A Mathematical Model of the Effects of Acetylcholine Pulses on Sinoatrial Pacemaker Activity

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SUMMARY. A mathematical model of dynamic vagus-sinus interactions was devised based on Hodgkin and Huxley-type equations of time- and voltage-dependent membrane currents. Brief vagal pulses were modeled with a concentration-dependent, acetylcholine-activated, potassium current. Single acetylcholine (vagal) pulses scanning the sinus cycle induced changes in pacemaker rhythm that depended on pulse magnitude, duration, and time of occurrence during the cycle. Phase-response curves summarizing these effects are strikingly similar to experimental results. Notably, appropriately timed acetylcholine pulses could produce an acceleratory response. With repetitive acetylcholine input, the model produced various patterns of synchronization of the sinus pacemaker. There was stable entrainment at harmonic (i.e., 1:1, 2:1, etc.) relations, as well as more complex arrhythmic patterns that depended on the relationship between the acetylcholine cycle length and the sinus pacemaker period. In some cases, shortening of the acetylcholine input cycle length led to "paradoxical" acceleration of the sinus pacemaker. Simulations suggest that many clinically observed sinus rhythm disturbances can be explained by dynamic vagus-sinus interactions. (Circ Res 55: 89–101, 1984)
membrane current \( I_m \) across such a patch is equal to the sum of the capacitative current \( I_c \) and the total ionic current \( I_T \):

\[
I_m = I_c + I_T
\]

The capacitative current can be described as a function of membrane capacitance \( C_M \) and membrane potential \( E \):

\[
I_c = C_M \cdot \frac{dE_m}{dt}
\]

so that the relationship becomes:

\[
I_m = C_M \cdot \frac{dE_m}{dt} + I_T
\]

For an isolated patch of membrane, it is assumed that the entire patch is at the same potential, and that there are no longitudinal currents to or from adjacent membrane patches. Thus, \( I_m = 0 \), and Equation 3 can be rearranged to:

\[
\frac{dE_m}{dt} = -I_T/C_M
\]

The total ionic current for the membrane patch is the sum of the currents carried by individual ions. Based on the results of voltage clamp experiments, Yanagihara et al. (1980) describe five ionic currents for the sinus node. The dynamic currents are: a slow inward current, \( i_s \); a sodium current, \( i_{Na} \); a delayed inward current activated by hyperpolarization, \( i_d \); and a potassium current, \( i_K \). In addition, the model requires a time-independent leak current, \( i_L \). Thus, the total ionic current \( I_T \) becomes:

\[
I_T = (i_s + i_{Na} + i_K + i_d + i_L)
\]

Based on the work of Hodgkin and Huxley (1952), the maximum current for a time- and voltage-dependent ion can be described by:

\[
\overline{I}_{ion} = g_{ion} \cdot (E_m - E_{ion})
\]

where \( \overline{I}_{ion} \) is the maximum membrane current for a given ion at a given membrane potential \( E_m \), \( g_{ion} \) is the equilibrium potential for that ion, and \( E_{ion} \) is the maximum conductance for that ion (i.e., the conductance when all channels for that ion are open). The actual membrane current \( i_{ion} \) is given by:

\[
i_{ion} = y \cdot \overline{I}_{ion}
\]

where \( y \) is a gating variable that corresponds to the fraction of ionic channels that are in the conducting state. The gating variable, \( y \), follows first order kinetics. Thus:

\[
\frac{dy}{dt} = \alpha_y (1 - y) - \beta_y y
\]

where \( \alpha_y \) and \( \beta_y \) represent the rate coefficients of opening and closing of the ionic channel, respectively, and \( y \) rep-

### Table 1

<table>
<thead>
<tr>
<th>Time- and Voltage-Dependent Membrane Currents</th>
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<tbody>
<tr>
<td>1. Slow inward current (( i_s )): ( i_s = (0.95d + 0.05) - (0.95f + 0.05) \cdot \frac{A}{E} )</td>
</tr>
<tr>
<td>( \alpha_s = 1.045 \times 10^{-7} \cdot (E + 35) )</td>
</tr>
<tr>
<td>( \beta_s = 4.21 \times 10^{-6} \cdot (E - 5) \cdot \exp[(E - 5)/2.5] - 1 )</td>
</tr>
<tr>
<td>2. Fast sodium current (( i_{Na} )): ( i_{Na} = m^3 \cdot h \cdot i_{Na} )</td>
</tr>
<tr>
<td>( \alpha_m = 9.44 \times 10^{-4} \cdot (E + 60) \cdot \exp[-(E - (29.5))/4.16] )</td>
</tr>
<tr>
<td>( \beta_m = 0.0007 \cdot (E + 37)/10 )</td>
</tr>
<tr>
<td>( \overline{I}_{Na} = 12.5 \cdot \exp[(E - 30)/15] - 1 )</td>
</tr>
<tr>
<td>3. Hyperpolarization-activated current (( i_h )): ( i_h = q \cdot \frac{A}{E} )</td>
</tr>
<tr>
<td>( \alpha_h = 9 \times 10^{-5} \cdot (E + 100) \cdot \exp[(E + 100)/4.4] - 1 )</td>
</tr>
<tr>
<td>( \beta_h = 2.25 \times 10^{-4} \cdot (E + 40) \cdot \exp[(E + 40)/13.3] - 1 )</td>
</tr>
<tr>
<td>( \overline{I}_h = 5 \cdot \exp[0.0277 \cdot (E + 90)] - 1 )</td>
</tr>
<tr>
<td>4. Potassium current (( i_k )): ( i_k = p \cdot \frac{A}{E} )</td>
</tr>
<tr>
<td>( \alpha_p = 9 \times 10^{-5} \cdot (E + 3.8)/9.71 )</td>
</tr>
<tr>
<td>( \beta_p = 2.25 \times 10^{-4} \cdot (E + 40) \cdot \exp[(E + 40)/13.3] - 1 )</td>
</tr>
<tr>
<td>( \overline{I}_k = 0.7 \cdot \exp[0.0277 \cdot (E + 90)] - 1 )</td>
</tr>
<tr>
<td>5. ‘Leak’ current (( i_L )): ( i_L = 0.8(1 - \exp[-(E + 60)/20]) )</td>
</tr>
<tr>
<td>6. Acetylcholine-activated potassium current (( i_{K.ACh} )): ( i_{K.ACh} = u \cdot \frac{A}{E} \cdot i_{K.ACh} )</td>
</tr>
<tr>
<td>( \alpha_u = 12.32 \times 10^{-3} \cdot \frac{1}{1 + (4.2 \times 10^{-5}/[ACh])} )</td>
</tr>
<tr>
<td>( \beta_u = 0.01 \cdot \exp[0.0133 \cdot (E + 40)] )</td>
</tr>
</tbody>
</table>

