Heart Rate

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Heart rate: a simple yet complex concept

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The assumption that cardiac automaticity is a simple phenomenon has given way to recognition of a complex system derived from multiple components and their kinetic and dynamic interrelationships. We describe the gross anatomical and electrophysiological features of the pacemaker (P) cells in the sinus node, and explain their ability to depolarize during electrical diastole in terms of the ionic and molecular determinants of their currents: these include the I(f) inward current, the putative initiator of phase 4 depolarization, activated not by depolarization of the membrane, as had been expected, but by hyperpolarization; the I(K) superfamily of delayed rectifier potassium currents; and the I_{Ca,L} and I_{Ca,T} calcium currents. Cloning of the molecular constructs of the individual currents has revealed four isoforms of the pacemaker channel, belonging to the hyperpolarization-activated, cyclic nucleotide (HCN)-gated family. The single most important modulator of sinus rate is the autonomic nervous system, comprising the sympathetic and parasympathetic neurons, the transduction pathways involved in autonomic signaling, and the biophysical targets of the autonomic channels, which are neurotransmitters. Although much investigation is still needed, it should ultimately be possible to replace or rejuvenate diseased pacemakers using techniques of molecular modification.

DEFINITION OF A PACEMAKER

A pacemaker is a device or tissue that initiates cardiac impulses. The physiological cardiac pacemaker, the sinus node, is an ovoid structure, about 1.5×0.5 cm in the normal adult human, and is found subepicardially, in the upper portion of the crista terminalis–sulcus terminalis region of the right atrium. The sinus node is not structurally homogeneous. Its blood supply is received primarily from an artery coursing through its center, and it has a rich sympathetic and parasympa-
thetic innervation, and some working myocardial cells as well as a preponderance of pacemaker fibers. The so-called “P” (for pacemaker) cells are pale-staining and are spindle-shaped or have polypoid branches that merge with slender, nonbranching, but also pale-staining cells that are increasingly numerous near the node’s periphery. As shown in Figure 1, enzymatically dis-aggregated sinus node cells are not only small, but polypoidal in comparison with cells of the myocardium, which are more rectangular and have an orderly array of striations. Hence, pacemaker cells of the sinus node are structurally distinct from other cell types.

Given the diverse population of cells within the sinus node that have the structural characteristics of pacemaker fibers, it should not be surprising that at various times the site of impulse initiation within the node may vary considerably. The pacemaker cells providing the primary impulse for the sinoatrial node initiate that impulse via diastolic depolarization during phase 4, electrical diastole, until a threshold potential is reached at which an action potential is initiated (Figure 2). This action potential, in turn, propagates to other cells, thereby permitting the conduction of the heart beat to the rest of the myocardium. Hence, each pacemaker cell incorporates two properties: that of spontaneous diastolic depolarization to bring the cell from its resting to its threshold potential, and that of carrying the depolarizing wave from cell to cell such that the impulse leaves the sinus node and reaches the entire heart. This form of impulse initiation is by no means unique in nature. Indeed, the rudimentary circulatory systems of invertebrates are endowed with this property that serves as their driver. Nor is it a property unique to myocardial tissue: for example, very similar impulse initiation occurs in pacemaker cells of the renal pelvis, initiating the rhythmic contraction of ureteral smooth muscle.

<table>
<thead>
<tr>
<th>ION CURRENTS</th>
<th>Description</th>
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<tbody>
<tr>
<td>$I_{K}$</td>
<td>outward potassium delayed rectifier current</td>
</tr>
<tr>
<td>$I_{K1}$</td>
<td>potassium inward rectifier current</td>
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<tr>
<td>$I_{K2}$</td>
<td>outward pacemaker current</td>
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<tr>
<td>$I_{K,ACh}$</td>
<td>outward potassium, acetylcholine-stimulated current</td>
</tr>
<tr>
<td>$I_{I}$</td>
<td>inward sodium pacemaker current (f for “funny”)</td>
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<tr>
<td>$I_{Kdd}$</td>
<td>outward potassium pacemaker current (dd, for diastolic depolarization)</td>
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<tr>
<td>$I_{Kss}$</td>
<td>outward sustained potassium current</td>
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<tr>
<td>$I_{Ca,L}$</td>
<td>large and/or long-lasting (L) calcium current</td>
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<tr>
<td>$I_{Ca,T}$</td>
<td>tiny or transient (T) calcium current</td>
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<tr>
<td>$I_{s}$ or $I_{q}$</td>
<td>cerebral variants of $I_{I}$</td>
</tr>
<tr>
<td>$I_{Kr}$</td>
<td>rapid delayed rectifier potassium current</td>
</tr>
<tr>
<td>$I_{ks}$</td>
<td>slow delayed rectifier potassium current</td>
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Figure 1. Photomicrographs of isolated myocytes from sinoatrial node (top) and ventricle (bottom). Cells were enzymatically dissociated from adult rabbit heart. The sinoatrial node cell shows the typical curved spindle shape with a paucity of myofibrils, and was beating spontaneously. The larger ventricle cell is rectangular with pronounced cross-striations, and was quiescent. The calibration bar represents 50 microns.
Hence, we are considering a process that is ubiquitous in nature, yet highly adapted to a particular type of function in mammalian heart. To understand the generation of this pacemaker function, we shall discuss the sinoatrial action potential and the ion channels that determine it, and review the effects of neurohumoral modification of sinus node function. In so doing, we shall develop a picture of how the sinus node maintains a rate and rhythm consonant with the needs of the body.

**THE CARDIAC ACTION POTENTIAL**

The cells of the sinus node are distinct from those of myocardium not only structurally, but electrophysiologically as well. The action potentials of sinus node cells differ from those of working myocardium, in having low resting membrane potentials ($\approx -60$ mV), and slowly rising action potential upstrokes ($\approx 2-4$ V$\cdot$s$^{-1}$), as shown in Figure 2. In fact, sinoatrial node action potentials are far more reminiscent of those of normal smooth muscle than those of working myocardium, and differ from the latter, as follows: working myocardial action potentials—whether atrial or ventricular—have very negative levels of membrane potential (negative to $-70$ mV) (Figure 2). Moreover, under normal circumstances, working myocardium does not depolarize during electrical diastole or develop pacemaker activity. As shall be discussed below, there are at least two factors that maintain myocardial cells in a physiological milieu that is not conducive to pacemaker activity. One is the primary pacemaker current itself, which, although present in working myocardium, is activated at a voltage range far negative to that which characterizes normal cardiac myocytes. The other is the inward rectifier, a potassium current that carries positive charge out of the cell over the more negative ranges of membrane potential, tending to maintain the voltage of the membrane at a negative level.$^5$

Although not automatic, cells of the normal, working myocardium are excitable, generating action potentials in response to a stimulus, such as that conducted from the node. Their action potential upstrokes are of rapid velocity ($\approx 200$ V$\cdot$s$^{-1}$ in ventricular myocardium) and are the result of inward current carried via a sodium channel. Working myocardium maintains a distinct action potential plateau, resulting from inward sodium and especially inward calcium currents, after which repolarization is effected by outward potassium currents, largely those carried by the delayed rectifier current, $I_{K}$, and, in atrium, the acetylcholine-stimulated delayed rectifier, $I_{K,ACh}$. $^6$

**THE HIERARCHY OF CARDIAC PACEMAKERS**

The sinus node is not the only cardiac tissue that is automatic. Many of the cells of the specialized conducting system (ie, the atrioventricular [A-V] node, coronary sinus, and the His–Purkinje system of the ventricle) have the property of automaticity as well. This results from qualitatively similar yet quantitatively different processes than those occurring in the sinus node.
sinoatrial node. Hence, these pacemaker tissues also have an inward rectifier current weaker than that in normal working myocardium and a pacemaker current that activates at voltages positive to those in the myocardium. However, in the normal heart, automatic activity is expressed in these tissues very infrequently. This, in part, reflects the higher rate of impulse initiation in the sinus node, with the intrinsic rate decreasing from atria through A-V junction to the Purkinje system.7 The rapid intrinsic rate of sinus node pacemaker cells, in itself, is not enough to explain why competing activity in secondary pacemaker sites is not often expressed. A major contributor to the tendency toward quiescence of these secondary pacemakers in normal hearts is the phenomenon of overdrive suppression.8,9 This is a property dependent on the function of sodium/potassium-activated adenosine triphosphatase (Na/K ATPase).10 This enzyme functions as follows: in normal myocardial and specialized conducting fibers, there is a high concentration of K+ inside the cell, and a high concentration of Na+ outside the cell. In contrast, intracellular Na+ and extracellular K+ are low. This disparity of ionic charge across the cell membrane, taken in light of the permanent negative charge on the inner surface of the lipid bilayer of the cell membrane, contributes to the largely negative transmembrane resting potential. Na/K ATPase, fueled by adenosine triphosphate (ATP) metabolism, pumps Na+ out of the cell in exchange for K+ into the cell in an approximate ratio of 3:2. The result is the accumulation of a negative charge intracellularly.

The major stimulus for the pump is the intracellular sodium ion, and a prime source of sodium is via its entry during phase 0 of the action potential. In contrast, there is little Na+ entry during the action potential upstroke in sinus node cells, as their action potential is largely Ca2+-dependent. Most other pacemaker tissues of the heart (especially those of the His–Purkinje system), have a large, fast, inward Na+ current, which induces significant pump stimulation. The resultant net outward movement of positive charge not only hyperpolarizes the cells, but provides an outward current that counters the inward pacemaker current, and suppresses the tendency toward automaticity in these cells. The value of having such secondary pacemaker cells in the heart is seen in settings such as sinoatrial block, where the sinus impulse cannot activate the remainder of the atrium, and high degrees of atrioventricular block, where the impulse cannot travel from the atrium to activate the ventricles. In either instance, the presence of lower, secondary pacemaker cells provides a mechanism for sustaining cardiac function and output.

To summarize, then, the normal heart has pacemaker cells and working myocardium whose fundamental structure and action potential characteristics differ from one another. To understand how pacemaker cells achieve their ability to depolarize during electrical diastole, it is necessary to consider the ionic and molecular determinants of their pacemaker currents. This is the subject of the next section.

ION CHANNELS AND PACEMAKING

The molecular events determining the function of normal pacemaker cells involve the function of several ion channels. Hence, the smooth slope of phase 4 depolarization in Figure 2 should be understood as representing the integration of a complex series of interrelated processes.

Before discussing the specifics of ionic control of the sinus node pacemaker, it is useful to consider what events might contribute to the generation of a pacemaker potential. Literally any circumstance in which net inward current increases and/or outward current decreases and there is some background inward current during electrical diastole would be sufficient to depolarize the cell during phase 4. In either case, the result would be the accumulation of positive charge in an otherwise negatively charged cell, the result being displacement of membrane potential in a positive direction. In fact, both events—increases in inward and decreases in outward positive current—occur during the normal sinus node action potential. Our understanding of these components has encompassed an often confusing history of experimentation on pacemaker currents. This difficult history derives in part from the fact that sinus node cells are small and difficult to isolate, and in part from the fact that limited experimental tools and difficult protocols produced data that could—and sometimes did—have more than one acceptable interpretation. Not only are the currents needed to induce phase 4 depolarization quite small and difficult to measure, but background currents are often difficult to distinguish from experimental artifact.

Despite the difficulties inherent in studying sinus node in particular and pacemaker currents in general, by the 1970s, the body of experimental evidence suggested that an outward pacemaker current, designated \( I_{K1} \) (to differentiate it from the outward, repolarizing inward rectifier, \( I_{K1} \)) operated at a high level at the end of phase 3 repolarization and then gradually declined during phase 4.11 Such a decline in outward current over time would move progressively less positive charge
from inside the cell to outside: hence, in the presence of a background inward current carried at a steady rate into the cell, the net accumulation of positive charge intracellularly would depolarize the membrane, creating a pacemaker potential.

In the 1970s, a fundamental series of observations was made that completely altered our concept of pacemaker current. It was noted that, when specialized fibers such as those in the Purkinje system or sinus node are hyperpolarized, this generates an increasing inward current carried by the sodium ion (Figure 3).

The hyperpolarization necessary to activate the current occurs at the end of phase 3 of the action potential, as it repolarizes to reach its maximum diastolic level. Moreover, this increase in inward current, leading to accumulation of positive charge in the cell, appeared to be the primary mechanism for initiating the slope of phase 4 depolarization. DiFrancesco and colleagues noted that it was unexpected that an inward current would activate on hyperpolarization of the cell membrane. They reacted this way because the major inward currents responsible for the upstroke (sodium) and plateau (calcium) of the action potential are activated, not by the hyperpolarization of the membrane, but by its depolarization (as during phase 0). Because this novel observation struck investigators as funny, the new pacemaker current was named $I_f$ (with the “f” standing for “funny”).

To consider the currents contributing to pacemaker function we shall review them in the sequence with which their onset occurs (Figure 4, next page). The current that is considered by many to be the primary pacemaker in heart is $I_f$. This current, initiated by the membrane hyperpolarization that characterizes the end of phase 3, is not specific for a single ion species. In other words, the channel—studied in isolation—can pass sodium or potassium ions. Nonetheless, at the level of membrane potential at which the pacemaker potential is initiated, the primary charge carrier is sodium (as this ion both carries positive charge and has a concentration and voltage gradient that favors its entry over that of potassium in the voltage range at which the channel opens). Studies in single cells and in multicellular preparations have shown $I_f$ to activate at about -35 to -65 mV, certainly within the range of voltages operating in sinus node. Once it activates, some studies have shown it deactivates during repolarization. As a result, $I_f$ appears to be the major charge carrier of inward current and the initiator of diastolic depolarization, but as it deactivates it carries less inward current with time.

There are several outward potassium currents whose contributions to pacemaker function have been and still are being explored. One such current, detailed thus far mainly in Purkinje fibers and not sinus node, is referred to as $I_{K_{dd}}$ (with “dd” meaning diastolic depolarization). Vassalle has championed this as a primary...
pacemaker current in the heart, and with a more important role than \( I_f \). Nonetheless, very cogent arguments have been made for \( I_f \) as well (see above and reference 18), and the general weight of present opinion is that the primary initiator of phase 4 depolarization is \( I_f \). However, given the deactivation that occurs in \( I_f \), as well as the demonstration that potassium current contributes to the pacemaker potential, the question of primacy must still remain open.

