Mechanisms for Impulse Initiation in Isolated Human Atrial Fibers

LUC MARY-RABINE, ALLAN J. HORDOF, PETER DANILO, JR., JAMES R. MALM, AND MICHAEL R. ROSEN

SUMMARY We used standard microelectrode techniques to study Tyrode’s-supersused human atrial fibers obtained at cardiac surgery. Two types of sustained rhythmic activity occurred. One resulted from slow phase 4 depolarization and had a spontaneous rate of 20–26 beats/min. Epinephrine increased and the slow channel blockers, AHR-2666 (AHR) and verapamil decreased both phase 4 slope and spontaneous rate. Acetylcholine (ACh) and lidocaine decreased the slope and phase 4 phase 4, but the slow phase 4 rate was less than that induced by AHR and verapamil. Tetrodotoxin (TTX) also decreased the phase 4 phase 4 and spontaneous rate, to an extent that was intermediate between the actions of AHR-verapamil and ACh-lidocaine. A second type of sustained rhythmic activity was triggered by delayed afterdepolarizations (DAD). DAD amplitude increased as stimulus cycle length decreased and, at critical cycle lengths, DAD initiated trains of spontaneous action potentials at rates > 70 beats/min. Spontaneously occurring DAD were suppressed by AHR and were transiently diminished by ACh. This effect of ACh was accompanied by hyperpolarization of the fibers. DAD also were induced by epinephrine. These DAD were unaffected by TTX, lidocaine, or ACh and were suppressed by AHR and verapamil. In summary, the slow inward current contributes to the sustained rhythmic activity that occurs with automaticity or DAD in human atrium. A TTX-sensitive current also contributes to automaticity. DAD that occur spontaneously are largely insensitive to the effects of agents that increase K conductance (although ACh has a transient effect) and those that are induced in the presence of epinephrine do not respond to agents which increase K conductance (ACh, lidocaine) or TTX.


HUMAN atrial specialized fibers develop spontaneous phase 4 depolarization and automaticity (Gelband et al., 1972; Trautwein et al., 1962; Mary-Rabine et al., 1978; Hordof et al., 1976) as well as delayed afterdepolarizations (Hordof et al., 1978). Phase 4 depolarization and automaticity occur in both normal and partially depolarized fibers; delayed afterdepolarizations are seen in normal fibers exposed to toxic concentrations of digitalis as well as in depolarized fibers. It may be that the phase 4 depolarization that occurs in normal human atrial specialized fibers results from a gradually diminishing iK2 current, the same mechanism that has been described in normal Purkinje fibers (Noble, 1975). However, other K+ currents, such as iK1 which appears to generate the pacemaker potential in frog atrium and in sinus node (Brown and Noble, 1969a, 1969b; Lenfant et al., 1972) have been implicated as having greater importance than iK2 in generation of the atrial pacemaker potential (Noble, 1975). The basis for automaticity in normal or diseased and partially depolarized human atrium has...
not been described, nor has the importance of background inward current to the human atrial pacemaker potential. Because we wanted to learn more about the mechanism or mechanisms that are responsible for impulse generation in human atrium and to determine how such impulse generation responds to pharmacological agents that modify various ionic conductances across the cell membrane we studied both automaticity and delayed afterdepolarizations in initially normal or partially depolarized atrial fibers.

Methods

Atrial tissue was obtained from the hearts of patients undergoing corrective cardiac surgery. Institutional and DHEW rules for the protection of human subjects were observed. For all patients receiving cardioactive drugs, therapy was terminated 24 hours prior to surgery. No patient was receiving digitalis or propranolol. At surgery, approximately 1 cm² of atrial myocardium was removed from the right atrium as part of the routine cannulation procedure for cardiopulmonary bypass. The tissue was immersed in cold Tyrode’s solution immediately after excision from the atrium and brought rapidly to the laboratory. It was mounted in a Lucite chamber holding a volume of 4 ml and superfused with Tyrode’s solution warmed to 37°C and equilibrated with 95% O₂-5% CO₂. The composition of the Tyrode’s solution (mm/liter) was: NaCl, 137; NaHCO₃, 12; NaH₂PO₄, 1.8; MgCl₂, 0.5; CaCl₂, 2.7; KCl, 4 and dextrose, 5.5 (pH = 7.30–7.35). For experiments with epinephrine, Na ethylenediaminetetraacetic acid (EDTA) was included in the Tyrode’s in a final concentration of 5 × 10⁻⁵M. This concentration has no effect on action potential characteristics or automaticity (Mary-Rabine et al., 1978). The superfusate flow rate was 15–17 ml/min.