Uppercase letters (K-ACh, S, Na, K, L, H) represent specific ionic currents. Lowercase letters represent activation (d, m, q, p, u) and inactivation (f, h) gating variables. Opening and closing gating rate coefficients are given by \( \alpha \) and \( \beta \), respectively. The bar over the current symbols (e.g., \( \overline{I}_s \)) indicates maximum current when conductance of that ionic channel is maximal. \( E \) represents membrane potential in mV.
represents the fraction (between 0 and 1) of the channels which are open. The specific equations for each of the ionic currents as described by Yanagihara et al. (1980) are given in Table 1.

**Computation**

To reconstruct the electrical activity of the sinus pacemaker and study the effects of various perturbations, computer programs were written using the equations of Yanagihara et al. (1980) for the time- and voltage-dependent membrane currents (see below). The computer programs were originally written in BASIC and run on an Apple II microcomputer. To improve the speed of the simulations, the programs were rewritten in FORTRAN and were run on a PDP 11/23 computer (Cyberchron Corp.). Graphic output during the computer runs was produced on a VT125 graphics terminal (Digital Equipment Corp.), and hard copy was produced with a Hewlett-Packard X-Y plotter.

For the present model, the surface area of the membrane patch is assumed to be 1 cm², and the capacitance of the membrane is assumed to be 1 μF/cm². Membrane and equilibrium potentials are given in mV, and ionic currents are in μA/cm². Pacemaker activity was reconstructed by integrating the current equations using a modified Euler method of integration (Randall, 1980). In contrast to the studies of Yanagihara et al. (1980), where the steady state values of the rate coefficients for the sodium channel were used throughout, all of the rate coefficients were calculated at each time step. In early simulations, firing of the pacemaker was achieved by stepping the membrane potential from a starting value of −50 mV to 0 mV. Once fired in this manner, the model continued to generate action potentials with an intrinsic period of 318 msec. However, it took two or three action potentials for the model to become stable. To ensure that the model had reached equilibrium, stable starting conditions were determined by recording the values of membrane potential and the gating variables at 1% intervals (0 to 99%) of the pacemaker cycle, after the model had stabilized for 10 beats. These parameters were stored in a "look-up table" which could be accessed by the program. Using this technique, it was possible to begin the simulation at any point in the pacemaker cycle.

In preliminary studies, it was found that varying the integration time step from 0.1 to 2.0 msec did not significantly affect the accuracy of the model. Thus, in the interest of computational speed, all the simulations reported here were done using an integration time step of 2 msec. Values for membrane potential and each of the membrane currents at each integration step were stored after calculation, and were available to be plotted later. The program measured firing times, period (cycle length), maximal diastolic potential, overshoot, and action potential amplitude.

**Perturbations of Pacemaker Activity**

Whereas it is generally agreed that the hyperpolarizing effect of acetylcholine (ACh) in cardiac tissues is due to an increase in potassium conductance (Trautwein and Dudel, 1958), the specific mechanism remains controversial. One group of studies supports the view that ACh opens ACh-specific potassium channels separate from the normal potassium channels (Katz and Miledi, 1972; Noma and Trautwein, 1978). Other studies suggest that the effects of ACh may be mediated by changes in the kinetics of the normal potassium channel (Garnier et al., 1978; Mubagwa and Carmelit, 1983). For the purposes of the present model, we have chosen the former alternative. Brief vagal pulses were simulated by adding an additional acetylcholine-activated potassium current (i_{KAC}) to the model. The model further assumes that ACh has no direct effect on any of the other ionic conductances. The kinetics of an ACh-activated potassium current have been described recently by Osterrieder et al. (1980) in studies on the rabbit sinus node. Their equations incorporate ACh concentration, diffusion, and hydrolysis. These equations, as modified by Yanagihara et al. (1980) are given in Table 1. In the model, the ACh-induced potassium current is added to the sum of the other currents, and the total ionic current is described by:

