Review Article

Biological pacemakers: Concepts and techniques

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ABSTRACT

The sinoatrial (SA) node is the dominant pacemaker of the heart which initiates the process of impulse generation in the cardiac tissue, thereby defining the rate and rhythm of cardiac contraction. The automaticity of the conduction cells in the SA node is due to ion channels which are inter-linked by molecular, histological and electrophysiological mechanisms causing spontaneous diastolic depolarization and generation of an impulse. The SA nodal action potentials are then transmitted to the ventricles by electrical coupling of the myocytes in different areas of the heart. Regulatory pathways overseeing cardiac impulse generation and conduction provide effective and safe pacing, and help maintain the rate according to the physiological demands of the individual's body. Failure of physiological pacing due to any pathology in the SA or atrioventricular node necessitates implantation of a permanent pacemaker. Implantable pacemakers, despite technological advances, are not without practical limitations including a defined battery life leading to lead and/or generator replacement at periodic intervals, vascular complications, occasional component failure, electronic interference from external/internal sources, e.g. myopotentials, electromechanical interference, etc., inadequate or incomplete physiological rate response to autonomic influences (devices have certain algorithms to address these issues) and most importantly the risk of infection. A biological pacemaker is therefore emerging as a promising technique to counter these challenges.

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INTRODUCTION

The sinoatrial (SA) node is the dominant pacemaker of the heart and contains specialized conduction cells capable of inherent automaticity. Closely linked intricate ion channels are responsible for initiating spontaneous diastolic depolarization and generation of a pacing impulse. Electrical coupling between myocytes of different areas of the heart helps propagation of action potentials generated in the SA node to the ventricles through the atrioventricular node, bundle branches and Purkinje fibre network. Normally, a stable balance between sympathetic and parasympathetic input maintains the heart rate in an organism. In rabbits, the dominant system is the sympathetic tone; whereas in canines and humans the parasympathetic tone is dominant.[1] The parasympathetic regulation involves modulation of cardiac currents by reducing cyclic adenosine monophosphate (cAMP) concentration as well as activation of IK, Ach.[2] Catecholamines activate the adrenergic receptor (AR), a G-protein coupled receptor that results in stimulation of stimulatory G-protein. This results in activation of adenylyl cyclase and generation of cAMP. Binding of cAMP to ion channels activates protein kinase A, which by phosphorylation influences the function of proteins. While AR-1 receptors only stimulate the G-proteins, AR-2 receptors stimulate as well as inhibit G-proteins.[3]

If AND HCN CHANNELS

A balance of inward and outward currents reflecting an interplay between the hyperpolarization-activated cation current, If, and the inward rectifier potassium current, IK1 initiates and maintains the normal cardiac rhythm. The If voltage-gated ion channels in the SA node are activated by hyperpolarization, exchange Na^+, K^+ and small amount of Ca^{2+} ions and contribute to the diastolic (phase 4) depolarization leading to the generation of an action potential. In the ventricular myocytes, the If current is masked by the large IK1, hence suppressing spontaneous pacemaker activity and leading to electrical quiescence. If is generated by hyperpolarization-activated, cyclic nucleotide-gated or HCN channels (HCN1, HCN2, HCN3 and HCN4), each having unique electrical and distribution patterns. The HCN4 is the dominant isoform in the SA node, while Purkinje fibres have equal amounts of HCN1 and HCN4 and in ventricles; HCN2 and HCN4 are present with no detectable levels of HCN1.

IK1 AND Kir2.1 CHANNELS

The inward rectifying potassium current, IK1, is nearly absent in the SA node while it is dominant in the ventricular myocytes, resulting in no diastolic depolarization in the latter cells. It is encoded by the Kir2.x (x=1, 2, 3, 4) gene family of which Kir2.1 is the predominant isoform in ventricular myocytes.[4]

Hence, SA node cells which depolarize spontaneously express high levels of If and low IK1 whereas electrically silent ventricular myocytes have an abundant IK1 and low If expression. Studies have shown that reducing IK1 conductance by 30%–40% and increasing If by three times can lead to the initiation of spontaneous pacemaker activity in electrically silent cells.

