., Maccherini,M., Giunti,G. & Mugelli,A. (1997) Characterization of the hyperpolarization-activated current, I_f, in ventricular myocytes from human failing heart. Circulation, 95, 568-571.

Cerbai,E., Pino,R., Sartiani,L. & Mugelli,A. (1999) Influence of postnatal-development on I_f occurrence and properties in neonatal rat ventricular myocytes. Cardiovasc.Res., 42, 416-423.

Cerbai,E. & Mugelli,A. (2006) I_f in non-pacemaker cells: Role and pharmacological implications. Pharmacological Research, 53, 416-423.

Sartiani,L., Bettiol,E., Stillitano,F., Mugelli,A., Cerbai,E. & Jaconi,M.E. (2007) Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: a molecular and electrophysiological approach. Stem Cells, 25:1136-44.

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SA8

An account of a ten year investigation into expression and distribution of ion channels in the mammalian sinus node

H. Dobrzynski, J.O. Tellez, I.D. Greener, H. Musa, N.J. Chandler, J.F. Yanni, E.S. H Abd Allah and M.R. Boyett

Cardiovascular & Endocrine Sciences, University of Manchester, Manchester, UK

In the healthy mammalian heart, the heart beat originates in the sinus node (SN). This specialised tissue of the heart is located at the junction of the right atrium with the superior caval vein and has unique structural and functional properties. Histologically, we have shown that in many mammalian species (mouse, rat, guinea-pig, rabbit, ferret and human) the SN tissue is embedded in a large network of connective tissue and contains cardiac cells that are smaller than working myocardial cells and not uniformly arranged. We have shown that this tissue can be delineated from its surrounding myocardium by some negative and positive immunohistochemical markers: ANP (atrial natriuretic peptide) and Cx43 (major cardiac gap junction channel protein) are negative markers whereas Cx45 (another gap junction channel protein) and NF-M (cytoskeletal protein) are positive markers. Over the last 10 years, using predominantly immunohistochemistry, but also Western blotting, quantitative PCR (qPCR) and in situ hybridisation on many mammalian species including human we have been studying expression and distribution of ion channel isoforms in the SN. We have shown that as compared to working myocardium: (1) HCN4 (the major isoform responsible for the pacemaker current, If) is more abundant in the rat and human SN; (2) Kir2.1 (responsible for the inward rectifier K+ current, IK,1) is less abundant in the mouse and human SN; (3) Nav1.5 (responsible for the cardiac Na+ current, INa) is less abundant in the rat and human SN; (4) Cav1.3 (responsible for the L-type Ca2+ current, IL,Ca) is more abundant in the rat and rabbit SN; (5) Kv1.4 and Kv4.3 (responsible for the transient K+ current, Ito) is present in the rabbit and human SN respectively; (6) Kv1.5 (responsible for the ultra-rapid delayed rectifier K+ current, IK,ur) is present in the rat and guinea-pig SN and (7) Kir3.1 and Kir3.4 (responsible for the acetylcholine-activated K+ current, IK,ACh) are present in the rat and human SN. In addition, we have shown that major Ca2+ handling proteins are differently expressed in e.g., the rabbit SN as compared to working myocardium; in particular RYR2 is less abundant in nodal cells. The unique ion channel expression profile of the SN can explain the unique electrophysiology of the SN.

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SA9

Multimodal biophotonic imaging of supraventricular pacemaker and conduction system

I. Efimov, V.V. Fedorov, W.J. Hucker, C. Ambrosi, C.M. Ripplinger, K.V. Foyil, M. McCain, M. Hemphill, J. Laughner, A. Voloshina and R. Schuessler

Biomedical Engineering, Washington University, St. Louis, MO, USA

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 cavas 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 10um 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 leading pacemaker on a beat-to-beat basis. Area of migration is variable from preparation to preparation and includes the entire intercaval region and the triangle of Koch. However, the isolated triangle of Koch shows remarkable lack of migration of the leading pacemaker that is located in the inferior AV nodal extension that also serves as the slow pathway input into the AV node. Thus, in contrast to the SA nodal region, the AV nodal pacemaker responds to both adrenergic and cholinergic stimulation without anatomic migration. Anatomic 3D reconstruction of this region in the human offers a venous pathway for novel therapeutic approaches.

Cholinergic stimulation of the FBZ that is located between the SA node and the septum provides a functional substrate for reentry that drives atrial flutter or atrial fibrillation in the canine and rabbit. Perfusion with acetylcholine (1-10uM) allowed induction of reentry around the SA node and FBZ in 15 rabbit and 5 canine preparations.