$$i_T = i_s + i_{Na} + i_K + i_{I_{H}} + i_L + i_{KAC} \quad (9)$$

During the time an acetylcholine pulse was to be applied, the ACh concentration, [ACh], was set to the desired value. To terminate the pulse, the opening rate coefficient, $\alpha_{op}$, was set to zero. This technique produced a membrane hyperpolarization similar in shape and time course to that seen experimentally (Jalife and Moe, 1979; Jalife et al., 1980). With different versions of the computer programs, three simulation protocols were used: (1) the application of a single, brief ACh pulse, (2) the repetitive application of brief ACh pulses at fixed ACh inter-pulse intervals, and (3) the repetitive application of brief ACh pulses at fixed sinus response-ACh pulse intervals (i.e., fixed coupling).

In some simulations, the intrinsic frequency of the reconstructed pacemaker was altered by adding an additional constant current to the model. Hyperpolarizing (outward) currents slowed and depolarizing (inward) currents accelerated pacemaker frequency. Studies of the effects of ACh pulses were conducted at various intrinsic pacemaker frequencies.

**Results**

**Effects of a Single Acetylcholine Pulse on Membrane Potential and Currents**

1. **Beating Mode**

The effects of a single acetylcholine (ACh) pulse on the activity of the simulated sinus pacemaker are illustrated in Figure 1. The top trace shows membrane potential and the lower traces illustrate the time course of the individual membrane currents. The first cycle shows control conditions. The pacemaker period was 318 msec, maximum diastolic potential was −59.6 mV, overshoot was 20.8 mV, and action potential amplitude was 80.4 mV. The algebraic sum of the six membrane currents described in the present model is represented by the total current ($i_T$). Positive deflections are outward current; negative deflections are inward. In the control cycle, there was a progressive increase in total inward current during diastole, until threshold was reached. As described by Yanagihara et al. (1980), under steady state conditions the major component of membrane current leading to pacemaker activity is provided by the slow inward current ($i_s$), and the contribution of all other currents is relatively small. However, the added changes ($i_{Na} + i_K + i_{I_{H}} + i_L$) resulted in a net reduction of inward current, so that $i_T$ was less than $i_s$ during the diastolic interval.
Increases, resulting in an almost constant slope of diastolic depolarization (see Yanagihara et al., 1980).

In summary, as shown in Figure 1, during the control diastolic interval and in the presence of a small but decaying outward current, a time- and voltage-dependent increase in \( i_T \) resulted in slow diastolic depolarization toward threshold.

The slow inward current is also the major contributor to \( i_T \) during the action potential upstroke (phase 0). As illustrated in Figure 1, during phase 0, \( i_T \) is inward and increases rapidly to a maximum of about 4.1 \( \mu A/cm^2 \) at the zero potential. Inward current then quickly decays and gives way to a relatively large outward current surge. This outward component of \( i_T \) (carried mainly by \( i_K \)) and the rapid inactivation of \( i_S \) are both responsible for membrane repolarization and for restoration of the MDP (Yanagihara et al., 1980).

In the example of Figure 1, a 50-msec ACh pulse was introduced during the second pacemaker period (horizontal bar, top trace). The phase (\( \phi \), defined as the time interval between the peak of the previous pacemaker discharge and the onset of the ACh pulse) was 280 msec and the ACh concentration was \( 1 \times 10^{-6} \) M. After a brief latency, the pulse activated specific potassium channels (see Methods) and induced a relatively large outward current (\( i_{K_ACh} \)), thus resulting in membrane hyperpolarization and in significant changes in the time courses of the other membrane currents. The changes in the reconstructed action potential record were qualitatively similar to those observed in the experimental situation immediately after a brief vagal burst, including a relatively large prolongation of the ensuing pacemaker period (548 msec) followed by abbreviation of the subsequent period (310 msec).

As shown also in this figure, after reaching its peak, \( i_{K_ACh} \) began to decay gradually upon termination of the pulse. The decay was accompanied by a gradual decrease in membrane potential toward threshold and by a transient loss of the time-dependent increase in \( i_S \). Hence, in spite of the fact that the time course of recovery from hyperpolarization was very similar to that followed by the control diastolic depolarization, \( i_S \) remained practically unchanged at a level that was higher than usual. Consequently, and in contrast to the control, slow diastolic depolarization following the ACh pulse was not dependent on the time course of \( i_S \), but resulted from the gradual decay in the outward ACh-induced potassium current in the presence of increased but relatively constant inward currents (\( i_S \) and \( i_H \)). The time course of \( i_{K_ACh} \) was reset by the ACh pulse.

An increase in \( i_S \) is apparent also during the first upstroke after the ACh pulse (Fig. 1). At this time, \( i_S \) reached a peak value that was 0.4 \( \mu A/cm^2 \) higher than control. However, this change was partially offset by a transient increase in outward currents (\( i_{K_ACh} \) and \( i_H \)) and resulted in a relatively small increase (0.26 \( \mu A/cm^2 \)) in peak inward current (\( i_T \)). Therefore, the resulting changes in membrane po-
tential were only minor and included small increases in maximum action potential amplitude and upstroke velocity.