There are other potassium currents involved in sinus node pacemaking. As stated above, the inwardly rectifying current in sinus node is weak, contributing to the low level of membrane potential. The major potassium current in the node is the delayed rectifier, \( I_K \), which has both rapidly activating and slowly activating components. Although the magnitudes and time courses of the delayed rectifier currents differ across species, it is clear that the time course of the deactivation of \( I_K \) over the duration of the cardiac cycle is such that it carries less potassium current outward over time. Hence, in the presence of a background inward current, it would contribute to the accumulation of positive charge within the cell and, with this, to diastolic depolarization. Finally, another outward potassium current that has received mention is a sustained current, referred to as \( I_{K_{sus}} \).

Two calcium currents, initially described in studies of neuronal tissues, are present in heart as well. These are \( I_{Ca,L} \) (with the \( L \) standing for large and/or long-lasting calcium current) and \( I_{Ca,T} \) (with the \( T \) standing for tiny or transient calcium current). \( I_{Ca,L} \) is the major charge carrier of the action potential plateau and the major determinant of the upstroke and plateau of the sinus node action potential. However, it activates at very positive voltages, such that it contributes at best to only the terminal portion of phase 4. \( I_{Ca,T} \) has been proposed to contribute to the terminal portion of phase 4 depolarization, based on the use of blockers of the current to slow phase 4 depolarization.

Therefore, at the simplest level, we can explain phase 4 depolarization in sinus node based on the operation of three currents: \( I_f \) turning on at the highest range of membrane potentials to carry positive charge into the
cell and then commencing to deactivate, the $I_f$ family of potassium currents carrying less positive charge from the cell during phase 4 in the setting of a sustained inward current contributing to further depolarization, and, finally, $I_{Ca,T}$ carrying inward current at the termination of phase 4, just prior to the onset of the nodal action potential carried by $I_{Ca,L}$.

Areas of uncertainty remain, as follows: first, the argument regarding the role of $I_{Kdd}$ remains active and unsettled, and must be kept open until complete resolution is attained. Second, other currents have been identified that may influence the pacemaker. These include a background inward sodium current, which is independent of $I_f$ and would provide a continual ‘leak’ of inward current during phase 4. It is this current that provides the essential inward component essential for a role for diminishing outward potassium current to induce phase 4 depolarization. However, this current and its contribution in the range of potentials operating in sinus node have not been demonstrated convincingly, so it remains more of a possible than a definitive contributor. Third, a different and ‘sustained’ inward current also carried by sodium, but perhaps via the L-type calcium channel rather than through a sodium channel, has been identified. However, given its demonstration in only highly specialized experimental circumstances, this current’s contribution remains conjectural.

More likely having a role in pacemaker function is the Na/Ca exchanger, which when operating at full efficiency will extrude one calcium for three sodium molecules, resulting in a net inward current at times of calcium loading (as would occur during the calcium-generated action potential of the sinus node). This current, too, could contribute to diastolic depolarization, with the extent of its contribution varying with the extent of calcium entry during the action potential and the extent of sarcoplasmic reticulum calcium uptake and release.

**MOLECULAR DETERMINANTS OF THE ION CHANNELS THAT INITIATE PACEMAKER FUNCTION**

One of the major advances of the 1990s was the application of molecular biological techniques to the study of the heart. These techniques have permitted an initial attempt to understand the structure of various ion channels and to relate the structure of the channels to the density and kinetics of the current they carry. It should be noted that this understanding of structure does not come from three-dimensional imaging (although such data are now forthcoming regarding some potassium channels). Rather, most data derive from the sequencing of the amino acids that provide the protein structure of the channels, and the use of techniques such as hydropathy analysis to estimate those portions of sequences that are cytosolic or extracellular as opposed to intramembranous. Such analyses provide the tools for extrapolating an image of what the three-dimensional structure might be like.

The $\alpha$ and $\beta$ subunits of channels have been one subject of focus. The $\alpha$ subunits are those which form the channel pores; that is, the ensemble of $\alpha$ subunits of any channel considered as a three-dimensional structure provides a passageway for ions dissolved in aqueous solution (which constitutes much of the extracellular space and cytosolic environment) to traverse the lipoprotein membrane of the cell. Initially, $\beta$ subunits were thought of as proteins that anchor the $\alpha$ subunits in the membrane, but have more recently been understood to have important physiological functions. These include the modulation of channel gating—the determinant of whether a channel is in its open state, during which it passes ions between a cell’s interior and exterior environment, and its inactivated or resting states.

Some data are now available concerning the molecular constructs of the pacemaker current, $I_f$. To a great extent the availability of this information derives from the fact that $I_f$ is found in brain as well (where it is referred to as $I_{Kr}$ or $I_q$), and has been actively investigated by neuroscientists. Four different isoforms of the channel that carries the pacemaker current have been identified. All four are of the HCN family (for hyperpolarization-activated, cyclic nucleotide-gated). We have discussed the hyperpolarization-induced activation of the channel above, and will consider the cyclic nucleotide gating below. The isoforms of the channel have been designated HCN1 to 4, of which HCN1 and 4 are the likely determinants of $I_f$ in the sinoatrial node.

With regard to potassium channels, $I_{Kdd}$ has not yet been cloned. The channels that contribute to the family of delayed rectifier currents have been the subject of intensive study, in part because of the association of both $I_{Kr}$ and $I_{Ks}$ with the congenital long QT syndrome. Both HERG (the human ether-a-go-go–related gene that is responsible for $I_{Kr}$) and KvLQT1 (the gene responsible for $I_{Kr}$) have been cloned and their relationship to other channel subunits has been defined. However, with the exception of the identification of KvLQT1 and HERG transcripts in ferret sinus node...
the data regarding these two proteins in sinus node of other mammalian species are uncertain. Finally, although several isoforms of $I_{\text{Ca,T}}$ have been cloned, there are no conclusive molecular data identifying the specific molecular subunits responsible for $I_{\text{Ca,T}}$ in sinus node.

**NEUROHUMORAL EFFECTS ON PACEMAKER FUNCTION**

Thus far everything discussed in this paper has related to the direct determinism of sinus node function and heart rate. We have reviewed the sinus node action potential and contrasted it with that of working myocardium, and have considered the biophysical and molecular determinants of the pacemaker current. Complexity derives from the number of components in this system and the interrelating of their kinetic and dynamic properties to initiate an action potential. Yet, the sum of the processes discussed thus far is a pacemaker capable of initiating a cardiac impulse whose rate is varied only by the level of membrane potential reached following complete repolarization of the action potential and the basal activity of the ion channels contributing to phase 4 depolarization. In other words, we have considered nothing so far that explains the variations that occur in sinus rate.

Probably the single most important modulator of sinus rate is the autonomic nervous system. In this section, we shall review first the function of the autonomic nervous system on a “macro” scale, on the heart in situ, considering the sympathetic and the parasympathetic nervous systems individually and together, then consider the transduction pathways involved in autonomically signaling to the heart, and, finally, the biophysical effects of autonomic stimulation.

**Autonomic effects on the heart in situ**

Even prenatally a profound influence of parasympathetic stimulation is demonstrable on heart rate. Schifferli and Caldeyro-Barcia\textsuperscript{43} demonstrated this by giving injections of atropine to pregnant women throughout gestation and noting that: (i) heart rate generally slowed with prenatal development; and (ii) the slowing could be reversed almost completely by atropine. Moreover, the use of fetal monitoring in the last decades of the twentieth century has demonstrated profound variations in heart rate that are attributable to vagal influences. Sympathetic maturation occurs later than parasympathetic maturation, well into the postnatal period (although there is marked species variability).\textsuperscript{44-46} Hence, the extent to which sympathetic and parasympathetic effects will influence the heart rate varies after birth. What is clear is that there are cardioaccelerator effects of sympathetic stimulation, resulting largely from the β-adrenergic action of norepinephrine released from sympathetic terminals and/or circulating epinephrine, and the cardiodecelerator effects of parasympathetic stimulation via the muscarinic effects of acetylcholine. Also important is the interrelationship of these two effects, such that in the setting of sympathetic stimulation, the action of acetylcholine in slowing heart rate becomes even greater. This effect is referred to as accentuated antagonism. Also important to the actions of neural stimulation are the effects of neuropeptide Y (NPY), which is colocalized with norepinephrine in sympathetic terminals\textsuperscript{47} and can be released during sympathetic stimulation.\textsuperscript{48,49} Hence, under conditions of intense sympathetic stimulation, not only is norepinephrine released from sympathetic terminals, but NPY is released as well. NPY affects heart rate by inhibiting transmitter release from both sympathetic and parasympathetic nerve terminals.\textsuperscript{50-52} This action, in itself, can modify heart rate significantly, depending on the extent of transmitter release at any given time and the net level of β-adrenergic and muscarinic input to the heart rate at that time. Importantly, NPY also has postjunctional effects, transduced via the Y receptors on cardiac myocytes.\textsuperscript{53} The expected effect is a decrease in the pacemaker current, $I_r$, and slowing of heart rate.\textsuperscript{54,55}

In complementary fashion, vagal stimulation releases vasoactive intestinal peptide (VIP), especially after intense neural stimulation.\textsuperscript{56} This peptide, too, interacts with cardiac receptors, with the postjunctional action of VIP being to increase $I_r$.\textsuperscript{54} Hence, each link of the autonomic nervous system incorporates within it the capacity to respond to intense stimulation by releasing a neuropeptide whose action on pacemaker current is opposite to that of the primary agonist released. Interestingly, while the action of VIP is consistently seen at times of intense vagal stimulation, that of NPY is more inconsistently seen. This difference may reflect distribution of the relevant receptors, experimental design, and other, as yet undefined, variables as well.

**Autonomic effects on the cellular electrophysiology of the heart**

The effect of β-adrenergic agonists on the sinus node is to hyperpolarize the maximum diastolic potential,\textsuperscript{57} which would shift the activation of $I_f$ in a way that increases net current and the slope of phase 4 depolar-

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**Notes:**
- $I_{\text{Ca,T}}$: Transient calcium current
- $I_r$: Resting current
- β-adrenergic: Beta-adrenergic receptors
- NPY: Neuropeptide Y
- VIP: Vasoactive intestinal peptide
- $I_f$: Fattreberg current
- $I_{\text{Ca,T}}$: Transient calcium current
- $I_r$: Resting current
ization, and speeds the rate of impulse initiation by the sinus node. Moreover, via its effects of increasing inward calcium current, norepinephrine may also enhance the rate of impulse initiation (see details below). In contrast, muscarinic stimulation has a generalized effect of increasing potassium conductance. This tends to hyperpolarize the membrane, which would also increase the fast sodium current. Yet, via mechanisms described below, muscarinic stimulation actually decreases the slope of phase 4 depolarization, its predominant action, and one that decreases pacemaker rate.

**Autonomic signal transduction and its effects on sinoatrial ion channels**

β-Adrenergic agonists exert their effects on sinus rate largely via a β1-receptor-mediated signal transduction system (for review see reference 58). Binding to the β1-receptor activates the enzyme adenylyl cyclase (Figure 5). Importantly, this activation depends on transduction via a stimulatory guanosine triphosphate (GTP) regulatory protein, referred to as Gs. Dissociation of the α and βγ subunits of Gs facilitates the activation of adenylyl cyclase, which then metabolizes ATP to cyclic adenosine monophosphate (cAMP) and P. The second messenger cAMP, in turn, activates protein kinase A, and this kinase can phosphorylate ion channels. Importantly, however, cAMP also has effects not dependent on channel phosphorylation, but central to impulse initiation.

β-Adrenergic stimulation increases sinoatrial pacemaker rate by inducing a positive shift in the voltage dependence of If activation, while also enhancing its kinetics (Figure 6). The result is an increase in the slope of phase 4 depolarization. Importantly, while...
this process is cAMP-dependent, it is not the result of channel phosphorylation. Rather, the effect of cAMP is direct here, leading to the designation of the channel as hyperpolarization-activated, cyclic nucleotide–gated (HCN). This effect of cAMP induces the positive shift of $I_f$ activation described above. Moreover, the likelihood of $I_f$ opening is increased by cAMP as well, increasing the net inward current density.

With respect to the delayed rectifier current, the component activated by β-adrenergic stimulation is $I_{K_s}$. Activation of this current would tend to accelerate repolarization, a process that appears dependent on protein kinase A–based channel phosphorylation. Although $I_{K_s}$ is a major component of the guinea-pig sinus node action potential, the extent of its involvement in humans is unknown at present. Therefore, no statement can be made regarding this component of modulation. Activation of $I_{K_s}$ would—by shortening the duration of repolarization—result in the earlier onset of diastole, an effect that could speed sinus rate. To the extent that the outward current persists during phase 4, this effect would tend to slow sinus rate.

With regard to calcium currents, only $I_{Ca,L}$ (not $I_{Ca,T}$) is activated by β-adrenergic stimulation. $I_{Ca,L}$ is activated via phosphorylation so that current density, but not kinetics, is increased. However, because the current is activated largely at voltages positive to the pace-maker potential range, $I_{Ca,L}$ modulation is not viewed as a primary contributor to pacemaker function. Hence, we are left with the effect of catecholamines on $I_f$ as being apparently dominant in increasing automaticity. Muscarinic stimulation (primarily via the M3 muscarinic receptor) activates the inhibitory GTP regulatory protein $G_i$ (for review see reference 62). There are two pathways whereby $G_i$ actions are expressed. One is seen in the direct channel interaction of the dissociated $\alpha$ and $\beta_7$ subunits of the protein (Figure 5). The other is via the effect of $G_i$ to inhibit adenyl cyclase activation—especially in situations where adenyl cyclase is being activated by $G_s$ secondary to β-adrenergic stimulation. This inhibition of effect that is magnified when there is baseline sympathetic stimulation is the basis for the accentuated antagonism of β-adrenergic by muscarinic effects. When adenyl cyclase activation is inhibited, all downstream processes dependent on cAMP activation and/or protein phosphorylation are also impeded.

Hence, either via direct actions determined by the subunit of $G_i$, or indirectly via antagonism at the level of adenyl cyclase activation, parasympathetic reductions can reduce sinus rate.