Multimodal biophotonic imaging of the rabbit and human AV junction revealed that AV conduction may bypass the compact AV node under certain physiological conditions. The lower nodal bundle expressing a high density of Cx43 exists below the compact AV node. Our data suggest that slow and fast pathway excitation leads to different patterns of engagement of the compact AV node and the bundle of His. Fast pathway excitation leads into the compact AV node followed by superior His excitation. Slow pathway excitation leads into the lower nodal bundle and inferior His excitation. Differing AV delays and distinct His electrogram morphologies confirm different patterns of excitation. Slow pathway stimulation allows AV conduction without AV delay, which suggests a bypass of the compact AV node via the lower nodal bundle.

APCS is an anatomically and functionally vast structure that includes tissues within the entire intercaval region of the right atrium and the triangle of Koch. Control of heart rate and AV delay is achieved via precise modulation of the APCS and cannot be understood within purely reductionist paradigm. Multimodal biophotonic imaging allows comprehensive investigation of control mechanisms of the heart rate and AV delay.

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SA10

AVN function: one node two pathways

J. Billette and M. Lavallée

Physiology, Université de Montréal, Montreal, QC, Canada

The rate-dependent properties of the AV node account for the filtering of atrial impulses during supraventricular tachyarrhythmias. These properties may generate important beat-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, fast and dual 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.

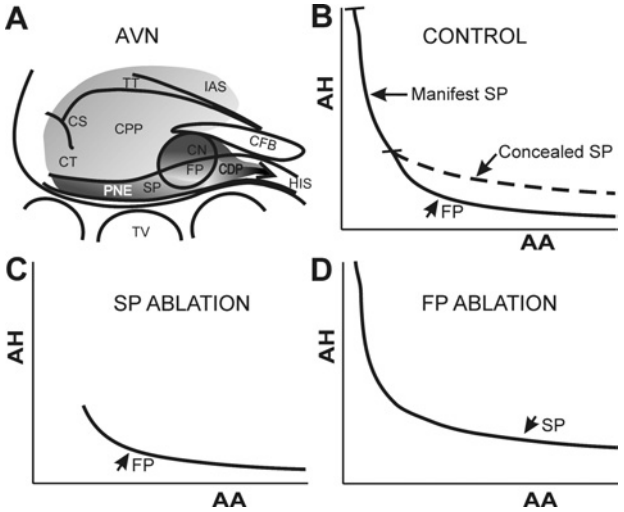


Figure 1: Schematic representation of dual AV nodal pathways. A, AV node structures and landmarks. B, dual pathway conduction curve (normal AV node). C, FP conduction curve after posterior extension ablation. D, SP conduction curve after FP ablation. FP, fast pathway. SP, slow pathway. CPP, common proximal pathway. CDP, common distal pathway. AH, atrial-His interval. AA, atrial cycle length.

Supported by Canadian Institutes of Health Research and Quebec Heart and Stroke Foundation.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA11

A 'model' of the atrioventricular node

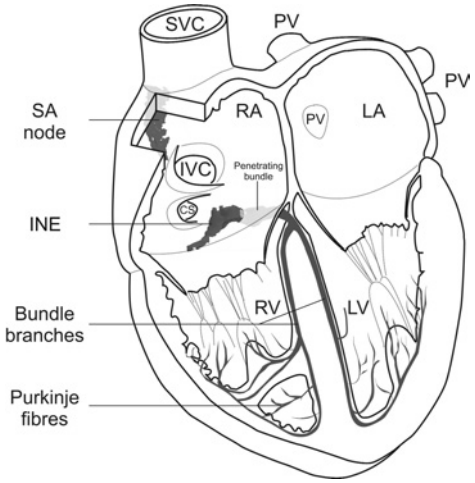
M.R. Boyett[1], I.D. Greener[1], J. Li[1], S. Inada[1], J.O. Tellez[1], R. Billeter[2], H. Zhang[1], J.C. Hancox[3], I.R. Efimov[4] and H. Dobrzynski[1]

[1]Cardiovasculoar and Endocrine Science, Manchester University, Manchester, UK, [2]Nottingham university, Nottingham, UK, [3]Bristol university, Bristol, UK and [4]Washington University, St. Louis, MO, USA

