Cardiac Pacing
From Biological to Electronic . . . to Biological?

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Abstract—The prevention and treatment of life-threatening bradyarrhythmias have been revolutionized in the last half century by electronic pacemakers. Because this represents a palliative therapy, attempts have begun to effect a cure with the novel tools of gene and cell therapy. Over time, the strategies used have coalesced to focus on achieving a stable and autonomously responsive cardiac rhythm in a setting that ultimately would require no implanted hardware. In this report, we review the history of the disease process being treated, approaches now in progress, and the demands that must be met if biological therapies are to be successful. (Circ Arrhythmia Electrophysiol. 2008;1:54-61.)

Key Words: atrioventricular block ■ cell therapy of bradyarrhythmias ■ gene therapy ■ gene therapy of bradyarrhythmias ■ hyperpolarization-activated, cyclic nucleotide-gated channels

More than 2 millennia ago, the Chinese physician Pien Ch’i10 reported what appear to have been variations on heart block. He wrote that if 1 heartbeat of 50 were dropped, the patient was normal and had no diseased organs. If the number increased to 1 in 40, then life expectancy was limited to 4 years, and a single organ was diseased (which organ was not specified). As the number of dropped beats increased further, life expectancy diminished and diseased organs increased progressively, until, at 1 dropped beat of 3 to 4, life expectancy was approximately a week.

That was about as good a description of a devastating and incurable arrhythmia as existed until 1761, when Morgagni described what later would be referred to as Adams-Stokes attacks. As quoted by Osler, Morgagni wrote: “My fellow citizen, Annastasio Poggi, a grave and worthy priest . . . was in his sixty-eighth year . . . moderately fat and of a florid complexion, when he was first seized with the epilepsy, which left behind it the greatest slowness of pulse, and in like manner a coldness of the body. But this coldness of the body was overcome within seven hours, nor did it return any more, though the disorder often returned; but the slowness of the pulse still remained.”2

The separate reports of Adams3 (1827) and Stokes4 (1846) further detailed the symptoms and presenting signs of patients. They did far less well when it came to therapy. In part, the perplexity over treatment stemmed from the fact that the origin and the conduction of the human heartbeat were then mysteries. The structure and beginning appreciation of the function of the sinus node as cardiac pacemaker awaited the work, respectively, of Keith and Flack5 in 1907 and Lewis and associates6 in 1910. The beginnings of comprehension of atrioventricular conduction were the work of His7 in 1893 and Tawara8 in 1906. Only Purkinje’s9 work on the false tendons in 1845 may have been available to Stokes, and this was an anatomic treatment of the ventricular conducting system.

If one does not know the origin of the heartbeat, where and how does one start to treat its dysfunction? In fact, the beginnings of electronic pacemaking anteceded the understanding of the mammalian sinus node and atrioventricular conduction. It appears that the first to stimulate mammalian hearts in a systematic fashion was McWilliam10 in 1889. He used induction shocks, the rate of which was regulated by a metronome at 60 to 70 bpm to generate pacemaker function in the ventricles of cats whose sinus rhythm had been depressed via vagal stimulation. He also emphasized the potential therapeutic value of the procedure for treating human subjects and described the type and placement of electrodes to be used.

Advancement was slow. In Australia in 1928, Lidwell11 reported using an electric power source to deliver shocks through a needle plunged into the heart to revive “a stillborn infant.” Hyman12 in Philadelphia used a hand-cranked generator to provide electric shocks in 1932 and coined the term cardiac pacemaker. The modern era in pacing was ushered in independently by Hopps et al13 and by Zoll,14 whose devices were used in the 1950s to deliver transcutaneous shocks. Weirich et al15 in 1957 brought back the intramyocardial needle electrode to treat postsurgical complete heart block, and in that same year Bakken16 fabricated the first portable external pacemaker.

Temporary transvenous pacing was reported in 195917 and permitted practitioners to dispense with thoracotomies for
intramyocardial electrode placement. Then, the early 1960s saw fixed-rate pacing via the transvenous route with the use of permanently implanted power packs. The units were bulky, their batteries had limited functionality, and their shocks too often competed with existing idioventricular rhythms. They were life saving, however. In the 1960s and 1970s, batteries, electrodes, and software all were improved. The resulting pacemakers incorporated a demand mode, could sense competing beats, and could reset to avoid arrhythmogenesis.