The results presented thus far are qualitatively similar to those recently obtained by Bristow and Clark (1983) in their simulations using a modified version of the McAllister-Noble-Tsien (MNT) model. These authors were also able to reproduce the initial delaying effects of brief vagal trains on pacemaker periodicity. However, their model was unable to mimic the secondary acceleratory behavior that is commonly observed experimentally (Jalife et al., 1983). Previous results have shown that acceleration of the first, and sometimes the second (Jalife and Moe, 1979; Spear et al., 1979), pacemaker period can indeed occur after a brief vagal input, depending on the time relationship between the initial hyperpolarizing response and the duration of the pacemaker period. If a given period is scheduled to end slightly after the termination of the vagally induced hyperpolarization, a postinhibitory rebound can advance the subsequent discharge and abbreviate that period (Jalife and Moe, 1979). This is precisely the case in our simulations using the Yanagihara model (see also Figs. 5 and 6). In the example of Figure 1, the second cycle after the ACh pulse was 8 msec briefer than the control, which corresponds to about 2.5% acceleration. This is well within the limits found in the experimental situation (Jalife and Moe, 1979; Spear et al., 1979). In the model, this paradoxical acceleration is the result of a relatively slow recovery of iK from the perturbing ACh pulse. As shown in Figure 1, the ACh-induced decrease in iK lasted beyond the first pacemaker discharge after the pulse. Consequently, after repolarization, the time- and voltage-dependent increase in inward currents (primarily i) became more dominant, thus leading to a slightly less negative (~1.5 mV) MDP and to a significant abbreviation of the third cycle. Thus, our results indicate that the secondary acceleration is not dependent on the multicellular nature of the sinus node (Bristow and Clark, 1983), but can be accounted for by the kinetics of the membrane ionic channels alone.

2. Quiescent Mode

To study the shape and time course of the ACh-induced membrane hyperpolarization, we performed a series of simulations in which the conductance for the slow inward channel was set to zero. Since this channel is responsible for spontaneous activity in the present model, its removal rendered the membrane potential quiescent. This situation is similar to experimental studies in which verapamil was used to block the slow inward current (Jalife and Moe, 1979; Jalife et al., 1980). We investigated the hyperpolarizing effects of ACh under these conditions (Fig. 2). In one series of simulations (Fig. 2, panel A), a 50-msec ACh pulse was applied at various ACh concentrations, ranging from $1 \times 10^{-8}$ to $1 \times 10^{-4}$ M. As ACh concentration increased, the magnitude and duration of the hyperpolarization increased, reaching a maximum at ACh concentrations around $10^{-4}$ M (inset). These results mimic very closely those obtained by Jalife et al. (1980) in verapamil-treated preparations when the vagus nerve was stimulated pre-ganglionically or when ACh was applied iontophoretically.

After peak hyperpolarization was reached, the membrane potential did not return immediately to its resting level. A rebound depolarization was apparent with a magnitude and duration that depended on the maximum level of the preceding hyperpolarization, and, hence, on the ACh concentration. This rebound effect is commonly observed experimentally in the verapamil-treated preparation (Jalife et al., 1980) and may play an important role in the accelerator effects of brief vagal input in the spontaneously beating preparation (see Fig. 6). Removal of the hyperpolarization-induced current (iK) prevented this secondary depolarization (not shown) and allowed for exponential recovery from the hyperpolarizing effects.

At a constant ACh concentration of $1 \times 10^{-4}$ M, increasing the duration of the ACh pulse from 5 to 400 msec (Fig. 2, panel B) increased both the mag-
nitude and duration of the resulting hyperpolarization. A plot of the maximum change in membrane potential vs. the log of the pulse duration (panel B, inset) showed a sigmoid relationship with a maximal hyperpolarization achieved at pulse durations of 200–400 msec. We also observed a secondary rebound depolarization whose magnitude and duration were directly dependent upon the magnitude and duration of the preceding hyperpolarization. As noted above, removal of i_{K1} eliminated this rebound effect (not shown). This rebound depolarization has been observed by others in the absence of verapamil (Glitsch and Pott, 1978; Mubagwa and Carmeliet, 1983; Baumgarten et al., 1984). Baumgarten et al. (1984) attributed this phenomenon to the extracellular accumulation of potassium. However, the present model does not permit extracellular accumulation of potassium. Thus, in the simulations, it would appear that the rebound depolarization is mediated by i_{K1}. Finally, in both panels of Figure 2, it is apparent that once the maximum hyperpolarization is reached, increasing the ACh concentration or the pulse duration serves only to increase the duration of the response. This occurs because there is a maximum activation (saturation) of the ACh-induced potassium channels, and also because there is a decrease in the driving force for potassium ions as the membrane hyperpolarizes (Glitsch and Pott, 1978).