With respect to $I_f$, very low concentrations of acetylcholine suppress the pacemaker current, even in the absence of sympathetic stimulation (Figure 6). A second mechanism for suppressing pacemaker function occurs via the activation of the outward potassium current $I_{K,ACH}$. This both accelerates repolarization (via G-protein–gated potassium conductance) and hyperpolarizes the membrane. The sustained net outward potassium current that is produced decreases the slope of phase 4 depolarization. Finally, by antagonizing the effects of catecholamines to increase cAMP levels, there is an action to decrease $I_{Ca,L}$.

Summing up, via an action primarily on $I_f$, catecholamines increase sinus rate. Via actions on $I_{K,ACH}$ and $I_f$, acetylcholine decreases sinus rate.

**CONCLUSION**

The simplicity of sinoatrial pacemaking processes is seen in the seemingly effortless repetition of the heart beat and the ability of the sinoatrial pacemaker to adjust rate in a variety of circumstances demanding more or less cardiac output. The complexity derives from the very detailed machinery that contributes to the generation of impulses in sinus node, the prevention of impulse generation elsewhere, and the modulation of these impulses. The microanatomy of the node and the large number of cells within the node that are capable of serving as the primary pacemaker contribute to the potential for pacemaker shifts, as does the heterogeneity of autonomic innervation and gap junctions.

Hence, while much of our understanding of sinoatrial control remains to come, much has already been done to indicate which areas we need to focus on. Areas currently being explored include the quantitative understanding of structure, function, and modulation, as well as the molecular/genetic determinants of pacemaker cells. When this work comes to fruition it is possible that diseased pacemakers can be replaced/augmented by molecularly modified biological pacemakers, rather than resorting to electrical implants.
THREE KEY QUESTIONS

After taking a look at the complex "machinery" underlying the deceptively simple concept of heart rate, the reader will now—hopefully—be better armed to appreciate the implications of the questions posed in the following section. Antonella Boraso asks "Why is reduced heart rate beneficial?" and takes us for a tour of the animal kingdom, tantalizingly concluding that the slower the heart beats, the longer one lives, thus opening up intriguing prospects for intervention. Gabriel B. Habib picks up from there to ask "Is heart rate a risk factor in the general population?" and highlights the fact that heart rate is significantly associated with clinical outcome both in the general population and in those at risk for cardiovascular disease, the good news being that evidence is accumulating in support of the cardioprotective effect of heart rate–lowering therapies. Finally, drawing the logical conclusion from these findings, Henry Purcell’s poses the question "Is heart rate a prognostic factor for cardiovascular disease?" and shows that alongside increasingly sophisticated techniques there still is room for refreshing simplicity: taking a patient’s pulse can go a long way toward helping the doctor determine the prognosis of the cardiovascular patient—but of course it took sophisticated epidemiological studies to prove this.

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In an outstanding editorial, Levine\(^1\) reviewed the theory of the relationship between heart rate and life span, which states that smaller animals have higher heart rates and shorter life spans than larger animals. The explanation is provided in part by a biophysical imperative in which the ratio of heat loss (a function of body surface area) to heat production (a function of body mass) increases as body size is reduced. Among mammals, except the human species, there is a linear inverse semilogarithmic relationship between heart rate and life expectancy.\(^1\) The reasons for the life expectancy of 80 years achieved in humans may reside in advances in science, medicine, and sociology.

HEART RATE “CONTROLS” THE BODY’S METABOLIC ACTIVITY

By adjusting its rate, the heart can control both the temperature and the energy requirements of the whole body. The heart is able to send “messages” and “talk” to most cells of the body through the circulatory system, mainly with the help of the endothelium. The “language” chosen by the heart could be the “heart rate,” via the intensity and frequency of shear stress, which thus exert an important regulatory role on endothelial function and vascular tone.\(^2\) The endothelium responds to shear stress by releasing nitric oxide and other vasoactive compounds, regulating the degree of vasodilation and thus the amount of blood and oxygen to peripheral muscles (Figure 1, see next page). The heart could be viewed as the connection between the central nervous system and the periphery, determining and regulating the activities of peripheral muscles through heart rate.

The metabolic rate is dependent on physical activity, which in turn is related to, and likely determined by, heart rate itself. Regression analysis on a logarithmic scale between body mass and metabolic rate among animals yields a straight line with the same slope as that between body mass and heart rate.\(^3\) There is, therefore, a close link between temperature, metabolism, and heart rate, and the question is, which is the primary control among these parameters? If heart rate determines metabolic rate, it follows that a relationship between heart rate and life span exists in the entire animal kingdom, including man.

THE TOTAL NUMBER OF HEART BEATS IN A LIFETIME IS CONSTANT

Calculations show that the number of heart beats per lifetime is remarkably constant among mammals, despite a 40-fold difference in life span.\(^1\) When the number of heart beats per lifetime is plotted against body weight, the difference in life span is even greater, in the order of 0.5 million, from hamster to whale.\(^1\) These considerations are important since, in terms of evolution, they point to a relationship between life span and available energy, which

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**Keywords:** heart rate; metabolic rate; cellular energetic requirement; life expectancy

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can be summed up as “the less the energy needed, the longer the life span.” Comparisons between homeotherm and hibernating animals yield further interesting information. In homeotherms, the fall in body temperature is prevented by an increase in metabolic rate, which, in turn, is associated with an increase in heart rate. In hibernating animals, the fall in metabolic rate is achieved by a drop in body temperature and heart rate. The mean heart rate, which in nonhibernating marmots is 150 beats per minute (bpm), drops to 3 to 5 bpm during hibernation.

Azbel stresses the concept that smaller animals have higher heart rates and shorter life durations than larger animals, with a 35-fold difference in heart rate and a 20-fold difference in life span, and suggests that life expectancy is predetermined by basic energetics of living cells, and that the inverse relationship between longevity and heart rate reflects an epiphenomenon in which heart rate is a marker or a determinant for metabolic rate and energetic requirements.4 Although the fact that the total number of heart beats per lifetime is constant has been demonstrated only among mammals, there is good reason to believe that this holds true throughout the entire animal kingdom. Thus, a giant (Galapagos) tortoise with a heart rate of 6 bpm and a life expectancy of 177 years will produce $5.6 \times 10^8$ beats per lifetime,5 which is similar to the figure obtained for rat ($6.3 \times 10^9$) with a heart rate of 240 bpm and a life expectancy of about 5 years.

Indeed, the number of heart beats per lifetime is quite constant in different mammals, despite a 40-fold difference in life expectancy.1

**HOW COULD LIFE BE PROLONGED?**

Whether heart rate reduction would determine a prolongation of life span is not yet known for certain in man. Coburn et al tested this hypothesis experimentally by feeding mice with digoxin,6 and reported that treated mice had a slower heart rate and lived significantly longer than control mice. However, because of various confounding factors, such as lower body weight in the treated mice, it was impossible to establish a clear cause-and-effect relationship. Humans, with a mean heart rate of 70 bpm and a life expectancy of 80 years, are an exception to the relationship between heart rate and life expectancy shown in mammals, as their life expectancy is higher than that predicted by their heart rate. It has been estimated that a decrease in heart rate from 70 to 60 bpm would further increase life expectancy from 80 to 93.3 years in humans. An established fact, however, is, that in the general population, the risk of death from all causes, including cardiovascular disease, augments as resting heart rate increases. Several clinical studies have demonstrated that heart rate is an important risk factor for cardiovascular morbidity and mortality, not only among patients with established heart disease7 or well-known cardiovascular risk factors such as hypertension,8 but also in the general population.9

**CONTRACTION OF THE HEART**

In order to contract continuously, the heart needs regular oscillations of intracellular cytoplasmic calcium,
the ultimate messenger of contraction. It also needs energy, provided as adenosine triphosphate (ATP), which enables—among other functions—the myofilaments to contract and relax. These mechanisms are finely regulated by a complex interplay that results in a continuum of systole and diastole, ie, the heart beats.

During each action potential, cytoplasmic calcium increases transiently and interacts with the contractile elements to result in contraction, ie, systole, in the process known as excitation-contraction (E-C) coupling (Figure 2). In the heart, E-C coupling involves two types of calcium channels: the L-type/dihydropyridine-sensitive calcium channels in the sarcolemma and the ryanodine-sensitive/calcium release channels in the sarcoplasmic reticulum. In the sarcolemma, the depolarization that initiates action potential causes an influx of calcium ions into the cell, proportional to their electrochemical gradient, mainly through channels sensitive to dihydropyridines and other calcium channel blockers.10 This influx is not sufficient to initiate contraction in the heart, but permits the release of other calcium ions from the sarcoplasmic reticulum through the ryanodine-sensitive/calcium release channels, via a mechanism called calcium-induced calcium release.10,11 The increase in intracellular calcium then leads to contraction.

In the conduction system, eg, the Purkinje fibers, other sarcolemmal calcium channels have been identified, such as the T-type calcium channels, which have a role in pacemaker activity. T-type calcium channels (also referred to as low-threshold or low-voltage–activated channels) open in response to a smaller sarcolemmal depolarization (approximately -40 mV from a resting potential of -80/-100 mV), whereas L-type calcium channels (high-threshold or high-voltage–activated channels) require depolarization to -20 mV to open and sustain contraction. T-type calcium channels, like sodium channels, participate in the early stage of pacemaker depolarization and, therefore, may contribute to the initiation of the heart beat.

At diastole, three main cellular mechanisms are involved in the extrusion of calcium from the cytoplasm. In the sarcolemma, the sodium/calcium exchanger and a calcium pump (the latter with a restricted capacity) are active, whereas a more powerful calcium pump stores calcium back in the sarcoplasmic reticulum. In cardiac muscle, the activity of the calcium pump in the sarcoplasmic reticulum is regulated by a phosphorylation/dephosphorylation mechanism involving a 27-kd protein associated with the membrane, phospholamban.12 Phospholamban phosphorylation involves protein kinase A for the removal of calcium pump inhibition, thus leading to a marked increase in calcium transport activity across the sarcoplasmic reticulum membrane and to cardiac muscle relaxation.10 Mitochondria are only involved in removing calcium from the cytoplasm under pathological conditions. There is, in fact, a competition between mitochondrial calcium transport and ATP production as the two processes utilize the same inner membrane potential (in the mitochondrion) in (Figure 3, see next page).13

**Figure 2.**
Schematic representation of the excitation-contraction coupling mechanism in cardiac muscle. CICR, calcium-induced calcium release.
In man, heart beats total on average 100,800 cycles per day. This figure corresponds to 36.8 × 10⁶ cycles per year and 29 × 10⁸ heart beats in a lifetime (80 years on average). The heart produces and consumes approximately 30 kg of ATP every day, i.e., nearly 11,000 kg per year and approximately 880,000 kg in a lifetime. It follows that the cost of each heart beat is approximately 300 mg of ATP. Slowing the heart rate by 10 bpm would result in a saving of about 5 kg ATP every day. To produce ATP, the myocardium needs oxygen, which is used by the mitochondria during oxidative phosphorylation. Azbel has calculated that, in all animals, the basal oxygen consumption/body atom is approximately 10 molecules of oxygen/lifetime, which, referred to heart rate corresponds to approximately 10⁻⁸ molecules of oxygen per heart beat. Astonishingly, the total number of heart beats per lifetime calculated with these data (10⁻⁶) is similar to the mean value observed among mammals (7.3 × 10⁸). Is heart rate reduction beneficial in man? Even though these calculations are based on simplified figures, they point to the pivotal role of heart rate, and suggest a significant potential benefit of heart rate reduction at the cellular level. Since oxygen delivery in the heart mainly occurs during diastole, via coronary flow, it is clear that the deleterious consequences resulting from conditions in which the heart is damaged and oxygen delivery is impaired, such as ischemic heart disease and certain types of heart failure, stand to be improved by agents that decrease heart rate. Because heart rate is a major determinant of oxygen consumption and metabolic demand, heart rate reduction would be

**Figure 3.** Schematic representation of mitochondrial adenosine triphosphate (ATP) production and calcium transport. ADP, adenosine diphosphate.
expected to decrease cardiac work-load. The use of β-blockers would therefore improve the myocardial energetic balance and result in a less negative force-frequency relationship. The saving of energy at myocardial level, therefore, represents just one aspect of the relationship between heart rate and life expectancy, the other being a reduction in the body’s metabolic rate. Interestingly, the increase in mortality in subjects with elevated heart rate is mostly attributed to a higher risk of death from coronary artery disease. Atrial fibrillation, especially in the postoperative period, is a common complication of cardiac surgery, and a combination of β-blockers and/or calcium channel blockers would be a logical treatment. Thus, the heart determines heart rate, and heart rate itself can be harmful for the heart. In other words, the heart is the cause and the target of the same paradigm. At present, it is not clear whether a primary reduction in heart rate may effectively prolong life in patients, although clinical studies suggest that agents that decrease heart rate do improve survival in patients with myocardial infarction. Hypertension, and heart failure Conversely, an increased chronotropic effect through β-adrenergic stimulation worsens prognosis. As suggested above, the heart itself could derive the greatest benefit from heart rate reduction.

CONCLUSIONS

Epidemiological studies suggest that low heart rate is associated with decreased cardiovascular and all-cause mortality. In particular, heart rate has been reported to be an independent predictor of outcome after myocardial infarction. Pharmacological interventions that reduce heart rate, eg, through the use of β-blockers and calcium channel blockers, generally reduce mortality and improve outcome. At present, the mechanism for this beneficial effect is not entirely understood, but most likely multifactorial. Although all the body’s cells may benefit, this is particularly true of the damaged cardiac myocyte with its impaired energy production, since reduction in heart rate will drastically reduce the cardiac myocyte’s energy requirements.

Heart rate also has prognostic value in the general population. In the animal kingdom, an inverse relationship has been observed between heart rate and life expectancy, and the likely explanation for this is that heart rate is a marker of metabolic rate. Thus, once again, the “saving” theory seems to be plausible. The question of whether human life can be extended by reducing heart rate remains unresolved, but is a fascinating one, which should encourage major efforts in this research field.