We now have nearly achieved a pacemaker nirvana: Atrioventricular (AV) sequential pacing permits the normal staging of atrial and ventricular contractions in patients with normal sinus nodes but with AV block. The coronary sinus permits access to the epicardial veins for biventricular pacing used in the setting of heart failure. Cardioverter-defibrillators that sense, shock, and pace when needed are life saving for patients with potentially lethal tachycardias. As if this were not enough, units now being used can vary heart rate to adjust to the exercise level of a patient, and leadless electrodes are being developed that may permit stimulation of the heart without incorporating a catheter.

Do the units have problems? Yes. These include (1) unresponsiveness to the autonomic nervous system (to the demands of exercise and emotion), although variable rate units are a potential answer to this need; (2) a requirement for monitoring and maintenance, including at times battery and/or electrode replacement; (3) infection or interference from other devices; (4) risk of heart failure evolving with right ventricular apical placement of electrodes (now being met by use of other electrode sites); and (5) problems in adapting equipment to the growth and development of pediatric patients.

Despite these issues, it is highly likely that with continued effort, electronic pacemakers will be made even better. As a result of all this, we can say that the electronic revolution has been astounding, and its future is bright. Why in the world would anyone want to build a biological pacemaker?

**Back to the Future: Why Build a Biological Pacemaker?**

An anecdote: A chief of medicine whom one of us (M.R.R.) knew had an outstanding peritoneal dialysis unit in his department. A generous donor offered to bankroll a hemodialysis unit, at the time a new and largely untested therapy. The chief declined the offer, stating that peritoneal dialysis was state of the art and was excellent in its outcome, and this new therapy would likely never work (as well) in any case.

We mention this story because the mistake it highlights is the Panglossian—accepting that which is available for one has the imagination, the beginning tools, the will, and the possibility that maybe, just maybe, one can recreate the normal function of the heart using entirely biological materials. Would that confer an advantage over electronics? Arguably, the answer is affirmative if we could construct a unit that does the following: (1) competes effectively in head-to-head competition with electronic pacemakers yet requires no battery, no electrodes, and no replacement; (2) not only creates a lifelong, stable physiological rhythm but also is responsive to the autonomic nervous system and as such to the demands of exercise and emotional status; (3) is implantable at a site that optimizes the pathway of cardiac (or at least ventricular) activation, such that contraction and cardiac output are similarly optimized; and (4) does not induce proarrhythmia or carry concerns about inflammation, infection, or neoplasia. In other words, the ultimate aim is to cure rather than merely palliate. And it is by curing, perhaps only by curing, that the biological unit can achieve superiority. In this case, there would be no electrodes, no batteries, no power packs, no software. Would any of us seriously argue the superiority of reclaiming normal function over the use of a prosthesis, regardless of how sophisticated that prosthesis might be?

Although this is a tall order and may take years to realize, if one wishes to advance beyond an outstanding therapy such as electronic pacing, one needs to aim for perfection. Such perfection need not be achieved at the outset: At such time as biological units become useful adjuncts to electronics, this would suffice for beginning their testing. As shall be summarized in the discussion of tandem pacing, this level of achievement may not be that far away.

**Building a Biological Pacemaker: What is the Template? What Are the Outcomes?**

The template for most of us working in this area is the sinoatrial node. The goal is not necessarily replicating the node structurally and functionally but replicating enough of its function to provide an effective mime. As reviewed in Figure 1, the sinus node depolarizes spontaneously during phase 4 until membrane potential reaches threshold and an action potential is generated. This event occurs rhythmically and regularly for the lifetime of most individuals. The slope of phase 4 depolarization results from a balance between inward and outward ion currents. The initial inward current, activated on hyperpolarization of the membrane at the end of repolarization, is referred to as $I_{K1}$. Other currents that are inward and contribute to phase 4 depolarization are the T- and L-type Ca currents (the latter largely responsible for the upstroke of the sinus node action potential). Providing outward current during the same time frame are the not yet completely decayed potassium currents $I_{K0}$ and $I_{Ks}$ and a weak $I_{K1}$. In addition, the Na–Ca exchanger operates during phase 4 to further influence the rate of depolarization of the membrane.

Modulating the ion channel contribution to pacemaker function is the autonomic nervous system. Catecholamine binding to $\beta$-adrenergic receptors operates via a Gs protein–linked pathway to increase cyclic adenosine monophosphate...
Strategies that have been used to generate biological pacemaker function are based on gene and/or cell therapy and are as follows.