Phase-Dependence of the Effects of Single Acetylcholine Pulses

To determine whether the computer model would mimic the phase-dependence of the effects of brief vagal pulses in experimental preparations, we introduced acetylcholine pulses at various times during the pacemaker cycle. Figure 3 illustrates typical results. Panel A shows the simulated pacemaker under control conditions where the basic cycle length was 318 msec. In panel B, an ACh pulse (conc. = 1 × 10^{-6} M; duration = 50 msec) was introduced at a phase of 127 msec (horizontal bar). The pulse produced a hyperpolarization of 4.9 mV and prolonged the cycle by 112 msec to 430 msec. In panel C, a similar ACh pulse applied at a phase of 280 msec (horizontal bar) hyperpolarized the membrane by 17.5 mV and prolonged the cycle to 548 msec. These results are similar to those found in experimental preparations (Jalife and Moe, 1979) in that pulses occurring late in the cycle produce larger hyperpolarizations and greater prolongations of the cycle length (phase shifts) than pulses occurring early in the cycle.

Since the effects of a brief ACh pulse are short-lived and, after the second cycle (see Fig. 1), the pacemaker returns to its intrinsic period, the change in the duration of the cycle during which the pulse occurs can be thought of as a phase shift of the new cycle with respect to the old cycle (Aschoff, 1965), and the phase-dependence of this shift can be summarized in a phase-response curve (PRC). PRC’s obtained in the model by scanning the simulated pacemaker cycle with single ACh pulses are shown in Figure 4. The phase shift (Δφ) is plotted as a function of the phase (φ) of the ACh pulse within that cycle. Three different ACh concentrations were employed: 1 × 10^{-6} M (solid line), 5 × 10^{-7} M (dotted line), and 1 × 10^{-9} M (dashed line). Pulse duration was 50 msec in all examples. For all concentrations, as the pulse was applied at progressively later phases, there was a progressively greater prolongation of the cycle during which the pulse occurred, up to a point after which pulses had no effect. From this point, a latent period began where the pulse was ineffective in changing the duration of the current cycle and ACh pulse effects were postponed to the following cycle. In the model, this latent interval (range 30–80 msec) was a function of the

![Figure 3](image_url)

**Figure 3.** Phase-dependence of cycle length prolongation produced by a single ACh pulse (1 × 10^{-6} M, 50 msec). Horizontal bars indicate pulse timing. Panel A: control; panel B: phase (φ) = 127 msec; panel C: φ = 280 msec.

![Figure 4](image_url)

**Figure 4.** Phase-response curves (PRC's) showing effects of a single ACh pulse applied at different times during the cycle. Phase shift (Δφ) is plotted as a function of phase (expressed as percent of basic cycle length) at three different ACh concentrations. Solid line: 1 × 10^{-6} M ACh; dotted line: 5 × 10^{-7} M; dashed line: 1 × 10^{-9} M ACh.
ACh concentration. Higher concentrations produced larger phase shifts at any given phase and had shorter latent intervals. These curves are similar to those obtained experimentally, with the exception that latent intervals in the model were considerably shorter than those observed experimentally (see Discussion for significance).

Influence of Intrinsic Pacemaker Period on Acetylcholine Effects: “The Vagal Paradox”

In experimental preparations different PRC’s are obtained at different intrinsic sinus periods (Jalife and Moe, 1979). When the sinus period is longer than the duration of the ACh-induced hyperpolarization, the vagal pulse can accelerate the next pacemaker discharge (Jalife and Moe, 1979). We investigated this phenomenon in the computer model by adding a constant current to the model equations. Constant hyperpolarizing (outward) current slowed the pacemaker, and constant depolarizing (inward) current accelerated it. These results with constant current application are similar to those found by Yanagihara et al. (1980). By selecting different values for this current, pacemakers with different intrinsic periods could be obtained. In Figure 5, a constant hyperpolarizing current of 0.463 μA/cm² greatly prolonged the cycle length of the first discharge (A) to 1522 msec. The oscillation seen under these conditions is frequently seen in experimental preparations (see Fig. 5, inset). When an ACh pulse (conc. = 1 × 10⁻⁶ M; duration = 50 msec) was applied at a phase of 630 msec (arrow), it produced a marked hyperpolarization and advanced the discharge (B) by 390 msec. This is a demonstration of the “excitatory” (i.e., acceleratory) effect of an “intrinsically inhibitory” (i.e., vagal) input. A similar result obtained from an experimental preparation is illustrated in Figure 5, inset, where the prolongation of pacemaker cycle length was achieved with repetitively applied, low frequency vagal stimuli. Under these conditions, a single high frequency (200-Hz) vagal train of brief duration (50 msec) also hyperpolarized the sinus nodal cells and accelerated the pacemaker discharge.

The phase-dependence of the effects of single ACh pulses on simulated pacemakers whose cycle lengths have been prolonged by the addition of constant hyperpolarizing current is illustrated in Figure 6. In all panels, a constant hyperpolarizing current of 0.45 μA/cm² was used. Panel A (control) shows that the cycle length of the simulated pacemaker has been increased to 774 msec. In panel B, an ACh pulse (conc. = 1 × 10⁻⁶ M; duration = 50 msec) applied at φ = 94 msec (arrow) produced a
hyperpolarization of 4.3 mV, and accelerated the next pacemaker discharge by 44 msec. In panel C, a similar ACh pulse at \( \phi = 466 \text{ msec} \) (arrow) hyperpolarized the membrane by 13.3 mV, and prolonged the cycle length by 170 msec. The phase-response curve for this simulation (not shown) was similar in shape to that seen in Figure 4. However, pulses introduced early in the cycle produced acceleration of the next discharge.