The tissues were impaled with 3 M KCl-filled glass capillary microelectrodes having tip diameters <1 μm and resistances of 10–25 MΩ. The electrodes were coupled by a 3 M KCl interface to an Ag-AgCl bar which led to an amplifier having a high input impedance and input capacity neutralization. The output was displayed on a cathode ray oscilloscope (Tektronics model 565) and a Brush recorder. The tissue chamber was connected to ground through a salt bridge and an Ag-AgCl junction. The methods used to stimulate the tissues and to calibrate the equipment have been described previously (Rosen et al. 1973a).

Experimental Protocols

All tissues initially were stimulated at a cycle length of 1000 msec through Teflon-coated bipolar silver wire electrodes. Measurements were made of action potential amplitude, maximum diastolic potential (MDP), and maximum upstroke velocity of phase 0 depolarization (Vₘₐₓ). Initially, transmembrane potential recordings for each tissue sample were obtained from 20–40 impalements in the first subendocardial cell layer. All impalements were made at least 2 mm away from the cut edges of the preparation. This mapping of the tissue was necessary not only to determine its electrophysiological characteristics, but to locate specialized conducting fibers. As previously described, human atrial specialized fibers may have normal or depressed “fast response” action potentials or “slow response” action potentials (Hordof et al., 1976). The specialized conducting fibers that have “fast response” action potentials are identified easily on the basis of a rapid phase 0 upstroke, a prominent plateau preceding phase 3 repolarization, and the occurrence of phase 4 depolarization and automaticity on discontinuation of electrical stimulation. By comparison, in atrial myocardial fibers, the plateau is very brief, and phase 4 depolarization does not occur (Gelband et al., 1972). For human atrial fibers having “slow response” action potentials, the distinction between working myocardial and specialized fibers is not made readily on the basis of the criteria described above. The action potentials described as “slow response” in this study fulfilled the resting and action potential criteria determined for slow responses (resting potential <–60 mV; phase 0 maximum upstroke velocity <20 V/sec) in a prior study of human atrium (Hordof et al., 1976).

To study phase 4 depolarization and automaticity, the drive stimulus was discontinued and the tissue was allowed to initiate spontaneous activity. Escape time was variable, and once fibers commenced to beat spontaneously, periods as long as 120 minutes were required for spontaneous rhythm to stabilize. In all preparations on which we report here, the fibers had the characteristics of a “pacemaker” cell; there was a smooth transition from phase 4 to the more rapid depolarization of phase 0. The following measurements were recorded: activation voltage (AV: measured from “0” reference potential to the inflection between phase 4 and phase 0), MDP (measured from the “0” potential to the point of maximum membrane potential occurring at the end of phase 3), AP amplitude (measured from the MDP to the peak of the overshoot), mean slope of phase 4 depolarization (measured by subtracting AV from MDP and dividing the difference (Δ, in mV) by the time (in sec) between the MDP and AV. Preparations in which the rhythm was irregular were not included in the study. Fifteen preparations in which no drug was studied were used as controls to ensure that spontaneous variation in rhythm was not a significant factor that might be confused with drug effect.

Delayed afterdepolarizations were studied in driven and nondriven preparations. In addition, in some experiments, we used previously described techniques to deliver premature stimuli at chosen intervals after the drive stimulus (Rosen et al., 1973a).
In initial experiments (which were used to prepare the concentration-response curves that are presented in Figure 2B) we determined a concentration of each of the drugs to be studied which markedly depressed phase 4 depolarization and/or delayed afterdepolarizations but did not markedly decrease action potential amplitude. The concentrations of the drugs used in these experiments were: verapamil (Knoll), $1.1 \times 10^{-6}$, $2.2 \times 10^{-6}$, and $3.3 \times 10^{-6}$ M; AHR-2666 [an inhibitor of the slow inward current (Lustig and Kirsten, 1974; Siegal et al., 1978)] (A.H. Robins), $1.8 \times 10^{-4}$, $2.4 \times 10^{-4}$, and $6.4 \times 10^{-4}$ M; acetylcholine (Sigma), $1 \times 10^{-6}$, $3 \times 10^{-6}$, $5 \times 10^{-6}$, and $1 \times 10^{-5}$ M; lidocaine (Astra), $1.9 \times 10^{-5}$, $3.7 \times 10^{-5}$, and $7.4 \times 10^{-5}$ M; epinephrine (l-epinephrine bitartrate, Sigma), $1 \times 10^{-7}$, $1 \times 10^{-6}$, $1 \times 10^{-5}$, and $1 \times 10^{-4}$ M, and tetrodotoxin (TTX) (Sigma), $3.1 \times 10^{-6}$, $6.3 \times 10^{-6}$, and $1.6 \times 10^{-5}$ M. Preparations were superfused with each drug concentration for 20 minutes before measurements of drug effect were made, with the exception of some experiments in which epinephrine was added directly to the bath without stopping the inflow to achieve an estimated concentration of $1 \times 10^{-4}$ M.