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Double-blind, placebo-controlled study of the effects of carvedilol in patients with moderate to severe heart failure.
Heart rate is an independent risk factor for cardiovascular disease. There is compelling evidence of a clinically meaningful and statistically significant association between heart rate and clinical outcome in the general population, as well as in elderly subjects and hypertensive patients. There is also increasing evidence supporting heart rate as a unifying hypothesis explaining both the favorable cardioprotective effects of heart rate–lowering β-blockers and calcium channel blockers and the unfavorable effects of calcium channel blockers that do not lower heart rate in patients recovering from myocardial infarction. The wider recognition of heart rate may help clinicians identify patients at an especially high risk for cardiovascular disease and target these high-risk subjects with cardiovascular therapies specifically designed to reduce heart rate.

EVIDENCE SUPPORTING HEART RATE AS A RISK FACTOR IN THE GENERAL POPULATION

Evidence that heart rate is a risk factor in the general population is supported by a growing body of large epidemiological observational cohort studies published in the last two decades. These epidemiological studies evaluated the role of heart rate as an independent risk factor for all-cause and cardiovascular and/or coronary heart disease mortality, and are summarized in Table 1 (see next page).1-12 Overall, these studies combined comprised over 116,000 apparently healthy male and female subjects varying widely in age (from 18 to 80 years), generally with no prior known cardiovascular disease, who were followed for 5 to 36 years. This large sample size of over 116,000 subjects from a large number of different populations worldwide supports the generalizability of the results of these studies to the population at large.

These studies1-12 and others13 have demonstrated that the risk of death from all causes, including cardiovascular disease, increases as resting heart rate increases2 or when heart rate exceeds 84, 90, or 100 beats per minute (bpm)1,3-13 Mortality was consistently associated with increased heart rate regardless of gender or ethnic background, and amounted to a 3-fold higher risk of death in subjects with a heart rate of 90 to 99 bpm compared with subjects with a heart rate <60 bpm.4 The excess mortality is mostly attributable to a higher risk of death from coronary artery disease. Resting heart rate was associated with an increased risk of both fatal and nonfatal manifestations of coronary artery disease.6 The significant 2- to 3-fold increase in all-cause and coronary heart disease mortality over 12 years, accompanying an increase in resting heart rate from <60 to >100 bpm in the Swedish Multifactor Primary Prevention Trial—one of the larger epidemiological studies—is illustrated in Figure 1, (see page 27).4

The three Chicago epidemiological studies—Chicago Western Electric, Chicago Peoples’ Gas, and Chicago
Heart rate was consistently associated with a higher risk of death, mostly attributable to an excess of cardiovascular deaths. The increase in mortality was quite clinically significant and amounted to a doubling of risk with every 40-bpm increase in heart rate.7,8,10-12 After adjusting for any other known risk factor, such as age, blood pressure, gender, race, diabetes mellitus, blood lipids, and body mass index, heart rate remained significantly predictive of an excess risk of death in all 12 studies.1-13

Now that we have examined the evidence supporting heart rate as a risk factor in the general population, we will specifically address the importance of heart rate in two large segments of the population, elderly subjects and hypertensive patients.

### IS HEART RATE AN IMPORTANT PROGNOSTIC FACTOR IN ELDERLY SUBJECTS?

In elderly subjects, the risk of developing new coronary events, such as sudden cardiac death or acute myocardial infarction, is 14% higher for every 5-bpm increase in heart rate, even after adjusting for confounding effects of other risk factors.14 This observation has important public health implications, since the elderly segment of the US population is growing at a dispor-
portionately higher rate than any other segment of the population. The Cardiovascular Study in the Elderly\textsuperscript{15} was an epidemiological study specifically designed to evaluate the independent contribution of heart rate to the risk of death in 1938 men and women aged 65 years or older. In men, cardiovascular deaths were significantly increased in those in the top quintile of heart rate (relative risk [RR], 1.55). After adjustment for baseline age, body mass index, hypertension, diabetes mellitus, angina or previous myocardial infarction, lipid levels, smoking, alcohol intake, and other confounders, the RR for cardiovascular death in men was 1.38 (95% confidence interval [CI], 0.94-2.03) for the top quintile of heart rate and 0.82 (95% CI, 0.52-1.28) for the bottom quintile. Cox multivariate regression analysis indicated that heart rate (\(P<0.001\)) was the most powerful predictor of cardiovascular death, followed by age (\(P<0.001\)), concomitant coronary heart disease (\(P<0.001\)), clinical heart failure (\(P=0.001\)), diabetes mellitus (\(P=0.001\)), hypertension (\(P=0.02\)), and triglyceride levels (\(P=0.04\)). Thus, an elevated heart rate is a strong and independent predictor of cardiovascular death in elderly men regardless of any other known coronary risk factor.

**IS HEART RATE AN IMPORTANT PROGNOSTIC FACTOR IN HYPERTENSIVE PATIENTS?**

Hypertensive subjects are another especially important group. They comprise 20% of the population and have a substantially greater risk for cardiovascular disease compared with the general population. In the 4530 untreated hypertensive subjects aged 35 to 74 years in the Framingham Heart Study,\textsuperscript{16} resting heart rate was significantly predictive of all-cause mortality, cardiovascular mortality, and coronary heart disease mortality. The odds ratios (OR) and 95% CI for each 40-bpm increment in heart rate after adjustment for age and systolic blood pressure level are shown in Table II.\textsuperscript{16} This striking increase in all-cause, coronary heart disease, and cardiovascular mortality with higher resting heart rates in the hypertensive Framingham patients is illustrated schematically in Figure 2 (see next page).\textsuperscript{15}

In the Framingham Heart Study\textsuperscript{16} as well as several other epidemiological studies,\textsuperscript{17-23} heart rate was significantly associated with systolic and diastolic blood pressure in men and women. Resting heart rate is consistently higher in hypertensive patients than in age-matched normotensive controls.\textsuperscript{16} Could the association of heart rate with cardio-

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### Table II. All-cause and cardiovascular mortality in hypertensive subjects in the Framingham Heart Study.\textsuperscript{16} OR, odds ratio; CI, confidence interval.

<table>
<thead>
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<th>OR (CI)</th>
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<tbody>
<tr>
<td>All-cause mortality</td>
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<tr>
<td>In men</td>
<td>2.18 (1.68-2.83)</td>
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<tr>
<td>In women</td>
<td>2.14 (1.59-2.88)</td>
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<tr>
<td>Cardiovascular mortality</td>
<td></td>
</tr>
<tr>
<td>In men</td>
<td>1.68 (1.19-2.37)</td>
</tr>
<tr>
<td>In women</td>
<td>1.70 (1.08-2.67)</td>
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**Figure 1. All-cause and cause-specific mortality and heart rate in the general population.** = total mortality, = coronary heart disease, = other deaths, = cancer, = stroke. All-cause mortality is 2- to 3-fold higher in subjects with heart rates higher than 90 bpm compared with those with heart rates <60 bpm. Similarly, coronary heart disease mortality is about twice as high in subjects with heart rates >90 bpm compared with subjects with heart rates <60 bpm.

Based on data from reference 4.
vascular and coronary heart disease mortality simply be attributable to the higher likelihood of hypertension among tachycardic patients?

This important question has been extensively addressed in at least 8 large epidemiological studies published between 1993 and 1999 involving about 172,000 patients (over 43,000 hypertensive subjects and over 129,000 normotensive subjects). These studies, as well as other large epidemiological studies, have clearly and conclusively established the independent contribution of heart rate as a cardiovascular risk factor after adjustment for the effects of a number of known coronary risk factors, particularly age and blood pressure. A high heart rate is a marker for a higher overall cardiovascular risk profile (including hypertension, elevated lipids, obesity, and diabetes mellitus, among others) in a large number of epidemiological studies (Table III). However, the association of heart rate with cardiovascular and all-cause mortality remains statistically significant and clinically relevant after all known coronary risk factors have been appropriately adjusted for.

WHY IS HEART RATE A RISK FACTOR IN THE GENERAL POPULATION?

Heart rate may have direct or indirect effects on cardiac function. Lowering heart rate may have the following important direct cardiac effects.

Decrease in myocardial oxygen demands

Heart rate is a key determinant of myocardial oxygen consumption (MVO₂). It is the most easily measured and one of the most readily modifiable of all known determinants of MVO₂. If all other determinants of MVO₂, including blood pressure, ventricular chamber size, wall thickness, and myocardial contractility remain constant, lowering heart rate alone may favorably increase the ischemic threshold.

Increase in coronary blood flow by increasing diastolic filling time

Diastolic filling time as a percentage of the cardiac cycle increases as heart rate decreases. Since coronary blood flow occurs almost exclusively during ventricular diastole, an increase in diastolic filling time should result in an increase in coronary blood flow. Thus, even in the absence of atherosclerotic coronary artery disease, marked increases in heart rate alone may result in myocardial ischemia. This is particularly meaningful clinically in patients with long-standing hypertension and elderly subjects who are substantially more likely to have developed compensatory left ventricular hypertrophy. In the presence of left ventricular hypertrophy with an inherent increase in myocardial oxygen demands, any decrease in diastolic filling time would have an even greater adverse effect on the balance of myocardial oxygen supply and demand.

Increase in ventricular fibrillation threshold

Experimentally, ligation of a coronary artery in an open-chest dog may cause ventricular fibrillation and sudden arrhythmic death. Reducing heart rate with BBs (prior to the experimental ligation) prevents ventricular fibrillation in these animals. Similarly, BBs have been shown to reduce the occurrence of...
ventricular tachyarrhythmias in humans with acute myocardial infarction. Early β-adrenergic blockade reverses the propensity to develop ventricular fibrillation in animals as well as in humans and may explain the favorable effect of BBs in patients with myocardial infarction. Furberg et al reported a much greater reduction in mortality with BBs in myocardial infarction patients with ventricular fibrillation or tachycardia compared with those with uncomplicated myocardial infarction. This observation, coupled with the decrease in the incidence of ventricular arrhythmias with BBs, supports the conclusion that an increase in ventricular fibrillation threshold may, at least in part, explain the favorable effect of BBs on myocardial infarction mortality. This may readily explain the higher risk associated with high heart rates and the favorable effects of lowering heart rate with BBs in survivors of a clinically recognized myocardial infarction. This may also explain the higher risk of sudden death associated with faster heart rates in hypertensive patients and in elderly subjects with prior clinically unrecognized—so-called silent—myocardial infarction.

Antiatherogenic effect

Heart rate may be an important factor in the pathogenesis of coronary atherosclerosis. Several findings in experimental animals support a direct antiatherogenic effect of a lower heart rate (whether spontaneous or pharmacologically induced):

- Coronary atherosclerotic lesions in primates with a low resting heart rate are one third the size of lesions in primates with a high heart rate despite similar blood pressures, serum lipids, and body weights.
- Slowing heart rate with propranolol in primates is associated with reduced progression of atherosclerosis, independent of lipid levels.

Similarly, heart rate is correlated with severity of coronary atherosclerosis in patients surviving myocardial infarction at a young age. The exact mechanism of this antiatherogenic effect of slowing heart rate is unknown. However, it has been hypothesized that heart rate changes may cause alterations in the velocity and direction of blood flow, which may have an important effect on the pathogenesis of atherosclerosis. Alternatively, heart rate may have important indirect effects that affect cardiac function.

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<th>Publication year</th>
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<td>Framingham Heart Study</td>
<td>4530</td>
<td>HT</td>
<td>1993</td>
</tr>
<tr>
<td>British Department Of Public Health</td>
<td>7735</td>
<td>NT</td>
<td>1994</td>
</tr>
<tr>
<td>University of Pavia, Italy</td>
<td>8811</td>
<td>8115 NT</td>
<td>1997</td>
</tr>
<tr>
<td>Finnish National Public Health Institute in Helsinki</td>
<td>3386</td>
<td>NT</td>
<td>1997</td>
</tr>
<tr>
<td>Centre d’Investigations Préventives (IPC) in France</td>
<td>100 000</td>
<td>NT</td>
<td>1999</td>
</tr>
<tr>
<td>Italian TensioPulse Study</td>
<td>38 145</td>
<td>HT</td>
<td>1999</td>
</tr>
<tr>
<td>Toulouse France Study</td>
<td>1175</td>
<td>NT</td>
<td>1999</td>
</tr>
<tr>
<td>Belgian Nationwide Survey</td>
<td>9177</td>
<td>NT</td>
<td>1999</td>
</tr>
<tr>
<td><strong>Overall</strong></td>
<td>129 588</td>
<td>normotensives and 43 371 hypertensives</td>
<td></td>
</tr>
</tbody>
</table>

Table III. Epidemiological studies assessing the relationship between heart rate and blood pressure in hypertensive and normotensive subjects. HT, hypertension; NT, normotension.

Poor health and/or physical fitness

High heart rate may be an index of poor physical fitness or poor overall health. It is well known that poor physical fitness may result in higher coronary and cardiovascular death rates. Resting heart rates are generally higher in physically de-conditioned and unfit individuals.

Autonomic nervous system abnormalities

A high resting heart rate may indicate increased sympathetic nervous system activity, reduced vagal activity, or both. In experimental studies, these factors have been shown to lower the threshold for ventricular fibrillation and may mediate the detrimental effects of higher heart rate and the beneficial effects of lower heart rate on cardiovascular morbidity and mortality.
SUMMARY

There is compelling evidence of a clinically meaningful and statistically significant association between heart rate and clinical outcome in the general population as well as in hypertensive patients and the elderly. At least 20 large epidemiological studies in over 288,000 subjects published in the last two decades provide compelling evidence supporting the important role of heart rate as a risk factor for cardiovascular mortality independently of any other well-established cardiovascular risk factor. This is particularly interesting in view of the widely divergent effects of cardiovascular strategies such as heart rate–lowering CCBs and BBs, which reduce cardiovascular mortality in survivors of myocardial infarction,27,38-41 and CCBs that do not lower heart rate, such as nifedipine, which increase mortality.42 The wider recognition of heart rate, a new easily measured cardiovascular risk factor, may help clinicians identify patients at an especially high risk for cardiovascular disease and target these high-risk subjects with cardiovascular therapies specifically designed to reduce heart rate. Future research should attempt to validate heart rate as a primary target for cardiovascular pharmacologic therapies in patients with, or at risk for, cardiovascular disease in large-scale prospective controlled clinical trials.

REFERENCES


Is heart rate a prognostic factor for cardiovascular disease?