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PROBLEMS WITH CURRENT IMPLANTABLE PACEMAKERS

The most important limitation of current electronic pacemakers is device-related infection. Complete explantation of the permanent pacemaker is recommended in such cases, with provision of temporary (transthoracic or transvenous) pacing for pacemaker-dependent patients along with a 2–3 weeks' course of parenteral antibiotics. However, external pacing is not suitable for longer term, and the use of temporary transvenous pacing in such situations is often associated with recurrence of infection when a new permanent device is re-implanted.[5] The other limitations of permanent pacemakers include: (i) a definite battery life needed for lead and/or generator replacement periodically; (ii) risk of technical failure; (iii) vascular complications, for example, bleeding, haematoma, pneuomothorax; and (iv) issues related to patient growth in children often necessitating multiple revisions.

WHY BIOLOGICAL PACEMAKERS?

The most desirable feature of an ideal biological pacemaker is the ability to generate a stable and spontaneous pacing rate that is physiological, non-arrythmogenic and has optimal autonomic modulation. In addition, it offers the ability to be self-sustaining, term durability without any need of battery/lead/electrodes, hence obviating the need for redo or revision procedures and lack of inflammatory or infectious potential. Creating a biological pacemaker involves imparting a tissue with no or zero net current flow in diastole, a brief net inward current causing diastolic depolarization and generation of an action potential. This can be done by augmenting HCN channels or inhibiting Kir2.1 channels or a combination of both. The following strategies can be helpful.

Cell-based therapy using natural pacing cells (sinus node cells)

The SA node cells can pace adjoining quiescent atrial myocytes and transmit impulses through gap junctions. Foetal canine atrial myocytes and SA node cells have been transplanted into adult canine left ventricles (LV) with successful coupling between the host and donor myocytes.[6] Human atrial myocytes (containing SA node cells) have been injected into porcine LV with successful pacing and optimal autonomic responsiveness.[7] Ethical issues, lack of availability of foetal myocytes, problems related to identifying a critical mass of SA nodal cells that can form effective gap junctions and concerns of ectopy and possible arrhythmogenesis have limited the role of this strategy.

Cell-based therapy using stem cells

Human embryonic stem cells (hESCs) can be induced to develop into cardiac myocytes expressing HCN channels.[8,9] These myocytes have been shown to functionally couple with neonatal rat ventricular myocytes, and generate action potentials. The use of hESCs can, however, be associated with tumourigenesis, immunoreactivity and pro-arrhythmogenesis. Inducing fusion of ventricular myocytes with genetically engineered fibroblasts can help form heterokaryons with pacemaker-like activity. Heterokaryons obviate the need for gap junction coupling and have been shown to be stable for long durations, providing long-term biological pacing.[10–12] Adult human mesenchymal stem cells (MSCs) can deliver the *HCN2* gene to ventricle myocytes and generate spontaneous pacemaker activity in these cells.[13–15] Since MSCs can migrate from injection sites, it may lead to gradual time-dependent loss of pacing. Ongoing studies are focusing on utilizing lencapsulated MSCs or biomaterials to anchor the cells for site-directed delivery of HCN2 and SkM1 ion channels. Human-induced pluripotent stem cell-derived cardiomyocytes (iPSC) have also been used to generate pacemaker activity because of their ability to differentiate into functional cardiomyocytes.[16] Using iPS enables an autologous approach and reduces the chances of immune rejection. Although previous studies have shown that hESC-derived cardiomyocytes (hESC-CMs) and iPSC-derived cardiomyocytes (iPSC-CMs) are capable of firing spontaneously and responding to autonomic signals, Mandel *et al*. characterized their dynamic firing pattern and their stability features over a 15-day follow-up period and showed that spontaneous electric activity of these cells exhibited beat rate variability behaviour comparable to that of human SA with similar power-law behaviour.[17] The ability to generate sinoatrial-compatible spontaneous cardiomyocytes from keratinocyte-derived iPSC therefore eliminated the need for immunosuppres-sion, making these cells an attractive cell source of biological pacemakers.