Based on the above experiments, we identified the following drug concentrations for use in the remainder of the study: verapamil, $2.2 \times 10^{-6}$ M; AHR-2666, $2.4 \times 10^{-6}$ M; acetylcholine, $3 \times 10^{-6}$ M; lidocaine, $1.9 \times 10^{-5}$ M; TTX, $6.3 \times 10^{-6}$ M, and epinephrine, $1 \times 10^{-4}$ M for further studies of automaticity alone and $1 \times 10^{-3}$ M for studies of automaticity and delayed afterdepolarizations.

Statistical analysis of the data was performed using analysis of variance and Mest for paired or grouped data (Snedecor and Cochrane, 1967). For the concentration-response curves in Figure 2B, a logistic curve was calculated using the logit transformation to the response variable, p, as described by Finney (1964). Results are expressed as mean ± SE.

### Terminology

The following terms are used to describe the membrane potential changes during phase 4 and during spontaneous rhythmic activity (Cranefield, 1975; Hoffman and Cranefield, 1960).

**After-hyperpolarization**

An afterpotential that is continuous with phase 3 repolarization, but that carries the membrane potential to a level more negative than that seen later in diastole.

**Delayed Afterdepolarization**

A depolarizing afterpotential that occurs after repolarization has brought membrane potential to the maximum diastolic potential. The amplitude of a delayed afterdepolarization was measured from the peak voltage attained by the afterdepolarization to the maximum diastolic potential.

**Automaticity**

Spontaneous impulse initiation that results from gradual depolarization during phase 4. This may occur in the presence or absence of afterdepolarizations.

**Triggered Activity**

Impulse generation in which action potentials are initiated by a delayed afterdepolarization.

**Sustained Rhythmic Activity**

This term includes triggered activity and automaticity (as well as repetitive activity arising from reentry).

### Results

#### Control Action Potential Characteristics

We studied 68 preparations from human atria (Table 1A). For nineteen preparations (group A) values for MDP, action potential amplitude, and $V_{\text{max}}$ were classified as “normal,” based on earlier studies (Gelband et al., 1972; Hordof et al., 1976). The distribution of patients in this group was: atherosclerotic heart disease, seven patients; tetralogy of Fallot, four; ventricular septal defect, three; aortic stenosis, three; transposition of the great vessels, one; anomalous left coronary artery, one. Mean age was 32 years, with a range from 3 months to 57 years. Thirty-seven preparations (group B) had lower values of MDP, action potential amplitude, and $V_{\text{max}}$ than group A. This group included eight patients with atherosclerotic heart disease, tetralogy of Fallot, seven; atrial septal defect, seven; rheumatic heart disease, four; ventricular septal defect, three; transposition of the great vessels, three; pulmonary stenosis, two; aortic stenosis, two; endocardial cushion defect, one. Mean age was 23

### Table 1A Transmembrane Potential Characteristics of All Fibers Studied during Electrical Stimulation at CL = 1000 msec

<table>
<thead>
<tr>
<th>Group</th>
<th>(19)*</th>
<th>(37)*</th>
<th>(12)*</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP amplitude (mV)</td>
<td>94.6 ± 1.9</td>
<td>80.5 ± 1.0</td>
<td>54.9 ± 3.0</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>79.4 ± 1.0</td>
<td>70.3 ± 0.6</td>
<td>53.4 ± 1.5</td>
</tr>
<tr>
<td>$V_{\text{max}}$ (V/sec)</td>
<td>227.2 ± 9</td>
<td>125 ± 6</td>
<td>&lt;10</td>
</tr>
</tbody>
</table>

### Table 1B Transmembrane Potential Characteristics of the Same Fibers Described in 1A, after Discontinuation of the Drive Stimulus and Attainment of a Stable Spontaneous Rhythm

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP amplitude (mV)</td>
<td>65.3 ± 1.7</td>
<td>63.5 ± 1.4</td>
<td>51.6 ± 2.6f</td>
</tr>
<tr>
<td>AV (mV)</td>
<td>44.7 ± 1.0</td>
<td>43.6 ± 0.9</td>
<td>41.9 ± 1.1</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>60.0 ± 1.0</td>
<td>56.6 ± 0.9</td>
<td>53.4 ± 1.3f</td>
</tr>
<tr>
<td>$\Delta t/f$ (mV/sec)</td>
<td>8.7 ± 1.3</td>
<td>10.8 ± 1.0</td>
<td>7.5 ± 1.1</td>
</tr>
<tr>
<td>Rate (beats/min)</td>
<td>25.5 ± 3.2</td>
<td>27.5 ± 1.6</td>
<td>21.0 ± 2.9</td>
</tr>
</tbody>
</table>

Results are expressed as mean ± SE.