Based on electrophysiology, histology, quantitative PCR, in situ hybridisation and computer modelling, we have constructed a 'model' of the rabbit atrioventricular node: a tract of neurofilament- (NF-) positive nodal tissue – the 'penetrating bundle' – plunges through the fibrous layer insulating the atria from the ventricles and emerges in the ventricles as the His bundle (Fig. 1). There are two inputs into the penetrating bundle: slow and fast pathways. The slow pathway is most likely the 'inferior nodal extension' (INE), a ~5 mm tract of small dispersed NF-positive nodal tissue. At its start it is connected to atrial tissue and at its end it is continuous with the penetrating bundle, but in the middle it may be insulated by a vein. Just like the sinus node and different to the working myocardium, the INE lacks expression of Cx43 and $Na_v1.5$ and expresses HCN4 and $Ca_v1.3$; the result is expected to be a slowly conducting 'Ca^{2+}-dependent' action potential. We suggest that the INE is made up of N cells (cells, identified using electrophysiology, with these characteristics). Paradoxically, the penetrating bundle (including the 'compact

node'), although NF-positive, expresses some Cx43 and $Na_v1.5$ 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 $Na_v1.5$ 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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SA12

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

M.E. Mangoni

Physiology, Institute of Functional Genomics, CNRS UMR5203, Montpellier, Hérault, France

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-/-)(4) 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 bardycardia, strong SAN dysrhythmia and display sporadic II-degree AV blocks (4). Atrial fibrillation and flutter were also observed in Cav1.3-/- mice. SAN dysrhythmia, but not bradycardia could be compensated by crossing Cav1.3-/- animals with mice lacking the cardiac IKAch (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.

Hagiwara, N.,Irisawa, H.,Kameyama, M. (1988). Contribution of two types of calcium currents to the pacemaker potentials of rabbit sino-atrial node cells. J Physiol 395:233-53

Mangoni, M. E. and J. Nargeot (2001). Properties of the hyperpolarization-activated current (I(f)) in isolated mouse sino-atrial cells. Cardiovasc Res 52(1): 51-64.

Mangoni, M. E., A. Traboulsie, A. L. Leoni, B. Couette, L. Marger, K. Le Quang, E. et al. (2006). Bradycardia and slowing of the atrioventricular conduction in mice lacking CaV3.1/alpha1G T-type calcium channels. Circ Res 98(11): 1422-30.

Mangoni, M. E., B. Couette, E. Bourinet, J. Platzer, D. Reimer, J. Striessnig and J. Nargeot (2003). Functional role of L-type Cav1.3 Ca2+ channels in cardiac pacemaker activity. Proc Natl Acad Sci U S A 100(9): 5543-8.

Wickman, K.,Nemec, J.,Gendler, S. J.,Clapham, D. E. (1998). Abnormal heart rate regulation in GIRK4 knockout mice. Neuron 20:103-14

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SA13

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

S.K. Maier

Medizinische Klinik und Poliklinik I, Universität Würzburg, Würzburg, Germany

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 bradyarrhythmias and tachyarrhythmias, and inappropriate responses of heart rate during exercise or stress.

Besides disease-associated reasons, one reason for developing idiopathic sick 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 I_f. 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 I_f. Knockout of the HCN2 gene results also in a reduction of I_f 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 'electric 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.

Dobrzynski H., Boyett M.R. & Anderson R.H. (2007). Circulation 115, 1921-1932.

Wilde A.A.M. & Bezzina C.R. (2005). Heart 91, 1352-1358.

Mangrum J.M. & DiMarco J.P. (2000). New England Journal of Medicine 342, 702-709.

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SA14

Tandem biological and electronic pacemaking

R.B. Robinson[1], P.R. Brink[2], I.S. Cohen[2] and M.R. Rosen[1]

[1]Pharmacology, Columbia University, New York, NY, USA and [2]Physiology and Biophysics, Stony Brook University, New York, NY, USA

Many laboratories are pursuing the use of gene and cell therapies to treat bradyarrhythmias, 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, I_f, 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 initial 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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SA15

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

D.A. Terrar

Pharmacology, University of Oxford, Oxford, UK

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 β-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 (CaMKII) 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 β-adrenoceptor stimulation. Evidence for the presence of Ca^{2+}-stimulated adenylyl 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 CaMKII 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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SA16

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

T.M. Vinogradova, A.E. Lyashkov, Y. Li, S. Sirenko, A. Younes and E.G. Lakatta

NIA, NIH, Baltimore, MD, USA

Rabbit sinoatrial node cells (SANC) possess localized, submembrane 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 SERCA2 labeling 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 μmol/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 Fluo-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 μmol/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 μmol/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 β-AR agonist isoproterenol (33%, 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 confine 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.