These data are consistent with previous experimental results (Jalife and Moe, 1979, Jalife et al., 1983), and indicate that a brief cholinergic input is capable of abbreviating the pacemaker period whenever the intrinsic cycle length is longer than the duration of the ACh-induced effect. These simulations also show that "paradoxical" acceleration can occur in single-cell pacemakers independent of external factors such as nodal reentry, vagally induced desynchronization, or underlying injury effects (Bristow and Clark, 1983).

**Effects of Repetitively Applied Acetylcholine Pulses**

In an attempt to simulate both the stable and the complex patterns of entrainment of the sinus pacemaker by repetitive vagal input, simulations were devised wherein acetylcholine pulses could be applied repetitively at fixed intervals. Results of these simulations are shown in Figures 7, 8, and 9. The control cycle length of the simulated pacemaker (Fig. 7, panel A) was 318 msec. In panels B-E, ACh pulses (arrows) with a concentration of \( 1 \times 10^{-6} \text{ M} \) and a duration of 30 msec were applied at various fixed interpulse intervals (IPI's), resulting in entrainment of the simulated pacemaker. In panel B, pulses were applied at an IPI of 240 msec. The pacemaker was entrained to a cycle length of 480 msec, exactly twice the duration of the IPI (pacemaker discharge:ACh pulse ratio = 2:1). The coupling interval (CI) between ACh pulses and the next pacemaker discharge was fixed during entrainment (CI = 456 msec for the first ACh pulse in a cycle, and CI = 216 msec for the second). At an IPI of 460 msec (Fig. 7, panel C), 1:1 entrainment occurred with the pacemaker cycle length matching the IPI. The coupling interval was again constant (CI = 228 msec). At an IPI of 540 msec (Fig. 7, panel D), an alternating pattern of pacemaker discharges was observed with an entrainment ratio of 2:3. The coupling intervals exhibited a periodicity, with the pattern of coupling intervals of 232 and 308 msec repeating every third ACh pulse. Note that the cycle length of the pacemaker was not constant and also exhibited periodicity (cycle length = 312, 304, 464, etc.). Finally, at an IPI of 740 msec, the pacemaker was entrained in a 1:2 manner with pacemaker cycle lengths alternating between 312 and 428 msec. The coupling interval was again constant at 248 msec.

The effects of repetitive ACh pulses for a complete range of interpulse intervals are illustrated in Figure 8. Simulations performed as described above for Figure 7 were done at two different ACh concentrations for the range of IPI from 200 to 1100 msec in 20-msec increments. ACh pulses (30 msec in duration) were applied repetitively for 10 seconds, and the mean pacemaker period (cycle length) was determined and plotted as a function of the interpulse interval. Panel A shows the results when the ACh pulse concentration was \( 1 \times 10^{-7} \text{ M} \). Diagonal lines connect points in zones where increasing the interpulse interval increased the mean pacemaker period by an equivalent amount, i.e., zones where stable entrainment was maintained. The ratio of the entrainment is given at the top of each of the diagonal lines. At the low concentration of ACh used for Figure 8A, the zones of entrainment at harmonic or subharmonic relations were very narrow, and were separated by large zones where the interaction of vagal pulses and pacemaker discharges was more complex.

When the ACh pulse concentration was increased to \( 1 \times 10^{-6} \text{ M} \) (Fig. 8, panel B), the zones of stable

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**FIGURE 7. Entrainment of the simulated sinus pacemaker by repetitively applied ACh pulses (conc. = \( 1 \times 10^{-6} \text{ M} \); duration = 30 msec) at various fixed interpulse intervals (arrows). For further explanation, see text.**
entainment became wider at the expense of the zones of complex, dysrhythmic activity. In both panels, in the zones of stable entainment, when the frequency of the inhibitory input (ACh pulses) is increased, the pacemaker frequency is also increased. In addition, in Figure 8B, the zones of 1:1, 1:2, and 1:3 entrainment show that at certain IPI's it is possible to entrain the pacemaker at stable rhythmic periods that are briefer than the intrinsic pacemaker period. This "paradoxical" acceleratory effect has also been observed experimentally (Jalife et al., 1983). As the model demonstrates, this effect can be a direct consequence of the phase advance produced on the first (Fig. 5) or the second (Fig. 1; top trace) period when the expected pacemaker discharge is scheduled to occur after the termination of the hyperpolarizing response.

Figure 9 shows an example illustrating the effects of repetitively applied pulses under such conditions. Panel A shows steady state pacemaker activity when a constant hyperpolarizing current of 0.45 μA/cm² prolonged the intrinsic pacemaker cycle length to 774 msec. In panel B, ACh pulses (arrows) at a concentration of $1 \times 10^{-6}$ M and a duration of 30 msec were applied at an IPI of 680 msec. The first ACh pulse occurred early in the cycle and accelerated the next pacemaker discharge. The next ACh pulse occurred soon after and advanced the following discharge. The process continued so that the pacemaker was entrained in a 1:1 manner to fire at an accelerated rate equal to the interpulse interval. This again demonstrates the "excitatory" effects of an "intrinsically inhibitory" input.