* Number of preparations (see text for discussion).

† $P < 0.001$ compared to Groups A and B.

‡ $\Delta t$: slope of phase 4.
years (range, 11 months–57 years). Group C consisted of tissues from 12 patients. Here, MDP was less than ~60 mV and the action potentials were consistent with the slow responses described earlier in human atrium (Hordof et al., 1976). This group included four patients with rheumatic heart disease, tetralogy of Fallot, three; atrial septal defect, two; atherosclerotic heart disease, one; transposition of the great vessels, one; left atrial myxoma, one. Mean age was 29 years (range, 8 months–64 years).

Electrophysiological Characteristics of Fibers that Developed Sustained Rhythmic Activity

Following cessation of the drive stimulus for the fibers described in Table 1A, and the attainment of stable sustained rhythmic activity we recorded the characteristics of the spontaneously occurring action potentials (Table 1B). On attaining this stable rhythm, the fibers in groups A and B both had depolarized to an identical maximum diastolic potential. Furthermore, no significant difference in the characteristics of the spontaneously occurring action potentials, phase 4 depolarization, or automaticity was observed comparing these preparations.

As contrasted with the fibers in groups A and B, those in group C did not depolarize further on cessation of the drive stimulus. Moreover, although the fibers in groups A and B showed a marked decrease in MDP, the value for MDP in group C still was significantly lower than those in the first two groups, as was the action potential amplitude. The spontaneous rate was not significantly different from that of group C. Because automatic impulse initiation was similar (i.e., no difference in spontaneous rate) among groups A, B, and C, fibers from the three groups were pooled to study automaticity. During the course of the experiments, it was apparent, as well, that fibers from one group responded similarly to fibers from another with respect to drug effects on impulse initiation.

The effects of verapamil, 2.2 X 10^-6 M, on action potential characteristics and automaticity were studied in eight spontaneously firing preparations (Table 2A). Action potential amplitude, the slope of phase 4 depolarization, and spontaneous rate were reduced markedly by verapamil. Table 2B shows the effects of AHR-2666, 2.4 X 10^-4 M, on 10 fibers. As with verapamil, action potential amplitude, the slope of phase 4 depolarization, and spontaneous rate decreased. There was no change in AV or MDP, and the change in rate apparently was accompanied by a decrease in the slope of phase 4.

Table 2C shows the effects of acetylcholine, 3 X 10^-7 M, on the action potential characteristics and automaticity of nine preparations. In all preparations, ACh increased MDP and AV and decreased the slope of phase 4 depolarization and spontaneous rate. On return to control solution, there was a sudden increase in automaticity, and spontaneous rate was transiently 30 ± 9% greater than the control (P < 0.05). During the subsequent superfusion with control Tyrode’s solution, rate again decreased to the values recorded before superfusion with ACh. This sequence is shown in Figure 1.

The effects of lidocaine 1.9 X 10^-5 M were determined in nine experiments (Table 2D). Lidocaine decreased the slope of phase 4 depolarization and the spontaneous rate. There was no consistent change in action potential amplitude, AV or MDP. In three instances, lidocaine induced periods of quiescence alternating with episodes of spontaneous activity during which the rate was irregular.

The effects of TTX were studied in five experiments (Table 2E). At a concentration of TTX of 6.3 X 10^-6 M, there was a marked decrease in spontaneous rate and in the slope of phase 4 depolarization.

### Table 2: Effects of Verapamil, AHR-2666, Acetylcholine, Lidocaine, and TTX on Human Atrial Fibers

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Drug</th>
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<tbody>
<tr>
<td>AP amplitude (mV)</td>
<td>63.2 ± 2.4</td>
<td>62.8 ± 2.5</td>
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<tr>
<td>AV (mV)</td>
<td>45.1 ± 1.1</td>
<td>44.8 ± 1.0</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>58.5 ± 1.2</td>
<td>57.4 ± 1.2</td>
</tr>
<tr>
<td>Δ/Δt (mV/sec)</td>
<td>7.7 ± 0.7</td>
<td>3.4 ± 0.7</td>
</tr>
<tr>
<td>Rate (beats/min)</td>
<td>26.4 ± 3.5</td>
<td>11.0 ± 2.7</td>
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</table>

Δ/Δt = slope of phase 4.
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CONTROL

ACh 3x10^{-6} M

1 MIN  15 MIN

WASHOUT

1 MIN  10 MIN

FIGURE 1 Effects of ACh on automaticity of a human atrial fiber. Control; MDP = -60 mV, spontaneous rate = 22.6 beats/min. ACh 3 x 10^{-6} M; MDP = -65 mV, spontaneous rate = 18.2 beats/min at 1 minute and stabilizes at 20.7 beats/min in 15 minutes. On return to control solution (washout), there is a transient acceleration of spontaneous rate before a return to control values.