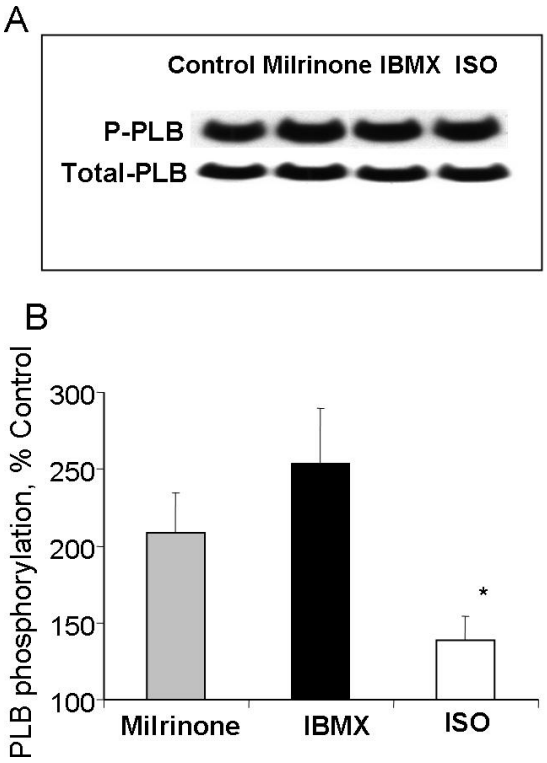

Figure 1. A, representative Western blot of the basal level of PLB phosphorylation at serine16, and total PLB in SANC prior to and following milrinone (50 μmol/L), IBMX (100 μmol/L) or β-AR stimulation (1 μmol/L, ISO). B, relative values of phosphorylated PLB normalized to total PLB (n=8).

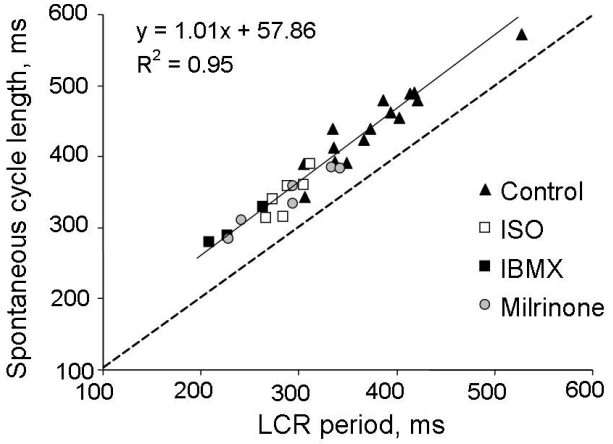

Figure 2. The effects of PDE inhibition (IBMX, 100 μmol/L or milrinone, 50 μmol/L) or β-AR stimulation (isoproterenol, 0.1 μmol/L) to alter the spontaneous cycle length are linked to their ability to control the LCR period.

Bogdanov KY et al. (2001). Circ Res; 88,1254-1258.

Lyaskov AE et al. (2007). Circ Res 100:1723-1731.

Vinogradova TM et al. (2004). Circ Res 94:802-809.

Vinogradova TM et al. (2006). Circ Res; 98,505-514.

Maurice DH et al. (2003). Mol Pharmacol; 64,533-46.

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SA17

Heart failure in the sinoatrial node

E. Verheijck, M. Van Borren, A. Verkerk, J. Zegers and J. Ravesloot

Physiology, AMC-UvA, Amsterdam, Netherlands

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 bradyarrythmic 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 internodal 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 arrthymia 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+}_i) has been recognized as an additional mechanism through which beta-adrenergic stimulation exerts its positive chronotropic action. Beta-adrenergic stimulation increases Ca^{2+}_i 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+}_i transients and Ca^{2+} releases during diastolic depolarisation in pacemaker cells. Moreover, the role of Ca^{2+}_i 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.

Janse MJ. Electrophysiological changes in heart failure and their relationship to arrhythmogenesis. Cardiovasc Res. 2004, 61:208–17.