**Fixed Coupling of Single Acetylcholine Pulses to Pacemaker Discharges**

The results presented in the previous section provide a very precise description of the phasic effects of brief ACh pulses on sinoatrial pacemaker activity. The data are empirically useful, since they can predict more complex interactions between the sinoatrial pacemaker and reflexly mediated vagal discharge.

Perhaps the most important prediction concerns the ability of the cardiac pacemaker to synchronize to the repetitive, reflexly mediated, efferent vagal input. Since efferent vagal discharges tend to be grouped at fixed phases within the pacemaker cycle, it can be demonstrated that each vagal burst should either advance or delay the subsequent pacemaker discharge, depending on phase relations. The rate and rhythm resulting from these servo-control interactions can be predicted from the phasic phenomena demonstrated using the present model.

The effects of the repetitive application of ACh pulses at various fixed pacemaker-ACh pulse coupling intervals (FCI's) on the simulated sinus pacemaker are illustrated in Figure 10. In all panels, the free-running pacemaker cycle length was 318 msec. The computer model was modified so that whenever the pacemaker fired, it introduced an ACh pulse after a predetermined delay (fixed coupling interval) to mimic reflexly induced vagal input. In panel A, when the FCI was 140 msec, the ACh pulses changed the simulated pacemaker period to 436 msec. The computer model was modified so that whenever the pacemaker fired, it introduced an ACh pulse after a predetermined delay (fixed coupling interval) to mimic reflexly induced vagal input. In panel A, when the FCI was 140 msec, the ACh pulses changed the simulated pacemaker period to 436 msec. Under these conditions, the interval between the ACh pulse and the next pacemaker discharge was constant at 296 msec. When the coupling inter-
The phase-dependence of these effects, (3) relative and absolute acceleration of the pacemaker by ACh pulses, and (4) simple and complex entrainment of the sinus pacemaker by repetitively applied ACh pulses. The slope and time course of the ACh-induced hyperpolarization seen when the membrane potential was rendered quiescent by the removal of the slow inward current are also similar to those observed experimentally. This suggests that the equations for the ACh-induced potassium current can reasonably approximate the biological situation. The hyperpolarizations are also similar to those seen by Bristow and Clark (1983) who modeled the vagal stimulus as a brief train of individual ACh pulses. The results can be used to suggest possible ionic mechanisms for the phenomena observed experimentally, and to provide important predictions regarding the dynamic vagal control of heart rate and rhythm.

It should be noted that, in its present form, the model is a very simplified interpretation of the biological system. Various aspects of the biological situation are not included in the model. For example there are no provisions for the extracellular accumulation of potassium (Spear et al., 1979; Baumgarten et al., 1984), or for the possible effects of an electronegative sodium pump (Noma and Irisawa, 1974). In addition, the model assumes that ACh opens a second potassium channel and has no direct effect on any of the other ionic channels.

**Mechanism of ACh-Induced Effects**

The simulation results suggest that the alterations in cycle length induced by a single ACh pulse are secondary to a hyperpolarization and to a change in the nature of the basic ionic mechanism underlying spontaneous depolarization. In the model of Yanagihara et al. (1980), and in a later version by Irisawa and Noma (1982), the major factor contributing to slow diastolic depolarization is the steady increase in the slow inward current ($I_s$). The contribution of the decrease in potassium conductance seen in other pacemaker models [e.g., McAllister et al., (1975)] is smaller. In contrast, following an ACh pulse (Fig. 1), $I_s$ remains unchanged at an elevated level, and there is a continuous decrease in outward current through the ACh-activated potassium channels. This decrease in outward current, in the presence of a relatively constant inward current, also depolarizes the membrane until threshold is reached. A rapid increase in $I_s$ results in an action potential, and the original pacemaker mechanism is then restored. The overall data thus indicate that phase control (Figs. 3, 6, and 10) and entrainment (Figs. 7–9) of the sinoatrial pacemaker by efferent vagal bursting may be direct consequences of the ACh-induced opening of specific potassium channels, and of the changes in other time- and voltage-dependent currents induced indirectly through ACh-receptor interactions (Fig. 1).
The Delay to Hyperpolarization and the Latent Interval

Yanagihara et al. (1980) found that their pacemaker model hyperpolarized by about 20 mV when the slow inward current was removed. They also found this to be true if the initial starting membrane potential was set at −45 mV. However, setting the initial membrane potential to −50 mV and holding it there for a single time step resulted in a stable, "quiescent" membrane potential that could then be used to study the hyperpolarizing effects of single ACh pulses. Under these conditions, the model mimicked closely the membrane potential changes induced by brief cholinergic input in quiescent preparations (Jalife et al., 1980). One discrepancy between the present model and the experimental results was the noticeably shorter latent intervals in the PRC's for the ACh pulses in our simulations (Fig. 4). In experimental preparations, latent intervals typically range from 100 to 200 msec. The latent interval in the simulations depends on the ACh concentration and ranges between 30 and 80 msec. The difference is partly the result of the shorter delay between the application of the ACh pulse and the beginning of the hyperpolarizing response, since, in our simulations, the concentration of ACh was changed in a step-wise manner. Experimentally, this delay has been reported to be on the order of 100 msec for iontophoretically applied ACh, but delays of 30 msec or less have also been observed (Osterrieder et al., 1980; Jalife, unpublished). On the other hand, after vagal stimulation, the delay is usually longer and ranges between 90 and 120 msec.