Interestingly, at this time there was no significant change in the amplitude of the action potential. These effects of TTX on phase 4 were reversible within 30 minutes after returning to control Tyrode's solution.

The effects of epinephrine, 1 x 10^{-6} M, were studied in 10 experiments (Table 3). Epinephrine markedly increased MDP, action potential amplitude, the slope of phase 4, and automaticity. It did not induce delayed afterdepolarizations or triggered activity.

Figure 2 summarizes the effects of verapamil, AHR-2666, ACh, lidocaine, TTX, and epinephrine on spontaneous activity of the human atrial fibers. No significant differences could be found among the control spontaneous rates of the different groups of fibers, between the effects of verapamil and AHR on automaticity (P > 0.05), or between the effects of ACh and lidocaine on automaticity (P > 0.05). However the slowing of rate induced by verapamil and AHR was greater than that induced by ACh or by lidocaine (P < 0.05). The extent of slowing with TTX was intermediate and did not differ from that of either AHR and verapamil, or lidocaine and ACh (P > 0.05). Epinephrine enhanced automaticity. B: Concentration-response curves of AHR, verapamil, lidocaine, and ACh effects on spontaneous rate of human atrial fibers. Vertical axis, spontaneous rate expressed as % of control, horizontal axis, log drug concentration (M/l). The numbers of experimental observations are, verapamil 4 (1.1 x 10^{-6} M), 8 (2.2 x 10^{-6} M), 8 (3.3 x 10^{-6} M); AHR 4 (1.8 x 10^{-4} M), 10 (2.4 x 10^{-4} M), 10 (6.4 x 10^{-4} M); lidocaine 9 (1.9 x 10^{-5} M), 9 (3.7 x 10^{-5} M), 4 (7.4 x 10^{-5} M); ACh 9 (1 x 10^{-6} M), 9 (3 x 10^{-6} M), 8 (5 x 10^{-6} M). The r values for the curves are verapamil, 0.94; AHR, 0.99; lidocaine, 0.97; ACh, 0.99.

verapamil and AHR was significantly greater than the slowing induced by ACh or by lidocaine (P < 0.05). The magnitude of rate change induced by TTX was intermediate between that caused by verapamil or AHR and that of lidocaine or ACh, and did not differ from either group (P > 0.05). To ensure that the greater magnitude of rate change induced by the slow channel blockers did not reflect only a greater potency, we calculated logistic curves

![Table 3](http://circres.ahajournals.org/)

**TABLE 3 Effects of Epinephrine (1 x 10^{-6} M) on Human Atrial Fibers**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Epinephrine</th>
</tr>
</thead>
<tbody>
<tr>
<td>AP amplitude</td>
<td>60.6 ± 2.8</td>
<td>75.5 ± 3.2†</td>
</tr>
<tr>
<td>AV (mV)</td>
<td>41.0 ± 1.0</td>
<td>44.4 ± 3.9</td>
</tr>
<tr>
<td>MDP (mV)</td>
<td>56.6 ± 1.4</td>
<td>67.3 ± 4.2‡</td>
</tr>
<tr>
<td>Δt/mV</td>
<td>8.7 ± 2.2</td>
<td>23.6 ± 17.8‡</td>
</tr>
<tr>
<td>Rate (beats/min)</td>
<td>22.1 ± 3.1</td>
<td>38.6 ± 19.4‡</td>
</tr>
</tbody>
</table>

Δt/mV = slope of phase 4.

* Ten experiments.

† P < 0.01 compared to control; ‡ P < 0.001 compared to control.
(Finney, 1964) for verapamil, AHR, lidocaine, and ACh (Fig. 2B). These concentration-response curves suggest a greater efficacy of verapamil and AHR in depressing spontaneous rate. Although a high concentration of ACh (1 × 10^{-5} M) depressed rate to an extent equivalent to that of AHR and verapamil, this effect was accompanied by hyperpolarization (to MDP > -70mV). The effects of the other drugs on rate occurred in the absence of changes in MDP. In summary, verapamil and AHR markedly decreased the slope of phase 4 and, thereby, the spontaneous rate, without changing AV or MDP. ACh in concentrations that did not markedly increase MDP and lidocaine were less effective in decreasing rate, whereas the effect of TTX was intermediate. Epinephrine markedly increased the automaticity of the atrial fibers.

