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Biological Pacemakers – A Review

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Abstract

Slow heart rates, due to sinus node disease or atrioventricular conduction block, are a significant problem for many patients. Currently, these patients are treated with electronic pacemakers, which provide effective therapy, but are also associated with many problems. Use of biological pacemakers is an attractive solution to these problems. Approaches for the creation of such pacemakers include either the injection of cells that have pacemaker activity (cell-based approach) or modification of cells in the heart to induce pacemaker activity by delivering genes (gene-based approach). This article reviews the progress in the development of biological pacemakers.

INTRODUCTION

Decades of innovation has produced and refined the modern electronic pacemaker, which has revolutionized the treatment of patients with slow heart rates. Currently, we stand on the cusp of the next major revolution in this field, which is the development of biological pacemakers that will replace electronic components with biological components with various attendant benefits. This article will review the progress so far in this field.

Normal Pacemaker Activity of the Heart

The heart is a muscular organ, yet unlike skeletal muscles, which are activated by nerves, it is activated intrinsically. Many of the cardiac muscle cells have the ability to generate an action potential after a certain period of time, a property called automaticity. In cells possessing this property, the membrane potential during diastole does not stay constant, yet gradually increases, eventually reaching a threshold when the cells are excited. While many cells in the heart show automaticity and can thus behave as pacemakers, a group of specialized cells in the right atrium form the Sino-Atrial Node (SAN), which is the dominant pacemaker of the heart by virtue of its higher rate. Excitation spreads to adjacent atrial muscle and then the entire atrium from this group of pacemaker cells. Electrical connections between cells, which facilitate this spread of depolarization between cells, are mediated by gap junctions. The depolarization wave-front is then conducted by atrioventricular node to activate the ventricles. Activation of the ventricles produces the final pumping function of the heart. When there is failure of pacemaker activity or failure of impulse conduction in the atrioventricular node, a slow heart rate ensues. This could result in inadequate blood flow through the body, which causes various symptoms and sometimes even death.

Electronic Pacemakers

Patients are currently treated by implantation of a pacemaker, which is a device capable of delivering timed electrical impulses to the heart muscle. The pulse generator, which is the electronic component generating the pulses, is placed under the skin while a lead connected to the pulse generator is placed in contact with the heart muscle by being passed through a vein. While pacemakers are a very effective form of treatment and many advances have made the treatment safe with few complications, there still remains various problems. The pacemaker is a foreign substance with a risk of infection, which is likely to occur at the time of implant, yet rarely happens many years later. The pacemaker batteries have a finite longevity, about 10 years, and surgery is needed to replace it at the end of this period. Therefore, a younger person may require multiple surgeries in his or her lifetime for battery depletion. The leads generally last longer, but they may also develop problems with wear and tear and may require replacement. Small children, who need pacing, are especially exposed to many problems. Rapid growth may result in the

need for leads to be changed. A lifetime of pacing will lead to multiple surgeries. In addition to these, the electronic nature of the devices results in occasional electromagnetic interference and inability to perform Magnetic Resonance Imaging (MRI) scans. Biological pacemakers are a possible solution to these problems, providing a new functioning pacemaker, which is entirely biological.

Principles of the Natural Biological Pacemaker

Since biological pacemakers attempt to replicate the heart's natural pacemaker, understanding how the natural pacemaker functions is important. Like the other working myocytes, SAN cells have various ion channels in their membrane, which result in variations of the membrane potential with each cycle, known as the action potential. Traditionally, the action potential is described with 5 phases (0 to 4) with 4 representing diastole, the period of rest between cycles when the membrane potential stays constant at a negative voltage is called the resting membrane potential (Fig 1).