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Many sophisticated techniques are employed in order to diagnose cardiovascular disease and assess long-term prognosis. It may be that the simple measurement of resting heart rate can provide useful prognostic information. Epidemiological studies show that a resting tachycardia is associated with increased risk of cardiovascular disease and sudden death. Elevated heart rate is also highly and independently predictive of 6-month and 1-year mortality following acute myocardial infarction. This article reviews some of these studies and supports the view that measurement of the heart rate at rest should be an integral and routine part of the clinical examination and that it should have a role in determining patient prognosis.

Keywords: heart rate; epidemiological study; patient prognosis; screening; hypertension; post–myocardial infarction

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and changes in heart rate during exercise in individuals who are candidates for coronary angiography. How useful is resting heart rate prospectively in the healthy population and in those with a high likelihood of, or with confirmed CAD? In other words, is resting heart rate a useful clinical marker?

**“NORMAL” HEART RATE**

Among mammals, there is an inverse relationship between heart rate and life expectancy, that is, generally speaking, the faster the heart the shorter the life span. For example, the giant (Galapagos) tortoise, with a heart rate of 6 beats per minute (bpm) can live up to 177 years, while a mouse, which ticks over at about 500 bpm, probably has a life expectancy of 1 year. Humans are said to have “stretched the boundaries of biology” to achieve a life expectancy of around 80 years. But what is a normal human heart rate? Normal sinus rhythm is generally regarded to range between 60 and 100 bpm, and is usually between 70 and 80 bpm. Epidemiological studies show that a high resting heart rate is associated with an increased risk of development of hypertension, CAD, and sudden death (see reviews in references 7, 8, and 9), and that it may be sensible to redefine the threshold of tachycardia to around 85 bpm. Some of the more pertinent epidemiological studies will now be reviewed.

**EPIDEMIOLOGICAL STUDIES OF HEART RATE**

A number of studies have enrolled and followed healthy men and women over a course of years.

**The Framingham Study**

The Framingham Study, which began in 1948, is probably the longest running prospective study of cardiovascular disease. Heart rates were determined at rest in all 5209 men and women entering the study. Analysis of the “heart rate effect” over 30 years in 5070 subjects, free of cardiovascular disease at entry, shows that rates tended to increase with age in both sexes, with rates in women exceeding those in men by 3 bpm. About 5% of heart rates were >95 and <60 bpm. While overall mortality increased progressively with resting heart rate (with no indication of “safe” or “hazardous” thresholds) in both young and old, the number of deaths at any heart rate was greater in men than in women. There was also a substantial excess in noncardiovascular death in the Framingham Study, and the authors commented that heart rate may be a nonspecific measure of health and mortality rates.

A further analysis from Framingham looked at 2037 male and 2493 female participants who, at the time of examination, had a blood pressure level which exceeded 140/90 mm Hg. Over 36 years’ follow-up, some 565 men and 367 women died from cardiovascular causes. Elevated blood pressure was positively correlated with heart rate. Adjusted for age and blood pressure, each 10-bpm increase in heart rate was associated with a 20% increase in overall mortality and a 14% increase in cardiovascular mortality in both sexes. Coronary mortality increased by 16% in men and 12% in women. Adjusting for coexisting risk factors and excluding those who died in the first 4 years of hypertension onset to eliminate those with rapid heart rates due to poor health did not substantially change the results. Increased heart rate, therefore, appears to be an independent risk factor for cardiovascular and coronary mortality in hypertension, and the effect is stronger for fatal than nonfatal events.

**The British Regional Heart Study (BRHS)**

This large prospective study of middle-aged British men followed 7735 men aged 40 to 59 years drawn at random from age-sex registers in general practices, for a period of 8 years. It showed that, in men without preexisting ischemic heart disease, there was a strong positive association between resting heart rate and age-adjusted rates of all major ischemic heart disease events (fatal and nonfatal), ischemic heart disease deaths, and sudden cardiac deaths (Figure 1, see next page). This association remained significant even after adjustment for age, systolic blood pressure (SBP), cholesterol, smoking, social class, heavy drinking, and physical activity, with a particularly high risk in those with heart rates >90 bpm. The increased risk in those with sinus tachycardia was five times higher than in those with heart rates <60 bpm.

**Coronary Artery Risk Development In young Adults (CARDIA) study**

The Coronary Artery Risk Development In young Adults (CARDIA) study examined whether baseline heart rate predicts subsequent blood pressure independently of baseline blood pressure. This US longitudinal study involved 4762 black and white men and women between 18 and 30 years without any history of cardiovascular disease, in whom baseline heart rate and blood pressure measurements were performed and repeated at 2, 5, 7, and 10 years. Heart rate was shown to be an independent predictor of diastolic blood pressure (DBP) over the next 10 years in white men and women and black men, regardless of initial blood pressure and other potential confounders, with a 0.7-mm Hg increase per 10 bpm.
Is heart rate a prognostic factor for cardiovascular disease? - Purcell

Thus, a high heart rate was considered a risk factor for development of high DBP in young adults. The authors suggest that some individuals who develop hypertension have increased sympathetic tone (manifested by higher heart rates prior to blood pressure elevation), which can lead to smooth muscle cell proliferation, with subsequent reduced compliance of the peripheral vasculature and, consequently, raised DBP.

Centre d’Investigations Préventives et Cliniques (IPC) Study

A larger study\textsuperscript{14} conducted at the Centre d’Investigations Préventives et Cliniques (IPC) in Paris assessed the effects of high heart rate on mortality in different subgroups in a “relatively low-risk” French population according to age, gender, and blood pressure levels. They studied 19,386 subjects (12,123 men and 7,263 women) aged 40 to 69 years undergoing routine health checks. Heart rates (HR) were divided into four groups (HR1 < 60 bpm; HR2 = 60-80 bpm; HR3 = 81-100 bpm; HR4 > 100 bpm), and mortality was recorded over the subsequent 20 years. Heart rate was shown to be an independent predictor of all-cause mortality for both genders and for cardiovascular mortality in men, the explanation for which “remains unclear.” Higher heart rate was associated with higher blood pressure and plasma cholesterol. In men, the relative risk for cardiovascular death after adjustment for age and other risk factors, in the HR2, HR3, and HR4 groups was 1.35, 1.44, and 2.18, respectively, when compared with HR1. Heart rate did not influence cardiovascular mortality in women, which may be explained by the relatively small number of cardiovascular deaths in the female population.

Spandau Health Test

The Spandau Health Test\textsuperscript{15} involved a survey among 1827 men and 2929 women aged 40 to 80, followed for 12 years, but in total some 6410 participants had at least one primary health record and a follow-up. Participants were examined on average three times. An almost linear increase in all-cause mortality with initial heart rate was observed for men. An increase in the age-standardized all-cause and cardiovascular mortality rates (Figure 2) was also seen in men, but only in 60-to-80-year-old women, whereas in women with heart rates < 70 bpm no cardiovascular mortality was noted. Again, low mortality rates are probably responsible for the absence of an association between heart rate and overall cardiovascular death in women. No significant association between heart rate and cancer was seen. The authors commented that the observed difference of about 3 to 5 bpm between former East Germany (which has higher mortality) and West Germany may partly explain the discrepancies in life expectancy between the two parts of Germany.

Cardiovascular Study in the ELderly (CASTEL)

The Cardiovascular Study in the ELderly (CASTEL) investigated whether a high heart rate is associated with mortality in elderly men and women.\textsuperscript{16} The study was carried out in 763 men and 1,175 women aged 65 or older, with a 12-year follow-up. It showed that an elevated heart rate is a strong predictor of cardiovascular death in elderly men. Its predictive power is greater than that of the classic risk factors. This effect was greater in men, but the association was not statistically significant in women. The authors
suggest that heart rates >80 bpm should be “considered hazardous” in elderly men.

**POST–MYOCARDIAL INFARCTION PATIENTS**

Hjalmarson et al\(^1\) studied 1807 patients admitted with acute myocardial infarction (AMI) and the relationship of heart rate to in-hospital mortality, post-discharge mortality, and total mortality from day 2 to 1 year in patients with and without heart failure. Both in-hospital and post-discharge mortality increased with increasing admission heart rate, and total mortality from day 2 to 1 year was:

- 15% for admission heart rates 50-60 bpm;
- 41% for heart rates >90 bpm;
- 48% for heart rates >100 bpm.

Severe heart failure carried a worse prognosis, and mortality was high regardless of admission heart rate. In patients with less severe heart failure, cumulative mortality for patients with admission heart rate >90 bpm was over twice as high as those with admission heart rates <90 bpm (39% vs 18%, respectively). The Gruppo Italiano per lo Studio della Sopravvivenza nell’Infarto Miocardio II (GISSI-2) database\(^1\) allowed the reevaluation of the prognostic significance of different levels of heart rate in a large number of patients all treated by thrombolysis. Heart rate values were increasing heart rate was seen at discharge, from 0.8% for heart rates <60 bpm to 14.3% for heart rates >100 bpm. Multivariate analysis confirmed the independent prognostic significance of heart rate.

In a smaller study\(^1\) of 576 MI survivors who were followed for 2 years, both heart rate and heart rate variability were stronger predictors of mortality than ejection fraction. Further mechanistic insights were provided by another small study\(^2\) of 56 men below the age of 45 years who survived AMI, which showed that high heart rate correlated positively with angiographic scores of global severity of diffuse atherosclerosis and stenoses, independently of other risk factors.

**DISCUSSION**

Some of the many epidemiological studies that involved baseline measurement of the heart rate taken from a standard resting 12-lead ECG, ambulatory Holter ECG, or some other technique, have been reviewed. A consistent finding in these studies is that among subjects who were apparently well on entry, the resting heart rate is a sensitive independent marker of cardiovascular disease. In some studies, the relationship is more robust for men than for women, in whom (in the population studied) the disease prevalence may be lower. In males, resting tachycardia in particular has been shown to be a strong, independent predictor of cardiovascular death, which persists into old age. High heart rates are also associated with the development of hypertension and are an ominous finding in both sexes. Heart rate is also a strong predictor of mortality in men and women after AMI, and this is compounded by congestive heart failure and other complications of infarction.
It seems sensible to revise the normal limits of heart rate. It is regrettable that heart rate, even though it is simple, inexpensive, and easily measured, has become somewhat clinically neglected in favor of more sophisticated noninvasive techniques. Perhaps we should look to the wise physicians of the past (Figure 3) who held that there is no substitute for a good medical history and physical examination and who could not resort to “hi-tech” investigations.

REFERENCES


Web sites of important health organizations often provide fresh perspectives on cardiovascular diseases with respect to those offered by professional societies. The web site of the World Health Organization (WHO) is a prime example. WHO’s Programme on Cardiovascular Diseases includes various strategies aimed at the prevention, management, and monitoring of the disease at a global level, and these are extensively described at www.who.int. The section on cardiovascular diseases is found under Health Topics among the list of Noncommunicable Diseases, and can easily be accessed at www.who.int/ncd/cvd/index.htm. This represents an enormous source of epidemiological data and facts on the impact of heart disease on health. The home page defines cardiovascular disease, and describes its risk factors and social and economic consequences. There are several subsections, including a Publications area, where it is possible to download an impressive amount of material on the subject found in WHO publications, and, under Country Profiles, the powerful Global Cardiovascular Infobase. The section entitled News provides comprehensive information about important selected topics. At the end of your visit, you will understand why, I quote, “heart disease has no geographic, gender or socio-economic boundaries.” A great web site!

The US National Institutes of Health, through the National Library of Medicine, has developed www.clinicaltrials.gov to provide patients, family members, and members of the public with current information about clinical research trials. This web site currently contains information on approximately 5200 clinical studies sponsored primarily by the National Institutes of Health and other US federal agencies. The powerful search engine can be utilized to find the needle in the haystack of available knowledge; the database can be interrogated using keywords or browsed by conditions. A similar web site would be useful in Europe!

The Internet is rapidly changing and new ways of communicating are constantly being developed. A webring is a series of linked sites about the same subject: here’s one dedicated to humor about science or scientists. In this ring, you can find out which ancient language gave rise to the chemical nomenclature, what happens if you eat cesium, why you should NOT allow your children to study chemistry, what is the newly discovered evolutionary transition between the car and the airplane, how NOT to write a scientific paper, and many other things. For more information or to submit material, go to http://www.xs4all.nl/~jcdverha/scihum/webring.html. This site is well worth a visit for scientists or physicians needing a break from a frantic day!

All sites accessed 24 May 2001
The path leading to the discovery and development of the first angiotensin-converting enzyme (ACE) inhibitor, captopril, as a valuable agent in the management of cardiovascular disease provides a typical example of the complexities and uncertainties that surround novel drug research. The trail starts in 1898 with Tigerstedt’s discovery of the pressor effects of a renal extract, renin, which was able to constrict resistance vessels in the arterial tree without altering cardiac output. He subsequently showed that renal extracts caused a marked pressor effect in nephrectomized animals. However, other investigators in the field were unable to repeat his observations, and so he abandoned studies on the action of renin. The reason for the failure to replicate Tigerstedt’s experiments is probably that the renin in the extracts of other investigators was easily destroyed by keeping it at room temperature with or without the additional effects of bacterial contamination.

Little further interest was shown in the biological effects of renal extracts until 1934 when Goldblatt showed, in dogs, that clipping the renal artery raised blood pressure with a hemodynamic profile similar to that in human hypertension. This was associated with an increase in plasma vasopressor activity. When plasma renin levels were subsequently measured in patients with essential hypertension, it was found that, in contrast to Goldblatt’s experiments, the plasma renin levels were normal or low. They were elevated in less than 10% of patients, and so Goldblatt’s renin hypertension hypothesis was rejected (Table I).

The field of renin and essential hypertension was confounded for over 30 years from 1950 onwards by methodological problems in measuring renin in human plasma. One immunoassay (Haber) did not control pH adequately, leading to a lack of sensitivity and accuracy. An alternative assay, in which plasma was routinely acidified, led to conversion of prorenin to active renin, so that small differences in the in vivo levels of renin were submerged by the in vitro formation of excess renin. Subsequently, an appropriate assay was developed.8

EARLY STAGES OF ACE RESEARCH

There are three interrelated research themes that led to the discovery of specific ACE inhibitors (Table I). These are: (i) the discovery of bradykinin in snake venom; (ii) the discovery of angiotensin I and II; and (iii) the discovery of carboxypeptidase-mediated conversion of angiotensin I to angiotensin II, as well as the degradation of bradykinin.