Kannel WB, Ho K, and Thom T. Changing epidemiological features of cardiac failure. Br Heart J 1994, 72: S3-9

Jose AD, Collison D. The normal range and determinants of the intrinsic heart rate in man. Cardiovasc Res. 970;4:160–167

Sanders P, Kistler PM, Morton JB, Spence SJ, and Kalman JM. Remodeling of sinus node function in patients with congestive heart failure: reduction in sinus node reserve. Circulation 2004; 110: 897-903.

Opthof T. The normal range and determinants of the intrinsic heart rate in man. Cardiovasc Res 2000; 45: 177-184, 2000

Witte K, Hub K, Swiatek J, et al. Experimental heart failure in rats:effects on cardiovascular circadian rhythms and on myocardial beta-adrenergic signaling. Cardiovasc Res. 2000;47:350–358.

Zicha S, FernÃndez-Velasco M, Lonardo G, L'Heureux N, Nattel S. Sinus node dysfunction and hyperpolarization-activated (HCN) channel subunit remodeling in a canine heart failure model. Cardiovasc Res. 2005 Jun 1;66(3):430-2

Verkerk AO, Wilders R, Coronel R, Ravesloot JH, Verheijck EE. Ionic remodeling of sinoatrial node cells by heart failure. Circulation. 2003 Aug 12;108(6):760-6. Epub 2003 Jul 28.

Kjaer A, Appel J, Hildebrandt P, Petersen CL. Basal and exerciseinduced neuroendocrine activation in patients with heart failure and in normal subjects. Eur J Heart Fail 2004;6:29–39

Maltsev VA, Vinogradova TM, Lakatta EG. The emergence of a general theory of the initiation and strength of the heartbeat. J. Pharmacol. Sci. 2006;100(5):338-69.

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Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA18

Development of the cardiac conduction system and the nodes

A.F. Moorman and V.M. Christoffels

Heart Failure Research Centre, Academic Medical Centre, Amsterdam, Netherlands

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 Hnc4 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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SA19

Ionic mechanisms underlying the pacemaker potential in the sinoatrial node cell

A. Noma

Physiology, School of Medicine, Kyoto University, Kyoto, Kyoto, Japan

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 deactivation 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, Ist 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 ICa,L
Activation of Ist
    Activation of If
    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 incorpolating 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, Jongsma, & 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.

Demir SS, Clark JW, Murphey CR, and Giles WR. A mathematical model of a rabbit sinoatrial node cell. Am J Physiol Cell Physiol 266: C832-C852, 1994.

Noble D and Noble SJ. A model of sino-atrial node electrical activity based on a modification of the DiFrancesco-Noble (1984) equations. Proceeding of Royal Society of London Proc R Soc Lond B Biol Sci 222: 295-304, 1984.

Sarai N., Matsuoka S., Kuratomi S., Ono K. and Noma A. Role of individual ionic current systems in the SA node hypothesized y a model study. Jap. J. Physiol. 53,125-134. 2003

Wilders R, Jongsma HJ, and Van Ginneken ACG. Pacemaker activity of the rabbit sinoatrial node. A comparison of mathematical models. Biophys J 60: 1202-1216, 1991.

Yanagihara K, Noma A, and Irisawa H. Reconstruction of sino-atrial node pacemaker potential based on the voltage clamp experiments. Jpn J Physiol (Lond) 30: 841-857, 1980.

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SA20

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

M. Lei[1], Y. Ke[2], T.P. Collins[3], S. Rakovic[3], P.A. Mattick[3], M. Yamasaki[3], M.S. Brodie[2], D.A. Terrar[3] and J.R. Solaro[2]

[1]School of Medicine, University of Manchester, Manchester, UK, [2]Department of Physiology and Biophysics and Center for Cardiovascular Research, University of Illinois at Chicago, Chicago, IL, USA and [3]Department of Pharmacology, University of Oxford, Oxford, UK

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. Overexpressing 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-I). 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.

Ke Y, Lei M, Collins TP, Rakovic S, Mattick PAD, Yamasaki M, Brodie MS, Terrar DA & Solaro RJ. (2007). Regulation of L-Type Calcium Channel and Delayed Rectifier Potassium Channel Activity by p21-Activated Kinase-1 in Guinea Pig Sinoatrial Node Pacemaker Cells. (2007) Circ Res 100, 1317-1327.