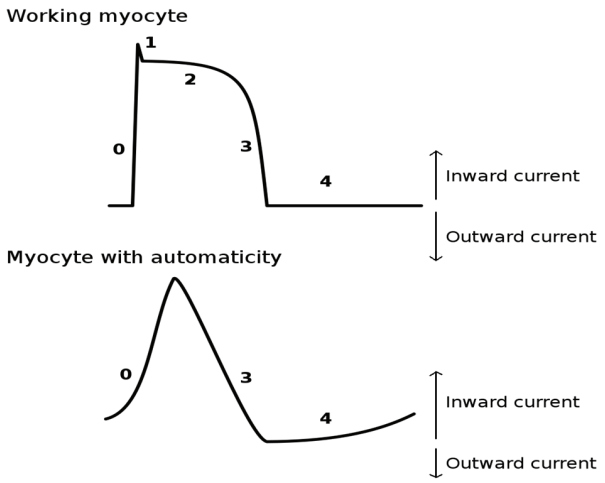

Figure 1: Schematic of Action Potential

Schematic diagram showing action potential in a working myocyte without automaticity (top panel) and in a myocyte with automaticity. In Phase 4, the membrane potential depends on a balance between inward and outward currents, which increase or decrease the membrane potential, respectively. In cells without automaticity, these currents are balanced and the membrane potential remains constant. In cells with automaticity, increased inward currents and/or decreased outward currents result in gradual increase in membrane potentials.

Pacemaker cells are different from other cells in exhibiting a gradual depolarization that is change in membrane potential to a less negative value, during phase 4. Once the membrane potential reaches a certain critical voltage, it initiates phase 0, which is depolarization. This is the basis of the property of automaticity. The genesis of this diastolic depolarization has been a subject of interest and many hypotheses have been placed forth [1-4]. Diastolic depolarization is due to an increase of the resting membrane potential during phase 4, which may be the result of a decrease in an outward current or an increase in an inward current (Fig 1). The dominant hypothesis at present is that diastolic depolarization is due to an inward current, which is activated at the hyperpolarized voltage during phase 4. Since activation of an inward current at lower (hyperpolarized) voltage is contrary to the usual behaviour, it was called a "funny" current and is hence known as If [5, 6]. As the outward repolarizing K+ current decays, this If current results in a net inward movement of positive ions resulting in gradual depolarization. Once a threshold potential is reached, a new action potential is initiated. The If current has also been shown to increase in the presence of adrenaline [7], explaining how adrenergic stimulation may increase the pacemaker rate. The channels mediating the funny current are called Hyperpolarization activated Cyclic Nucleotide (HCN) gated ion channels. Four HCN alpha subunits have been found in mammals, of these, three (HCN1, HCN2 and HCN4) are expressed in various regions of the heart. All are expressed to a high degree in the SAN, with HCN1 and HCN4 being specifically found in the SAN [8, 9]. Conversely, a decrease in inward rectifying potassium current (Ik1), which is an outward current, can also generate diastolic depolarization. This is mediated by ion flow across the Kir channels, with the Kir2.1 isoform being dominant in the ventricles. The Ik1 is very low in the sinus node and allows the inward if current to have significant effect on membrane potential. On the other hand, in ventricular myocytes, a strong Ik1 current and weak or absent If current leads to inhibition of automaticity. An alternative hypothesis for the generation of cardiac pacemaker activity implicates oscillations in intracellular calcium levels. According to this theory, Ca2+ release by Ryanodine receptors during late diastole activates a Na-Ca exchanger, which enhances diastolic depolarization and hence results in the next action potential [10-12]. *In this model, adrenergic stimulation increases heart rate by recruiting additional Ryanodine receptors for calcium release [13, 14].* From the sinus node, the action potential has to propagate to the rest of the atrium. This occurs via connections between cells, known as gap junctions. These junctions are formed by two hemi-channels on the cell membrane of two adjacent myocytes [15]. These hemi-channels are called connexons and are each comprised of six connexins (Cx). Connexin types identified in the heart include Cx40, Cx43, and Cx45. These are differentially distributed in various regions of the heart, yet Cx43 is the dominant form in the SA nodal region. Desmosomes are another form of intercellular connections, which do not mediate conduction of ions, yet instead form adhesive bonds. They may have a role in signalling in addition to providing mechanical strength alone [16]. Recently, it has also been recognised that desmosomes may play an important role in normal pacemaker activity [17]. The significance of this in the light of biological pacemakers is yet unknown.