BRADYKININ

Snake poisons were known to cause hypotension, shock, and hypovolemic death. In 1949, Rocha e Silva identified bradykinin in snake venom and he proposed that this was responsible for the vascular collapse.8 Bradykinin appeared to be rapidly broken down in venom, making it difficult to study. Thirteen years later, Ferreira,9 working in the same laboratory, used metal-
binding agents to inhibit the hypothesized enzyme, carboxypeptidase, as suggested by Erdos. Not only did these agents reduce bradykinin breakdown, but, subsequently, Ferreira found that the venom itself could potentiate the action of bradykinin, which suggested that the venom contained an inhibitor of the degrading enzyme. Two years later, Ferreira was then working in Vane’s laboratory in the Royal College of Surgeons, London, examining the disappearance of bradykinin from vascular beds, including the pulmonary circulation. Bradykinin was rapidly destroyed in a single passage through the lungs. He subsequently isolated 22 protein fractions from the snake Bothrops jararaca (Figure 1), and eventually isolated and synthesized a small peptide (BPP5A), which not only prolonged the action of bradykinin in the circulation, but also increased the amount of angiotensin I that had to be administered to raise blood pressure in anesthetized rats. At the same time, Bakhle, also working in Vane’s laboratory, showed that the original bradykinin-potentiating factor (BPF) of Ferreira was a competitive inhibitor of the enzyme that converted angiotensin I to angiotensin II in dog lung and also protected bradykinin against inactivation.

**ANGIOTENSIN**

The generation of a pressor substance by the action of renin on plasma was described in 1939 and 1940, both by Page and Helmer, working in Indianapolis City Hospital, and Braun-Menendez from the Institute of Physiology in Buenos Aires. Page called the pressor substance angiotonin and Braun-Menendez called it hypertensin. This dual nomenclature caused continuing confusion among workers in the field and was only resolved by renaming the subsequently characterized peptide as angiotensin in 1958.

Pivotal studies by Skeggs et al., working in Cleveland from 1950 onwards, showed that the dialysate of plasma from patients with malignant hypertension contained hypertensin, but only some patients with essential benign hypertension had hypertensin in the plasma. During their studies, these authors made a vital, serendipitous, discovery. In preparing hypertensin from blood, the purification...
The discovery ACE inhibitors

The discovery ACE inhibitors

The procedure involved dialysis against distilled water. For a reason that has never been clear, in one experiment, dialysis was against 1.05 mol/L sodium chloride. The material resulting from this incubation had a very different distribution pattern from the usual distribution curve. Skegg’s et al concluded that hypertensin was present in plasma in two forms, and that one was converted to the other by ACE, which is a chloride-activated enzyme. They showed that when hypertensin I is injected into the perfused rat kidney, there was no change in pressure, whereas hypertensin II caused a marked rise in pressure. In the same time frame, Page’s group in Cleveland described the structure of angiotensin II obtained from hog plasma, and Elliott and Peart, in London, described the structure of hypertensin isolated from bovine plasma. The discussion in the paper of Skegg’s et al, 17 1956, contains some prophetic remarks. Despite the general view that renin was not important as a cause of essential hypertension, they wrote:

It is of paramount interest to discover a therapeutic method of lowering the blood pressure of human beings afflicted with hypertensive cardiovascular disease. Owing to the knowledge now available, concerning the structure of hypertensin I, hypertensin II, and the converting enzyme, it becomes possible for the first time to approach this problem upon rational grounds. It may now be possible to discover and protect by structural analogues the bond which the enzyme renin dissociates when hypertensin I is formed from renin substrate. It may also be possible to prevent the formation of hypertensin II from hypertensin I by the converting enzyme, for example, by providing a structural analogue of the phenylalanyl-histidyl-leucine bond. Finally, it may be possible to prevent the vasoconstrictive action of hypertensin II upon smooth muscle.

Over the past 25 years, all three approaches have been explored by different research groups within the pharmaceutical industry. Subsequently, Skegg’s group spent much time exploring approaches to finding an inhibitor of renin.

TEPROTIDE AND CAPTOPRIL

In 1968, Vane, a consultant for Squibb, had just published two key papers showing that angiotensin I was converted to angiotensin II when passing through the canine lung, and speculated that the enzyme responsible for the conversion was a carboxypeptidase. He met with the Director of Pharmacology, Horowitz, Head of Peptide Chemistry, Ondetti, and Cushman, a gifted biochemist. Based on that discussion, it was decided to mount a speculative research program in order to find an inhibitor of ACE with potential application in essential hypertension. This was a courageous decision for several reasons. Despite Goldblatt’s demonstration in 1934 that renal artery clip hypertension in dogs had the same hemodynamic profiles as patients with essential hypertension, studies in humans suggested that blood renin values were mostly either normal or low and that only a minority had truly high values. With few exceptions, most investigators assumed that renin had nothing to do with the etiology of most forms of essential hypertension. The basis for such opinions is exemplified by Gross, of Heidelberg, an authority on the renin-angiotensin system, who concluded that the renin-angiotensin system had little, if any, significance in the pathogenesis of essential hypertension and that interference with the renin-angiotensin system would not lead to new antihypertensive drugs. He based his opinion on the fact that angiotensin II antagonists such as saralasin caused vasoconstriction and only lowered blood pressure when patients were in negative sodium balance, and that the ACE inhibitor teprotide had to be given by injection.

The research program in Squibb made rapid progress for two reasons. Firstly, Cushman developed a spectrophotometric assay that permitted careful structure–function studies on any peptides made by Ondetti. Secondly, the chemistry program could be based on the published findings of Ferreira et al, who had shown that snake venom contained inhibitors of the breakdown, not only of bradykinin, but also angiotensin I. The Squibb group was the first not only to characterize, but synthesize, specific peptide inhibitors isolated from snake venom. One of them, SQ20881, was shown to be the same as the bradykinin-potentiating factor described by Ferreira. This nonapeptide was called teprotide and taken forward for human studies. In 1972, Laragh’s group used teprotide to undertake a series of studies of its effects in animals and humans. This group was the first to show that inhibition of ACE with teprotide could reduce blood pressure in patients with hypertension. Structure–function studies showed that the minimum requirement for inhibition was a tripeptide, Phe-Ala-Pro and its acylated derivatives. Attempts to discover an orally active inhibitor led to repeated failures, and even random screening of 2000 compounds did not provide any lead. So, in 1973, the ACE-inhibitor project was stopped by the research directors. One of the factors influencing the decision to stop the ACE-inhibitor program was that the provisional sales estimates for an orally active ACE inhibitor were $20 million annually. The current sales exceed several billion dollars.

In March 1974, Cushman read a paper by Byers and Wolfenden on carboxypeptidase inhibitors, showing that benzylsuccinic acid was a potent inhibitor. Horowitz called a meeting with Ondetti and Cushman, and it was decided to make some custom-designed molecules without official sanction. The then Director of Pha-
macology, Goldberg, recollects that, as he was entering a meeting room to give a strategic research presentation to the senior management, the Vice-President of R&D said: “Do not discuss anything about the ACE work, these guys have been told the program is dead and buried.”

Cushman and Ondetti firmly believed that they could achieve an orally active ACE inhibitor based on drug design considerations. They speculated that the compound, α,δ-methylsuccinyl-L-proline would be active, and this proved to be the case. Based on theoretical considerations, Ondetti and Cushman also wanted to make a sulfhydryl-substituted analogue. From the chemical synthesis point of view, this was very difficult to do. Progress was only made possible by the chance reading of a biochemical product brochure showing that there was a new reagent that would make it much easier to introduce sulfhydryl groups on proteins. Using this new reagent, a compound, 3-mercaptopropanoyl-L-proline, was made, which was 3000 times more potent than the initial lead compounds and was active orally as well as intravenously. The α-methyl analogue was named captopril. By 1976, studies in human volunteers showed that 1 to 10 mg orally could block the pressor effects of angiotensin I for several hours. Captopril was taken into full clinical development, and after following several blind alleys in the clinical studies, it was eventually shown that low doses of captopril were safe and efficacious in controlling blood pressure in 50% to 60% of hypertensive patients. In April 1981, captopril was approved for the treatment of heart failure. Finally, in July 1985, it was approved for treatment of all types of hypertension.

The publication of the hypotensive effects of teprotide, in man, triggered the interest of many other pharmaceutical companies, especially Merck in the US and Servier and Hoechst in Europe. The Merck research program started in 1974, but it was 5 years before enalapril and lisinopril were identified. The target was to identify a compound with a much longer duration of action than captopril and without sulfhydryl groups, since it was believed that some of the side effects (rashes, taste alteration) were due to this substituent. In 1981, it was shown that enalapril maleate (Vasotec), 20 to 40 mg once daily, controlled blood pressure. I had twice the systemic bioavailability of the analogue lisinopril. Enalapril maleate was marketed in 1985 and lisinopril in 1987. By 1990, perindopril (Servier) and ramipril (Hoechst) had been marketed and there were 30 other ACE inhibitors in clinical development.

| 1967 | • Squibb peptide research redirected by Welch from gastrointestinal hormones |
| 1968-70 | • Vane suggests ACE as a target  
• Horowitz = Biology Director, Ondetti = Chemist, Cushman = Biochemist at Squibb  
• Cushman devises spectrophotometric assay for ACE  
• Ferreira et al isolate inhibitory peptide from Bothrops jararaca snake venom (<Glu-Lys-Trp-Ala-Pro)  
• Ondetti et al confirm and synthesize 6 specific peptide inhibitors, of which SQ20881 = Ferreira’s bradykinin-potentiating factor (BPFLa) = TEPROTIDE  
• Erdö shows bradykinin and angiotensin I are identical substrates for ACE |
| 1971-72 | • TEPROTIDE lowers blood pressure in Goldblatt rat model of hypertension as does 1st angiotensin II antagonist saralasin |
| 1973 | • Collier, Robinson, and Van show in humans that TEPROTIDE inhibits angiotensin I rise in blood pressure |
| 1974 | • Laragh’s group show TEPROTIDE lowers blood pressure in hypertensive patients  
• Search for oral TEPROTIDE fails after 2000 compounds are tested  
• Cushman sees Byers and Wolfenden paper on carboxypeptidase inhibitor  
• Ondetti agrees to make analogues of succinil-L-proline in antibiotic program! |
| 1975 | • Chance information that a new reagent (propiothiolactone) enhances synthesis of sulfhydryl groups on proteins  
• 3-mercapto propanoyl-L-proline found to be 3000 times more potent as ACE inhibitor: α-methyl analogue was CAPTOPRIL |
| 1976 | • CAPTOPRIL lowers blood pressure in volunteers |
| 1981 | • CAPTOPRIL marketed |
| 1981 | • CAPTOPRIL gets chronic heart failure indication |
| 1991 | • CAPTOPRIL shown to decrease chronic heart failure morbidity |

Table II. Angiotensin-converting enzyme (ACE) inhibitor program.
CONCLUSION

The history of the discovery of the ACE inhibitors provides interesting examples of certain general principles in speculative drug research. One of the more important principles is that most breakthroughs in therapeutics are preceded by lengthy incubation periods. The 1956 paper by Skeggs et al. laid out the target options for modulating the renin-angiotension system, but it was 25 years before captopril was marketed. Another feature is the need for persistence and commitment from drug researchers so that, even when the projects are stopped because of lack of progress, attention remains focused on solving the problem some time, even when working on a different therapeutic target. The relevance of the Byers and Wolfenden therapeutic target. The relevance of time, even when working on a different focused on solving the problem some lack of progress, attention remains the projects are stopped because of marketed. Another feature is the need for persistence and commitment from drug researchers so that, even when the projects are stopped because of lack of progress, attention remains focused on solving the problem some time, even when working on a different therapeutic target. The relevance of the Byers and Wolfenden-2 paper was grasped by Cushman, which led to the drug design model that he and Ondetti devised. A further lesson is the critical importance of clinical scientists as exemplified by Laragh’s group in not only studying the pathophysiology of disease, but also having a willingness to undertake proof-of-concept studies to validate novel drug target concepts. The award of the Lasker prize to Ondetti, what we need, and what we expect, and also having a willingness to undertake proof-of-concept studies to validate novel drug target concepts. The award of the Lasker prize to Ondetti and Cushman not only studying the pathophysiology of disease, but also having a willingness to undertake proof-of-concept studies to validate novel drug target concepts. The award of the Lasker prize to Ondetti and Cushman († in 1999) provides dilatory, but welcome, recognition of their great contribution to cardiovascular therapeutics.

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The architecture of the sinus node, the atrioventricular conduction axis, and the internodal atrial myocardium

R. H. Anderson, S. Y. Ho


It is well established that, following initiation of the impulse in the sinoatrial (S-A) node, propagation through the atrial musculature is asymmetric, demonstrating regions of fast and slow conduction. Anatomists and pathologists have long argued whether there is a fixed anatomical substrate that underlies this functional difference. With the increasing therapeutic use of catheter ablation to introduce precisely localized lesions within the atrium, this at times arcane debate has taken on new relevance. For this reason, Anderson and Ho readdressed the issue in 1998.

Unlike in the ventricle, where the specialized conducting tissue is separated from the ventricular myocardium by insulating sheaths of fibrous tissue, the atrioventricular (A-V) and S-A nodes are not electrically isolated from the atrial musculature. As such, they do not fulfill the criteria of a conducting tract. Anderson and Ho state that transitional cells from the S-A node extend for only short distances out from the node, where they interdigitate with atrial myocytes of the terminal crest. There are no histologically prominent extensions from the node into working atrial muscle, and no insulated tracts arising from the S-A node that are equivalent to those extending from the A-V node to the ventricular musculature. Thus, there are no histologically identifiable conducting tracts between the S-A and A-V nodes, isolated from surrounding myocardium by insulating fibrous tissue. Rather, they argue, any preferential conduction probably reflects the specific cellular electrophysiologic properties and interconnections of the cells lying between the nodes. Near the A-V node, in the region of the triangle of Koch, there is a zone of transitional cells that joins the A-V node from multiple directions. These cells are intermediate in morphology between those of the compact node and the working atrial myocardium, making the precise definition of the extent of this region difficult.