Delayed Afterdepolarizations and Triggered Activity

In 12 preparations, discontinuation of the drive stimulus was followed by the appearance of delayed afterdepolarizations after the last driven action potential. These occurred in six fibers from group A, five fibers from group B, and one fiber from group C. The transmembrane potential characteristics for these 12 fibers on development of delayed afterdepolarizations were action potential amplitude, 70.9 ± 2.9 mV; AV, -43.3 ± 2.1 mV; and MDP, -62.5 ± 1.9 mV. The rate of the automatic rhythm occurring at the time of appearance of the delayed afterdepolarizations was 26.2 ± 2.7 beats/min. The amplitude of the delayed afterdepolarizations was 13.5 ± 3.0 mV. In these preparations, small increases in stimulus rate or occurrence of a single premature depolarization (spontaneous or stimulated electrically) could trigger the fiber into sustained rhythmic activity of a higher frequency (rate: 62.8 ± 4.0 beats/min). In all instances, the first triggered action potential arose at or near the peak of a delayed afterdepolarization (Fig. 3).

Hashimoto and Moe (1973) showed that ACh decreased the magnitude of digitalis-induced delayed afterdepolarizations in canine atria. We compared the effects of AHR and ACh on non-digitalis-induced delayed afterdepolarizations in five experiments. Figure 4 shows that both AHR and ACh abolished these delayed afterdepolarizations. Whereas AHR did not alter the MDP, ACh hyperpolarized the membrane (P < 0.05). Of particular note is that, in the presence of only a slight subsequent decrease in membrane potential, there was nevertheless a recurrence of delayed afterdepolarizations in the presence of ACh. Hence, the suppression of delayed afterdepolarizations in the presence of ACh was transient and accompanied an initial increase in membrane potential. In the presence of AHR, the suppression of delayed afterdepolarizations was persistent and not related to a change in membrane potential.

Epinephrine-Induced Delayed Afterdepolarizations and Triggered Activity

We studied 22 fibers in which stable automatic activity resulted from slow depolarization during phase 4 and in which neither delayed afterdepolarizations nor triggered activity could be demonstrated during superfusion with Tyrode's solution. We superfused these fibers with Tyrode's containing epinephrine, 1 × 10^{-4} M, because lower concentrations induced delayed afterdepolarizations only inconsistently, and at 1 × 10^{-4} M delayed afterdepolarizations and triggered activity invariably occurred (Table 4). In the presence of epinephrine, the MDP increased and delayed afterdepolariza-

![Figure 3](http://circres.ahajournals.org/FIGURE 3Delayed afterdepolarizations (DAD) and triggered activity in human atrial fiber. Note the occurrence of DAD and phase 4 depolarization in the same cell. The first triggered AP (the sixth action potential on the trace) arises near the peak of an afterdepolarization.)

![Figure 4](http://circres.ahajournals.org/FIGURE 4 Effects of AHR and ACh on five preparations in which delayed afterdepolarizations and automaticity occurred spontaneously. AHR suppressed delayed afterdepolarizations and did not change the MDP. ACh also suppressed the afterdepolarizations and hyperpolarized the membrane, but ACh failed to prevent the reappearance of delayed afterdepolarizations later in the superfusion.)
The initial value of 2000-4000 msec. At each cycle length was decreased progressively from an initial cycle length of 1150 msec. The stimulus was discontinued after the third beat. In B (cycle length, 700 msec), the stimulus is discontinued after the fourth beat, and the amplitude of the delayed afterdepolarization was increased.
Effects of stimulus cycle length on the amplitude of delayed afterdepolarizations. All preparations were superfused with epinephrine, $1 \times 10^{-4}$ M. Each symbol designates a different preparation and each point represents the mean ± SE for five cells in the preparation. In five preparations, delayed afterdepolarization amplitude increased progressively as cycle length decreased. In one preparation (filled triangles), cycle length was decreased to 2000 msec before afterdepolarization amplitude began to increase. The shortest cycle length shown for each experiment is the cycle length which induced triggered impulses. The addition of epinephrine, $1 \times 10^{-4}$ M, to the bath. There were no significant differences among the control values for the different subgroups. There were no significant effects of ACh, lidocaine, or TTX on delayed afterdepolarization amplitude or triggered rhythms. The results of these experiments are summarized in Figure 9. The controls for this figure are values obtained for delayed afterdepolarizations and sustained rhythmic activity after the addition of epinephrine, $1 \times 10^{-4}$ M, to the bath.
TTX on the magnitude of the delayed afterdepolarizations that occurred or the rate of the sustained rhythmic activity. However, in the fibers superfused with verapamil or AHR-2666, delayed afterdepolarizations could not be induced and the sustained rhythmic activity that occurred was the result of effects of epinephrine on phase 4 depolarization and automaticity rather than on the triggered activity observed in the control. The spontaneous rate following verapamil and AHR was significantly lower than the control rate that was due to triggered activity (P < 0.02).