Biological Pacemakers – An introduction

The term "biological pacemaker" refers to cellular components that could replace the natural pacemaker cells to provide electrical stimulation after being implanted or injected in specific regions of the heart. A functioning biological pacemaker would provide many advantages over electronic pacemakers as discussed previously. Biological pacemakers would not need to be replaced once they are integrated and function. They will not be associated with problems due to leads, such as infection and thrombosis or mechanical prob-

lems, like lead fracture. A biological pacemaker is also expected to be responsive to autonomic stimuli whereby heart rate increases with physiological factors that produce sympathetic stimulation. This would be physiologically superior to the rate response functions provided in current pacemakers. From the preceding discussion, it is clear that for a functional biological pacemaker, a group of cells with the property of automaticity is required. This corresponds to a net inward current in phase 4, mediated by modulation of inward or outward current. Cells with the requisite characteristics may be injected in the heart (cell-based approach) or existing cells in the heart may be induced to become pacemakers by modifying their genes (gene-based approach). These cells should be connected to the rest of the myocardium by gap junctions for the activation to propagate. In addition, it is desirable for the automaticity to show autonomic responsiveness so that the heart rate would appropriately increase with exercise.

Cell-Based Approach

The first successful use of biological pacemakers was reported from Germany. These investigators used transplanted fetal canine atrial muscle cells [18]. Implanted in the adult canine heart, the cells were demonstrated to survive, integrate and drive an escape rhythm after the creation of atrioventricular block. Similar results were also shown using human fetal atrial myocytes [19]. This approach has been limited by the need to obtain the cells from atria of aborted foetuses, which limits the quantity that could be attained but also raises significant ethical concerns. Embryonic stem cells are derived from an early stage pre-implantation embryo. These cells are pluripotent, showing the ability to differentiate to any cell type. Initial attempts were directed at producing functional SAN cells from these stem cells. It was shown that it is possible to use CD 166 expression to select SAN precursor cells [20]. However, attempts to autologously graft the SAN or inject the myocytes failed to provide sustained biological pacemaker activity [21, 22]. Instead, human Embryonic Stem Cells (hESC) derived myocytes were studied as the source of cells for creating biological pacemakers. Various approaches have tried to differentiate pluripotent stem cells into spontaneously beating myocytes [23]. Excitable hESC-derived cardiomyocytes were shown to be capable of functional integration in vivo [24], forming gap junctions and providing a sustainable biological pacemaker in 50% of pigs with AV block [25] When implanted in the guinea pig ventricle, spontaneous action potential generation was documented [26]. However, ethical issues are involved because the cells are obtained from early human embryos. In addition, immuno-reactivity with potential for graft-versus-host disease is present. Also, a major concern has been the ability of these cells to produce tumours [27]. These concerns are bypassed by using adult human mesenchymal stem cells or human induced Pluripotent Stem Cells (iPSC).

Human Mesenchymal Stem Cells (hMSC) are multipotent stromal cells, which can be isolated from various sources, such as the umbilical cord, amniotic fluid, dental pulp, adipose tissue, etc. Brown adipose tissue has also been found to be a good source of mesenchymal stem cells [22]. They are a suitable candidate, being relatively immunopriveleged and expressing two cardiac gap junction proteins, connexins 40 and 43 [28]. Once these gap junctions establish electrical connection with host cells, an inward current can propagate to depolarize them. However, these are electrically quiescent and do not have the property of automaticity. Potapova et al. used human Mesenchymal Stem Cells (hMSC), transfected with a pacemaker gene (mHCN2) by nucleoporation as a suitcase to deliver an If-like current to canine ventricular myocytes [29]. The spontaneous beating rate increased from 93 to 161 bpm, showing effective pacemaker activity. In a canine model of induced atrioventricular block, Plotnikov et al. delivered hMSC-HCN2 cells to the left ventricle [30]. The biological pacemaker was shown to be functional during a 6-week follow up period with efficiency correlated with number of cells injected. Two other experimental studies confirmed [31, 32] these findings. As with other approaches using stem cells, concerns have been raised about the risk of infection, neoplasia, and further differentiation over time [33, 34]. Another approach is to not use stem cells, and instead reprogram adult cells to become undifferentiated cells. This raises the premise of autologous regenerative therapy without the need for immunosuppression. Takahashi et al. reprogrammed adult mice fibroblasts by introducing 4 transcription factors into their genome [35]. This technique has been extended to human cells [36, 37]. Mandel et al. used the patient's own hair to generate cardiomyocytes from iPSC and showed that these cells were spontaneously active with intrinsic heart rate variability and ability to respond to isoprenaline and carbamylcholine [38].