**Gene Therapy**

Gene therapies either overexpress a gene that will increase pacemaker rate in cardiac myocytes (by increasing inward current) or knock out the function of a gene that would otherwise decrease pacemaker rate (and decrease outward current). These approaches modify the electric signals generated by cardiac myocytes and/or specialized conducting cells to transform them into dominant pacemakers. The modified current, in concert with the endogenous “package” of ionic currents in a myocyte or specialized conducting fiber, creates a pacemaker in a cell that previously had little or no pacemaker function.

The first biological pacemaker created was based on overexpression of $\beta_2$-adrenergic receptors in porcine atrium. The result was an increase in basal atrial rate as well as responsiveness to catecholamine. While providing proof of concept that biological pacing might be workable, this approach also carried the potential proarrhythmic complications of excess catecholamine action on the heart. The first attempt to manipulate ion channel activity was performed by injecting a dominant negative construct of the repolarizing K current $I_K$ into guinea pig ventricle. This resulted in an increased automatic rate in situ, as well as increased phase 4 depolarization in isolated myocytes. However, reducing repolarizing K current also leads to prolonged repolarization, as was reported subsequently by the same authors. This outcome is potentially arrhythmogenic.

The other ion channel approach that has been explored and largely adopted by most groups now working in the area is to increase or to create a variant on the inward current $I_f$. Because this approach now holds center stage, some additional explanation of the ion channels that generate pacemaker function. The channel also closes rapidly on depolarization of excess catecholamine action on the heart. The first attempt to manipulate ion channel activity was performed by injecting a dominant negative construct of the repolarizing K current $I_K$ into guinea pig ventricle. This resulted in an increased automatic rate in situ, as well as increased phase 4 depolarization in isolated myocytes. However, reducing repolarizing K current also leads to prolonged repolarization, as was reported subsequently by the same authors. This outcome is potentially arrhythmogenic.

All HCN isoforms have cAMP binding sites near the carboxy terminus of the channel. The binding of cAMP to these sites shifts activation positively, resulting in a faster rate of phase 4 depolarization. This interaction with cAMP is responsible for the autonomic control of the aforementioned pacemaker current.

To date, the use of HCN2 and HCN4 to fabricate biological pacemakers has been reported, with most work having been done with HCN2. In addition, mutant and chimeric channels have been reported. Two viral vectors have been used in the gene therapy experiments. The first is adenovirus, which is

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**Figure 1.** A. Representation of sinoatrial node action potential (control, solid lines) and some of the ion channels and exchangeers contributing to it. $I_f$ is activated on hyperpolarization, providing inward current initiating phase 4. T-type and L-type Ca currents are initiated toward the end of phase 4. The latter also contributes the major current to the action potential upstroke. Na-Ca exchange current also influences the phase 4 slope. Delayed rectifier current $I_K$ is responsible for repolarization. The acceleratory effects of norepinephrine (NE) are represented as broken lines. A prominent increase in phase 4 reflects the actions of norepinephrine on $I_f$. B. An HCN (hyperpolarization-activated, cyclic nucleotide-gated) pacemaker channel. There are 6 transmembrane spanning domains. When the channel is in the open position, Na is the major ion transmitted. cAMP binding sites are present near the carboxy terminus. $\beta_2$-Adrenergic (H1-AR) and M2-muscarinic receptors, which provide norepinephrine and acetylcholine (ACh) binding sites, respectively, are shown. Via G-protein coupling, these receptors regulate adenylyl cyclase (AC) activity, which in turn regulates intracellular cAMP levels, determining the availability of the second messenger for binding and channel modulation. Adapted from Biel et al. with permission from Elsevier Limited. Copyright 2002 Elsevier Limited.
expressed episomally only and is useful for proof-of-concept studies lasting 2 to 4 weeks at most. The second is lentivirus, which is incorporated in cells genomically; although it is slow to express, it should be permanent in expression for the life of the cell.

HCN2 in an adenoviral construct expresses well in atrium and ventricle. When injected into the left atrium, it generated pacemaker function that was sensitive to both catecholamines and vagal stimulation, increasing and decreasing the rate, respectively. When injected into the left ventricular specialized conducting system of dogs whose sinus rate and/or AV conduction was suppressed by vagal stimulation or in dogs having AV block secondary to radiofrequency ablation of the AV nodal region, the result was an escape pacemaker that fired at 50 to 55 bpm in the resting state. This was responsive to catecholamine infusion such that rate could increase to 90 bpm. No attempt was made to infuse acetylcholine, but preliminary experiments on heart rate variability suggested both a vagal and a sympathetic component to the function of this biological unit.