Discussion

We have demonstrated the occurrence of two types of sustained rhythmic activity in atrial fibers from normal and diseased human hearts. One rhythm is automatic and depends on slow diastolic depolarization during phase 4. The other is triggered by delayed afterdepolarizations and occurs either spontaneously or as the result of epinephrine superfusion. Moreover, we have shown that these two types of sustained activity can occur in the same cell over a narrow voltage range. Intrinsically induced depolarization of fibers need not be invoked to explain the occurrence of triggered or automatic rhythms in fibers such as these. As suggested by Cranefield (1975, 1977), triggering, when it occurs, could be intrinsic rather than extrinsic and the inciting influence for ectopic activity in a single cell could come from that cell itself.

The membrane potentials at which both phase 4 depolarization and delayed afterdepolarizations were observed ranged from −70 to −40 mV. These are within the range of atrial pacemaker potentials reported by Noble (1975) but are lower than the pacemaker potentials reported by Gelband et al. (1977) in normal human atrial specialized fibers during exposure to epinephrine. However, differences in technique may explain the differences in our results and those of Gelband et al. Gelband et al. stimulated the fibers they studied at a cycle length of 1000 msec and interrupted stimulation only briefly to record pacemaker activity. In our preparations, electrical stimulation had been discontinued prior to the study of automaticity and delayed afterdepolarizations. The decreases in resting potential and maximum diastolic potential that occurred on discontinuation of the drive may be explained by assuming that active sodium extrusion was reduced (Carpentier and Vassalle, 1971). Similarly, canine coronary sinus fibers have been reported to lose resting potential when they remain quiescent (Wit and Cranefield, 1977). With respect to the low resting potential that occurred in the diseased atrial fibers, Ten Eick and Singer (1979) have suggested that K+ conductance is not the principle determinant of this variable in diseased human atrium.

The ionic mechanisms for slow diastolic depolarization and delayed afterdepolarizations in atrium are still controversial. Whereas, in normally polarized mammalian Purkinje fibers, automaticity currently is said to be governed primarily by a time-dependent decay of a voltage- and time-dependent potassium current iK2 (Noble, 1975), Brown and Noble (1969a, 1969b) suggested that diastolic depolarization in frog atrial cells is produced by the decay of an outward current similar to iK1 against a steady background inward current. More recently, Brown et al. (1976a, 1976b) described three outward currents with some degree of inward-going rectification. Since there is a substantial difference between the pacemaker range of potentials in the human atrial fibers in this study and normal Purkinje fibers, it may be that pacemaker activity in these atrial fibers is controlled by a K+ current resembling iK1 rather than iK2, against the background of a steady inward current (Cranefield, 1975; Noble, 1975). Pacemaker activity also could be due to an anomalously rectifying time-independent K current (iK1) (Noble, 1975).

The currents underlying delayed afterdepolarizations have been studied by Tsien and his associates. Tsien and Carpenter (1978) reported that such afterdepolarizations induced by digitalis result from a transient inward current that either is absent or immeasurable in Purkinje fibers not treated with digitalis. This current, which they interpret as superseding the normal iK1 pacemaker, is carried in the main by Na+, although its effect appears to be mediated by the oscillatory release of Ca2+ from intracellular stores. The characteristics of digitalis-induced delayed afterdepolarizations in mammalian Purkinje fibers (Rosen et al., 1973; Ferrier et al., 1973) are generally similar to those we have reported in digitalis-toxic human atrial fibers (Hordof et al., 1978) and to those which Hashimoto and Moe (1973) reported for canine atrium. However, they are, in one way, dissimilar to the characteristics of the delayed afterdepolarizations that we report in this study; that is, when digitalis induces delayed afterdepolarizations in canine Purkinje fibers and canine and human atrium, the cycle length for peak magnitude and triggering usually is ≥700 msec (Ferrier et al., 1973). In contrast, the peak magnitude of the delayed afterdepolarizations and the occurrence of triggering in the present study occurred at cycle lengths of 450–1750 msec. These differences are sufficient to lead us to speculate that either different mechanisms, or different kinetics, or both, are involved in producing non-digitalis and digitalis-induced delayed afterdepolarizations.