Receptor-based therapy. Over-expression or upregulation of exogenous β2-adrenergic receptors is known to increase heart rate by 40% in right atria of mice and by approximately 20% in porcine atria.[18,19] However, partial uptake, unreliable durability of pacing and potential for arrhythmogenesis limit its long-term use. The intrinsic potential of ventricular cells for generation of rhythm can be used by raising cAMP levels through over-expression of adenylyl cyclase. In porcine models of AV-block, over-expression of adenylate cyclase type VI is known to induce pacing capability in quiescent ventricular cells.[20]

Gene-based therapy. Manipulation of *HCN* and *Kir2.1* gene expression using single or double gene constructs (reducing outward current, Ik1, increasing inward current, If or co-expression of both). Inhibition of IK1can be achieved by over-expression of negative mutant of Kir2.1 (Kir2.1AAA) in guinea pig ventricular myocytes has shown to induce pacing in these cells.[21] Spontaneous pacing activity has been noted in cells if IK1 was suppressed below 0.4 pA/pF. However, the Kir2.1AAA mutant can reduce membrane stability and create pro-arrhythmogenic electrical heterogeneity.[22,23] This can be offset by co-expression of human ether-a-go-go-related gene (HERG) which encodes for the rapid delayed rectifier K^+ current.[24] Enhancement of If to initiate spontaneous pacing can be done by using the *HCN* gene (isoform HCN2) to over-express the inward depolarizing current (If), even in the presence of a large background IK1 in the atrial or ventricular myocytes.[25,26] Creating HCN1 mutant (three deleted residues: HCN1-ÄÄÄ) which has activation kinetics similar to that of the native SA node has been also shown to generate pacing activity in porcine models.[27] Manipulation of the microRNA pathway was attempted to over-express HCN2 and HCN4. Gene-specific oligodeoxynucleotides have been used to mask microRNA binding sites on HCN2/HCN4 mRNA resulting in over-expression of HCN2 by 70% and HCN4 by 45% in cultured ventricular cardiac cells.[28]

The limitations of HCN-based biological pacing include heteromultimerization with endogenous HCN channels, immunogenicity of the mutated channels and lack of complete autonomic sensitivity. Adenoviral dual gene constructs that lead to IK1 inhibition as well as If potentiation have also been used successfully to initiate pacing activity in experimental models.[29,30] The *HCN2/SkM1* adenovirus dual gene constructs are noted to have no dependence on electronic backup pacing along with better autonomic responsiveness.

CONCLUSIONS

Implantable pacemakers have major limitations prompting the need for developing biological pacemakers. The key criteria for a

biological pacemaker are the ability to produce a depolarizing current at the end of repolarization, cessation of current after depolarization is over, and adequate and physiological electrical coupling with adjoining host cells by gap junctions. An ideal biological pacemaker allows replication of physiological cardiac conduction with optimal autonomic regulation. Biological pacemakers can act as 'bridge to device'; alternatives in patients who need a pacemaker but have contraindications to indwelling hardware or in pacemaker-dependent patients with infections. While initial strategies targeted over-expression of β2-adrenergic receptors, subsequent studies have focused on manipulating ion channels (enhancing If and reducing Ik1) to generate pacemaker activity in electrically silent cells.

More enhanced understanding of mechanisms that control the gene expression and coupling between the donor and host cells in the future is likely to make the use of biological pacemaker a clinical reality. Irrespective of the cell type used to create biological pacemakers, the ideal cell template is the intrinsic SA nodal cell. In addition to regular automaticity, the pacemaker cells should also exhibit beat rate variability similar to that of the SA nodal cells.

A new development in this field is the use of human iPSC-derived cardiomyocytes (iPSC-CMs) as a potential biological pacemaker. Using this technique, human somatic cells such as hair follicles or skin cells can be programmed to become pluripotent stem cells that get differentiated into cardiomyocytes. This approach is immunocompatible since it facilitates the creation of pacemaker cells from a patient's own tissue and hence is capable of surmounting various ethical issues associated with the use of hESC-derived cardiomyocytes (hESC-CMs).

More studies in large animal models are required to assess the ideal gene construct, optimal site of implant, mode of cell delivery and stability of biological pacing.

Conflicts of interest. None.

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