Attempts to influence the rate of biological pacemakers by creating mutant or chimeric channels have met only modest success. The E324A mutation of HCN2 resulted in increased catecholamine sensitivity in biophysical studies but showed no physiologically relevant difference in effect from the wild-type channel, HCN2. An HCN2 chimera incorporating the pore-forming unit of HCN1 (which has more favorable activation kinetics than HCN2) and the amino and carboxy termini of HCN2 (which has more robust cAMP responsiveness than HCN1) resulted in an “overshoot” of function, in that ventricular tachycardias well over 220 bpm in rate were the result. A mutation of a K channel to give it the inward current–carrying capability of HCN2 has been reported, but this has no cAMP-binding capacity and, with this, no potential for autonomic responsiveness. Therefore, although it is clear that mutating wild-type channels and creating chimeras and artificial channels result in phenotypic expression, the right combination of characteristics to provide a basal heart rate of 60 to 70 bpm and autonomic responsiveness capable of reaching 150 bpm has not yet been reported.

Cell Therapy
Because viral vectors for gene therapy can carry various risks (with the greatest concern being the transmission of viral-based illnesses), other means of creating biological pacemakers have been sought. One approach uses human embryonic stem cells (hESCs) that are placed in cell culture and “forced” into a pacemaker lineage. The other approach uses adult human mesenchymal stem cells (hMSCs) or other cell types as platforms for delivering HCN genes.

Human Embryonic Stem Cells
hESCs coaxed into a pacemaker lineage have the advantage of not requiring the overexpression of pacemaker genes. Rather, they have a full complement of these. When injected...
into the ventricles of pigs in complete heart block, these hESCs have delivered acceptable pacemaker function for several months.\textsuperscript{38} The cells have coupled with the porcine myocytes such that the signal transfer from hESCs to the heart has been robust. One problem has been the need for immunosuppression of the animals (and this is a general problem with hESCs). Other issues have been the general concern that hESCs might eventually evolve into an inimical cell type (including the possibility of neoplasia) or even into a cardiac cell that does not persist in having good pacemaker function, such as a ventricular myocyte.

**Human Mesenchymal Stem Cells**

As opposed to hESCs, the multipotent hMSCs do not have the machinery to generate a cardiac action potential; the necessary ion channel components are not present. Present, however, is a robust population of connexins (Cx43 and Cx40) that are the protein building blocks of gap junctions that provide the low-resistance pathways for current to flow among cells.\textsuperscript{42} These properties led us to hypothesize that (1) hMSCs might couple electrically to one another and to myocytes (they do with great efficiency); (2) electroporation could be used to overexpress HCN2 in hMSCs (the cells loaded well, obviating the need for viral vectors); and (3) an hMSC would be hyperpolarized by any repolarizing myocyte to which it is electrically coupled. This hyperpolarization would generate inward current that initiates depolarization and an action potential in the myocyte. In that way, the 2 cells would constitute a single functional unit, with the hMSC providing the depolarizing stimulus and the myocyte the action potential (Figure 2).\textsuperscript{21,22}

hMSCs have been injected into the anterior left ventricular walls of dogs in complete heart block and have been studied through 6 weeks (Figure 3).\textsuperscript{40} Because there is evidence that hMSCs are immunoprivileged\textsuperscript{43,44} and are not necessarily rejected across species, these experiments were done without immunosuppression. The experiments were successful, showing pacemaker firing at $\approx 50$ to 60 bpm in these animals.