The use of pharmacological methods provided us with a means to study further the sustained rhythmic activity in human atrial fibers. As expected, the slope of phase 4 depolarization was greatly enhanced by epinephrine (1 × 10−6 M). This effect also has been observed in frog atrial fibers in which an increase in a component of iK may be responsible for the hyperpolarization and an increase in slow inward current may account for the
enhanced spontaneous activity (Noble, 1975; Brown and Noble, 1974). Higher epinephrine concentrations (1 × 10^{-4} M) were necessary to induce delayed afterdepolarizations and triggered activity in human atrial fibers. The slow channel blockers AHR (Siegal et al., 1978) and verapamil (Cranefield, 1975) decreased the slope of phase 4 depolarization markedly at concentrations which did not affect the maximum diastolic potential, an action of these drugs that has been reported previously (Siegal et al., 1978; Wit and Cranefield, 1974). Verapamil and AHR also reduced the magnitude of spontaneous and epinephrine-induced delayed afterdepolarizations, a finding consistent with that reported for verapamil (Cranefield, 1977). It is important to note that, in preparations superfused with AHR or verapamil, epinephrine failed to induce delayed afterdepolarizations but was capable nevertheless of inducing phase 4-dependent automaticity. Although it is possible that higher concentrations of AHR and verapamil might have suppressed phase 4 depolarization here as well, such concentrations also depressed the action potential and therefore were not studied. It is reasonable to interpret these observations as indicating that there are differences between the current(s) required to initiate afterdepolarizations and the currents required for phase 4 depolarization in these fibers.

Agents that increase potassium conductance, lidocaine (Arnsdorf and Bigger, 1972) and ACh (Noble, 1975; Rayner and Weatherall, 1959), decreased the slope of phase 4 depolarization and automaticity. Lidocaine has been shown to increase \( i_{K1} \) and to decrease the background inward current of sheep Purkinje fibers (Weld and Bigger, 1976). Although lidocaine did not induce hyperpolarization, ACh did so, and decreased not only automaticity but also the magnitude of the delayed afterdepolarizations. In interpreting this effect, it must be kept in mind that ACh reduces calcium conductance and inward current (Giles and Tsien, 1975; Ikemoto and Goto, 1975, 1977; Ten Eick et al., 1976). Whether it was a decrease in inward current induced by ACh or the hyperpolarization of the membrane itself that suppressed the afterdepolarizations is uncertain. In contrast to its action on spontaneously occurring delayed afterdepolarizations, ACh had no effect on epinephrine-induced afterdepolarizations. The sudden increase in rate on return to control solution and the reappearance of delayed afterdepolarizations during continuous superfusion with ACh might possibly be the result of catecholamine release.

As occurred with ACh, lidocaine and TTX did not alter epinephrine-induced delayed afterdepolarizations. The failure of TTX to depress these delayed afterdepolarizations is in contrast to its action on digitalis-induced delayed afterdepolarizations (Vassalle and Scida, 1979; Rosen and Danilo, 1980). This observation suggests that, even if delayed afterdepolarizations are caused by a transient inward Na^+ current (Tsien and Carpenter, 1978), the current that induces them in human atrium is relatively TTX insensitive and differs from that which induces automaticity.

In summary, agents that reduce calcium influx (verapamil, AHR) decreased automaticity and suppressed spontaneous and epinephrine-induced delayed afterdepolarizations more effectively than did lidocaine or ACh. Given these observations, it seems reasonable to conclude that both automaticity and delayed afterdepolarizations in human atrial fibers may be caused, at least in part, by slow inward current, and that a low potassium conductance is important for their generation. It must be emphasized that the inward current associated with phase 4 depolarization in human atrium may well be different from the inward current involved in the genesis of delayed afterdepolarizations. This interpretation is strengthened by the results obtained with TTX. Although TTX decreased automaticity, it had no effect on the delayed afterdepolarizations. That TTX induced a decrease in automaticity is somewhat perplexing because of experimental evidence that slow sodium entry is not blocked by TTX, whether this is a slow sodium component of the plateau (Shigenobou et al., 1974) or a component of a delayed afterdepolarization (Tsien and Carpenter, 1978). However, more recent studies have shown that TTX blocks a background sodium current that contributes to the plateau of the Purkinje fiber action potential (Coraboeuf et al., 1979) and depresses ouabain-induced delayed afterdepolarizations in Purkinje fibers (Rosen and Danilo, 1980). In addition, in lobster and squid axons, TTX has been shown to decrease the resting membrane conductance to sodium (Narahashi, 1974). If these phenomena occur, as well, in human atrium, they might explain the action of TTX on automaticity.

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References


Brown HF, Clark A, Noble SJ (1976b) Analysis of pacemaker...
and repolarization currents in frog atrial muscle. J Physiol (Lond) 258: 547-577


Finney DJ (1964) Statistical Methods in Biological Assay, ed 2. London, Griffin


Hashimoto K, Moe GK (1973) Transient depolarizations induced by acetylchophosphatidin in specialized tissue of dog atrium and ventricle. Circ Res 32: 618-624


Wit AL, Cranefield PF (1974) Effect of verapamil on the sinoatrial and atrioventricular nodes of the rabbit and the mechanism by which it arrests reentrant atrioventricular nodal tachycardia. Circ Res 35: 413-425

Mechanisms for impulse initiation in isolated human atrial fibers.
L Mary-Rabine, A J Hordof, P Danilo, Jr, J R Malm and M R Rosen

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