Gene-Based Approaches

An alternative to cell-based techniques discussed already is to use genes delivered, usually using a viral vector, for the myocytes to induce the development of pacemaker activity. This was first described by John Hopkins University in 2002 [39]. An initial approach was overexpression of beta adrenergic receptors [40]. This produced an increase in sinus rate by 20%. However, this approach was limited because a functional pacemaker is still required to respond to the adrenergic stimulation. This approach also increases the risk of tachyarrhythmias and hence was abandoned. The two main approaches are the use of genes that inhibit Kir2.1 or genes that express HCN channels, which mediate the dominant inward and outward currents, respectively. The Kir2 gene encodes the Ik1 potassium current. Expression of a dominant negative subunit reduced the outward current. This converted a quiescent ventricular preparation with no automaticity to one with spontaneous depolarization [41]. However, this approach had to be abandoned because it led to the appearance of a prolonged QT similar to the phenotype of Andersen Tawil syndrome with an increased risk of dangerous ventricular arrhythmias. Overexpression of genes encoding HCN channels that mediate the inward depolarizing current If [42] is more attractive because increasing If current would not have a significant effect on action potential duration. Furthermore, HCN2 is specifically preferred because of its intermediate action kinetics and strong response to cyclic AMP producing a response to sympathetic stimulation [43].

Qu et al. reported increased spontaneous beating rate in neonatal rat ventricular myocytes infected with adenoviral HCN2. Increased escape rate was demonstrated by injection of adenovirus carrying HCN2 in canine left atrium [44] and in the canine left bundle branch [34]. In a canine model of

atrioventricular block, implanted with a pacemaker set at a rate of 45 beats per minute, requirement of pacing was reduced after injection of the wild-type HCN2 [45]. Another group showed reduction of pacing requirement in a porcine model of sick sinus syndrome with a pacemaker set at 60 beats per minute. HCN1 was overexpressed in the guinea pig ventricle, after which sinus node was ablated using radiofrequency energy. Large If current with activation kinetics mimicking sinus node was detected [46]. A different approach has been utilized employing Adenylate Cyclase type VI gene delivered with adenoviruses. When injected in the left ventricle in pigs, this produced an escape rhythm denoting pacemaker activity [47].

Co-expression of two genes together, typically HCN with another gene has increased the efficacy of the biological pacemaker. Combination of HCN2 with adenylate cyclase gene [48] or with Kir gene [49] has been examined and resulted in improved outcomes. A more favourable response was obtained by expressing HCN2 with SkM1. Expressing this combination in adenoviruses and injection in the left bundle branch in dogs indicated an adequate resting rate and good response to autonomic stimulation [50]. Most studies using gene transfer use Adenoviruses as vectors. Adenovirus-based protein expression, however, is not expected to last beyond four weeks. Lentivirus as a vector can result in long-lasting changes, but has been associated with a risk of neoplasia. It is likely that in the future, improved understanding of host-vector interactions may allow safe use of lentivirus vectors [51]. Tbx is a gene that is important for SAN specification during early development. Kapoor et al. used Tbx 18 loaded adenovirus to reprogram rodent ventricular myocytes in spontaneously active cells similar to sino-atrial nodal cells [52]. Persistence of pacemaker activity was shown up to 6 to 8 weeks after gene transfer. This is at present a promising possibility for gene therapy using transient viral vectors to become a permanent pacemaker therapy, yet large animal studies are required.

Current Status and Concerns

As it stands now, the technology of biological pacemakers is still in its very early stages. Only a few animal experiments have been performed and there is no data on the long term stability of pacing function and no studies have been done in humans. The therapeutic approaches available at present also offer single site pacing only and this does not provide atrioventricular synchrony. There are also concerns regarding the risk of infection and neoplasia. Despite these limitations, with technological improvements, biological pacemakers appear to be destined to replace electronic pacemakers in the future for the treatment of patients with slow heart rates.

The development of biological pacemakers is a relatively new field, yet progress has been rapid and a few different viable approaches have been developed. Most of these approaches still have limitations in different forms that have precluded transfer to clinical practice, yet methods are being developed to overcome these limitations. Although electronic pacemakers are currently available for the treatment of patients with slow heart rates, there is no doubt that the emergence of effective biological pacemakers will provide a better solution for many of these patients. Seeing the rate of progress in this field, one cannot feel anything but certain that this day is not far off.

CONFLICT OF INTEREST

Neither of the authors had any conflicts of interest in relation to this manuscript.

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