The debate on the existence of specialized internodal conducting paths within the atrium has been going on since the discovery of the nodes, but in modern times probably since the studies of James in the early sixties. Despite the thorough review of the issue by Anderson and Ho, it is unlikely that the issue will be considered entirely resolved, nor should it. This is because there is no question that asymmetric conduction exists, and there must therefore be an underlying basis for that asymmetry. The actual question we should focus on is not whether discrete anatomical pathways of specialized conducting tissue exist—the prevalence of evidence suggests they do not. Rather, the question we should focus on is, what is the structural, cellular, and molecular basis of the asymmetric conduction? To what extent does it reflect regional disparity in fiber packing and orientation, gap junctional connections, specific ionic currents, and/or neural input? Depending on the answers, we also will gain insight into the associated important question of whether the asymmetry and preferential conduction are fixed or variable, and if variable, what factors impact on the conduction pathways.

1998

Mika Häkkinen beats Michael Schumacher to win the Japanese Grand Prix and the Formula One World Championship;

Bob Kane, cartoonist and creator of “Batman,” dies aged 83;

and President Mugabe of Zimbabwe orders the seizure of 841 white-owned farms
Early in this century, anatomical and electrical studies suggested that the sinoatrial (S-A) node was the site of origin of the heartbeat (see Keith and Flack [1907] and Eyster and Meek [1921] summaries in this section). However, even half a century later there remained questions as to whether there was a single fixed origin and whether the impulse propagated out from this site along a fixed or constant pathway. In attempting to address these questions, the study by Boineau and colleagues was the first to apply the technique of simultaneous multiple electrode mapping to the in situ canine atrium.

This study employed an array of over 100 bipolar electrodes to construct isochronal maps of atrial activation, while perturbing heart rate with either vagal stimulation or autonomic agents. The agents most often used were isoproterenol to increase heart rate, and propranolol to decrease heart rate. They focused explicitly on modest P-wave changes and changes in initiation site within and near the node, rather than perturbations associated with changes in the P axis or PR interval. They first confirmed that, for a constant P-wave morphology, the activation sequence through the atrium was also constant, and each distinct P wave was associated with its own activation pattern. They also found that when heart rate changed, so did the activation pattern in the atrium and the corresponding surface P wave. Thus, their data demonstrated a reliable correlation between the surface P wave and atrial activation pattern, confirming that one could infer changes in the latter from the former. Control experiments ruled out the possibility that the changing activation pattern was secondary to an effect of their perturbation (ie, vagal stimulation or infused agents) on local conduction in the atrium. Rather, these changes resulted from abrupt shifts in the point of earliest activation, which shifted between several fixed sites surrounding the superior vena cava, some of them outside the defined sinus node. However, even when some of these sites activated with a delay relative to the first, they were frequently surrounded by even later activation times. This suggested, in the words of Boineau et al, that these sites continued to be “points of depolarization origin.” Since they were only mapping the epicardial surface, they could not definitively distinguish between two possibilities: either a distributed (ie, multifocal) pacemaker, or multiple specialized pathways arising from a single focus, but exiting at distinct epicardial locations.

By using a fixed, reproducible grid of closely spaced electrodes in large numbers, Boineau et al achieved a degree of resolution that permitted them to simultaneously directly measure from multiple origin points—something earlier investigators had been unable to accomplish. This allowed them to argue against a “wandering” or migrating pacemaker. Rather, their data were more consistent with the existence of a pacemaker complex, within which the area of earliest activation could abruptly shift. The alternative interpretation of their data, namely a single focus with specialized conduction pathways exiting on the epicardium at their mapped origination points, was not well supported by the anatomical data of the time (which suggested many random connections between the S-A node and atrium, rather than a few distinct pathways). However, this issue continued to be a source of debate (see Anderson and Ho [1998] summary in this section).

In Oslo, Menachem Begin and Anwar Sadat accept the 1978 Nobel Peace Prize; India’s former PM, Indira Gandhi, is released from jail; and chanting “Allah is great,” anti-Shah protesters pour through Tehran
Voltage-clamp investigations of membrane currents underlying pace-maker activity in rabbit sino-atrial node

H. Brown, D. DiFrancesco

*J Physiol (Lond)*. 1980;308:331-351

This paper and another study appearing the same year (see Yanagihara and Irisawa [1980] summary in this section) provided the first detailed analysis of the current known as $I_f$ or $I_h$. What is particularly noteworthy about this paper is the emphasis on the relation between the activation range of this current and the diastolic potential voltage range. The authors argue for the contribution of $I_f$ to normal pacemaker function and to the effects of adrenergic agonists and temperature to increase the slope of the pacemaker potential and sinus rate. In this sense, they reach a quite different conclusion from that of the study of Yanagihara and Irisawa.

As with the study of Yanagihara and Irisawa, these authors first considered the question of whether $I_f$ is indeed a separate current system from $I_K$. They addressed this by comparing the kinetics of the two currents, demonstrating that they could be readily distinguished. They then turned to the question of the physiologic role of $I_f$. One of the more striking experiments in this study involved recording spontaneous action potentials in a small piece of sinus node tissue, then immediately voltage-clamping the same specimen and recording $I_f$ for a series of voltage steps within the measured diastolic potential range. This experiment clearly demonstrated that, at voltages within the normal diastolic potential range, $I_f$ was activated with a magnitude and time course that was consistent with a direct contribution to diastolic depolarization. The authors went on to demonstrate that norepinephrine, which accelerates spontaneous rate in the sinus node, also increased $I_f$ when measured at voltages in the diastolic potential range (subsequent studies have shown that this effect on $I_f$ results from a shift of the activation voltage range toward less negative potentials, rather than an increase in conductance). They similarly showed that an increase in temperature, another positive chronotropic factor, increased both the slope of the diastolic depolarization and $I_f$. In addition, while they did not fully explore it in this study, they reported an effect of elevated external potassium to increase the current; subsequent studies have demonstrated a potassium dependence for $I_f$ conductance. The final portion of the paper, not central to the present discussion, focused on a possible calcium dependence for $I_h$, thereby providing a mechanistic linkage between the slow inward calcium current and the delayed rectifier potassium current.

This paper is significant because it was the first to lay out the arguments, in detail, for $I_f$ to be the major current contributing to the pacemaker potential in the mammalian sinus node, as well as for it being the explanation for regulation of spontaneous rate by catecholamines or temperature. As such, it suggested that $I_f$ might be the critical current in control of heart rate. The matter is still unresolved, in part because the current required to modulate pacemaker slope is exquisitely small (and therefore susceptible to errors in measurement), and in part because of the limited availability of pharmacological probes. However, now that the molecular correlate of $I_f$ has been determined (see Santoro et al [1998] summary in this section), one can hope that we may be in sight of a resolution of this contentious issue.

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1980

Steve Ptacek in Solar Challenger makes first solar-powered flight;
Sadahura Oh, legendary batter with the Yomiuri Giants, retires;
and Hollywood actor Steve McQueen dies of cancer, aged 50
A new interpretation of the pace-maker current in calf Purkinje fibres

D. DiFrancesco

J Physiol (Lond). 1981;314:359-376

Throughout the seventies, the ionic basis of the pacemaker potential responsible for diastolic depolarization and spontaneous activity in cardiac Purkinje fibers was thought to be well understood. The relevant current, $I_{K2}$, was considered a potassium current. Its deactivation time course upon repolarization, combined with a background inward current, accounted for the time-dependent increase in net inward current during diastole. However, although the apparent reversal potential of the current and its potassium dependence more or less followed the calculated reversal potential for potassium, they deviated somewhat from prediction. This was explained as being due to potassium depletion effects in the clefts immediately outside the Purkinje cells. However, this depletion effect also impacted on $I_{K1}$, a known background potassium current that could contaminate the recording of $I_{K2}$ and introduce an apparent potassium dependence even if the $I_{K2}$ channel were not entirely potassium-selective. In 1980, two reports appeared detailing the existence of a time-dependent inward current activated on hyperpolarization in the sinus node, designated $I_t$ or $I_f$ (see Yanagihara and Irisawa [1980] and Brown and DiFrancesco [1980] summaries in this section). It was inevitable that these findings would lead to reexamination of the ionic nature of $I_{K2}$.

In 1981, DiFrancesco published the results of such a reexamination, and concluded that $I_{K2}$ was indeed $I_t$, a time-dependent inward current activated on hyperpolarization and not selective for potassium. The problem faced in this study was in distinguishing between two situations that might occur on hyperpolarization: an increasing inward current, or a decreasing outward current combined with a time-independent background inward current. He was able to demonstrate that the former situation applied by the use of somewhat selective blockers. Using a low concentration of cesium, he preferentially blocked $I_{K2}$ (i.e., $I_f$) relative to $I_{K1}$, and showed that net current shifted in the outward direction, suggesting that the current being reduced was an inward current. Then, he used barium to selectively block $I_{K1}$ and demonstrated that the remaining time-dependent current ($I_{K2}$ or $I_t$) no longer reversed near the potassium equilibrium potential. In fact it showed no reversal potential in the voltage range studied, even with external potassium as high as 48 mM. This indicated that the supposed reversal potential of $I_{K2}$ was in fact an artifact resulting from depletion of external potassium when large inward currents flowed through $I_{K1}$ at very negative potentials, and that $I_{K2}$ was not potassium-selective. Finally, he showed that during hyperpolarization in the presence of barium net membrane conductance increased, further arguing against a decreasing outward current as the explanation of $I_{K2}$.

The reason $I_t$ was first identified in sinus node is that nodal tissue has little $I_{K1}$, and so provided less contamination of $I_t$. Thus, its true nature as an increasing inward current was more apparent. However, application to Purkinje fibers was more than a trivial extension to another tissue. $I_{K2}$ in Purkinje fibers had been extensively studied and characterized, and incorporated into computer simulations of pacemaker activities, it was one of the fundamental underpinnings of cardiac electrophysiology for over a decade. Demonstrating that conventional wisdom was entirely wrong was, to say the least, unsettling. Further, various studies that depended on $I_{K2}$ being a potassium current and, for example, drew conclusions on potassium gradients based on its reversal potential, now had to be reconsidered.

1981

Reggae singer Bob Marley dies of brain and lung cancer, aged 36;
François Mitterrand defeats Valéry Giscard d’Estaing for Presidency of France;
and Andrew Lloyd Webber’s musical “Cats” premieres in London
The origin and conduction of the heart beat

J. A. E. Eyster, W. J. Meek

Physiol Rev. 1921;1:1-43

In the 40 years from 1880 to 1920, numerous electrical and histological studies considerably advanced our understanding of the initiation and propagation of the heartbeat. In 1921, Eyster and Meek summarized and synthesized those 4 decades of progress in this seminal review. They began by reviewing the anatomical literature and its contribution to identification of the sinoatrial (S-A) node as the site of impulse initiation and the atrioventricular (A-V) node as the region through which the signal propagates from atria to ventricles. However, they devoted much more attention to what were at the time recent advances using electrical methods, and in particular the string galvanometer, to identify the site of primary negativity during electrical activity. In a sense, their reliance on—and faith in—electrical measurements to provide answers to the questions of the day ushered in the era of cardiac electrophysiology.

Many of the early functional studies they reviewed attempted to demonstrate the primary function of the S-A node in impulse initiation by disturbing cardiac rhythm via local application of heat or cold, or by surgical excision. While local application of cold or excision of the node region slowed the rhythm of the entire heart, the fact that the heart continued to beat led many to conclude that the S-A node was not clinically significant. Clearly, this is an example of the dangers of reductionism. In fact, Eyster and Meek had the emphasis right in the footnote on the first page of their review, when they asked instead why the automatic region dominated the remainder of the heart and kept automaticity of these other regions in check—50 years before development of the concept of overdrive suppression (see Vasalle [1977] summary in this section). Studies beginning around 1910 (many of the key ones by Eyster and Meek), used the string galvanometer to map the region of primary negativity and, with minor variations, localized it to the general region of the S-A node. These early electrical experiments, in addition to identifying the site of impulse initiation, attempted to plot the propagation of the impulse as it spread throughout the atria. This era thus also represented the inauguration of the field of cardiac mapping. In fact, in a 1914 paper, Meek and Eyster recorded simultaneously from two sites using a pair of galvanometers, no doubt a technically heroic effort at the time, and probably the first example of simultaneous multisite mapping of the atrium. One of the conclusions from these mapping efforts was that there was no well-defined and localized path by which the impulse spread from the S-A to the A-V node—an issue that has been regularly revisited since then (see Anderson and Ho [1998] summary in this section). The remainder of the review dealt with the question of conduction in the ventricle, an area outside the focus of this overview.

When reviewing literature from 80 to 100 years ago, one does not expect the original authors to have gotten everything right. In fact, it is obvious from the articles summarized by Eyster and Meek that, even at that time, errors in observation and interpretation were present. Eyster and Meek were at times quite harsh in their criticisms of the methods, details (or lack thereof), and interpretation of contemporary investigators. Yet they were able to see through the confusion to identify some essential truths: the S-A node is the site of initiation of the heartbeat, and questions concerning electrophysiologic functionality are often best addressed by employing electrical methodologies.

1921

- Cuba’s Jose Capablanca defeats Dr Emmanual Lasker to take the World Chess Championship;
- “The Kid,” starring Charlie Chaplin and Jackie Coogan, is released;
- and Irish Free State gains independence from Britain.
When Keith and Flack undertook their extensive survey of vertebrate hearts, the site of origin of the human heartbeat was unknown. What was known, largely from studies of simpler hearts, was: "(i) that the heart's impulse is conducted by the cardiac muscle tissue; and (ii) that normally the impulse arises in the musculature of the sinus, setting the heart's rhythm, and then passes to the auricle and ventricle, finally reaching the bulbus cordis." What Keith and Flack did was to conduct a systematic and detailed anatomical study of the hearts of a range of vertebrate species (including, but not limited to, eel, dogfish, frog, turtle, sparrow, mole, dolphin, kangaroo, whale, mouse, kitten, and pony), as well as both normal and malformed human hearts, with the intent of elucidating anatomically identifiable structures in the human heart that could account for the initiation of the heartbeat in the sinus and its propagation through the atria.