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SA21

Ageing to arrhythmias: Conundrums of the ageing heart

S.A. Jones

Institute of Membrane & Systems Biology, University of Leeds, Leeds, UK

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 showed that the rodent intrinsic heart rate decreased with age to 70-80% of the rate observed in the young animal, paralleling a comparable decrease reported in humans by Opthof (2000). Protein analysis by immunofluorescence and Western blot showed a decline in expression of the Cav1.2 channel within the SA node during ageing, functionally exhibited as an increased sensitivity of the SA node to nifedipine (a Cav1.2 channel blocker), resulting in an age-dependent decrease in the EC50 for complete abolition of SA node activity (Jones et al. 2007). Analysis of protein expression also demonstrated a substantial decline in Cx43 expression, accompanied by remodelling of expression of the remaining Cx43 protein in the aged SA node tissue. This was associated with an alteration in the conduction properties within the node and postulated to contribute to pacemaker degeneration with age (Jones et al. 2004). The decline of Cx43 protein within the SA node correlated with increasing levels of activated JNK in the heart implicating JNK in the regulation of Cx43 protein and SA node remodelling during progressive ageing (n=5 per age group; ANOVA $p<0.01$; Linear regression $y=1.0305X-0.3484$; $R2=0.96$).

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.

Lakatta EG. (2002) Heart Fail Rev 7, 29-49.

Ross RA & Kenny RA. (2000) Age Ageing 29, 13-15.

Fleischmann KE et al. (2006) Heart Rhythm 3, 653-9.

Opthof T. (2000) Cardiovasc Res 45, 177-184

Jones SA et al. (2007) Circ 115, 1183-90

Jones SA et al. (2004) J Physiology 560, 429-437

Research was performed in collaboration with Dr. Matthew K. Lancaster.

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SA22

Fibroblasts and cardiac electrophysiology

P. Camelliti and P. Kohl

Physiology, Anatomy & Genetics, University of Oxford, Oxford, UK

Fibroblasts form one of the largest cell populations in the heart (Fig. 1), outnumbering cardiomyocytes in healthy myocardium by a factor of at least two [1]. The relative proportion of fibroblasts in the heart increases with age, and shows pronounced regional differences. Cardiac pacemaker and conduction tissue, for example, has a high connective tissue content even under physiological conditions, as highlighted by Arthur Keith and Martin Flack in 1906: '*Purkinje fibres […] are isolated from the rest of the musculature […] by well-developed connective-tissue sheaths*' [2]. Many pathological conditions cause further fibroblast proliferation and/or invasion, and give rise to either diffuse (fibrosis) or localized (scarring) elevation in connective tissue content.

As apparent from the quote above, and somewhat paradoxically given its name, connective tissue in the heart was and is usually assumed to form a barrier, i.e. to divide. This has also dominated the view on its role in cardiac electrophysiology, even though isolated fibroblasts and myocytes show abundant heterogeneous coupling *in vitro*, as has been seen from the very beginning of cardiac cell culture work [3].

Assessment of fibroblast-myocyte electrical interaction *in situ* is not as straightforward as it may seem, since i) cardiac fibroblasts have a very high membrane resistance (10^9-10^{10} Ohm), and ii) they form very long sheet-like processes in native tissue [4]. This means that i) it is nearly impossible to identify individual fibroblast-myocyte coupling in native tissue by microelectrophysiological means (as any myocyte-coupled fibroblast will simply follow the myocyte's trans-membrane potential changes, including mimicry of action potential waveforms), while isolation of intact fibroblast-myocyte pairs, or complete individual fibroblasts, is difficult (due to the spatial extent of fibroblasts, which will cause truncation during the process of tissue digestion, mincing, and agitation for cell release).

Alternative experimental approaches include i) the use of dyes to study heterogeneous cell coupling in intact tissue, or ii) the mapping of electrical propagation in cardiac scars. Both methods have provided clear evidence for heterogeneous fibroblast-myocyte coupling [5, 6]. The probable substrate for this interaction has been identified in native rabbit sino-atrial node as small gap junctions connecting the different cell population [5].

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 conductions – 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, refractoriness and electrical load (which are key determinants of arrhtythmogenic 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.