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**Figure 3.** A and B. Biological pacemaker function in canine heart in situ. Top to bottom, ECG leads I, II, III, AVR, AVL, and AVF. A shows a control that received a saline injection, and B shows an animal that received hMSCs loaded with HCN2 and green fluorescent protein (GFP). Left panels. Pacing from hMSC injection site showing ECG configuration. Center panels, Spontaneous (Spont.) rhythms about a week later. In saline, the QRS configuration is independent of impulses initiated at the injection site, and rate is low. In hMSCs+HCN2, the rhythm pace-maps to site of injection, suggesting that it is initiated at the injection site. Right panels, Last 2 beats of overdrive pacing (shaded) at 80 bpm followed by escape rhythm. Escape time is 4 seconds in saline and 1.3 seconds in hMSC+HCN2. Adapted from Potapova et al\textsuperscript{39} with permission of Lippincott Williams & Wilkins. Copyright 2004 American Heart Association. C, hMSCs 6 weeks after left ventricular anterior wall intramyocardial injection. Hematoxylin-eosin (H&E) identifies basophilic cells. These are CD44 positive, reflecting human origin, and GFP positive (via peroxidase stain). These findings indicate that they are, in fact, the cells we injected 6 weeks earlier. These hMSCs do not display labeling/binding of canine IgG to their surface, evidence against humoral rejection. CD3-positive T lymphocytes are rarely noted in association with clusters of hMSCs, evidence against cellular rejection. Staining is negative for activated caspase-3, evidence against apoptosis. Magnification $\times 400$. Reproduced from Plotnikov et al\textsuperscript{40} with permission of Lippincott Williams & Wilkins. Copyright 2007 American Heart Association.
Figure 4. Tandem pacing. A, A dog with an adenoviral HCN2 construct in the proximal left bundle branch and an electronic pacemaker with electrode implanted in right ventricular endocardial apex and in complete heart block induced by radiofrequency ablation. Note that the animal is being paced biologically; the electronic unit then intervenes, and the biological unit then takes over. B summarizes results from 7 dogs. Those animals receiving electronic pacing have >80% of beats initiated electronically. In contrast, those with tandem pacing see only 30% to 40% of beats as electronically initiated. C, Electroanatomic mapping of left ventricle in a dog with complete heart block, an electronic right ventricular apical endocardial pacemaker, and an HCN2 adenoviral construct administered into the proximal left bundle branch. Panels demonstrate 4 projections, showing early (red) through late (blue) activation. Left panels show an impulse activating left ventricular endocardium at several sites simultaneously, reflecting arrival of an impulse initiated in the left bundle branch system. Right panels show early activation of left ventricular septum via the electronic pacemaker. AP indicates anteroposterior; LAO, left anterior oblique; RL, right lateral; and PA, posteroanterior. Adapted from Bucchi et al33 with permission of Lippincott Williams & Wilkins. Copyright 2006 American Heart Association.

We and others have obtained sufficient evidence from both viral and stem cell approaches to state that proof of concept has been demonstrated. We also have evidence that trials can be designed that permit us to test biological versus electronic pacemakers in relative safety in patients who are protected from failure of the biological unit (see reservations below, however). The likely way to proceed clinically when ready (perhaps in a patient population with chronic atrial fibrillation and complete heart block) would be to implant both a biological pacemaker and an electronic demand pacemaker in the same individual. The design used here is referred to as tandem pacing.

We have tested this in dogs in complete heart block to which we delivered an adenoviral HCN2 construct (into the left bundle-branch system) and an electronic demand unit, the electrode of which was placed in the right ventricular endocardial apex (Figure 4).33 The biological pacemaker fired ~70% of the time and was catecholamine responsive. Moreover, when the biological unit slowed, the electronic unit took over seamlessly; similarly, the electronic unit sensed the biological unit well and discontinued its function when the biological function emerged.

Extrapolating this tandem system to the clinic, one can envision a situation in which the biological pacemaker provides the majority of beats to the heart, conserving the battery of the electronic unit, and is autonomically responsive, adding this component of function to the patient’s heart. Via placement at a site in the conducting system that maximizes cardiac output, the biological unit provides another benefit not seen with electronics to date. At the same time, the memory function of the electronic unit can track the
function of the biological unit, providing a record for the cardiologist.

Issues for the future go well beyond learning how to test biological versus electronic units. More pressing problems include understanding whether the biological approach is in fact superior to the electronic pacemaker in terms of adaptability to the body’s physiology and duration of effectiveness; the long-term incidence of inflammation, infection, rejection, and neoplasia; the long-term proarrhythmic potential; the issue of whether there is localization versus migration of the viral vector or cells administered; and, for stem cells, the persistence of administered cell types versus their differentiation into other cells. In addition, other toxicities, not yet conceived of, must be watched for, and delivery systems must be optimized. All of these issues must be laid to rest before one passes judgment on clinical application.

An additional issue must be considered. Biological pacing came on the scene as a disruptive technology. In an era of excellence in electronic pacing, its goal was and remains to supplant this with a biological alternative. Given the imperfections that still reside with electronics, the possibility of a system with no wires, no hardware, and a software that is of the body’s own ion channels and autonomic nervous system offers something more appealing, if it can be made to function at the level needed and for the time required. Nevertheless, the biological approach does not arrive at a time when electronics are static. As mentioned above, rate responsiveness is here, and improved and leadless systems have arrived as well. Therefore, we see 2 competitive approaches evolving. Which one wins out, traditional electronics upgraded to achieve still newer levels of success or biologics, is frankly irrelevant. What is important in this, as in all areas of competition, is that the better approach be identified and brought to bear to the benefit of the patient in need.

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