One must remember that this study predated detailed electron microscopic analyses that identified the P (or pale) cells, lacking in abundant myofibrils, within the atria. Keith and Flack were working at a more macroscopic scale. In addition, although some electrophysiological studies of propagation had been made by this time, the present paper relied entirely on anatomical correlations from simple to complex hearts for its conclusions. Despite these limitations, they did a remarkable job of achieving their two objectives: to identify the region of impulse initiation based on anatomical characteristics, and to infer whether the propagation from this site to the atrial musculature is anatomically restricted, and might therefore account for sinoatrial block. With respect to the first objective, they identified a region at the sinoatrial junction that was striking for its abundant arterial blood supply and dense nerve plexus, and that contained "white" type muscle fibers (ie, pale fibers) analogous to those previously identified as composing the atrioventricular node. They concluded that this specialized area was where the heartbeat originated. With respect to the second objective, they found no evidence of restricted anatomical connections from this region to the atrial musculature, and concluded that there was most likely no fixed anatomical basis to sinoatrial block, arguing instead for a functional cause related to vagal influence.

It must be reemphasized that their conclusions were based entirely on circumstantial evidence—they carried out no functional experiments. Yet they identified and recognized the significance of a constant occurrence of specialized pale fibers, heavily innervated, that appeared in the analogous region throughout vertebrate evolution. They further recognized that studies by others associated the initiation of the heartbeat with this same general region and that the heartbeat was in fact known to be heavily influenced by neural activity. From this, they reasonably argued that they had anatomically identified the site of origin of the human heartbeat, the sinoatrial node—and in fact they were correct.

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Russian chemist Dmitri Ivanovich Mendeleyev, creator of the periodic table, dies aged 72;
English suffragettes storm British Parliament and 60 women are arrested;
and Finland becomes the first European country to give women the right to vote
to understand the time- and voltage-dependence of specific currents flowing during a cardiac action potential, one must employ the voltage clamp technique (which, by effectively making voltage a constant, eliminates one of the two variables that define the properties of individual ionic channels). Today, the most common method employed involves the use of some form of patch clamp, and is generally employed on enzymatically dissociated single cells. Prior to the development and widespread application of this approach, typical methods were either the sucrose gap or the one- or two-microelectrode voltage clamp. The sucrose gap was employed on the sinus node with mixed success (due to problems with leakage currents) in the early seventies. This study by Noma and Irisawa in 1976 was the first to use the two-microelectrode voltage clamp method on the sinus node. Success required ligating the tissue to limit it to a very small size ($\approx 0.3 \times 0.3 \times 0.3$ mm) to minimize spatial decay of voltage.

In this study, the authors first conducted a careful validation of their methodology, defining the extent of spatial uniformity of voltage, and adequacy of voltage control, in tissue specimens ligated to progressively smaller dimensions. Once they had defined a specimen size that both preserved spontaneous activity and showed adequate spatial uniformity, they conducted a survey of ionic currents present upon depolarization and repolarization. Limitations of the methodology prevented rigorous isolation of individual currents, or precise quantitative determination of their biophysical properties. However, this study still demonstrated all the key currents involved in the sinus node action potential and diastolic depolarization. They recorded a transient inward current on depolarization that they recognized as most likely analogous to the Ca$^{2+}$-dependent slow inward current of other cardiac preparations. While they were uncertain of the ion specificity of this current, they noted the absence of a fast inward current analogous to the sodium current that accounts for depolarization of the ventricular or Purkinje fiber action potential. They also recorded a delayed outward current on depolarization that they recognized as comparable to the delayed rectifier potassium current, and they studied the deactivation of this current upon repolarization and discussed the potential contribution of that deactivation to the diastolic depolarization. However, they also pointed out that while a deactivating outward current could contribute to a positive after-potential following an action potential and thus to subsequent diastolic depolarization, such a mechanism alone could not explain the initiation of spontaneous action potentials after a period of quiescence, as they observed in their specimens. In this regard, it is intriguing that they also observed a slow, time-dependent inward current upon hyperpolarization. We now know that this current, which they had difficulty explaining, is the pacemaker current $I_f$, but at the time of this study that current had not yet been identified.

Thus, this paper provided a qualitative understanding of the ionic basis of automaticity in the mammalian sinus node. The subsequent two decades of electrophysiologic and molecular research have yielded critical additional details and provided significant quantitative insights into the biophysical characteristics of the individual ionic channels involved, but have not invalidated the important general observations of this seminal study.

Membrane currents in the rabbit sinoatrial node cell as studied by the double microelectrode method

A. Noma, H. Irisawa

Pflugers Arch. 1976;364:45-52

Oil magnate J. Paul Getty dies in London, aged 83; student uprisings begin in Soweto, South Africa; and Syrian troops enter Lebanon to put down civil war
Identification of a gene encoding a hyperpolarization-activated pacemaker channel of brain


Cell. 1998;93:717-729

The cardiac pacemaker current, a time-dependent inward current active during diastolic depolarization, was originally coined $I_f$ because of its "funny" behavior. Unlike other voltage-gated channels, which open on depolarization, $I_f$ opens on hyperpolarization (a useful feature for activating the current during diastole, after action potential repolarization). As subsequent characteristics of the current were identified, it continued to earn its name as a "funny" or unique current. For example, regulation of the current by cyclic adenosine monophosphate (cAMP) occurs largely from direct nucleotide binding, rather than by the more common phosphokinase A–dependent phosphorylation (although the latter also may occur). Also, the current demonstrates a tremendous tissue- and age-dependent voltage activation range, with threshold values in the sinus node around −50 mV, but threshold values in the adult ventricle as negative as −145 mV. This ≈100-mV range in threshold for one current is without precedent. Further, the extremely negative values in the adult ventricle are well outside the physiologic range, raising the inevitable question of why an apparently nonfunctional protein is expressed.

Despite the efforts of many labs, the gene responsible for this current proved intractable to cloning until 1997. It then continued to live up to its “funny” reputation, being cloned serendipitously by a group that was not even trying. Rather, Santoro et al (in the predecessor to the present paper), using a yeast two-hybrid system to identify proteins that interacted with nSRC, identified a protein with several intriguing characteristics: (i) high-sequence homology to the family of voltage-gated potassium channels; (ii) a pore sequence that was similar to, but not identical to the prototypical potassium channel sequence ($I_f$ exhibits mixed $K^+/Na^+$ permeability); and (iii) a cyclic nucleotide–binding domain in the carboxy tail. From this, they speculated that the cloned sequence represented a member of the $I_f$ family. In their subsequent paper, discussed here, they confirm this speculation by expressing the cloned protein in oocytes and generating an $I_f$-like current that: (i) activates on hyperpolarization, (ii) is cesium-sensitive, inward, and time-dependent, and (iii) is (barely) cAMP-dependent—all characteristics of native $I_f$. They also provided partial sequences for three other members of this family and investigated isoform-specific expression in brain and heart—two tissues where the current plays an important role. Two of the isoforms (BCNG2 and BCNG3, now termed HCN2 and HCN4, respectively) were detected in all regions of the heart, consistent with prior electrophysiologic studies. In brain, BCNG1 (HCN1) showed region-specific expression, while BCNG2 was more ubiquitously expressed. BCNG3 was also detected in brain.

This paper thus opened up a new field of research that is likely to keep investigators interested in regulation of excitability busy for years to come. In fact, subsequent studies by this and other groups, in which several of the other isoforms have been expressed and tissue distribution more thoroughly analyzed, have probably succeeded in raising more questions than they have answered with respect to the molecular basis for the unique characteristics of $I_f$. For example, the dominant isoform expressed in sinus node is the slowest activating in heterologous systems, despite the native current activating more rapidly in the sinus node than in cardiac regions expressing other isoforms. This sort of anomaly is just what one might expect from a “funny” current.

Europeans agree on single currency, the euro; a powerful car bomb severely damages Florence’s Uffizi Gallery; and violence breaks out in Tibet during demonstrations against the Chinese
The relationship among cardiac pacemakers. Overdrive suppression

M. Vassalle

Circ Res. 1977;41:269-277

The sinus node is the normal site of cardiac impulse initiation, and thus serves as the dominant, or primary, cardiac pacemaker. The heart also contains secondary, or subsidiary, pacemaking tissue. Cells in these areas have a slower intrinsic rate than that of sinus node cells, and so are normally discharged by a propagated impulse from the sinus node before they can themselves reach threshold. However, the slower rate alone does not explain the behavior of the subsidiary pacemakers, which fail to intercede in response to a simple missed beat. Rather, there must be a sustained quiescent period before a subsidiary pacemaker initiates a beat, and then its rate gradually increases until reaching its intrinsic (but slower than the sinus node) rate. This is actually a very clever system, which provides a necessary safety mechanism for such an essential physiologic function as cardiac impulse initiation, yet at the same time does not overreact to every little hiccup in cardiac rhythm.

In this paper, Vassalle reviewed a century of research and explored the underlying mechanisms that control the reluctant but critical subsidiary pacemaking function of the heart. In doing so, he firmly established the phrase “overdrive suppression” in the lexicon of cardiac electrophysiology. The early history is fascinating for its moments of occasional insight, which, of course, are only recognized by us in hindsight. So although the concept that the sinus node suppresses subsidiary pacemakers specifically by means of its faster rate appeared intermittently in the early literature, as Vassalle notes, it “never became a general notion.” In the sixties, studies of atrial pacemakers determined that, if driven at rates they can follow, atrial pacemakers are suppressed, and that the sinus node was less sensitive to overdrive suppression than other regions. Further, there was an element of vagal stimulation to atrial suppression. This is not the case for ventricular overdrive suppression, so when both the sinus node and latent atrial pacemakers are suppressed by excess vagal stimulation, ventricular subsidiary pacemakers are still available to maintain the heartbeat. In this review, Vassalle discussed several mechanisms that might contribute to overdrive suppression in the ventricle, including increased external K⁺, increased intracellular Ca²⁺, and stimulation of the Na⁺/K⁺ pump. The latter mechanism is now recognized as the main contributor. When driven at a fast rate, an automatic cell in the ventricle experiences more net Na⁺ influx and K⁺ efflux than if firing at its own intrinsic rate (since there are more action potentials per unit time). This results in greater Na⁺/K⁺ pump activity, as this is the main mechanism for restoring concentration gradients. Since the pump is electrogenic, extruding 3 Na⁺ ions for every 2 K⁺ ions pumped into the cell, it generates a current that hyperpolarizes the cell and drives it away from threshold, thereby suppressing automaticity.

An ideal review article summarizes and synthesizes disparate and at times contradictory studies, using hindsight to identify the kernels of truth and more significant observations. It then focuses the reader on the remaining issues and concisely puts forth the arguments for and against various explanations of those issues. In doing so, it creates a new consensus and stimulates new research directed toward the outstanding questions. This 1977 review of cardiac pacemakers, and its formalization of the concept of overdrive suppression, meets all those criteria.

South African black student leader Steven Biko dies in police custody; the celebrated opera singer Maria Callas dies of a heart attack in Paris, aged 53; and Cambodian leader Pol Pot meets Chinese Prime Minister and Communist Party Chairman Hua Guofeng
Inward current activated during hyperpolarization in the rabbit sinoatrial node cell

K. Yanagihara, H. Irisawa

Pflugers Arch. 1980;385:11-19

Following the successful application of the two-microelectrode voltage clamp technique to sinoatrial node tissue (see Noma and Irisawa [1976] summary in this section), there were increased efforts to define the characteristics of the ionic currents present in the mammalian node. In 1980, two independent papers appeared that provided detailed analysis of the pacemaker current, referred to as either $I_h$ (this paper) or $I_f$ (see Brown and DiFrancesco [1980] summary in this section). This paper (and some others that preceded it) follows from the initial 1976 study of the same group, in which the current was evident in the data, but was not characterized or otherwise defined. The paper of Brown and DiFrancesco follows on from a short study in Nature in 1979 in which that group first identified the current as a distinct one and coined the name $I_f$.

One of the difficulties in the early identification of $I_h$ or $I_f$ was distinguishing this time-dependent inward current from the time-dependent deactivation of $I_K$ that also occurs as the membrane potential becomes more negative. In this study, Yanagihara and Irisawa used a series of protocols and external solutions to definitively demonstrate that $I_f$ was distinct from $I_K$ and represented a separate current system. By using a series of progressively more negative hyperpolarizing steps, they were able to distinguish the deactivation and activation voltage ranges of $I_K$ and $I_f$, respectively.

More convincingly, they demonstrated that they could selectively block $I_K$ with 5 mM external barium, thereby isolating $I_f$. External barium has become a standard component in all subsequent studies of $I_h$ or $I_f$. This study also defined several characteristics of $I_h$ that have subsequently become hallmarks of the current: (i) activation kinetics become faster with more negative voltage steps, (ii) the fully activated I-V is linear, (iii) the reversal potential is positive to $E_K$ (~25 mV in this study), and (iv) activation threshold is in the diastolic potential range (~50 mV here). The authors gave considerable attention to the question of the physiologic role of $I_h$, employing a Hodgkin-Huxley model in an attempt to quantify the extent of any contribution. They conclude that, because threshold was around ~50 mV and maximal diastolic potential in the sinus node is ~70 mV, any current activated during diastole would be small. Further, because $I_h$ activation kinetics are slow near threshold, they further conclude that the current would exhibit little time-dependence during the cardiac cycle. Rather, they argue that the main role of $I_h$ is to provide an inward current that fights the tendency of surrounding atrial muscle to drive the sinus node toward $E_K$ and away from threshold. In this sense, the current would contribute to automaticity, but only indirectly, by maintaining the cell in the diastolic potential range.

The conclusion of these authors with respect to the contribution of $I_h$ to sinus node pacemaker activity is in marked contrast to that of the other group (see Brown and DiFrancesco [1980] summary in this section) who published in the same year. That group concludes that the time- and voltage-dependence of $I_f$ is appropriate for it to make a significant contribution to the pacemaker potential. Twenty years later, this debate rages unabated.

1980

English rock vocalist Ian Curtis (Joy Division) commits suicide at 23; siege at Iranian Embassy in London ends when British commandos and police storm building killing three of the five hostage takers; and World Health Organization announces the worldwide eradication of smallpox.
# Heart Rate

## Bibliography of One Hundred Key Papers

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