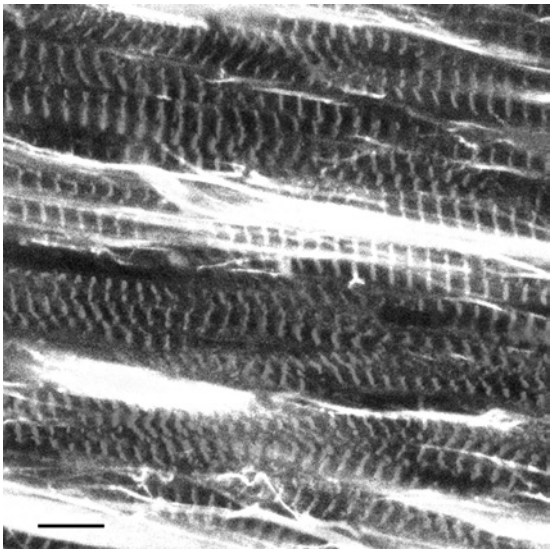

Figure 1: Confocal micrograph of rabbit right atrial myocardium, immunohistochemically labelled for cardiomyocytes (myomesin label, giving rise to striated cell appearance) and fibroblasts (vimentin, revealing brightly stained cell sheets between myocytes). Scale bar 10 μm.

Zak R. Cell proliferation during cardiac growth. *American Journal of Cardiology* 1973;**31**:211-219.

Keith A & Flack MW. The auriculo-ventricular bundle of the human heart. *The Lancet* 1906; August 11:359-364.

Goshima K, Tonomura Y. Synchronized beating of embryonic mouse myocardial cells mediated by FL cells in monolayer culture. *Experimental Cell Research* 1969;**56**:387-392.

Kohl P, Kamkin AG, Kiseleva IS, Noble D. Mechanosensitive fibroblasts in the sino-atrial node region of rat heart: interaction with cardiomyocytes and possible role. *Experimental Physiology* 1994;**79**:943-956.

Camelliti P, Green CR, LeGrice I, Kohl P. Fibroblast network in rabbit sino-atrial node: structural and functional identification of homo- and heterologous cell coupling. *Circulation Research* 2004;**94**:828-835.

Walker NL, Burton FL, Smith GL, Cobbe SM. Mapping of epicardial activation in a rabbit model of chronic myocardial infarction: response to atrial, endocardial and epicardial pacing. *Journal of Cardiovascular Electrophysiology* 2007; (in press).

Gaudesius G, Miragoli M, Thomas SP, Rohr S. Coupling of cardiac electrical activity over extended distances by fibroblasts of cardiac origin. *Circulation Research* 2003;**93**:421-428.

Jacquemet V. Pacemaker activity resulting from the coupling with nonexcitable cells. *Physical Review E* 2006;**74**:011908.1-011908.7.

Kizana E, Ginn SL, Smyth CM, Boyd A, Thomas SP, Allen DG, Ross DL, Alexander IE. Fibroblasts modulate cardiomyocyte excitability: implications for cardiac gene therapy. *Gene Therapy* 2006;**13**:1611-1615.

Porter KE, Turner NA, O'Regan DJ, Balmforth AJ, Ball SG. Simvastatin reduces human atrial myofibroblast proliferation independently of cholesterol lowering via inhibition of RhoA. *Cardiovascular Research* 2004;**61**:745-755.

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

U. Ravens

Pharmacology, TU Dresden, Dresden, Germany

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.

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

SA24

A Tale of Two Arrhythmias

M.J. Janse

Experimental Cardiology, Academic Medical Centre, Amsterdam, Netherlands

I will give a brief overview of the history of two arrhythmias in which the atrioventricular (AV) node is involved.

1. Atrioventricular reentrant tachycardia.

After describing circulating excitation in ring-like preparations of hearts of a variety of species, G.R.Mines (1) wrote in 1913: "I venture to suggest that a circulating excitation of this type may be responsible for some cases of paroxysmal tachycardia as observed clinically". One year later, he repeated this suggestion after reading Stanley Kent's description of a connection between the right atrium and the right ventricle (2): " Supposing that for some reason an impulse from the auricle reached the main A-V bundle but failed to reach this "right lateral" connection, it is possible then that the ventricle would excite the ventricular end of this right lateral connection, not finding it refractory as it normally would at such a time. The wave spreading up to the auricle might be expected to circulate around the path indicated "(3) This was written 16 years before Wolff, Parkinson and White described the clinical syndrome that now bears their name (4), 18 years before Holzmann and Scherf (5) ascribed the abnormal ECG in these patients to pre-excitation of the ventricles via an accessory AV bundle, 19 years before Wolferth and Wood (6) published the first diagrams showing the pathway for orthodromic and antidromic reentry, and 53 years before the first studies in patients employing intraoperative mapping and programmed stimulation during cardiac catheterization proved Mines, predictions to be correct (7). It is remarkable that none of these studies quoted Mines.