Some authors have attributed this delay to some kind of metabolic rate-limiting factor intervening between muscarinic receptor activation and the onset of hyperpolarization (Hartzell et al., 1977; Hill-Smith and Purves, 1978; Pott, 1979). However, recent voltage clamp experiments have suggested that the major portion of the delay can be attributed to the time required for diffusion of ACh molecules to the muscarinic receptor (Osterrieder et al., 1980). To account for this in their simulations, Bristow and Clark (1983) assumed the ACh concentration to be time-dependent, and they were able to mimic quite successfully both the delay to hyperpolarization and the latent interval in the PRC.

The Postinhibitory Rebound

These data provide strong support for the hypothesis that the acceleratory effect of brief vagal input on sinoatrial pacemaker activity (Brown and Eccles, 1934; Jalife and Moe, 1979) is the result of a postinhibitory rebound leading to a transiently increased rate of diastolic depolarization, and to acceleration of the first pacemaker discharge after the input. As demonstrated in Figure 2, a postinhibitory rebound is apparent after a brief ACh pulse, even in the absence of the slow inward current. In the "quiescent mode" (Fig. 2), this rebound is provided in large part by \( i_{K} \), which increases soon after the pulse. In the presence of a decreasing \( i_{K,ACh} \) and a significantly reduced \( i_{K} \), \( i_{H} \) provides inward current for depolarization beyond the resting membrane potential. In the "beating mode" (Figs. 3–6), the presence of \( i_{S} \) can contribute even more inward current during diastole and can produce a marked increase in the slope of phase 4. In this case, the final effect on pacemaker cycle duration will depend, of course, on the magnitude of the ACh-induced hyperpolarization, and on its rate of decay and duration in relation to the duration of the intrinsic pacemaker period.

The present model also shows evidence of acceleration in the second pacemaker cycle following the ACh pulse. This was not observed by Bristow and Clark (1983) in their simulation studies, but is a common finding experimentally (Jalife and Moe, 1979; Spear et al., 1979). The current records suggest a possible mechanism for this relatively small acceleratory effect. The effect is most noticeable for ACh pulses that occur late in the preceding cycle, so the change in the mechanism of spontaneous depolarization (see above) with a resulting increase in the slope of phase 4 depolarization may be involved.

The Triphasic Effect of Brief Vagal Input

The response of sinus pacemaker cycle length to the effects of a brief vagal pulse have been shown to follow a triphasic time course (Brown and Eccles, 1934; Levy et al., 1970; Jalife and Moe, 1979; Spear et al., 1979) over a period of several seconds. The triphasic effect of brief vagal stimulation consists of a primary inhibitory effect, a secondary acceleration, and a secondary inhibitory component. Most investigators agree that the primary inhibitory effect is due to the hyperpolarizing effects of ACh, and this model supports that view. Spear et al. (1979) have proposed that the acceleratory component is due to a direct increase in sodium conductance produced by ACh. The present model does not allow for direct effects of ACh on ionic channels other than the ACh-specific potassium channel. Nonetheless, the acceleratory component is observed in our simulations (Figs. 1, 4, 5, and 6). Finally, the secondary inhibitory effect has been attributed to the extracellular accumulation of potassium (Spear et al., 1979). The model does not account for changes in extracellular potassium, nor does it produce significant secondary inhibition. These simplifications do not allow us to address the mechanisms that have been proposed to account for the secondary inhibitory component in the triphasic curve (Spear et al., 1979). More sophisticated versions of the model will be required to deal adequately with these issues.

Entrainment Phenomena and Their Implications

There is a striking similarity between simulation results of entrainment of the pacemaker by repetitively applied ACh pulses and experimental observations. The zones of stable entrainment at which
the pacemaker period becomes equal or harmonically related to the period of the ACh pulses are determined by the PRC. These zones of simple, stable entrainment patterns are separated by zones where the pacemaker is entrained to more complex, dysrhythmic patterns. These results add further evidence for the suggestion that such dynamic vagous-sinoatrial interaction may be the basis of some cardiac rhythm and conduction disturbances (Jalife et al., 1982; Michaels et al., 1983).

Finally, the results with fixed coupling simulations suggest that properly timed, repetitive bursts of vagal activity may provide the basis for the servo-control mechanisms involved in baroreceptor reflex-pacemaker interactions. These simulations show also that changes in phase relations induced by alterations in any of the processes involved in this feedback loop may lead to complex rhythm disturbances that are usually attributed to other etiologies. Indeed, as illustrated in Figure 10, the model can readily reproduce patterns of sinus pacemaker activity indistinguishable from patterns found with sinus node dysfunction (sick sinus syndrome) and other supraventricular arrhythmias. The small amplitude of some of the pacemaker discharges (Fig. 10C) might prevent these impulses from being conducted to the atria, resulting in a pattern that resembles intermittent sinoatrial exit block.

The results of simulations done with the present model are in general agreement with previously described experimental and mathematical studies (Jalife et al., 1983; Michaels et al., 1983). This model should be of value in further investigations of the ionic basis of dynamic vagus-sinus interactions and their potentially dysrhythmic consequences. In addition, the model should be useful for investigating the mechanisms of mutual synchronization of the electrically coupled cells of the SA node (Michaels et al., 1984).

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