The era of surgical ablation of the accessory pathway started in 1967, and was initially hampered by not realizing the correct anatomy of the accessory pathway, which was quite different from what Kent had described (2). In 1944 Öhnell showed that the accessory bundle did not penetrate the fibrous annulus, but coursed in the epicardial fat surrounding the coronary arteries. After Sealy and colleagues in 1976 developed a "fish hook" to scrape through the epicardial fat, surgical treatment became successful. It also paved the way for the hugely successful catheter ablation.

2. Atrioventricular nodal reentrant tachycardia.

Mines (1) also was the first to describe AV nodal reentry, which he called a reciprocating rhythm. He postulated that the different fibres in the AV node " are ordinarily in physiologic continuity, yet it is conceivable that exceptionally, as after too rapid stimulation, different parts of the bundle should lose their intimate connection… A slight difference in the rate of recovery of two divisions of the A-V connection might determine that an extrasystole of the ventricle, provoked by a stimulus applied to the ventricle shortly after activity of the A-V connection, should spread up to the auricle by that part of the A-V connection having the quicker recovery process and not by the other part. In such a case, when the auricle became excited by this impulse, the other portion of the A-V connection would be ready to take up transmission again back to the ventricle. …the condition once established would tend to continue, unless upset by the interpolation of a premature systole" (1). It took more than half a century before upsetting AV nodal reentry by "premature systoles' was accomplished in patients and in isolated rabbit heart preparations. Both papers did quote Mines.

Although all authors working on AV nodal reentry agree that that the lower level of the junction of antegrade and retrograde pathways is above the level of the His bundle, controversy has existed regarding the question whether or not the atrium forms part of the reentrant circuit. The fact that it is possible, both by surgery and catheter ablation, to abolish AV nodal reentry by destroying tissue far away from the compact AV node whilst preserving AV conduction seems clear evidence that the atrium must be involved. However, in the canine heart the reentrant circuit during atrial and ventricular echo beats is confined to to the compact node and regions immediately adjacent to it, and atrial tissue is not involved. To quote Zipes, who borrowed the words that Churchill used to characterize Russia : "The AV node is a riddle wrapped in a mystery inside an enigma".

1. Mines GR (1913). On dynamic equilibrium of the heart. J Physiol 46: 349-382.

2. Kent AFS (1914). Observations on the auriculo-ventricular junction of the mammalian heart. Q J Exp Physiol 7: 193-195.

3. Mines GR (1914). On circulating excitations in heart muscle and their possible relation to tachycardia and fibrillation. Trans R Soc Can 4: 43-52.

4. Wolff L, Parkinson J, White PD (1930). Bundle-branch block with short P-R interval in healthy young patients prone to paroxysmal tachycardia. Am Heart J 5: 685-704.

5. Holzmann M, Scherf D (1932). Ueber Elektrokardiogrammen mit verkürzten Vorhof-Kammer Distanz und positiven P-Zacken. Z Klin Med 1932; 121: 404-423.

6. Wolferth CC, Wood FC (1933). The mechanism of production of short PR intervals and prolonged QRS complexes in patients with presumably undamaged hearts. Hypothesis of an accessory pathway of atrioventricular conduction (bundle of Kent). Am Heart J 8: 297-308.

7. Durrer D, Roos JR (1967). Epicardial excitation of the ventricles in a patient with a Wolff-Parkinson-White syndrome (type B). Circulation 35: 15-21.

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SA25

Moderate hypothermia facilitates termination of spiral wave reentry in the ventricle

H. Honjo, M. Harada, M. Yamazaki, Y.S. Ishiguro, K. Kamiya and I. Kodama

Research Institute of Environmental Medicine, Nagoya University, Nagoya, Japan

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 nornothermia 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

P. Molenaar

Medicine, University of Queensland, Chermside, QLD, Australia

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 β2-adrenoceptors or 100 nM CGP 20712A to block β1-adrenoceptors 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 otherhand 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).

Where applicable, the authors confirm that the experiments described here conform with The Physiological Society ethical requirements.

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The Purkinje cell 2007 style

P. Boyden[1], W. Dun[1], B. Stuyvers[3] and H.E. ter Keurs[2]

[1]Pharmacology, Columbia University, New york, NY, USA, [2]Medicine, University of Calgary, Alberta, AB, Canada and [3]Memorial University, St. Johns, NF, Canada

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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